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Review Papers

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PREFACE

Forensic Science continues to play an ever increasing role in solving crimes and assisting investigations throughout the globe. For years, INTERPOL has fostered international police cooperation by facilitating the sharing of valuable forensic science information and data. The 17th Triennial INTERPOL International Forensic Sciences Managers Symposium serves as a unique opportunity for forensic science managers across the world to share and exchanges ideas and best practices.

The purpose of the symposium is to bring together senior managers from member states in a forum that facilitates:

- the presentation of advances made in scientific methods over the previous three (3) years, and to provide a look into future forensic needs and advances;
- the exchange of information which will enhance scientific methods in criminal investigation and the administration of justice;
- the discussion of problem areas encountered by member states and the possible provision of solutions; and
- the exchange and pooling of ideas for future progress.

The symposium *Proceedings* on this CD concentrate on the Review Papers prepared by the Coordinating Laboratories, which highlight and summarize advances in the various evidence types. The various evidence areas are grouped into five (5) major categories:

- **Criminalistics**
  - Firearms;
  - Toolmarks;
  - Paint and glass;
  - Fibers;
  - Forensic geology

- **Identification Sciences**
  - Fingerprints.
  - Biological evidence (DNA);
  - Document examination

- **Media Evidence**
    - Audio Visual;
    - Video;
    - Imaging;
    - Digital evidence

- **Forensic Chemistry**
    - Fire investigation;
    - Explosives;
    - Drugs; and
    - Toxicology

- **Management**

In addition to the discipline specific reviews, three (3) thematic sessions will be held at the 17th IFSMS on the following topics:

- *Management,*

- *Scene of Crime, and;*

- *State of the Art Forensics*

The 17th IFSMS is only possible with the support of Interpol and the General Secretary, Ronald Noble. Interpol staff coordinated all aspects of Interpol's involvement to include distributing meeting announcements; organizing registration; arranging the meeting venue, and publishing the meetings' s proceedings. In particular, the Organizing Committee is grateful for the efforts of Mr. Simon Dzidrovski, Mr. Antonio Farelo and Dr. Serge Eko who worked closely with the Organizing Committee in every step of the process

Also, IFSMS would not be possible without the significant work of the Organizing Committee, each Coordinating Laboratory and the review paper authors.

Lastly, a special acknowledgment goes to Prof. Niamh Nic Daéid who compiled and edited the submitted papers and prepared the proceedings that are contained on this CD.

Nelson Santos
Chair
17th IFSMS Organizing Committee

CRIMINALISTICS

Examination of Firearms

Review: 2010 to 2013

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Introduction

This review paper covers the advances in scientific methods and general discussions applied to firearms reported since the 16th International Forensic Science Managers Symposium in October 2010.

A literature search was conducted covering articles published in the main forensic journals since mid-2010.

1. Firearms Identification

Strengthening the scientific foundations of firearms identification should be an ongoing activity for all examiners. Following the recommendations made in the National Academy of Science's 2009 report (1) several articles have been published.

Saks has opted for three research strategies which could prove a step forward for the identification sciences (2), namely;

a) The DNA model
b) The black box model
c) The basic research model

The DNA model would focus on setting-up databases to assess the variation of attributes in a reference population. Enabling the examiner to determine the probability of an incidental match. Following the black box model the assessment would still be a subjective one performed by an examiner. But the accuracies of the different types of examinations should be well studied and reported. By performing specific studies to test hypotheses based on beliefs about the nature of evidence, these beliefs may turn out to be correct or incorrect. An example of the basic research model approach is the comparison of the frequencies of consecutive matching striae in pairs of bullets that are known matches versus known non-matches. Through this model – somewhere in between the DNA and the basic research model – the error-rate of a minimum number of consecutive matching striae can be stated (2;3).

The problems of the lack of a strong scientific foundation in firearms investigation are also seen in court. The transformation in the admission of forensic identification evidence in the United Stated following the U.S. Supreme Court decisions in *Daubert v. Merrell Dow Pharmaceuticals Inc.* and *Kumbo Tire Co. Ltd. V. Carmichael* shows a number of exclusion and limitations of forensic evidence. A total of 37 (17.9%) of all analysed challenges to firearms and toolmarks testimony resulted in an exclusion or limitation of the evidence by the court. For these cases, reliability concerns

were mentioned in 52.8% of the cases (4). The given reasons for the exclusions or limitations of forensic identification evidence in court could be used to refocus the research and to adjust applied methods to overcome the criticism. Exclusions and limitations were specifically based on unfounded statistics, error rates and certainties, a failure to document the analytical process and of following standardised procedures, and the existence of observer bias (5).

Apart from the possibilities of future research, changes in the overall approach of interpreting and concluding forensic evidence might help in showing the restrictions of firearm examinations. Changing conclusions from an 'absolute certainty' to a 'practical certainty' has been proposed (6), but this will not solve the main issues. A fundamental change from concluding a categorical or an inconclusive opinion to a reflection of the evidential value under two hypotheses following Bayesian inference might give the court a better understanding of the actual evidence given by the firearms examiner (7).

As an example Wevers et al. studied a potential model to increase the objectivity of the interpretation of toolmarks through the use of both consecutively matching striae (CMS) and Bayesian inference. Given the probabilistic nature of the data, standard statistical thinking suggests that Bayesian inference is likely to be the most powerful method for interpretation. The resulting likelihood ratios from the used model show some, but incomplete separation between known match and known non-match conditions. Although promising, these results are thought to represent the limitations of the CMS summary of the complete striae pattern and the limitations of the modelling used (8).

1.1 Validation studies and statistical foundations

Traditionally, validation studies within the field of forensic firearms examination have been based on:

a) Reproducibility of markings
b) Individuality of markings

1.1.1. Reproducibility of markings

Reproducibility has been studied by shooting large amounts of ammunition through one firearm and comparing the markings to check whether they stay the same.

Mikko et al. states that it was possible to find sufficient matching individual striations in the bullets after firing 20.000 rounds through a M240 machine

gun barrel, but that after 10.300 rounds some of the prominent striae started to change significantly (9).

Grom & Demuth studied the reproducibility of a Glock firearm. Showing that the IBIS system was able to properly correlate the known matches within the top twenty percent results entered in the system. The study showed that the breechface and firing pin markings didn't significantly change over 500 rounds (10).

Before and after castings of the chambers of one FN Browning model High Power and one Hi-Point model C pistol showed that the toolmarks in the chambers didn't change significantly over 1.440 rounds. Finer markings may have filled more rapidly with deposits than the gross chamber markings, masking details in the cartridge cases from around the 100th round. The markings were identifiable up to 960 rounds (11). The material of the cartridge case had a large influence on the availability of striae.

1.1.2. Individuality of markings

Individuality has been studied by comparing the markings in cartridges cases and bullets fire by consecutively produced firearm components.

A study based on ten consecutively finished Hi-Point model C9 slides showed that the variations in the breechface marks resulting from the process of sanding were unique and identifiable. A blind test performed by the author showed that no mistakes were made (12).

Fadul studied the individuality of Glock EBIS barrels. Fifteen questioned bullets were matched to one of the groups of two known test fires from ten consecutively produced barrels, by 183 examiners. There were 11 incorrect answers made by a total of 7 examiners. The error rate for the comparison of bullets fired through Glock EBIS barrels was established to be 0.4%. As a limitation the author states that not all of the barrels showed the same barcode-like patterns although they were consecutively produced (13).

A study based on obturation markings from ten consecutively reamed chambers from three manufacturers (Ruger, Kel-Tec and Hi-Point) showed that 178 out of 192 comparisons resulted in a correct identification, 11 in an inconclusive opinion and 3 in an incorrect identification. A total of 64 examiners participated in this study (14). These types of blind examinations fulfil the demand for known accuracies as proposed by Saks' black box model. To be able to relate the resulting error rates, double blind testing (the examiners does not know he is being tested) will be even better.

Sarıbey & Grace Hannam studied the markings left by ten pistols with consecutive serial numbers from two Turkish pistol manufacturers. They state that for each make of pistol (Kırıkkale and Fatih 13) the individual

characteristics within the firing pin impression, the ejector and the breechface markings of all ten pistols were significantly different (15).

1.2 Parameters that affect the identification process

While comparing markings left on spent bullets or cartridge cases different parameters could influence the quality and quantity of these markings. During the last years a few studies have been performed on different parameters.
When focussing on striae transferred from a barrel to a bullet the construction of the bullet plays a major role. James studied two non-lead rimfire cartridges manufactured by CCI/Speer and Winchester. The .22 Long Rifle Winchester bullets, made of tin, appeared to take matchable striae from the rifling lands. The .22 Long Rifle CCI bullets, from compressed powdered-copper don't take any matchable striae (16).

Chumbley et al. studied the influence of different ammunition manufacturers and the primer hardness on the transfer of microstamped identifiers from the firing pin. They illustated that both brand of ammunition and type of firearm play a role on the transfer. But no primary parameter of the ammunition could be identified as ensuring complete identifier transfer. Lacquered ammunition showed to degrade identifier transfer (17).

In addition to the previous study the possibilities of using scanning electron microscopy (SEM) to distinguish the alpha-numerical identifiers as well as the gear code structure was investigated. The authors say that this technique showed a good optical imaging of the microstamped identifiers from the firing pins (18).

1.3 Identification based on unusual markings

When comparing markings on spent bullets and cartridge cases there are a few aspects which can be considered, namely;

a) What is the source of the markings?
   1) From a firearm component?
   2) From the ammunition?
   3) From the manufacturing of the ammunition?

b) How was the firearm component responsible for the markings produced?

c) Is there any subclass present in the markings?

All mentioned aspects play a roll in the interpretation of the evidential value of the compared markings.

1.3.1 What is the source of the markings?

When comparing markings left on bullets and cartridge cases it should be considered whether the markings are the result of the firearm or whether they result from the ammunition itself or were already present on the cartridge before firing due to manufacturing of the cartridge.

1) From a firearm component?

A number of articles was published on comparisons based on peculiar markings or on previously unpublished markings resulting from firearm components.

Barrel
Windsor states that it is possible for a smooth bore firearm (Winchester model 37, calibre .410) to produce striated patterns on bullets that are reproducible and sufficient for comparison (19).

Additionally a case report from Pendleton et al. states that is was possible to compare the markings left by a rough spot on the muzzle of a sawed-off .410 shotgun with those on three 000 Buck pellets. Due to different compressions of the pellets during firing it was even possible to determine the order of the pellets (20).

In contrary to a rough spot on the muzzle of a sawed-off shotgun used for identification, a choke tube might be the cause of an erroneous elimination. Firing .45 Colt calibre ammunition through a Thompson/Center Arms Contender model single shot pistol with the choke tube in place - enabling the pistol to fire .410 bore shotshells – will cause the bullet to deform significantly. And might even cause the rifling characteristics to be different (21).

Comparison of the striae present on the plastic bases of two successively fired 40mm less lethal projectiles illustrated that it was possible to match the markings present in the land engraved areas (22).

Even though the questioned bullets had very poor rifling impressions that were devoid of nearly all fine striae Collins was able to relate them to the barrel of a Russian Nagant M1895 revolver. The large amount of lead fouling in the barrel produced markings on the test fired bullets having the same general appearance as on the questioned bullets. In combinations with axial engravings on the compared bullets and silicone casts from the revolver’s bore the author was able to make an identification (23).

McCombs stated that is was possible to use striae left by the chamber throats of various types of firearms for comparison. The resulting striae from the chamber throat will run parallel to the axis of the bullets and not

with the rifling and might be seen in both land and groove engraved areas (24). Similar striae were found in the bullets fired from a FEG 9mm Luger pistol. In this case the beginnings of the striae were already present when just chambering the cartridge (25).

Ejection port

Ejection ports might transfer markings to spent cartridge cases, but also to unfired cartridges. The striae present on the bullet's ogive from a .22 Magnum cartridge could be matched to the ejection port markings from a .22 Magnum calibre Stirling Model 15 bolt-action rifle (26).

Breechface

Breechface markings are usually referred to as markings from a firing process. Clow showed that they can also be transferred to an unfired cartridge by chambering the cartridge. This was seen in Hi-Point firearms where the impressions were probably caused due to the mass of the slide or breech block and the strength of the recoil spring (27).

On the rim of cartridges chambered in Glock and Smith and Wesson Sigma pistols it is often possible to find striae. These reproducible cycling markings are the result of the left underside corner of the breechface recess when the cartridge is released from the magazine lips (28).

Similar striae from the breechface recess were also seen on cartridges fired by Walther model P99 pistols. Azahidi performed an reproducibility study with five pistols. The results showed that after 742 to 802 rounds the breechface recess toolmarks retained their characteristics (29).

Although breechface marks are usually transferred from the breechface to the cartridge it might also be the other way around. A negative of the cartridge case headstamp impression was discovered on the breechface after test firing a rusty homemade submachine gun (30). This type of crossover might be expected in breechfaces made from softer metals.

Carrier

A case report by Hunsinger showed that the carrier of a Maverick by Mossberg model 88 shotgun had a couple of defects. Striae resulting from this defect could reproducible be found on cycled shotgun shotshells (31).

Firing pin hole

Zidon et al. published on striae produced during chambering of a cartridge in a FEG pistol model WALAM 48, calibre 380 APC. The upper circumference edge of the firing pin hole created striae at the 12 o'clock position on the base of the cartridge. These striae can be used for comparisons (32).

Miscellaneous
Giverts et al. gives an overview of possible extraneous markings – not resulting from lands and grooves - on projectiles. These markings can provide an investigator with information about the used firearm and the crime scene. Features such as the addition of elements to the barrel (silencers), ammunition manufacturing marks and intentional markings in the barrel are addressed (33).

2) From the ammunition?

A case report illustrated that it might be possible to match a lead core to its bullet jacket. Test marks were produced with the base of the jacket and compared to the striae present in the lead core (34).

Again matching jackets to other bullet components, Clow illustrated that it was possible to compare the impressed toolmarks on a copper disk associated with Prvi Partizan .40 S&W TMJ ammunition, with the jagged edges of the rolled over base of the bullet jacket. When no copper disk is present this type of markings from the jacket might also be transferred to a lead core (35).

3) From the manufacturing of the ammunition?

In certain brands and lots of 12 gauge shotgun shotshells parallel manufacturing markings are present on the primers. This markings show subclass characteristics from the original tool. The Integrated Ballistics Identification System (IBIS) is not able to differentiate between these manufacturing markings and markings transferred by the breechface of a firearm. Without the interpretation of the IBIS technician this might cause erroneous matches (36).
Parallel manufacturing markings are not only seen on the primers of shotgun shotshells, but are also seen on calibre .30-06 Golden Bear ammunition (37) and on the base of .45 AUTO (38) and .357 SIG Speer ammunition (39). When unknown by an examiner these markings could be interpreted as breechface markings from a firearm.

Irregular impressions on the primers of unfired calibre 9mm Luger Winchester cartridges were thought to be the result of the manufacturing process, but turned out to be caused afterwards during packaging and marketing (40).
Raised concentric rings were noted on calibre 25 ACP and .40 S&W cartridges from CCI Blazer. These rings are fairly similar to fingerprint ridge details but are the result of the final washing process during manufacturing (41).

Production toolmarks – such as markings from drawing, primer pocket formation, headstamp formation and flash hole formation -can be used to

determine whether the cartridges originated from the same production line, within a (relatively) short period of time. Markings found in crime scene cartridge cases might be matched to those present in cartridges found in the house of a suspect (42).

1.3.2 How was the firearm component responsible for the markings produced?

In order to make a sound assessment on the evidential value of found matching and non-matching markings it is of importance to understand how the tool (firearm component) creating the marking was produced.

General
The effect of the machining process on the discriminative power of toolmark surfaces is explained by Monturo. The resulting markings left by a tool on a firearm component are the result of an interaction between the wearing of the cutting edge of the tool, the built-up edge of the material being removed and the machining conditions such as feed rate, cutting speed and vibrations (43).

Furthermore grinding of tools introduces a surface topography of a random nature. The 'self-sharpening' grinding wheel gives an essentially infinite combination of topography due to self-sharpening, plowing (plastic deformation), side flow and vibrations (44).

Rifling
Through a literature search and direct contact with the manufacturers Smith has made a list of over fifty different companies and their rifling methods. Broach, button, hammer forged, electrochemical, hook and scrape methods have been studied. The broach and the button method combined accounted for over 75% of the 1.7 million firearms imported/manufactured in the United States in 2007 (45).

Bolton-King has written an article on the manufacturing methods of SIG Sauer 9mm Luger pistols. The different component of the pistols are highlighted with special attention on the two used methods of rifling. The land transition profiles from the hammer forged and the electrochemical rifled barrels differ significantly (46).

Metal injection molding
A fairly new production manufacturing method used for firearm component is metal injection molding (MIM). This is a multi-step process that combines metal powders into a solid metal part through molding, debinding (removal of polymer or paraffin binder through evaporation by heating) and sintering (heating to a temperature near the melting point of the alloy, which hardens the resulting component (47).

Based on a molding technique MIM has a potential to introduced subclass characteristics in the resulting components. Comparisons of the markings of five extractors from a calibre .40 S&W M&P pistols illustrated that there was some presence of subclass. The electropolish finish or the melonite process after production of component seemed to diminish the possibility of subclass (48).

The extractors from Para-Ordnance are also the result of MIM without additional finishing. Other component of their firearms are either finished by pressing, heating and tumbling or are made by different methods such as cut-broaching of barrels, and slides are cast and the machined by CNC machine (49).

1.3.3 Is there any subclass present in the markings?

According to the 5th edition of the AFTE Glossary subclass characteristics are defined as:

"discernable surface features of an object which are more restrictive than class characteristics in that the are:

- Produced incidental to manufacture
- Are significant in that they relate to a smaller group source (a subset of the class to which they belong)
- Can rise from a source which changes over time
- Examples would include: bunter marks, extrusion marks on pipe, etc." (50)

Lightstone studied the breechfaces of SW40VE Smith & Wesson Sigma pistols. From Mikrosil casts from the breechfaces of ten slides it was found that they showed gross subclass characteristics resulting from the broach that produced them successively. Not all of the markings transferred to the spent cartridge cases and additional fine striae were visible enabling the examiner to differentiate between the firearms. It is speculated that the granular finish caused by a combination of the hand-sanding, hand-blasting and glass-beading individualised the otherwise subclass characteristics on the slides, because the abrasives changed the planar structure of the surface of the toolmarks on the breechfaces (51).

Subclass characteristics were also present in the firing pins from Smith & Wesson, SW40E and SW9VE Sigma pistols. A seemingly characteristic marking in the firing pins was found to be similar in a pistol of both models. The firing pins are produced by Metal Injection Molding (52).

1.4 Proficiency testing

The ENFSI Expert Working group Firearms/GSR sent out the 2nd edition of their proficiency test. The test consisted of ten sets of castings, from which five contained bullets and five contained cartridge cases. Each set consisted of one questioned item and two known items from the same firearm. Sixty-four laboratories (mostly European) returned the answer form, giving a total of 637 conclusions (the three missing conclusions were from examiners who submitted the specific test sets). In total twenty-six conclusions (4%) were false identifications and thirteen conclusion (2%) were false exclusions (53).

To ensure that every examiner is provided with exactly the same markings to compare castings can be used. The quality of the polymer replications from two Standard Reference Material (SRM) bullets produced by the National Institute of Standards and Technology (NIST) have been tested. Using the Max phase correlation scores no significant difference in the imaging performance between the SRM bullets and the replica bullets was found (54).

Collaborative Testing Services, Inc. remains well known as a provider for their interlaboratory tests. From their website new test can be ordered and reports from past tests can be downloaded (55).

1.5 Instrumental methods

Forensic firearms examinations are traditionally based on the fairly subjective comparison work of people. Although these examiners are highly trained the call for more objective methods keeps coming up. Different approaches to objectify the firearm comparisons through the use of 2D and 3D instruments have been studied in the past years.

Pyramidal Technologies Ltd. introduced a portable, measurement instrument and analysis tool to create, compare and analyse 3D volumetric models of fired cartridge cases and bullets; Advanced Ballistics Analysis System (ALIAS) (56).

Furthermore different studies have been performed to assess the available 2D and 3D techniques and their potential in forensic firearms comparisons. Gerules et al. give an overview of the nowadays available systems. The necessary steps to reach a conclusion and the validity of the methods are described. They conclude that due to the large amount of variations within the firearms field the available techniques are still in their early stages (57).

Bolton-King compared four available 3D imaging techniques for their potential application in forensic firearms and toolmarks comparisons. She concluded that from the four studied techniques – point laser profilometry,

vertical scanning interferometry, confocal microscopy and focus-variation microscopy – the latter two were the most promising for the forensic field. From these two, focus-variation microscopy might by the most promising because of its relatively low-cost access to 3D technologies (58).

Three types of microscopy were addressed for the comparison of the markings in a 9mm Luger bullet fired from the polygonal rifled barrel of a Glock 17 pistol: optical microscopy, comparison scanning electron microscopy (CSEM) and virtual (confocal) microscopy. Optical microscopy resulted in an 'inconclusive opinion', CSEM in a positive identification and virtual microscopy in the conclusion that 'regions of interest and commonality' were found. They conclude that optical microscopy is the most efficient, CSEM the most advanced but hardly ever necessary and that virtual microscopy is promising but needs more development (59).

Scanning electron microscopy is also addressed as a possible supplement for the traditional optical microscopy. The high magnification, the large depth of field and the independence from oblique lighting issues might make it possible to perform better comparisons (60).

Although new 2D and 3D techniques might be promising, one of the main issues in automated comparisons is the huge amount of data that needs to be compared. Chu et al. have proposed a way to pre-assess the availability of striae in bullet land engraved areas. Through an automated determination of the *striation density* a bullet can be assessed by a quantitative criterion as having sufficient or insufficient striae for reliable identification (61).

A study using effective correlation area based method for cartridge case image matching shows that the proposed method has a high discriminative power. The authors advocate that the method will enable forensic science to compile image database on a large-scale to perform correlation of the markings present in cartridge case bases (62).

Another study focuses on the automated segmentation of the markings present on the base of the fired cartridge case. This system is proposed as a preliminary step for the matching process. Based on a 3D image the firing pin impression and headstamp could be distinguished (63).

As a proposed objective method to support the subjective conclusion of an examiner the striae present in the matched land engraved ereas from bullets were translated to a barcode. The barcode was based on the distance of the striae from one shoulder of the land engraved area. Through Principle Component Analysis and Support Vector Machine error rates varied between 19.444% and 1.149%. The second result generated by the majority of the analysed bullets indicates the correct grouping based on

barcodes was possible, supporting the examiner's subjective identifications (64).

A study using the NIST Standard Reference Material focused on a quality system for ballistic identifications within the National Integrated Ballistics Information (NIBIN) of the U.S. Twenty-four periodic image acquisitions and correlations performed over a year were processed. This resulted in control charts and control limits for the proposed quality system and for promoting future assessments and accreditation for firearm evidence in accordance with the ISO 17025 Standard (65).

1.6 Court rulings

Firearms and toolmarks examiners in the USA are particularly prone to court challenges focusing on the scientific principles of the discipline. The Scientific Working Group Firearms (SWGGUN) is keeping track of these court rulings on their website (66).

2. Firearms & ammunition miscellaneous reports

2.1 Firearms

Class characteristics
The underside ejector markings, found on the rim of cartridge cases fired from Glock pistols, can be used as a means to differentiate the Glock's class characteristics from the class characteristics of the S&W Sigma pistols (67).
The class characteristics and their comparison value of the Israeli Tavor assault rifle, TAR 21, are discussed. Most class characteristics resemble the M-16 class characteristics, but they are distinguishable. The most prominent markings are the shape of the extractor cut-out mark, the presence of the neck mark and the ring around the firing pin (68).

Sound levels
In this final article of three on sound levels Haag presents some sound level measurements for unsuppressed and suppressed firearms fitted with professionally and home-made suppressors. The sound levels of supersonic bullets in flight near ear witnesses is discussed and common high amplitude sound levels are related to sound levels of gun shots. Earlier articles in the series describe the requirements for sound level meters and how to verify proper performance (part 1) and the effect of different variables on sound levels (part 2) (69).

Serial number recovery
Acid etching techniques do not work well when recovering obliterated laser etched serial numbers in aluminium alloy frames. Relief polishing might work properly when trying to recover these serial numbers (70).

Big bore airguns
Popularity of big bore airguns has risen in the United States. Two articles about their history, development and manufacturing discuss these airguns from a forensic viewpoint. Additionally two popular American made big bore airguns are studied and their rifling characteristics are given (71;72).

Hunting fatalities in Sweden
An overview of the unintentional firearm fatalities due to hunting between 1983 and 2008 in Sweden shows that there were 48 such fatalities. Restrictive firearm legislation combined with the introduced mandatory hunter's exam accounts, at least partly, for a decrease in the average number of fatalities over the last few decades. Of all fatalities, human error was found to be the main cause (73).

2.2 Ammunition

Chemical composition
Fragments of the plastic tips of bullets which might be found in wound tracts can be used for forensic class determination. Due to the high quality control used by the manufactures, discrimination between the polymers found in common commercially available plastic-tipped bullets is possible using colour. The authors made use of Fourier transform infrared spectroscopy analysis coupled to the statistical power of discriminate analysis and x-ray fluorescence spectrometry (74).

Apart from the plastic tips of bullets it is also possible to compare the chemical composition of bullet fragments to each other and to different ammunitions lots. The evidential value of matching compositions is highly dependent on the composition variability within and between different ammunition boxes (75). Another publication stated that XRF of the bullet core of 9mm Luger FMJ (DM1 1A1B2) ammunition was a good technique to determine variability between batches (76).

Compositional features
Compositional features of major ammunition parts can be used to differentiate between commercially and home-made cartridges. Some features such as markings from a lathe producing the cartridge case and of the headstamp can be used to trace the origin of the home-made cartridges (77).

Alpha characters
Alpha characters can be found on some of the bases of calibre .22 Remington bullets and inside the base of cartridge cases. Due to blending of the component prior to assembly these can not be used to match a particular bullet and cartridge case (78).

Crimp marks
While most of the discernible class characteristics of a fired and deformed bullet might be missing, the crimp marks could still make it possible to distinguish between a 9mm Luger or a .357 SIG calibre. The crimp marks are located further from the base of the bullet in .357 SIG bullets (79).

Reducing powder charge
In reconstruction work it is sometimes necessary to reduce the speed of bullets by reducing the powder charge. For rimfire cartridges the use of a kinetic bullet puller might be dangerous so another method was proposed. Filing a small slot on the side of the cartridge and opening the resulting metal foil with a surgical knife makes it possible to remove some of the powder charge. When the desired amount of powder has been removed the foil can be close again. Using this method the powder charge of a rimfire cartridge can be decreased in a safe way (80).

Ammunition components
Aspects of ammunition can be used to make a statement about the manufacturer of found ammunition components. Windsor addresses two new shotgun shotshells and their components (81), while Huang focuses on the properties of two different brands of expanding bullets (82).
A case report shows the match made between the black polyethylene particles recovered from a gun shot victim and the buffer material present in two Remington 12 gauge shotshells. The buffer material was compared with respect to size, shape, colour, texture, pigment distribution and chemical characteristics through microscopic and Fourier Transform Infrared Spectroscopy examination (83).

Jacket thickness variation
When testing a ballistic armour standard the specified reference projectile has to be defeated. Due to this working method it is important to be able to establish the variations in bullet jacket dimension, which could influence its effect upon impact. The use of non-destructive X-ray computed tomography is explored. Thickness variation in the order of up to 200 μm were found commonly across all the bullets along the length and an angular variation of up to 100 μm was found in a few bullets (84).

3. Legislation

Even though the existence of strict UK legislation concerning hunting with air rifles and pistols and legal power limitations on the possessions of air weapons (air pistol max 8 Joule, air rifle max 16 Joule), an increase in the number of deaths has been reported. The weapons are fairly easy to buy through mail order or on the internet and the pellets become more sophisticated and therefore more dangerous (85).

In Turkey blank cartridge firing pistols have often been used during the recent years. The arising problem as the result of removing the barrel obstruction after which projectiles can be fired from these firearms has led to new legislation. The 5729 Act (2009) concerning the manufacture and sale of blank cartridge firing guns has resulted in a reduced number of converted and unconverted pistols used (86).

According the Turkish law ‘mole guns’ used for pest control can be considered to be prohibited weapons provided they are of the correct dimensions. Between 2006 and 2008 eighteen ‘mole guns’ were examined of which thirteen were 12-gauge bore and five were 16-gauge bore (87).

4. Technical examinations

Technical examination of a firearm can be useful to be able to assess the evidential values of markings on spent bullets or cartridge cases. But apart from this it might also be very useful for the assessment of statements made by victims, eye-witnesses or suspects about the functioning of the firearm. Different articles were published on altered or self-made firearms and on the reconstruction of possible events.

4.1 Altered or self-made firearms

Sometimes the question arises whether a firearm is still functioning under its present conditions. One such an example is shown by Schreiner, examining a Springfield model XD-40 without a guide rod (88). Tunnel went a bit further, explaining and showing that with some additional support it is still possible to produce test fires with a firearm that has been cut into multiple pieces as long as the bare necessities are present (firing pin assembly, breechface and chamber) (89).

Missing grip plates of a calibre .380 Auto, Bryco model 38 pistol will prevent the firearm from firing when pulling the trigger. The cam will not properly engage with the sear necessary to release the hammer. When holding the pistol extra tight, enough external pressure will overcome this problem

ensuring a proper engagement causing the pistol to fire when pulling the trigger (90).

Apart from questions about the operability of a firearm technical examination can also visualise changes made to a firearm. One such an example is given by a Ruger revolver from which the barrel was replaced with a Colt barrel. This resulted in different rifling characteristics than would be expected (91). Changes could also result in the firearm being able to fire automatic instead of semi-automatic (92;93).

Without making changes to a firearm it might also be possible to use it in a different way than originally intended. For instance, it is possible the fire numerable different calibre cartridges with a smooth bore, calibre .410 Winchester model 37 (94).

When a firearm is not available it is also possible to manufacture one. An example of this is shown by Giverts et al., they examined an improvised shotgun which fired improvised ammunition. The ammunition consisted of rim-fire 6mm blank cartridges with an attached drinking straw filled with lead pellets (95). Other examples are the attempted conversion from a 25mm flare gun to a rifle (96) and a home-made 20-gauge firearm disguised as a Super Soaker (97).

The Aydin Regional Criminal Department sees a lot of home-made reproductions of commercially made firearms. In 2006, 2007 and 2008 around 13% of all their cases consisted of these firearms. Although they are reproductions the class characteristics of the firearms are usually very different from the original version (98).

Home-made and converted blank firing pistols are usually less reliable compared to commercially manufactured firearms. But still bullet velocities can be more than enough to penetrate the skin. The velocities might differ significantly from shot to shot and due to unstable bullets it is sometimes hard to predict the possibility to penetrate the skin (99).

Borgers & De Ceuster report on a fatal incident with a converted blank firing pistol in combination with 8mm Knall ammunition, fitted with a steel ball. Velocity measurement with this combination have indicated a high variance, but threshold energy densities for skin perforation are easily exceeded (100).

4.2 Reconstruction of events

When a statement is given about the accidental discharge of a firearm or when it malfunctioned it might by possible to investigate the possible cause of this by examining the firearm.

Haag showed that the point of a 7,62x39mm cartridge can function as a firing pin. When trying to chamber a second round from the magazine of a Chinese Type 56 into the chamber the point of the bullet hit the primer of the first cartridge. Upon discharge a fragment of the cartridge case was blown from the chamber and penetrated the victims chest cutting a major heart vessel. The second cartridge hit the first one in the centre instead of on the side due to damaged magazine lips of an aftermarket magazine (101). Haag also investigated an accident with a calibre 7mm Remington, Browning model 81L rifle. The use of fast burning pistol propellant in a hand loaded cartridge caused a peak pressure around 150.000 to 160.000psi instead of the designed peak pressure of 61.000psi. This caused the bolt of the rifle to dislodge (102).

Other publications on technical examinations on firearms focus on the possibility of a slide action shotguns – such as the Winchester model 1300 - to extract and eject shotshells without manually sliding the forearm when firing or dropping the firearm. This could cause the shotgun to work in a semi-automatic way (103;104).

Unexpected discharge of a firearm can be the result of the forces related to discharge or to alterations of the firearm. A double fire is possible with a Smith & Wesson model 500 revolver due to recoil. During recoil the hand moves slower backwards than the revolver. Contact with the trigger is (partially) lost, resetting the firearm. When trying to regain control the trigger might incidentally be pulled causing a second discharge (105).

Modification of firearm components might make them less reliable and safe. A bend firing block plunger of a calibre .45 AUTO Ruger model P90DC pistol might cause the hammer to be released when pulled slightly back in up-side-down position (106). Modifications to a Walther model P38, caused the hammer to be released when cocked in single-action due to a slight impact (107).

Trigger pull forces are sometimes measured to make a statement about the ease of firing. Lawrence & Lee state that for long guns especially the location of the applied force relative to the trigger’s axis of rotation and the direction of the trigger rotation influence the magnitude of force. A change in hand grip angle of 20-30° to the bore axis had no significant impact on the outcome of the tests (108).

Another force which can be applied is a force against the direction of travel of the slide, holding the slide closed. For a calibre 9mm Luger, Glock model 17 approximately 2 pounds ensures that the cartridge case will not be ejected from the firearm while firing (109).

Smith discusses how it would have been possible to find a fired bullet fragment imbedded in the frame of the rear of the sear, without external frame damage (110).

5. Shooting Incident Reconstruction

5.1 Research

While reconstructing a shooting incident a lot of factors have to be taken into account. Research within this field has especially focused on reconstructing bullet trajectories, bullet deformation and estimating shooting distance when shots are fired with a shotgun.

Bullet trajectories
For the reconstruction of a shooting incident the determination of the direction of impact plays a major role. Some layers of paint and sealant on for example sheet metal may cause the absence of the normally visible pinch marks and wave points (111).

When reconstructing bullet trajectories from irregular surfaces such as cars the use of baselines or the so called “boxing” of a car makes it possible to relate trajectories to fixed positions (112). By using this system the azimuth of trajectories can be measured by viewing from straight above on the intersection of the “box’s” string and the trajectory rod. This technique can be replaced by using a laser. When the trajectory is illustrated by a laser, two different locations along the trajectory can be used to make a laser ‘plump bob’ using mirrors. This technique allows the investigator to measure the azimuth between the two resulting points (113).

When reconstructing a bullet trajectory from a hole in drywall it is important to know that the ejection of displaced calcium sulphate from the exit side of a panel of drywall usually takes place orthogonal to the surface of the drywall and not to the trajectory of the bullet. Because the perforation of drywall hardly influences the speed and the deflection of the bullet a secondary impact can be used to determine the trajectory (114).

Comparable to the behaviour of drywall, the expelled glass fragments as the result from a perforation do not always follow the trajectory of the bullet. When shooting on glass from an angle the glass fragments will travel away orthogonally to the surface of the glass plate (115).

A study on the behaviour of bullets after water impact showed that the highest variability of azimuth angle after ricochet occurs at the lower post-ricochet velocities. The critical ricochet angles for the studied projectiles (K50 BMG, 0.5-cal Ball M2, 0.5-cal AP-T C44, 7,65mm Ball C21 and 5,56mm Ball C77) were ranging from 15° to 30°. The average ricochet angles (approximately 8° and 13°) were close for all projectiles at respectively 2,5 and 10° incident angles (116).

Bullet deformation

Haag and Jason explain and show why it is possible that no recognisable bullet (fragments) are found at a crime scene despite obvious bullet damage. Orthogonal impact of bullets ranging from lead air rifle pellets to .50-calibre projectiles all performed similar on unyielding surfaces once impact velocity exceeded approximately 600 ft/s. Soft lead bullets and at a higher velocity copper jacketed bullets effectively disintegrate upon impact (117).

Further research on the impact on unyielding targets has resulted in some equations where bullet deformation, velocity and strain are taken into account (118).

Orthogonal impact with handgun calibre ammunition on smooth unyielding surfaces can results in a mirror-like finish on the nose of bullets (119).

Shooting distance

Estimations of shooting distances based on pellet dispersion from shotguns have been studied by Arslan et al. They show that different parameters such as shot number, choke type and barrel length influence the dispersion. Furthermore they showed that the best estimations of shooting distances result from regression formulas fitted to a specific shotgun-ammunition combination. An overall regression formula resulted in a decreased precision of the estimation (120).
Impact patterns from pellets are not always complete. In case of incomplete patterns it might be possible to relate the visible distribution to the pattern distribution at different distances. Using a neural network trained on test samples a fine estimation can be made of the shooting distance (121).

When shortening the barrel length of shotguns no specific relationship between barrel length and the resulting distribution size was established. The influence of cutting off the choke of a shotgun is clearly visible (122).

Apart from the pellet distribution the wad drop-off might also be used to estimate the shooting distance. Different designs of wads seem to have an independent level of consistency that may be useful while estimating shooting distance (123).

Doppler Radar measurements
Because the drag coefficient C_d is the most important aerodynamic coefficient to enable ballistic calculations it has been studied how well the coefficient can be determined through Doppler Radar measurements. This study showed that is was possible for bullets that travel in the supersonic range. For bullets around Mach 1 and within the subsonic range the technique is not very reliable (124).

5.2 Case reports

Apart from studies on different parameters that influence reconstruction work some casework examinations also give useful information for future examinations.

Thompson examined a heavy padlock damaged by a bullet impact. At the centre of the impact a small hole was visible. Test showed that some hollow point bullets with a copper disk at the base show this phenomenon. Dr. Planka suggests that this might be the result of pressure and tension stress wave interference in target mass (the Hopkinson Effect) (125).

When reconstructing a crime scene it is sometimes possible to compare striae on a bullet to markings present in a hit surface. An example of this is shown by the impact on a shower door frame (126).

When an apparent suicide incident is found but the location of the firearm suggests that there might have been a third party present recoil test with the firearm might help. When firing, the recoil might cause the firearm to fly away for some distance when not held properly. This might explain the location of a firearm some distance away from a suicide victim (127).

In some cases wounds on a victim might be related to parts of a firearm. One such an example is given by the comparison of abrasions in the face of the victim with the rear sight of a pistol (128).

When determining whether a gun shot incident was the result of a suicide or a homicide it might be important to examine the hand of the victim. Two cases have been discussed were the firearm grip impressions were visible on the hand of the decedent. In one case a ‘negative’ of the grip pattern was visible and in the other case this was visible in dried blood (129).

Cascini et al. have written an article on an accident in which the victim was killed due to an overpenetrated bullet. The bullet perforated a wild boar after which it hit the victim aligned in the shooting path (130).

5.3 Trace analysis

Microtraces on bullets and on impacts can help in establishing a relation between a bullet and the impact site. Foreign material from the impact can be embedded or adhere to the bullet or the other way around. Examination using SEM/EDX on lead round nose and full metal jacket bullets fired into MDF, greenboard, gypsum fibreboard, glass and steel showed that in most cases traces of the target material were found on the bullet for both perforations and ricochets. Only perforation of MDF with FMJ bullets and ricochets on glass without breaking the glass didn't result in particles on the bullets. When shooting through multiple targets the sequence of perforation could be established by examining the deposition of the materials on the bullet. Traces of the bullets themselves can also be found on the target materials (131).

When bullets hit or perforate bone, particles of the bone might be imbedded in or adhere to the bullet. When the bone particles are large enough they might be recognisable using optical microscopy. Possible other, more objective methods to qualify the bone particles are SEM-EDS, polarised light microscopy and magnetic levitation (132).

6. Wound ballistics

During the examination of firearm related incidents multiple factors may be taken into account. The field of terminal ballistics is of importance when reconstructing the trajectories of bullets through the examination of deformation, perforations and ricochets, but there is also another source of information: wound ballistics. As a specific part of the terminal ballistics the results from examinations might give more insight in the lethality of firearms under specified conditions.

6.1 Research

Less-lethal ammunition
Less-lethal projectiles are used by agencies all over the world. A study using post-mortem human subjects showed that 50% risk of fractures occurred at 79.2 m/s on the forehead, 72.9 m/s on the temporal, 72.5 m/s on the sternum and 76,7 m/s on the tibia when using hybrid ammunition (133).

A review of the literature demonstrated that the feature of injuries appeared to be related to the type of less-lethal projectile. Less-lethal projectiles are meant to incapacitate but not kill, for which it is very hard to impossible to find an optimum fulfilling both criteria (134).

Simulants

To be able to test the effect of new less-lethal ammunition a surrogate was established to predict the risk of penetration. The 50% risk of penetration conditions established in previous studies were correlated with various combinations of materials. The validated surrogate consisted of a Laceration Assessmnet Layer of natural chamois and 0.6cm of closed-cell foam over a Penetration Assessment Layer of 20% ordnance gelatine (135).

For questions about lethality 10% ordnance gelatine at 4° Celsius is often used. A relatively new product, Perma-gel, has been tested. The author states that it allows easy and reliable testing of different types of pistol calibres (136).

The wound channels simulated in ordnance gelatine can be used to determine the energy transferred from a bullet to the surrounding tissue. The total crack length (TCL) can be used for this determination. The TLC can easily be obtained and measured using computed tomography (CT) (137).

Airsoft guns

The Criminal Code of Canada's definition of a firearm states that it is a barrelled weapon that is capable of causing serious bodily injury or death to a person. As a threshold, courts have used the criteria of "penetration or rupture of an eye". When using conventional 6mm airsoft ammunition the airsoft gun should be capable of achieving velocities in excess of 99m/s. The energy densitity parameter for a typical 6mm plastic airsoft projectile is 4.3 to 4.8 J/cm^2 (138).

The potential lethality of various airsoft guns using plastic pellets was studied according to the criteria posed by the Israeli law. For replicas of pistols, machine guns, assault rifles and bolt action rifles the muzzle velocity was measured. By calculating the available penetration energy of each pellet is was established that none reached the level of energy to penetrate or even superficially injure the skin (139).

Contact wounds

The bursting effect, defined as the disruption of at least 50% of the head due to contact wounds with a firearm occurred in 25 out of 35 examined cases. The effect was associated with available energy. The bursting effect occurred in 12 out of 22 case with energy <2700 ft-lbs and in 13 out of 13 cases with energy >2700 ft-lbs. The volume of gunpowder gas injected into the wound was considered as a contribution to the bursting phenomenon (140).

To test the wounding capacity of muzzle gases a test using acryl spheres filled with 10% ordnance gelatine was set-up. The damage along the bullet

path was compared between contact shots and shot from different distances (9mm Luger). Depending on the section of the bullet path, crack lengths were 31% to 133% longer in contact shots compared to larger distance shots (141).

Unconventional weapons and ammunition
When comparing typical guns shot wounding with the effect of captive bolt guns it is stated that in the latter no temporary cavity was observed. Nevertheless the transfer of kinetic energy to the head could cause secondary skull fractures in thin parts of the skull due to hydraulic burst effect (142).

The effect of scare guns used for scaring away menacing animals has been tested using gelatine. The possible injuries range from abrasions to contusions, lacerations and fractures (143).

The trauma potential of direct-acting, powder-actuated fastening tools (nail guns) has been studied. The average velocities ranged from 400 to 580 m/s, while average kinetic energy of the projectiles ranged from 385 to 547 J and mean energy density from 9 to 18 J/mm^2. These findings might make the comparison with ballistics parameters of calibre 9mm Luger pistols appropriate (144).

The trauma potential of unconventional projectiles was studied by using a M-16 assault rifle in combination with 5.56mm blank cartridges. The potential energy was calculated and found to be potentially lethal when over 33 J/cm^2. Using this set-up and threshold a piece a tree branch, stone (pebble), disposable foam earplug, cotton applicator swabs and un-used chewing-gum could potentially be fatal. Tumbling of “projectiles” decreases the potential trauma potential (145).

6.2 Case reports

Blank firing and home-made firearms
Although blank firing pistols can be legally obtained in some countries this does not mean they are harmless. Multiple articles have been published on fatal injuries caused by these firearms.

Three deaths were reported by Zdravkovic et al., all as a result from contact shots. The ignition of a powder load results in a pressure wave ranging from 1200 to 1500 m/s creating a gas volume of 950mL/g for nitrocellulose, leading to a pressure of 100 to 200 bar at the muzzle (146).

Additional to the review and summarisation of eighteen previously reported injuries due to blank firing pistols a fatal neck injury which led to exsanguination is reported (147).

Conversions or home-made firearms might show wound characteristics which are not typical for 'normal' firearms. A close examination of the injuries in relation to the firearm might help in explaining the findings (148).

Entry wounds, exit wounds and wound channels
Within the field of wound ballistics it is important to be able to differentiate between entry and exit wounds, to establish correct wound channels through a body and to be able to differentiate between close and distant shots. Naik et al. have reported on a case showing multiple variations from common findings (149).

Matching the number of entry and exit wounds with the number of bullets that should be present in a body might sometimes be difficult. Tandem bullets going through the same entry hole might cause a higher number of exit wounds compared to the number of entry wounds. When a body shows more entry wounds than exit wounds and not enough bullets in the body to explain this difference, a blank firing pistol should be considered (150).

Establishing the direction of fire from a body might be difficult especially when the body is already partially-skeletonised with adipocere formation on the upper part of the body. Postmortem changes and destruction of soft tissue made the determination of direction of fire impossible (151).

A case involving a victim with two bullets trapped in between the inner and the outer table of the cranium is discussed. Linear fractures were only visible in the inner table and no brain injury was seen. The bullets and fractures were made visible using computed tomography (152).

Farrugia et al. discuss the ricochet of a bullet in the spinal canal and give a review of the literature on bullet migration in bodies (153).

Remarkable self-inflicted gun shot wounds
Große Perdekamp et al. report on an exceptional suicide case were two firearms were fired at the head and give a literature overview of previous reported suicide cases with two firearms (154).

A few others report on suicide cases with multiple shots from the same firearm showing that instant incapacitation is not always the case with gun shot incidents. The ability to fire a second shot with a captive bolt gun is dependent on the depth of penetration of the first shot (155). Henja has published two articles on multiple self-inflicted gun shot wounds and discusses to possibility to inflict these (156;157).

7. Training Material and Books

Since 2008, the NFSTC has put a firearms examiner training course online (158). This course was made in collaboration with AFTE members and is based on the AFTE training manual.

Haag and Haag published the second edition of *Shooting Incident Reconstruction*. Three new chapters are introduced: gun sound levels, projectiles and glass, and working the shooting scene (159).

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Examination of Firearms – Gun Shot Residue

Review: 2010 to 2013

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Introduction

This review paper covers advances in scientific methods applied to Gunshot Residues reported since the 15th Interpol Forensic Science Symposium in October 2010. A literature search was conducted covering articles published in the main forensic journals since mid-2010.

During discharge from a firearm, primer and gunpowder residues as well as metal particles from the projectile and the cartridge case are expelled from the muzzle and other openings of the firearm. These residues are referred as primer residues, firearm discharge residues or gunshot residues (GSR). During the last three years, a couple of articles dealing with the basic principles of GSR were published (see ref (1) and (2), for example). In particular Murtha and Wu gave the reasons of finding GSR particles on a suspect, but they reminded that the absence of GSR does not indicate the suspect did not discharge a firearm. They concluded that GSR is a piece to the investigative puzzle in conjunction with other evidences. Trimpe (2) discusses case-acceptance criteria that are strongly dependant of the politic of each laboratory, taking the example of the FBI Laboratory that no longer accepts GSR cases because of a re-dispatching of the resources towards fighting terrorism. Contamination and testimony issues are also discussed in this article, since interpreting the results can sometimes or even often be challenged.

This field has also been reviewed in 2010 by Dalby et al. (3). The review begins with some issues related to GSR analysis, pointing out the article of Mejia (4) and examples of high profile cases in the U.K., such as the Jill Dando murder trial that have brought the evidential value of GSR analysis into question. Organic and inorganic parts of GSR are both discussed in the review. Concerning GSR collection technics, stubbing using tape lifts is the most commonly used procedure for the collection of inorganic residues. Scanning Electron Microscopy – Energy Dispersive X-ray microanalyses (SEM-EDX) still is the method of choice for the identification of inorganic GSR on samples. This technique is well adapted to the detection of small particles containing heavy metals such a lead, barium and antimony coming from primer with a classical composition. On the other hand several technics such as GC, HPLC and capillary electrophoresis have been tested, and for some of them used in casework to analyse the organic fraction of GSR. However until now these technics have not been widely applied to routine analysis in real cases. Finally a large part of the review is dedicated to the interpretation of results, in particular environmental sources of GSR-like particles, the problematic of GSR from ammunition with lead free primers leading to false negatives, and the transfer, persistence and contamination of GSR. The authors recommend the adoption of a “case by case” approach, when possible performing comparison of samples

collected from a suspect and/or a victim to references such as the cartridge case(s) found on the crime scene.

1 Inorganic GSR

1.1 Non-GSR sources of GSR-like particles

Since a number of years, concern has existed about GSR-like particles originating from a non-ballistic origin, which could lead to false-positive interpretation of the results at the source level. A number of publications have already described particles produced by used brake pads, detonated fireworks and exploded airbags. These particles are similar to GSR but do not originate from the use of primers. Grima et al. (5) investigated more deeply the problematic of GSR-like particles from firework exposition. Firework residue was collected at a display site, from amongst spectators. The authors identified some particles having a composition similar to GSR (mainly BaSb and BaAl), but no three-component particles considered as characteristic of GSR were found. Surprisingly, the authors only focussed on the presence of the “consistent with” particles (two-component particles), taking some conclusions about the impact on GSR evidence. They do not mention the fact that no characteristic particles were found; this is the main conclusion that should be pointed out.

Another potential source of GSR-like particles is cartridge-operated tools. Gerard et al. (6) investigated 17 different types of contemporary powder loads. For each type of powder load, most of the particles consisted of PbBa particles, and no characteristic PbBaSb particles were found. However since some rimfire ammunition (especially .22 calibre) have a PbBa-based primer, according to the authors the residues produced by these ammunition cannot be distinguished from the residues produced by cartridge-operated tools.

1.2 Interpretation of analysis results and the application of Bayesian principles

Ditrich investigated the formation of the plume after firing for several different firearms with high speed-video analysis (7). As stated before, the author shows that a vast scope exists between revolvers, pistols and shotguns. These differences are then deeply discussed in terms of interpretation of results, since a gap exists between the initial request of the court (e.g. has a given shot been fired by a suspect) and the type of response that can give the analyst/expert. In fact a lot of energy has been put to assure a high level of precision in detection, prevention of contamination and misinterpretation of a given particle (source level). Nevertheless less effort has been taken to critically evaluate the interpretation of the analytical level (activity level), since a lot of reasons

can explain why someone is contaminated while he did not shoot. The author concludes that even if this type of issues is known to experience analysts, the court may not be fully aware of these limitations, and finding the right decision should not entirely be left to the court. This could be done by analysing the specific circumstances of the case following a case-by-case approach, instead of using a generalized protocol.

In 2009, Biedermann et al (8) offered a rigorous discussion of the implementation of the Bayesian principles and networks in the GSR field. The part II of their study published in 2011 (9) concentrates on Bayesian parameter estimation, allowing GSR-experts to combine prior knowledge with newly acquired experimental data. The sensitivity of the likelihood ratio due to uncertainty in parameters is also discussed.

Gallidabino et al. (10) propose a probabilistic model using Bayesian principles to estimate the time since discharge of spent cartridges, and illustrate this model by applying it to a hypothetical scenario. The analytical method proposed to estimate this time is the analysis of residual quantity of naphthalene using solid-phase micro-extraction followed by gas chromatography.

As a following work of a previous study (11), Brozek-Mucha (12) studied the properties of GSR particles produced by a P.64 pistol with a 9mm Makarov ammunition and deposited on various substrates and locations in the vicinity of the shooting gun. Samples were collected from targets placed at various distances in the range 0-100cm from the gun muzzle as well as from hands and clothing of the shooter. Results revealed a dependence of the number of particles, the proportion of the chemical classes and dimensions as a function of the distance from the gun muzzle, both in the direction of shooting and in the opposite one. For instance the chemical composition on the targets is strictly related to the composition of the primer and to some extent to the bullet that has been used. On the side of the shooter, a small fraction of the particles has a composition that can be related to a memory effect of previous shots done with other types of munitions. According to the author, in some favourable circumstances a close look at these particles can be helpful in the reconstruction of shooting incidents.

Lindsay et al. (13) investigated the number of GSR particles on shooters and on bystanders in close proximity to the shooter (1 meter). They observed that GSR particles may be deposited on the hands of the bystanders, and the concentration found on the hand of these bystanders sometimes overlaps the concentrations found on the hands of the shooters. Extended to typical casework, according to the authors it is usually not possible to distinguish a shooter from bystanders, especially when the number of GSR particles detected is low.

In another study, Gerard et al. (14) performed tests in an indoor firing range to establish the distance GSR particles can travel. Sampling was performed up to a distance of 18m.The authors observed that the number of GSR particles detected at 13.5m of the muzzle was in excess of the number of GSR particles found adjacent to the ejection port. As a consequence an individual near the path of the projectile and within 13.5m of the muzzle may be more contaminated than the shooter.

Another matter of concern consists in the evaluation of the risk of contamination of GSR from police officers or from facilities to suspects. Some studies published in the past show that secondary transfers of GSR by conventional police forces seems to be quite negligible (see references (15) and (16)); however Charles and Geusens (17) studied the particular case of special units of the police, for which the level of contamination of GSR during the arrest of a suspect can be evaluated as high, depending on how the arrest was performed. By performing simulations of the interception of individuals by these special police forces, they observed that the major contamination occurs during the frisking step, with an average contamination of 7 PbBaSb particles found on the coats of the individuals. The risk of contamination was also indirectly pointed out by Diaz et al. (18) who measured the level of airborne lead, barium and antimony in a ballistics laboratory. If the highest values were found at the firing range, some airborne lead was also detected in other facilities such as some experts' offices. This phenomenon can, by potential secondary transfers, affect the level of contamination of other facilities for which it is critical to have a null-contamination (e.g. SEM laboratories or sample preparation facilities).

In order to evaluate the potential contamination of people in a high contaminated environment, Lindsay et al. (19) collected tapelift samples from the hands of employees of two firearms factories, but who did not discharge a firearm on the day of sampling. If employees involved in handling firearms were found to be sometimes contaminated (up to 400 particles), the number of GSR particles observed on the hands of staff who had no direct contact with firearms ranged from zero to two.

1.3 Quality

Due to international requirements (e.g. ENFSI) or national legislations, Quality assurance is more and more implemented in GSR laboratories, with a crucial step consisting in the accreditation in the domain of GSR of the analytical method used to detect GSR, following the ISO 17025 norm. The reference norm is the ASTM 1588 (20) revised in 2010; compared to the previous version, the major change consists in the introduction of a new class of particles (e.g. TiZnGd and CuSnGa) that are produced by some police forces (in Germany and in The Netherlands) when using marked ammunition. These particles are considered as characteristic of GSR.

Moreover, since 2010 a high compatibility exists between the ENFSI guide (21) and the ASTM norm (20). In 2011, the Scientific Working Group GSR (SWGGSR, mainly composed of members belonging to US-Laboratories) published a detailed guide for GSR analysis by SEM-EDX (22). Beside usual information and procedures that can also be found in the ASTM norm and the ENFSI guide, the SWGGSR guide contains a chapter Interpretation that takes into account some reporting considerations, and the way to write some interpretations as a function of, for example, the number of particles found on the hands of a suspect. Procedures describe how to sample, and how contamination at scenes and in the laboratories should be prevented. Finally, testimony issues are also discussed in this guide, and some typical questions and proposition of answers are presented.

Proficiency tests are conducted every year. They are organised by the ENFSI Expert Working Group "Firearms", and consist of the detection by SEM-EDX of 150 to 200 three-element particles (lead, barium and antimony) distributed in 4 size classes (0.5-2.5 µm). Three proficiency tests were conducted during the period of interest (GSR2011, GSR2012 and GSR2013).

2. Instrumentation and methods

The last couple of years has shown a renewed interest in the development of new analytical techniques for use in the field of GSR research, either to detect the presence of GSR components, or to characterize their composition and differentiate between types of ammunition. Also the advent of new “Heavy Metal Free” ammunition has spurred researchers to look into new analytical techniques, either focused on light element detection or organic component analysis. A final class of research interest goes to the equipment that can be used “in the field”, typically to allow the local police forces to perform a screening test of suspects, possibly to be confirmed by SEM-EDX analysis in the lab later on.

2.1 Use of Raman Spectroscopic techniques in GSR analysis

Raman spectroscopy is one of the techniques which has recently known a large and broad introduction in the forensic science disciplines. Also in the GSR field researchers are implementing Raman in the lab, for example by Bueno et al. who in (23) discuss the possibilities of using Raman spectroscopic analysis in combination with advanced statistical chemometric methods for the detection of GSR and the differentiation between ammunition types and calibers/firearms used in producing the GSR particles. Their method is demonstrated on macroscopic particles, produced by controlled close-range shooting experiments on targets, on which they are able to distinguish between characteristic spectra from 9mm and .38 ammunition. These preliminary results should be further elaborated

both for confirmation of the experiments and to study the different effects of ammunition composition which may enter into play. The direct application of these techniques would be the direct exclusion of a firearm/ammunition of a suspect as the source of GSR found at a crime scene. In order to provide positive identification however, a database of the Raman signatures of numerous ammunitions and firearms would need to be compiled in order to calculate the probabilities of such a positive identification.

Lopez-Lopez et al. studied in (24) the use of Raman spectroscopy on burned and unburned ammunition propellant pellets. They were readily able to characterize and differentiate between several types of ammunition, based on Raman spectra. Differences were observed between the GSR and unburned corresponding pellets, probably because of the different burning effects of the firing process. Also, other typical trace materials which are often found on a crime scene, such as blood, soil and ballpoint ink, were shown to be readily discernible from the GSR particles. Raman is therefore shown to be a good technique for fast screening of clothes of victims and suspects for the presence of (organic) GSR particles. Moreover, with the advent of metal-free ammunition, the analysis of organic GSR components by Raman spectrometry would become a complimentary technique to the now common SEM-EDX analysis method for GSR. In any case, more extensive research is necessary to determine the relevant parameters which can influence the Raman spectra, study of the GSR distribution as a function of shooting distance, heterogeneity of ammunition/GSR composition etc. To this end, a library of Raman spectra obtained from ammunition is needed, which would by itself already be a great forensic tool and probably useful in identifying the ammunition/firearm used in a crime.

2.2 Use of Mass-spectrometric analysis techniques in GSR analysis

Although Mass Spectrometry was used before in GSR – for example in organic component analysis – we now see a come-back of the MS as a detector coupled to an old companion technique: ICP. Although the ICP can be coupled to some rather novel sampling devices, like in Abrego et al. who in (25) used Laser ablation coupled to ICP-MS to detect and characterize GSR particles. By moving the Laser probe in a raster geometry over the surface of a carbon-coated SEM stub, which had been previously applied to the hands of a person who had just fired a gun, the authors were able to detect the presence of GSR in comparable amounts to the classic SEM-EDX technique. By measuring the coincident MS signals of the different characteristic isotopes of Pb, Sb and Ba as the Laser beam hits a GSR particle, they define the nature of the analytical species under observation as a GSR particle, along with its position on the stub. In this way, GSR particle samples pertaining to four different weapon/ammunition combinations – both revolver and pistols – were studied. The weapons were fired between one and six times, after which the palms and backs of

the gloves worn by the shooters were sampled separately. GSR particles could be detected on samples from all weapons, even after one single shot had been fired. The results with LA-ICP-MS confirm and correspond with the findings of earlier published studies regarding abundance of GSR on samples from different types of firearms, contamination levels of hand palms versus backs etc.

The authors conclude that the technique they developed has a number of strong advantages over the classic SEM-EDX technique such as its scan speed of about an hour per stub, the possibility to detect particles covered by epithelial cells, the simultaneous measurement of different elements, which can be used to characterize primers in detail, etc. Of course, additional study by forensic specialists is still necessary in order to test the technique in real-life situations.

Morelato et al. in (26) developed a screening method for GSR using Desorption Electrospray Ionisation-Mass Spectrometry (DESI-MS) and SEM stubs as a sample carrier. They note that apart from the inorganic GSR particles, the firing of a gun liberates also a number of very specific organic compounds such as methyl and ethyl centralite, which may be used to exclude a non-ballistic source of GSR-like particles found in a case. As the SEM sample stub needs to be searched for organic GSR prior to its SEM-EDX analysis, a rapid and non-destructive screening technique is necessary. The authors propose a system based on Desorption Electrospray Ionisation sampling, which can be applied on stubs, skin or other items such as clothing in their native state. Coupled with a mass spectrometer, this will provide real-time information on the composition of the sample surfaces and its deposits, enabling a comprehensive examination of a SEM stub for both organic (with DESI-MS) and inorganic (with subsequent SEM-EDX analysis) GSR compounds.

Although the authors succeeded in devising and optimizing a procedure to detect the presence of organic GSR compounds in unburned powder samples pertaining to thirteen different types of ammunition, it was not possible with this procedure to obtain good results from stub samples acquired after real shooting experiments. This can, according to the authors, be attributed to either the intrinsic variable nature of the production, deposition and persistence of organic GSR in the shooting process, or to the specificities of the adhesive surface of the SEM sample stubs, which could inhibit desorption of the analytes by the DESI probe. In any case, further investigation is necessary before the procedure can be put into routine use on real samples. It could, however, be shown that subsequent SEM-EDX analysis of the stubs still showed the presence of inorganic GSR particles, proving that the principle of a combined organic screening followed by SEM-EDX analysis is feasible. However, since only a manual search by SEM-EDX could be performed – and therefore no particle count statistics were obtained – the practical applicability of this part of the

procedures still needs to be investigated as well. When successful, however, this technique would improve the probative value of the presence of GSR particles significantly.

Freitas et al. in (27) propose a technique using ternary graphs to interpret ICP-MS data of GSR particles on fabric. As the case load of shooting incidents is very high in Brazil, a fast procedure to discern between shooters and non-shooters is needed. Conventional techniques such as SEM-EDX, although having the advantage of permitting morphological analysis and particle identification, take too long to accommodate the needs of the Brazilian police, who have had to treat more than half a million firearms-related homicide cases in the period 1979 to 2003 alone. In this study the capability of this ICP-MS technique in identifying the type of firearm used is demonstrated. This information may be valuable in such cases where a suspect needs to be tested and no firearm was recovered.

In this study, two types of pistols (.40 and 9mm caliber) and one type of revolver (.38 caliber) were used to produce test firings at 0.5m on different types of fabric targets. All firearms were collected from real police apprehensions, and thus represent a realistic image of the situation of firearms use in Brazil. The shooting at 0.5m is also inline with the modus operandi of the firearms criminality encountered by the Sao Paulo police forces. The bullet holes in the fabrics were cut out, extracted and their contamination levels of Pb, Sb and Ba were measured. The data was subsequently plotted and interpreted using ternary graphs. Although classical statistical treatment of the results does not permit to make clear distinctions between the shooting incidents, the authors show that using the ternary graph representation, a distinction can be made between the patterns originating from the pistols on one hand and the revolver on the other. The authors acknowledge, however, that further testing is necessary to establish the robustness of the technique.

Arndt et al. in (28) demonstrate a use of Ion Mobility Spectrometry (IMS) in sampling and analyzing (organic) molecules which are formed in the gas phase and subsequently condense in the shooting environment – among others on the hands of the shooter. As many of these molecules are lipophilic, they may tend to adhere onto the skin surface and show a longer persistence and lower secondary transfer rate than the inorganic particles. In this study diphenyl amine (DPA) was used as the trace compound to be analyzed. The technique used was ion mobility spectrometry with a ^{63}Ni ionization source, a nitrogen or air gas counter flow, and detection of positive ions only (“positive mode”).

Test shooters were swabbed with acetone swabs, which left no residual material on the skin after sampling. Test persons were sampled after 1 to 4 hours after shooting, so that the persistence of the DPA target could be

ascertained. Target molecules could be observed up to four hours post-shooting.

It was shown that hand washing with soap completely removed the target compounds from the hands of the test shooters. Secondary transfer of organic GSR, simulated by firmly hand shaking of a shooter with a non-shooter, was however not observed.

According to the authors, IMS can be developed into an effective field screening technique for firearms discharge evidence. Future work will focus on the use of more sensitive detection capabilities and the development of chemometric and pattern-matching techniques to make the distinction between shooters and non-shooters based on the whole ion mobility spectrum.

2.3 Use of Milli X-Ray Fluorescence techniques in GSR analysis

A novel technique in the GSR field is the milli-XRF – not to be mistaken with the micro-XRF – as used by Schumacher et al. who have been using it for some time in the estimation of shooting distances. In (29) they give an overview of their findings with the Spectro Midex M. In order to make the standard equipment suitable for the application of shooting distance estimation, new sample stages had to be built and extra software was developed to interpret the resulting XRF elementary mappings. The collimators were furthermore equipped with filters to enhance signal to background and thus sensitivity for some of the elements of interest. The authors show some typical results from tests carried out on targets of jeans/cotton cloth which had been shot at from several distances using different types of ammunition. The used ammunition had classic lead type as well as lead-free TiZn type primers.

From their six years of experience working with m-XRF equipment, the authors conclude that the method is indeed useable in the GSR laboratory as an alternative to the chemographic printing methods, and in cases where no chemographic coloring agent exists (such as for Ti and the light elements used in metal-free primers). In cases where the clothing is bloody or stained/soiled the m-XRF method can be used as well, as the X-rays easily penetrate the masking agents. Finally, the method can be easily used to determine the type of primer by analyzing the area around the bullet hole or wipe ring and can thus provide important indicative information regarding the elements of interest in the SEM-EDX GSR analysis in a case.

Latzel et al. in (30) show in a follow-up article some more detailed examples of using the m-XRF technique in the application of shooting range estimation. The authors discuss in more detail the spectral background, which is among others dependent on the exact elements

present in the GSR, on the target material and its configuration, and the need for correction of the spectrum information to provide correct interpretations.

The influence of the spectrum treatment on the element identifications, and indirectly on the range estimation itself, is discussed and demonstrated with a few examples using both Sintox (TiZn) and doped (TiZnGd) German police ammunition. For these primers no specific chemographic coloring agents exist (Ti, Gd), making the m-XRF technique invaluable in these cases. The applicability of m-XRF is still dependent however on a skilled operator, as the spectra need to be evaluated and the background correction optimized as a function of the case materials at hand. Finally, the usefulness of the technique is augmented by the fact that the target material is not damaged or altered by the procedure, enabling subsequent sampling and/or analysis by any other means.

2.4 Visualization of GSR using alternate light sources

It has been widely known that the use of special light sources can help in finding macroscopic GSR particles, for example on clothing. Still, sometimes one comes across an interesting trick, spurred by a fortunate incident. To illustrate, Windsor shows in (31) that it is possible to increase the number of spots obtained by the Modified Griess Test (visualization of nitrites) using a standard black light. After an initial incidental observation of this phenomenon on a test material during a training session, the authors conducted a series of test shootings to verify their observations. After processing of the targets following the standard Griess procedure, the authors used a Philips fluorescent black light to detect and count the reaction spots observed on a desensitized photo paper transfer, and compared this to the result obtained under normal room light conditions. A striking increase in the number of spots ranging from 50% to 950% on the test samples was noted. Further research will be conducted to test if this phenomenon is specific to photo paper, as used in their procedures, and if the use of different ammunition and powders affects the observed phenomenon.

Lake et al. in (32) tested the use of the Video Spectral Comparator 2000 (VSC2000) in determining GSR particles on clothing. The VSC2000 is a computerized camera system equipped with different lighting and filtering possibilities, enabling it to visualize small objects in IR range wavelengths, and to observe fluorescence effects. It is therefore most often used in document examination.

As was already studied, authors could verify the usability of the equipment in making IR and fluorescence images of macroscopic GSR deposition patterns, thereby aiding in the shooting distance estimation and/or the search for ballistics-related damage to the clothing. The authors also tested

the usability of the VSC2000 on blood-stained clothing and gravel and glass contaminated pieces with positive results.

Some final notes from the authors are that, although glass may be visible in IR images, the shape of the shards makes them easily discernible from the GSR particles. Care must be taken though with bloody or wrinkled clothing, as these areas will appear dark in the IR image, and so may mask the presence of GSR particles. Finally, the possibilities of using fluorescence spot mode should be used with caution, as there may be fluorescing features present on the fabric (in the fabric itself, glass etc.) which have nothing to do with the GSR particles or powder grains of interest.

2.5 *The use of micro-CT for shooting distance estimation*

A completely novel technique for GSR research is the application of micro-CT, an X-ray microscope developed in Belgium, which has now made its way into GSR research via forensic medicine. Cecchetto et al. in (33) have used such a micro-Computed Tomography system to study the deposition of GSR particles in and around gunshot wounds from shots fired at several mid-range distances. Using micro-CT 3-dimensional images can be acquired from sample objects, by means of X-ray shadow effects, which have a spatial resolution of a few μm. The authors used this technique to map the deposited GSR particles – defined by their elevated density in comparison with the surrounding soft tissues – in and on the surface of gunshot wounds, fired in surgically amputated human legs using 7.65mm ammunition.

The shooting distance was varied between 5 and 40 cm, at distances which are forensically interesting to evaluate different homicide scenario's, including suicide. A clear distinction could be observed between the shots fired at close range – where the GSR particles were both present inside as well as on the surface of the wound – and at medium ranges, where the GSR particles were only found near or on the surface of the wounds. The authors used a Gaussian model to estimate the percentage of GSR particles with relation to the complete imaged volume as a function of shooting distance. Likewise, the inverse function yields the most probable firing distance as a function of the observed GSR particles density. In this manner it was possible to estimate an unknown firing distance given a known percentage of the GSR deposit, as measured on the micro-CT scan.

A critical point described by the authors is that reference shooting experiments need to be conducted with the litigious weapon and ammunition used in the crime in order to reliably model the curves, so that the availability of the litigious ballistic evidence is at this moment a prerequisite. Although the technique still needs some further development, notably in the proper definition of the GSR particles, the authors see their

work as an inexpensive and rapid tool to determine the (short and mid-range) shooting distance in an objective manner.

2.6 Field testing equipment for GSR

As stated above, testing equipment, usable by the police or local labs in the field, receives ample attention:

Ceto et al. in (34) report on the development of a novel technique in which a combination of Square Wave Voltammetry and chemometric data treatment has led to a possible road-side test equipment for a suspect's involvement in a shooting incident. Using a new data treatment method based on Canonical Variate Analysis (CVA) of Fast Fourier Transform filtered electrochemical measurement data, the authors were able to classify test persons involved in a variety of typical shooting-related scenarios. The classes or scenarios were: "Shooter" (subject fired a firearm), "Loader" (secondary contamination from firearm), "Presence" (secondary contamination from the shooting environment), "No Contact" and "Washed" (subject washed hands after contamination).

In order to sample the test subjects, the authors used a newly-developed carbon sensor-strip disposable electrode with which a low cost and reliable electrochemical fingerprint is obtained from the sample. For comparison purposes, a more traditional diluted acid-assisted swab sampling was employed. Sampling was carried out on test persons in and around a shooting range with good results.

Vuki et al. in (35) describe the development of an electrochemical technique capable of measuring both inorganic and organic GSR components in a single run, using Cyclic Square-Wave Voltammetry. The authors hope to develop this work further to produce a portable GSR detection system which records highly specific fingerprint patterns from inorganic and organic GSR constituents. This equipment could then be used at the crime scene by police officers in order to rapidly record firearms exposure of subjects.

2.7 Separation and Identification of gunpowder and additives using electrochemical methods

Finally, there are a number of developments of techniques using electrochemical methods, a large number of which could also have been classified among the "field techniques" on which we have reported in the previous paragraphs, were it not that the focus of the research group was more on the chemical separation and identification features of the method than on the portability aspect of the equipment. De Perre et al. discuss for instance in (36) the development of a separation and identification method

of smokeless gunpowder additives by Capillary Electro Chromatography (CEC).

Detection of the components of smokeless powders was developed using both UV and Mass-Spectrometric methods. Their method should, besides for the characterization of powders and their additives used in rifle ammunition, also be usable in the analysis of unburned explosives pellets which are left over after explosion of Improvised Explosive Devices (IEDs). The small injection volumes necessary for CEC permit for the analysis of the small sample sizes which are often encountered in a forensic context.

Gilchrist et al. in (37) show that separation of the lower molecular weight inorganic and organic anions in GSR is possible by suppressed anion exchange chromatography. The specific detector peak height ratios of these materials could be used to discern between different ammunition types using samples from the spent cartridges. On the other hand, different matrices such as the sweat of a suspect's hands could be forensically relevant to show involvement with a firearms incident. Finally, tests showed that the results of GSR-like residues coming from non-firearms related sources such as brake linings showed a marked difference with real GSR material.

Erden et al. in (38) show that it is possible to obtain roughly the same or even better working ranges for the simultaneous detection of lead and antimony in GSR with Differential Pulse Cathodic Adsorptive Stripping Voltammetry (DPCAdSV) and Square Wave Cathodic Adsorptive Stripping Voltammetry (SWCAdSV) as with the classic Graphite Furnace AAS. The electrochemical methods have the added advantage over AAS that they can detect several elements at once and are simple to operate using compact equipment – again making their deployment possible even in the field.

Salles et al. in (39) show that the amount of lead in GSR samples can be quantified using a gold micro electrode and stripping analysis. The results of the analysis of hand samples from shooters at a shooting range were compared with results from GF-AAS. The results were shown to be identical to GF-AAS at the 95% confidence level.

Paixão et al. in (40) show that it is possible to construct a voltammetric electronic tongue to distinguish between GSR from handguns and long-barreled guns used with several kinds of ammunition. For this, they combined voltammetry with non-supervised pattern recognition methods.

3 Shooting distance estimation

3.1 Shooting distance estimation – Comparison of gunshot injuries

Sometimes interesting information can be obtained during the autopsy of the victim through medical morphological examination of the bullet wound and damage to the clothing. Lepik et Vassiljev in (41) continue their study of the morphology of the damage inflicted by close range shots to skin and fabrics. Using the most common weapons in Estonia – the Tokarev (TT), Makarov (PM) and Glock 19 (G) – shots were fired at cotton and polyester woven cloth and fresh human skin originating from autopsies. After shooting, the damage to the fabric and the wounds to the skin tissues was documented in terms of material defects, deformations and tears, as well as the distribution and density of gunpowder residue particles. The skin was fixed and sectioned for further microscopic investigation.

3.2 Using pellet dispersion for shooting distance estimation

In those cases involving shotguns, the pellet dispersion pattern can be used to estimate shooting distances at larger ranges. Still, although no chemical tests are involved, also this method has its pitfalls, requiring great scrutiny and care by the expert. There is, for example, a large dependence of the dispersion patterns on the length of the barrel – which was in many cases shortened for reasons of easy transport and concealment of the weapon – and thus also the choke. Arslan et al. in (42) investigate the dispersion pattern of shotgun pellets fired from different shotguns, having the same caliber but a different choke. The barrel length of the three weapons was identical to rule out this well-known influencing factor in the pellet dispersion pattern. In their experiments, two 12-gauge – one with full choke and one with no choke (or cylinder bore) – and one 16-gauge shotgun with cylindrical barrel was used. The ammunition used was #2 and #5 pellets in Winchester brand 12-gauge and 16-gauge shotgun shells. Targets were made from coarse calico in 2x2m wooden frames. Firing distances were 75, 100, 300, 500 and 1000cm, perpendicular to the targets. Using a “mean enclosing circle” to define the pellet dispersion, regression curves were calculated from the compiled data for each gun individually and for all the data taken together as one set. The authors show that, although for each gun individually very good correlation coefficients can be obtained, the data taken as a whole offers but poor correlation (82%).

They conclude that this result shows that general formulae cannot be used to estimate shooting distance based on pellet dispersion – even when the barrel lengths of the guns are the same. However, good results can be obtained if tests are conducted with the litigious weapon and ammunition.

3.3 Robustness tests of shooting distance estimation

It is well known that shooting distance estimations are prone to many uncontrollable factors, forcing the use of large brackets in the reporting distances. Logically, many researchers are interested in determining the extent of the effects of various factors on the robustness of the techniques used. Crego in (43) investigates whether firearms with the same make, model and barrel length show the same GSR deposition patterns using visual inspection and chemographic tests. Nine different rifles with in total five different barrel lengths were used to fire at fabric targets at close ranges varying from 7.6cm to 61cm. The ammunition was always the same (Federal 115 grain FMJ). After firing the deposition of powder particles was visually evaluated and represented by the largest radius of the dispersed gunpowder particles. Subsequently, Griess and Rhodizonate tests were carried out on the targets and their dispersion radii documented. The author finds that the results of similar firearms lie within 5 cm, which is smaller than the uncertainty attributed to the distance estimation in their reports.

The author concludes therefore that, when compared with firearms of the same caliber, barrel length, make and model, the results of shooting distance estimation in this distance range, corresponds. Reference shots can therefore be performed by using similar firearms if the litigious weapons are not available. The author does advise to make it clear in the report that the findings are based on references obtained from a similar firearm, and not from the firearm recovered from the crime, and to increase the brackets of the results until further statistical studies on this method have become available.

Stuart in (44) demonstrates in the discussion of a case the importance of using reference target fabric which as closely as possible resembles the victim's clothing in determining the shooting distance. Not only the type of material but also wear and age degradation of the fabric of the victim's clothing can have a profound influence on the damage caused by the bullet's impact. The estimation of the shooting distance can in extreme cases be influenced by these factors. In the case at hand, only when using the original garment, was it possible to obtain similar impact images for shooting distance reference purposes.

Goater in (45) tested a number of common nitrite/nitrate-containing materials like cured meats, cleaning agents plant fertilizer and tooth paste for the potential danger in producing previously reported false-positive reactions of the Griess test. Also a number of other possible interfering substances like marijuana and a herbicide were tested.

The results of these tests showed either no or only a very light coloration of the test material. Some of the tested agents, like the plant fertilizer and herbicide, did however produce a discoloration which was shown to be due

to the material itself, rather than a Griess reaction product. The author concludes that these alleged false-positive producing contaminants do not react substantially with the Griess reagents. However, there can still be a danger in the fact that their coloration masks the light colorations produced by the Griess reaction of GSR which may be present on the test material, or that these products are involved in competitive reactions or other interferences with the Griess reagent, rendering the method less responsive.

Jeffres in (46) reports on the validation of an alternative method of producing positive controls for the Griess reagents. As described by Dillon, swabs are normally produced by soaking in a $NaNO_2$ solution, and then dried until used. Before use, the swabs are moistened with acetic acid and spotted on the photo paper to yield a positive test of the Griess reaction. In the adapted method, the swabs are not prepared before, but rather they are produced fresh at the moment they are needed. Results of the tests show that the freshly prepared swabs consistently yield a better coloration than the rehydrated swabs. The main disadvantage is that a solution of $NaNO_2$ solution needs to be prepared anew prior to each test.

In another paper (47), Jeffres reports on the effects of various types of manipulation of shot garments on the results of the Griess test. Test targets were shot at close distances of 12” and 18” and subjected to several types of handling/contamination. The objective was to test the effect of these (mis)handlings on the overall outcome of visual GSR search and Griess tests. Of the array of manipulations, the improper packaging (crumbling the fabric and putting the targets together in a vigorously shaken paper bag) has the largest detrimental effect of destroying and deforming the GSR patterns. Other manipulations tested were: shaking of the bag, proper packaging (wrapping in paper, folding and storage in separate envelopes), staining with blood followed by horizontal or vertical drying, contamination with grass/dirt/insects, and finally removal of GSR by tape lifting. All these manipulations had the effect of removing and/or deforming the GSR pattern, but to a lesser extent than the improper packaging of the items. The author concludes that the effects of handling of victim’s clothes are not under control of the examiner, which makes the use of large brackets in the reporting of shooting distances very important. The reporting of muzzle-to-target distances with precisions of inches is only possible when applied on test targets, treated in laboratory conditions. These results also reinforce the need for proper handling of evidence garments by all who come into contact with them prior to the GSR examiner, who should be made aware of all the handling the clothing has been subjected to previously in order to avoid potential investigative pitfalls.

Williams and Silverstein in (48) report on the use of blood elimination solutions in the treatment of bloody victim’s clothes prior to the application of Griess and Rhodizonate chemographic tests. The authors used

ammonium hydroxide, sodium chloride and the commercial cleaning agent Haemo-Sol, which was previously found to be the best blood removing solution. According to the results of this validation study, however, the ammonium chloride solution works best in removing blood while leaving nitrite and lead traces undisturbed on the clothing. Sodium chloride was slightly less effective in removing the blood, while the Haemo-Sol solution, although best at removal of the blood stain, showed a clear removal of lead and nitrite traces – making it the least interesting product for this application. Although this is in contradiction with the results earlier reported by Haag in 1991, the authors point out that a number of the variables in their study differ from the work by Haag, for example the use of bovine blood instead of human blood, as well as the temperature of the blood when applied. Also, it must be noted that the composition of the commercial Haemo-Sol product might have changed considerably over the 20 year period that has passed since Haag performed his tests. The ammonium hydroxide on the other hand forms a simple and effective method for blood removal, using only chemicals which are readily available in most laboratories.

3.4 Doped ammunition

Since the commercial introduction of doped ammunition, the interest on this topic has grown. Not only do we need more types of dopants, they must also be readily detectable – if possible with a screening technique in the field. Weber et al. in (49) report on the development of doping agents for use in ammunition based on Metal-Organic Frameworks (MOF), comprising lanthanide ions (Tb and Eu). The presence of the lanthanides renders these complexes photoluminescent, which means they can be easily made visible by the use of UV-lights. As the lanthanides also are incorporated into the microscopic GSR particles, they can be readily identified using standard SEM-EDX analysis. Ammunition doped with these compounds can therefore be used both for rapid screening of subjects and materials by the police, and provide a characteristic signature in the lab. Finally, the cost of these compounds is minimal and the toxicity of the lanthanide ions is also lower than that of lead ions. However, a detailed investigation into the toxicity of the complete doped ammunition products still needs to be performed.

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The Forensic Examination of Marks

Review: 2010 to 2013

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1 Introduction

The examinations of contact marks and related topics, covered by this review, are among some of the core issues of forensic science. This review covers advancements in scientific methods applied to the forensic examination of various marks, since the Interpol 16th International Forensic Science Symposium (IFSS) in October 2010.

This Review is based mainly on a literature review, which covers articles published in the principle peer-reviewed forensic science journals and other relevant sources over the review period. In addition, the National Clearinghouse for Science, Technology and the Law (NCSTL) Forensic Database (1) was used. This is an Internet-based service, provided free-of-charge by the Stetson University College of Law, FL, US.

Manuals, guides and standard operating procedures of various forensic science laboratories and organizations, many of which are relevant to this Review, may also be found on the Web, like those of the Virginia Department of Forensic Science (VA-DFS) (2), the Scientific Working Group on Shoeprint and Tire Tread Evidence (SWGTREAD) (3), the Scientific Working Group for Firearms and Toolmarks (SWGGUN) (4) or the Scientific Working Group on Imaging Technology (SWGIT) (5).

2 Footwear and Tire-Tread Impressions

Footwear impressions may be considered as one of the most common types of evidence, and are found, practically, in every crime scene. As a significant form of physical evidence, impressions left behind at the crime scene may provide valuable information on where the crime occurred and the direction the suspect travelled while committing the crime. This information may place the suspect at the crime scene or eliminate him as having been there.

The Scientific Working Group on Shoeprint and Tire Tread Evidence (SWGTREAD) continues its effort for setting professional guidelines for the documentation, collection, preservation and examination of footwear and tire tread impression evidence (3). The Group's Internet web-page provides a vast amount of information, including some recently-approved guides:

- Range of Conclusions Standard for Footwear and Tire Impression Examinations (March, 2013),
- Standard for Terminology Used for Forensic Footwear and Tire Impression Evidence (March, 2013).

These guides, along with the previously-published ones, may be downloaded free-of-charge from the SWGTREAD web-page. Other standards, not approved yet, are posted at the Group's web page, awaiting comments by the forensic community, and will be finalized later this year:

- Standard for the Examination of Footwear and Tire Impression Evidence,
- Standard for Report Writing for Footwear and Tire Impression Examinations.

Useful guides, regarding various aspects of footwear impressions photography and imaging, are also found at the Scientific Working Group on Imaging Technology (SWGIT) web-page (5).

The European Network of Forensic Science Institutes (ENFSI) Expert Working Group Marks (EWGM), established in 1995, also runs an active web-page (6). Unlike many other ENFSI EWGs, this resource is publicly accessible, for the benefit of the forensic community. One of the remarkable features of this web-page is its “Wanted Page”, enabling the exchange of information regarding unknown shoeprints. Since the beginning of 2011, more than 175 queries were submitted by shoeprint examiners from all over the globe, many of which (more than 50%) were successfully answered by other peers. In addition, this Group’s newsletter, “*Information Bulletin for Shoeprint/Toolmark Examiners*” (*IBSTE*) is also posted on this web-page.

The Virginia Department of Forensic Science (VA-DFS) prepared a procedures manual of various laboratory methods for footwear and tire tread impressions, as well as a training manual for experts in this field (2). These documents, as other VA-DFS manuals, are available on the Internet.

2.1 Detection and Recording

2.1.1 Photography and Image Processing

Photography (either on film or digitally) is still the fundamental tool for the documentation of footwear impressions at crime scenes. As mentioned earlier, both SWGTREAD and SWGIT published several standard guides for photographing shoeprints and tire impressions. General guidelines for proper shoeprint photography was recently published by Hammer (7), stressing the need for correct camera positioning and oblique illumination.

A method for recording dust tire impressions (applicable for footwear impressions as well) on fabrics, using polarized illumination and colour separation digital photography, was presented by Jin (8). This method reduces the reflectance from the background fabric fibres, and thus enhances the impression itself. The article includes several examples of successful applications of the proposed method for impressions on dark and coloured clothes.

Difficult lighting situations that lead to challenging photographic conditions are common at crime scenes. High dynamic range (HDR) photography, already mentioned in our 2010 Review, is a method for processing a series of photographs into one image that captures the fullest range of highlights and shadows present in the originaly photographed impression. HDR is a method used to increase the span between shadows and highlights in an image by taking more than one picture of the same scene – shots that maximize shadows, maximize mid-tones, and maximize highlights – and then merging them into one unified picture with wide tonal range. Thus, the application of HDR photography for footwear impression was evaluated by Rogahn (9, 10). This project found that HDR processing of multiple images does not produce a significant increase in detailed information compared with viewing the same images in Photoshop. However, exposure auto-bracketing increases the ability to capture more detailed images of footwear impressions than a single image alone, and allows the use of HDR software for rapid processing and comparison.

Hyperspectral imaging (HSI) integrates conventional imaging and spectroscopy, to obtain both spatial and spectral information from a specimen. This technique enables investigators to analyse the chemical composition of traces and simultaneously visualize their spatial distribution. HSI offers significant potential for the detection, visualization, identification and age estimation of forensic traces. The rapid, non-destructive and non-contact features of HSI mark its suitability as an analytical tool for forensic science. A paper by Edelman *et al* (11) provides an overview of the principles, instrumentation and analytical techniques involved in HSI. These authors describe recent advances in the HSI technology motivating forensic science applications, e.g. the development of portable and fast image acquisition systems. Reported forensic science applications, including one for footwear impressions by Miskelly and Wagner (12), are reviewed. Challenges are addressed, such as the analysis of traces on backgrounds encountered in casework, concluded by a summary of possible future applications.

A device for three-dimensional (3D) scanning of footwear impressions and tire tracks at crime scenes was developed by Tuceryan, Zheng and their colleagues (13 - 15). 3D images of tire track and footprint impressions at crime scenes can be captured with high fidelity, using this device, while capturing high resolution 2D colour texture images simultaneously. The developed device is portable, easy to use, and non-destructive of the evidence, and it saves time at crime scenes. The same technique can also be used in the laboratory to create 3D depth images of suspect tires or shoe soles. Computer-based pattern matching technology can be used to assist in matching and comparison tasks. According to these authors, the device produces better quality data at a close range obtained in a larger field (or span in the case of tire impressions) compared to existing devices. It avoids problems related to occlusions by using two lasers and can digitize long spans of impressions in one scan. The method includes a calibration method which is integrated into the scanning process on site, thus avoiding

problems with pre-calibrated configurations becoming stale during transportation and setup.

Polynomial Texture Maps (PTMs) are simple representations for images of functions instead of just images of colour values. In a conventional image, each pixel contains static red, green or blue values. In a PTM, each pixel contains a simple function that specifies the red, green or blue values of that pixel as a function of two independent parameters, specifying the direction of a point light source. PTMs are typically produced with a digital camera by photographing an object multiple times with lighting direction varying between images. Even a low-end digital camera provides enough resolution to produce good PTMs, and almost any light source can be used, such as a light bulb, LED or a flash. Hamiel and Yoshida (16) applied this imaging technique for shoeprints and other impression evidence, including the use of a portable unit for field studies. The PTM images were compared to conventional sidelight and casting techniques. The application of this technology could be more cost-effective than conventional methods and provide higher-quality data. Results of the evaluation reveal that PTM technology can successfully be used in the forensic field and has the potential to produce better resolved images for the comparison of known shoe soles or tire treads to crime scene impressions. Specific results indicated that PTM images and enhancements improved the visibility of detail in some of the impressions under analysis when compared to traditional photography techniques, including improvement of the visualization of texture within a shoe or tire impression. As reported, PTM technology thus gives the examiner the best opportunity for visualizing unique characteristics in impression evidence. PTM technology has also the advantage of being cheaper to operate than traditional sidelight and casting techniques.

Andaló and co-workers present a method for capturing 3D footwear impressions, based on Computer Vision, multiview stereo, which yields an accurate 3D model and provides some benefits over existing methods (17). These authors evaluate the results comparing their reconstructed 3D models with the ones acquired by 3D scanning, and examine the advantages and drawbacks of each method. The proposed method utilizes several software packages, "Bundler" recovers camera parameters for each image, PMVS generates a dense point cloud and SSD reconstructs the surface. Despite the simplicity for set up and acquisition, the reconstructed surfaces proved to be comparable with 3D scanning, a high-end technology used in practice, providing accurate 3D models of the shoeprints. A digital camera is the only equipment required to recovery the evidence, which makes the process convenient and fast.

Reflected ultra violet (UV) imaging is used for many forensic science uses, including latent fingerprint visualization, bruises and bitemarks imaging, etc. (18). Richards and his colleagues discuss the use of this imaging methods for in-situ shoeprints photography (prior to lifting, or instead of it), among various other such applications, and demonstrate its advantages over conventional methods (19, 20). The earlier article presents a discussion

about the difficulties of reflected ultraviolet photography and the use of digital reflected ultraviolet photography with cameras like the Fujifilm camera. The benefit of using a Baader UV Venus filter in lieu of other barrier filters is also explained. The later work examines the different types of UV light sources and explains how and when to use different imaging techniques to visualize hidden evidence. The authors explain, in detail, the wavelength of light required, the image capturing equipment, and the type of evidence that can be examined using these techniques, including faint footwear impressions in dust.

De Jong reviewed the application of 3D visualization methods in forensic pathology (21). This article describes methods like computed tomography (CT), magnetic resonance imaging (MRI), photogrammetry and computer aided design (CAD), and several of the examples are of footwear and tire tracks impressions on human subjects. Since the impressions in the presented cases were of very limited size and quality, this article does not refer to the question whether the resolution of those methods is practically sufficient for the comparison of the impressions to the footwear or tire tread.

LaMay (22) presented a case where a large outdoor crime scene with 143 footwear impressions in a dirt and gravel driveway was documented using photographic and diagramming techniques. There were 22 known individuals, including suspects, victim, witnesses, paramedics and police officers, who had entered the scene, potentially leaving footwear impressions. The author was able to associate 136 of the footwear impressions to the shoes of those 22 individuals. A colour-coded diagram was produced to illustrate the locations of the footwear impressions at the crime scene and the shoes that could have made the impressions.

2.1.2 Casting and Lifting

Following photography or scanning, shoeprints found on various surfaces are usually lifted or being cast, according to their nature. The common methods, used by crime scene investigators (CSIs) and forensic scientists are dental stone (or plaster of Paris) casts for 3D impressions and adhesive lifters, gelatin lifters (sometimes being referred to as "Gellifters") or electrostatic lifters (ESLs) for two-dimensional (2D) prints.

Dental stone is used as the major material for recovering 3D shoeprints and tire tracks from crime scenes. The procedure for using dental stone sparsely changed over the years. There are two common methods for mixing dental stone: either a premeasured amount of dental stone is put in a zip-lock bag to which water is added, or the water and dental stone are mixed in a bucket. Cohen and her colleagues suggested a novel, rapid and efficient method of mixing dental stone and water in a plastic bottle (23). These methods were compared at equal conditions. The parameters measured were the number of air bubbles, the strength of the cast, the ease of use, and the sharpness and quality of the accidental characteristics present in the cast. The proposed "Bottle Method" has the advantages of both the bucket and the zip-lock methods, hence it combines strength,

sharpness, high quality, and ease of use. This study proves that despite the vigorous mixing in the bottle, not many air bubbles were noticed in the casts. Moreover, the great advantage of the bucket method is the ability to add the powder to the water and to let it soak—this process increases the strength of the cast. In this experiment, a similar process was performed in the "Bottle Method", reaching the same affect.

As for 2D impressions, Wiesner and her colleagues compared two lifting methods, namely the ESL and the gelatin lifter, and concluded that the gelatin lifter was the method of choice for most substrates (24). Several substrates were chosen, and on each material a set of dry dust shoeprints was made, and a set of wet prints was made on paper as well. The shoeprints were approximately of the same quality, and the only variable was the nature of the material. On substrates indifferent to the method used, the preferable sequence was tested. Gelatin lifter was superior on most substrates and for wet prints. The superior sequence for using both methods is ESL followed by gelatin lifting.

Milne reported the development of a wireless ESL apparatus for crime scene use (25). This article describe the physical principal behind ESL, gives an overview on the development of a low-budget, three-electrode wireless instrument, that is commercially available today, and provide guidelines for using the ESL method. The author also suggests using ESL for in-situ cleaning up marks, before taking their photographs and prior to gelatin or adhesive lifting.

Vinyl static cling films (VSCFs) are used as signs, decals, window graphics, door coverings, and protective masking, and are manufactured in all sizes, colours, and degrees of opacity. LeMay and colleagues examined the application of such VSCFs for lifting shoeprints in dust from various types of surfaces, and evaluated this methods comparing to other lifting methods (26). These authors concluded that the use of VSCF is an effective, affordable, and simple method for the lifting of dust impression evidence at crime scenes and off of evidence. The results of their study show that on some surfaces it performs better than ESLs. It can be packaged and preserved well in simple manila folders, which can in turn be packaged and sealed in paper bags or larger manila envelopes. VSCF can be used on virtually any surface, with no threat to the health or safety of the user. The matte surface of the VSCF is also less reflective than that of ESL film and photographs well with less specular highlights. Because of the affordability and ease of use, it may also be likely that the use of VSCF for lifting and preserving dust impressions at crime scenes may result in more footwear and tire track evidence being collected and preserved. Examinations and comparisons may also yield more favourable results because of improved detail and contrast when VSCF is used to collect and preserve dust impressions.

2.1.3 Chemical Enhancement

Chemical enhancement of footwear impressions is performed mostly on dust prints, and is usually based on the chemical composition of the print-forming substance. One of the most active research groups in this field (and in the study of enhancing shoeprints in blood, as well) is led by Prof. Nic Daéid at the Centre for Forensic Science, University of Strathclyde, Glasgow, UK (27-29).

A recent review by Nic Daéid describes various methods for the chemical enhancement of footwear impressions on fabrics, in blood and urine, as well as in soil (27). These methods are reviewed more thoroughly in the following paragraphs.

Croft *et al* performed a feasibility study on the chemical enhancement of soil-contaminated footwear marks (28). Investigations into the application, including the advantages and limitations of processes available for the enhancement of footwear marks in soil, were carried out as part of this study. This included a comparison of current enhancement solutions such as potassium thiocyanate, ammonium pyrrolidine dithiocarbamate, potassium ferrocyanide, and bromophenol blue. The solutions were compared on the basis of sensitivity, sharpness of the colour reaction, and their application to a range of commonly encountered substrates. According to this study, the best-performing chemical enhancement technique for footwear impressions in soil was found to be potassium thiocyanate, on both porous and nonporous surfaces tested. Potassium thiocyanate was further explored to study the effects of aging on the mark deposited as well as assessing the stability (shelf life) of the solution. The age of the mark appeared to have no significant effect on its ability to be chemically enhanced using potassium thiocyanate. The stability study of potassium thiocyanate revealed that, although aged solutions still enhanced footwear marks, background staining, fading, and deterioration in colour sharpness were all observed.

A study by Farrugia and his colleagues investigated the enhancement of footwear impressions prepared with soils from different locations on a variety of fabric surfaces with different morphology (29). Preliminary experiments using seventeen techniques were carried out and the best responding reagents were evaluated further. Results indicated that the soils investigated (a cross-section of soils from Scotland, UK) are more likely to respond to reagents that target iron ions rather than calcium, aluminium or phosphorus ions. Furthermore, the concentration of iron and soil pH did not appear to have an effect on the performance of the enhancement techniques. For the techniques tested, colour enhancement was observed on all light coloured substrates while enhancement on dark coloured fabrics, denim and leatherette was limited due to poor contrast with the background. Of the chemical enhancement reagents tested, 2,2'-dipyridil was a suitable replacement for the more common enhancement technique using potassium thiocyanate. The main advantages are the use of less toxic and flammable solvents and improved clarity and sharpness of the enhanced impression. The surface morphology of the fabrics did not have a

significant effect on the enhancement ability of the reagents apart from a slight tendency for diffusion to occur on less porous fabrics such as polyester and nylon/lycra blends.

McNeil and Knaap also compared the performances of the bromophenol blue (BPB) indicator to those of potassium thiocyanate, for the enhancement of dust footwear impressions (30). In contrary to the above-mentioned findings, these authors found that BPB performed better than potassium thiocyanate on most shoeprints tested.

Ross and Gorn studied the application of Pyridyldiphenyl-triazine (PDT) for chemical enhancement of soil and dust impressions (31). PDT is mainly used for detecting ferrous traces on suspects' skin surfaces (32), and these authors tested it as an alternative to conventional chemical enhancement techniques (i.e., ammonium thiocyanate) of soil or dust footwear impression evidence. The PDT in the commercially-available Ferrotrace reagent reacts with ferrous iron, and was compared with ammonium thiocyanate using ferrous solutions and produced results that were sensitive. Soil samples were then tested with Ferrotrace and ammonium thiocyanate, and the results were compared. After sensitivity testing, Ferrotrace and the laboratory-prepared 0.1% PDT solution seemed to be viable alternatives to chemical enhancement of soil or dust footwear impressions as compared to ammonium thiocyanate. Experimentation with soil samples collected from across the country (USA) revealed that these alternatives were not as successful as the sensitivity study. Based on the results from these studies, it is recommended by the authors that the forensic community continue using ammonium thiocyanate as a chemical enhancement technique for soil or dust footwear impressions that may contain levels of iron.

Not surprisingly, the main lesson derived from these studies (27-31), as well as from the Israeli experience (33), is that chemical enhancement of footwear impressions in dust is highly location- (and probably geology-) dependent, so each laboratory is encouraged to analyse the dust in its area and evaluate several deferent reagents, in order to achieve the optimal methods mostly applicable to its local conditions.

Ahmad *et al* took this issue into a more practical domain, by developing a reagent test kit for the enhancement of shoeprints at crime scenes (34). These authors suggested a field kit that contains chemical reagents both for prints in blood and for those in dust and soil: the reagents for prints in blood are Leucomalachite Green (LMG) and Patent Blue, and for soil and dust prints - potassium ferrocyanate and Sudan Black. The reagents in this kit are not novel, but the authors compared several formulations for each of the reagents and recommend a specific set of those.

2.1.3 Shoeprints in Blood and Urine

The recovery of footwear impressions in blood is similar generally to the recovery of fingerprints in blood, and indeed many of the relevant

references cover both these areas of interest. This section will focus mainly on those articles referring to shoeprints in particular.

Most footwear marks made in blood on a surface such as fabric tend to be enhanced in-situ, rather than physically recovered using a lifting technique prior to enhancement. Since bloody footwear impressions are found sometimes on multi-coloured fabrics, the in-situ enhancement provides in such instances less-than-desired results. A work by Farrugia *et al* reports on the use of an alginate material to recover the impressed footwear marks made in blood and deposited on a range of fabric types and colours (35). The lifted marks were then enhanced using Acid Black 1 (AB1) and Leucocrystal Violet (LCV) with excellent results. The use of alginate casts for lifting impressions in blood from various surfaces had already evaluated by Adair (see our 2007 Review), and this article pursues this novel method further. These authors recommend the use of AB1 for this purpose, due to the safety issues involved when using LCV.

A similar approach was taken by Wiesner and her colleagues, who used several types of the alginate casting material and applied several reagents prior to lifting, during the casting process, and on the lifted footwear impressions (36, 37). These researches report that the best results were achieved using Aroma Fine® alginate, combined with enhancement of the shoeprints by Amido Black (Acid Black 1). This method had already been used successful at the authors' laboratory in casework.

Farrugia and his colleagues preformed a comprehensive and detailed study on chemical enhancement of footwear impressions in blood on fabric, using protein stains, peroxidase reagents and amino acid staining (27, 38-40).

In the 1st article in this series (38), a range of protein stains were utilised for the enhancement of footwear impressions on a variety of fabric types of different colours with blood as a contaminant. A semi-automated stamping device was used to deliver test impressions at a set force to minimise the variability between impressions; multiple impressions were produced and enhanced by each reagent to determine the repeatability of the enhancement. Results indicated that while most protein stains used in this study successfully enhanced impressions in blood on light coloured fabrics, background staining caused interference on natural fabrics. Enhancement on dark coloured fabrics was only achieved using fluorescent protein stains, as non-fluorescent protein stains provided poor contrast. A further comparison was performed with commercially available protein staining solutions and solutions prepared within the laboratory from the appropriate chemicals. Both solutions performed equally well, though it is recommended to use freshly prepared solutions whenever possible. The results clearly showed that Acid Yellow 7 (AY7) is the most suitable protein stain for footwear impressions made in blood and deposited onto dark fabrics. Limited fluorescence was observed for similar marks on denim and leather. Comparable but weaker results were obtained with other fluorescent protein stains.

These researchers also studied the optimisation of peroxidase based enhancement techniques for footwear impressions made in blood on various fabric surfaces (39). Four different haem (heme) reagents: LCV, LMG, Fluorescein and Luminol were used to enhance the blood contaminated impressions. The enhancement techniques in this study were used successfully to enhance the impressions in blood on light coloured surfaces, however, only fluorescent and/or chemiluminescent techniques allowed visualisation on dark coloured fabrics, denim and leather. Luminol was the only technique to enhance footwear impressions made in blood on all the fabrics investigated in this study.

Amino acid staining reagents are not commonly used for chemical enhancement of shoeprints in blood. Nevertheless, Farrugia and his co-authors studied this approach as well (40). Ninhydrin and two of its analogues were used for the enhancement of footwear impressions in blood on various types, colours and porosities of fabric. As in their previous work, test footwear impressions on fabric were prepared using a specifically built rig to minimise the variability between each impression. Ninhydrin enhancement of footwear impressions in blood on light coloured fabric yielded good enhancement results, however the contrast was weak or non-existent on dark coloured fabrics. Other ninhydrin analogues which have the advantage of fluorescence failed to enhance the impressions in blood on all fabrics. The sequential treatment of impressions in blood on fabric with other blood enhancing reagents (e.g. protein stains and haem reagents) was also investigated.

Another extensive review on methods for chemical enhancement of impressions in blood on non-porous surfaces was published recently by Velders (41). The purpose of this research was to explore the possibilities to improve visualization of blood traces, after they were detected with Luminol, by exposing them to other chemicals, such as LCV, AY7 and Hungarian Red. The results show that LCV appears to be a less suitable chemical to enhance visibility of blood impressions on non-porous substrates.

In this context of chemical enhancement of marks in blood, Leintz conducted a study to determine whether investigative personnel walking on a surface that had been contaminated with blood and then cleaned would transfer the deposited haem onto a non-bloody surface, thus causing chemiluminescence on the non-bloody surface (42). It was found that crime scene investigators can feel confident in finding chemiluminescence, and a presumptive positive result for cleaned up blood, with the chemical Bluestar Forensic, even after the tested area has been travelled over by investigative personnel. This experiment showed that chemiluminescence, and the presumptive presence of haem, was not transferred by the movement of shoes through the tested area.

Many other articles, focusing on the chemical enhancement of fingermarks in blood, were published during the review period. One that is worth mentioning here is a review by Bossers and her colleagues (43), that examines techniques and materials that may be used to enhance and

record fingermarks deposited in or by blood. A large number of techniques are presented and discussed from a chemical as well as practical perspective. It is concluded that an optimized sequence of techniques targeting both latent (non-bloody) and bloody fingermarks must be applied to detect and enhance the maximum number of marks, and therefore optimize the information content from exhibits that may bear marks in blood. This article may serve as a guide for chemical enhancement of footwear impressions in blood as well.

Zarate and Morden describe the use of the application of the recently-developed Zar-Pro fluorogenic lifting strips for lifting, enhancing and preserving bloody impression evidence (44). The Zar-Pro strips are manufactured in sheets and cut to size according to use. Smaller strips can be cut from a large sheet for fingerprints or the large sheets can be left uncut for larger handprints or footwear impressions. The strips are composed of white nylon transfer membranes with a specialized chemical formulation that is bonded to the membrane and is activated with a 50:50 methanol and water solution. These easy-to-use strips successfully lift and enhance bloody impressions from a variety of substrates, regardless of porosity or background colour. The lifting strips are highly sensitive and fluoresce when coupled with proteins and excited with an alternate light source.

Thomas and Farrugia discuss the application of two novel reagents for blood, genipin and lawsone, both natural products, for the enhancement of fingermarks in blood on paper (45). The abilities of these two reagents to enhance blood contaminated fingermarks on papers of various porosities and colour were investigated and compared to the routinely used amino acid reagents, ninhydrin and 1,8-diazafluoren-9-one (DFO). Results indicated that while genipin showed some potential as a reagent for the enhancement of latent fingermarks, it displayed no suitability for the enhancement of fingermarks in blood on paper. Lawsone also failed to successfully enhance either type of fingermark. Upon comparison of the results with those of ninhydrin and DFO, it was found that ninhydrin displayed the highest success rate of development of these marks.

Another bodily fluid that might be encountered in crime scene environments is urine. Farrugia and his colleagues (46) utilised a range of chemical techniques for the enhancement of footwear impressions deposited on a variety of fabric types of different colours with urine as a contaminant. Multiple impressions were produced and enhanced by each reagent to determine the repeatability of the enhancement. Urine samples from different donors were analysed using a spectrofluorophotometer revealing differences between individuals. Results indicated that the enhancement of footwear impressions in urine was possible using amino acid staining techniques whereas protein stains failed to achieve successful enhancement.

As a summary of the chemical enhancement issue, an article regarding the comparison of enhancement techniques for footwear impressions, in blood and urine, as well as in soil, on dark and patterned fabrics, is to be

published by Farrugia *et al* (47). A range of readily available chemical and lighting techniques, already discussed in previous work by these authors, were utilized to enhance footwear impressions made in blood, soil, and urine on dark and patterned fabrics. In most cases, results demonstrated that fluorescent chemical techniques were required for visualization as non-fluorescent techniques provided little or no contrast with the background. Occasionally, this contrast was improved by oblique lighting. Successful results were obtained for the enhancement of footwear impressions in blood; however, the enhancement of footwear impressions in urine and soil on dark and patterned fabrics was much more limited. The results demonstrate that visualization and fluorescent enhancement on porous substrates such as fabrics is possible in many cases.

2.2 Manufacturing Processes and Outsoles Design

As in many areas of forensic comparisons, understanding of manufacturing processes is essential for shoeprint examinations. Good sources of information for this aspect are the footwear industry newsletters, like the *SATRA Bulletin*. In the Review period, several articles have been published in this *Bulletin* regarding the use of PVC in shoe soles, thermoplastic rubber soles, the manufacturing of vulcanised rubber soles, deck shoes and polyurethane soles (48-52). Although the addressees of these articles are mainly members of the footwear industry, forensic scientists dealing with shoeprint comparison may gain great benefits from them as well.

2.3 Tire Tracks

Jin discusses the forensic importance of tire track impression examinations (53). In this paper, the characteristics of vehicle tire pattern and its application in forensic science was analyzed and summarized, based on the analysis of manufacturing processes and of acquired individual characteristics during use.

Several other articles dealing with the examination of tire tracks impressions are included in other sections of this Review (8, 21 and xx1).

2.4 Test Impressions

During the course of their extensive work on chemical enhancement of footwear impressions, Farrugia and his colleagues developed a device for producing controlled test impressions (54). Since the comparison of enhancement techniques for the visualisation of footwear impressions may be hindered by uncontrolled variables such as the force applied when the impression is created, a test rig was constructed for overcoming this

difficulty. The footwear impressions prepared from the test rig limit some of the variables introduced during the production of test footwear impressions and allow for a more robust evaluation of the enhancement techniques to be made. This footwear rig has been utilised for the preparation of test footwear impressions for the evaluation of chemical techniques for the enhancement of footwear impressions in blood, urine and mud on fabric. Such trials can only demonstrate the potential of a specific technique and its operational use must still be evaluated in contextualised trials. According to these authors, this rig was suited for the preparation of test footwear impressions with a stamping action to approximate the action of stamping on clothing. A similar rig can potentially be utilised to observe bruise patterns on skin produced at different forces.

2.5 The Evidential Value of Shoeprints Examination

A textbook on forensic comparative examinations, not available to this Reviewer when preparing the previous Review, had been published in 2009 by Vanderkolk (55). The author focuses mainly on the scientific process and methodology of comparative examinations in general, with emphasis on various disciplines (for instance fracture matching, toolmarks and firearm identification, fingerprints and lip mark). This publication covers, among other topics, the issue of footwear impression and tire tracks comparisons (55, chapter 9, pp. 149-168), including manufacturing processes, acquired features in shoe soles and tires, etc.

Several studies addressed the issue of class- and individual- (or acquired-) characteristics in shoeprints analysis. Hancock and colleagues conducted a survey of five hundred shoeprints taken from volunteers in Auckland, New Zealand (56). These prints were compared against each other for the presence of any pattern correspondences. Comparisons were undertaken of the full outsole and of smaller portions of the more common patterns. Of the 500 shoe impressions collected, 488 (97.6%) were ultimately represented only once in the survey. The greatest number of corresponding patterns was for the most common brand of shoe (Converse Chuck Taylor All Star) and occurred in 3 of 500 observations. No instances of an imitation brand matching the authentic brand were found. Smaller sections of the common patterns showed a greater number of corresponding prints. However, the greatest number of matching partial patterns was again for the most common brand of shoe and occurred in 29 of 500 observations. These researchers conclude that pattern match alone is of considerable evidential value even when the print is partial.

Another article dealing with class characteristics, written by Gross *et al*, examined 402 test prints, collected by the Minnesota Bureau of Criminal Apprehension (BCA), US, and compared each one to all others (57). These prints originated from footwear of 127 different manufacturers. Using the manufacturing characteristics of general design element type, outsole

design, and design element size-relationship, 99% of the impressions could be distinguished. In addition, utilizing the class characteristic of wear, all 402 BCA footwear impressions (80,601 possible pairs) were easily differentiated. It should be noted that not all outsole designs persist over such a long time span (twenty years), the population size was relatively small, and the impressions were of high quality. However, it still demonstrates that class characteristics are quite variable in casework and therefore can be very significant in evaluating footwear. The more variation one has with the class characteristics present on the outsole, the more significance it may have when evaluating two impressions. This study also reiterates and expands upon previous literature that the examination of class characteristics can be an extremely significant tool for footwear comparisons.

Bodziak and his colleagues wrote an extensive article on the significance of outsole wear characteristics in the forensic examination of shoeprint evidence (58). These authors defined terms used in the forensic footwear examination and comparison of outsole wear, summarized past research in the area of wear, and discussed the various considerations that should be taken into account when evaluating general wear in casework comparisons. Considerations include factors that limit clarity of the impression, manufactured characteristics, and time intervals between when the impression was deposited and when the shoes were seized. A variety of general wear is encountered in footwear casework and can be used to limit the population of shoes that could have made the impression. However, general wear may appear similar on shoes of the same person and between shoes belonging to different people and therefore general wear alone should not be used to identify a shoe as the particular source of an impression. A survey conducted as part of this project indicates that general wear is not used to individualize footwear impressions by the international community of footwear examiners.

Wilson conducted a study on 39 pairs of running shoes (Adidas Supernova Classic, men's size 12) that were worn by one individual over approximately an 8-year time period, on similar surfaces for a similar number of miles (59). These shoes were examined for the presence of individual characteristics to determine whether they were able to be individualized. The total number of individual characteristics in each shoe varied from 1 to 61, with an average of 11.23 for the right shoes and 20.31 for the left shoes. In comparing the number of individual characteristics in each area for each shoe, it was found that no shoe had the same number of individual characteristics in the same area in every area as another shoe. Therefore, each shoe was different from every other shoe. The results of this study support the premise that all individual or accidental characteristics are random and happen by chance, and that by using these characteristics, footwear impressions are able to be identified to a single source.

The Bayesian approach, advocated in the last decades by many forensic scientists for its application in various disciplines, has not yet been fully

studied for footwear impression analysis. However, several recent articles addressed this issue.

Skerrett and his co-authors proposed a model for evaluating shoemark evidence in a more transparent manner, based on the Bayesian approach (60). The model is currently limited to sole pattern and wear characteristics, and it does not account formally for cuts and other accidental damages. Furthermore, it requires the acquisition of relevant shoemark datasets and the development of automated comparison algorithms to deploy its full benefits. These are not currently available. Instead, these authors demonstrate, using casework examples, that a pragmatic consideration of the various variables of the model allows us to already evaluate shoemark evidence in a more transparent way and therefore begin to address the current scientific and legal concerns. This study expands equations from previous research to refine a model for evaluating shoemark evidence. While focusing their development on the source-level elements, the formal mathematical development and the examples offered in this paper clearly highlight how this research relates to the offence-level LR. The contribution of the pattern and degree of wear to the weight of shoemark evidence was considered, while accounting for the time difference between the acquisition of the mark and print impressions. The LRs obtained using this pragmatic approach, and by assigning only very conservative probabilities, show strong to very strong support to the prosecution hypothesis for the chosen examples. This demonstrates the high evidential potential of the combination of pattern and wear features; and shows that there is a significant benefit to better exploit these features before attempting to account for more volatile features such as cuts and other accidental damages.

Juchli and colleagues took this a step forward and assessed the combined evidential value of several types of evidence (including footwear impressions) by using Bayesian networks (61). This paper discusses graphical probability models, i.e. Bayesian networks, as framework within which the joint evaluation of scientific evidence can be approached in some viable way. Based on a review of existing main contributions in this area, the article here aims at presenting instances of real case studies from the author's institution in order to point out the usefulness and capacities of Bayesian networks for the probabilistic assessment of the probative value of multiple and interrelated items of evidence. A main emphasis is placed on underlying general patterns of inference, their representation as well as their graphical probabilistic analysis. Attention is also drawn to inferential interactions, such as redundancy, synergy and directional change. These distinguish the joint evaluation of evidence from assessments of isolated items of evidence. Together, these topics present aspects of interest to both, domain experts and recipients of expert information, because they have bearing on how multiple items of evidence are meaningfully and appropriately set into context.

Koehler used footwear impression evidence as an example for the problematic way (according to this author) scientific evidence is presented

in court (62). This article uses a likelihood ratio (LR) approach to identify the probative value of forensic science evidence. It argues that the “evidence” component should be characterized as a “reported match,” and that the hypothesis component should be characterized as “the matching person or object is the source of the crime scene sample.” This characterization of the LR should force examiners to incorporate risks from sample mixups and examiner error into their match statistics. In addition, this work includes a controlled experiment with 315 jury-eligible jurors who received a shoeprint match statistic in a hypothetical burglary case finds that, contrary to normative theory, people are more persuaded by statistical testimony that ignores various error risks than by testimony that is objectively stronger by virtue of taking those risks into account. That experiment also found that jurors are relatively unresponsive to exposure of those risks by a defense attorney on cross-examination. These results support and extend previous research that finds many people are confused about how to evaluate the risk of error associated with expert forensic testimony.

Nordgaard and his colleagues (63) present a method to develop an ordinal conclusion scale for the value of evidence that can be applied to any type of forensic findings. The method is built on probabilistic reasoning about the interpretation of findings and the number of scale levels chosen is a compromise between a pragmatic limit and mathematically well-defined distances between levels. The application of the unified scale, used by the Swedish National Laboratory of Forensic Science (SKL), is illustrated by a number of case studies (unfortunately, none of which is of shoeprint examination). One of the features with the type of scale presented in this paper is the possibility to use the translation of intervals of likelihood ratios to scale levels backwards. Once a scale level has been decided for some particular findings, it is possible (though maybe conservative) to find a lower limit for the likelihood ratio, which in turn may be used if these findings are to be combined with other findings (conditionally independent of the former) of the same criminal case. Instead of leaving the issue of combination to the court, the forensic laboratory may thus investigate the total evidentiary strength of the findings addressed at activity level propositions.

The 2009 US National Academy of Science - National Research Council (NAS-NRC) report, "Strengthening Forensic Science in the United States: A Path Forward" (64), stressed the issue that "...there is no consensus regarding the number of individual characteristics needed to make a positive identification, and the committee is not aware of any data about the variability of class or individual characteristics or about the validity or reliability of the method. Without such population studies, it is impossible to assess the number of characteristics that must match in order to have any particular degree of confidence about the source of the impression..." (64, p. 149).

In order to tackle this issue, ENFSI EWGM conducted several collaborative tests, where participants were given footwear impressions and test prints,

compared them and summarized the results using the six-step conclusion scale developed by this Group several years ago. Two such tests were held since 2010, distributed the same way as earlier exercises were, on a DVD containing image files and other information. In the 1st one (2010), test samples were prepared using a right bicycle shoe, placing prints on a sheet of paper and on a towel (65). The print on the sheet was lifted with a gelatin lifter and the print on the towel - with an electrostatic device. Both lifted prints were photographed. Test prints were also made from that pair of bicycle shoes. Answers were received from 52 participants, and the spread of the results for this test was smaller than that of previous ones. It seems that the print on the towel was more difficult than the on the paper sheet, since the conclusion spread was larger in this one, and the average level of confidence - lower.

For the 2nd test (2012), one pair of long time worn Adidas running shoes was used to produce prints on a flat smooth surface. The prints were enhanced with grey powder, photographed and then lifted using black gelatin lifters (66). The gelatin lifters were photographed, and these images were sent to the participants. Answers were received from 73 participants. Here, again, the spread of the results for this test was even smaller than that of previous ones, and the average level of confidence - higher.

The variability in footwear impression comparison conclusions was studied also by Hammer and her colleagues (67). Six footwear simulated case studies were created and sent to 60 certified footwear examiners in North America. The examiners were asked to independently assess each case, based on features that were clearly marked on each impression, and were directed to use a specific scale of conclusions to report their findings (identification, probably made, could have made, inconclusive, probably did not make, elimination, and unsuitable). Forty participants completed the task and provided their input. The results of this study, in contrast to those of previously published ones, were that when experienced examiners used the same conclusion scale and compared the same features, there was little variability within their stated findings. This indicates that there is significant agreement among trained footwear examiners regarding the level of associative value of corresponding characteristics and that a standard scale of conclusions may facilitate the expression of consistent opinions. To further explore the variations observed in the conclusions expressed by the certified footwear examiners, the examiners were divided into those who do not use the SWGTREAD guidelines regarding footwear impression evidence and those who do. Following statistical analysis it was determined that overall, the values reported by these subgroups of examiners were not significantly different, and demonstrating that prior experience with the SWGTREAD guidelines is not required for them to be appropriately used during a footwear evidence comparison.

Cognitive bias in forensic science was addressed in a workshop held in the Northwestern University School of Low in September 2010, where Hammer introduced the issue of footwear and tire track impressions comparison (68, pp. 11-14). Various aspects of these types of examinations were presented,

including the examination process, potential problems and error rates. There was a broad recognition among participants of the workshop that issues related to cognitive bias in the forensic sciences, including shoeprints examination, is an important policy issue. Meaningful progress can only be made with the cooperation and good will of both the broader forensic science community and funding agencies that prioritize this interdisciplinary research.

Izraeli and his colleagues describe a simple, yet powerful and efficient, method for assisting the presentation of shoeprint comparisons in court (69). This method uses Adobe Photoshop Elements (Adobe Inc., San Jose, CA), or other similar software for image processing, and Microsoft PowerPoint for the presentation in court. The PowerPoint presentation enables the expert to show the test impressions overlapping the prints, gradually change the opacity of the test impression on the print, and slightly move the test impression to imitate in great accuracy the comparison and evaluation process done in the laboratory. It is these authors' opinion that the quality of the presentations to judges and juries alike will prevent misunderstandings by non-scientists involved in the judicial system. This method can assist tool marks and firearms comparison experts as well and might simplify the expert's job.

2.6 The Judgement of Regina v T

In October 2010, the Appeal Court of England and Wales issued a redacted judgment in Regina *v* T (R *v* T, 70), which caused considerable concern amongst forensic scientists and statisticians who supported the approach to evidence interpretation and evaluation by the Bayesian or likelihood approach. Due to the importance of this decision, numerous articles were published, discussing this subject, and a special issue of *Law, Probability and Risk* was dedicated to it (71-83). Thus, a special emphasis is given to this matter here as well.

In this case, an expert with extensive experience in the examination of footwear marks, carried out a comparison with pieces of footwear, including trainers found in the defendant's house after his arrest. The expert concluded that there was a "moderate degree of scientific evidence" to support the view that the trainers recovered from the defendant had made the marks in question. His reports contained no statistical information or reference to use of a likelihood ratio or the formula used in calculating.

A detailed description of the case is outside the scope of this Review, but in general, the Court of Appeal stated that:

> "In our judgment, an expert footwear mark examiner can therefore in appropriate cases use his experience to express a more definite evaluative opinion where the conclusion is that the mark "could have been made" by the footwear. However no likelihood ratios or other

mathematical formula should be used in reaching that judgement for the reasons we have given" (70, §95).

"It is essential, if the expert examiner of footwear expresses a view which goes beyond saying that the footwear could or could not have made the mark, that the report makes clear that this is a view which is subjective and based on his experience. For that reason we do not consider that the word "scientific" should be used, as, if that phrase is put before the jury, it is likely to give an impression to the jury of a degree of precision and objectivity that is not present given the current state of this area of expertise" (70, §96).

"The process by which the evidence was adduced lacked transparency. This is no personal criticism of [the expert witness], as he was simply following practice. However, it is simply wrong in principle for an expert to fail to set out the way in which he reached his conclusion in his report" (70, §108ii).

The Court accepted, however, the practice of using LRs, when used properly:

"The practice of using likelihood ratios was justified as producing "balance, logic, robustness and transparency"... In our view, their use in this case was plainly not transparent" (70, §108iv).

The Court then found the conviction as unsafe, and quashed it. At a subsequent retrial, the accused was found 'Not Guilty' (71).

Berger and his colleagues discussed several of the issues raised in R *v* T (84). According to these authors, although the judgment concerned with footwear evidence, more general remarks have implications for all disciplines within forensic science. Their concern is that the judgment might be interpreted as being in opposition to the principles of logical interpretation of evidence. They re-iterate those principles and then discuss several extracts from the judgment that are potentially harmful to the future of forensic science.

In the light of the R *v* T Court opinion, Biedermann and his co-authors discuss issues that pertain to the choice of relevant databases for assigning values to the components of evaluative likelihood ratio procedures at source level, and argue, from a methodological point of view, that there are additional levels of qualitative evaluation that are worth considering prior to focusing on particular numerical probability assignments (72). Analyses are proposed that intend to show that, under certain assumptions, relative numerical values, as opposed to absolute values, may be sufficient to characterize a likelihood ratio for practical and pragmatic purposes. It is further argued that, even if numerical evaluation can be made, qualitative considerations may be valuable because they can further the understanding of the logical underpinnings of an assessment. In the second part of this article, a parallel is drawn to R *v* T by concentrating on a practical footwear mark case received at the authors' institute. This case serves the purpose of exemplifying the possible usage of data from various sources in casework and help to discuss the difficulty associated with reconciling the

depth of theoretical likelihood ratio developments and limitations in the degree to which these developments can actually be applied in practice. These authors conclude that it would be simplistic to believe that dealing with a case involving footmark evidence could be reduced to the task of drawing a single numerical value from a database. The actual challenge is far more subtle than this because it involves a detailed consideration of the competing propositions of interest, a critical examination of available data and incorporation of information from the framework of circumstances.

Similarly, Nordgaard and Rasmusson argue that the scientific framework for forensic findings interpretation stems from Bayesian theory (73). The resulting likelihood ratio, which may be expressed using a verbal or a numerical scale, compares how frequent are the obtained results given that one of the propositions holds with how frequent they are given that the other proposition holds. A common misunderstanding is that this approach must be restricted to forensic areas such as DNA evidence where extensive background information is present in the form of comprehensive databases. In their article, these authors maintain that the approach with LRs is equally applicable in areas where the results rely on scientific background data combined with the knowledge and experience of the forensic scientist. In such forensic areas the scale of the LR may be rougher compared to a DNA case, but the information that is conveyed by the likelihood ratio may nevertheless be highly valuable for the court. It is interesting to note that the LR verbal conclusion scale used by these authors is somehow different than the one used by the shoeprint expert in the R *v* T case.

Similar approach was take also by Sjerps and Berger (74), seeing the likelihood ratio framework and Bayesian networks as tools to promote transparency and logic, and arguing that transparency requires making clear whether a conclusion is a consensus and reporting diverging opinions on request. These authors recommend that reporting guidelines explicitly address transparency of expert reasoning.

Thompson, while defending the LR calculation approach, laid the responsibility for the outcome of the R *v* T case in the hands on the prosecution's expert (75). Thompson states that LR calculations are far more transparent than the intuitive, experience-based, judgements of the "traditional" practice of presenting evidence in court. In addition, if the expert’s judgement rests on a weak scientific foundation, that fact becomes more apparent when the expert explicitly computes likelihood ratios, than when the expert makes the kind of global evaluative judgement favored by most forensic scientists outside Europe.

Bodziak, on the other hand, presents the "traditional" way of presenting footwear impression evidence in court (76). The R *v* T Court’s comments and the values used by the footwear mark examiner as applied to his Bayesian evaluation and likelihood ratio are discussed and contrast is drawn to this method versus the traditional footwear mark evaluation used by footwear examiners in the USA and most other countries. This author conclude that when conclusions are supported with documented and confirmable characteristics, including supporting photographs embedded

within or attached to the report, and containing traditional wording to clearly express those observations and conclusions, a more thorough and transparent way of transmitting an opinion to both the investigator and a jury in a clear concise and fair manner is accomplished.

According to Ligertwood and Edmond (77), forensic science evidence must be presented in a form that can be accommodated within the process of proof employed by judges and juries. This is a non-mathematical inductive process that seeks ‘the inference to best explanation’ to a standard of proof beyond reasonable doubt. The question posed is not the mathematical probability of the prosecution hypothesis but whether having regard to all the evidence before the court the prosecution hypothesis is the only explicable hypothesis, in the sense that no reasonably possible defense hypothesis remains open. The challenge is to present forensic science evidence in a form that can be accommodated within this non-mathematical inductive standard of proof. It is argued that this is most effectively achieved if that evidence is tendered as a frequency rather than as a likelihood ratio.

Kaye wrote another review on the R *v* T case (85), analyzing the various ways scientific evidence can be presented in courts (the "traditional" way, the "extreme Bayesian" approach, the "mild Bayesian" approach and LR). The author concludes that "any expert who reasonably can testify to a degree of confidence in a source hypothesis reasonably can testify to likelihoods", and that all the expert has to inform the fact-finder (either a judge or a jury) is the strength of the evidence, namely how more likely it is that the impression in question was made by the examined shoe rather than by any other shoe in general.

As it seems, the ongoing debate raise by the R *v* T judgement, between Bayesian and "traditional" forensic scientists, is far from resolution, and will probably be further discussed by these and other scholars as well.

2.6 Databases, Reference Collections and Automated Classification

With the numbers of both footwear outsole designs as well as shoeprints documented at crime scenes rapidly increasing, the need for computerized mean of keeping these records is becoming more and more crucial in forensic laboratories.

In addition to the ENFSI EWGM “Wanted Page” mentioned earlier (6), several commercial firms manufacture footwear sole pattern databases.

Foster & Freeman Ltd. (UK) provide its footwear and tire tread databases, SoleMate®, TreadMate® and SICAR® 6 (86).

Laboratory Imaging s.r.o. (Czech Republic) manufacture a scanning system for shoeprints and fingerprints - Lucia TrasoScan™, that incorporate the ability to scan the prints and the footwear soles with high resolution (1000

DPI) with a computerized system for on-screen comparison of the crime scene prints with known test prints from suspects' footwear (87).

Chochol and Świętek present examples of several such shoeprints databases used around the world, as well as practical applications of a database developed at the Institute of Forensic Research (IFR), Krakow, Poland (88). The authors carried out an analysis of the shoe market in Poland and collected information in the form of sole imprints as well as photos of shoes, which gives a chance of creating, in cooperation with other laboratories, a large database of soles for forensic purposes.

The practical use of a crime intelligence database in Switzerland (including mainly situational information, DNA, shoeprints and images) covering the years 2009-2011, is described by Rossy *et al* (89). The database, shared by intelligence units of six states of the western part of Switzerland since 2008, analyzes, filters and classifies events reported to the police on a daily basis, to detect crime repetitions and interpret the crime environment. Several forensic outcomes are integrated in the system such as matches of traces with persons, and links between scenes detected by the comparison of forensic case data. Systematic procedures have been settled to integrate links assumed mainly through DNA profiles, shoemarks patterns and images. This article contains detailed statistical analysis of the links developed by the database, including an interesting observation that the ratio of series where shoeprints were used to link crime scenes (23%) is higher than those with DNA (8%) or images (6%). The results suggest that forensic outcomes have a great potential to detect crime series. DNA and shoemarks mainly detect burglaries, while images are better at detecting series of distraction thefts and pickpocketing. It is then worth relying on a diversified set of forensic case data to gain better insight on the different types of crimes series. The vast majority of events are linked through only one forensic link type (99.2%), further demonstrating the necessity to use all types of marks for a better detection of crime repetitions. The integrated processes of shoemarks patterns at state level and regional level have been also compared. It shows that the detection of shoemarks patterns links at the regional level takes more time than at the state level. Nonetheless they have the same potential to detect series. The regional level links have even a better potential to increase already detected series.

The development of computational methods for use by forensic footwear examiners may address several tasks encountered by the forensic footwear examiner: determining the type of sole (and that of shoe) that produced a given print, linking this print to other scenes where similar prints were found, linking potential suspects to crime scenes and eventually assessing the evidential value of a match.

Deshmukh and Patil present a shoeprint matching algorithm invariant to rotation and to intensity variations (90). The multiresolution features of a shoeprint have been extracted using Gabor transform, while rotation of the shoeprint image is computed using Radon transform and is compensated by rotating the features in opposite direction. The performance of the proposed algorithm was compared with the algorithm in which the features

have been determined using Fourier transform and its power spectral density. Euclidian distance classifier was used to find a suitable match. The performance of the proposed algorithm has been evaluated in terms of Correct Recognition Rate computed using best Match Score for rank '1' and cumulative match score for the first four matches, on a database of 200 sets of prints. It is observed that a good matching performance is achieved - starting at 91% for first rank on full prints and rising to 100% for best four matches. Performance of the proposed approach was even better for the rotation, intensity and mixed attacks on full prints, and even for partial shoeprints. It is not, however, invariant to scale variations, and was tested only on test prints, and not on crime scene impressions.

Dai and Tang present content-based image retrieval method, applied for developing a system for automatic retrieval of questioned shoeprint images from a reference database of shoeprint images (91, 92). The organizational structure, functional features, shoeprint query of the system and its application to footwear impression examination and identification is introduced in details. Comparison experiment was made to demonstrate its ability of retrieving similar images and identifying shoeprints. The author state that experimental results show that the retrieval speed and performance of the system are satisfying. The developed system is widely used in many police stations throughout China. The system proofs to be quite effective from case solving practice and the utility of footwear impressions increases greatly as convincing physical evidence.

The use of the scale-invariance feature transform (SIFT) approach for recognizing and retrieving incomplete shoeprints was investigated by Wei, Li and their colleagues (93, 94). These researches proposed and evaluated scale-invariance feature transform for recognition and retrieval of partial and noisy shoeprint images, applied to a dataset of 430 full-size prints, about 1,700 partial prints and thousands of noisy prints (all produces with 86 different shoes). The proposed method first constructs different scale spaces to detect local extrema in the underlying shoeprint images. Those local extrema are considered as useful key points in the image. Next, the features of those key points are extracted to represent their local patterns around key points. Then, the system computes the cross-correlation between the query image and each shoeprint image in the database. Experimental results show that full-size prints and prints from the toe area perform best among all shoeprints. Furthermore, this system also demonstrates its robustness against noise because there is a very slight difference in comparison between original shoeprints and noisy shoeprints.

Rathinavel and Arumugam (95, 96) proposed an integrated technique for shoeprint recognition system, based on pass band discrete cosine transform (DCT) components analysis in Fisher linear discriminant (FLD) with principal component analysis (PCA). These authors claim that the proposed system perform better than other published solutions in terms of computation time as well as in noise reduction. Fourier transforms (FT), invariant to translation and rotation, were used for classification of partial prints.

Tang and colleagues describe a clustering approach, based on common primitive patterns (97). Shape features consisting of lines, circles and ellipses are extracted from database prints using variations of the Hough transform. Then an attributed relational graph (ARG) is constructed for each known print, where each node is a primitive feature and each edge represents a spatial relationship between nodes. A footwear print distance (FPD) between ARGs is used as similarity measure. The FPD is computed between each known print and pre-determined patterns to form clusters. The use of the methodology is demonstrated with a large database of known prints.

Taking this approach further on, these researchers used the proposed method for crime scene, partial and degraded prints (98, 99). like in the previous work, prints in the database were clustered based on outsole patterns, and each footwear print pattern is characterized by the combination of shape features and represented by an ARG. Similarity between prints is computed using Footwear Print Distance. It was demonstrated that the proposed system is invariant to distortions like scale, rotation or translation, is insensitive to noise and degradations of the prints, and works well with the partial prints, color prints and crime scene marks. Sensitivity analysis of FPD was performed to show its robustness. Experiments show that the approach outperforms other state-of-the-art footwear print retrieval systems.

This system for footwear impression retrieval (97-99) is described in more details by Srihari (100). This report compares several methods for image retrieval, like the SIFT and ARG mentioned above, and conclude that the performance of the ARG-based system is significantly better than other published methods. Several data sets were used in the research: simulated prints, photographs of outsoles retrieved by a web crawler from shoe-vendor websites, and 350 actual crime scene prints and over 5,000 known prints. Since results with simulated images tend to be over-optimistic, most of the reported results focused on the real crime scene prints. However, this system hasn't matured yet to a fully operational one.

Gao and Allinson present a multiresolution-based hybrid approach for 3D outsole feature classification and extraction (101). Their system is able to extract information-rich 3D outsole patterns and produce 2D shoeprints regardless of different degrees of wear. Based on pattern characteristics, outsoles are categorized into Convex-Pattern-Dominant Outsoles (Convex-PDOs) and Concave-Pattern-Dominant Outsoles (Concave-PDOs). Initial work for extracting 3D Features from Concave-PDOs is reported in this paper. Outsole models are first captured using a 3D scanner. Patterns corresponding to higher and lower curvature variations are subsequently classified using a multiresolution-based curvature analysis approach. Visual analysis on current experimental investigations shows promising results for further 3D feature extraction and 2D shoeprint generation.

Cervelli dedicates a chapter in his PhD dissertation to the automatic retrieval of footwear impressions from crime scene images (102). The author reviews the existing systems for shoeprints retrieval, and compares

their algorithms. Then, systems based either on the Mahalanobis distance map feature to tackle the noise affecting real shoe marks, or on the translation and rotation properties of the Fourier transform to realize a translation and rotation invariant system suited for comparison of uncontrolled images, were developed. It was found that the Mahalanobis distance method, coupled with modified phase-only correlation (MPOC), is well performing with real shoe marks, thanks to its robustness to noise, but the system is not invariant to translation and rotation. On the other hand, the performance of the Fourier phase correlation (FPC) system degrades with noisy real shoe marks. Despite these results, the translation and rotation invariance of the system would make it more suitable in real cases, where the uncertainty of the aforementioned parameters would make both the MPOC and the Mahalanobis based systems less effective.

Huang *et al* proposed a novel algorithm based on Gabor wavelets and support vector machine (SVM) for recognition of tire tread patterns (103). Input tire images are first preprocessed by morphological opening to enhance the features (or textures) on tire surface. The features of tire tread patterns are then represented by Gabor wavelets, and feature extraction is further carried out by principal component analysis (PCA). Finally, the matching processes are achieved by the classifiers of the SVM, Euclidean distance and cosine distance. Result shows that the recognition rate of 60% for tire images can be obtained by the SVM classifier when 15 tire tread patterns are used

Another effort that is worth mentioning here, although it is not yet completed, is a research project conducted at the Hadassah Academic College, Jerusalem, Israel, in conjunction with the Israel Police DIFS (funded by the NIJ, US), for the development of a computerized expert system for supporting shoeprint experts in evaluating the degree of certainty in 2D footwear impressions. The system has already been presented in several international conferences (104, e.g.), and a workshop was held during the last ENFSI EWGM Meeting, June 2013 (Bled, Slovenia), where a beta version of the system (Statistic Evaluation of Shoeprint Accidentals - SESA) was demonstrated and distributed to 20 police agencies to be practiced and evaluated.

3 Toolmarks

Toolmark examinations (in the sense of the examination of marks produces by surfaces other than firearms) are, in a way, the “poor family-member” of firearm identification. Many of the professional groups, as well as published articles, are dedicated mainly to firearm examinations, and toolmarks are referred to matter-of-factly. Since firearm identification issues are covered by another review in this Symposium, this section of my Review will focus mainly on those aspects more relevant to toolmarks per-se.

SWGGUN (4) continues publishing guidelines and statements, covering many aspects of firearm and toolmark examinations. One of these documents, relevant for toolmark examinations, written by the SWGGUN Scientific Committee, is "The Foundations of Firearm and Toolmark Identification" (105). This article concludes that "the discipline of Firearms/Toolmark Identification is scientific and reliable" and that "sufficient validation testing by competent examiners and collaborating scientists have been conducted to affirm the theory of firearm and toolmark identification over the past ninety years for it to be considered a legitimate science pursuant to the criteria set forth in the scientific method" 105, p. 6). Quality assurance guidelines were also published by SWGGUN (106), designed to provide a framework of standards for quality and integrity in the firearms and toolmarks examination processes, evidence handling, evidence evaluation, reporting and testimony. Other guides that may be found at the SWGGUN web-site are the "Criteria for Identification" and the "Code of Ethics".

A new book exclusively dedicated to the examination of toolmarks was recently published by Petraco (107). This text is divided into two main sections, with the first devoted to the rationale and methodology behind toolmark examination (optics, microscopes and measurement, collecting and documenting toolmarks, the preparation of toolmark standards, etc.) while the second section explores the wide range of tools commonly encountered in casework. The book also includes a chapter on the application of statistical pattern comparison to the examination of toolmarks.

3.1 Casting and Reproduction Methods

Many cases of toolmark examinations involve the need for duplicating the impression marks, found at the crime scene, in order to facilitate their examination at the laboratory. As discussed in our previous Review, silicone rubber is the method of choice for this purpose.

Athanasopoulos and co-authors studied the use of magneto-rheological fluids as an agent for capturing impressions in situ (108). These materials are fluid under most conditions, but solidified when a magnetic field is applied to them, and can be used in lieu of silicone rubber for collecting impression evidence. The fluid compositions were developed through trial and error by adjusting the concentration of the components in the fluid. According to these researchers, the solution used created long lasting, durable, and high resolution casts, which enabled the visualization and analysis of small details not discernible on the original object. The downside of this method is that the casting substrates need to be non-porous and non-magnetic.

3.2 Observation and Imaging Methods

The comparison microscope is by far the most commonly used tool for toolmarks examination. Petraco's book, mentioned above (107), contains a section on basic microscopy for toolmarks examination, including that of the comparison microscope, which may serve as a good starting point in experts' training programs.

Lamagna, on the other hand, criticize the almost-100-years-old use of optical comparison microscopes by toolmark examiners, and claims that more modern sophisticated tools, like 3D optical microscopes, white-light interferometers, confocal laser scanning microscopes and scanning electron microscopes, should be employed (109).

However, several such techniques were already been evaluated for this task, with some reported success. Heikkinen and co-authors studied the potential of scanning white light Interferometry (SWLI) for the examination of toolmarks (110). According to this work, SWLI allows rapid and non contact measurements of millimeter-size objects with nanometer vertical resolution, without any sample preparation.

This group reported also the application of SWLI, as well as of confocal microscopy (CM), for determining the chronological order of creation of crossing lines and of overlapping marks (110-112). It was demonstrated that 3D imaging techniques, like SWLI and CM, can determine the chronological sequence of creation of crossing toolmarks on a copper surface by looking at the depth profiles of the engraved lines, and that the engraving direction of the last groove can be determined. In addition, 3D imaging may provide a partial solution to confidence issues related to expert forensic evaluation of overlapping marks.

An extensive survey, regarding image processing techniques used for examining striated and impressed toolmarks, was conducted by Gerules and his colleagues (113). These authors review 2D and 3D imaging techniques, as well as many of the algorithms used for matching images, and discuss the strength and weakness of these methods for both image matching and statistical uniqueness. Although focused only at firearm identification examinations, this paper may serve as a reference point for toolmark examiners as well.

Scanning electron microscopy (SEM) had already been proposed for toolmark examination (see, for instance, our previous Review). The high depth-of-field required sometimes for toolmark examination (especially on rough and un-even surfaces), and the high magnification needed, led to several attempts for using SEM for that purpose. Scanlan and Reinholz (114) have recently studied this issue by using a SEM equipped with a firearms comparison stage that allows cartridge cases or bullets to be held and manipulated in two independent holders. The samples tested were both spent cartridge cases and copper wires cut by diagonal cutters. It was concluded that the SEM is not a replacement for the optical comparison microscope, rather it is a valuable specialized tool to supplement it for

firearms component and toolmark comparisons. SEM comparison can make the difference between an inconclusive and conclusive finding if the optical comparison microscope lacks enough magnification, or because visualizing detail is difficult or impossible due to lighting/shadowing problems.

Zhang and Chumbley (115), together with their student Ekstrand (116), used an infinite focus microscope (IFM) for producing virtual manipulative 3D images of sequentially manufactured screwdrivers tips, and compared these images to virtual "toolmarks" that were produced using these tips. It was demonstrated that given the right conditions this approach can be adopted for quantitative and objective toolmark characterization. Factors affecting the correct identification include the quality of the marking, suitable noise cleaning techniques, suitable virtual mark making approach, and the suitable statistical routine. Moreover, this method presents a unique opportunity to improve tool mark analysis by saving examiners' time and reducing the possible damage to the evidence.

3.3 Marks Produces by Various Types of Tools

Knowledge of the manufacturing processes of various types of tools is an essential part in the examination of toolmarks. Montero published a series of articles, relating to this issue, covering a variety of processes relevant both to firearm identification as well as to toolmark examination. The first article is dealing with the machining process and its influence on the produced surface (117). According to this paper, many factors involved in producing surface irregularity of machined surfaces. The tool has a continuously changing edge due to its interaction with the workpiece, resulting in random surface contours. Additionally, the speed of the tool and the feed rate at which the work piece is moved under the tool effect the resulting surface finish. The manufacturing process leaves many machine marks, which vary depending on the conditions of the tool and machining parameters. Outside influences, such as vibrations, may cause additional marks.

Another article in this series describes grinding processes (118). Grinding is a vital process in the manufacture of tooling and finishing of metal products. The grinding process is a material removal process which yields marks resulting from the contact of the wheel and the workpiece surface. The grinding wheel is a self-sharpening tool with essentially an infinite combination of topography. In addition to the marks made from cutting material, there are marks caused by plowing, side flow, and vibrations. These all attribute to the random nature of the surface topography of machined items.

The third paper, about drilling processes (119), illustrates several phenomena, like vibration, that influence the final surface.

Sevigny studied the possibility of identifying toolmarks made by filing (120). Experiments were performed using different types of files, on metal

surfaces of various hardness ratings, and toolmarks made by the same file were compared in an attempt to reach identification. This experiment showed that it is sometimes possible to identify toolmarks made by filing. However, only the filed toolmarks produced under very controlled circumstances exhibited sufficient individual characteristics for identification.

Several case reports, regarding marks produced by unusual tool, were published during this Review period. Clark describes a case where a mattock was positively linked to a clod of soil found at a gravesite (121). This clod, displaying a striated toolmark and collected during the excavation of the grave, was preserved, and the striation mark was cast using silicone rubber. Shovels and a mattock, which had been discarded by the suspects, were subsequently found at another location. A toolmark comparison identified the hoe end of the mattock head as having produced the striated toolmark.

Kumar *et al* present the identification of toolmarks found on a telephone cable to a sickle found at the possession of a suspect, during the course of investigating the theft of that cable (122). The cable contains about 100 pairs of thin insulated copper wires inside a metal sheath and plastic jacket. The cut end of the cable was found at the scene, and a sickle was recovered from a suspect. Following the detection of copper on the sickle blade (using chemical spot test), a test mark was produced on lead and compare to the mark found on the metal sheath of the cable, resulting in a positive identification.

A positive identification of an angle grinder to an abrasive cutting disk was also reported by Newton (123). It was found that the use of these high speed tools produced striae on the metal collar of the cutting discs, that these striae were found to be reproducible, and that identification and exclusion were possible. It was possible to conclude that the disc found at the crime scene could be excluded as being used on the suspect's angle grinder. Although of no forensic significance to this investigation, it was also possible to conclude that the disc found on the suspect's angle grinder had been used on that angle grinder.

Shooting cases involve sometimes the examination of toolmarks produced by surfaces other than firearms. Such a case was presented by Clow (124), who compared striation marks on a lead core to the marks produced by the base edge of the bullet jacket, both found at the scene. Test marks were prepared by pressing the jacket edge to a sheet of lead. The conclusion of this examination was that the marks on the lead core were produced by the edge of the bullet jacket, and that these two items were pieces of the same bullet.

Barnes (125) examined striation marks, unrelated to rifling, on a deformed bullet found at a shooting scene, and compared them to a damaged area on an aluminum shower door frame. The examination revealed that the marks on the bullet nose were impressed there by contact with the door frame, and were actually extrusion process marks of that frame.

In cases where a firearm had been used, but no weapon was available for examination, it might be needed sometimes to compare bullets or cartridge cases found at the crime scene to ammunition rounds found during the search of suspects' residence. Then, comparison of manufacturing process marks may turn out useful. Hebsgaard (126) studied thoroughly the characteristics of toolmarks induced to cartridge cases during production processes, and presents several casework examples for such examinations. It should be noted that although the toolmarks can be used as a supplemental analysis which increases the strength of the evidence, it cannot be used to “prove” that cartridge cases from a crime scene are the same as those found in subsequent searches of a suspect. As a result, a match of these marks merely increases the probability that cartridges come from the same production line.

Marks found inside locks and on keys may indicate the way the locks were picked, or the keys duplicated. Clausing and co-authors used a confocal microscope for the contactless acquisition of toolmarks on cylinder locks pins (127). The purpose of this study was the development of an automated system for detecting marks on picked cylinder locks pins, in order to identify the opening method. Several picking methods were used, like raking and single pin picking, and the marks produced were compared to those of regular use (wear) of the lock. It was found that the automated system is able to differentiate picking marks from normal usage wear to some extent, but further improvement is still needed.

Jin studied the characteristics of the marks found on concave keys reproduced by key duplication machines (128). It was found that marks produced by duplication machines can be distinguish from those on normal wear.

Eckardt and her colleagues presented the examination of joint edges and faces on protective foils of identity documents, using standard equipment in forensic toolmark laboratories (129). It was demonstrated that cutting patterns of foils as thin as 0.1 mm can be examined and attributed to comparison cuts. The features on the joint edges provide only few details and complexity. They alone do not allow an identification of the applied blade. But they assist finding the correct positions for a comparison of the patterns on the joint faces. The matching patterns on the joint faces are complex sequences of striae, representing the accidental production characteristics (grinding striae) of the blade. With these matching patterns it was proved that the identity document was cut with one of the rotary cutters of the cutting board.

3.4 Examination of Consecutively-Manufactured Tools

One of the ways of demonstrating the uniqueness of toolmarks is by studying the marks produced by consecutively-manufactured tools or firearms. Grieve used 50 sequentially-manufactured slip joint pliers for

cutting copper and lead wires (130). The cut ends of the copper wires were scanned using an infinite focus profilometer, at 10x magnification, and the marks compared using the algorithm previously developed by this group for screwdrivers striated marks. It was found that the algorithm may be applied to quasi-striated marks such as those made by the shear edge of slip-joint pliers, by changing the comparison parameters, specifically the sizes of the search and validation windows, and produce successful identification of known match/non-match comparisons.

3.5 The Examination of Stabbing and Cutting Marks

Many of the studies, conducted in the Review period in the field of identification and comparison of marks, deal with stabbing, cutting or sawing marks to the human body. Such marks may be generally divided into saw marks on one hand, and knife (cutting and stabbing) marks in the other.

Symes and co-authors developed an extensive manual, containing standard definitions, documentation protocols, and analytical methodologies that enable more accurate and reliable analyses of saw- and knife- marks in bone and other hard tissues (131). In developing the content of the manual, the project first relied on the creation, analysis, and documentation of a comparative sample of human remains cut with various serrated tools among a spectrum of the main commercial saw types and classes. The results can serve as a baseline comparative sample for future students and experimental designs on saw-mark analysis. Further testing of the protocols in the manual and the reliability of various proposed markers for the analysis of basic tool parameters (class characteristics) was performed through inter- and intra-observer studies, controlling for the degree of experience and exposure of the participants to the instructional materials. The experimental component of the project also examined some common misconceptions regarding the evidentiary value of some major saw-mark elements.

Saw marks on bone have been routinely reported in dismemberment cases. When saw blade teeth contact bone and the bone is not completely sawed into two parts, bone fragments are removed forming a channel, or kerf. Therefore, kerf width can approximate the thickness of the saw blade. Bailey *et al* evaluated 100 saw kerf widths in bone produced by ten saw types, to determine if a saw can be eliminated based on the kerf width (132). The cuts were examined with a stereoscopic microscope utilizing digital camera measuring software. Two statistical cumulative logistic regression models were used to analyze the saw kerf data collected. In order to estimate the prediction error, repeated stratified cross-validation was applied in analyzing the kerf mark data. Based on the two statistical models used, 70–90% of the saws could be eliminated based on kerf width. Saw characteristics affecting the kerf width and bone surface adjacent to

the kerf include style and design of the teeth, width of the teeth, teeth per inch (tpi), degree of wear on the teeth, saw cutting speed, blade vibration, defects in the blade and erratic sawing motion. These authors state that analyzing kerf mark measurements can be an effective method for predicting and eliminating possible saw blades by comparing the width of the blade to the width of the kerf.

Love *et al* presented a study on an independent validation test of microscopic saw mark analysis (133). The method, as published, was replicated without deviation and an ample sample size was generated for statistically sound analysis. Four morphologically different saws were used to make 58 partial and 58 complete saw marks in human femurs. The saw marks were examined independently by three doctoral level anthropologists using a digital microscope. Fifteen variables were documented for each saw mark. Analysis of the class characteristics was done using Random Forest (machine learning technique) classification, built by constructing a large number of classification trees on a set of training data and passing new cases down each tree. This study presents a statistically sound approach to evaluating the reliability and accuracy of a class characteristic recognition method.

Love and her colleagues also designed a study for establishing the potential error rate associated with the generally accepted method of tool mark analysis of cut marks in costal cartilage (134). Three knives with different blade types were used to make experimental cut marks in costal cartilage of pigs. Each cut surface was cast, and each cast was examined by three analysts working independently. The presence of striations, regularity of striations, and presence of a primary and secondary striation pattern were recorded for each cast, and the distance between each striation was measured. The results showed that striations were not consistently impressed on the cut surface by the blade's cutting edge. Also, blade type classification by the presence or absence of striations led to a 65% misclassification rate. Use of the classification tree and cross-validation methods and inclusion of the mean interstriation distance decreased the error rate to about 50%.

Pounder *et al* studied the class characteristics of serrated blade knives to cartilage (135). They produced a total of 136 stab wounds in cartilage, with 8 serrated knives and 72 stabs with 4 non-serrated knives. The walls of the stab track were documented by photography, cast with dental impression material, and the casts photographed. The class characteristics that might be determined from the marks are the overall pattern of coarse and/or fine serrations, the distance between the spine of the blade and the first serration point, the distances between the spine of the blade and the subsequent serration points, whether the blade was right side or left side ground (scalloped), and the shape of the tip of the blade if a chatter mark is present.

Pounder and Reeder concentrated on striation patterns in serrated blade stabs to costal cartilage (136), and found that all stabs with all 13 serrated blades produced striations on the cartilage cut surfaces, as anticipated by

previous studies, while unusual and distinctive blade serration patterns produced equally distinctive wound striation patterns. The striations were easily visible to the naked eye on both the cartilage and the casts, but photography was easier with the casts as previous experience has shown.

Puentes and Cardoso assessed how certain variables influence the ability of human cartilage to retain the class characteristic of the blade in sharp force trauma (137, 138). With some exceptions, this study was able to show that cartilage is able to retain the class characteristic of the blade (mean distance between teeth) used to cut it, quite faithfully, in a forward cutting motion when the direction of the cut is parallel to that of the axis of the knife's teeth. In addition, this study also showed that quantification of these class characteristics could be highly repeatable and reproducible. However, the blade's penetration angle and inter-individual variation in costal cartilage affect the identification of the tool class characteristics from the striation pattern observed in a kerf wall, although this fact seem to be intimately related to the degree of calcification of the costal cartilage of the individual under analysis.

Another study on the analysis of serrated and non-serrated sharp force trauma to bone was performed by Tegtmeyer (139). Results of this study indicate that the identification of width, kerf shape, and presence of striations are useful for distinguishing between serrated and non-serrated knife classes. As such, these characteristics may be useful for assisting in the exclusion or inclusion of suspects and weapons in a forensic context

Shaw and co-authors designed a chopping stage with a gravity accelerator and a fixed bone platform, in order to describe tool marks on bone tissues that had been chopped with knives (140). A digital microscope was also used to measure the knife angle and the retained V-shape tool mark angle in a pig skull. The elasticity coefficient was derived and recorded after the knife angle and the accompanied velocity were compared with the proportional impulsive force of the knife on the bone. The constant impulsive force revealed a correlation between the V-shape tool mark angle and the elasticity coefficient.

Rutty *et al* published a review on the use of x-ray micro computed tomography (micro-CT) in forensic investigations (141). One of the proposed applications of this technique was for the examination of toolmarks on bone. An advantage of this method is that it is nondestructive, so other observation or casting methods may be utilize subsequently. It seems that even individual characteristics can also be visualized with micro-CT.

Cases of postmortem dismemberment in two Mediterranean countries, and the analysis of the tool used, are presented by Kahana and her colleagues (142). The attribution of a suspected specific tool to a dismembered body was possible in four cases where the dismemberment was performed using a single-edged blade knife and in one performed with an electric saw (rotating disk) by casting the bone edges bearing cut marks and the suspected tool's cutting edge. High-magnification photography was applied

to make the match. In all other cases, the specific tool was not retrieved, although an examination of the cut marks indicated the type of tool used.

Other articles dealing with the characterization of trauma caused by various types of tools were found to be outside the scope of this Review.

3.6 Evidential Value of Toolmark Examination

For the last 10 year or so, Toolmark examinations (as well as firearm identification) have gone through the same scrutiny as other "classic" identification areas. The way forensic scientists are stating that a questioned mark was made by a specific known tool (with the exclusion of all others), drew a lot of criticism, mainly from non-forensic-science scholars (143-145, for instance).

On the other hand, the relevant scientific community is pursuing its efforts to provide the legal system with as accurate and reliable expert opinions as possible. The Association of Firearm and Toolmark Examiners (AFTE) Committee for the Advancement of the Science of Firearm & Toolmark Identification published a revised version of the AFTE Theory of Identification, stating that "...Agreement is significant when **the agreement in individual characteristics** exceeds the best agreement demonstrated between toolmarks known to have been produced by different tools... The statement that 'sufficient agreement' exists between two toolmarks means that the agreement **of individual characteristics** is of a quantity and quality that the likelihood another tool could have made the mark is so remote as to be considered a practical impossibility" (146).

Arendse and Mustard reported that the 2009 NAS report (64), along with findings generated during an internal audit of their Lab's policies and reporting practices, initiated an internal review of report wording when associations are made at the Firearms and Toolmarks and the Documents Units of the Centre of Forensic Sciences (CFS), Toronto, Ontario, Canada (147). After reviewing the NAS report conclusions and the relevant scientific literature, this Lab updated its reports, and statements that conveyed absolute certainty were replaced with statements of “practical certainty". The definition of “practical certainty” is explained and incorporated into this lab's reports where an association (“identification”) had been previously made.

Historically, firearm and toolmark examiners have rendered categorical or inconclusive opinions and eschewed probabilistic ones, especially in the US. Bunch and Wevers proposed that this practice may no longer be necessary or desirable, and outlined an alternative approach that is within a comprehensive logical, or Bayesian, paradigm (148). Hypothetical examples are provided, and the strengths and weaknesses of both approaches are considered. These authors discuss the influence of laboratory errors on the estimated LRs and argue that there are no scientific or logical advantages to the traditional approach, but only deficits,

and that when using the LR approach there is less risk of a contextual bias. Although all examples are firearm-related, implications on toolmarks examination is obvious. It is recommended by these authors that examiners worldwide, and especially in the US, begin moving toward the likelihood approach and toward standardization of verbal scales and training. Similar arguments were also raised by Kerkhoff *et al* (149).

In an earlier article, Wevers and co-authors explored a potential model for increasing the objectivity in the interpretation of toolmarks by using consecutively matching striae (CMS) and Bayesian inference (150). Given the nature of the data, standard statistical thinking suggests that Bayesian inference is likely to be the most powerful method of interpretation. The unavoidable paucity of data for high CMS runs for the known non-match (KNM) condition is handled using a small advance in modeling. The resulting likelihood ratios show some, but incomplete separation between the known match (KM) and KNM conditions. Although promising, the resulting incomplete separation between KM and KNM is thought to represent limitations of the CMS summary of the complete pattern and limitations of the modeling used.

An interesting application of this Bayesian framework has been demonstrated recently by Newton, in a case where the association of paint flakes to a wheelbarrow was estimated using LR (151). Although no physical match was found between the paint flakes recovered from the crime scene and the wheelbarrow tray, and the toolmarks impressed to the flakes were of sub-class characteristics, the author estimated that the evidence provided extremely strong support to the suggestion that the red paint was from the submitted wheelbarrow tray.

Petraco *et al* conducted a study that focused on striation patterns left by screwdrivers and on cartridge casings from firearms, using confocal microscopy (152). Since all impressions made by tools and firearms can be viewed as mathematical patterns composed of features, their study used the mathematics of multivariate statistical analysis in order to recognize variations in these patterns (“machine learning"). Mathematical details also enable the estimation of extrapolated identification error rates and, in some case, the calculation of rigorous, universal random-match probabilities. This research succeeded in composing a set of objective and testable methods for associating toolmark impression evidence with the tools and firearms that produced them. Estimated toolmark identification error rates were on the order of 1% using these algorithmic methods.

This project group studied also reproducible sets of ideal striation patterns, made with nine slotted screwdrivers, encoded into high-dimensional feature vectors, and subjected to multiple statistical pattern recognition methods (153, 154). The specific methods employed were chosen because of their long peer-reviewed track records, widespread successful use for both industry and academic applications, rely on few assumptions on the data’s underlying distribution, can be accompanied by standard confidence levels, and are falsifiable. For partial least squares discriminant analysis (PLS-DA), correct classification rates of 97% or higher were achieved by retaining only

eight dimensions (8D) of data. Principal component analysis, combined with support vector machines (PCA-SVM), required even fewer dimensions, 4D, for the same level of performance. Finally, it was shown how to use conformal prediction theory to compute identifications of striation patterns at a given level of confidence.

This group also maintains a publicly-accessed web-site, containing their research findings and enabling other scholars to evaluate their results, "in order to assist in developing and improving the science behind toolmark and firearm analysis" (155).

Instead of using time-consuming and scale-dependent cross-correlation techniques for measuring the similarity of two striation toolmarks, Lin and Wen (156) converted striation marks manufactured by screwdrivers into patterns of alternative bright and dark lines (similar to "barcodes"). The authors built striation pattern features based upon the distance of adjacent bright lines, and denoted the feature by a sequence. Then they used the longest common subsequence (LCS) method to compare the similarity of sequences of striation marks. The LCS method provides a good and efficient way for measuring the similarity between sequences. The 1D strings can also reduce the storage space of database. Based on the experimental results, the LCS method provides feasibility to describe the similarity between two striation marks.

Spiegelman and Tobin (both are not qualified firearms or toolmarks examiners, to the best of this Reviewer's knowledge) critically evaluated the experiments used to justify inferences of individualization and ‘near-zero’ rates of error claimed by firearm and toolmark examiners (157). The authors review two of the articles that defend the statements of certainty rendered by firearms examiners, and point out intrinsic methodological weaknesses, like the absence of standard operating procedures (SOPs) or detailed criteria for identification, the small size of the examined population, and that the tests were not blind. They also proposed approaches for establishing statistical foundations and experimental setup for proper studies in this field, including one for error rate estimation.

Another argumentative article, by Tobin and Blau (158), is rising similar claims, by comparing firearm identification and toolmarks examination to comparative bullet-lead analysis (CBLA). It is stated that existing studies in the domain literature, typically presented as support for specific source attributions, have no external validity for extrapolation to universal assumption. They are, thus, of no value for validation of the critical premise of discernible uniqueness in real-world forensic scenarios and are largely irrelevant to any particular criminal judicial proceeding. Another issue criticized in this article is the validity of proficiency tests as they are preformed today, and the use of these tests for estimating error rates.

The issue of proficiency tests and their role in estimating the potential error rates of forensic science examinations has been addressed recently by Koehler as well (159). The author discusses the specific factors influencing the tests' outcome, like the composition of the test designers and

administrators, the features of tests and reference samples, the composition and selection of test participants and the use of blind test protocols, and proposes practical solutions that should be implemented by the forensic science community.

3.7 Miscellaneous Issues

Maxwell and Williams studied the effect of humidity on the dimensions of toolmarks in wood (160). In many cases, particularly residential burglaries, toolmarks are left in a wood medium. The hygroscopic properties of wood leave it particularly susceptible to the effects of changes in relative humidity. This study investigated the effect of differences in relative humidity on the measurements of tool marks in wood. The wood samples were placed in six separate locations with different levels of relative humidity. After the samples acclimated, marks were made and measured; the wood samples were then collected and placed in a laboratory hood to simulate storage in an air-conditioned evidence storage room. Subsequent measurements were made after one and two weeks. Results obtained showed that all marks changed in size to some degree; some marks actually disappeared, while others became visible by a change in moisture levels. It is evident that moisture can significantly change toolmarks made in wood, hinder identification to their source, and even prevent marks made by the same tool from being linked to each other.

Wakefield describes a case where marks left on a cut window and door screens enabled the investigators to determine which side the screens were cut from (161). It was found that after making numerous test cuts on similar screen material, then directly observing the cuts under magnification, and comparing these results with the cuts from the questioned screens, the orientation of the tool making the cuts could be established.

The forensic analysis of knot evidence is an uncommon examination type. Nevertheless, Chisnall published several articles in this field, dealing with knot-tying habits, tier handedness, and experience (162-164). Knot-tying behavior of hundreds of subjects was observed over a period of 25 years. A number of key principles applicable to forensic knot analysis emerged, many of which have been confirmed by other studies. Most notably, tying behavior is consistent and reproducible. The results of these studies did not indicate an exact correlation between the principal manipulating hand and the chirality of resultant knots. These results serve as a foundation for future research in forensic knot analysis. Obtaining more information about the latent tying habits of inexperienced knotters would be valuable.

4 Physical Match

Physical match, namely linking two or more objects by the morphology of fractured or torn surfaces, is usually viewed as one of the strongest ways for establishing common origin (165). The evidential value of such physical matches, and their admissibility in court, seem to be taken for granted, considering the limited number of articles published during the Review period regarding this area of forensic science.

Jayaprakash discuss the general issue of individualization in forensic science, with a special emphasis to physical match examinations (166). This article describes case examples illustrating physical matching and other pattern matches to support the practical relevance of individualization based on the premises of uniqueness. Arguably, proving uniqueness or individuality by exhausting examination of every other related object in the world would never be possible. Uniqueness, as a paradigm for forensic science practice, is proposed based on the indeterminacy in the causal pathways of patterns as evidenced in the fields of sciences to which these patterns originally belong. While uniqueness enables individualizations, it does not vouch for eliminating errors. As a prime requirement during criminal investigations, individualizations are of practical relevance as they offer conclusive decisions that eliminate confusion during investigation, and, this essay seeks to support continuing the practice of individualization wherever the physical evidence types permit. Instead of dismissing uniqueness and individualization, accepting errors as human or system failures and seeking remedial measures would benefit forensic science practice and criminal investigation.

Yekutieli *et al* (167) demonstrated a prototype system used for physical matching in 2D. The system has two main functions: One is to assist forensic experts in performing physical matching in an objective manner, and the second - collecting statistics and build confidence levels regarding physical matches. The probability distribution functions (PDFs) of matching error values, for correct matches and for non-matches were estimated. This analysis was applied for different fracture line lengths and three different materials. Eventually, these authors were able to calculate error rates much more reliably than previous estimates. With the results of this research, an expert can express his findings in a more numerical way, and the Daubert criteria for a potential or known error rate can be fulfilled. Surprisingly, statistical results were much lower than initially expected, probably because the authors used only the 2D fracture lines and not any additional information commonly used in fracture match comparison, such as the 3D nature of some fractures or any existing texture and graphic patterns on the surface or outer border of the pieces to be compared.

Following their work on duct tape end matches, mentioned in our 2007 Review, Bradley and her colleagues performed also a similar study on vinyl electrical tapes (168). The present study was designed to determine the validity and error rate associated with conducting end-match (fracture, or

physical, match) examinations on vinyl electrical tape. Test designs varied the source roll of tape, test preparer, or mode of separation from the roll. Results indicated that each affected the resulting severed tape ends. The analysts examining the end matches also had an effect on the results. Eight end matches in the study were not identified by the initial analysts and were considered inconclusive. One end match was misidentified, resulting in one false positive and an error rate of 0.049%. These results support a comprehensive physical and chemical tape comparison regardless of indications of an end match. It is interesting to mention that recognizing the inherent difficulty in accurately determining end matches on an amorphous polymer, such as tape, the FBI Laboratory modified its tape comparison protocol in 2003. The revision mandated that for all cases where there was an end match of value, after the end match was confirmed by a second qualified individual, the full complement of examinations (physical and chemical analyses) would also be conducted on the reconstructed tape specimens.

Another study on adhesive tape end matching, this time - duct tapes, was conducted by Tulleners and co-authors (169, 170). This study was designed to statistically evaluate the error and accuracy rates associated with duct tape physical end matching. The experimental design consisted of a blind study in which three researchers independently analyzed eight types of tape subjected to four methods of separation. The lowest mean accuracy observed was 98.15%, the highest mean false-positive rate observed was 3.33%, and the highest mean false-negative rate was 2.67% (the relatively-high error rate observed in this study may be due to the fact that the participants were inexperienced graduate students, and not qualified examiners). Overall, high accuracy with low false-positive and false-negative error rates were observed. This study confirms the use of physical end matching in identifying duct tape samples as matching or non-matching and that the differences between analysts, brands, tape grades, tape color, and methods of separation have varying contributions to misidentifications and inconclusive results. This study also demonstrates the importance of peer review in duct tape analysis.

Weimar *et al* presented a new method of examining cut edges of polyvinyl chloride (PVC) electrical tapes (171). These authors cut tapes using scissors, in a controlled manner, and heat treated the tapes in approximately 100°C hot water (as described by Weimar, see our 2010 Review). Following heat treatment, silicone rubber casts of the tape joint faces were prepared, and the casts were examined microscopically, under a comparison microscope, using oblique illumination from opposite directions. The proposed procedure enabled the correct association of all cut tapes.

The aforementioned articles dealt with cutting or tearing of ductile substance (plastic tapes). Several other studies focused on brittle material (like glass or metal).

Claytor and Davis studied the topography of fractured hacksaw blades (172). Two consecutively manufactured hacksaw blades were each

fractured eleven times and inter-compared. Two hundred fifty-three topographical comparisons were conducted between 44 fractured edges, and each fracture produced two surfaces discernible from any other. In addition, a series of proficiency style tests were made from consecutively manufactured blades and sent to participants throughout the United States and abroad. A total of 66 answer sheets were returned, providing 330 test results for evaluation. This research demonstrated that not only will two consecutively manufactured hacksaw blades fracture uniquely, but also the same blade, when fractured multiple times will also fracture uniquely.

Tulleners and colleagues documented the controlled fracture patterns of 60 glass panes, 60 glass bottles, and 60 plastic tail light lens covers (173). Two methods were used to initiate the fractures - dynamic impact from a dropping weight and static pressure from an Instron® 4204 Tensile Tester. The fracture patterns were then documented in great detail in such a manner that allowed the analyst to inter-compare the fracture patterns. This subsequent comparison illustrated the uniqueness of all of the fracture patterns observed in the examined samples.

Whenever a machined metal object is broken, and its fragments are subject to physical match examination, striation marks present on the fractured pieces may also serve for individualization. Streine describe a murder case, where pieces of a broken knife blade, recovered at the crime scene, were submitted to the laboratory for physical fracture comparison in order to determine if they had been, at one time, joined together as an intact, unbroken unit (174). In addition to the agreement of physical contours along the fracture line that was sufficient for identification, striated marks, some of which were consistent with being manufacturing marks, as well as other apparently incidental, non-manufacturing, marks, were also observed. This author reports that the agreement of these striated marks was also sufficient for positively linking the broken pieces together.

The reverse illumination method, mentioned earlier (171), was used by Garcia for fracture matching of wooden knife handle in a police-involved shooting case (175). A broken knife handle, found at the scene, was compared to a piece of foreign material found embedded in the shooting victim's hand. The foreign material was positively identified as having originated from the broken knife handle, indicating that the victim was actually holding the knife when shot.

5 Restoration of Obliterated Marks

The visualization of obliterated serial numbers, on chassis and engine of vehicles, on firearms frames, or on other objects of value, may provide important forensic evidence during criminal investigations, when items (stolen or otherwise) have their serial numbers obliterated in an attempt to conceal their identity or origin.

The methods applied for such examinations may be generally divided usually into two groups: Non-destructive methods (like magnetic particles, Eddy current or x-ray radiography), and destructive methods (like chemical and electrochemical etching or thermal annealing). The appropriate method, or combination of methods, suitable for each surface, is dependent mainly on the surface composition and manufacturing history, on the marking methods and on the obliteration process. Many metallurgical tests may be required for each type of exhibit, for determining the proper procedure to be used.

Comprehensive guidelines for various methods for restoration of obliterated marks, including formulations and procedures, may be found, for instance, at the Virginia Department of Forensic Science web-site (2).

Collaborative Testing Services (CTS), US, distribute proficiency tests on the restoration of obliterated marks (176). The final reports, available over the Internet, include lists of methods used by the participating laboratories. It is interesting to see that many laboratories are using the magnetic particles method, prior to, or instead of, using chemical etching.

5.1 Aluminium alloy surfaces

Kuppuswamy, an active researcher in this, published an extensive review on the restoration of obliterated marks on aluminum alloy surfaces (177). This article, available on-line, presents background information on serial number restoration and etching techniques applied to recover the obliterated markings on aluminum and especially two of its important alloys, Al-Zn-Mg-Cu and Al-Si. The etching results arising from some of these surfaces are illustrated. For the sake of completeness, some brief notes on the classical recovery of obliterated marks on iron and steel surfaces and use of methods other than chemical etching are also added.

Uli and colleagues performed a survey of etching reagents for the restoration of erased marks on Al-Si alloys surfaces (178). These authors conclude that plastic deformation introduced into the alloy by the original engraving could be revealed by alternate application of 10% sodium hydroxide (NaOH) and 10% nitric acid (HNO_3). This procedure was found to be the most desirable one, as it was able to show the metallic disturbance, which is, unlike in stamping, very minimal in case of engraving. The contrast provided by the reagent was also good.

Two articles on the recovering of obliterated engraved vehicle identification numbers were published by Jin (179, 180, available only as abstracts). The first paper deals with the restoration of obliterated engraved vehicle identification number on vehicle frame surfaces by an etching technique (179). Results of this study indicate that for vehicle frames made of Al-Si alloy, the use of concentrated hydrochloric acid (HCl), acetic acid (AcOH) and ethanol (EtOH) (2:1:1 by volume) solution showed good effectiveness;

For other frames tested, the use of concentrated HNO_3, AcOH and EtOH (1:1:1 by volume) solution was preferable.

The other article by this author (180) discusses a method for recovering obliterated engraved vehicle identify number on aluminum engine surfaces by alkaline etching technique. Results indicate that a 25% NaOH solution not only can restore the original numbers on aluminum engine effectively, but also is easier to prepare and is less volatile than traditional methods that use acid solutions. With aluminum motor engines becoming more common, this method is recommended by the author.

A case report, regarding the successful restoration of a motorcycle engine number was presented by Dower *et al* (181). Following an unsuccessful attempt to restore erased characters on an engine block using standard polishing and etching procedures (60% aqueous HCl with intermittent rinsing with 40% aqueous NaOH solutions), the top layer of the surface was carefully removed by hand filing and the surface was repolished and re-etched. Successful restoration was then achieved.

5.2 Steel and Iron surfaces

It is agreed by most sources that chemical etching has been established to be the most sensitive technique for detection of metal deformation present under stamped numbers. Heating of the obliterated surface using oxyacetylene flame is an alternative recovery treatment, suggested in the literature and used in practice.

Abdul Wahab and co-authors investigated several etching reagents for restoring obliterated stamped marks on cast iron engine blocks (182). This work investigated the suitability of some common etchants, mostly copper containing Fry's reagent and its modifications, on cast iron surfaces with a view to determining the most suitable one for revealing the obliterated marks. The stamped numbers (varied in depth between 0.2mm and 0.3mm) were completely ground off manually using a metal file. The grounded surface was then polished smooth using emery papers and etched with a few selected reagents mostly by swabbing. Experimental results showed that a modified Fry's reagent, consisting of 45g cupric chloride ($CuCl_2$), 100mL HCl and 180mL water, restored the numbers with better contrast at a reasonably shorter time. Preliminary testing has shown that the proposed reagent was effective to render visible the obliterated engraved marks on low (0.1% carbon) and medium (0.3% carbon) carbon steel surfaces. The above reagent is a slightly modified form of one of the Fry's original compositions – 45g $CuCl_2$, 180mL HCl, and 100mL water. The most widely used Fry's composition (90g $CuCl_2$, 120mL HCl and 100mL water), although recovered the obliterated numbers, did not cause the desired contrast.

Another study was performed by Richa and her colleagues (183). These researchers examined ten different reagents, most of them copper and iron containing, for the restoration of erased marks. The erased surfaces (obliterated serial numbers on iron keys) were etched with every one of these etchants using the swabbing method. The relative sensitivity and efficiency of these reagents in recovering marks obliterated by grinding are described on the basis of experimental results observed. The best results were achieved with the use of an etching solution containing 25g of ferric chloride ($FeCl_3$), concentrated 25ml HCl and 100ml distilled water.

5.3 Restoration of Laser-Engraved Numbers

Da Silva *et al* present three cases of obliterated laser-engraved serial numbers of pistols that were recovered using a combination of fine relief polishing and digital imaging microscopy (184). Since the laser engraving process leaves no pronounced subsurface deformation, like crystalline structure dislocation, chemical etching methods may not be successful is such cases. These three cases illustrate the importance of microscopy and use of relief polishing for recovering obliterated laser etched serial numbers in aluminum alloy firearm frames.

5.4 Glass and Plastic Surfaces

The restoration of obliterated marks on glass surface is not a common practice in forensic science labs, therefore only limited research had been done on this topic. Miller conducted a study into the effectiveness of hydrofluoric acid (HF), a known etchant for glass (185). Character sequences previously etched into panes of vehicle glass were sanded to varying depths and attempts were made to restore the sequences by polishing and using a range of concentrations of HF. A concentration of 30% HF gave at least a 50% restoration of the sequence if up to approximately 30μm of glass had been removed during obliteration. Recovery improves if less glass is removed, but not if the concentration of the acid is increased. It appears that removal of glass below the level of the original characters makes subsequent restoration using this technique impossible. Based on the results of this research, when more than 90μm of glass has been removed there would be little need to go ahead with a restoration. However, should characters be etched particularly deeply into the surface of the glass, there may be more opportunity to recover these characters after a removal of greater than 90μm of glass. If there is any doubt as to whether a successful result can be achieved, the preference should clearly be to attempt restoration.

As for plastic and polymers, Christen and his co-authors (186) compared the known methods for the recovery of erased markings in polymers, under

consistent and controlled conditions. Preference was given to methods that had led to good results in the past. In order to find the best strategy for each kind of polymer, the selected methods were applied to all of the selected polymers. It was found that the restoration of erased markings in polymers can be problematic. Good results can be reached with the chemical swelling methods. However, it is difficult to control the reaction and to stop it at the right time. Additionally, these methods may produce vapors which are hazardous to health. For these reasons, the combination of relief polishing and heat treatment should be preferred - this combination led to the best or nearly the best results for all examined polymers.

Conlan *et al* (187) used imaging secondary ion mass spectrometry (SIMS) to investigate the recovery of erased serial numbers from polypropylene, polycarbonate and polyvinylchloride substrates. The recovery of the obliterated numbers was initiated by a swelling mechanism due to the application of two swelling agents - methyleugenol and cinnamaldehyde. The localization of the characteristic molecular ions for the swelling agents is observed in regions associated with erased characters. This study examines and evaluates SIMS images to discover the optimum combination of the polymer and solvents. The results are discussed in reference to the Hildebrand solubility parameter and comments upon the limitations of this suggested indicator.

5.5 Non-Destructive Methods

Due to inherent destructive nature of the methods described above, non-destructive methods would have been preferable for this purpose in forensic science labs. Nonetheless, the only frequently used non-destructive methods are the magnetic techniques. The different kinds of magnetic restoration methods are discussed in an article by Weimar and Hermann (188). In the experiments described, the applicability of magneto-optical methods for the restoration of obliterated markings was examined. The results show that the methods are suitable and the required equipment is not too costly. Very good results, comparing to those of etching methods, were obtained with a stainless steel sample, even though the sample was made of austenitic steel which is normally not ferromagnetic.

These authors also describe a simple version of the classical magnetic particle method, where the magnetic field is generated by cheap and handy permanent magnets, and the fluid with the dispersed ferri- or ferromagnetic particles is welded densely between two plastic foils (189). This device is called "fluidpad". The examined object is not contaminated, the fluidpad can be used several times and the examination can be conducted in a short amount of time. This method is used at the Bundeskriminalamt (BKA), Germany, as a standard procedure for the first examination of objects with erased markings. In many cases the quality of the restoration is sufficient so that no further (destructive) technique has to be applied.

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Examination of Paint

Review: 2010 to 2013

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Introduction

1. General considerations

Paint is defined as a coating that is applied to a surface in order to provide protective and/or decorative properties. Paint is commonly encountered in everyday life (on vehicles, building walls, tools, art paintings, furniture…) and can be transferred between objects or between people and objects. It is therefore referred as “trace evidence” by the forensic scientists.

This review covers relevant publications in forensic paint analysis since the last review presented at the 16th International Forensic Science Symposium Interpol in 2010 by M.J. Bradley, A.H. Mehltretter and D.M. Wright from the United States Federal Bureau of Investigation (FBI) laboratory. We would like to congratulate them for their great job.

Based on the model of our predecessors, this review is divided into the following headers:

1. Innovation and changes in the paint and coatings industry as well as in pigment manufacturing.
2. Key publications which outline relevant forensic science research for chemical paint analysis. This section also includes analytical data treatment by chemometric methods.
3. Studies regarding the application of Bayes’ Law for interpreting the analytical paint results in the context of the case.

1.1 Forensic aspects

The examination of paint evidence for forensic purposes principally concerns:

- Automotive paints in cases involving of traffic accidents, hit and run accidents, or ram-raids against jewelers’ shops or banks.
- Tool paints and/or house paints in case of burglaries or assaults.
- Spray paints in case of vandalism.

Paints from artworks are often submitted to scientific examination but forensic paint examiners are less concern about this subject. The aim of such analysis is more concerning by art conservation and restoration, long-term preservation and manufacturing technology. However, some forensic institutes applied such analysis in case of false.

When paint smears found at a crime scene are sent to a forensic laboratory, the request may be formulated at one of the following levels:

- Evaluate if there is a common source between the recovered paint and a control material: comparative/evaluative level.
- Establish the origin of the paint flake or give other interesting information that could help the investigation: investigative level.

For people who start in this area of expertise, we recommend to have a look on the paint and coating entries in “The second edition of the Encyclopedia of forensic sciences”, edited in 2013. It is a judicious complement of those included in the first version of this Encyclopedia edited in 2009 [1, 2]. The paint and coating entries make up five articles. The first one, written by Bender, contains an overview of the chemical characteristics of paints in general [3], while the two following ones focus on architectural paint and automotive paint respectively [4, 5]. The fourth article, written by Muehlethaler et al., describes the current methods available for paint analysis [6]. The authors illustrate a sequence of examinations for the comparative analysis of two red paint samples. The sequence of examinations depends on the nature of the samples, the kind of request and the equipment available in the laboratory. Not all methods must be applied for each case. In the fifth article, Bender focuses on decision criteria concerning the main question: “Can the know paint and the questioned paint be differentiated or not based on the differences observed” [7]. Bender considers both the variation within the samples (intra-sample variation) and variation between samples (inter-sample variation). As paint is a heterogeneous substance, in his view, a certain number of particles should be sampled from the known source (preferably of the same size as the questioned sample) and analyzed in order to detect any variation.

Since 2010 two other general papers focusing on forensic paint examination were published:

- “Fundamentals of forensic science” (second edition) where the chapter 16 is dedicated to the forensic paint analysis [8].
- “Crime reconstruction” (second edition) where the chapter 10 presents various aspects of trace evidence in crime reconstruction including paint as commonly encountered forms of trace evidence [9].

2 Industrial / economical evolution of paint and coating

Paint is produced in very large quantities and in various colors and shades. Moreover, chemical composition varies in function of the type of surface coated, the paint application method and the purpose. Whatever the paint is, the chemical composition is based on a resinous binder in which a pigment is dispersed and some additives are added to modify the paint’s

film properties, application or storage characteristics. In the liquid state, solvent is also present and constitutes the volatile portion of the paint [1].

However, the chemistry of paint is in constant evolution. The paint & coatings industry always develops new engineering applications and new materials (including pigments but also binders, additives and fillers) to keep up with the evolution of the market (trends, fashion, …), the environmental requirements and cost reduction constraints. This chapter proposes a summary of the major evolution in the paint & coatings market since 2010.

During the review period, the following books have been edited:

- "Paint and Coatings Testing Manual: 15th edition of the Gardner-Sward Handbook": This book has been thoroughly rewritten and extended as compared to the 14th edition.
- "European Coatings Handbook 2nd Ed": Update of the 1st Ed of 2000, covering the full spectrum of coatings formulation from chemistry to engineering, safety to quality control and regulations to application of coatings.
- "Coatings Formulation 2nd Ed": This book teaches paint formulation through binder composition, formulation advice and analysis of existing recipes.
- "Acrylic Resins": Latest knowledge of acrylic resins in solvent-borne and water-borne systems, including radiation curing, production methods, properties and applications.
- "Fillers for Paint, 2nd Ed": overview of the working mechanisms and application areas of most common fillers, including nanoscale types.
- "Functional Coatings": Overview of functional coatings (anti-graffiti, antifouling, soft-feel, anti-ice, …) and the principles they are based on.
- "Coatings for Plastics": Compact and practical handbook presenting classical and modern coating technologies for plastics.
- "Powder Coatings, Chemistry and Technology, 3rd Ed": All about powder coatings in one book: types, chemistry, formulation, production and application technology, REACH.
- "The automotive body manufacturing systems and processes": All about manufacturing cars, from metal forming to plant layout.
- "Epoxy polymers": This is a reference source, collecting scientific and technological breakthroughs otherwise spread over hundreds of publications, patents and reports.

2.1 Guidelines in the paint & coating industry: environment, performance & cost challenges

2.1.1 Environmental challenges

The European legislation REACH imposes volatile organic compounds (VOC) to slope down and/or to stop (even though some local markets as the Italian one still do prefer solvent borne systems [10]). Anyhow, the solvent demand in the paint & coating industry is predicted to increase by about 3% each year until 2019 especially related to dynamic economic developments in the BRIC (Brazil, Russia, India and China) emerging markets [11].

Reach legislation has triggered the replacement of most of the organic solvents in solvent-borne basecoats by demineralized water. This has created challenges to the waterborne chemistry : 15% by weight of waterborne paint consist of water as compared to solvent contents of 50% by weight in solvent-borne paints [12]. In 2011, more than half of worldwide car manufactures are water based, although locally as i.e. in Ukraine the majority of paints are solvent based [13]. The water based paint proportion will globally increase. Worldwide, more than 100 million vehicles in use at the beginning of 2012 already incorporated waterborne basecoats. Car producers in Europe in particular and also increasingly in Asia are committed to these environmentally-friendly paints. The waterborne paint systems have to be improved more and more to attain solvent borne paint systems qualities. (Overview of heterogeneity control in waterbased coatings as a tool of achieving optimal properties [14]).

One of the challenges of formulating waterborne coatings is to achieve an acceptable balance of properties both during the film application and the drying process as well as in the final film. The period in which irregularities in a freshly applied coating can be repaired without leaving brush marks is referred to as the open time, while the period in which a coating can be applied over an existing paint film without leaving lap marks is called the wet edge time. Aqueous coatings generally employ dispersed high molecular weight polymers as binders. These binders often have short open times when drying because the dispersed polymer particles tend to be immobilized quickly in the edge region of an applied coating. References [15, 16] describe the process by which new, low VOC additives were developed to improve open time and wet edge in aqueous coatings.

Other industrial challenges invoked by the Reach legislation and VOC reductions are:

- Cleaning and priming of the substrate and/or the use of adhesion promoters is more critical when using waterborne systems (paint performance is more affected by poor adhesion).

- The coating/paint application lines have to be modified to resist corrosion and to deal with the increased drying requirements (higher energy consumption).
- Exterior durability is to be enhanced.
- Especially for waterborne applications, high performance additives e.g. wetting and dispersing agents are needed that are both ecologically and economically accepted [17].

A questionnaire (April 2013) "What is the biggest challenge in developing zero VOC waterborne coatings" to 279 readers of Paint & Coating Industry [18] yields answers like: eliminating co-solvent(s) (28.7%), zero VOC coalescing agents (24.0%), zero VOC polymerization surfactants (6.5%), Zero VOC pigment dispersants (5.4%); zero VOC rheology modifiers (3.2%), and zero VOC defoamers (2.5%).

REACH regulations are also the driving force for the application of new technologies such as powder coatings and hyper-branched polymer thickeners in VOC-free paints [19].

2.1.2 Materials shortage and price raising challenges

Raw materials shortages (particularly TiO_2) and rising prices of raw materials (25% TiO_2 price increase in 2010) and energy compels paint producers to look for alternative partners and products to make their processes more efficient.

The reasons for this evolution include low inventory, high capacity utilization levels at producers, rising production costs and a persistent under-supply situation [20]. For example the TiO_2 market stagnated in 2012 although the market studies forecasting 3% per year increasing in Europe [21]. Another example of shortage in supply is the effect of natural disasters (as Japan earthquake in March 2011) on the "supply chain" of effect pigments for automotive paint [22].

Due to the current tight supply and cost run-ups of TiO_2, paint companies are looking into options to minimize the effect of the cost increases and reformulate for more efficient utilization of TiO_2. Technological solutions are treated in paragraph 2.3.

2.2 The Market evolution

In 2012, the coatings market has fully recovered to the total global (worldwide) sales level of 2008, reaching 95 billion USD. Decorative paint, the largest segment, accounts for almost half of the market. The second largest segment concerns automotive OEM and refinishing paints (15%). The marine & protective coatings segment makes up another 12% [23].

2019 forecasts estimate the paints and coatings consumption at 48 millions of tons which would correspond to a growth rate acceleration compared to the years 2004-2012. The Asia-Pacific region will raise its demand and will keep its leadership in consumption [24].

2.2.1 The automotive Paint Industry

Globally, automotive OEM and refinishing sales continue to face significant cyclicality. Trends go towards light and compact cars that need a lower amount of coating per car. Moreover, performance quality of coatings increases (e.g. self-healing paint) while at the same time anti-collision systems will reduce the need for repairing [23].

In particular in Germany and China original equipment manufacturer (OEM) automotive paint production is reduced compared to refinish automotive paints for which the demand is growing [25]. It is predicted that the Chinese automotive market will triple over next decade [26].

Last 10 years witnessed an expansion in the use of powder coatings in the automotive industry. As it works well with the specialized and organic pigments that are being used for popular colors, some brands use powder coatings in their topcoats. Recently powders have begun to be used in clearcoat applications due to developments in weatherability and temperature performance [27].

The 2012 Dupont Automotive Color Popularity Report lists the most popular car colors [28]. White and White Pearl dominate for the second consecutive year (23 %). Black and Black Effect move to the second place (21 %) due to its increased popularity in the Asia Pacific market and because this color has a worldwide perception of high quality and luxury. Silver fell to third place (18%) due to its overall gradual decline, especially in the large markets of Europe and Asia (18 %). Gray (14 %), Red (8 %) and Brown/Beige (6 %) gained each a percentage point compared to 2011. Blue (6 %) maintains its market share. Green accounts for 1 %, Yellow/Gold for 1 % and others for 2 %.

In comparison, it is interesting to note that, according to PPG's annual survey of global color popularity [29], white ranked first (22 %) and silver was second (20 %), followed by black (19 %), gray (12 %), red (9 %), natural (8 %), blue (7 %), green (2 %) and other colors (1 %).

By region: In North America, white ranks first (21 %), followed by black (19 %), silver and gray (16 % each), red (10 %), blue (8 %), natural (7 %) and green (3 %). In Europe, white is also the most popular (23 %), followed by black (21 %), gray (17 %), silver (13 %), blue, natural and red (7 %each), other colors (3 %) and green (2 %). In the Asia-Pacific region, white and silver tied for most popular (23 %), followed by black (19 %), natural (10 %), red (9 %), gray (8 %), blue (7 %), and green (1 %).

2.2.2 The High Performance Pigment (HPP) market

In the High Performance Pigments (HPP) market, the inorganic effect pigments represent about 80% by tonnage and about 60% by value. Organic HPP represent 20% by volume but about 40% by value (2011). This distribution is expected to remain unchanged until 2017. The largest HPP volume is used in coatings. Plastics, inks and cosmetic are three other big market applications. Currently the European and Asian markets have the same size but the fast growth rate forecasts for Asia will show Asia market bigger than the European one in 2017. China remains the largest producer and exporter of HPP [30].

2.3 New materials and tendencies

2.3.1 Alkyd paints

Driers paint additives are used to promote curing of alkyd solventborne and waterborne coatings. In waterborne formulations, standard driers are used in excess. Another possibility is to use driers dedicated to waterborne systems. Much research has been done to develop environmentally safer substitutes for cobalt drier. The most promising results are obtained with iron and manganese complexes [31].

A new alkyd paint with low content of volatile organic presents better protective performances and lower permeability than conventional alkyd paint [32].

Tetra (2,7-octadienyl) titanate is used as an active diluent in air-drying solvent-borne alkyd paints. The resulting alkyd–reactive diluent formulations exhibit low viscosities and extremely fast dry times in comparison to neat alkyd resins. The results indicate the potential utility of this compound to achieve lower VOC formulations with equivalent performance properties [33].

2.3.2 Acrylic paints

A series of UV curable highly branched waterborne polyurethane acrylates and diacrylates were developed in order to enhance photopolymerization performance, water and solvent resistance [34].

Incorporating hyperbranched polyester polyols in urethane-acrylic wood coatings provides excellent adhesion and high gloss [35].

2.3.3 Powder Coatings

Powder coatings are among the best performers related to carbon footprint. High efficiency and the absence of solvent and water are other very positive

aspects. Current developments aim at reducing the curing temperature and film thickness. If renewable raw materials can be used in the organic part of the powder coatings (binders, crosslinkers and additives) their carbon footprint will be further reduced. The development of renewable binder systems is anyhow facing the very limited availability of renewable dicarboxylic acids, which provide hardness to the coating. Globally, the first renewable powder coating resins are expected to become available within the next five years [36].

Dielectric studies of epoxy/polyester powder coatings reveal detailed information of the homogeneity of the film. All systems exhibit heterogeneity and in the case of a pigmented system, charge carriers can be introduced into the system by the pigment. The effect of TiO_2 obtained from the chloride process is distinctly different from TiO_2 from the sulfate process [37].

PPG patents (Patent No. U.S. 7,468,401 B2) a procedure to introduce flake-like color effect pigments into a powder coating [38].

2.3.4 Additives & fillers

TiO_2 and derivatives

Trends include scattering pigment partially replacing TiO_2, or pre-composite polymer particles improving the wet and dry hiding efficiency of TiO_2 [39]. Other benefits, such as reduced formulation cost, improvements in barrier film properties, and better eco-profile of both interior and exterior waterborne paints, are also possible [40].

Surface treated, submicron TiO_2 particles can be used to toughen epoxy resin formulations. A small amount of TiO_2 submicron particles (1 %) improves the flexural, abrasion and pull-off strengths, while amounts up to 5% significantly enhance tensile properties only [41].

Silica/silicon additives

Silicone additives can improve paint consistency by preventing flooding and floating [42] and also contribute to improve the sustainability of decorative coatings, for both architectural and wood coating applications [43].

Adding a nanodispersion of surface-functionalized fumed silica to an acrylic paint gave significantly lower dirt pick-up and good cleaning behavior while retaining other paint properties [44].

Rice husk ash (RHA) —a carbon neutral waste product that is an abundant source of silica, increased the wear resistance, scratch resistance, and elongation of an epoxy coating [45].

A UV-cured hybrid anti-glare coating was formulated using modified silica and dipentaerythritol hexaacrylate as a binder [46].

The use of synthetic hectorite ($Na_x(Mg_{3-x},Li_x)Si_4O_{10}(OH)_2$) in multicolor paints opens up new fields of use, for example imitation granite finishes that closely match the real stone. Clay mineral colloids allow the formulation of environment-friendly water-based low-VOC multicolor coatings [47].

Using a novel silane-acrylate macromonomer and organically modified montmorillonite ($(Na, Ca)_{0,3}(Al, Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$) in aqueous PUR coatings increased their water resistance [48].

Aqueous fumed silica dispersions are a new form of additive that can improve a variety of performance attributes in waterborne coatings. Pre-dispersed fumed silica provides the potential to lower VOCs by improving film formation. They also serve as a potential way to gain enhanced durability of waterborne coatings by helping to replace plasticizers [49].

Superior nanoscale fumed-silica dispersions with uniform distribution are reported to maximize its performance. Optimizing the silica distribution can potentially help water-borne coatings to reduce the performances gap compared to solvent-borne coatings [50].

Various

Epoxy- and amino-functionalized $BaSO_4$ particles can improve the performance of high solids, powder, waterborne and UV coatings systems [51]. In general, $CaCO_3$ can replace feldspar ($XAl_{(1-2)}Si_{(3-2)}O_8$ with X being either Na, K and/or Ca) with no loss and, in most cases, with an improvement in physical properties. It results in overall formulation cost savings and leads to comparable physical and exterior durability properties [52].

Reference [53] reports surface modification of extender calcite with silicone to give surface functionalized calcite. Coatings were formulated by incorporating this functionalized calcite into an epoxy polymer matrix, and their properties were compared to coatings containing untreated calcite. The effect of the functionalized calcite to physico-mechanical properties, anticorrosion efficiencies, UV resistance and chemical resistance were studied in detail. The results revealed a remarkable enhancement of the coating performance.

2.3.5 Catalyst

A new cobalt-free catalyst helps to improve the cure time of water-based alkyds, high solids alkyds and alkyd-modified resins. The catalyst provides the missing piece in new, low-VOC alkyd formulations. Alkyd resins still offer excellent performances in the decorative and light-end industrial paint markets [54].

A new water-soluble, hydrolytically stable catalyst provides fast dry times and very good physical properties for waterborne 2K polyurethane formulations. Use of the new catalyst offers wide application latitude. The selectivity of this catalyst is promoting the reaction of isocyanate with hydroxyl groups with respect to its reaction with water [55].

2.3.6 Self-healing technology

Self-healing materials have the structurally incorporated ability to repair damage caused by mechanical wear over time. Self-healing polymer coatings are already used in automotive topcoats (Nissan) [56]. One of the self-healing mechanisms is achieved by incorporating a powder additive consisting of metal microcapsules with a Ni:Zn alloy shell and a diisocyanate monomer resin inside. When scratched, the capsule breaks and the resin flows to the damaged zone and cures upon exposure to ambient humidity to repair the coating [57]. As an alternative, a supramolecular elastomer with high biochemical content is used. The liquid elastomer precursor can be cured on a metal substrate by a simple procedure, producing a very strong adhering thick (>100 μm) coating. This coating exhibits self-healing behavior, excellent vibration dampening and notable corrosion resistance [58]. Finally, Andersson and Wilson evaluated the application of polydimethylsiloxane (PDMS) based chemistries in the development of self-healing coatings for heavy-duty industrial and marine applications [59].

The pros and cons of each healing mechanism are debated by Garcia et al. They also highlight the potential of development of non-explored areas of coatings technology [60].

2.3.7 micro- & nano- technology

Micro and Nano technology like microencapsulation, nanocomposite materials, and carbon nanotubes help to improve chemical, mechanical and physical properties of paints.

Microencapsulation makes it possible to add materials that would have shorter useful lives if mixed conventionally. Microencapsulation creates potential for new applications, like a wall coating that helps moderate the temperature of a room [61].

Nanocomposite materials improve water resistance, corrosion resistance and color retention [62, 63].

Incorporation of predispersed nanoparticle additives increases the rub resistance against MEK and IPA as well as improving humidity and weathering resistance [64, 65]. Nano oxides provide better wear and UV protection, and are not released as nanoparticles into the environment [66]. Nano zinc particles can stop the formation of cracks in the film during the

cathodic electrodeposition of paint films. It reduces the photodegradation of the aromatic polyurethane binder. Particles in the films reduces the tendency of the films to yellowing [67]. Nano-silica particles were incorporated in an automotive OEM clear-coat based on acrylic-melamine chemistry. It was found that there is a close relationship between the surface chemistry of the silica nanoparticles and the nanomechanical behavior of the baked film [68, 69]. The same observations are applied to 2 K isocyanate/polyol clear coats. The nano-silica particles at the surface of the coating increase the mar resistance by increasing the surface hardness [70]. Two-pack acrylic urethane paint filled with hydrophobic nano-silica provides enhanced barrier properties as compared to pure PUR [71].

Carbon nanotubes (CNT) can be incorporated into paints to enhance their conductivity. They are more efficient than carbon black or metal fillers [72, 73]. Nano-clay incorporated in polyurethane coatings enhances dry adhesion and impact resistance [74, 75].

2.3.8 Biocide action

In order to predict the useful service life of exterior coatings subject to fungal and algal growth, it is desirable to be able to measure the rate at which biocides leach out of paint. The radiotracer technique was examined by preparing a small quantity of radioactive “diuron” biocide. The pigment volume concentration (PVC) of the paints appears to be an important factor determining biocide retention [76].

Release from coated surfaces is slower when biocides are formulated into the paint system in microspheres, compared to the customary direct addition [77]. Microencapsulation enables coatings to be infused with longer-lasting biocides [78]. Use of modified nano-clay particles as a controlled release system for biocides from building materials are studied in reference [79].

Anti-fouling is particularly important in marine paints & coatings. New development include the reduction of the amount of biocide in the paint [80] and the development of alternative anti-fouling coatings with new natural products as biocides [81]. Other alternatives include biocide polymeric materials [82], waterborne polyurethane resins enriched in silicon [83], self-stratified siloxane–polyurethane coatings [84] and the replacement of traditional polishing pigments (ZnO, Cu_2O) by a starch/enzyme combination [85]. As final article, Buskens overviews the toxin-free anti-fouling marine paint systems under research to date, giving both their strengths and drawbacks [86].

A second trend focuses on antimicrobial action. Kugel reviews studies on antimicrobial surface treatments and coatings in which the antimicrobial agent is covalently bound to the surface or coating matrix. This constitutes an environmentally friendly option for replacing antimicrobial coatings that

release biocides [87]. The problem of silver or copper nanoparticles (NPs) stability was solved by the development of silica nanospheres containing immobilized NPs. These nanospheres can be applied as the effective antibacterial or antifungi additives for architectural paints and impregnates [88]. Reference [89] describes a water-based latex paint that can be disinfected upon chlorination with dilute household bleach.

2.3.9 Anticorrosion

Effective protection of metal structures against corrosion generally requires two or more layers of paint, each one with different properties. The development of a waterborne binder system providing effective metal protection in only one single coat is described in [90].

Pigments and additives are effective agents to face anti-corrosion problem. Since 2010, new candidates are proposed on the market like talc [91], Si nanoparticles [92], cerium (IV) oxides treated with SiO [93], wollastonite (calcium metasilicate, $CaSiO_3$) [94], cloisite 15A clay [95], calcined kaolin or diatomites [96, 97], dispersion of nano polyaniline particles [98], different kinds of nano materials with various forms (layered Na-montmorillonite (Na-MMT) and mesoporous silica particles) [99], and calcium-exchanged silica (Si/Ca), hydrotalcite/vanadate and calcium bentonite [100-102].

Nanotechnology plays an important role in the anticorrosion efficiency of paints in case of abrasion and scratching. The release of corrosion inhibitors encapsulated within nanocontainers or the application of microcapsules filled with film former can prevent further corrosion [103, 104].

The amelioration of paint to face anticorrosion problems can be approached by the binder. Zinc-rich 2K water-borne epoxy primers are now possible using new amine technology. At the same zinc loading, the corrosion performance using a new curing agent with solid epoxy dispersions is comparable to, or better than, that achieved with traditional solventborne epoxy/polyamidoamine binders [105]. More flexible glycidyl carbamate coatings can be synthesized based on linear monomers and the anticorrosion performance depends on the monomers used [106].

The replacement of non-sustainable and toxic substances used as corrosion inhibitors is also studied.

A major obstacle to chromium replacement in thin organic coatings is corrosion performance, as non-chromium coatings are generally less protective than chromium-containing ones. A novel, non-chrome, thin organic hybrid coating for coil coating applications on a variety of metal substrates has been created. The coating is based on a combination of unique structural, metal-binding and redox features that are tied to its performance properties [107].

The performance of different replacements to chromates are studied. Zinc molybdenum phosphate, zinc polyphosphate and aluminium polyphosphate have good protective behavior, independently of the resin used. Zinc pyrophosphate only shows a good anticorrosion behavior in epoxy paints. Calcium ferrite has a low performance in outdoor tests regardless of the resin employed [108].

Intended to replace phosphate pigments in anticorrosive paints, a modified zeolitic rock was obtained by grinding followed by ionic exchange with molybdenyl ions. This “composite” has an intelligent behavior because molybdenum compounds are leached from the zeolite particle by the corroding species [109].

2.3.10 Pigments

New pigments

New special-effect pigments based on natural mica are suitable for automotive, plastics and architectural paints [110].

The new Eckart effect pigments distinguish themselves from traditional natural mica-based pigments by their extraordinary luster, sparkle effects and glamorous look. The smooth metal oxide coating of the calcium sodium borosilicate leads to very high transparency and pure interference colors [111].

New developments include a heat-resistant yellow iron oxide with a completely inorganic encapsulation that makes it stable up to 240 ºC, and zinc ferrite based pigments [112] These new pigments are able to replace standard yellow iron oxides. Although they are ideal inorganic pigments to develop a wide range of color shades, their use is limited in powder, coil coating or other high-temperature coatings because of their shade turning darker and browner under the curing conditions used.

Lead chromates have been predominantly replaced by organic/inorganic pigment blends. This is being accelerated by the classification of these pigments as SVHC (substances of very high concern), and their phase out is expected by 2015 [113]. BASF will stop producing lead chromate pigments by the end of 2014. Even if substitution is not perfect, organic as well as inorganic solutions are proposed [114]. New pigment chemistry, niobium tin pyrochlore yellow (PY227), has been developed and expands the durable colors available in paints and coating. It has the chromaticity and brightness of organic pigments and the opacity and durability of inorganic pigments. The new yellow is supplemented by improvements in rutile tin zinc to increase its red value. Together these pigments provide an alternative to lead chromate pigments and expand the durable colors available in yellow and orange shade [115, 116].

A new inorganic black pigment has been developed with an extraordinary IR-reflecting ability. Multilayer systems can be found in automotive OEM or refinishes, as well as in other industrial coatings applications. Positive

findings for lower heat build-up of thermal insulation systems using IR-reflecting black pigments instead of carbon black or black iron oxide have been reported in lab and outdoor tests [117-119]. Being a sustainable solution, heat-management pigments can be used in a variety of applications, including architectural, industrial, transportation and automotive. They enable a more environmentally efficient use of resources and help to extend the shelf life of exterior coatings [120]. In Reference [121], Huntsman Corporation presents in turn its new coating pigment giving high infrared reflectance.

Formulation

The interplay of various pigments types is to be considered in the strategic color design. Mixing interference and solid-color pigments revive the color palette. Pigments choice is crucial as mixtures can either increase or reduce color effect [122].

In pigmented epoxy- and acrylic-urethane films, films with poor particle dispersion and highly photoreactive pigments exhibit the most severe degradation, whereas little or no degradation occurs in films with good particle dispersion containing pigments with low photoreactivity [123].

Scattering by rutile pigment is treated and a method is proposed that can support the formulator in evaluating whether the hiding power of a white paint formulation should be improved by increasing the amount of pigment or by improving the spatial dispersion state [124].

The evolution of the “flip-flop” effect in the European OEM silver car color shades from different manufacturers between 1950 and 2010 has been evaluated in an attempt to understand the trends of the “metal look” for the automotive industry [125].

2.3.11 Degradation problems

Many efforts are done to improve the scratch and mar resistance of automotive coatings, and to reduce degradation due to bird droppings, tree gums and those due to weathering.[69, 126] Many alternatives are available for today’s paint formulators to explore and use.

The degradation of automotive clearcoats by bird droppings is mainly due to enzyme catalyzed hydrolysis reactions [127]. To face this problem, the following solutions are proposed during this review period:

- Additives like reactive polysiloxane [128, 129] or trialkoxysilane treated nanoparticles of silica or alumina [130] give substantial improvements.
- Change in the polymeric backbone [129]

Clearcoat degradation by tree gums can be reduced by using Acrylic/melamine clearcoats with higher melamine content (higher crosslink density) [131] .

The scratch and mar resistance of clearcoats can be improved by using a binder consisting of acrylic polyol resin, with butylated melamine and silane modified blocked isocyanates, higher isocyanate loadings being very favorable through increased network density [132].
Adamsons reviews paint defect and depth profiling studies of automotive paint systems exposed to environmental conditions [133]. Publication [134] reports tests comparing inorganic and organic light stabilizer efficiency in waterborne clearcoats.

In multilayer automotive coating systems, it is often the e-coat layer which degrades by atmospheric weathering, leading to adhesion problems, peeling and finally to corrosion of the metal substrate. The photooxydation of the e-coat is influenced by thermocatalytic effects, so e-coats of dark colored automotive coatings systems are exposed to enhanced photodegradation because of the increased heat uptake [135]. The basecoat pigmentation has also an effect on the chemical structure and surface topology of its attached clearcoat during weathering exposure. A black basecoat induces more post-curing reactions in the attached clearcoat in the early stages of weathering. A silver basecoat imposes higher degrees of photodegradation to its clearcoat during the whole weathering exposure [136].

Others kind of paint face also this kind of problem. The increasing use of deep colored finishes on façades has revealed problems of color fading [137]. It is shown that the reason for this is not purely fading of the organic color pigment by UV exposure, but also erosion of the binder leading to more titanium oxide becoming exposed on the surface. These paints are normally formulated above the critical pigment volume concentration (PVC). The use of lower PVC levels might improve the situation, but could impair the required water vapor permeability. A new acrylic binder was developed [138]. In parallel, the development of PVDF hybrid latex technology now allows the coatings industry to take advantage of the properties of the PVDF fluoropolymer for field and factory-applied coatings on concrete and other cementitious substrates. Because these coatings are low VOC to begin with, and are extremely long-lasting, repainting is not needed nearly as often. The ability of PVDF-based coatings to resist UV degradation, water and chemical attack, allows these coatings to more easily resist dirt, staining and mildew/algae growth [49].

2.3.12 Flame retardant

Solvent base alkyd and emulsion paint formula were made flame retardant by incorporation of hexachlorodiphosh (V) azane of types (I–III). Oxygen index value results indicate that solvent based alkyd and emulsion coatings

with these compounds containing chlorine, nitrogen and phosphorus exhibit a very good flame retardant effect. The gloss and the impact strength of the paints are decreased by the additives, the hardness and adhesion resistance on the other hand increase [139]. Coatings with Cyclodiphosph(V)azane exhibit a very good retardant effect, when blended with polyurethane varnish [140].

Bio-based polymer nanocomposites have a unique niche of their own in the domain of green technology: improvement of flame retardancy of the nanocomposites is possible. The results indicate the potential of these bio-based epoxy/clay nanocomposites for multifaceted advanced applications [18].

A water-borne intumescent fire retardant varnish based on phosphate resin acid (PRA) cold cured amino resin was synthesized. The flame retardancy tests demonstrate that a higher phosphorus content is beneficial to the intrinsic flame retardancy of painted films, but the high quality char formation is another key of fire retardancy of painted films [141].

3 Forensic analysis of paint

The analytical scheme for the comparison of a paint smear with a known sample does not evolve considerably. Fourier Transform Infrared Spectroscopy (FTIR), UV-Visible icropectrophotometry (MSP), Scanning Electron Microscopy Energy Dispersive X-ray spectroscopy (SEM-EDX), X-Ray Fluorescence (XRF) and Pyrolysis Gas Chromatography coupled with Mass Spectrometry (Pyr-GC/MS) are still the methods of choice for organic and inorganic analysis of paint smears.

FTIR spectroscopy stays the most useful technique for paint analysis since the 1970s, requiring only a small quantity of sample to achieve rapid analysis and high quality spectra. Since 2010, the American society of Trace Evidence Examiners publishes a peer-reviewed journal dedicated to the analysis of trace evidence. They established a working relationship with the Scientific Working Group on Materials Analysis (SWGMAT) (http://swgmat.org/). This group proposes an interesting standard guide to assist a paint examiner in the selection of appropriate sample preparation methods and instrumental parameters for the analysis, comparison and data interpretation of paint samples by FTIR [142]. Techniques as FTIR microspectroscopy, diamond cell and attenuated total reflectance are discussed in term of requirements, benefits, limitations and proper use of IR accessories as well as sampling methods. Complementary information on this technique can also be found in the Second Edition of the “Encyclopedia of Spectroscopy and Spectrometry”, in a chapter dedicated to forensic science application of infrared spectroscopy [143].

In addition to the above mentioned methods often available in most of the forensic laboratories, others exist but are not routinely used by forensic paint examiners. These are proposed in the literature in typical casework and can provide novel solutions to particular problems. As already

underlined in the previous review, literature shows an increasing interest in some techniques that emerged during these last years:

- Raman spectroscopy becomes more important in the analytical scheme for pigment identification.
- Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) gain popularity for the elemental analysis of paint flakes.

Other new trends concern applications of chemometric tools for data treatment and the use of imaging technology to obtain 2D or 3D chemical images of a paint flake or of a paint layer sequence.

In parallel, many publications focus on the discrimination capabilities of classical techniques for a specific set of samples like black or colored spray paints, clearcoat car paints, household paints…

The organization of this chapter is based on the trends explained above. As some research papers incorporate several trends, these will be mentioned repeatedly. This chapter also includes a summary of the principal studies done in artwork.

3.1 Data treatment

Many publications on forensic paint investigation during the last three years concern data treatment. Lavine et al. review the most significant developments in the field of chemometrics from December 2009 through October 2012., including pattern recognition [144]. Pattern recognition allows the classification of samples using techniques such as Principal Component Analysis (PCA), Hierarchical Clustering (HCA), K-nearest neighbor, and others. Applications of these methods dominate the literature including the field of forensic paint analysis. Lavine proposes the two following publications as illustration.

In the first one, also written by Lavine, the authors develop search prefilters to search the Paint Data Query (PDQ) database in order to differentiate between similar but non identical FTIR spectra [145]. Normally, the PDQ database uses text based fields (color, chemical text codes, layer sequence) for searching with the aim of obtaining a preselection of spectra that are then checked manually. However, unlike the undercoat and colored paint layers, clearcoats do not provide a characteristic color nor include inorganic fillers that can serve to further discriminate between them. Lavine develops prefilters to face this failure and the inability of the PDQ database to accurately search IR spectra. Prefilter is a quick test to identify library spectra that are dissimilar to the unknown. In this case prefilters are based on chemical information.

The second application is reported by Muehlethaler et al. who apply PCA and HCA to the analysis of the infrared and Raman spectra of 34 red household paints, and compare the result to visual comparison [146]. Six

distinct clusters were detected from the spectroscopic profiles by chemometric tools. This allows for a quick classification of the samples comparable to the visual classification. Combining the results of both spectroscopic methods, all samples were individually separated yielding a potential discriminating power of 1. However the authors are cautious: “It is safer to remain at a higher level and not to conclude that all samples are categorically different. By using this methodology we can rapidly form groups of samples with similar properties, and this process is repeatable until a defined level of discrimination is reached, so at a brand level. At a lower level, the mechanisms of the separation are more difficult to understand and more work has to be performed as batch variation is yet too arbitrary to be used systematically”. This is particularly important with Raman spectra for which there are reproducibility problems. Future works is to define objective criteria for sample comparison.

During this review period other published papers discuss data treatment by chemometric tools. Three papers describe the use of multivariate statistics on a large population of automotive clearcoats based on MSP spectra [147], Pyr-GC-MS spectra [148] or ATR-FTIR spectra [149].

The first one focuses on the detection of ultraviolet absorbers in clearcoats that are added in order to protect the vehicle against UV light and weathering. Liszewski et al. used UV microspectrophotometry for the comparison of this kind of samples. They studied 71 clearcoats and applied agglomerative hierarchical clustering and principal component analysis for classification. Three mains groups of spectra are identified corresponding to spectra with one, two and three maxima. These results showed no correlation to the make, model and year of the automobile. So this method is only of interest when comparing questioned and known samples and cannot be used for investigative purposes. As environmental factors such as exposure to sunlight can affect the clearcoat and its UV spectrum, care should be taken when comparing an unknown sample with a corresponding known sample not collected at the same time. Also various parts of the automobile body can be affected differently by external degradation.

The second study was authored by Zadora et al. They propose a methodology based on the likelihood ratio model to compare paint data: “could two samples have originated from the same object?” The model is applied to the Pyr-GC/MS data of 36 acrylic clearcoats that are indistinguishable in terms of their infrared spectra and elemental composition. The performance of the model is discussed in order to check the level of false positive and false negative answers. The results were satisfactory, with only 3.0% false positive answers and 2.8% false negative answers.

The last publication concerns 130 clearcoats coming from Australian and some European and Japanese manufacturers representing a total of 18 car makes and 60 different models. Based on FTIR spectra and PCA results, samples are classified into 9 classes. They differ in the relative amounts (absence and presence) of styrene and melamine. The authors make a link

between the classes and the origin (manufacturer) of the samples and even the manufacturing site. However, this conclusion has to be treated with care: the amount of styrene in a clearcoat is related to the quality of the clearcoat. Low cost clearcoats contain more styrene than high durability clearcoats in which styrene is replaced by larger monomers. The same manufacturer can use two clearcoats: one for low cost vehicles and a second one for premium class vehicles.

The use of LIBS in car paint analysis and the subsequent use of nonparametric testing method for the pairwise comparison of these samples is reported by McIntee et al. [150]. Their study focuses on 90 automotive paint samples encompassing a range of automobile makes, manufacturers and colors from production years 1987 – 2006. The paint chips are divided into sets before LIBS analysis according to the following characteristics: color, presence or lack of effect pigments, and number of layers. The capacity of the method is evaluated in terms of its discriminating power but also in terms of Type I error (failure in intra sample comparison). Inter-sample discrimination was 100 % for all color paint groups but with occasional intra-sample discrimination (Type I error) meaning that there is a risk for false discrimination. The black colored set gives a discriminating power of 95.8% with one Type I failure. In the other hand, LIBS failed to discriminate between white paint samples with a DP of 86.56 % only. However, no Type I errors occurred in this set.

Two remaining papers involve other kinds of samples. Orellana et al. discuss the analytical treatment of LA-ICP-MS data obtained from various samples in the field of forensic sciences [151]. Recently, Staniszewska et al. applied univariate and hierarchical cluster analyses on data coming from chemical imaging of cross sections of glass painting by FTIR and Raman spectroscopy [152]. This article is principally focused on the processing of data coming from the imaging process.

3.2 Emerging techniques

3.2.1 Raman spectroscopy

Raman spectroscopy is a non-destructive analytical technique that gives the vibrational spectrum and physical or chemical information of virtually any matrix in any state of matter. For these reasons, Raman spectroscopy has increased in popularity in the forensic sciences since 10 years. Das and Agrawal review Raman spectroscopy including a summary of the basic principles of the technique, recent technical developments in instrumental design and sampling methodology. Various applications are also presented in different fields of science including forensic science [153]. This review is a good start for beginners. In the paint area, Raman spectroscopy is particularly well suited to the characterization of the pigments. Here are the most relevant publications about Raman spectroscopy in paint analysis during the last three years.

Zieba-Palus et al. applied Raman spectroscopy to the analysis of green paint samples including 13 solid automotive paints and 2 household paints [154]. The 785 nm excitation wavelength gives the best discriminative power. The identified pigments are copper phthalocyanine, Prussian blue, chrome yellow, chlorinated copper phthalocyanine, brominated copper phthalocyanine and titanium dioxide. Some paint samples contain a mixture of these pigments. Zieba-Palus also proceed with the characterization of automotive paints of various colors by combining the information coming from Raman spectroscopy and infrared spectroscopy [155]. Three yellow paint samples with similar binder (phthalic resin with or without melamine) give quite similar FTIR spectra while Raman spectra are totally different because of different pigment composition.

Another study by Muehlethaler et al. compares FTIR and Raman spectroscopy as complementary tools for the analysis of red household paints [146]. The Raman spectra could be separated into 5 groups according to the pigments or combination of pigments. The most frequently used pigments were Pigment Red 112 and Pigment Red 170. Others are less used and sometimes mixtures of two red pigments are detected.

As illustrated in the previous references, pigment identification based on Raman spectroscopy is very useful and involves the comparison of the spectrum of the paint trace to spectra in a reference database. Large spectral libraries are required and publications presenting such databases are of interest to the forensic community (e.g. Scherrer et al. [156]). However, Raman spectra can contain many bands or show a mixture of compounds, and searching can become quite complex. That is the reason why automatic methods are proposed as by Vandenabeele or by Khan.

The model of Vandenabeele is based on a multivariate comparison of Raman band position rather than the spectral intensities as in classical chemometric algorithms [157]. This approach overcomes problems such as the presence of fluorescence background radiation or spikes, spectra recorded at different spectrometer with different laser wavelengths and power. Moreover, this model permits using non-digital database such as those presented in literature. The model is illustrated with unknown paint samples containing organic pigments.

Khan et al. develop a similarity measure specific to Raman spectroscopy. They propose a modified Euclidean metric algorithm to handle the problem of spectra of substances mixtures [158]. The method takes into consideration not only the intensity at a given wavenumber but also the contribution from its nearest neighbors to assess the resemblance of query and reference spectra of mixture of substance. They discuss in detail the principle of their method and propose to evaluate the performance of their new model against the performance of other similarity methods. Their dataset however consists of liquid chlorinated and non-chlorinated solvents and is not of direct interest to the forensic paint scientist.

The popularity of Raman spectroscopy leads to the development of portable systems combined to spectral preprocessing methods and library search algorithms that give an “answer box” : a Raman spectrometer that could be used by everybody and attach a product name to a spectrum [159].

In the artwork field Raman spectroscopy is also a very interesting tool and is widely used.

3.2.2 Elemental analysis

X-ray based techniques like SEM-EDX, XRF and XRD are commonly encountered in forensic paint laboratories since many years. During the last three years, some authors publish new research on these techniques [127, 160-165]. Most of the time, these elemental techniques are used in combination with others with the aim to obtain a maximum specification on the paint. All the cited references are developed elsewhere is this review.

In addition to these traditional techniques, LA-ICP-MS and LIBS are developing. LA-ICP-MS is already increasingly used for routine analysis in forensic laboratories with the main application developed for glass and paint samples. LIBS could become a fast and relatively inexpensive alternative to LA-ICP-MS.

However, few publications were published since 2010 about these emerging techniques. Orellana et al. review the LA-ICP-MS technique and two papers were focus on LIBS analysis.

Orellana et al. propose an interesting review on LA-ICP-MS in chemical analysis of forensic evidence [151]. The authors explain the basic principles of the technique and present advantages and drawbacks. They also review the application of LA-ICP-MS to the elemental analysis of glass and paint. All references applying to application in paint analysis cited by the authors date before 2010.

McIntee et al. study the capability of LIBS in discriminating between automotive paint samples [150]. The study focuses on 90 automotive paint samples. The major drawback of LIBS technology is its poor reproducibility. Twelve LIBS spectra were recorded on each paint sample, each an average of five single shot “drill down” spectra from consecutive laser ablations at the same spot on the sample. This procedure takes time and destroys the sample.

Staicu et al. optimize the best working condition of LIBS like the laser fluence and the number of pulses in order to use LIBS for depth elemental profile of multilayered paints [166]. They sample the paint by a consecutive number of laser shots applied at the same spot and record the spectra of the ablated material. They use “homemade” samples consisting of several stacked layers of known composition and thickness. The proper choice of the main ablation parameters allows them to determine painting layer sequence and the elemental composition of each layer.

3.3 Comparison of specific sets of samples

In casework, the use of only one technique is rather rare. Techniques are often combined to obtain the more information and to either confirm or exclude that two samples are indistinguishable. Many publications compare the discriminating capabilities of several techniques or emphasize the complementary of them.

Ryland et al. evaluated the discrimination power of a series of four analytical techniques on a sample set of seventy-one black household spray paints acquired at retail stores in the United States in 2001 [161]. Samples were initially inter-compared by FTIR (using a spectral library approach) and only 23 pairs out of a possible 2,485 pairs are indistinguishable. These samples were then compared by microscopy and two additional pairs on the 23 were discriminated. SEM-EDX distinguished between an additional 5 pairs; leaving 16 pairs undifferentiated. Finally, paints still indistinguishable were compared by pyrolysis gas chromatography giving a final 14 indistinguishable pairs. The discriminating power for the combination of the four techniques was 99.4 percent. This study underlines that good discrimination capability is obtained for the analysis of black household spray paints using a classical analytical scheme.

An important study has been done by 11 laboratories of the European Network of Forensic Science Institute (ENFSI) Paint and Glass working Group to determine some batch-to-batch variations in spray paints [165]. This question can help forensic expert when evaluating the chance of matching between two distinct batches when he is confronted with undifferentiated paint samples. The study concerns four color groups (black, white, red and papaya) and includes seven analytical techniques (optical microscopy, FTIR, Raman spectroscopy, Pyr-GC/MS, elemental analysis and MSP). In a first step, each laboratory has compared the data visually. Additionally, spectroscopic data (MSP, FTIR and Raman) have been compared by chemometric analysis. The results also include calculation of discriminating power of the techniques. Differences between batches of colored samples are principally detected by methods that give information on pigment composition (optical microscopy, Raman spectroscopy, MSP). FTIR is more adapted to discriminate white samples, detecting changes in binder composition. Black samples are not easily differentiated. Pyr-GC/MS was the only way to provide some difference between these last samples.

The FBI reports a big study focused on architectural paint samples randomly collected in the United States and Canada. The samples are analyzed by stereomicroscopy, FTIR, SEM with both backscatter electron imaging (BSI) and energy dispersive spectroscopy (EDS), and Pyr-GC/MS [164]. A table summarizes the level of discrimination achieved subsequent to each method that was utilized. At the end, no random pairs of samples

remained indistinguishable thanks to the combination of all classical techniques. The results underline the strength of stereomicroscopic examination. Actually, for the off-white group of samples, the discrimination power was 99,86 % following microscopic examination alone.

Stone et al. use UV-MSP for the comparison of clearcoat paints in combination with stereomicroscopy and FTIR [167]. Their works focuses on the degradation of UV absorbers in clearcoats due to exposure to the environment. The selected samples come from vehicles from the same manufacturing plant, with the same paint color but of different model years (from 2000 to 2008). They underline the fact that there exists a UV absorption gradient in function of the depth to the surface. As already mentioned earlier by Liszewski et al. [147], false distinction can be made between a questioned and a known sample if UV spectra are not recorded at the same layer depth. More work is necessary to check the reproducibility of the results.

Lv et al. take another direction to paint discrimination based on Infrared and Raman spectroscopy [168]. They propose to investigate different kinds of clay such as kaolin and bentonite. For each kind of clay, characteristic peaks are noted. For example, they are able to discriminate between kaolin and another clay (not specified) based on paint Raman spectra in the 3000 – 4000 cm^{-1} region. However, their study is very limited (3 clays and few paint samples) and deserves to be extended.

3.4 Imaging

Imaging is widely used by scientists since many years, principally in medicine and biology. The first techniques developed only give visual information of the object (optical microscopy, scanning electron microscope...). Chemical imaging, that includes additional elemental or molecular information, has become a powerful tool for generating detailed chemical images based on the point-by-point mapping of a sample.

We note that, during this review period, Imaging is more and more used by forensic scientists for paint analysis, particularly for detecting additional layers of a multilayer coating that are not visible under optical microscope.

3.4.1 Molecular imaging

Zieba-Palus et al. use Raman imaging to differentiate additional layers of a multilayer automotive coating that are not visible under the optical microscope because they have the same color. The layers differ only in terms of chemical composition. Additionally, Raman imaging can give data on the distribution of pigments of a particular paint layer [155]. Stewart et al. also used Raman imaging to chemically map a cross-section of a multilayer white household paint chip by lateral scanning Raman spectroscopy [169]. In this feasibility study, the authors treat the homogeneity of the individual

layers, the influence of degradation over time, and the optimization of Raman parameters. They also show that, due to diffuse scattering, there is no sudden transition from one paint layer to another in the sequence of spectra. The boundary regions give several spectra that have characteristics of both paints. However, this typically occurs over length dimensions much smaller than the paint layers. Further work of these authors will involve examination of samples representative of multilayer white paint casework.

Two groups present Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy to obtain chemical images of multi-layered paint cross section. Joseph et al. [170] illustrate the potential of Macro-ATR-FTIR with the study of the paint cross section of three historic samples, while Sloggett et al. [171] illustrate the potential of micro-ATR-FTIR coupled to a synchrotron IR source in a study of a paint cross-section of an exterior household paint. The high spatial resolution of the last system (5 µm) emphasizes the pigment distribution inhomogeneity in a paint layer. Unfortunately, this kind of technique is not easily accessible to forensic laboratory and so it is very difficult to transpose this method in current expertise. Joseph et al. obtain a spatial resolution of about 15 µm which is also good results, and the macro-ATR-FTIR being much more available for forensic experts. Inorganic compounds and organic substances are characterized and localized within a paint layer.

3.4.2 Elemental imaging

Nakamo et al. present the recently developed confocal µ-XRF techniques combined with polycapillary X-ray lenses that enables elemental depth profiling and mapping images. They apply this technics to the analysis of three kinds of automotive paint fragments [163]. The instrument allows for a nondestructive elemental analysis of the sample in 3 dimensions with a spatial resolution of 10 µm. The data obtained on the 3 paint flakes are compared to those obtained by conventional µ-XRF of cross-section of the paint flakes. The results are in good agreement except for light elements such potassium and sulfur. These elements are not detected by confocal µ-XRF because the XRF intensity from the low-Z element is strongly absorbed by the sample. De Nolf et al. propose the combination of microscopic X-ray fluorescence and microscopic X-ray diffraction for the tomography of an automotive paint flakes. The advantage of this combination is the possibility to determine the elemental and crystal-phase content of each layer of a paint flake without physically sectioning the questioned sample [162]. They give an example of the data obtained for a paint flake originating from a VW Passat car. The paint flake is characterized by eight layers of different chemical composition relating to the inorganic components of the paint. We also suggest the reading of an article coming from the art field expertise. The authors achieved the elemental mapping of a painting with high definition (large area, high spatial

resolution) thanks to XRF microscopy using synchrotron radiation [172]. The method has the ability to reveal metal distribution in the pigments in spite of the presence of the highly X-ray absorptive lead white paint used by the artist. For a complement information about XRF imaging we suggest the review done by Janssen el al [173]. The authors present an overview of the instrumental and methodological improvements of the Micro X-ray fluorescence technics and compare it to other microanalytical methods. Authors propose some examples as the investigation of cultural heritage materials or the investigation of industrial materials such as the characterization of car paint multilayer systems.

Edelman et al. review the hyperspectral imaging instrumentation (HSI). This is a method that combines conventional imaging and spectroscopy to obtain both spatial and spectral information from a sample [174]. Like spectroscopy, HSI can be applied in different parts of the electromagnetic spectrum such as ultraviolet (UV), visible (Vis), near infrared (NIR), mid infrared (IR) ant the thermal infrared range. HSI makes comparisons of different specimens easier and reduces the analysis time. The potential of HSI has been compared to point measurements performed with traditional spectrometers. The benefit of HSI in forensic science is principally the detection of latent fingermarks. However, Edelman also reported the analysis of paint samples done by Flynn with such techniques [175].

K. Macuchova et al. propose a special device for non-destructive examination of forensic samples by enabling simultaneous visualization of examined samples and their spectroscopic measurement in visible and UV light [176]. The instrument is specified for the color characterization of an object as well by visual as by spectroscopic measurements. This device can image a sample using an optical imaging system, the image can be digitally processed and in parallel the reflected light in the integrating sphere enters an optical fiber that brings the light to the aperture of the spectrometer for recording the spectrum. The device has been applied to various samples as sea shore sand, gem and solid paint samples. The authors mention that tests demonstrated the ability of the device to meet requirements with sufficient precision and reliability but without additional comments. They do not give additional information of what they exactly checked. A drawback is that spectroscopic measurements seem to be done only in reflected mode which has limitation due to surface features and illumination angle.

3.5 Artwork

The field of art paint has similar criteria to those required by the forensic expert. Due to conservation ethics, there is a need for noninvasive methods or methods that can be applied to very low amounts of material sampled from the artwork. This is the reason why forensic paint examiners must be attentive to this area of research as the methods can be applied to forensic samples. However, the approach done in the art field is quite different from

that in the forensic field. The authors often focus on a specific artwork and combine several techniques in order to obtain a fully characterization of the pigments and/or binder and/or additive in the object [177-180]. Their aim is to provide information about the authenticity of a paint or to provide support to the restoration of a piece of art.

Two major trends dominate this field in the last three years: the use of Raman spectroscopy (including SERS) to identify artists‘ pigments [152, 177-179, 181-185] and the analysis of proteinaceous and lipid binders by various techniques.

The first trend can be completed by the review of Berrie concerning the history of analysis of artists' pigment [186]. This review provides an overview of the analytical methods widely used in this area of expertise and focuses on application to art paints.

The second trend is of great importance in the field of cultural heritage where paint binders are of animal or vegetable origin. Liuveras presents a new extraction procedure for the simultaneous characterization of glycerolipids, natural waxes, and proteinaceous, resinous and polysaccharide materials by GC-MS [187]. Miguel et al. use FTIR combined with chemometric tools for the characterization of medieval paints [188]. Van der Werf propose a simple protocol, based on Bligh-dyer extraction followed by MALDI-TOF-MS analysis for the analysis of a 15th century Italian panel painting [189]. This method allows also the simultaneous extraction of lipids and protein in pigmented paint layers. Sandu reviews optical microscopy of cross sections, including fluorescence and staining techniques, for investigating natural organic materials in paints [190]. The principle is the use of dyes able to form colored compounds with organic materials, such as proteins, polysaccharides, resins and oils based on the interaction with specific functional groups and/or on the characterization of specific properties of chemical functions of these materials. He lists and explains the most reported stains and their preparation together with the specific positive responses for organic paint material.

Finally, Targowski et al. propose an alternative to the traditional method to reveal the stratigraphy of easel paintings [191]. The traditional method is to collect a small sample, embed it in resin, and then analyze its cross section by microscope. The alternative method proposed is Optical Coherence Tomography (OCT), and comes from diagnostic medicine. Infrared radiation penetrates the paint and is partially reflected at interfaces of layers of different refractive indices, or sometimes scattered from sites of inhomogeneity in its structure. Returning light is collected and the time of propagation from the given depth of the structure is determined thus providing a measurement of the optical path to this structure. It is a noninvasive, noncontact method of optical sectioning of partially transparent objects, with micrometer-level axial resolution.

3.6 Various

3.6.1 Clearcoat degradation/modification

In the previous Interpol review, an article was cited treating the interaction of the basecoat and clearcoat within a refinish system. The data showed evidence of strong interaction between the clearcoat with the basecoat. In a new study by Maric et al. the migration of melamine and low molecular weight organic pigment from the basecoat to the clearcoat of some Mazda vehicles was confirmed [192]. They used synchrotron FTIR microspectroscopy to map paint sections in transmission mode using X-Y step size of 2.5 µm. The images obtained clearly show a significant decrease in melamine abundance in the clearcoat going from the basecoat to the surface. The consequence of these results can be extremely significant as they can affect the analysis and characterization of paint layers especially when multivariate statistics are used to compare samples of when searching a database [149]. However, the authors noted that they only observed this phenomenon with Mazda vehicles painted with the newly developed “wet paint system” which is a one-step baking and drying method consisting of the successive application of the primer surfacer, basecoat and clearcoat all whilst wet. This technology was initially developed by Mazda but will be used by other manufacturers in the future.

Yari et al. conducted an interesting study about the mechanism of degradation of a typical automotive clearcoats (acrylic melamine clearcoat) by bird droppings [127]. The study shows that in addition to humidity and sunlight, various biological substances such as bird droppings can have an impact on the appearance of a car body due to the degradation of the clearcoat. This etching is the result of an enzyme-catalyzed hydrolytic degradation of ether and ester bonds. This information is interesting for the forensic expert comparing chemical comparison of automotive samples. Actually, this degradation has consequences on the FTIR spectra (carbonyl bands and etheric bands) and on SEM-EDX spectra where additional peaks of Na, K, Ca, Mg and Cl are detected from the degraded part of the clearcoat. These elements are present in bird droppings.

3.6.2 Pigment identification

Since the development of Raman spectroscopy, this technics is particularly used for pigment identification in paints or other objects. However, scientists try to develop alternative techniques to achieve this work.

Lomax presents the diffraction pattern for over 200 synthetic organic pigments [193]. While organic pigments are generally poorer diffractors of X-Rays than mineral pigments, this study shows that many of the pigments have distinctive diffraction pattern, including pigment within the same class. Lomax shows also many pigments which have very similar infrared spectra are easily differentiated by this technique. X-ray diffraction can also help to distinguish polymorphs of pigments especially copper phthalocyanine α and

β form and quinacridone (β and γ forms). However, when the method is applied to commercial artists' paints, it is quiet more difficult [160]. Actually, in this case, the diffraction pattern of the pigment is not necessary detected. Depending of the binder, the results are more or less interesting. The best result is obtained with acrylic and alkyd binders, where the pigments could be identified in more than half of the samples examined. In the other cases, the problem comes from the low amount of pigment in the paint or the presence of high filler or extender contents. Moreover, in case of mixtures of pigment, not all pigments are detected. While FTIR is currently used to characterize binders in paint, Von Aderkas et al. used Fourier-Transform PhotoAcoustic Infrared Spectroscopy (PAS), a variant of the classical FTIR method, to analyze 12 inorganic pigments commonly used by artists today [194]. The paper presents the PAS spectra of the 12 inorganic pigments selected for the study with the aim to build a database. The identification of pigments was previously confirmed by Raman spectroscopy. While the approach is original, the authors do not test their method on paint samples whose binder can complicate the detection of pigment by PAS.

Russell et al. use Pyr-GC/MS for the identification of synthetic organic pigments currently found in modern paintings [195]. They start with the analysis of pure pigments and study the fragmentation patterns that help the chemist in classifying pigments by class. They report pyrolysis products of 70 organic pigments including diazo pigments and phthalocyanine pigments. Many fragments are produced by more than one pigment but the combination of pyrolysis products will allow most pigments to be uniquely identified. However, the application of this method to paint samples is quite complex because of the presence of binder signals masking those of the pigments in low concentration. In this case, pigment must be separated using dichloromethane. The method is long and quite complex compared to FTIR and Raman spectroscopy. It could be interesting to compare these methods to better understand the contribution of Pyr-GC/MS for pigment identification.

3.6.3 Paint and fire

Robert et al. studied the modification of the infrared spectra of paints subject to a gradual warm-up with the aim of correlating the heating temperature to the spectral changes of the paint samples [196]. Consequently, this article is more addressed to fire investigation. However, forensic paint scientists could be confronted with the comparison of a reference paint to a burned paint (for example car on fire). In a first conclusion, the authors state that:

- The loss of (C=O) absorption indicates T > 300°C
- Appearance of water bands on cooling indicates T > 500°C, with intense water bands indicating a temperature closer to 700°C.
- In clay-based paints, changes to (Si–O) bands indicate T > 700°C.

- In $CaCO_3$-containing paints, loss of (CO_3) bands indicates T > 950°C.

However, as explained by the authors, the article is of a preliminary nature. Before obtain good conclusion and a trend, much wider range of paints and on various surfaces are needed. Repeatability and reproducibility must also be checked and other parameters as for example the influence of smoke in addition to heat.

3.6.4 Polystyrene characterization

Yang et al. focus their study on the development, optimization and validation of a method to quantify polystyrene in paint by Pyr-GC/MS with the aim of enhancing the evidential value obtained from other techniques like FTIR in the case of very similar paints [197]. The quantification of Polystyrene resin is based on the production of styrene monomer. Their optimized method yields more than 99 % of styrene monomer. The authors however illustrate this only on three samples and they do not state its contribution with respect to the FTIR method.

4 Interpretation

In the previous Interpol review, the FBI laboratory insisted on the need for better standardization of definitions across laboratories in report writing, interpretation of results, and significance assessments. Unfortunately, literature on paint interpretation is rather rare in comparison to other trace evidence like fibers or glass. Paint interpretation is often based on the frequency of occurrence of the measured characteristics to define if these characteristics are common or rare. These data are sufficient if paint examiners work on source level hypotheses [2]. Additional data have to be taken into account if paint examiners want to work on the activity level, like transfer, persistence and background parameters. Muehlethaler et al. and Bender developed this point in their respective article published in the second edition of the Encyclopedia of Forensic Sciences [6, 7]. They particularly focus on the importance of building appropriate databases and or reference materials for interpreting the results.

4.1 Population studies

Since 2010 only the study of the FBI laboratory was published on this issue. The study involved analysis of architectural paint samples from homes, offices or others buildings. They collected about one thousand samples in the United states (34 states) and Canada. Multiple samples from a location are included and no specification were given with regard to the substrate (wall, door, window) [164]. The aim was to determine the discriminative

power of a classical sequence of examination (FTIR – microscopy – SEM/EDX and Pyr-GC/MS) but also to determine if any random matches were possible. Actually, the final 11 undifferentiated pairs (the total number of comparison pairs possible is 464,166) proved to originate from the same source.

4.2 Transfer, persistence and background

We have not found studies on the transfer and persistence of paint flakes during this review period. Muehlethaler et al. specify that phenomena of paint transfer are not fully understood, especially the transfer of vehicle paint. Transfer mechanism is very important and dry paint is normally transferred by direct contact and if sufficient force is involved because paint is designed to bind strongly to its substrate. The case of wet paint is quite different. Paint can be transferred by direct contact as well as by splashing or spraying. For example, spray paint droplets can drift during spraying onto nearby surfaces like shoes, clothes or the skin of the person who makes "graffiti"[6].

An interesting study was done by Moore et al. to determine the background level of paint flakes on the clothing of persons suspected of involvement in crime [198]. The aim is to determine how likely paint flakes of a certain color and layer sequence will be found at random on an item of clothing. They focused their study on 100 garments submitted for casework examination of other particulate type. The presence of paint flakes was recorded separately for the surface and the pockets of each garment. The flakes were characterized regarding their size, color and layer sequence. The authors summarize the data by several distribution studies like number of paint flakes on garments, distribution on the garments, size distribution, color distribution, numbers of layers. However no information is given regarding the similarity of paint flakes coming from the same garment. They compare their results to a previous study done in 1971 by Pearson [199]. It would be interesting to compare these results to the result coming from persons not involved in a crime.

5 Case reports

We have only found two significant caseworks to expose here during this review period.

The FBI laboratory presents a case in which the Paint Data Query database (PDQ), automotive paint supplier contacts and refinish color pages and internet were used as resources to provide information in a make-model-year investigative automotive paint examination. The case was a hit-and-run fatality involving a motorcycle and a "blue car" as described by the contributing agency [200]. The authors clearly explain the approach to

finally propose a Volvo 850, S70, V70 models car coming from the Belgium/Ghent manufacturing site and produced between 1993 and 1998. The author underlines information available from internet is plentiful and can supplement information from other resources to aid in developing investigative lead information.

Schrag et al. report a non-common case. In accidents involving pedestrians, we always look for paint transfer from the vehicle to the victim. In this case however, the deposit of make-up particles from the pedestrian onto the vehicle impact zone was considered . The presence of make-up on the upper part of the truck's front panel was the only way to check the testimony of the truck driver and the witnesses because of autopsy revealed extensive mutilations making it impossible to give information about the pedestrian's position at the moment of the first impact [201].

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The Forensic Examination of Fibres and Textiles

Review: 2010 to 2013

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1 Introduction

This report should t be considered a ‘follow-on’ from that produced by the *author* [1] in 2010 as it catalogues the research, and other activity relating to the forensic examination of fibres since the 16th INTERPOL Forensic Science Symposium held in Lyon, October 2010. This report consists of a literature review of published articles in forensic science journals, as well as the results of research and other activities reported by the proceedings of various working groups between May 2010 and June 2013. It also contains references from other sources such as the internet.

The articles in this report have not been cited in chronological order as it was felt it more appropriate to group these according to ‘theme’.

2 General

As in previous reports by the author, the European Fibre Group (EFG) of the European Network of Forensic Science Institutes (ENFSI), the Fibre subgroup of the Scientific Working Group for Materials Analysis (SWGMAT) led by the FBI, and the healthy collaboration between the Australian Federal Police and academic institutions, continue to be the main drivers in promoting, developing and conducting research in this evidence type worldwide. Many of the citations in this document originate from the activities of these groups and/ or their members.

Europe

The EFG remains committed to disseminating best practice and over the last 3 years members have delivered workshops and presentations at a variety of meetings and symposia.

The revisions to the EFG Fibre Examination Guidelines – Manual of Best Practice document (originally published in 1998) were completed in 2012. This document is available on request.

The results of a pan-European target fibre study initiated by the group in 2011 are presently being collated [18].

Representatives from working groups in the USA and Australia continue to attend meetings. In 2012, a representative from the Asian Forensic Sciences Network joined also the group.

In 2011, the ENFSI hair working group was merged with the EFG and as a consequence, the working group name was changed to The European Textile and Hair Group (ETHG), for the sake of clarity.

The last two years have seen the closure by the UK government of the Forensic Science Service (FSS) of England and Wales, leaving the provision of forensic science wholly within the private sector in these countries. Given that the FSS at its peak was arguably the most proliferate source of research and development in all aspects of forensic science, its demise is a cause of concern not only within the UK but globally.

At the time of writing, the main commercial forensic science providers in the UK are enduring smaller and smaller profit margins and not surprisingly, engaging in areas of research unlikely to confer a commercial advantage to them (but nevertheless of value to practitioners and the criminal justice system), appear to be very low or non-existent in their list of priorities.

Despite recommendations by a UK government report for the funding of research and development in forensic science to be prioritised by research councils, almost two years later, this has yet to come to pass.

With the present state of the global economy and the austerity measures in place in many countries, difficulties encountered in obtaining funding for research is an issue not likely to be confined to the UK.

USA

Koch [2, 3] provided an update on the activities of the SWGMAT fibre subgroup. This group has been working on updating the chapters of the fibre guidelines and the most recent chapter updates have been posted on their website [4].

Additionally, the fibre sub-group has created an admissibility presentation for fibre examiners facing court hearings on the admissibility of fibre examinations which is also posted on their website. A companion document is currently being worked on with supporting references.

The 2009 National Academy of Science (NAS) report on forensic science: Strengthening Forensic Science in the United States [5] causes the forensic community to take a closer look at current practices, and seek ways to improve the scientific foundation of forensic analysis.

The White House Office of Science and Technology initiated Interagency Working Groups (IWG's) to look at the field of forensics and offer suggestions to improve the varied disciplines found in forensic laboratories within the United States. The SWGMAT fibre group prepared a response to the questions posed by the IWG for Standards and Technology and that response and bibliography is again posted on the SWGMAT website.

Based on the NAS and IWG reports, SWGMAT along with many of the specialist working groups for other forensic disciplines will be shifted to fall under the jurisdiction of NIST - the National Institute of Standards and Technology. SWGMAT will continue to work to provide guidance documents and has the next meeting scheduled for September 2013.

At the present time, interpretation reporting is a big focus in the US and there are some labs that include a scale for their report findings and others that prefer a descriptive interpretation section. Accordingly, the FBI trace evidence unit has a section that discusses transfer and persistence in their reports, as well as limitations to fibre associations due to their manufactured nature.

Australia and New Zealand

Roux [6, 7] reported on the situation in Australia and New Zealand and outlined the various forensic groups e.g. ANZFSS [8], and discussed the NIFS innovation strategy. This has involved surveying laboratories to identify the current status and identifying what was emerging. This exercise identified a lack of funds, lack of research skills, etc.

A “forensic standards development” project is being developed, as there are currently no mandatory defined standards for forensic science in general in these countries.

There are a number of proposals relating to reporting structures/ formats presently under discussion, amongst which is the adoption reporting of a Bayesian system similar to that applied in Europe.

Representatives from the University of Canberra, University of Technology, Sydney and the Australian Federal Police formed a fibres and textiles research group in 2013. At the first meeting the gaps and future needs for fibre and textile research in forensic science were discussed and several areas of possible focus were identified. These included, but were not limited to;

- Research on environmental impact on textiles and fibres from macro aspects to ultra-trace analysis
- Understanding background fibre populations and how these may play a more important role for intelligence
- Transfer and persistence especially on footwear
- Improving the evidential value of fibres
- Textile damage

- Improved analytical approaches, including a rethinking about the role of Pyrolysis GC-MS
- Continuing work on Raman spectroscopy for dyes and dye classification

Asia

Lim [9] provided a summary of the Asian Forensic Sciences Network (AFSN), the Asian equivalent of ENFSI which has been running for about 3–4 years, which has a trace evidence working group (TEWG) comprising 36 members from 8 Asian countries and 10 organisations [10]. The aims of this group are to;

- Promote the use of trace evidence
- Develop best practices, quality assurance and guidance documents
- Foster research and development, and collaborations among member institutes and with other networks

The TEWG completed a review "Tracking standards and trends in trace evidence". A summary of this article has been published in the 2nd issue of Forensic Asia (2010). Review focused on: current state of trace evidence, standards and guidelines, evidence interpretation and evaluation, technology and manufacturing trends, as well as education and training.

Within this working group a fibres/textile damage sub-group was formed in November 2012.

Colleagues in the various working groups continue to collaborate and representatives from each group attend the annual meeting of those of the others.

3 Case Reports

As in the 2007- 2010 report [1], there have been numerous instances where fibre evidence has proved crucial in the investigation of complex major inquiries, and/ or added value to other evidence types employed.

Karoly [11] reported on an alleged rape of a young girl after she left a nightclub.

12 blue cotton fibres and 15 black cotton fibres were found on her underpants, which matched with the cotton fibres of the suspect's blue jeans and T-shirt. A further 15 blue cotton fibres and 10 black cotton fibres were recovered from the inside of the victim's trousers also matching the suspect's blue jeans and the suspect's T-shirt. The victim's underpants and trousers had no shedding potential.

These findings were disputed by the suspect who claimed they must have been as a consequence of secondary transfer; "*We only talked for a while and danced together. I never grabbed her or forced her on the floor. I didn't rape her. Matched fibres probably transferred from my hand to her hand by handshake and than from her hands to her underpants."*

The secondary transfer scenario was tested at the laboratory using 40 experiments involving the actual donor garments. None of these experiments resulted in a secondary transfer of fibres near the magnitude of that observed in the results of the casework examination. These findings therefore supported the victim's version of events rather than those of the suspect.

Nehse [12] described a double homicide where fibre evidence complemented that of DNA evidence. DNA evidence was found linking the suspect to one of the victims, and fibre evidence providing a link to the other victim. The case demonstrates the value of a holistic approach to casework examination and evidence recovery in providing effective outcomes.

A case involving the examination of textile fibres recovered during the exhumation of a corpse was described by *Was-Gubala* [13].

The analysis and identification of textiles recovered during the exhumation of a corpse, as well as investigations into the causes of the textile damage, are potentially helpful in determining the circumstances surrounding the death of the individual.

Temperature, moisture and a biological activity of the soil in graves are important factors in the evaluation of the potential degradation of buried textiles fibres over time.

In some cases, degradation of textiles is so minimal, that a comprehensive analysis is constituent fibres is possible. One example of such a case, was the results of the examination of clothing secured during the opening of the sarcophagus of General Wladyslaw Sikorski, the Prime Minister of the Polish government in exile during the World War II.

This was carried out in order to help determine the cause and circumstances of his death. In addition, other multifaceted examinations of

the body were performed: an x-ray computed tomography, medical studies; a determination of the mitochondrial DNA profile; anthropological examinations of the deceased's facial appearance (freehand and computer drawing), detection of organic or inorganic toxins in the organs.

This example illustrated the usefulness and limitations of similar research in the future.

4 Textile/Fibre damage

In the second of a two part study, *Was-Gubala* [14] studied the colour changes in several types of textiles due to the long-term effects of exposure to laundry detergents. A 14-day study was carried out using blue, red, and grey/black cotton, wool, acrylic and polyester textiles.

The spectrophotometric measurement of colour changes in fabric samples and test solutions, as well as the microspectrophotometric analysis of colour changes in single fibres were described. An evaluation of the observed colour changes from a forensic fibre analysis expert's point of view, as well as that of an average user/consumer of the textiles and laundry detergents is also provided.

The results presented from this investigation of the effects of detergent solutions on various textile products can also be used to predict colour changes that may occur when laundering in a domestic situation.

A study on the effect of ionising gamma radiation on natural and synthetic fibres and its implication for forensic examinations was carried out by *Colella et al* [15].

The effect of exposure to 1–1000 kGy radiation doses in natural and synthetic fibres was noticeable using comparative forensic examination methods, such as optical microscopy, microspectrophotometry, and thin-layer chromatography. Fourier transform infrared spectroscopy analysis showed no signs of radiation-induced chemical changes in any of the fibre structures.

The outcome of the comparative methods highlighted the risk of "false negatives" associated in comparing colours of recovered fibres that may have been exposed to unknown radiation doses.

Consideration of such results supports the requirement to know the context, including the environmental conditions, as much as possible before undertaking forensic fibre examinations.

An ongoing study into the effect and identification of unknown chemicals in cases of textile damage was described by *Morison et al* [16].

The aims and goals of this study were to; identify any discriminating visual or analytical features of the damage caused to the fabrics and dyes which may identify the reagent and to identify any traces of the reagent still present on the fabric. A number of corrosive reagents, chosen from casework history as well as ease of retail access to the general public, were dripped onto various textile fabrics and allowed to dry undisturbed.

The fabrics were then sampled at intervals of 30 min, 2 hours, 1 day, 3 days, 1 week, 2 weeks, 1 month, 2 months and 4 months. Visual examinations, microscopy, microspectroscopy, FT-IR, IC, SEM imaging, SEM-EDX were carried out and the damage features for a given reagent/ textile fabric were noted.

This study is continuing, using more reagents, longer exposure times and further instrumental analysis.

Krauss [17] described a number of unusual fibre plastic fusion marks encountered in casework.

The increased use of modern occupant restraint systems in cars (airbag, safety belt tensioners, and safety belt buckle tensioners) has meant that the number of cases involving the investigation of fibre plastic fusion marks decreased considerably within the last years.

Nevertheless, this particular approach to the investigation of road traffic incidents is still highly probative. Some of the more unusual marks encountered in recent casework included;

- Fibre plastic fusing marks on left front car door panelling. The embedded fibre material originated from the driver's seat cover.
- Fibre plastic fusion marks and fabric impressions were found on the seat bench of a motorcycle.
- Smeared acrylic fibres of a jumper were found on the windscreen of a roadster.
- Smeared polypropylene material from the safety belt buckle was found on the safety belt.

5 Significance of evidence

Whilst there can be no doubt that the development of new analytical techniques and methodologies are an extremely important aspect of fibre evidence, even the most sensitive discriminating analytical technique is rendered ineffective if its results cannot be applied to answer specific case work related questions.

Over the last 3 years, the use of a Bayesian approach to casework assessment and interpretation continues to be used in Europe and there are signs that similar approaches are beginning to be employed in the USA and the Antipodes [2,3,6,7].

Over the last 3 years, work has continued to provide data which assists the practitioner (and the courts) in evaluating the results of analysis – particularly when a Bayesian approach is employed.

Much of this work cited in this section has been directly driven through questions arising from operational casework.

Jochem [18] reported preliminary results for the pan-European target fibre study carried out by members of the European fibres group in 2011-12.

This study involved 4 violet coloured target fibres (2 different cottons, 1 rayon and 1 PET). Participants were asked to recover extraneous fibres from different areas/ garments at home, work or public places. The tapings were then searched and any possible matches with the target fibres were recovered and analysed. The results were then sent to the BKA for collation. Reported matches are checked by the BKA.

The preliminary results indicate:

- Routine methods for fibre examination are sufficient for discrimination in the vast majority of cases

- (Still) Highly unlikely to find matching fibres by chance

The results of a fibre population study using cinema seats and cars were reported by *Dufros et al* [19]. The results of their study were compared to that obtained from previous similar studies carried out in a variety of countries and found to be broadly similar.

Coyle et al [20] published the results of a study examining the evidential significance of car seat fibres. Thirty six samples of car seat fabric were

examined and the fibres catalogued according to their morphology and characteristics.

The majority of car seat fibres were black or grey thick polyester fibres that were either dyed or pigmented. The MSP spectra produced were unlike those usually obtained from black or grey polyester fibres used in clothing.

Tapings taken from car seats were examined for car seat fibres, various types were found showing that these fibres are expected to shed from the fabric albeit in low numbers, unless the vehicle is older.

No fibres that matched the samples of the car seat fabric were found on the tapings of the car seats. One hundred garments were examined for car seat fibres, 10% of garments had populations of such fibres present and 41% had at least one car seat fibre present. None of these fibres matched the samples of the car seat fabric or those from the car seat tapings.

Bennett et al [21] published a case study illustrating the importance and significance of fibre transfer in homicide inquiries.

In April, 1995 the body of a young woman was found in a suburb of Sydney, Australia. The body was fully clothed and bore a number of injuries to the neck, face and fingers. There were no signs of sexual assault and she appeared to have been strangled. The only physical evidence located at the scene was a number of dark, coarse fibres adhering to the soles of her shoes.
These fibres consisted of nine grey polypropylene, 12 blue polypropylene and 50 black polyester fibres. The source of these fibres was found to be the carpet of a 1991 Honda CRX that belonged to the suspect. Almost all other possible sources of these fibres were eliminated.

At trial, the source of the fibres was not disputed by the defence. Instead the issue became how long these fibres had persisted on the shoe soles.

A number of experiments were conducted to investigate the factors influencing the transfer and persistence of carpet fibres to shoe soles and the results of these experiments became a critically important part of the prosecution.

Palmer & Polwarth [22] carried out a study to investigate the persistence of fibres on skin in an outdoor deposition crime scene scenario.

Textile fibres were transferred to pig skin carcasses and their persistence determined at daily intervals for up to a 12 day period during which time the carcass was left outdoors exposed to the prevailing weather conditions and animal activity.

In the absence of strong winds and precipitation, the loss of fibres was found to be exponential. Stronger winds and heavier precipitation caused an increase in the rate of loss of fibres.

The results of this study showed that the majority of fibres transferred to a body deposited outdoors, can be expected to be lost after the first 2 days, however, none of the experiments performed resulted in a complete loss of fibres, even after 12 days exposure.

These persistence characteristics differed from those observed in a similar study using small sections of skin, rather than carcasses. The implications of the results of the present study in relation to the examination of fibre evidence in cases of homicide are discussed.

CCTV and other camera surveillance systems are often useful in identifying the perpetrator(s) of a crime by providing details of clothing (colour, construction, labels/ logos etc.) worn at the time of the incident.

A study by *Dillinger* [23] demonstrated that different camera systems, particularly those that operate in the infra-red range, can give misleading information regarding the colour and other details of garments. The study showed that the degree to which a camera system can mis-represent a particular colour depends on the particular dyestuff employed and was particularly evident with certain reactively dyed cotton fabrics.

The results show that where camera surveillance data is being considered for intelligence purposes, caution must be employed when attempting to ascertain the colour and other distinctive features of a perpetrators clothing.

De Wael et al [24] reported on the frequency of an unusual type of polyester fibre encountered in blue denim garments. In a double murder investigation, the victims were found after a prolonged stay in a drainage canal. In spite of the expectations, fibre examination established a multitude of primary and secondary transferred fibres.

One of these fibre types was a colourless polyester fibre possessing a blue coloured molten fibre end. These matched one of the types present in the suspect's blue denim trousers.

The aim of this study was to verify the rarity of this peculiar fibre type and more precisely its presence in blue denim textiles.

Over five hundred different blue jeans textiles were examined and only one of these presented exactly the same type. The comparison involved microscopy, microspectrophotometry in the visible range and Raman spectroscopy.

The results indicated that this fibre type is extremely rare in a blue jeans fabrics and that "standard" blue denim should not be disregarded in case work.

An investigation into the he evidential value of fibres used in ‘high visibility’ work wear was carried out by *Coyle et al* [25].

This study investigated whether the finding of fluorescent fibres, typical of those seen in high visibility (‘Hi-Vis’) work-wear, have any evidential significance.

The study was performed by combining a colour block study (examining a number of samples of ‘Hi-Vis’ work-wear and assessing the extent to which they can be discriminated from each other), a population study (examining tapings taken from the general public to assess the extent to which ‘Hi-Vis’ fibres are present on a person’s clothing at random) and a target fibre study (examining tapings taken from the general public to assess whether there are any fibres present that are microscopically and chemically indistinguishable from an individual sample of ‘Hi-Vis’ clothing).

Two case studies are also presented involving the examination of ‘Hi-Vis’ fibres.

This study concludes that whilst it is possible to discriminate between garments constructed from ‘Hi-Vis’ fabrics, there were instances where significant numbers of samples were found to be indistinguishable from each other.

On that basis, caution is recommended in the interpretation of findings involving transfers from ‘Hi-Vis’ work-wear.

A study by *Szewcow et al* [26] investigated the influence of various factors on the redistribution of extraneous fibres on garments during machine washing.

Cotton T-shirts were seeded with known numbers of acrylic, wool and viscose target fibres in controlled positions and laundered in top-and front-loading machines, both individually and accompanied by undergarments.

The persistence of target fibres was low (generally <10%), but never zero. Between 50% and 100% of recovered fibres were redistributed away from the primary contact area. A secondary transfer of target fibres always occurred to at least one undergarment, 90% of experiments resulting in fibres transferred to the inside surface of the undergarments.

This implies that whilst valuable fibre evidence may be recovered from garments after machine washing, the location/ distribution of recovered

fibres should not be relied upon to corroborate alleged scenarios when it is known or suspected that the garment under investigation has been laundered.

Hellwig [27] investigated the effect of textile construction on the shedding rate of knitwear. As a consequence of issues raised in casework, the purpose of this project was to investigate the influence of basic textile construction characteristics on the shedding rate of acrylic knitwear.

The construction of knitted garments is a very important factor affecting its sheddability. The sheddability of basic knitting constructions (PJ, PR, PC, Interlock, RHCS) is very different from that of complex knitting constructions or ribbed garments.

Other factors that mainly influence the sheddability of knitwear garments are:

- Construction of single yarn or twisted yarn
- Number of stitches per area
- Staple length of single textile fibres

The sheddability of damaged or heavily worn garments is very different from that of new or undamaged garments.

The author points out that the results of this study only represent experiments carried out under defined conditions and only for a few knitting construction types. Nevertheless, it does provide information useful in forming expectations of fibre transfers in casework.

An investigation into the evidential value of fibres on hands is currently being carried out by *Almazrooei et al* [28]. The results of this study (to date) show that it is not uncommon to find fibres on hands and that fibre present tend to be extraneous rather than related to garments being worn by the recipient.

The majority of fibres recovered have been found to be natural, with cotton being the most predominant. Black-grey and blue cottons were the most prevalent fibre type/ colour combinations. Approximately 50% of the man-made fibres were delustred. Approximately 90% of the recovered fibres were 3mm or less in length.

Deviterne-Lapeyre et al [29] presented some preliminary approaches/ data from an ongoing study into the use of chemometric analysis in the forensic discrimination of fibres.

This study is principally concerned with using these statistical tools in an to attempt to remove some of the subjectivity presently inherent in the evaluation and comparison of microspectrophotometry spectra produced in the forensic examination of textile fibres.

6 Instrumental Analysis

Over the past three years many institutions/ organisations have faced cuts or other restraints to their budgets. Consequently the purchase of new equipment or adoption of new methodologies involving a financial implication has more than ever been the subject of cost-benefit analysis scrutiny.

In addition, the economic situation has likely been a driver for making better use of, or extending the scope of existing instrumentation.

The articles cited in this section provide information likely to be of assistance in the above two scenarios.

De Wael et al [30-35] carried out a series of studies into the utility of dichroism measurements in the forensic analysis and comparison of textile fibres;

Part 1- Dyed polyester fibres

One hundred and twenty dyed polyester samples were examined with plane polarized light on their dichroic behaviour by optical light microscopy (OLM) and microspectrophotometry in the visible range (MSP Vis).

It was found that most of these disperse dyed polyester fibres possess a strong dichroism, which fall into two broad categories. Either a decrease of intensity (hypochromic effect) or a change of hue (hypsochromic or bathochromic shift of absorption bands) is noted. These dichroic effects are related to the orientation of the dye structure with respect to the polymer chains.

Part 2 - Dyed polyamide, wool and silk fibres

A number of dyed polyamide, wool and silk samples were examined with plane polarized light on their dichroic behavior by optical light microscopy (OLM) and microspectrophotometry with plane polarized light (MSP-PPL).

It was found that most of these acid dyed peptidic fibres possess dichroism, but these are weaker than the effects previously described for polyester fibres. The small effects may be not observed, especially for wool, but these can be measured using MSP-PPL.

In the three peptidic fibre classes, for the first time, a so called “inverse dichroism” is observed which appears in the absorption spectra as a hyperchromic effect.

Part 3 - Dyed cotton and viscose fibres

A number of dyed cellulosic fibres were examined with plane polarized light on their dichroic behavior by microscopy and microspectrophotometry (MSP-PPL).

Significant dichroic effects (mostly hypochromic effects and hypsochromic bands shifts) were reported. The effect is related to the chemical structure: some dye structures always possess dichroism (azo, stilbene, thiazole and oxazine), some dyes demonstrate sometimes dichroic effects (anthraquinoid, indigoid) while other structures never demonstrate dichroic effects (sulphur, diphenylmethanes, triarylmethanes, phthalocyanines).

In some cases a different dichroic behaviour was found for the same dyes applied on cotton and on viscose.

Part 4 - Dyed acrylic and acetate fibres

A number of dyed acrylic and acetate fibre samples were examined with plane polarized light on their dichroic behaviour by optical light microscopy (OLM) and microspectrophotometry with plane polarized light (MSP-PPL).

It was found that most of these low birefringent fibres possess weak dichroic effects that are often difficult to observe with microscopy. However, using MSP-PPL, the linear dichroism could be measured.

A comparison between the dichroic effects found for the same disperse dyes on triacetate (TrAc), diacetate (Ac), polyester (PES) and polyamide (PA) shows that the linear dichroism follows the order: PA > PES > > TrAc, Ac.

Part 5 - Pigmented fibres

A number of pigmented fibre samples were examined with plane polarized light on their dichroic behaviour by optical light microscopy (OLM) and microspectrophotometry with plane polarized light (MSP-PPL).

It was found that about half of the samples show a strong dichroic effect and another 20% have a weak dichroism. Both regular (80%) and inversed dichroic effects (20%) occur. The dichroic characteristics of pigmented fibres can be compared to these of sheet polarizers.

It is suggested that the dichroic behaviour of pigmented fibres depends strongly on the crystal structure (shape of the pigment grains) and the draw ratio (orientation of the polymer chains).

Part 6 – Validation and Practical aspects

This paper summarizes the results of previous work on the microscopic observation of linear dichroism found in dyed fibres (polyesters, polyamides, wool, silk, cotton, viscose, acrylics and acetates) and in pigmented fibres as well as the measurements on these fibre classes using microspectrophotometry with plane polarized light (MSP-PPL).

The validation of this method is discussed and a practical tool is proposed for comparing fibre traces with control fibres using this method. The limitations and strengths of this method are also discussed.

Research into the application of Raman spectroscopy to the forensic examination of textile fibres has continued since the last review, with members of the European Fibre Group being the most active in this area [36-38]. Despite this research, adoption of this technique into the majority of operational laboratories is still poor, possibly because it has still to demonstrate substantial advantages over the combination of existing techniques and in many respects has been shown to be complimentary;

Massonnet et al [36] carried out a study into the analysis and detection limits of Raman spectroscopy and microspectrophotometry on reactively dyed cotton fibres.

This collaborative study was carried out by members of the ENFSI (European Network of Forensic Science Institutes) European Fibres Group (EFG) on different dyed cotton fabrics. The detection limits of the two methods were tested on two cotton sets with a dye concentration ranging from 0.5 to 0.005% (w/w).

This survey shows that it is possible to detect the presence of dye in fibres with concentrations below that detectable by the traditional methods of light microscopy and microspectrophotometry (MSP). The MSP detection limit for the dyes used in this study was found to be a concentration of 0.5% (w/w). At this concentration, the fibres appear colourless with light microscopy.

Raman spectroscopy clearly shows a higher potential to detect concentrations of dyes as low as 0.05% for the yellow dye RY145 and 0.005% for the blue dye RB221. This detection limit was found to depend both on the chemical composition of the dye itself and on the analytical conditions, particularly the laser wavelength.

Furthermore, analysis of binary mixtures of dyes showed that while the minor dye was detected at 1.5% (w/w) (30% of the total dye concentration) using microspectrophotometry, it was detected at a level as low as 0.05% (w/w) (10% of the total dye concentration) using Raman spectroscopy.

This work also highlights the importance of a flexible Raman instrument equipped with several lasers at different wavelengths for the analysis of dyed fibres. The operator and the set up of the analytical conditions are also of prime importance in order to obtain high quality spectra. Changing the laser wavelength is important to detect different dyes in a mixture.

A study by*Yu et al* [37] used principal component analysis and analysis of variance to investigate the effect of 'ENTELLAN NEW' on the Raman spectra of textile fibres.

During the forensic examination of textile fibres, fibres are usually mounted on glass slides for visual inspection and identification under the microscope. One method that has the capability to accurately identify single textile fibres without subsequent demounting is Raman microspectroscopy. The effect of the mountant Entellan New on the Raman spectra of fibres was investigated to determine if it is suitable for fibre analysis. Raman spectra of synthetic fibres mounted in three different ways were collected and subjected to multivariate analysis.

Principal component analysis score plots revealed that while spectra from different fibre classes formed distinct groups, fibres of the same class formed a single group regardless of the mounting method. The spectra of bare fibres and those mounted in Entellan New were found to be statistically indistinguishable by analysis of variance calculations.

These results demonstrate that fibres mounted in Entellan New may be identified directly by Raman microspectroscopy without further sample preparation.

Zieba-Palus et al [38] investigated the use of micro-RAMAN spectroscopy for the analysis of car paints and single textile fibres.

The aim of the study was to determine the degree of discrimination between fibres coloured by defined chemical dye classes and to differentiate between paint samples on the basis of pigment/dye content.

Samples of coloured cotton fibres and samples of green car paints were examined. It was found that the majority of the obtained Raman spectra provided information about the main dyes present in the sample. However, in some cases fluorescence of the samples made dye identification impossible.

Spectral libraries for examined paint samples and single fibres were created in order to facilitate quick recognition of similar forensic traces using this analytical method.

A study by *Lepot* [39] considered the use of Raman spectroscopy in the analysis of dye mixtures on cotton fibres.

Recent work has shown that Raman spectra of dyes depend on the excitation laser wavelength used (resonance effects) and on the scattering ability of the dye molecule itself. Both factors together with fluorescence emission may affect the detection of a dye, especially within a mixture.

In order to obtain a better understanding of their Raman behaviour binary mixtures at various ratios have been prepared using five known dyes showing different scattering and fluorescent abilities. Their spectral features at 514 and 785 nm highlights the complementarity of these two resonant and non-resonant sources and the limitations of the Raman technique in the detection of both major and minor components of a dye mixture.

Other investigations have been performed on binary and ternary known dye mixtures on cotton fibres by Raman spectroscopy and MSP-Vis. The combination of two laser sources leads in most cases to the detection of both or two out of three dye components.

This Raman information reinforces clearly the confidence in MSP-Vis results. Indeed the contribution of the minor dye component is sometimes very small in the MSP spectrum and a visual inspection of the spectra in addition to inhomogeneous dyeing on cotton may result in difficult interpretation.

For these reasons Raman spectroscopy is a very convenient technique to confirm or perhaps clarify MSP results, especially for fibre types with common MSP spectra. Furthermore, MSP-Vis also showed some limitations with very light or very dark coloured fibres whereas Raman spectroscopy could still discriminate between fibre types.

The use of confocal Raman mapping in the analysis of bicomponent fibres was described by *Weimer et al* [40, 41].

Bicomponent fibres, those composed of at least two polymer types, were examined using Confocal Raman mapping to determine chemical composition and cross-sectional shape.

Cross-sections were prepared for the bicomponent fibres of known composition and compared to the Raman results. Confocal Raman mapping provided chemical compositions and indications of cross-sectional shape for bicomponent fibres without any sample preparation.

For an accurate shape determination and/or comparison, however, preparation of a cross-section is still recommended.

Johansson [42] reported on the methods of fibre cross sectioning used at the Swedish National Laboratory of Forensic Science (SKL).

At the SKL, there was a requirement for a cross-sectional method applicable to all fibre types. Two methods suitable for manual sectioning were tested. The acetate sheet method was used for fibres other than acetate and the polyethylene method was used for acetates and other fibres with a melting point over approximately 140^0C.

The methods employed hoped to achieve quality sections with the microtome already available at the laboratory. The acetate and polyethylene embeddings were thus cut perpendicular to the fibres and placed in a special mould standing on double-sided tape, with the cut fibre cross-sectional side down, to further embed the acetate/polyethylene in the liquid plastic Technovit 2000 LC (mono- and difunctional methacrylate). A microtome adapter was put on top. This "double embedding" was left to cure for 10 min in an UV-chamber. Cross-sections (5-10 microns) were made and straightened by being placed on a drop of water on a glass slide and dried in an oven at 60^oC for a few minutes. The sections were then ready to be mounted in mounting media on the glass slide.

It was discovered that the polyethylene embeddings were too soft to stay in the methacrylate embedding. Polypropylene was tested as an alternative and was successfully used to further embed in methacrylate. Good quality sections for acetates and other fibres with a melting point over approximately 170^oC were obtained. As a result, the laboratory now has a combination of methods which, depending on the fibre types and the quality needed in each specific case, give good cross-sections.

Markstrom et al [43] evaluated the use of a liquid crystal tunable filter microspectrophotometer for obtaining visible absorbtion spectra from single textile fibres.

Spectra obtained from this instrument compared well with results from a conventional instrument. Some advantages include very fast and simple sample preparation and easy comparison of multiple fibres at the same time. Advantages over extraction-dependent methods include the fact that it is applicable to extremely small sample size, not susceptible to artefacts induced by variable extraction efficiencies, non-destructive, and much easier. Because an immense amount of information is collected in one experiment, good signal averaging is possible, along with multiple comparisons for each data set.

The addition of a camera, computer, and liquid crystal tunable filter can transform a standard microscope into a microspectrophotometer capable of performing similar work.

A method development for high-sensitivity analysis of acid dyes in nylon fibres was investigated by *Zhou et al* [44] using time-of-flight-secondary ion mass spectrometry.

As a minimally destructive technique for the determination of dyes in finished fibres, it provides an important tool for crime scene and other forensic investigations.

The analytical power and the minimal sample consumption of time-of-flight-secondary ion mass spectrometric (TOF-SIMS) analysis provides the ability to obtain definitive molecular and elemental information relevant to fibre identification, including identification of dyes, from a very small volume of sample. For both fibre surface analysis and, with the aid of cryomicrotomy, fibre cross-section analysis, TOF-SIMS was used to identify various dyes in finished textile fibres. The analysis of C.I. Acid Blue 25 in nylon is presented as a representative example.

The molecular ion of C.I. Acid Blue 25 with lower than 3% on weight-of-fibre (owf) dye loading cannot be identified on dyed nylon surfaces by TOF-SIMS using a Bi(3)(+) primary ion beam. Sputtering with C (60)(+) provided the ability to remove surface contamination as well as at least partially remove Bi-induced damage, resulting in a greatly improved signal-to-noise ratio for the Acid Blue 25 molecular ion.

The use of C(60)(+) for damage removal in a cyclic manner along with Bi for data acquisition provided the ability to unambiguously identify Acid Blue 25 via its molecular ion at a concentration of 0.1% owf from both fibre surfaces and cross sections.

The use of Terahertz Time Domain Spectroscopy for the identification of cellulosic fibres with similar chemical composition was investigated by *Yan* et al [45].
The distinct terahertz spectra of ramie and bamboo fibres were obtained by means of terahertz time-domain spectroscopy. Numerical simulation for glucose based on density functional theory has been performed to interpret the observed THz features theoretically.

The results indicate that the intramolecular motions do make partial contribution to experimental features of cellulosic fibres, but most of the features are attributed to intermolecular modes.

The investigation suggests that THz spectroscopy is a promising candidate for distinguishing bamboo and ramie fibres, and this will open new prospect to identify textile fibres especially those with similar chemical composition.

An Investigation into the provenance of un-dyed spun cotton fibres using multi-isotope profiles and chemometric analysis , was carried out by *Nic Daeid et al* [46].

The analysis of un-dyed spun cotton fibres can be challenging within a forensic science context where discrimination of one fibre from another is of importance.

Conventional microscopic and chemical analysis of these fibres is generally unsuccessful because of their similar morphology.

This study explores the potential of isotope ratio mass spectrometry (IRMS) as a tool for spun cotton fibre analysis in an attempt to reveal any discriminatory information available.

Seven different batches of un-dyed spun cotton fibre from four different countries were analysed. A combination of the hydrogen and oxygen isotopic data facilitated the correct association of the samples, demonstrating, for the first time, the applicability of IRMS to fibre analysis in this way.

Kato et al [47] investigated the discrimination of white cotton fibres through the detection of residual surfactants.

This study evaluated a new method for the discrimination of white cotton fibre by the detection and comparison of residual surfactants from detergents using liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS).

Twenty three brands of powder-type laundry detergents were collected from 10 manufacturers and used for the study. Standard samples of the surfactants contained in the powdered laundry detergents were offered by the manufacturers of raw materials for detergents.

A sample of a washed textile was prepared after washing a T-shirt (cotton 100%) with one of the detergents and drying it. The surfactants in a cotton thread (5 mm in length) taken from the washed T-shirt were extracted into 30 mu l of methanol and analyzed by LC/ESI-MS. Analyses of surfactants by LC/ESI-MS was also performed on a detergent itself after the extraction of surfactants into methanol by mixing 400 mg of detergent and 8 ml of methanol followed by centrifuge and subsequent dilution of supernatant 50 times by volume.

The powder detergents could be classified into 14 groups on the basis of the difference in the combination of 5 surfactants, polyoxyethylene alkyl ether (POE), linear alkylbenzene sulfonate (LAS), Isulfonato fatty acid methyl ester (ISF), alkylsulfuric ester (AS), and fatty acid (FAT).

Residual surfactants in the washed T-shirt could be detected using 5 mm of thread. The patterns of residual surfactants were found to be similar to those of the detergents except the absence of peaks for some surfactants with relatively short alkyl chains. Mass chromatograms of POE's fragment ion measured at m/z 133 and cone voltage of 50 V in the positive mode allowed the simultaneous detection of POEs with a different length of alkyl chain at a higher sensitivity than those obtained by measuring the molecular ion of each POE at cone voltage of 20 V.

Comparison of residual surfactants patterns obtained by the present method was significantly useful for the discrimination of white cotton fibres, which were difficult to differentiate by the morphological characteristics, when the fibres had originated from textiles washed by different detergents.

A method for the microscopic identification and sourcing of ancient Egyptian plant fibres, using longitudinal cross section was described by *Borojevic et al* [48].

The goal of this study was to design a simple and accurate method of identifying archaeological plant fibre sources.
Twenty-two fibre samples from two sets of ancient Egyptian botanical artefacts were examined under both a stereomicroscope and a compound microscope, and compared to a large reference collection and to previously published research.

By examining longitudinal thin sections of the ancient plant specimens, plant fibres from the following species: *Hyphaene thebeica* , *Cyperus papyrus* , *Desmostachya bipinnata* , *Imperata cylindrica* , *Phragmites australis* and *Linum usitatissimum* were identified. The identification of these plant fibres reveals essential information about the materials used for producing ropes, baskets, sandals, mats and fabric.

The results of this study demonstrate the value of longitudinal thin sectioning and light microscopy as a major means of identifying the source material of botanical artefacts, and advance our knowledge of ancient Egyptian plant exploitation as well as the associated technologies involved in constructing these types of artefacts.

The identification of natural fibres using the ‘Herzog effect’ was described by *Hess* [49]. This technique exploits the anisotropic behaviour of ‘S’ and ‘Z’ twist fibres which allows them to be differentiated using a first order

(530nm) plate with polarising microscopy. A 3% solution of sodium hydroxide improves/ enhances the effect making identification easier.

Hiroma et al [50] described the use of laser ablation ICP-MS for the use of discriminating single PET fibres obtained from a variety of car trunk mats.

The purpose of this study was to establish a forensic analytical method for the discrimination of samples of different origins. The analytical conditions of LA-ICP-MS equipped with a 213 nm Nd : YAG laser were optimized to analyze trace elements, such as Cu, Sb, and Ba at ppm levels.

A total of 31 samples produced by 7 car manufactures in Japan were used for analysis. The concentrations of Li, Mg, Al, P, Ca, Ti, Co, Cu, Ge, Nb, Sb, Ta, and Pb were successfully measured from a single fibre sample with a diameter of c. 20 mu m.

The study showed it was possible to discriminate all 31 samples based on the analytical results of a single fibre by LA-ICP-MS combined with those of FT-IR and SEM-EDS. LA-ICP-MS has good analytical sensitivity, and requires a much shorter preparation time and a smaller sample size than any other, conventional element analysis methods.

This study demonstrated for the first time that this method is practical, useful tool for the forensic identification of a single car trunk mat fibres.

7 New Fibres

A summary of the optical characteristics of some ‘new/ modern’ biodegradable fibres now being increasingly used in various end products is given by *Brisko* [51].

Fibres that are termed "eco-friendly" or "biodegradable" by manufacturers are increasingly being used in textile products such as apparel and carpeting to appeal to the ever more environmentally aware public. As such, these modern fibres are expected to begin showing up more often in forensic casework, and it is important that the forensic examiner recognize them.

This study employed polarized light microscopy (PLM) and Fourier transform infrared (FTIR) microspectroscopy to characterize selected fibres of azlon, polylactic acid (PLA), cellulose composites of alginate or chitin, and bamboo (viscose rayon).

Fibre cross-sections, refractive indices, melting points, solubilities, and FTIR measurements were conducted.

Results indicate that the azlons and PLA fibres are easily distinguishable from other textile fibres by their optical and chemical properties. The cellulose composites show only small differences in comparison with other cellulose-based fibres, while bamboo viscose rayon is indistinguishable from normal viscose rayon.

8 The Textile Industry

The present worldwide economic climate means that more than ever, the textile industry is in a state of flux, with availability and price or raw materials, increased costs of labour, transportation etc. influencing the industry. Despite this, all predictions are that the industry will continue to expand. By far the most useful tool in monitoring developments in the textile industry (including the development and/ or emergence of new fibre types) is the internet. To this end, the URL's listed in the reference section [52-68] are useful, but by no means exhaustive.

9 The Future

The present global economic situation has meant that all aspects of forensic science provision (whether in the public or private sector) are likely to become under even greater scrutiny in terms of effectiveness/ delivering value for money. The key to this is in better case assessment as well as a more transparent, robust, context sensitive interpretation reporting of casework results.

The process of logical, evaluative reasoning in the interpretation of forensic evidence needs continued support through the provision of data from basic research into the factors governing the dynamics of a particular evidence type.

Whilst funding for research continues to be an issue in many countries, it needs to be borne in mind that much of the cogent research in the forensic examination of fibres is of low cost, but high value.

International collaboration between the various working groups/ agencies will continue to be crucial in delivering this basic research.

10 Summary

Despite financial and resource constraints, the considerable amount of research and activity relating to the forensic examination of fibres and textile materials over the last three years, continues to demonstrate the dedication of practitioners world-wide, in promoting and progressing knowledge and raising standards in this field.

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Forensic Geology
Review: 2010 to 2012

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Introduction

The objective of this review is to provide publications, presentation abstracts, and other activities since the previous review in 2010 (1). This review is based on articles in academic journals, meeting abstracts, reliable internet resources, academic societies' web pages and publications, police and forensic magazines and publications. The number of cited articles and abstracts was over 200. We have also included many presentation abstracts because this study field is experiencing a rapid change and expansion and we therefore considered that published articles alone cannot cover all of the advances in forensic geology being made. In the 20th century, it was rare to find forensic geology papers and presentations in any media, but now it's much easier to reach them as the numbers increased significantly and as a consequence it is not impossible to cite of them. One of the reasons of this expansion in the development of forensic geology is due to the establishment of professional organizations such as the Geological Society of London Forensic Geoscience Group (FGG) (2) and the International Union of Geological Sciences (IUGS) (3), Initiative on Forensic Geology (IFG) (4). These were established specifically to promote and develop forensic geology throughout the world.

The term 'forensic geology', also known as 'geoforensics' and 'forensic geoscience' is still subjected to some confusion. However, as Ruffell (5) has reviewed these definitions and pointed out, that forensic geology now includes a range of sciences which are related and/or applied to forensic purpose

The boundary between forensic geology and taphonomy is very obscure and it isn't possible to decide if a paper should be included or not in this review because of the increasing number of papers on this issue, for example change of soil property with decomposition of a carcass or what happens to a buried body according to different soil environment (6-21). The relationship between forensic botany or microbial DNA and forensic geology is also useful for soil discrimination and provenancing although the boundary is indistinct. Environmental forensics is another field of forensic science on natural substance drawing attention these years, and some part is related to forensic geology, but their names, i.e. 'environmental forensics' and 'forensic geology' are considered to be categories of different concepts. Because it's impossible to follow all of the related fields of the science, most of the studies of this field were excluded. We would like to note that it was decided whether a paper was to be included or not in this review by authors alone, and NOT by any institutions, groups, or other individuals.

IUGS-IFG and GIN

A meeting on forensic geology was held in London, at Westminster Palace in 2002, by Dr Laurance Donnelly (4). This was followed by a forensic geoscience conference at the Geological Society of London in 2004 (22). The Geological Society of London, Forensic Geoscience Group was established soon after, in 2006 by Dr Donnelly. By 2008, Dr Donnelly then established an international working group on Forensic Geology, as part of the International Union of Geological Sciences (IUGS) Commission for the Geoenvironmental Management (GEM) (23). As part of this group, the Geoforensic International Working Group formed (GIN). The international working group was elevated by IUGS to the status of an 'initiative', and the IUGS Initiative on Forensic Geology, chaired by Dr Donnelly, held its inaugural meeting in Rome on 18-19 September 2011 (3, 4).

The aim of IUGS-IFG are to develop forensic geology internationally and promote its applications. The specific objectives of IUGS-IFG are as follows:

1 Collate and disseminate data and information on forensic geology applied to policing and law enforcement, criminal, environmental and civil investigations.
2 Promote international meetings, seminars, conferences and training.
3 Develop a 'Committee' to act as principal advisers, collaborators and active participants.
4 Develop an international network whereby each 'member' will act as a principal contact in their respective country for the collation and dissemination of information on forensic geology.
5 Collate, make available and where appropriate review any existing documentation and publications in forensic geology.
6 Produce a document endorsed by the Committee to be called A Guide to Forensic Geology.

IUGS-IFG achieves the aim and objectives by various activities. These include for example the delivery of knowledge transfer, training and outreach events throughout the world, by publications and by the provision of information on the IUGS-IFG web site (4).

1 Meetings

1.1 Academic sessions

The numbers of presentations and conferences have increased more rapidly than the previous review period. The success of each event was due to the hard work and commitments of the hosts and organizers. These events have also benefited from the increasing interests in forensic geology

and related sciences. A brief summery of meetings, which included sessions on forensic geology is presented on Table 1 (24-51).

The numbers of presentations are also introduced in this review to illustrate the scope of forensic geology in terms of the techniques, discussions, and internationally growing recognition of the importance of forensic geology. Most of the presentations were presented in English in the previous review, however the number of non-English language has increased significantly.

During the review period, the 3rd and the 4th International Soil Forensic Conferences were held. The 3rd International Soil Forensics Conference was held as "Soil Forensic" session in ASA, CSSA and SSSA 2010 International Annual Meetings (24), and 25 oral and 8 poster presentations were reported. The 4th International Soil Forensics Conference was included in the 6th European Academy of Forensic Science Conference and 13 oral and 18 posters (25) were reported.

The European Geosciences Union General Assembly held in Vienna, Austria in 2011 (26) held a session on "Forensic and Archaeological Provenancing with Light and Heavy Isoscapes" on utilization of isoscape techniques to forensic issues such as estimating origin of unidentified corpse of archaeological material and study of lead isotopes in glass for forensic discrimination.

In 2012, a session on forensic geology was held in Brisbane, Australia as part of the 34th International Geological Congress (IGC), which is the largest international academic meeting of geoscience, in Australia, and 9 oral and 2 poster presentations were reported (27). There was over 100 audience including police officers.

Table 1 List of Conferences which included forensic geology.

Year	Date	Conference and Session Names	Venue
2010	22 - 27 Feb.	AAFS Annual Meetings	Washington, USA
	9 - 16 July	VIII Congresso Nacianal de Geologia	Portugal
	18 Sep.	Earth Science Teachers' Association Conference 43rd Annual Course and Conference	Leicester, UK
	15 -16 Nov.	Annual Meeting of JAFST	Tokyo, Japan
	31 Oct - 3 Nov	GSA Annual Meeting	Colorado, USA
	31 Oct - 4 Nov	ASA, CSSA, SSSA International Annual Meeting, Soil Forensic (Third International Soil Forensics Conference)	Long Beach, USA
	16 Dec.	Environmental and Criminal Forensics. Forensic Geoscience Group Conference	London, UK
		GeoNZ 2010 Conference	New Zealand
2010 - 2011	20 - 21 Dec. & 7 Jan.	Geologia e Botânica Forenses, Workshop	Porto, Portugal
2011	6 - 11 Feb	AXAA Workshop, Conference and Exhibition	Sydney, Australia
	21 - 26 Feb.	AAFS Annual Meetings, Workshop	Chicago, USA
	03 - 08 Apr.	European Geosciences Union General Assembly, Forensic and Archaeological Provenancing with Light and Heavy Isoscapes	Vienna, Austria
	24 – 26 July	International Network of Environmental Forensics Conference	Cambridge, UK
	8 - 11 Aug.	Trace Evidence Symposium: Science, Significance and Impact	Kansas, USA
	14 - 19 Aug.	Goldschmidt Conference	Prague, Czech
	24 - 28 Sep.	Congresso Ibérico de Geoquimica	Castelo Branco, Portugal
	25 - 30 Sep.	The 48th Annual Meeting of The Clay Minerals Society	Lake Tahoe, USA
	9 - 12 Oct.	GSA Annual Meeting	Minneapolis, USA
	16 - 19 Oct.	ASA, CSSA, SSSA International Annual Meeting	San Antonio, USA
	17 - 18 Nov.	Annual Meeting of JAFST	Tokyo, Japan
2012	7 - 10 Feb.	Australian Regolith and Clays Conference	Australia
	20 - 25 Feb	AAFS Annual Meeting	Atlanta, USA
	29 - 30 Mar.	Congresso Investigação Criminal	Coimbra, Portugal
	27 - 29 May	GAC-MAC･AGC-AMC Joint Annual Meeting, Forensic Geology	St. John's, Canada
	5 - 10 Aug.	IGC, Forensic geoscience	Brisbane, Australia
	20 - 24 Aug.	6th European Academy of Forensic Science Conference, the 4th International Soil Forensics Conference	The Hague, The Netherlands
	23 - 27 Sep.	ANZFSS International Symposium on the Forensic Sciences	Hobart, Australia
	4 - 7 Nov.	GSA Annual Meeting, Progress in Forensic Geochemistry	Charlotte, USA
	11 - 12 Nov.	Annual Meeting of JAFST	Tokyo, Japan

American Academy of Forensic Sciences (AAFS) (28) hosted a two-day workshop “What Did You Just Step In?! Use Forensic Soil Examinations to Find Out” (29) and also there were 4 presentations in criminalistics session. The Geological Society of America (GSA) Annual Meeting (30) had ‘Progress in Forensic Geochemistry’ session with 12 presentations. GAC-MAC·AGC-AMC Joint Annual Meeting (31) held Forensic Geology session with 7 presentations.

1.2 Training & Workshops

Forensic geology training was held in Mexico on 19-23 July 2010. The purpose was to demonstrate and draw attention to forensic geology in policing and law enforcement (32).

A workshop on Geologia e Botânica Forenses (33) was held in Portugal in 2010.

“Trace Evidence Symposium: Science, Significance and Impact” co-sponsored by National Institute of Justice and Federal Bureau of Investigation held sessions on Soil and Soil Analysis (34). A session on microscopy was also held in the symposium. The symposium was designed to share information and collaboration among the trace evidence, law enforcement, and legal communities.

An outreach program on forensic geology was organized by IUGS-IFG and the Geological Society of London, as part of the British Science Festival, held in Bradford, UK for public (52).

IGS2012 in Brisbane (27) was followed by an IUGS-IFG and the Australian Federal Police (AFP) organised event which included a two-day training course on search, held at the Queensland Police Training Centre in Brisbane, Australia, on 8-9 August 2012 (53). The training course was delivered to approximately 25 selected delegates comprising forensic and major crime investigators, anthropologists, archaeologists, detectives and forensic scientists. Attendees represented the AFP, Queensland Police, Western Australia Police, Victoria Police, Brazilian Federal Police, Netherlands Police Agency and Japan Criminal Investigation Bureau.

2 Books

Murray (54) revised his text book published in 2003 and produced a second edition, in which some of newly introduced techniques were included. Hiraoka (55) published the first forensic geological reference book in Japan. Bergslien (56) published a textbook with some practical study on simulated case works for college students who had knowledge of only general natural science.

2.1 Articles on overview and outreach

Four books of forensic science included chapters on forensic geology. Murray (57) wrote about overview of soil examination in Forensic Chemistry Handbook. Pirrie and Ruffell (58) described the applications of forensic geology and soils in Forensic Ecology Handbook, in which some related sections such as archaeology, diatoms, palynology and botany were also included.

A section in the publication Encyclopedia of Forensic Sciences was written by Ruffell (59). This included an overview of forensic geoscience. Molina (60) also wrote a chapter on forensic geology in Enciclopedia “Criminalística, Criminología e Investigación”.

Dawson and Hillier (61) provided wide range of information on the studies of forensic geoscience including microscopy, and electrorical/chemical analytical methods of inorganic and organic components, as well as future perspectives of databases utilizing quantitative and digital profiles.

Pringle et al. (62) described the advance of geoscientific techniques of search and detection methods for forensic purposes with case studies. It covered from the identification of search area by geological techniques as well as search dogs, reconnaissance and site investigations to excavation.

Suárez-Ruiz et al. (63) reviewed application of organic petrology and they shared a section to forensic geology describing usefulness of pollen, spore and organic components as indicators for discrimination and identification.

In academic sessions of conferences, overview and brief summary of progress were presented by many scientists (64-70).

Many articles were published on forensic geology and related sciences in magazines for students and teachers, police officers, forensic services, non-forensic geologists/soil scientists, and public (70-86). This included the possibility of geological techniques and knowledge was explained to non-forensic geologists in various occasions.

Donnelly et al. (73) described usefulness of geographic information system (GIS).

Guedes (74) described a brief history and overview of forensic geology, and explained its importance to academic community of Portugal, and Molina (76) described its contributions to Colombian forensic community. Mazhari (77) described the applications of forensic geoscience to Iranian society and suggested two approaches to prepare specialists for implement of forensic geology.

Ruffell et al. (86) provide information of many fake and fraud cases related to geology such as gems, mining, faked fossils, and art fraud.

To utilize forensic geology in policing, Donnelly (71) and Donnelly and Harrison (72) provided fundamental information to recognize its importance to police and law enforcement. Steck-Flynn (81) described importance of soil evidence and proper collection and handling. Bryant and Mildenhall (78) and Bryant and Mildenhall (79) stated the situation of forensic palynology in the United States of America and how useful it is to solve crime with some case examples.

Fitzpatrick (81, 85) presented how forensics could attract students and effective for education.

Donnelly published approximately 15 items on forensic geology, which included peer review papers, conference papers, posters, abstracts, posters and magazine articles. This included a paper on the renaissance in forensic geology and explored the possible reasons for the global increase in interest in forensic geology (87)

3 Analysis for discrimination and provenancing

Studies on various analytical techniques were reported during the review period including unfamiliar techniques in forensic geology such as utilization of high-energy radiation, mid-infrared and Raman spectroscopies. Studies on organic components were increased very much which had been only a few in former reviews. Conventional methods such as color analysis, grain size distributions, and pH measurement were re-evaluated by statistical analysis.

3.1 Analysis of bulk soil

Combination of grain size distribution and pH measurement with Principle Component Analysis (PCA) was presented by Bonelti and Quarino (88).

The applications of micro spectrometer was presented by Guedes et al. (89) and Woods et al. (90).

Quantitative analysis of trace heavy elements in soil by high-energy synchrotron radiation (HE-SR) X-ray fluorescence (XRF) was developed by Bong et al. (91) for forensic purpose. Furuya et al. (92) described details of HE-SR-XRF quantification method and applied to develop a chemical map of soil for forensic purpose. Bong et al. (93) described development of database on heavy mineral and heavy elements using HE-SR-X-ray diffraction (XRD) combined with HE-SR-XRF quantification method developed by Furuya et al. (90). Fitzpatrick et al. (94, 95) and Raven et al. (96) provided information about laboratory and SR XRDs in their presentations. The application of wavelength dispersive spectrometry X-ray mapping was presented by Schwandt (97). Application of nuclear techniques in geochemistry such as neutron activation analysis and X-ray fluorescence was explained by Rodrigues et al. (98). Jantzi and Almirall (99, 100) applied laser-induced breakdown spectroscopy (LIBS) to surface soil elemental analysis. Favorable result comparing to LA-ICP-MS was obtained and discrimination of soil from 2

sites was successful. Application of rare earth element analysis was examined by Rodrigues et al. (101).

Jantzi and Almirall (102) presented on difference of elemental composition between soils of similar lithologies. They also presented inter-laboratory study of bulk soil analysis (103).

Utilization of mid-infrared (IR) spectrometry with chemometric analysis for discrimination of soil was performed by Baron et al. (104), and discrimination based on land-use type was possible. Edwards et al.(105) developed an oxidative sample preparation procedure for near-infrared Raman spectrometry. Soil samples were treated with hydrogen peroxide which gave a good result in enhancement of peaks of both inorganic and organic components. Application of visible near and IR, diffuse reflectance spectroscopy was studied by Kobylinski et al. (106) to fingerprint soil.

Dawson et al. (107, 108) conducted analysis of 16 kinds of polycyclic aromatic hydrocarbon (PAH) by gas-chromatography mass spectrometry (GC-MS) as an indicator to locate the origin of soil. Gas chromatographs showed different patterns according to soil types and vegetation, and they also differed between samples which collected more than the order of square kilometers apart although similarity was found within the order of square meters. Lee et al. (109) examined soil organic matters by thermally assisted hydrolysis and methylation pyrolysis-gas chromatography/mass spectrometry (THM-PyGC/MS). Evaluation of the result was conducted by chemometrics method and 40 soil samples were correctly discriminated with less than 3 mg of samples. Application of liquid chromatography - tandem mass spectrometry (LC-MS/MS) analysis of organic compounds was presented by Hupfer and Wetzel (110), and use of high performance liquid chromatography (HPLC) was studied by McCulloch et al.(111). Morrisson (112) also presented potential of organic component for forensic purpose.

Studies on magnetic susceptibility were presented by Ribeiro et al. (113), Carvalho et al. (114) and Guedes et al. (115). Automated mineralogy applied to beach sand was examined by Pirrie et al. (116). Characterization of sediment was presented by Guedes et al. (117). Ogle (118) described application of carbonate isoscaping for forensic purpose.

3.2 Analysis of mineral grain

Morgan et al. (119) described the usefulness of quartz analysis in forensic geology. Konopinski et al. (120) and Morgan et al. (121) examined atomic force microscopy (AFM) to investigate quartz grain surface. Konopinski et al. (120) described that various empirical measures such as surface roughness, skewness and height distributions could be obtained from analyzing the topography scans, and self similarity of surface texture across the scale was demonstrated. The method of automated quartz grain surface analysis to images obtained by scanning electron microscopy was developed by Newell et al. (122) and nearly 99% of the texture was successfully classified, and the method was applied for forensic purpose (123).

Bailey et al. (124) combined analyses of elemental and surface texture for provenance estimation of quartz grains. Particle-induced X-ray emission (PIXE) and particle-induced *γ*-ray emission (PIGE) were applied for analyses of elements in their study. Surface texture, trace elemental mapping, and chemical composition of inclusion could provide information related to provenance estimation. Dalpe et al. (125) studied trace elements in quartz for developing database.

Dalpé et al. (126) described discrimination of sources of rough diamonds by analysis of trace elements using laser ablation-induced coupled plasma-mass spectrometry (LA-ICP-MS). Results of experiment were statistically analyzed and samples from two different sources were well discriminated. Oliveira et al. (127) introduced the Diamond "DNA" Project of Brazil to determine the origin of uncut diamonds.

Purcell et al. (128) described usefulness of cathodoluminescence (CL), and application of CL to feldspar and calcite was presented by Hasbrouck et al. (129). Brokus et al (130, 131) and Buscaglia (132) also studied luminescence of feldspars and calcite for forensic purpose.

Examination of volcanic glass was performed by Schneck (133). MicroRaman spectroscopy to identify small mineral grains was studied by Harris (134) and Mamedov and Darling (135). Source attribution of material type including geological origin was studied by Stoney and Stoney (136).

Differentiation techniques under the microscopes of asbestos were described by Solebello (137), Solebello and Tomaino (138) and Van Orden et al. (139).

3.3 DNA and botanical matters in soil

Papers on DNA analysis were constantly published following to the previous two review periods. All the papers suggested need of further investigations to understand the nature before applying bacterial DNA examination to case work although favorable results were provided. Botanical matters in soil were also studied not only by macro- and microscopic observations but also by biomolecular and isotopic methods.

Use of biomolecular analysis as complement technique to conventional soil examination was described by Macdonald et al. (140). Lenz and Foran (141) applied terminal restriction fragment length polymorphism (T-RFLP) method to nitrogen fixing bacteria DNA for discrimination of soil. A stable result was obtained through one-year period and discrimination using samples from the vicinity of control sites gave a good result. Macdonald et al. (142) examined potential of bacterial and fungal DNA profile by T-RFLP to discriminate similar land use and/of geographic location. DNA profiles of microbial communities were different among the locations, and patch discrimination was also evident within several sites, which would be useful for site-specific matching. In the examination of Quaak and Kuiper (143), distribution of distances between DNA profiles obtained by T-RFLP analysis of bacterial DNA in soil samples

were calculated using several estimation methods, and Bray-Curtis distances could discriminate better than others. Moreno et al. (144) analyzed bacterial DNA which had extracted from control and grave soil using length heterogeneity polymerase chain reaction (PCR). Difference was found in microbes especially existence and amount of anaerobic and nitrogen fixing bacteria between control and grave soils. It was considered to be caused by decomposition of cadavers. Application of Rhizobial profiling was examined by Smith and Foran (145). DNA barcoding and next generation sequencing for forensic soil examination was presented by Young et al. (146). Handling and storage of soil for DNA analysis was studied by Larson et al. (147, 148).

Geobotanical characterization of a river beach was presented by Carvalho et al. (149). Hawksworth et al. (150-152) described application of fungal study to forensic examination. Application of palynology was presented by Weber(153), Adekanmbi and Ogundipe (154), and Mildenhall and Wiltshire (155). There were also studies of biomolecular and isotopic analyses on input of plants to sediments in relation to environmental forensics (156-159).

4 Search for burials

The numbers of publications in search increased significantly in the period 2010-2012. The applications of geology to search has taken place since the 1990s, when Dr Laurance Donnelly, applied geological methods to search for burials in the United Kingdom. As a result, this work has significantly advanced police and law enforcement ground searches for graves and other buried items. Donnelly, and Donnelly and Harrison published a number of papers and articles on search, some of which have been reviewed in the previous report (1). These included the development of the conceptual geological model, the determination of ground search strategies and methodologies, determination of ground diggability, the usage of the Red-Amber-Green (RAG) prioritization maps and the determination of search assets that include geological mapping and observations, remote sensing, geochemistry, soil and groundwater analysis, deployment of victim recovery dogs (VRD) and geophysical surveys (160).

Mackinnon and Harrison (161) described interdisciplinary approach to this issue in UK.

Detection of metallic materials was examined using different equipments, and the choice of appropriate method suitable to the target was considered to be important. Rezos et al. (162) has tested a basic all-metal detector to buried firearms and weapons of various kinds. It was suitable to detect metallic weapons in relatively shallow depth, but considered to have advantage to other geophysical equipments such as ground-penetrating radar (GPR) because of its easiness of handling and equipping. Marchtti and Settimi (163) examined three geophysical methods, namely magnetometric survey, electrical resistivity tomography (ERT), and multifrequency frequency-domain electromagnetic (FDEM) induction survey. ERT could mainly detect the change of terrain by digging, and combination of magnetometry and FDEM

survey could detect actual steel drums buried as items for search experiment. Dionne et al. (164) presented a paper on controlled search of buried metallic weapons by a conductivity meter, and suggested a guideline for the use of conductivity meter by comparison with another study of the same site using different equipments (165).

Schultz and Martin (166) performed experiments on detecting a grave containing a pig carcass using 250MHz and 500MHz antennas of GPR. Both frequency antennas could detect graves with and without a pig carcass. From the pig carcass grave, reflection originated the pig trunk was obtained but only poor reflection was detected from the control grave. In this study, 500MHz antenna provided more detailed image of the grave and suggested to use 500 than 250 to investigate the similar soil type but they also noted when the soil with stumps, roots, and cobbles to be investigated it would provide too much reflection because of its high resolution. Solla et al. (167) tested GPR to obtain information for crime scene investigation using various items which were likely to involve in crime. As the result, relatively large sized items were easily found but items with small size were not. They suggested conditions of antenna, spatial resolution, and modeling for better results. Pringle et al. (168) collected data for three years by bulk ground resistivity, electrical resistivity imaging, multifrequency GPR, and 'soil-water' conductivity of clandestine grave in which a naked pig and a pig wrapped with plastic sheet located. As the result, resistivity and GPR were considered optimal methods if wrapping was unknown in the season of winter to spring. Other methods should be added depending on the condition of the site. Application of GPR was also studied by Fletcher et al. (169), Hawkins et al. (170), Barone et al. (171) and Forbes (172).

Pringle et al. (173) studied on search methodologies and examined GPR, electrical resistivity and magnetic susceptibility for the simulated clandestine grave in coastal beach environments. Lovestead and Bruno (174, 175) applied organic chemistry for the search of burial site. Head-space sampling method with porous layer open tubular (PLOT) columns was examined to detect ninhydrin-reactive nitrogen gas (NRN) produced by decomposition of cadaver from soil of a simulated clandestine grave using rats. They could successfully detect NRN, and described the quantity of gas recovered from the top soil as a function of time.

Application of reflectance measurement to differentiate gravesoil to fertilization treatment was presented by Herzog and Kalacska (176). Relationship of vegetation and clandestine grave was examined by Caccianiga et al. (177) in which vegetation dynamics was used as a tool for forensic search. Kalacska et al. (178) studied remote sensing to detect clandestine grave.

5 Database

Guedes et al. (179) applied multidisciplinary approach to fingerprint a site in a region using a set of data obtained by analyses of color, particle size,

magnetic susceptibility, pollen and statistical analysis combined all the results. Scheunemann et al. (180) and Guedes et al. (181) also presented development of soil database for forensic purpose. Studies of Bong et al. (93, 182-184) were parts of database establishment by HR-SE XRD and XRF in Japan. Dalpe et al. (125) presented on preliminary development of database of race elements in quartz.

6 Transfer and recovery

Morgan et al. (185) performed experiments to investigate the processes of reincorporation and redistribution of geoforensic particulates on clothing. They used UV particulate as simulated trace particles of geological origin and pollen.

Extraction method of diatoms from clothing was examined by Uitdehaag et al. (186).

Ruffell and Sandiford (187) described sampling of a small amount of soil from shoes and socks. The amount of soil adhered to a sock was very small but successfully recovered by suspending, vibrating and centrifuging in a tube with de-ionized water for comparison.

Bowen (188) presented about differential transfer and persistence, and Mckinley et al. (189) described on spatial sampling approaches in forensic geosciences.

7 Case report

Concheri et al. (190) combined chemical elemental analysis using ICP-MS and ICP-OES, and DNA for the discrimination between evidence and control samples in a murder case. As results of cluster analyses of two different methods, evidence soil was the most similar to the sample collected from the scene of crime.

A case of robbery and kidnap in which soil was investigated was presented by Ruffell and Sandiford (187). Soil samples taken from shoes and socks were discriminated to scene of crime.

There were two reports about case works utilizing geophysical methods to the search of clandestine graves. Pringle and Jervis (191) reported a case using electrical resistivity for a recent clandestine burial of a homicide, in which search by conventional method such as cadaver dogs had been unsuccessful. Anomalies were found as candidates for further investigation. Victim was found from out of the searched area later. Novo et al. (192) tested three-dimensional (3D) GPR in mountainous environment, and applied the condition obtained to the search of a clandestine grave. They could obtain a good 3D map by 250MHz and found several anomalies as candidates of the burial site.

A case work in Colombia was introduced by Gallego (193). A case, in which study of geomorphological and fluvial dynamics had been useful to a thirty-year old cold case, was presented by Scoles (194). Isoscaping technique was applied to a cold case of murder by Kamenov et al. (195). Two cases were introduced in which white mica was utilized as trace evidence by Hanna and Bradley (196). A case of murder in which sand as aggregate in cement had utilized for discrimination was introduced by Hashimoto (197).

Clark (198) reported a case of physical matching had been effective. Toolmark on soil clod was compared to a mattock and successfully identified

In 'Methodological Proposals for Documenting and Searching for Missing Persons in Colombia', articles on applications of hydrology, archaeology and remote sensing were included (199-201).

Case works were also presented by Di Maggio and Nuccetelli (202), Fitzpatrick et al. (203), Uitdehaag et al. (204) and Vinayak et al. (205).

8 Miscellaneous

Ruffell (5) discussed definition of terminology related to forensic geology, i.e. forensic geology, forensic pedology, geoforensics, forensic geosciences, and soil forensics.

Bowen (206) discussed a general introduction of foraminifera, whose various shapes of walls are identical each other and sometimes included as microfossil in soil with several case examples. Importance of microscopy was presented by Bowen (207) and Palenik (208). Topics on urban soil were presented by Isphording (209), and Ruffell and Pirrie (210).

Application of age assessment method of ivory using 14C and 96Sr was introduced Schmied et al. (211). Discrimination between bioapatite and geoapatite was described by Bergslien (212, 213). Use of man-made materials found in soil was presented by Schneck (214).

Importance of selection of suitable methods to present data was described by Miskelly et al. (215) and application of Bayesian interpretation was presented by Sandiford and Powell (216).

Situation of soil forensics in Russia was described by Nesterina et al. (217). Need and importance of guideline of forensic geology was discussed by Fitzpatrick and Raven (218) and Gradusova et al. (66). Guidelines for forensic investigations was published from Centre for Australian Forensic Soil Science by Fitzpatrick and Raven (219).

Scientific Working Group for Geological Materials (SWGGEO) chaired by Bill Schneck was started in 2011 (220, 221).

9 Conclusion

As the authors mentioned in introduction that the development of forensic geology is very fast, and this situation will continue. But geological setting, availability of equipments and/or database in each nation are different, and therefore optimization of developed techniques and knowledge to the region is also very important to utilize forensic geology effectively for criminal investigation. It obviously requires more attention on it from public and law enforcement to achieve this in every nation.

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FORENSIC CHEMISTRY

Fire Cause Investigation And Fire Debris Analysis

Review: 2010 to 2013

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1 Introduction

Investigating a large fire scene resulting from a severe fire is often difficult, and there is a pressure to define the nature of the fire as soon as possible provide a course to the criminal investigation. Fire cause analysis has traditionally been conducted in the context of fire scene investigation by defining technical and structural errors and faults, photographing and taking chemical and technical samples for laboratory analysis. It is very important to establish the area of origin of the fire correctly. For example, accelerant detection canines are used in many countries for indicating or corroborating the areas for fire debris sampling. Studies concerning changes on painted surfaces caused by fire may also be of assistance in detecting the seat of fire.

The forensic nature of the fire needs to be established quickly, if there are e.g. human remains found on the scene. Autopsy results often provide important information as they may explain the progress of the fire. Similarly, systematic and scientific evaluation of eyewitness statements may provide valuable intelligence-information for criminal investigators. A suspected arsonist may be linked to the fire scene by damage to the person's clothes caused by fire. In respect to technical fires, new energy forms and technical development in general pose new kind of challenges to fire scene investigation and fire cause analysis. This type of fire is often examined using reconstructions and more and more often, with computational modelling. It is often used also in examining vehicle fires, such as car fires and train fires.

In addition to defining the correct spot for sampling, proper sampling tools are also needed. Various kinds of packaging materials have been developed for sampling, and properties of these materials have been compared. Taking samples from the suspected arsonist's hands, in particular, is about to become an important method in fire cause analysis. Pre-treatment of samples in the laboratory is mainly conducted by using standard methods, but there are also new ideas developed for the purpose. GC/MS technology with various applications has become significantly common in analyses, and the results are more and more often interpreted using various statistical analysis methods. For example, statistical analyses have been used to identify residues of ignitable liquids as well as for classification and definition of the source of these liquids.

Bio fuels have become more and more common, and this is also reflected on fire debris analysis. New generation bio fuels may be compounds similar to traditional fuels, so identifying their source is often difficult. New approaches have been developed for handling problems caused by the background matrix of a fire debris sample, and the changes in ignitable liquids caused by weathering and microbial degradation have proved to be an interesting and useful research topic.

In some cases, deliberately set fire may be motivated by suicide or homicide. Investigation of this kind of fire may not always be straightforward, as comprehensive analyses and thorough evaluation of the evidence material is required for identifying the true motive. When examining fire-setting

behaviour, a lot of attention has been paid in personal characteristics of juvenile fire-setters. Differences between the sexes as well as personal differences caused both mental health problems and physical injuries in fire-setting behaviour have been studied as well. Several studies have been made on fire-setting behaviour, too.

International cooperation between fire cause analysts has been busy with several conferences during the period of past three years. In these congregations, many lectures were given and posters presented. Various expert groups exchanged new information in the field and examiners from many countries were able to share their experiences within the science community. Different kinds of proficiency tests were found as specifically important means of self-assessment.

2 Fire Scene Examination

2.1 Site examination techniques

The primary aim in forensic fire scene investigation is to establish the origin and the cause of the fire(1). However, it has been suggested that information arising from these investigations would be useful in mapping risks of fires and in general, in raising public knowledge about fires. Collecting such collated information may well require new methods and tools. Experiences gained by fire scene investigators may help in observing certain fire-related risk factors. Identification of such risks could also be beneficial for fire scene investigators in searching for common factors in the cases under investigation.

Eyewitness statements are often very important in fire scene investigations. Therefore, systematic analysis of this type of material has been developed(2). The scientific method recommended by the NFPA 921 has been applied into this purpose. Versatile and extensive material consisting of witness statements was utilised in the study, and on the basis of the material, applying the method into analysing the correctness of the hypotheses relating to the origin of fire could be analysed. Questions used in the study were summarized and focused on the layouts of the fire scenes. The advantage of the method was the scientists were able to note trends in the witness statements extensively and no information evaluated as insignificant was left out. The method also gave a clear understanding how the fire had progressed, even with no eyewitnesses.

Clothes of an arsonist suspect may be searched not only for ignitable liquid residues, but for further evidence as well(3). Evaporation of ignitable liquid causes vapours that mix with the surrounding air. At the start of a fire, these vapours form a flame front that flashes through the vapour. A flash burning may cause superficial heat damages on the surrounding materials. On this basis, microscopically small heat damages in the clothes may indicate that the person wearing them was present at the start of the fire. An ability to identify damages caused by combustion of ignitable liquids, in particular, is needed for obtaining such evidence. This type of evidence has been successfully used in the United Kingdom already.

Establishing the seat of fire is an important part of fire scene investigation. Studies focusing on paints can be utilised for this purpose(4). There is often paint on various surfaces on the fire scene. The effects of fire and high temperatures on various kinds of paints was studied using attenuated total reflectance Fourier transform infrared, Raman, X-ray fluorescence spectroscopy and powder X-ray diffraction. The study aimed to assess the temperature affecting the paint on the basis of spectral changes. The paints included in the study were car paints, metallic paints, matt emulsion and clay paint. So called temperature markers i.e. changes in the consistency of the paints caused by certain temperatures were observed.

When investigating a new fire scene, it is useful for the investigation to find out in good time advance whether or not the nature of the investigation is forensic, as it defines the further progress of action in the case(5). For example, human remains may indicate that it is a question of a forensic fire scene. However, detecting human remains, e.g. severely burnt bones, may be difficult. A special method utilising a YAG laser has been developed and tested for the purpose. In the study, a fluorescence yttrium garnet laser was used to take photographs of severely burned pig bones. Next, it was studied how the burnt meat in the bones affects the observing fluorescence of the burnt bone. In both cases detecting burnt bones was possible, and the detection was clear.

Accelerant detection canines (ADC) are of valuable assistance for fire scene investigators(6). Ignitable liquid residues have been detected with the help of these dogs for a long time. However, their findings are feasible only after they have been verified by using laboratory methods. This gave a ground for a study in which accelerant detection dogs' sensitivity for detection was compared to the sensitivity of the gas chromatography-mass spectrometric (GC/MS) analysis method. There were two Labrador retrievers in the study tasked to find residues as small as possible of five different ignitable liquids, and the same samples were also analysed using the GC/MS. The ignitable liquids used in the study were methylated spirits, thinner, E10 petrol, kerosene and mineral turpentine. Small amounts of these liquids were injected in a rug that was used as background material. The outcome of the study was that the both ways of detection i.e. the dogs and GC/MS were effective ways to detect ignitable liquids, and that they also gave very similar results.

Examining volatile hydrocarbons in the blood of a victim who died in a fire may reveal information on the fire and on the circumstances relating to the victim's death(7). Concentrations of e.g. benzene, styrene and toluene in the victim's blood were monitored quantitatively, and on the basis of these concentrations, it was possible to infer what the person has inhaled at the time of his/her death. For example, a high concentration of toluene in the victim's blood may be an indication of inhaled petroleum vapour. Concentrations of hydrocarbon were assessed together with e.g. concentrations of carboxyhemoglobin (CO-Hb) in blood and the amount of ash in respiratory ducts. Another study focused on establishing whether residues of petrol may be detected from the victim's heart blood and lung tissue(8). Questions such as are this type of samples suitable for analysis and whether samples can be collected in the

context of autopsy without a risk of contamination were assessed. The conclusion was that lung tissue and heart blood were suitable laboratory samples for detecting ignitable liquids and thus, for fire scene investigation.

In addition to detecting ignitable liquid residues, analysing the soot produced by the fire could be useful(9). Zhi et al. studied various kinds of soot from ignitable liquids. On the basis of consistency of the soot, they were able to establish the ignitable liquid which the soot was from. They studied soot from diesel and petrol, and found clear differences in their consistencies. However, it was also noted that circumstances relating to the fire had significant impact on the consistency of the soot.

2.2 Sampling and sample packaging

There are many alternatives available for packing fire debris samples. Properties of three commercially available fire debris bags have been assessed and compared. For example, permeability, durability and background interferences of new AMPAC bags were compared to FireDebrisPAK® bags that have been considered previously to have good properties(10). The results showed that the compared properties were similar due to the similar materials. Both bags had the advantage that they were impermeable for the studied compounds. Weakness of the both bags was that they should not be exposed to temperatures above 80°C. Four commercial alternatives for fire debris bags (DUO, ALU, AMPAC and NYLON) were compared to each other in another study(11). The composition of the polymer bags and their properties important for fire scene investigation were analysed. The NYLON bag was found susceptible e.g. for cross-contamination and leakage whereas the ALU bags had some volatile compounds disturbing the analysis. The AMPAC bag was found the most feasible for preserving fire debris samples in respect to several properties. In respect to analysing ignitable liquids, the problem with polymer bags was their sorbtive capacity of the polymeric layers(12). This generally known phenomenon was studied using three different fire debris bags, namely AMPAC, NYLON-11 and Duogasbags. The result was that the sorbtive capacity of all the bags was poor, which should be taken into consideration when selecting the bag material and analytical circumstances for fibre debris analysis.

Collecting ignitable liquid residues from the suspect's hand would be very useful in arson investigations. Sampling such residues is difficult, but there are studies introducing a method to collect the samples at the fire scenes or at the police stations for laboratory analysis(13,14). After adding a small amount of petrol in a person's hands, the petrol was detected using this method even after three hours. The introduced method utilises passive adsorbtion (activated charcoal strips). The suspect's hands were warmed up by keeping them in a fire debris bag for a suitable time at the temperature of 45°C. The method has been tested in some police stations in Israel. In another study, white absorbent material used in cleaning chemical leaks (PIG ® Oil-Only Mat, Product MAT 423, New Pig Corporation, One Pork Avenue, Tipton, PA, USA) was tested(15). The material was compared to cotton pads used in collecting ignitable liquid residues from different surfaces. This white

absorbent material was found to be more efficient for collection of ignitable liquid residues from surfaces than cotton. Based on the result, a prototype field test kit for collection of ignitable liquid residues from hands was developed. Detection of ignitable liquid residues in hands may have a crucial role in fire scene investigation, but there is no generally approved method for sampling yet(16). Evidential value of this kind of sampling was researched and evaluated. At the same time, a need for developing a generally approved method was emphasised.

Sometimes it is not possible to bring fire debris samples to the laboratory, so ignitable liquid has to be extracted at the fire scene(17). In this respect, four different methods of extracting ignitable liquid from a concrete surface were experimented. The extraction was made using cotton pads, absorbent matting and cat litter. Each of these conditions required an additional extraction in the laboratory. In respect to the fourth technique experimented, the extraction procedure took place at the crime scene and the sample was then ready for analysis. The method in question is called Passive Headspace Residue Extraction Device (P.H.R.E.D.). The results of this experiment indicated that cat litter and the P.H.R.E.D. are capable of extracting ignitable liquid residues from a concrete surface one hour after a fire has self-extinguished. In respect to sensitivity, P.H.R.E.D. was found to be more sensitive than the cat litter.

3 Vehicle fires

Road traffic accidents often result in motor vehicle collision fires(18). The association between collisions and fires was analysed and their general characteristic researched in a study in which data was derived from the Kentucky State Police Records State for 2000-2009. Collisions involving vehicles of different types and different ages were compared with each other, and the results showed that large vehicles were at a higher risk for a collision involving a fire. Also the vehicle’s age (> 6 years) was a linking factor, collisions were often caused by a single vehicle.

Motor vehicle fires often involve serious injuries and deaths(19). For example, nineteen persons died in a fire resulting from a collision of a bus and a lorry, and burning was so extensive and heavy that identification of the victims was very difficult. Various kinds of vehicle fires have been reconstructed and numerically simulated in order to increase passenger safety and to understand how the fires progress. For example, cars have been fire-tested and especially computational modelling of vehicle fires has become popular in recent years(20). These methods are particularly useful in investigating fires in parking lot and road tunnels, as reconstructing such fires is difficult and expensive(21, 22). In addition to motor vehicle fires, also train fires were with numerical simulation to gain understanding on both accidental fires and arsons(23, 24).

4 Technical fires

Electrical appliances may initiate fire in various ways. Circumstances relating to this type of fire were studied and various possible reasons for them were established(25). In this study, e.g. hazardous conditions caused by arcing faults or overheating of electrical connections were researched. The results showed that in hazardous conditions, material used for wire insulation often ignite first. PVC containing e.g. PVC resins and plasticizers is commonly used as insulation material. According to the study, risks relating to the decomposition of wire insulation caused by overheating included the production of igniting gas and charcoal. Due to the ignition sensitivity of PVC, properties of wire insulation containing PVC were studied in overload and fire conditions using thermal analysis(26). The method was found feasible, as when using thermal analysis, different properties were found for inner and outer layers of insulation in different conditions. With this method, it might be possible to deepen our knowledge on wire insulation and thus, develop fire scene investigation methodology further.

In addition to examining wire insulation, studying the electrical distribution wires is one of the methods used in establishing the cause of fire. For this aim, melting of electrical distribution wires in the context of an electrical appliance fire was studied(27). Melted distribution wires were subjected to composition and microstructure analyses using x-ray photoelectron spectroscopy (XPS), video microscopes and optical microscopes. Comprehensive analysis showed that plain visual examination was not always the most accurate way to establish what role the melted wire had had in the fire. The methods were used to establish the consistency of the melted wire in more detail and based on that information, it was possible to conclude whether the fire was caused by the melted marks or whether they were just caused by the flames. In another study, copper molten marks were analysed using optical microscopy (OM) and atomic force microscopy (AFM)(28) whereas molten marks are usually examined by OM and electron microscopy (SEM). The reason for experimenting AFM was that with the method, 3D images even of very small microstructures may be taken. The results showed that AFM suits well for analysing copper molten marks due its particularly good resolution.

An attention was paid on detecting electrical fires and circumstances relating to fire scene investigation especially by emphasising the proper way of sampling and documentation(29). Phases of proper investigation in electrical fires are iterated in these guidelines: comprehensive documenting, interviewing, photographing, sampling and reconstructing fire scenes. Also, typical samples from electrical appliances and their correct interpretations are introduced. These include e.g. phenomena relating to electrical conductors melting, arcing, eutectic and chemical degradation. Laboratory methods used in examining electrical appliances, e.g. microscopy and X-ray are explained.

Electrical fires take place in industrial facilities, too, and one of the likely places where a fire may start is the electrical cabinet(30). Investigation of such fires has been scarce up to now and it has concerned almost exclusively the

nuclear industry. The Institut de Radioprotection et de Sûreté Nucléaire (IRSN) conducted fire tests involving electrical cabinets, and investigated phenomena relating to their burning under a calmetric hood. The experiments showed e.g. that the size of the vents of the electrical cabinets had a great significance to the way the fire spreads. A switchboard damaged in an industrial fire in Taiwan in 2006 was analysed in the context of the fire scene investigation(31). A carbonized steel plate and an electrical wire with a molten bead were found in the switchboard. Metallographic analysis showed that the fire had initiated from a short circuit. The fire was also computationally modelled. It showed that the polyethylene insulator of the electrical cable had allowed the fire to spread fast.

Another kind of industrial has also been researched. There was a fire in a hydroelectric power plant which initiated in the transformer room with transformer oil as ignitable material(32). The study is a numerical modelling of a major lethal accident in Taiwan with a purpose to establish employees' possibilities to escape the facilities at the start of the fire. A major part of industrial accidents is due to technical problems or human errors. However, industrial accidents may be triggered by external factors such as deliberate human action or acts of god(33). Lightning strikes are the most frequent cause of major accidents triggered by natural events, and therefore such accidents have been studied with an intention to analyse various kinds of typical accidents. The study introduces various accidents caused by lightning and with the help of them, identifies equipment vulnerable to lightning and failure dynamics. For example, various kinds of storage tanks for liquid fuels, were found very vulnerable to the lightning damage.

Fires caused by lithium ion batteries serve as an example for another kind of electrical fire(34). As new energy forms such as solar energy and wind energy or for example electronic vehicles become more common, storage of the relating energy must be considered from the point of fire safety. Lithium ion batteries are good for storing energy, so the related risks have been studied using simulations and reconstructions. The safety problem of lithium ion battery is mainly contributed by thermal runaway caused fire and explosion, and the study in hand introduces the related chemical reactions, thermal models, simulations and reconstructions.

Broken mobile phones have been studied by subjecting them to microwaves(35). The study focuses in analysing damage patterns caused by microwaves and accurate causes for fire. Acknowledging these patterns is useful when deliberate breaking of mobile phones for financial interest is investigated. A man claimed in the media that his new mobile phone had exploded while being charged(36). In this study, combustion of the mobile phone was studied using microwave.

Electrostatic discharge may cause fire e.g. when fine-grained material ignites(37). For example, there was a fire in a dust filter of a secondary pharmaceutical powder-transfer operation. The cause of the fire was traced back to a faulty design, because had there been a suitable filter in use, the electrostatic discharge would not have ignited the powder.

5 Fatal fires

In respect to fatal fires, an attention has been paid to suicides committed by burning. It is a relatively rare form of suicide in Western cultures and we do not know much yet about its characteristics or people who commit such act.(38). Death records of the King County Medical Examiner's Office in Washington State over thirteen years were analysed to address these questions. Twenty-five cases of suicide by burning were identified, and they were used to characterize decedent demographics, motivating factors and circumstances of death. Suicide by burning demonstrated a significant over-representation of certain factors relating to descendants' age, sex and background. The act often occurred at the decedent's home, but no unifying theme in motivating factors was found.

Autopsy data was utilised in analysing epidemiological and injury characteristics in criminal and suicidal immolation cases(39). The study focuses in finding characteristics of injuries relating to criminal immolations on one hand, and to suicidal immolations on the other hand. There were twelve autopsy reports used as material for the study covering a period of 18 years. An important outcome was that a classic indication of fire as the cause of death, i.e. high blood CO-Hb level, was not a self-evident indicator, as it was not measured in some cases, although the cause of death was known to have been fire.

Another study focuses on establishing the existence of warning signs predicting self-immolation(40). The study compares warning signs prior to suicide attempt by self-immolation versus suicide attempt by self-poisoning. In the interviews, both demographic and psycho-social warning signs were identified. The comparison showed that warning signs for suicide by self-immolation differed from warning signs for suicide by self-poisoning. There were also less warning signs for suicide by self-immolation, and it was assumed that prevention of this type of suicide is more difficult. Byard et al. introduce a few with homicide connected to suicide(41). Arson may be committed not only for suicidal purposes, but to commit homicide or to cover a murder. The authors claim that finding a possible arsonist dead in the fire scene does not always indicate suicide. It may be a question of arson committed to cover a murder in which the arsonist accidentally died. It is also possible in cases where petrol was used to ignite the fire. Expansion of fire ignited with petrol may be unexpectedly heavy.

A case study of a similar crime scene investigation in Rajasthan, India, was made to establish whether a fire was a homicide or suicide(42). The study emphasizes the importance of reconstruction in investigation in this type of offences. The researchers found reconstruction very useful if started on the preliminary stage of the investigation and continued during the investigation. Reconstructions may be used to estimate e.g. reliability of statements given by eyewitnesses, victims and suspects.

Post-mortem burning may have been committed to cover up a murder(43). The issue is investigated in a study focusing on characteristics of thirteen

murders. All these cases involved post-mortem burning after suspected homicide. The cases were analysed with regard to e.g. gender, age and place of death as well as autopsy findings and manner of death.

Bushfires are the leading cause of death from natural disasters in Australia, and it has been shown that a half of these fires are deliberately set(44). Much of the research on the phenomenon has focused on identifying motivations of potential arsonists. The study by Beale et al. assesses the utility of this approach in reducing the number of bushfires. Other approaches feasible for the purpose are also introduced. The conclusion of the study is that concentrating preventive work and collection of data in the high risk communities and individuals would give the best result. Better data on bushfire ignitions would yield for better possibilities to prevent arsons.

In relation to bushfires, studies have also been made on victim identification and determination the cause of death(45). Data on victims of various bushfires, circumstances of their deaths and relating diagnostics were studied. In these cases, there were many factors hindering the examination, such as effects of weather and animal predation and heat. Fire is a common cause of major disasters especially in densely populated areas, and therefore this kind of disasters were studied with the aim to prevent them in the future.(46). One example is a study on an alleged arson and during the investigation, all victims underwent forensic autopsy.

Burning of a human body and its role in fires have also been studied(47). It is often assumed that a human body is only an object of fire that gets damaged due to circumstances relating to the fire. However, they may well serve as a fuel package maintaining the fire. This was studied by conducting tests to analyse circumstances in which human cadavers may serve in this capacity. The test environment was made to simulate circumstances of a natural fire, and the fire burned non-accelerated until it naturally extinguished. The tests showed that human bodies can support a modest-sized fire for some 6-7 hours. By that time the torso where the greatest amount of subcutaneous fat resides, resulted in extensive destruction. The head and limbs suffered less damage. History knows several cases in which a human body has burned in the way described above. This phenomenon is known as spontaneous human combustion (SHC)(48). Levi-Faict et al. have studied the history of the phenomenon and looked for similar cases from the near past. They also looked for similarities between reported cases and found several of them. For example, one victim was burnt so that the vicinity of the cadaver was almost intact and the burning of the body had taken place post-mortem in the way described above only in part. Burnt bodies were often found with high concentrations of alcohol in the blood, and there was often a source of heat near the body. The cause of death was usually natural, but a versatile and comprehensive analysis was needed for establishing that. Unlike the phenomenon is called, the burning of the body was not spontaneous in these cases, so for the sake of clarity, the phenomenon should be re-named.

6 Laboratory Analysis

6.1 Sampling and sample preparation techniques

Extraction and concentration of ignitable liquid residues is hindered by the fact they have different types of chemical and physical properties. Burning may also change the consistency of ignitable liquid residues. Many ways have been developed for pre-treatment of fire debris samples. One of the most recent devices developed for sampling pollutants is called Radiello Passive Air Sampler(49). It is a passive headspace method which properties have been reported to have good level repeatability, sensitivity and adsorption capacity. Suitability of Radiello Passive Air Sampler for sampling fire debris has been assessed by comparing it to the commonly used ACS extraction method. According to the results, Radiello produced more stable chromatograms from various consistencies of residues of ignitable liquids than the ACS. In other words proportions of light hydrocarbons were not enhanced in the samples, but the profiles remained stable. At least in the test environment in question, the ACS was found the most effective way of extraction. Radiello's disadvantage was that it did not allow the analysis of heavier components (>n-C16). Therefore, detecting residues of heavy petroleum distillates in fire debris may be difficult.

In recent years, ACS (i.e. activated charcoal strip) has been a common way of pre-treatment(50 51 52 53 54 13). The method has been utilised in several studies concerning detection of ignitable liquids and establishing the origin as well as in assessments of the effects of weathering on ignitable liquids and background matrix. It has also been part of sampling when a sampling method was developed for detecting ignitable liquid residues on suspected arsonist's hands. In the context of several tests, part of ACS was placed in a sample bag and then the sample was heated for several hours. Subsequently, the compounds adsorbed into the ACS were dissolved e.g. in pentane, dichloromethane or carbon disulfide or subjected to thermal desorbtion (activated charcoal trap). Afterwards the compounds were analysed using chromatography.

There are also examples of using Tenax TA® tubes in the development work made for fire scene investigation(10 11). The tubes have been used at least to compare properties of commercially available fire debris bags. In these studies, compounds adsorbted by Tenax TA® were analysed using GC/MS with an Automated Thermal Desorber (ATD). White et al. have studied operational conditions suitable for Tenax TA® with a view of detecting ignitable liquid residues(55). GC/MS with an ATD was used in the study. The use of the ATD was also optimized for sampling. According to preliminary results, the suitable temperature for Tenax TA® is 90˚C and residues of ignitable liquids were successfully detected during the adsorbtion of 3-9 hours. When compared to the ACS, the reported advantage of Tenax TA® was i.a. the fact that it contained a smaller number of work phases resulting also in a smaller risk of contamination. Tenax TA® and other different extraction techniques, such as the ACS and Solid Phase Microextraction, have also

been compared with each other in the analysis of pyrolysis products derived from bone(56).

The Solid Phase Microextraction (SPME) is a solventless extraction technique that is commonly used in fire debris sampling(57). It is particularly useful in extracting very small residues of ignitable liquids. When extracted, injection of the examined compounds e.g. to GC/MC was simple. The SMPE fibre that adsorbed the examined compounds was placed in the GC/MS injector where the compounds were released due to the heat for analysis. However, using expensive and fragile extraction fibres with limited lifespan were required for benefiting from the SPME technique. The Liquid-Phase Microextraction (LPME) and the Single-Drop Microextraction are similar extraction techniques in which a small amount of organic solvent is used in extraction that is based on solubility differences between the aqueous phase and the organic phase(58). A technique known as Headspace Single Drop Microextraction (HS-SDME) has been developed for the purposes of analysing fire debris samples. In this study, curtain fabric was used as the sample matrix and fire accelerants such as diesel, kerosene and gasoline were analysed. The study showed that the HS-SDME coupled with GC-FID is an easy, rapid and sensitive method for the analysis of accelerants in fire debris samples.

A new, alternative device for extraction and concentration of volatile organic compounds is a polymer particle-packed extraction needle tested in pre-treatment of fire debris samples(59).

For example, polymer of divinylbenzene was packed in a needle which was then attached to a commercially available vacuum sampling device. When the handle of the vacuum sampling device was manually pulled, the analytes were adsorbed in the headspace on the polymeric particles in the needle. Then the extraction needles were attached to an injection syringe containing N2 gas and inserted into a heated GC injection port. The polymer particle-packed extraction needle technique is effective, and detection of residues of ignitable liquids in very small consistencies is easy. Kabir et al. have prepared a comprehensive article introducing sample preparation methods applied in modern forensic applications of chemistry(60). In respect to fire scene investigation, they also give an overview of recent advances made in the field of chemistry and introduce e.g. the methods and techniques described in this chapter. Analysis of ignitable liquid residues has been studied from another angle as well(61). Residues of ignitable liquids may be extracted e.g. from soot with the above described adsorbtion techniques, but volatile compounds in the sample are concurrently destroyed, so analysis of spatial distribution of the residues i.e. layers of the soot is not possible. This alternative type of analysis considers layered soot deposits as a potential source of information about fire scenes. Samples of soot from the combustion of petrol were analysed using so-called layered soot analysis and the technique was assessed as a promising means to detect petrol in fire debris residues without destroying the sample. Laser-induced thermal desorption (LITD) coupled with Fourier transform mass spectrometry (FTMS) was used as a method in analyzing the soot samples. The purpose of the study was to find out optimal sampling conditions for analysing soot from burning petrol.

6.2 Methods of analysis

It may be concluded from the studies described in this report that a significant number of chemical analyses relating to fire scene investigation published within past three years have been made using GC/MC technology often a HP-1 or HP-5 or their equivalent attached to the gas chromatograph, and the quadruple mass analyser as the most commonly used detector. Performance of the commonly used GC columns for analysis of fire debris residues has also been evaluated(62, 63). Comparisons included HP-1 (25 m x 0.20 mm id x 0.5 µm film thickness), HP-5MS (30 m x 0.25 mm id x 0.25 µm film thickness) and HP-5 (25 m x 0.20 mm id x 0.5 µm film thickness). Performance of each of the columns was optimized and then used to analyse the matrix matched Grop mixture used in the ASTM E1618-06 technique and an ignitable liquid mixture containing petrol and diesel. The results showed that the HP-1 column extracted most effectively many of the hydrocarbon compounds under analysis and therefore, it was the most suitable way to extracting compounds in ignitable liquid mixtures.

Evaluation of usability of GC/MC technologies published by the American Society for Testing and Materials (ASTM) for detection of residues of ignitable liquids in fire debris has also been further developed e.g. in respect to sample conservation techniques, extracting ignitable liquid residues, GC-MS analysis and interpretation of the results(50). A new procedure based on a valid internal standard technique was developed in the context of this study for evaluation of analytical methods following standards ASTM E1412-07 and E1618-10. The developed procedure proved to be adequate and easy to apply. Another study developed further the standard procedure ASTM E-1618-06 of National Centre for Forensic Science (NCFS)(64). The new method is shorter in respect to analysis time, and it produced better extraction and sensitivity. In addition to the internal standard, a metrological procedure has been proposed for evaluation of measurement performance of the extraction methods of ignitable liquids following ASTM E 1412-07 and ASTM E 1618-10 standards(65).

From the angle of fire scene investigation, the most unusual application involving mass spectrometry proposed is the procedure in which an isotope ratio mass spectrometry (IRMS) is attached to the gas chromatograph.(66). In this way, extracting paraffin compounds of different lamp oils, candles and matches was possible on the basis of δ2H and δ13C values. Muccio describes application of IRMS technology in forensic purposes, including analyses of residues of ignitable samples in more detail in his PhD dissertation(67).

In addition to IRMS, another application of mass-chromatography not so commonly used is transform mass spectrometry (FTMS)(61). Laser-induced thermal desorption (LITD) coupled with Fourier transform mass spectrometry (FTMS) was used as a method for analyzing soot samples with the aim to detect soot from the burning petrol in the samples.

In addition to GC/MC, two-dimensional gas chromatography coupled with a flame ionization detector (GC x GC-FID) has been used to estimate weathering(68). In the study, samples of petrol were weathered, and exposure times of the samples for weathering were defined. Two-dimensional gas chromatography coupled with quadruple mass spectrometry (GCxGC-qMS) has been used in analysing hydrocarbons of ignitable liquids(69).

In another case, fire debris was analysed using the Raman spectroscopy(70). The method was applied in identification of materials and pyrolysis products in the background matrix even when the sample had ignitable liquids present in the sample, and the burning changed the chemical consistency of those materials. Identified compounds were e.g. aromatic and polyaromatic compounds and alkanes. Although Raman spectroscopy has not been widely applied in the fire scene investigation yet, it is considered as a rapid and inexpensive analysis method. The acquisition costs are also reasonable, and the device is easy to take along.

A method known as Hyperspectral Imaging (HSI) has been tested for detecting ignitable liquid residues on typical clothing fabrics and carpets(71). The identification is based on detecting markers and dyes of the liquids commonly used for igniting fires, such as petrol, diesel and kerosene. After evaporation of hydrocarbons in the ignitable liquid, only these markers and dyes are left and they may be detected with fluorescence.

In his PhD dissertation, Jang experimented a colorimetric sensor array with a pre-oxidation technique in detecting ignitable liquid residues(72). He was able to detect ignitable liquids both from a nylon carpet and equivalent fire debris.

6.3 Individualization of ignitable liquids

Fire examiners have paid a lot of attention to classification of ignitable liquids and determining the source of origin. Various kinds of statistical approaches are an essential part of studies in this field. For example, fresh petrol of different brand names was analysed with the SPME-GC-MS method, and the data was then subjected to Principal Component Analysis (PCA) and Discriminant Analysis (DA) with the aim to differentiate between the brand names. In fact, multivariate analysis methods in question did produce the classification. In another case, results of GC/MS analyses of petrol of different brand names were classified in a similar manner using e.g. Fuzzy Rule-building Expert System (FuREs) and Projected Difference Resolution (PDR)(73). Similarly, a method of individualization based on GC/MS analyses and target compound analysis has been developed for kerosene and petroleum distillate products (MPD products)(74).

Samples simulating fire debris samples have been classified in a similar manner even more often. Fire debris samples have contained ignitable liquid residues, and the studies have considered also inferences caused by the background matrix and weathering of the ignitable liquid. For example, PCA, PPMC and HCA have been applied together with GC/MS results for identifying ignitable liquids from their residues despite the effects caused by

burning, weathering and the background matrix(51 52). Self Organising Feature Map (SOFM)(75 76 77) and Bayesian soft-classification(78) have been applied for the same purpose. In addition to the above, likelihood ratio has been used in detecting the origin of petrol residues(79).

An essential part of identification of ignitable liquids is evaluating changes in the compound consistency of the ignitable liquid under analysis. For example, the amount of time that ignitable liquid is subjected to weathering, i.e. the exposure time for volatile conditions, was evaluated using a simulation of weathering conditions and monitoring the respective effects(80). For the weathering experiment, an evaporation chamber that permits control of airflow and temperature was constructed. Composition of a model nine-component hydrocarbon mixture was monitored first using GC/MS. Consequently, the study was expanded to using the GCxGX-FID method in estimating and monitoring various kinds of petrol(68). Statistical analyses were used in both studies to classify the results.

Microbial degradation has an effect on the consistency of the ignitable liquid. The effects of microbial degradation and weathering on petrol have also been studied(53). Petrol samples were intentionally weathered using nitrogen evaporation. In order to study microbial degradation, petrol was added in potting soil and the samples were placed in closed paint pots for suitable time. The methods used for analysis included passive headspace concentration and GC/MS. The results showed that weathering and microbial degradation affect the consistency of ignitable liquids in different ways. Microbial degradation on lamp oil, turpentine and tiki torch fuel has been studied also. Small amounts of the ignitable liquids in question were added in the samples of soil. Samples were then stored at room temperature(81). Ignitable liquid residues were subsequently analysed using passive headspace concentration and the GC/MS technology. Tiki torch fuel alkanes proved to be vulnerable to degradation. In respect to lamp oil, the amount of cyclic and branched nature of alkanes decreased whereas in respect to turpentine, terpenes, such as limonene, o-cymene and β-pinene, turned out to be more vulnerable to microbial degradation. Also microbial degradation of petrol residues from incendiary devices has been evaluated in respect to time with the help of both visual and statistical methods(82). Results of the study indicate that the way in which the consistency of petrol changes e.g. in various kind of glass samples, differs from the way it changes in soil samples. Also the season had an effect on the outcome.

The most commonly methods used in identification of hydrocarbon mixtures and paraffin mixtures are GC and GC/MS. Compound-specific 13C and 2H isotope ratios have also been used as an MS application (IRMS)(66). Isotope rations have previously been analysed from the mixture of hydrocarbons as a whole, and when combined with GC and GC/MS, it is an effective method for analysis. However, usability of compound-specific 2H and 13C C isotope rations as an extraction mechanism has also been tested, and the study in question validated the GC-IRMS/MS method for separation of paraffin waxes. Paraffins were extracted from candles, matches and lamp and the isotope ratios were then subjected to statistical analysis. In respect to candles, brand-

name based extraction was possible in 10 cases out of 15. In respect to matches, differences were found both between different brand names and between individual packages. The burning of a match did not have any significant impact on the isotope rations. When the lamp oils were analysed, differences were found between various retail sellers, between bottling years and even months.

Definitions for distillation curves have been used for a long time in the analysis of properties of complex fluids(83). The distillation curve gives a graphic presentation of the boiling temperature of the fluid as the distillation progresses. The method has been recently developed further, and it is considered useful also in the analytics of liquid fuels. The Advanced Distillation Curve method (ADC) could be used to develop and evaluate analyses applied in defining ignitable liquids, and it could provide more information on weathering of ignitable fluids. Examples of applying ADC e.g. in evaluation of weathering have been given, too.(84).

In addition to traditional petroleum-based ignitable liquids, fire debris may also contain residues of biomass-based ignitable liquids(85). Common examples of such ignitable liquids include ethanol and the first-generation bio diesel that is made of vegetable oil and contains fatty acid methyl esters. However, vegetable oils and animal fats can be used to produce renewable fuel of better quality, which is in fact commercially available already e.g. as lamp oil and diesel. This fuel consists of similar branched and linear paraffins as the petroleum-based products. Therefore, identification of renewable fuel requires special attention, especially when it is a question of a blend of traditional and renewable fuels. Both of these fuels have been analysed using the GC/MS technology, and differences in them were analysed with the help of extracted ion profiles (EIP).

Several methods have been developed for forensic identification of blends of bio diesel and diesel for extracting various fuel components (e.g. fatty acid methyl esters, aromatics and aliphatic hydrocarbons) using solid-phase extraction (SPE) (86 87). After extraction, the GC/MS method was used to analyse detailed consistencies of the fuel fractions. Characterization of diesel has also been made using Compound-Specific Isotope Analysis (CSIA) for analysis(88). The study focuses on evaluating the feasibility of the method in identification of the origin of the diesel. Establishing the origin is very important especially in forensic investigations into environmental crime.

6.4 Statistical methods in analysis

Statistical analysis methods have become an important part of chemical fire cause analysis. There are many recent examples on the application of various kinds of multivariate analysis methods, such as Principal Component Analysis (PCA). Primarily, they have been used to interpret results produced by the GC and GC-MS methods, and the studies have aimed to reduce the effects of factors hindering the detection of ignitable liquids in fire debris samples. For example, interference by pyrolysis products in the background matrix, weathering of ignitable liquids and microbial degradation for interpretation of

the results have been attempted to reduce(53). They have also been used in individualization of ignitable liquid residues and the source of non-volatile ignitable liquids and in identification such liquid as a commercial product(51 57). In addition to multivariate analysis methods, feasibility of e.g. neural networks(76) and the Bayesian decision making theory(78) have been tested. Principal aims in these studies were in identification of ignitable liquid residues in complex background matrices as well as classification of ignitable liquids and identification of the source.

Multivariate analysis methods have been used particularly often in interpretation of the results produced by the GC/MS methods. For example, PCA was used in comparing effects of weathering and microbial degradation on the consistency of petrol(53). Components of petrol were identified from samples subjected to weathering and microbial degradation by comparing mass spectres and retention times of the samples to certain standards and to the data in reliable mass spectre data bases. Then extracted ion profiles (EIP) were formed from the total ion chromatograms (TIC) as they can be used for detecting compounds typical to petrol, such as alkanes and aromatics. Peak areas from the compounds of interest were then normalized and auto-scaled for PCA, where changes caused by weathering were easily differentiated from those caused by microbial degradation. The study concluded that compounds lower the boiling point of 155˚C were vulnerable to weathering whereas less substituted aromatics and long chain alkanes were vulnerable to microbial degradation. Compounds that were not particularly vulnerable to weathering or microbial degradation could also be classified with PCA.

In another study, fresh petrol samples were analysed using the GC/MS techniques and the results were processed using PCA and Discrimintant Analysis (DA)(57). Today, the ability to identify the origin of the ignitable liquid detected in the sample is evermore important in forensic analysis. The study aimed to classify petrol samples on the basis of their commercial brand names. Fifty samples presenting five commercial products were analysed in the study. Peak areas of certain compounds were semi-quantitatively analysed from total ion chromatograms of the samples and the results were normalised for statistical analyses. Classification of the petrol samples per brand name was possible with PCA. It was noted consequently that aromatic compounds were more feasible for the purpose than straight-chained compounds DA is also a useful classification tool, particularly for aromatic compounds. In this case, samples were classified under their commercial brand-names with 100% certainty. It has been reported that the group of the above statistical analysis methods is a promising tool kit in tracing fresh petrol samples to their commercial brand-names or refineries.

Other chemometric methods in handling GC/MS results were tested in the study of identification of petrol and kerosene samples(73). The projected difference resolution (PDR) mapping was applied in quantitative measuring of the differences between the samples and Fuzzy Rule-building Expert Systems (FuREs) was used in classification of ignitable liquids. According to the study, the overall performance achieved was over 90% correct classification of both petrol samples and kerosene samples. The new and so far the relatively little

used method PDR produced results consistent with the FuREs results in the same context.

Multivariate analysis methods have proved to be useful in handling GC/MS results also in identification of ignitable liquid residues in the presence of matrix interferences hindering interpretations of the results(51). Visual interpretation of chromatograms is difficult. When the effect of background matrix, weathering of ignitable liquid or burning temperature on the results is evaluated, interpretation is often influenced by the examiner's own view. In this study, PCA and Pearson product moment correlation (PPMC) were used for statistical analysis. Six ignitable liquids (petrol, diesel, fresh paraffin lamp, adhesive remover, torch fuel and paint thinner) were injected in pieces of rug serving as the background matrix, and the samples were then burned in various conditions. Total ion chromatograms scaled so that the retention times were aligned and then normalised to consider the injection capacities and instrument sensitivity, were been used for statistical interpretations. When PCA was used, ignitable liquids were extracted from fire debris on the basis of their alkanes and aromatic compounds. When both PCA and PPMC were used, all ignitable liquid residues were traced to their original products. It is noteworthy that the burning circumstances did not have any effect on traceability.

Feasibility of PCA, PPMC coefficients and hierarchical cluster analysis (HAS) has been experimented also in another study in which ignitable liquid residues were extracted from simulated fire debris samples, and the residues where then traced to the clean original product(52). Both clean and partly evaporated ignitable liquids (petrol and kerosene) were injected in pieces of nylon carpet and the samples were then burned for a determined time. The preferred compounds were identified from total ion chromatograms (TICs) before statistical analysis and the data was then processed to minimise the sources of error irrelevant to the study. The results were then aligned per retention times and normalised to correspond to each other. All three statistical methods were applied to the results, and using each of the methods, they were traced to original ignitable liquid, although there were certain limitations due to the level of evaporation of the ignitable liquid. Of the three multivariate analysis methods, PCA was found the most useful, the interference caused by the background matrix in using PPMC coefficients and HCA strongly affected the results. In respect to PCA, only those background matrix components that were observed with the same retention times as the analysed compounds affected the results. It was concluded on this basis that PCA was the method excluding the interfering background matrix to the greatest extent.

Multivariate analysis methods have also been used in defining the age of weathered petroleum products. Previous research on evaporation of hydrocarbon compounds has often related to forensic investigations of oil spills. The aim of the research has been to examine whether a fresh oil sample could be artificially weathered to give an observed composition in a genuine oil sample taken from soil. However, definition of age or retention time may have many uses e.g. in examinations relating to environmental crime or fire cause analysis. Zorzetti et al. have experimented weathering of

light petroleum mixture in two articles with the aim to predict the amount of time for which a hydrocarbon mixture was exposed to weathering(80 68). The mixture was monitored over time using both GC/MS and GCxGC-FID with partial least squares (PLS), nonlinear PLS and locally weighted regression (LWR) as chemometric tools. In the first study, a simple nine-component hydrocarbon mixture was used, and changes in its composition were monitored over time under simulated weathering conditions. The subsequent GC/MS results were then processed with the above statistical analysis methods and by combining these methods, it was possible to predict the amount of time for which the hydrocarbons were exposed to weathering i.e. to evaporating circumstances. Using the partial least squares discriminant analysis (PLS-DA), it was possible to predict whether a sample was less than 12 hours old or more highly weathered (> 20 hours). Using LWR as the regression method, the age of the hydrocarbon mixture could be predicted even more precisely. Prior to the regression analysis, the data was pre-processed using the y-gradient generalized least square weighting (GLSW). In the other article, the above statistical analysis methods were applied in estimating the age of weathered samples of commercially available petrol using GCxGC-FID for monitoring. In respect to these slightly complex samples, PLS-DA proved to be a useful method in estimating whether the sample was relatively fresh or significantly older. Using the LWR subsequently, fresh and highly weathered samples were predicted to within 30 minutes and 5 hours of exposure, respectively.

Many of the chemometric methods have been found feasible e.g. in interpretation of chromatographic data. However, Sinkov at al .have paid attention to certain problematic aspects relating to their use. The first problematic issue is data alignment: for the data to yield to statistical analysis, chromatograms must correspond to each other. For each analyzed compound, the peak areas must be recorded at the same coordinates in the matrix for each and every analyzed sample so that it is possible to identify them as the same compound. There are several procedures available for the purpose, and they work well when the samples have very similar background matrices. If the background matrix for a series of samples to be analysed is highly variable, these methods are not as effective. The other problem is to find a relatively small amount of features for the statistical model with the help of which e.g. extracting ignitable liquid residues from the background matrix would be possible. The problem was demonstrated in the study by using simulated forensic fire debris samples, as in the field of chemical fire cause analysis these challenges are remarkably often present. GC/MS results were interpreted using partial least squares discriminant analysis (PLS-DA). To solve the first problem, the authors experimented deuterated alkanes which could act as retention anchors making the alignment of the peaks easier. An automated feature selection and model construction routine were applied in finding features characteristic to ignitable liquids to be used in the PLS-PDA model. The routine proved to be feasible, as it found a relatively small and adequate amount of usable features.

In fire debris analysis, multivariate analysis methods have also been utilised in processing spectroscopy data(70). Principal component analysis (PCA) was

used further processing of fire debris analysis results when experimenting feasibility of Raman spectroscopy. This multivariate analysis method was used to experiment whether information given by a Raman spectre taken from fire debris could be used to identify and classify components in the background matrix even when the samples were burnt and various kinds of ignitable fluids had been used to start the fire. The results showed that classification of the background matrix components of fire debris was possible using PCA.

A self-organizing feature map (SOFM) that is also known as Kohonen Neural Networks (Kohonen-NN)(75) has been introduced as a potential tool for identification and classification (GC/MS) of ignitable liquids. The method has been used to identify both fresh and varying degrees of weathered lighter fluids and to classify them to their origin, and in another case, lamp oil, paint brush cleaner and white spirit. In both studies, SOFM's feasibility was compared to two much used classification methods, namely PCA and HCA. When examining lighter fluids, the results showed that determining the origin of the weathered products was possible using HCA and SOFM. For application of these classification models, chromatographic data had to be pre-processed, and the best results were achieved using normalized fourth root transformation data. In another study, classification of weathered samples was possible due to good pretreatment of the data even when there were significant changes observed in chromatograms(76). The SOFM proved to be the most effective classification tool in both of the studies. In addition to its good classification abilities, its greatest advantage is a possibility for good visualization means to present features of each classified group. Later on, SOFM was applied to samples containing other ignitable liquids and different kind of background matrices(77). Even these studies validated SOFM as a potential tool for fire cause analysis in classifying ignitable liquids per their origin and possibly, in extraction of components from the background matrix, too.

A method for detecting ignitable liquid residues despite interferences caused by various kinds of background matrices has been developed on the basis of the Bayesian decision-making theory(78). In this study, the Bayesian soft classification method was combined with the target factor analysis (TFA) based on the interpretation of the average total ion spectra (TIS) of multiple parallel samples taken from the same fire seat. Total ion spectres (TIS) derived from the reference collection of ignitable liquids were used as target factors in forming TFA. Identification of ignitable liquids was based on the classification following the American Society for Testing and Materials (ASTM) in which the Bayesian calculates the probability in which a finding made from a sample falls to some ASTM category of ignitable liquids. When the method was tested in practise, the overall performance achieved was approximately 80% correct classification. Some other chemometric methods, such as the component analysis (PCA), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), cross-validation (CV) have also been tested in the context of ASTM classification.(89).

Vergeer et al. presented likelihood ratios (LR) for petrol comparison in the European Academy of Forensic Science conference in 2012. Unlike several other automated petrol comparisons, this LR method takes also weathering and effects of the background matrix into account. Preliminary results indicate that the LR may be a feasible tool for a fire cause analyst in petrol comparisons.

6.5 Spontaneous combustion

Oxidation reactions and spontaneous ignition of linseed oil has been studied in respect to spontaneous combusting of vegetable oils(90). Due to its drying properties, linseed oil has been used e.g. in paints. When drying, especially in the presence of metal salts, linseed oil induces heat and even spontaneous ignition. Chemical mechanisms relating to these phenomena were examined in the study. In particular, the study focused on the effect of transition metal salts on the oxidation of linseed oil. The authors propose that in the presence of a metal catalyst, the oxidation process involves the formation of metal-dioxygen (superoxide) adducts. The results showed that without the presence of metal, autoxidation process was slower and e.g. polymerisation and fragmentation of the compounds induced the observed oxidation products.

An attention has been paid to spontaneous combustion characteristics of coal, because it is a serious risk factor e.g. in underground coal mines(91). For example, the quality of air in mines has been monitored for a long time now in order to detect early indications of self-combustion by tracing gas products induced by spontaneous heating and self-combustion and assessing their amounts in the air. The study focused on the accuracy of the determination of coal spontaneous combustion and related circumstances in laboratory conditions. In another study, reducing pollution caused by coal waste gobs was examined. The proposed solution was covering coal mine waste gobs with soil(92). According to the study results, it was assessed to be a good way to prevent self-ignition of coal and pollution. Self-ignition of coals causes also problems for storing(93). A recent study focuses on risk factors in handling of coals and biomass resources using the isothermal oven procedure and analyses their tendency to self-ignite. The results give some guidelines on the optimum volumes and storage times of the materials considering their tendency to self-ignite.

Storing sulphide concentrates may also be problematic due to the tendency of the material to self-ignition, because they start to form oxidation products and release heat over long periods of storage(94). Therefore, self-ignition of various kinds of sulphides was studied. The study focused on sulphide concentrates rich in sulphur and iron. Self-ignition was studied by the method of crossing point temperature (CPT), and the sulphide concentrate rich in iron turned out to be more sensitive to self-ignition. In another similar study, tendencies of three different kind of sulphide concentrates from a metal mine to self-ignite were evaluated(95). In addition to a sulphur-rich sulphide concentrate and iron sulphide concentrate, the study included a sulphide concentrate rich with copper, and their corresponding apparent activation energies required for their self-ignition, respectively, was examined.

According to the study, sulphur-rich sulphide and iron sulphide concentrates had lower apparent activation energy than copper sulphide concentrate within certain temperature range, so they were evaluated to be more inclined to cause spontaneous combustion.

Certain chemical substances may self-ignite as mixtures(96). Self-ignition of mixture of petrol and pool chlorinators has been reported, and this study focused on examining the tendency of the mixture of these two substances to self-ignite. An organic pool chlorinator was combined with petrol in varying proportions in an attempt to form a hypergolic mixture. None of the combinations resulted in self-ignition, although larger quantities of chlorinator produced vigorous light-coloured smoke and the temperature of the mixture arose rapidly. Other pool chlorinators were also tested in the experiment. The above mixture could be used as a so-called Molotov Cocktail i.e. a petrol bomb(97). Chemical reagents that enable its self-ignition of this kind of Chemical Ignition Molotov Chemical (CIMC) were defined using capillary electrophoresis (CE) for anions in order to identify reagents used to produce the petrol bomb.

Hydrogen is generally considered as a clean source of energy and a possible next-generation fuel(98 99 100). It has been used by the industry for years, and today it is also considered for new energy solutions. However, the use of hydrogen as a source of energy requires special attention and security arrangements as pressurized hydrogen may self-ignite when it is released quickly to the air.

6.6 Background matrix studies

Volatile compounds of the background matrix of fire debris often interfere the identification of ignitable liquids(101). These volatile compounds are often from pyrolysis i.e. thermochemical decomposition of organic material almost in the complete absence of oxygen. It is specifically problematic that decomposition of material through pyrolysis may produce the same compounds as those that are typically used in identification of ignitable liquids. This is the reason why pyrolysis has been used an examination method and in generating volatile compounds produced by different kinds of background materials. In the study, a temperature programmable steady-state tube furnace was used to generate pyrolysis products from e.g. softwoods, carpet, paper and vinyl sheet flooring. A suitable temperature profile of the tube furnace was assessed with the aim to create the same pyrolysates as those found in real fire debris. The method is used in the preparation of various kinds of reference samples already. Another study utilizing pyrolysis focused on potential interferences from pyrolysates of a dishwashing liquid used as a fire-extinguishing agent in identification of ignitable liquids from fire debris samples(102). The dishwashing liquid contained linear alkylbenzene sulfonates that broke down to various kinds of substituted aromatic compounds at pyrolysis temperatures. These aromatic compounds may also be present in ignitable liquids.

In addition to chromatography, spectroscopic methods may be good alternatives for fire debris analysis and examination of background matrices(70). For example, Raman spectroscopy is a widely used method in analysing degradation products of e.g. plastics and polymers in material sciences. The method is also used to study various kinds of polymers in the field of forensic fire debris analysis. Samples of polypropylene, nylon and polystyrene were burnt as such and with ignitable liquids. Extracting the background materials in question from fire debris samples was possible even in cases where the samples were burnt, although chemical properties of the plastic were significantly altered in the fire. In addition, classification of different materials even in samples containing ignitable liquids was possible using statistical analysis.

The problem caused by interferences from background matrices in detection of ignitable liquids has been attempted to solve by utilising various kinds of statistical analysis methods, too. For example, there are two articles reporting studies on detection the origin of ignitable liquids(51 52). In the first study, six ignitable liquids (paraffin lamp oil, diesel, gasoline, paint thinner, torch fuel and adhesive remover) were spiked in pieces of nylon carpet (5x5 cm). The pieces of carpet were then burned with a blow torch for 10 seconds and when simulating heavier circumstances of combustion, for 20 seconds. Ignitable liquids were added in some of the samples before burning. Ignitable liquid residues were successfully classified into the matrix using statistical analysis methods (PCA and PPMC), regardless of the burning circumstances. As a result of lighter burning, mainly isoparaffinic and naphthenic compounds were extracted from the carpet. They were however blanketed by controlling ignitable liquid residues. Due to heavier burning, interference from the matrix was more severe, and e.g. styrene and benzaldehyde were extracted. In another similar study, gasoline and kerosene were spiked into pieces of nylon carpet, and the samples were then burned to cause the maximum interference from the matrix. PPMC coefficients, PCA and HCA were used as statistical analysis methods. In relation to PPMC coefficients and HCA, the matrix hindered interpretation of the results, whereas when PCA was used, compounds eluting in a similar way to the ignitable liquid under analysis hindered the identification. Therefore, PCA was the most feasible method in extracting components from the background matrix.

The Bayesian soft classification method used in combination with the target factor analysis (TFA) has been experimented in analysis of interferences from the background matrix and fire debris samples containing ignitable liquids(78). The method in question was used to classify ignitable liquids into groups. In the experiment, volatile compounds were collected both from laboratory-scale tests and fire reconstructions made in shipping containers. Background material for laboratory-scale tests was acquired from home improvement and furniture stores. In larger-scale tests, the burning material was plywood and wood/vinyl laminate. There were also sofas, chairs, beds and tables as well as smaller things made of plastic and paper in the containers. The overall performance achieved was approximately 75-80% correct classification of fire debris samples despite the interferences from the background matrix.

In addition to all the above mentioned studies, numerical likelihood ratio (LR)79 and Self Organizing Feature Map (SOFM)(77) have also been used as tools when comparing petrol and petroleum distillate products. In both studies, classification was possible despite interfering components in the background matrices, respectively.

7 Reconstruction And Modelling

In regard to fire scene reconstruction, a forest fire presumably caused by an arsonist has been reported(103). A small area of land close to a small village in Italy was destroyed by fire in the summer of 2010. Remains of a tool used in setting the fire were found in the point of origin of the fire, and the way in which the fire was actually set was examined and demonstrated using chemical analysis methods. Results from using GS/MC and μ-FTIR showed that a candle was used in starting the fire. When the fire was reconstructed, it was discovered that the arsonist may have aimed to burn a small brush glade for cultivation or farming cattle in the area.

Today, computational modelling is often used in reconstruction of various kinds of fire scenes(104). For example, fire dynamics simulator (FDS), a program used for modelling, has been developed over the years and, ultimately it is a relatively useful method in modelling various kinds of fire incidents. However, for the successful use of FDS, entering thermal parameters relating to reactions taking place during the fire into the mathematic model is also required and therefore, in recent studies, FDS is often used in combination with fire reconstructions and actual fires.

Modelling is most typically used in cases of fires in various kinds of buildings. For example, simulation of heat detectors used in detecting fires in buildings has been experimented with FDS(105). Small-scale and large-scale practical experiments focused on measuring the time the heat detector needed in detecting the fire, and the results were then compared to the results simulated with FDS. The electrical cable failure model in FDS suited well for the purpose in question, and results of the small-scale test made in the controlled environment, in particular, corresponded to the simulated results.

FDS has been used to simulate actual fires, too. For example, a disastrous fire in an inn was simulated on the basis of the official fire investigation report and data following the NFPA921 regulations(106). Using FDS, it was possible to demonstrate reasons why so many casualties resulted from the fire. FDS was also evaluated as a good tool for planning fire safety. Another study focused on analysing the development of a fire in a five-storey building in Germany where two people died(107). The fire was analysed numerically and experimentally. A special attention was paid on combustion characteristics of the building materials. FDS v.4 was used to model and compare the progress of the fire between ignitable and non-ignitable roof materials. The outcome of the study was that several building materials were not compliant with the regulations, and therefore, the arsonist was not responsible for the deaths. FDS and smokeview (visualization tool) were used to simulate the fire scenario of another fire leading to the deaths of two firemen(108). There were

two simulations made with the purpose to analyse the effect that the wind conditions contemporaneous with the fire had on the progress of the fire. The first simulation was made without the effect of the wind and in the second simulation, the wind was included as an element affecting the development of the fire.

The National Institute of Standards and Technology (NIST) has also used FDS when conducting an extensive investigation and reconstruction of a fire scenario that resulted in the collapse of the World Trade Center towers(109). It established in the investigation that a complex fire in a big building may be reconstructed even if the building itself does not exist anymore. However, the investigation called for an extensive collection of analysis material e.g. photographs, interviews, videotapes and other such documents so that there was adequate amount of information on the progress of the fire available. FDS was used to evaluate the magnitude and spread of the fire in all WTC towers. The results were in agreement with the understanding of the fire given by the photographs. This study was one of the first cases where travelling fires were used in simulations of structural failures.

Various kinds of vehicle fires, such as car fires, have been computationally simulated and reconstructed(20). Although the number of fires caused by self-ignition and technical malfunction is in a slight decrease in general, the number of car fires has increased in many countries. One reason for the increase in the number is, especially in big cities, arson. It is good to subject cars to fire experiments at regular intervals, because car technology is improving all the time and changed in respect to the respective fire characteristics. For example, materials used to build cars may differ from the materials used earlier on, and it means that thermal energy released by the car during a fire may be significantly different, too. Weisenpacher et al. conducted a fire experiment on Kia Ceed passenger car and monitored the progress of the fire graphically by studying the temperature in various parts of the car as a time function. The car fire was then computationally modelled on the basis of the results. The authors have presented possibilities for computational modelling of modern car fires more extensively, too(21). Fire scenarios typical to car fires in particular, such as a fire spread from the passenger compartment of the next car, and computational modelling using FDS simulation are described in the article. Even a complex fire starting in the engine compartment was simulated successfully with the method.

Fires in road tunnels have been fairly common in recent years, which has given a rise for analysing them e.g. by simulating fires of heavy goods vehicles (HGV fires) in road tunnels using computational fluid dynamic (CFD) and a much used CFX code by ANSYS(22). HGV fires were simulated in curved bi-directional road tunnels and e.g. the effects of tunnel geometry, point of origin of a HGV fire and traffic flow on the risk factors for fire hazards, such as formation of toxic gases, were analysed.

Computational modelling relating to car fires has been used also in train fires(23). The train is one of the most important transportation means in China. Train accidents and arsons have become a significant problem from

the view of passenger safety. Therefore, there have been various kinds of fire scenarios simulated for trains in order to improve safety. Computational Fluid Dynamics (FDS 4.0.6.) was used for simulation, and the conclusion of the study was e.g. that small enclosed spaces in train compartments are risk factors for fire hazards. A method based on numerical modelling was developed in another study in which small-scale test data was combined with a simplified flame spread model(24). The method was used as so-called initial mapping to assess flame spread performance of interior finish materials used in train compartments, and the analysis may be continued with more sophisticated methods. For example, if the outcome of the initial mapping is that finish materials are more likely to spread flames than curb the fire, more sophisticated methods may be applied to continue the analysis.

The NIST's Fire Dynamics Simulator (FDS) has also been used to analyse an electrical fire in a factory(31). Pieces of metal of a burnt switchboard were subjected to metallographic analysis with a view to identify the cause of the fire. As the fire had spread rapidly and caused severe damages, FDS was used for simulation in order to gain better understanding of the fire incident. The simulation was based on the heat estimated to have been released from the switchboard in the fire. The simulation indicated that thermal insulation polyethylene played an important part in the rapid fire spread. FDS was used to reconstruct a hydroelectric power plant in Taiwan(32). The simulation was used to assess risks relating to fires in hydroelectric power plants and to evaluate the safe escape time considering possible fire risks. The rate in which heat is released from burning materials is of great importance in numerical simulations of fire accidents. In this study, the simulation was based on the fact that the fire had started in the transformer room of the plant and that the burning material was transformer oil.

In addition to the Computational Fluid Dynamics program, a so-called stochastic fire safety engineering tool has been used to analyse the fire spread, too(110). The aim in using the Stochastic Computation and Hybrid Event Modelling Approach-Sécurité Incendie (SCHEMA-SI) developed by the French Scientific and Technical Construction Centre was to simulate incidental circumstances of the fire as well. Various fire scenarios were generated using this tool utilizing actual documented events and monitoring physical quantities, such as temperature and heat release rate (HRR) in relation to time. The simulation was experimented in one actual fire incident, and the aim of the study was to identify the ones that correspond to the actual circumstances observed in the fire accident in question among thousands of hypothetical fire scenarios generated.

A numerical modelling method has also been developed for predicting the fire spread in building fires(111). The method would be useful for fire-fighters, in particular, as there would be some other way to predict the progress of the fire than their personal experiences alone. Sophisticated computational fluid dynamics are too coarse and slow for the purpose, so the authors tested a simplified model using only a few constant parameters and data produced by various kinds of fire sensors. Sensor data was assessed in respect to e.g. location, density and sensor type. The study concluded that data produced by

these simplified models could be used to replace details of more sophisticated numerical models and thus, to simplify and streamline the computer modelling.

Feasibility of numerical modelling in forensic fire cause analysis has been evaluated and attention has been paid on related misconceptions(112). Modelling has stimulated a plenty of interest in the field of forensic fire cause analysis, but there has not been enough discussion about feasibility of the method yet, nor there has been reached an understanding in the matter. The study concludes that the aims of the computational modelling programs in use to day are different from the ones of fire cause analysis, and therefore utilising the programs is difficult.

8 Educational Aspects Of Fire Investigation

A review for fire cause investigators discusses basic matters relating to fire incidents and fire itself(113). The article reiterates the elements required for a fire to start and the life cycle of fire, and introduces several types of fire in respect to various kinds of burning materials. Combustion products, such as gases, and their effects are also explained. A few specific fire incidents, e.g. a fire in a room and the way it spreads and combustion of a human body are also described. Flame colour and flame types are introduced. The article concludes that in general, a fire is a complex process to which many factors effect. Although all fires are different, they all develop following certain predictable patterns and understanding these patterns helps the fire cause analyst and provides an scientific angle to the analysis.

Quintiere et al. reported of their study aimed to give information and understanding about spontaneous ignition(114). Three scenarios for spontaneous ignition are considered in the report, namely: cold material in hot surroundings, material on a hot surface, and hot material in cold surroundings. In relation to these scenarios, circumstances typical to spontaneous ignition and methods how to monitor these circumstances are also introduced. Using the introduced analytical methodology, a fire cause analyst may evaluate the possibility of spontaneous ignition in practical analysis situations.

Several books in the field of fire cause analysis have also been published during the past three years, e.g. the 7th edition of Kirk’s Fire Investigation(115) and a new edition of Scientific Protocols for Fire Investigation(116). The National Fire Protection Association has also published a guide: NFPA 921 Guide for Fire &Explosion Investigation, 2011 edition(117).

9 Explosions

Arsonists often use volatile ignitable liquids as accelerants in setting fire(118). A mixture of gasoline vapour and air may cause a devastating blast in suitable circumstances and thus, pose a significant risk to the arsonist. A case demonstrating the risk relating to the explosive nature of ignitable liquids was

given as example of the phenomenon. A 49 year old male was found in ruins of a destroyed house. The blast had destroyed the house, and there were hardly any signed of burning. Although there may be several reasons why a body was found at the fire scene, findings made both in criminal investigation and post-mortem examination indicated that the victim was the arsonist. Residues of petrol were found at the fire scene and the victim was holding a cigarette lighter in his hand. There were e.g. flash-type burns in the body. The study concludes that perpetrators often underestimated the explosive nature of ignitable liquids when setting the fire, which then turned out to be fatal for them. In some cases, fire was set using a mixture of petrol and kerosine(119). Petrol addition to kerosene caused the evaporation rate of the mixture to fall, and consequently ignitable fuel mixture was formed on the smaller area. Evaporation and diffusion behaviour of mixtures of petrol and kerosene were studied with a view to predict the behaviour of fuel mixtures of petrol and kerosene in an arbitrary mixture ratio.

Explosion of a natural gas fired oven has been given as an example of ignition of gas mixtures(120). The cause for the explosion was established and reported in order to avoid similar cases in the future. The industrial oven in question had a fully-automated control system and flame security guard. The established fire cause was a failure mode of the flame detector and malfunction of the control system. Due to a malfunction of the flame detector, the automated control system established normal operating status, but without a flame. So, natural gas entered the oven and did not burn in the flame as it should have been. Over time, there was a great amount of gas accumulated in the oven, and an attempted restart caused the gas to explode. The study emphasizes that testing and renewing these systems at regular intervals is important.

Vapour cloud explosion and flash fire caused by hydrogen gas were studied in circumstances typical to chemical industry(121). Potential damage to buildings and people were assessed by modelling the overpressure from the explosion with TNO multi energy, TNT mass model and Baker-strehlow model to estimate the pressure e.g. at the centre of explosion and 25 metres away from the centre.

Gas and coal dust may explode in combination or separately in mines(122). Gas explosions are often due to oxidation reactions between methane and oxygen at appropriate temperature whereas the cause of dust explosions is complex oxidation reactions of between a dust coal cloud and oxygen. In this case, the pressure wave velocity may be as high as 2000 m/s. Explosion of the combination of gas and coal dust is much more devastating than either of the above alone, as the flame propagation speed is approximately 2-3 times higher than in the gas explosion. This type of explosions often results in the loss of life over a wide area due to its wider sphere of influence. The study sheds light on the theoretical background of the explosions in question and reports results from practical analysis e.g. by studying explosion circumstances and gas composition after explosions.

Dust explosions are great risk factors in various kinds of industrial circumstances, such wood industry, food industry and pharmaceutical industry(123). For example, during the production process different kinds of hot surfaces can create a layer of dust that can heat up, ignite and explode. Many mechanical and electrical equipment may have hot surfaces either due to normal operations or malfunctioning. Dust explosions were analysed in respect to the minimum ignition temperatures of dust clouds and the minimum ignition temperature of dust layer. Tests were performed for e.g. different materials and medicinal herbs used in the food industry in accordance with EN 50281-2-1. The study revealed that dusts of sunflower husk and medicinal herbs ignited the most easily. In the case of sunflower husk, it was due to small bulk density and high heat of combustion whereas regarding medicinal herbs, thickness of the dust led to a lower ignition temperature.

An explosion in a sugar refinery was reported as an example of a dust explosion in food industry(124). In 2008, a series of sugar dust explosions at a sugar refinery in Port Wentworth, and consequent fires caused serious damages to various parts of the refinery. Fourteen workers died and several injured. According to the U.S. Chemical Safety and Hazard Investigation Board (CSB), the series of explosions initiated in an enclosed steel belt conveyor located below the sugar silos. Concentrations of sugar dust had accumulated inside the enclosure and the sugar dust ignited and caused a violent explosion. The first explosion was followed by several other blasts. As the cause of the fire, the investigation identified e.g. faults in designing the equipment and inadequate cleaning to minimize the release of sugar dust on the floors and other surfaces. Another article reported about investigation of the explosion at a flour mill in Italy in 2007(125). There was a mill producing wheat flour situated in an old multi-storey building. The accident happened in the context of loading a tanker that had come to collect the flour.

Fine wheat flour exploded in the pneumatic duct connecting the tanker and the silo, the explosion was ignited because of an electrostatic arc that occurred in the duct. The triggering discharge and the resulting primary explosion occurred between the flour and the metal wall at the joint of the duct. Grounding the tanker may have minimized the risk for the electrostatic charge and possibly, prevented the explosion. Magnesium dust is used for various industrial purposes, but as it highly flammable and explosive, it has caused devastating dust explosions(126). A scientific test was performed to study magnesium dust in respect to its explosion severity, flammability limit and solid inerting as well as to influences of particle size, dust concentration, ignition energy and initial pressure. The conclusion of the analysis was that magnesium dust causes a higher risk for explosion that coal dust and that KCl and CaCO3 addition attenuates the explosive properties of magnesium dust.

10 Criminological Aspects Of Fire Setting

Both theoretical knowledge and practical understanding of the motives at background of deliberate fire-setting are valuable in assessing and treating patients with symptoms of fire-setting behaviour(127). Theories and motives explaining the fire-setting behaviour were thus reviewed and synthesized with

the aim of further development of forensic-clinical psychology. Prime parts of this information were compiled into a new comprehensive Multi-Trajectory Theory of Adult Firesetting (M-TTAF) integrating of current theories and other important theoretical factors. Theories i.e. cognitions that are likely to relate fire-setting behaviour of adults are compiled and presented in another study(128). Structures, contents and etiological functions of five different theories are reported. Cognitive similarities between fire-setters and other offender types are also described. The aim of this study was also to aid clinical assessment and treatment of adult fire-setters.

Empirical studies on so-called intentional fire-setters and pyromaniacs were presented and examined critically(129). The research material included research data on both adult and juvenile arsonists, and the review article describes typological work on arsonists, as well as psychological and psychiatric interventions and their results. For example, low economic status and unstable childhood were found to be associated with fire-setting. It was further noted that these developmental and demographic factors combined with e.g. alcohol abuse are very common to other offender groups as well. In respect to arson treatments, there appear to be few effective interventions available for arsonists despite the continuing cost of arson.

Fire-setting by young people results in great financial loss and personal injuries every year(130). The article in question compiles the past 30+ years of studies e.g. models of fire setting behaviour, prevalence rates of fire setting, diagnostic issues and assessment tools. The article concludes that juvenile fire-setters form a heterogenic group the behaviour of which is affected by many variables. which at the individual level may be divided into fire-specific and general mental health variables. Fire-setting juveniles have often many general characteristics indicating behavioural problems. However, their personal history in fire-setting and developments in it are often indications of a pathological condition. Literature in the field offers alternatives in treatment young arsonists and for assessment tools, but they are relatively seldom applied into practise. The authors concluded that the research in the field is still quite preliminary.

Another literature review considered the existing body of research, theories and practices concerning young arsonists(131). For example, typologies, risk factors and treatment relating to the phenomenon were reviewed. The review is also an overview of the current research evaluating the relation between theoretical and empirical research as well as their strong points and weaknesses. The review concluded that different theories contradict each other and so do empirical studies, too. The surmised reason for this contradiction was that the fire-setting behaviour is a complex pattern and the arsonists form a versatile group of juveniles.

It is known that juvenile fire-setters form a heterogenic group, but hardly any more specific empirical classification has been done(132). This study approached the problem using cluster analysis to develop a classification of juvenile fire-setters based on both their general individual and environmental characteristics and fire-specific variables associated to fire-setting severity

and recidivism. Based on these factors, it was possible to empirically separate juvenile fire-setters into three classes: home-instability-moderate, conventional-limited and multi-risk-persistent fire-setters.

Motivation has been found as an important factor for juveniles' tendency to set fires and in their subsequent treatment(133). This study attempted to classify the motives for fire-setting by gaining insight into how both the fire-setters and their custodians understood motivation for such behaviour. Data was collected by interviewing 18 male youths engaged in fire-setting and 13 parents. Based on qualitative analysis of the data, young fire-setters were found to have certain motives such as experimenting, anger, peer pressure and boredom whereas according to the parents, factors relating to family history had influenced the child's fire-setting motives. The study concluded that there were often multiple motivations involved making classification of fire-setters difficult.

There are intervention programs available for children and adolescents engaged in deliberate fire-setting(134). Often these programs involve educational and psychosocial approaches. Effectiveness of these interventions is usually evaluated in respect to the recidivism rates, but Lambie et al. have made a study analysing the strengths and weaknesses of a certain intervention process from the perspectives of the program consumers involved. Data was derived from in-depth interviews with children and their parents/caregivers and qualitative analysis methods were employed The results indicated that the intervention program in question was generally regarded as a positive thing, but there were some points for development found as well.

Gender differences in fire-setting tendencies have also been analysed(135). Data for the study were derived from National Survey on Alcohol and Related Conditions with a specific focus on the subjects' lifetime histories in fire-setting. Based on multivariate logistic regression analyses, both genders associate often with psychiatric and addictive disorders, although women were more likely to have such diagnoses than men. Characteristics, psychopathologies, and current treatment efforts with female arsonists have also been reviewed(136). Meanwhile previous research on female arsonists was evaluated with the conclusion that it would serve the purpose to develop further the research on female arsonists.

Fire-setting behaviour is often evaluated from the perspective of mental health, but there is not so much literature on fire-setting related forensic evaluations available(137). In their study, Burton et al. have dealt with fire-setting, the diagnosis of pyromania and the crime of arson on the basis of research made in respect to fire-setter characteristics, recidivism, classification systems and treatment. Using examples, the study reports several types of fire-setting-related evaluations referred to forensic mental health experts. Evolving medico-legal concepts of arson and pyromania are monitored in another study focusing in mental health with the aim to raise current knowledge about mental disorders in the context of arson(138).

An example of a more rare fire-setting behaviour due to brain dysfunction was also reported(139). It is the first case of fire-setting behaviour caused by focal brain lesion reported. A 47-year-old man had been arrested for arson and he had complained of memory impairment and difficulties in concentrating. Medical examinations indicated that he had had a lacunar stroke just before setting the fire. The study concludes that the reason for the bizarre fire-setting behaviour was the brain dysfunction.

11 International Co-Operation

11.1 Working groups and conferences

International working groups aim to share knowledge, share experiences, collect information, develop methods and give advice to working group members. The European Network of Forensic Science (ENFSI) is recognized as an expert group in the field of forensic sciences. One of them is the Fire and Explosion Investigation Working group (FEIWG). The sphere of activities of the working group is field investigation, technical investigation and chemical analyses of fire debris samples. FEIWG strives to ensure the quality of development of fire and explosion investigation. There are three sub-committees: Fire Scene, Accelerants and Explosion. FEIWG has organised the New Science Seminars in Switzerland in 2010 and the topic was fire debris analysis. The Working Group published the Guidance on the identification criteria of ignitable liquids in 2012 (140).

In the USA, the Technical Working Group of Fire and Explosion (TWGFEX) maintains co-operation among personnel in the Forensic laboratory, public safety, private investigation, and legal communities. The Working Group develops protocols and/or guides for collection and analysis of fire and explosion debris, training and quality assurance and introduces new techniques in the field of forensic fire and explosion investigation and laboratory analyses. TWGFE has three active scene committees: Education/Training, Fire Modelling Database and Scene Protocol Committee, and six laboratory committees: Explosives Education and Training, Explosives Database, Explosives Standard Protocols, Fire Education and Training, Ignitable Liquids Database and Fire Standard Protocols (141). TWGFEX has published a Programs Certifying Fire Investigators (September 2010) and Self Heating Processes, Training guidelines for the Fire Debris Analyst (November 2012)(142).

The Ignitable Liquids Reference Collection (ILRC) and the Substrate Databases are developed jointly by TWGFEX and the National Center for Forensic Science (NCFS). The Ignitable Liquids is a compilation of reference materials used by forensic analysts to conduct fire debris analysis. The ILRC consists of a comprehensive set of ignitable liquids and accompanying characterization data used in the analysis of fire debris samples in accordance with the American Society for Testing and Materials (ASTM) E-1618 standard test methods(143). Another TWGFEX database is a substrate database, which is a tool designed for screening purposes only. It does not replace the need for obtaining comparison samples to evaluate the matrix, but it gives

good tools for estimating the results(144). These two databases are easy to use and all information is valuable for regular work.

Other databases are Thermal Properties databases, which are hosted by National Center for Forensic Science. The Burning Item Database is a collection of fire test data for commonly used household/office furniture (i.e. chairs, sofas, mattresses, bookcases, etc.) From the websites it can be find articles, books and technical websites(144). Material Thermal Properties Database is a small collection of thermal properties for materials used to construct common objects found in households and offices(145).

Other important forensic discussion forums are international conferences. The European Academia of Forensic Science (EAFS) organises Triennial Meetings, stimulates OOS workshops and effective transfer of knowledge between institutions. In the EAFS conference organised in 2012, the Fire and Explosion Investigation working group (FEIWG) had several oral presentation and posters (146). The American Academy of Forensic Science (AAFS) organised annual meetings including workshops, presentations and posters(147). The Australian and New Zealand Forensic Science Society (ANZFSS) holds an International Symposium every two years. The meeting and sessions cover the major disciplines of forensic science(148). The International Association of Forensic Sciences (IAFS) brings together academics and practicing professionals of various disciplines in forensic sciences and organizes triennial meetings(149).

11.2 International Collaborative Tests

Quite many forensic laboratories are accredited and they need a collaborative or proficiency test to prove the reliability of the test results. One possibility is Collaborative Testing Services (CTS)(150), which combines experience with forensic interlaboratory tests as Flammable Analyses. All participants report the results and methodology used in case it may help others to develop their own analyses. For the Flammables tests, examiners provide the detection, identification and comparison of flammable residue evidence. In 2012, the Flammables Analysis Test was sent to 355 participants and 298 participants (84%) returned the data. In approximately 89% of the replies the identification of the flammable substance was correct(151).

The ENFSI Fire and Explosion Investigation Working Group is the provider of a Collaborative Testing Program for ignitable liquid analysis and fire scene investigation to Working Group members. The collaborative tests are designed to share and exchange knowledge on subjects such as techniques, products used as accelerants in various countries, matrix and weathering effects and so on. They are not designed to monitor the performance of individual laboratories like the proficiency tests. Participation in this ENFSI collaborative testing programme gives the laboratories an opportunity to review their original methods by taking advantage of the varied information derived from these exercises. The overall review of the laboratories' results is carried out by the organising committee and represents one of its valuable outputs(152). In the test organized in 2012, forty one laboratories were listed

to participate, 39 of them (95%) returned their analysis results for evaluation and 49% of these are accredited. Thirty four (87%) laboratories gave the correct conclusion and five (13%) laboratories gave the wrong conclusion according to the evaluation(153).

Table 1. Results of CTS-tests.

CTS-test	2010(154)	2011(155)	2012(151)
Participants	367	358	355
Returned	305 (83%)	309 (86%)	298 (84%)
Correct answer from the returned	97%	98-99%	81-97%

Table 2. Results of ENFSI/FEIWG-tests for ignitable liquid.

ENFSI/FEIWG-test	2010(156)	2011(157)	2012(153)
Participants	44	26	41
Returners	42	24 (92%)	39
Correct answer from the returned	varies per element of the test	19 (79%)	87%

12 Summary

Road traffic accidents often result in motor vehicle collision fires and they can involve serious injuries and deaths. Detection of electrical fires and establishing circumstances relating to fire scene investigation have been paid special attention especially by emphasising the proper way of sampling and documentation. As new energy forms such as solar energy and wind energy and as e.g. electric-powered vehicles have become more common, forms of energy storage must be considered from the point of fire cause investigation.

Various kinds of packaging materials have been developed for fire debris sampling and properties of these materials have been compared with each other. The analysis results show that the AMPAC bag is one of the most suitable for packing fire debris. Taking ignitable liquid samples from the suspected arsonist's hands for analysis has raised a lot of interest within the scientific community. There seems to be an understanding on the need for developing a generally approved user-friendly method for the purpose in the near future. Ignitable liquids are also extracted from a suspected arsonist's hands using passive adsorbtion (i.e. activated charcoal strips) and absorbent material used in cases of chemical leakage has also been tested for adsorbtion.

SPME, ACS and Tenax TA® are all well-established methods for extraction and concentration ignitable liquids from fire debris bags. However, new methods have been developed and tested for the purpose, e.g. Radiello Passive Air Sampler, Headspace single drop microextraction (HS-SDME) and the polymer particle-packed extraction needle.

Ignitable liquids are very often analysed in laboratories using gas chromatography-mass spectrometry (GC/MS) with multivariate analysis methods, such as PCA. A self-organizing feature map (SOFM) is a potential way to classify and visualize, in particular, differences between samples. In addition to identification, changes in ignitable liquids caused by weathering and microbial degradation have proved to be an interesting and useful research topic. For example, Advanced Distillation Curve (ADC) has been proposed as a method to analyse the changes. Statistical analysis methods and e.g. pyrolysis technology have been used in analysing interferences caused by the background matrix.

In addition to reconstructions made in the context of fire cause analysis, numerical modelling has got a lot of scientific attention, as it is easier and more inexpensive to use than reconstructing a whole fire scene. However, computational modelling programs have not been designed for solely forensic purposes, and therefore some expertise is required for using these computer programs. It is likely that modelling will provide more possibilities for forensic analysts as the computational power and capacity grow.

Both theoretical knowledge and practical understanding of the motives for deliberate fire-setting are valuable in assessing and treating patients with symptoms of fire-setting behaviour. Empirical studies indicate that a low economic status and unstable childhood are associated with fire-setting behaviour.

Importance of international cooperation between fire cause analysts has grown due to the global economic crisis. Research resources are reducing, and a need for the exchange of information and experience by examiners from different countries has been recognised throughout the scientific community.

13 Books And Other Publications

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Analysis and Detection of Explosives and Explosives Residues

Review: 2010 to 2013

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1 Introduction and Coverage of the Literature

This current review starts with the previous paper covering explosives analysis from 2007-2010 presented in 2010 by Richard Strobel. It was extensive due to "the improved computer networking, the web, and the growth of abstracting services utilized by library technicians worldwide." [31] This review is no different. Despite the known austerity measures imposed nearly worldwide on governmental organizations, academia, and even on private research organizations and companies, there are still "hundreds of citations listed…because of result of the ability to survey specialty journals which were previously unknown to the forensic practitioner" [31] and because of continuing research. Forensic science, especially where it is based in the ever-developing sciences of theoretical and applied chemistry and physics, will hopefully continue to opt for the newest and best technology if it serves the purpose of the analyst, such as ease of use and covering a wide range of analytes. The forensic explosive analyst community is well served by surveying the available literature in all aspects of explosives and not just to borrow techniques, but also in an academic sense. Many aspects of explosives, including explosive properties, behavior, chemical structure, and physics should be a part of the overall continuing education of a good forensic analyst.

There are many applications in the overall field of explosives which may be of interest to the forensic analyst tasked with examining explosives for law enforcement purposes. The explosives detection field, which is primarily for security purposes, is both the fastest growing and most proliferated area from which forensics can draw. There are a host of references from this area, ranging from theoretical research to applied systems that are already in field use. Some of these papers may seem esoteric or limited on the surface but are worth perusing, especially if the technique can practically be more broadly applied. There are also hundreds of projects dedicated to the area of environmental explosive remediation, environmental effects, and the analysis therein. This is another area worth exploring.

There are 1341 references in this review. Most of these abstracts are hyperlinked when possible, thus a direct pathway to the abstracts of the articles are included. Another feature is the "bookmark" feature in Microsoft Word. This will aid the navigation of the bibliography section, starting on page 20 of this document. Many of these references could fall into two or even three categories. They will not be presented in multiple places, so it would be advantageous for the reader to peruse all of the text and the reference titles.

2 Review Articles

Several review articles were published in the last three years in the field. Some concentrate on very broad schemes of analysis employed in operational forensic science laboratories, some give a decent historical perspective of same, and many are concentrated on a specific type of instrumentation, explosive, or analysis.

Several reviews and overviews are given in the Encyclopedia of Forensic Sciences (2nd Edition). Speer, Otieno-Alego, and Ritchie cover Clandestine Explosive Laboratories [30], Beveridge covers Improvised Explosive Devices [5], Murray covers Military and Commercial Explosives [25,26], while S Doyle covers Improvised Explosives.[11]

An excellent overview and indispensible primer of explosives analysis as applicable to the forensic laboratory is provided by Tamiri and Zitrin.[34] Current techniques for forensic and detection work are reviewed and highly recommended. In addition, the authors offer insight into proper sample preparation and extraction techniques, as well as schemes of analysis especially for limited samples.

Similarly, Gruznov et al present a very detailed paper on progress in methods in Russia for the identification of explosives and it covers a wide range of chromatographic, spectral, and nuclear methods.[16]

An overview of several detection techniques are given by Lehnert and Kearfott and is well worth seeing which emerging techniques can be applied to forensic usage.[21] Likewise, Caygill, Davis, and Higson present a comprehensive overview of current trends in detection technology.[9]

A very detailed and theoretical exploration of Ion Mobility Spectroscopy (IMS) is presented by Buryakov [8] and one by Morelato, Beavis, Kirkbride and Roux give an overall review of DESI-MS for forensic applications including explosives.[23] Likewise, Mäkinen, Nousiainen, and Sillanpää provide an exhaustive review of IMS for the detection of ultra-traces of explosives.[22]

Izake gives us a thorough review of Raman Spectroscopy including theory, applications for stand-off detection, resonance enhanced Raman, spatially offset Raman (SORS), and surface enhancement Raman (SERS).[18]

Skvortsov and Maksimov review laser photothermal spectroscopy for stand-off detection of trace explosives and many of the existing stand-off configurations are explored.[29]

3 Explosive Standards and References, Laboratory Quality Control, Contamination Prevention

Crowson and Cawthorne describe upgrading the Quality Assurance program in a trace explosives laboratory by including peroxide explosives monitoring.[38] They use an LC/MS/MS system for HMTD and TATP.

Staymates et al use precision particle fabrication to make monodisperse polymer microspheres that contain high explosives with a decent degree of encapsulation.[40]

4 Sampling and Concentration of Explosive Traces

Sampling in both the forensic and detection environments remains crucial to the successful outcome of the mission. Much research has been devoted to optimizing the sampling process.

Fan et al adapt a planar solid-phase microextraction process (PSPME) to an IMS detector for the fast detection of TATP.[51] This seems to be an improvement for this analyte over traditional SPME.

Fryš et al report the optimal time (*20 minutes*) and amplitude (*35%*) of focused ultrasonic extractions of various amounts of smokeless powder samples.[52]

De Tata, Collins, and McKinley compare common swabbing material for the optimal recovery of both organic and inorganic explosive residues. Cotton ball swabs seem to perform consistently well with both the acetonitrile and ethanol:water solvents. Also reported is that sonication greatly enhances the removal of organic residues from the swabs over time.[49] Another study by De Tata, Collins and McKinley compared the efficacy of solvent extract cleanup procedures for organic explosives. Here they present the extraction efficiency of SPE, adsorbent resins such as Chromosorb-104 and also silica and Florisil, under simulated contaminated samples.[50] It is an excellent resource for those dealing with difficult real world samples.

Oxley et al describe how explosives bond to hair, involving the 18-methyleicosanoic acid lipid layer, and the possible melanin granular surfaces as a site for TATP crystals.[63]

Song-im, Benson and Lennard studied the storage effects (30 days) on a glass surface of four organo-nitrate explosives and two inorganic anions on swabs taken with 60% methanol based polyester wipes. They recommend low temperature, low light environments for the samples, especially TNT and TATP.[67] In another study the same researchers propose a “universal” swabbing and clean-up procedure that covers both organic and inorganic analytes after testing four commercial SPE cartridges and assessing the efficacy of acetone, acetonitrile and methanol with water of various ratios. They recommend the ABS ELUT Nexus® cartridge and a 60% methanol to water solution.[66] Finally, the same authors report that two types of commercial skin cleansing alcohol wipes performed better than conventional cotton and polyester swabs with various solvents in recovering both organic and inorganic analytes. They tested a variety of substrates here as well.[65]

Hansson et al use HPLC/UV and GC/MS in accelerated aging studies of post-blast samples of C4, a Swedish military explosive containing PETN and mineral oil (85:15) and an EGDN/NG dynamite. On a sand substrate the screening methods revealed that most residues were at low or undetectable levels after eight weeks of accelerated aging.[54]

5 Identification of Explosives, Explosive Residues and Explosive Properties

There are several papers reporting properties of explosives and theoretical modeling of explosive behavior.

5.1 General:

Aydemir and Ulas provide an extensive mathematical model of the thermal initiation of a confined explosive in 2-D geometry.[75]

Chaffee-Cipich, Sturtevant and Beaudoin look at the adhesion properties of TNT, RDX and PETN on three surfaces with atomic force microscopy-based colloidal probe microscopy where the explosive particles were mounted on AFM cantilevers.[85]

Zhang and Weeks construct a device for testing thermal impact sensitivity of explosives. [174]

Castro et al use several techniques (SEM-EDS, FTIR, and Raman) to investigate “liquid” explosive fireworks.[84]

5.2 TATP:

An interesting project was conducted by Fitzgerald and Bilusich [190] comparing headspace samples with SPME GC/MS of sulfuric, hydrochloric and nitric acid catalyzed samples of TATP where chloroacetone and 1,1,-dichloroacetone were detected in addition to the TATP for the HCl catalyzed samples only and could give investigators or prosecutors additional information. They further investigated whether they could see these by products in aged TATP and reported that they were successful.[189]

The behavior of TATP alone and when combined with TNT, AN, and nitroguanidine were investigated by DSC and Raman. Typically, they show an upward temperature shift for TATP decomposition as well as decomposition of the nitrated compound initiated by the well known decomposition of TATP. [196] (Ramírez et al)

Oxley, Brady, Wilson and Smith investigate TATP formation with various levels of hydrogen peroxide and acetone concentrations and report that when mixing dilute solutions of both (3% peroxide and 7% acetone) that no significant amount of TATP will form, and parts per million of TATP may form if an acid catalyst is introduced. Other results are given in useful tables. [194]

Zhang, Zhang, and Chen use an In_2O_3 nanoparticle sensor (developed in a one step glucose-assisted process at low temperature) for the detection of TATP [200].

5.3 Urea Nitrate:

Chemists are frequently asked if a finished homemade explosive (HME) can be linked to recovered precursor chemicals. Aranda et al show that the “isotopic composition of reactants in UN, along with a significant variability in isotopic composition of reactants, indicate that isotope analysis may be used to test if urea or nitric acid collected during an investigation is a possible reactant for a specific UN sample.”[201]

5.4 PETN

A unique case study is presented by Brust, van Asten, Koeberg, van der Heijden, Kuijpers, and Schoenmakers where they discriminated post-blast degradation product profiles of PETN (PETriN, PEDN, and PEMN) versus simple aged degradation profiles using LC-MS and determined a safe cracking thief was exposed to post-blast PETN.[81]

5.5 ANFO

A study of ANFO detonations in a high-sound-speed, shockless aluminum conifer is reported where the aluminum transports detonation energy *in front* of the detonation front.[230] (Jackson, Kiyanda and Short)

5.6 Peroxide Explosives (General):

A good primer on the remediation and destruction of many types of peroxide explosives is provided by Oxley, Smith, Huang and Luo which investigates use of metals and metal salts applied to solutions of peroxide energetics.[210]

5.7 Other Explosives including Novel or New Explosives:

One of the fastest growing technologies in the field of new explosives is the use of nanoparticles incorporated into well known explosives or employed on their own. Several studies report on the behavior of these explosives.

One paper shows that nanocomposite microparticles of RDX have reduced shock sensitivity (Qiu, et al [291]). Wang et al similarly report that the shock sensitivity in the Small Scale Gap Test that both RDX and HMX are reduced by 45% and 56% respectively versus microparticles of each explosive.[297]

Qiu et al report a single step spray drying nanocrystal production of HMX.[292]

Ermoline, Schoenitz and Dreizin investigate the reactions of “aluminum-metal oxide energetic compositions with components mixed on the nano-scale” and find them “substantially more reactive than conventional thermites”.[282] These have possible future use as propellants.

Vignes et al report that aluminum nanopowders have increased potential for lowering minimal energy needed for ignition as size of the particles decrease but also show lower explosion “severity” for particle sizes less than 1 micrometer.[296]

Dubey, Srivastava, Kapoor, and Singh synthesize copper nanoparticles and show via TG and DSC that these particles lower the energy of activation for thermal decomposition and energy for ignition of ammonium perchlorate and composite solid propellants as well as for HMX and NTO (5-nitro-2,4-dihydro-3H-1,2,4-triazole-3-one).[281]

Lewis et al present a very good research project on comparing RDX charges with RDX and nanometer diameter aluminum and micrometer diameter aluminum, and find that the early temperature of explosion fireballs was hotter for both aluminized RDX mixtures with the nanopowder charges being slightly hotter. Aluminum nanopowders yielded higher early temperatures but of less intense and shorter later emissions while the aluminum micron powders produced lower early temperatures but longer more intense later emissions.[285]

Bouillard et al report that as nanopowders decrease in size the minimum ignition temperature (MIT) and minimum ignition energy (MIE) decrease while the minimal explosion concentration did not do so and plateaued both in a theoretical model and experimentally. They also report that carbon nanopowders have a low propensity to explode but that metallic nanopowders are reactive and vulnerable to explosion.[277]

Kozak et al investigate the explosive transformations of HMX and benzoyl peroxide mixed with aluminum and find that the state of the aluminum introduced and the explosive temperature influence the resultant aluminum oxide structure.[234]

Koch et al use near infrared (NIR) to investigate the spectra of what they term “break-out” of PETN explosives doped with aluminum and silver particles, and silver coated aluminum particles.[233]

An attempt at identifying more environmentally friendly explosives is reported by Thottempudi and Shreeve. [258] The authors discuss the synthesis and properties of high density energetic salts of 5-nitro-3-trinitromethyl-1*H*-1,2,4-triazole and 5,5’-bis (trinitromethyl)-3,3’-azo-1*H*-1,2,4-triazole.

Another interesting new explosive is synthesized by Jin et al. They synthesize polymer polyvinyl acetate azide (PVAA) and evaluate its properties. They conclude that it is resistant to thermal decomposition up to 200 degrees C and is insensitive enough to be safely used in cast explosive(s).[231]

6 Instrumental Analysis of Explosives

6.1 LC/HPLC/UPLC

Much of the work of the forensic scientist in the laboratory is devoted to utilization of instrumental techniques to identify explosive traces. LC /HPLC/UPLC is an excellent separation technique and can be a part of a positive identification if coupled with specific detection methods, or by using orthogonal methods.

Tarvin, McCord, Mount, and Miller have investigated two recently developed HPLC methods for the analysis and confirmation of the TATP and HMTD pre-cursor, hydrogen peroxide, by employing fluorescence detection using post-column derivatization and electrochemical detection in field samples [308] and another publication dealing with optimization of these techniques [309]. Similarly, de Perre and McCord describe an LC-UV/Fluorescence method for the specific identification of urea nitrate as an entity.[301]

Tyrell et al have coupled reversed-phase HPLC with an IC system which provides, in under 25 minutes, a full suite of inorganic and organic analytes. The organic phase used a reversed- phase silica based Dionex Acclaim® Explosives E2 column with 210nm detector and the ionic phases were analyzed employing a hyperbranched anion exchange column and detected fluoride, chloride, chlorate, benzoate, nitrate, azide, sulfate, phosphate, thiocyanate, and perchlorate using suppressed conductivity detection.[310] Similar work is described by Xie et al.[311]

HPLC-UV was followed by photo-assisted electrochemical detection (PAED) in determining RDX and its degradation products in work by Fedorowski, LaCourse and Lorah.[302]

An excellent technical note by Cummins, Hull, Kitts, and Goodpaster describe using a porous graphitic carbon stationary phase for HPLC coupled to an electrospray mass spectrometer for the analysis of inorganic anions commonly found in post blast explosives.[300] It separates six common anions in five minutes.

6.2 Ion Chromatography

The technique of Ion Chromatography is frequently used in forensic post-blast analysis with a specific detector, the mass spectrometer. Traditional detection is still used as well.

López-López et al differentiate between smokeless powder nitrocellulose and colloidal nitrocellulose by alkaline hydrolysis and ion chromatography with suppression on conductimetric detection using the concentration of nitrate and nitrite ions in the hydroysate.[315] Gel permeation chromatography still exists as a method for differentiation of nitrocellulose types. Fernández de la Ossa et al review several techniques including gel permeation chromatography to study highly nitrated nitrocellulose.[313]

6.3 Gas Chromatography

GC continues to play a role in the separation of explosive compounds from various matrices prior to detection by a variety of means. Many references will be found in other sections.

6.4 Capillary Electrophoresis

While not as common as HPLC and IC are, CE still enjoys a prominent place in the suite of instruments for analysis of explosives. Its advantages are that it can be employed in both organic and inorganic analysis relatively quickly. Theoretically, CE offers advantages over IC in the sheer number of species it can analyze simultaneously.

Sarazin et al studied the use of CE in the identification of inorganic ions including azide and separated 19 anions in less than 20 minutes with diode array detection.[337]

Sarazin et al also report three distinct CE methods for use in a simulated suicide attack by looking at anions, cations, and carbohydrates, which may be useful in looking at some improvised mixtures such as those attacks where flour or sugar are used. All three systems are thoroughly described.[334]

Blanco et al look at inorganic explosives collected from an actual explosion using sequential injection CE and contactless conductivity detection.[328]

6.5 General Spectroscopy: Fluorescence, Luminescence, Spectrophotometric, UV, Chemiluminescence

Many of the techniques offered in the spectroscopy area fall into distinct categories of spectrum. Many of the techniques apply to specific explosive compounds or processes. Some of the techniques are applied to detection as

opposed to applications for the forensic laboratory. Nevertheless, they could have a future application in the forensic field.

An interesting paper is presented by Crespy et al describing the optimization of energy dispersive X-ray diffraction (EDXRD) to identify explosives.[349]

Li et al describe a novel recyclable aggregation-induced emission luminogen to detect picric acid in water.[366] Venkatramaiah, Kumar and Patil develop a novel chemosensor for picric acid.[388]

Li et al present a rapid portable detection and identification system for several organic explosives using a UV reflected fiber optic sensor and nanoliter droplets.[368]

Bouhadid et al compare three different fluorescent materials for their efficacy in detecting explosive vapors.[341]

Guenther et al propose using pulsed laser fragmentation (PLF) and a q-switched UV microchip to detect organic nitrated explosives by their NO:NO_2 concentration ratio. [360]

Wei et al use organic-inorganic hybrid polyphosphazene microspheres for the trace detection via fluorescence of nitroaromatic compounds.[391] Microspheres appear to exhibit better thermal stability, photobleaching stability, as well as solvent resistance.

Abdelhamid et al use LIBS with optical catapulting to analyze explosive residues (in the form of solid aerosols) in fingerprints on glass surfaces.[338] They report an advantage over traditional LIBS because of the absence of contamination of the sample and spectral contribution of the substrate.

6.6 Mass Spectrometry

The variety of mass spectrometric techniques continues to grow in the last three years. There are many possibilities in the selection of type of mass detector, how to achieve fragmentation, and the ionization of compounds of interest.

de Perre, Prado and McCord published an excellent study using 18-crown-6 ether to detect urea nitrate and ammonium nitrate using electrospray ionization and time-of-flight mass spectrometry. [407] The study also repeats earlier studies using various complexing agents to get optimal results with AN and UN analytes.

Nilles, Connell, Stokes, and Durst study a variety of explosives and substrates (75 combinations) using direct analysis in real time (DART) in a very comprehensive paper.[420]

Joshi, Rigsby and Almirall conducted a study of the headspace composition of smokeless powder samples by GC-MS, GC-uECD and SPME IMS finding 2,4-dinitrotoluene, diethyl and dibutyl phthalates, ethyl and methyl centralite, and diphenylamine indicating possible detection of smokeless powder constituents *in situ*.[415]

Rowell et al report the detection of several nitro-organic and peroxide explosives in fingerprints from six commonly encountered surfaces by using DART and surface-assisted time of flight mass spectrometry (SALDI-TOF-MS).[424]

Brady, Judge and Levis use laser electrospray mass spectrometry (LEMS) (Time of Flight) to do direct spectral analysis of explosives at atmospheric pressure on steel surfaces. A variety of explosives are explored including DMNB, RDX, HMTD and TATP.[403] They also show a multidimensional detection of explosives with LEMS as well.[402]

Kozole et al interface an IMS explosives trace detector to a triple quadrapole mass spectrometer.[417]

Improvements on DESI mass spectrometry were reported by Soparawalla et al. [427] Here, the researchers improve the desorption/ionization area by reportedly 200 times.

In a very interesting paper, Sokol, Jackson, and Cooks uniquely apply DESI mass spectrometry to detect traces of inorganic oxidants. [426] The versatility of this technique is apparent to the authors.

Téllez, Vadillo and Laserna use secondary ion mass spectrometry (SIMS) to directly detect explosives deposited on sticky tape.[430]

Takada et al present a detection portal using an AP chemical ionization ion trap mass spectrometry to detect TATP vapor.[429]

6.7 Isotope Ratio Mass Spectroscopy, IRMS

Carames-Pasaron et al propose the development of a dual-isotope procedure for the tagging and identification as it applies to the manufacturing of explosives.[434]

Gelman et al researched GC-IRMS for the precise and accurate compound-specific carbon and nitrogen isotope analysis of RDX by minimalizing the thermal decomposition of RDX.[435]

An excellent and forensically useful primer on the use of isotopic comparisons between precursors and the final product of HMTD is presented by Lock et al.[436]

6.8 FTIR

Fourier Transform Infrared Spectroscopy, and prior to that, IR, has had a long history in forensic explosives analysis.

Open path FTIR detection of explosive solids on metallic surfaces is explored by Castro-Suarez et al.[439] This could also be applicable in stand-off detection as the technique seems to be viable at 8 meters.

Osborn, Burns, Green and Reeve propose an “optical nose approach to explosive detection” by using spectral methods (IR, Pb salt diode lasers, DFG laser system, etc) to look at volatile vapors and specifically at C-4.[442]

Shishkov et al present two papers on the investigation of long-term aged explosives (of TD-50 and tetryl Bulgarian explosives made in 1961) with UV-VIS and FTIR.[445]

6.9 Raman Spectroscopy

The largest application of the Raman technique is in stand-off detection. Some applications are noted here.

There are some additional Raman uses such as surface-enhanced Raman to look at trace levels of explosives on a variety of surfaces by Botti et al.[449, 450]

Using Raman and mapping to detect every part of the whole sample of heterogeneous dynamite is discussed in a technical note by López-López, Ferrando, and García-Ruiz.[466]

Tripathi et al use Raman to detect explosives (e.g. RDX here) in fingerprints on a variety of substrates including those with active Raman spectra such as polystyrene and polycarbonate.[474] A semi-automation process for this is included in another paper.[475]

A hand held Raman spectrometer (in this case a ReporteR®) is also useful for determining the *concentration* levels of hydrogen peroxide in aqueous samples (Stewart et al [472]).

6.10 DSC, Thermal Analysis, TG

Sućeska et al look at the kinetics and activation energy of nitroglycerin evaporation by isothermal thermogravity [489] and the figures might be useful in comparisons of propellants.

Shock sensitivity of RDX from five manufacturers is described in work by Bellitto et al. [483] They analyzed RDX with DSC and atomic force microscopy (AFM) and found that there is no statistical relationship between HMX impurity and surface roughness impacts the shock sensitivity of RDX. The AFM data likewise showed the same result. However, the DSC curves are different with differing levels of HMX impurity in the RDX

7 Nanotechnology

One of the most exciting aspects in explosives in the last decade has been the development of nanotechnology. The microsensing field will be applicable both in field and laboratory testing of explosives. For that reason it is included here, just prior to the review of detection systems and technology.

A miniaturized DESI instrument using negative chemical ionization mass spectroscopy for trace explosives detection (TNT, tetryl, and HMX)is reported in work by Sanders et al.[517]

Much of the sensing technology overlaps detection with environmental testing and involves fluorescent detection techniques. Woodka and Schnee use a commercial fluorescent polymer to make a sensor array for detection of a variety of explosives in water.[527] Wang, Guo, Li, Chen and Sun describe using amphilphilic cellulose nanoaggregates for use in water.[524] Heller et al at M.I.T. apply secondary structure peptide modulates on carbon nanotube sensors for detection of nitroaromatics.[501] Riskin et al explore molecularly imprinted gold nanoparticles for the surface Plasmon resonance detection of the nitrate esters PETN, NG, and EGDN.[513] Ruan et al show that in situ synthesized carbon nanotubes on a microcantliever system can detect 2.4 pg of TNT.[514]

Cottineau et al synthesize vertically aligned titanium dioxide nanotubes on microcantilevers and report a “surface enhancement factor of circa 70 and an explosive molecule absorption improvement by 100” for TNT.[496]

A photoplastic microcantilever sensor platform for explosive detection with optical transduction and resistance piezoresistive film is described by Seena et al.[518]

Dobrokhotov et al use novel nanotechnology by using silica nanosprings coated with ZnO and metal nanoparticles to detect, via conductance, the explosives TATP and TNT and flammable vapors from toluene, acetone, and ethanol.[498]

A *chemiresistive* (as opposed to fluorescence) immunosensor using carbon nanotubes for the detection of nitroaromatics (in this case TNT) is detailed by Park, Cella, Chen, Myung and Mulchandani.[511]

Zhu, Park, Sessler and Gaitas combine a colorimetric detector, a tetrathiafulvalene-functionalized calyx pyrole, with a polyimide microcantilever to detect TNB vapors.[536]

Wang et al synthesize a metal-organic nanocrystal for the detection of nitroaromatic compounds. Here, they treat Cd(II) ion with the sodium salt of 2-aminoterphthalic acid).[525]

8 Detection

There are many references included here that are probably not directly applicable to forensic analysis but may have the propensity to be useful if borrowed. There are many investments in this industry, and some systems are better than others both for their intended purpose of detection and possible overlap with forensic laboratory usage. It has been shown that IMS and other spectrographic techniques can make the transition somewhat easily. The references are arranged as follows:

8.1 Canine Explosives Detection

Though not explicitly the detection of explosives, there is a study showing the viability of using canines detecting human scent (i.e. the bomber) in post-blast debris ([636] Curran, Prada, Furton). This could prove useful for on scene investigators.

Moore, MacCrehan and Schantz describe using automated training aid simulation materials for canine training.[644]

8.2 LIBS detection

Lucena et al describe some approaches to avoid secondary ionization in laser induced breakdown spectroscopy.[663] Roberson and Sausa use a two laser technique to look at TNT and RDX in real time and ambient conditions. The second laser at 226nm will photofragment and ionize NO.[671]

Morton, Torrione and Collins develop a theoretical chemometric technique for the use of laser induced breakdown spectroscopy (LIBS) on various substrates for the detection of explosives.[668]

8.3 Neutron

Papp and Csikai use neutron techniques to detect and identify illicit drugs and explosives.[708]

Laikin and Platovskikh explore the theory of optimizing spectrometric data by neutron-radiation and inelastic neutron scattering, based on the ratioing of nitrogen, oxygen, and carbon (comparing explosive signatures to non-explosive materials).[698]

8.4 Terahertz

Karam and Meyer report a methodology to determine the *type* of explosive detected with a non-imaging polarized terahertz passive system by using a set of algorithms (using dielectric content/refractive index values) and comparing them to a known database of explosives.[723]

8.5 Nuclear Techniques

Espy et al report progress using NMR and magnetic resonance imaging (MRI) in the ultra-low field (ULF) to detect liquid explosives and other liquids that DHS lists as excluded from airplanes.[747]

8.6 X-Ray

Work at the National Institute of Standards and Technology (USA) by Hudson et al reviews a newly constructed standards infrastructure for the detection of bulk explosives using x-ray or gamma-ray screening. They explore existing safety and imaging standards and questions.[765]

8.7 Ion Mobility Spectroscopy

Zhang et al describe using desorption electrospray ionization DESI-MS of aromatic amines and observations of surface reactions with same.[790]

Gilbert-López et al describe an ambient diode laser desorption dielectric barrier discharge system for sample for IMS for nonvolatile chemicals including the explosives HMX and RDX.[777]

Planar solid-phase microextraction IMS with a novel diethoxydiphenysilane coating is employed in TNT, DNT, and EGDN sampling in work reported by Mattarozzi et al.[783]

Najarro et al report improving the IMS signal for TNT and HMX by a factor of 5 and RDX and PETN by a factor of 10 by optimizing the desorber temperature.[784]

Staymates, Smith and Windsor study the transfer of explosive analytes from the swabbing material in IMS instrumentation and find that the limiting factor in a thermal desorption unit is probably the flow field around the swipe and not necessarily the heterogeneity of the heat transfer to the swipe.[788]

8.8 Novel Detection

Sausa and Cabalo investigate the detection of TNT and RDX with laser (near-infrared) and sound monitoring with photoacoustic overtone spectroscopy.[872]

Freeman et al use functionalized CdSe/ZnS quantum dots (with electron donating ligands) as fluorescent probes for the analysis of TNT and RDX.[854]

Fujiyama-Novak, Gaddam, Das, Vander Wal, and Ward couple a pre-concentration and separation system to a micro-hollow glow discharge

(MHGD) plasma detector and looked at TATP and DNT. The system is described as miniaturized and portable.[855]

Poling et al report on the use of trained Giant African pouched rats in the detection of land mines.[869, 870]

Shiou-jyh Ja reports on a novel detection system using surface plasma-couple emission by using the spectroscopic information generated by the surface Plasmon coupling emission and has the reported ability to detect and classify several explosives with two sensing materials in a prototype.[858]

8.9 Stand Off

Misra et al use a compact stand-off Raman and 85mm camera to detect targets at 50 meters. The targets are precursors of homemade explosives and many types of oxidizers including ammonium nitrate, potassium nitrate, potassium perchlorate as well as many fuels.[912]

Kendziora et al describe advances in the systems for IR photo-thermal stand-off detection.[903]

Bernacki et al write about visible hyperspectral imaging for stand off detection.[881]

Morales-Rodríguez describe UV actuated decomposition at the surface for stand-off IR spectroscopy.[915]

Zachhuber et al describe a pulsed stand-off Raman system they built for the qualitative and quantitative detection of a variety of explosives.[933]

9 Environmental

Another major contributor to forensic techniques are analytical techniques specifically tailored to environmental analysis of explosives. Environmental requirements mandate the monitoring of explosive compounds and by-products during the manufacturing process and later in the environment at large. References in this section are thus presented: Environmental (general), Soil, Water and Wastewater, Bioremediation and Biodegredation.

Interesting work by Tye Langston uses a europium/thenoyltrifluoroacetone sensor for photoluminescent detection of dissolved nitroglycerin in sea water.[992]

10 Other (Safety, Definitions, Etc):

An interesting analysis of the Semtin (Pardubice, currently in the Czech Republic) disaster of 1984 involving the detonation of smokeless powder is covered.[1070] It blames the breakdown in the safety programs of the manufacturing facility on the totalitarian construct of the government which pushed for production over safety. Another paper looks at the disaster with defined root cause analysis.[1069]

Sorensen and McGill provide a useful study on engineering factors in a blast scene, comparing different materials and structures to observations after an explosion.[1082]

Gill, Horgan, and Lovelace investigate the problem with often conflicting definitions of Improvised Explosive Devices and the way various agencies and academics define them worldwide [1071] while Barker analysis statistics from IED incidents in Afghanistan and Western Pakistan from 2002 to 2009.[1064]

Kim et al do a very thorough job of injury analysis by dissecting contemporaneous accounts of the Bath, Michigan, school bombing in 1927 by applying modern analytical paradigms.[1109]

Daniel Pope has an interesting paper on the development of a quick prediction tool for the assessment of human injury in terrorist attacks.[1122]

Kirkman, Watts and Cooper describe in great detail a blast injury research model encountered in theater and propose new forward resuscitation strategies for victims.[1110]

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Drug Evidence

Review 2010 – June 30, 2013

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Preface Notes:

1. With the exception of synthetic cannabinoids and cannabimimetics, all references are subdivided by individual drug, drug group or class, or general topic, then chronologically, and finally alphabetically within each year (first author's last name). Individual synthetic cannabinoids and cannabimimetics are included in that drug group (i.e., not as individual drugs). In addition, and in contrast to past reports from this laboratory, references are organized as much as is practical by specific drug or drug group/class. This change is necessary because of the large numbers of similar types of "designer drugs," most notably the synthetic cannabinoids and cannabimimetics, the cathinones and related amphetamine-type-stimulants, and the methylenedioxyphenethyl-amines and related hallucinogens.

2. References from January 1, 2010 to June 30, 2010 are included because many were either not cited in the last review (because they had not yet been abstracted or printed), or were cited as "Ahead of Print" (i.e., without volume, issue, or page numbers). Some of the references from January 1, 2013 to June 30, 2013 in this report are similarly cited as "Ahead of Print;" all such references were still in "Ahead of Print" status as of June 30, 2013. Readers should be aware that the year listed with "Ahead of Print" may not reflect the eventual year of publication; however, the article's author(s), article title, and journal should remain the same regardless of the actual year of publication, allowing the full citation to be easily found by Internet searching.

3. Note that the following reference is law enforcement restricted, and is not available to the general public: *Journal of the Clandestine Laboratory Investigating Chemists Association* (all years). All other references cited in this report were acquired from the "Forensic Chemistry" sections of *Chemical Abstracts*, and to the author's knowledge are non-restricted. [Please also note that the second quarterly issue of the 2013 *Journal of the Clandestine Laboratory Investigating Chemists Association* (i.e., 2013; 23(2)) had not been published by the reference cutoff date, June 30, 2013.]

1 General Overview:

Production, trafficking, and use of illicit drugs are not static situations, but rather are undergoing continuous change. The worldwide situation is significantly different since the last Symposium, and dramatically different versus 10 years ago. The most noteworthy change over the past decade has been the explosive expansion of so-called "designer drugs" (aka "legal highs"); i.e., substances that mimic the effects of controlled substances but that are not themselves controlled upon first appearance. Produced by semi-legitimate or rogue laboratories, widely sold over the Internet, and marketed under deliberately innocuous and misleading labels such as "research chemicals / not for human use," or as "smoking blends," "bath salts," "plant foods," etc., such drugs can either be structurally similar analogues of a controlled substance, or structurally dissimilar but still mimicking the effects of

a controlled substance. While nearly all such substances are eventually controlled, the legal processes for doing so are slow in comparison with their appearance and recognition as drugs of abuse – and new substances often appear immediately upon the control of existing substances, in obvious, direct response to the scheduling action(s).

In considering this situation from a law enforcement perspective, it is important to recognize that *most* controlled substance statutes (worldwide) specifically name every compound being scheduled; therefore, it would be quite challenging – though not impossible – to craft general statutes that would control abused substances based on their pharmacological / physiological effects as opposed to their names or structures. To date, however, no nation has enacted or to the author's knowledge even attempted to draft such legislation. In the U.S., the Controlled Substance Analogue Act (1986) and the Positional Isomer clause for Schedule I hallucinogens (2007) were efforts towards broader control of abused substances based on structural similarities; however, both are somewhat subjective with respect to interpretation and enforcement, often result in lengthy legal proceedings upon prosecution, and (more importantly) cannot address structurally dissimilar mimic compounds. Analogous statutes in other nations are either overly broad or restrictively narrow – and are similarly subjective. As a whole, this situation is now a major issue for forensic laboratories tasked with analyzing drugs of abuse. Long predicted as the wave of the future, "designer drugs" have arrived – and are here to stay.

Although a wide variety of "designer drugs" have appeared over the past decade, the most notable are the synthetic cannabinoids and cannabimimetics – of which over a hundred have already been identified. Others include the cathinones, the methylenedioxy-, dimethoxy-, and trimethoxy- phenethylamines, and the piperazines; these latter drugs, however, are for the most part utilized at only low to moderate levels.

Synthetic cannabinoids are compounds that are structurally similar to delta-9-tetrahydrocannabinol (THC), the active component of marijuana. Cannabimimetics are compounds with chemical structures that bear no resemblance to THC but that mimic the pharmacological / physiological effects of THC in the body. While many law enforcement personnel and forensic chemists use the terms interchangeably, the distinction is important because most synthetic cannabinoids are automatically controlled under U.S. law, whereas virtually all cannabimimetics are not controlled upon initial appearance and must be scheduled on a case-by-case basis (which is, as noted above, a process that can take many months and in some cases several years).

Although these compounds are occasionally seized in bulk quantities (i.e., as pure chemicals), in the vast majority of cases they are encountered as so-called "synthetic marijuana;" that is, in trace to low-level quantities laced onto mixtures of plant materials, intended for smoking similarly as marijuana. Such materials are commonly marketed in small foil packets with attractive labeling and naming. As an additional complication, it is routine for such products to

contain mixtures of synthetic cannabinoids and/or cannabimimetics, and further a few submissions have been found to consist of synthetic cannabinoids and/or cannabimimetics laced onto marijuana, Salvia divinorum, Kratom, or other psychoactive plant materials.

Nearly all of these compounds were originally developed by legitimate scientists who were researching the CB1 and CB2 cannabinoid receptors in the human body. In many / most cases, they are significantly more potent than THC – and in some cases far more potent. For this reason, their use was initially difficult (and for the cannabimimetics, impossible) to detect via standard marijuana drug screening tests. This, along with the non-controlled status of the cannabimimetics when first marketed, were quickly recognized and widely publicized by the drug-using communities, resulting in explosive growth in the use of "synthetic marijuana" type products. This use has since begun to level off, due to the passage of laws that controlled known compounds, the publication of numerous cases of negative and sometimes bizarre experiences resulting from their use, new drug tests, and a major, continuing law enforcement effort (in the U.S.) against the manufacturers and suppliers. New compounds and products are appearing continuously, however, and this situation is expected to continue for many years to come.

Actual marijuana use continues to grow steadily throughout the U.S., with both massive imports and domestic production filling an appetite among domestic consumers. The average potency (percent THC) of federally-seized marijuana continues to increase, and in 2012 was around 15 percent (due to budgetary contraints, however, this figure does not include any state or local seizures, which are usually of significantly lower potency).

The second most noteworthy change over the past decade has been the dramatic increase in the abuse of pharmaceutical opioids, especially those containing oxycodone, hydrocodone, and hydromorphone. Very widely prescribed for a variety of physical injuries, and improperly considered as "safe" with respect to abuse potential, their use has resulted in a large population of addicts, and many thousands of deaths. Efforts to restrict production and use (including the development of "abuse-resistant" time-release formulations) have resulted in a thriving black market, with genuine pharmaceutical products selling for extreme markups (as much as $50 USD *per tablet* for high-dose products). Clandestinely-produced mimic tablets containing heroin, fentanyl, or other narcotics are sold nationwide, expanding the problem and resulting in additional overdoses and deaths from multi-drug intoxications. In addition, "traditional" heroin abuse is rising very rapidly, as street-level heroin is easily obtained and at lower expense versus prescription opioids. As a result, heroin overdose deaths are rapidly increasing throughout the U.S., and several surges in fentanyl overdose deaths have also occurred over the past decade (including some involving more potent fentanyl derivatives).

The production and use of methamphetamine continues at a high level in the U.S. Over the past decade, high-purity, Mexican-produced methamphetamine has essentially taken over the U.S. markets. Small-scale

domestic laboratories are still in widespread operation, but their percentage of the domestic market is, on a relative basis, tiny. Across the U.S., the percentage of bills (currency) contaminated by methamphetamine is approaching and in some areas exceeding that by cocaine.

The domestic use of Ecstasy continues at recent levels, but with a notable difference in tablet composition. Although the term “Ecstasy” has historically referred to tablets containing 3,4-methylenedioxymethamphetamine (MDMA), or to a lesser extent 3,4-methylenedioxyamphetamine (MDA), the continuous increase over the past 15 years of combination or mimic tablets containing any number of active ingredients has effectively rendered this term almost meaningless. Indeed, it is now common for forensic laboratories to receive “Ecstasy Tablets” that contain no MDMA or MDA whatsoever. For this reason “Ecstasy Tablets” are now more properly categorized as “Polydrug.”

Other rising drugs of abuse in the U.S. include Attention Deficit/Hyperactivity Disorder (ADHD) pharmaceuticals (Adderall, Ritalin, Vyvanse, etc.), erectile dysfunction (ED) pharmaceuticals (Cialis (tadalafil), Levitra (vardenafil), and Viagra (sildenafil), etc.) and their many counterfeits, heroin, Kratom, phencyclidine (PCP), and “poppy tea.” The abuse of ADHD medications (as a study aid) is widespread among college students, and to a lesser extent among high school students, especially during examination time periods. In addition to their intended use – which constitutes a huge and quite lucrative market – ED medications are commonly abused as performance enhancing drugs in various sports, are commonly (illegally) added to “traditional” aphrodisiacs and similar folk remedies, and are widely counterfeited worldwide, sometimes with controlled substances, including with amphetamine and other ATSs, methylenedioxyphenethylamines, and/or similar designer drugs.

Drugs that appear to have leveled off somewhat in the U.S. over the past decade include clandestinely-produced amphetamine, GHB/GBL, khat, MDA, and Psilocybe mushrooms. In most such cases, however, the leveling is due to abusers turning to other less expensive or more easily obtained substitutes. Regardless, usage spikes and dips are routine with these and other, more obscure drugs.

Of some encouragement, however, while few drugs ever disappear completely, the use of certain drugs of abuse has faded somewhat in the U.S. over the past decade. Some of these include ayahuasca “tea”, flunitrazepam, LSD, Salvia divinorum, and two of the pro-drugs for GHB, i.e., 1,4-butanediol (BD) and tetrahydrofuran (THF). The ongoing scarcity of LSD dates from the seizure of a large-scale clandestine laboratory in Wamego, Kansas in 2000, which appears to have driven the major LSD production and trafficking group in the U.S. into deep seclusion – and given the passage of time, to have possibly faded altogether. In addition, the rise of many highly potent substitute hallucinogens has quite probably reduced the demand for LSD, since most of these substances are far more easily synthesized, at lower personal risk and and at much lower costs. The reduction in the use of Salvia divinorum is due to a combination of factors, including overharvesting in

Mexico and the U.S. desert southwest, the steadily increasing availability of high-potency marijuana, the continuing availability of synthetic cannabinoids and cannabimimetics, the rise of Kratom, and the negative (sometimes highly negative) experiences reported by some users in on-line chat-rooms and websites.

An interesting development for monitoring illicit drug use in a community is the analysis of municipal wastewater (sewage) for trace levels of abused drugs and their metabolites. First reported approximately 15 years ago as a mechanism for determining contamination of the environment by pharmaceuticals and their metabolites, such analyses were a curiosity until about 5 years ago, when focused interest and advances in isolation techniques and analytical sensitivity allowed for identification of select compounds associated with controlled substances. However, evaluation of the data from such analyses is somewhat subjective – consider, for example, whether the presence of cocaine metabolites in the wastewater stream of a small city is the result of 1,000 “hard-core” addicts or 25,000 “casual” users, or the effects of different water purification chemicals, co-contaminants, time, temperature, bacteria, algae, etc., on such metabolites.

Significant advances in instrumentation have also been reported. The use of near-infrared (NIR) and/or Raman based instrumentation for non-destructive, non-invasive “stand-off” analyses are quickly becoming mainstream techniques, particularly for identification of counterfeit medications and for quality control in pharmaceutical production. Of particular note, the ability of Raman to analyze substances enclosed within clear plastic packaging or glass containers (even those made of dark glass) is of particular utility for law enforcement screening of suspect products. Portable, high quality NIR and Raman instruments are now widely available. In addition, specialized techniques such as Surface-Enhanced Raman (SERS) and Attentuated Total Reflection – Fourier Transform Infrared (ATR/FTIR) allow for analyses of minute amounts of material. In the laboratory, the development of Ultra-High Performance Liquid Chromatography (UHPLC, aka UPLC) offers resolution approaching capillary GC, and tandem LC/MS techniques (HPLC/MS, CE/MS, UHPLC/MS) and tandem LC-MS/MS techniques, enable mass spectral analyses of thermally sensitive compounds that do not survive heated injection ports. Similarly, a number of ambient pressure mass spectrometry instruments (API, DART, DESI, etc.) allow for very rapid screening of materials, even trace amounts on surfaces or on wipes. Finally, although still hindered by a lack of authentics, isotope ratio and stable element analyses are slowly becoming mainstream in source determination programs, for determining both geographic and/or synthetic origins.

1.1 Routine and Improved Analyses of Abused Substances

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new "designer drugs."

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general fields of analytical chemistry and toxicology, provide new and/or improved methods of analysis for abused substances. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

1.A – General Reviews and Overviews
1.B – Individual Compounds or Substances
1.C – Common Groups or Classes of Compounds or Substances
1.D – Polydrug A: Mixed or Unrelated Named Compounds or Substances

1.A – General Reviews and Overviews

2010 INTERPOL Triennial Report on forensic science (1); brief overview (2); **2011** *Analytical Chemistry* biannual review of forensic science (3); brief, conversational overview (4).

1.B – Individual Compounds or Substances
(except individual synthetic cannabinoids and cannabimimetics)

Alprazolam: 2011 analysis by DART-TOF-MS (5);

Amphetamine: **2010** 2H and 13C isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane (6); impurity profiling (7); **2011** by Raman and SERS, with spectral analyses by ab initio calculations (8);

1-Benzyl-4-methylpiperazine: 2012 identification by MS, after derivatization with trifluoroacetic anhydride, and by NMR (9);

Buphedrone (2-(methylamino)-1-phenylbutan-1-one):
2013 characterization with GC/MS, HPLC-DAD, and LC-MS/MS (10);

Buprenorphine: 2011 by GC/MS (11);

2-(4-Chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe): 2013 characterization by GC-EI-MS (with and without derivatization with TFAA), LC-ESI-QTOF-MS, FTIR, and NMR (12);

meta-Chlorophenylpiperazine (m-CPP): **2011** characterization by easy ambient sonic-spray ionization, XRF, IMS, and NMR (13);

Citalopram: **2012** determination by chromatographic and spectrophotometric methods (14);

Cocaine: **2010** detection on clothing using Raman (15); transacetylation of benzocaine by acetylsalicylic acid to create N-acetylbenzocaine in cocaine (16); comparison of corona discharge ionization-IMS versus AP-CI-MS for detection of cocaine (17); a 20 year survey of cocaine seized in France (year range not specified in the abstract) (18); detailed evaluation of the mass spectrum of cocaine (19); **2011** detection of cocaine solutions in sealed bottles of (nominal) alcoholic beverages by Raman (20); determination on banknotes using an aptamer-based electrochemiluminescence biosensor (21); detection of 2,6-diisopropylnaphthalene as an adulterant in cocaine by GC/MS (22); detection of cocaine solutions in wine bottles by 1H-NMR (23); detection by TLC and cobalt thiocyanate (24); detection based on strand-displacement polymerization and fluorescence resonance energy transfer (25); analysis and classification using GC/IRMS to determine d13C values (26); use of the gold chloride microcrystalline test to identify cocaine and certain adulterants (27); temperature-dependent elimination of benzoic acid during pyrolysis of cocaine (28); analysis by TLC coupled to easy ambient sonic-spray ionization MS (29); use of metastable state nanoparticle-enhanced Raman for highly sensitive detection of cocaine (30); **2012** determination of phenyltetrahydroimidazothiazole enantiomers (present in cocaine) by chiral GC (31); detection by structure-switch aptamer-based CZE (32); determination of the time lag between coca leaf harvest and the seizure and analysis of illicit cocaine (33); analysis using differential mobility spectrometry-MS (34); by electrochemical detection (35); detection using a specialized fluorescence sensor (36); analysis of cocaine smuggled by dissolution in polyvinyl alcohol in a dance pad (37); quantification of binary mixtures of cocaine and adulterants using dispersive Raman, FTIR, and Principal Component Regression (38); analysis of Brazilian "oxi" cocaine (analytical methods not specified in the abstract) (39); **2013** by electrochemical determination (40); by GC/FID (41); detection of hygrine and cuscohygrine as possible markers (to distinguish coca chewing from cocaine abuse) by GC/MS (42); comparative analysis of solvent impurity profiles obtained by HS-GC/MS (43);

Diazepam: **2010** detection in spiked alcoholic beverages by fluorimetry (44);

3,4-Dimethylmethcathinone (3,4-DMMC): **2012** characterization by GC/MS, LC/MS, 1D- and 2D-NMR, IR, and UV (45);

2,5-Dimethoxy-3,4-dimethyl-beta-phenethylamine (2C-G): 2012 by GC-EI/MS (including after derivatization with trifluoroacetic anhydride), LC-ESI/QTOF-MS, LC-ESI/QTOF-MS/MS, FTIR, and 1H- and 13C-NMR (46);

2,5-Dimethoxy-4-nitro-beta-phenethylamine (2C-N): 2012 characterization by GC-EI/MS, LC/ESI-QTOFMS, FTIR, and NMR (including after derivatization with trifluoroacetic anhydride) (47);

2-(Diphenylmethyl)pyrrolidine: **2011** by GC-EI/CI-ion trap-MS and HPLC/DAD-ESI-MS (48);

N-Ethyl-alpha-ethylphenethylamine: 2013 characterization by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (49);

Ethylphenidate: **2011** characterization by MS, IR, and 1H- and 13C-NMR (50);

Fentanyl: **2012** impurity profiling using UHPLC-MS/MS (51);

Flunitrazepam: **2011** detection using a photocatalytic reaction with ZnO particles with monitoring by UV-Vis (52); **2012** detection in alcoholic beverages by DESI-MS (53);

Glaucine: **2010** detection in “legal highs” (54);

Heroin: **2010** a probabilistic approach to heroin signatures (55); profiling and classification of illicit heroin by GC/MS of acidic and neutral manufacturing impurities (56); by optimized GC/FID (57); analysis by FTIR (58); **2011** identification of levamisole and lidocaine acetylation reaction impurities in heroin (59); rapid and semi-quantitative presumptive testing (60); converting GC/MS heroin profiling to a UHPLC-MS/MS method (61); identification of adulterants and diluents in heroin by IR and/or Raman (62); **2012** analysis of trace elements by ICP-MS (63); comparative evaluation using a simplified clustering analysis (64); impurity profiling by GC (65); by GC (66); analysis of heroin containing aspirin, paracetamol, caffeine, theophylline, codeine, acetyl codeine, and monoacetylmorphine, by GC/MS (67); purification of street samples by prep-HPLC (68); analysis by ICP/MS (69); by reflectance NIR (70); impurity profiling based on the major alkaloids (acetylcodeine, 6-monoacetylmorphine, papaverine, noscapine, codeine, and morphine) (71);

Human Growth Hormone (HGH): **2010** analysis by CE-ESI-TOF/MS (72);

Ketamine: **2010** study of the fragmentation pattern of ketamine-heptafluorobutyramide by GC/MS (73); **2012** detection in beverage residues by LC/MS and MS/MS (74); (see also Methoxetamine, below, and Reference # 528);

Khat (Catha edulis): **2010** preservation of cathinone in khat via drying (75); **2012** qualitative and quantitative analysis of cathinone, cathine, and phenylpropanolamine by GC/MS and GC/FID (76); **2013** analysis by CE (77);

Kratom: **2012** quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine, and phenylephrine in a kratom cocktail using HPLC (78); by

HPLC/ESI-MS (with comparison of 3 different extraction techniques) (79); **2013** by HPLC- DAD (80);

LSD: 2010 quantitation by HPLC (81); **2012** LSD (and 9,10-dihydro-LSD) – by color testing, TLC, EASI-MS, HPLC-UV (82);

Marijuana and Marijuana-Derived Cannabinoids: 2010 tracing geographic and temporal trafficking patterns for marijuana in Alaska using stable isotopes (83); differentiation of fibre- and drug type seedlings by GC/MS and chemometrics (84); tracing retail cannabis in the U.S. using hydrogen and carbon isotope ratios to determine geographic origins, cultivation parameters, and trafficking patterns (85,86); evaluation of an experimental indoor hydroponic cannabis grow operation using the Screen of Green method (87); evaluation of an experimental indoor hydroponic Cannabis grow operation, using the "Screen of Green" yield estimation program, THC analysis, and DNA analysis (88); survey of the potency trends of THC and other cannabinoids in marijuana from 1993 to 2008 (89); analysis of marijuana seized in Novi-Sad, Serbia in 2008 (90); determination of THC, CBD, and CBN in edible oils by UHPLC-MS/MS (91); **2011** use of DNA collection cards for in-the-field sampling (92); differentiation of seedlings by GC/MS and Linear Discriminant Analysis, Partial Least Squares Discriminant Analysis, Nearest Neighbor Classification, Learning Vector Quantization, Radial Basis Function Support Vector Machines, Random Forest, and Artificial Neural Networks (93); a survey of cannabinoid ratios in marijuana seized in California from 1996 to 2008 (94); profiling and source determination by GF AAS and ICP OES (95); differentiation of drug and non-drug marijuana using a single nucleotide polymorphism assay (96); analysis of THC in industrial hemp crops in Morocco (97); differentiation of drug-type and fiber-type by multiplex PCR analysis (98); determination of the long term stability of select cannabinoids (method not reported in the abstract) (99); a formula for determining the yield and quality of indoor grow operations (100); semi-prep scale isolation of tetrahydrocannabinolic acid A (THCA) using two flash chromatography systems (101); **2012** determination of THC by voltammetry (102); investigation of potential interferences by other drugs with the Fast Blue B and Duquenois-Levine color tests (103); a survey of the potency of marijuana grown in Albania (survey range not listed in the abstract) (104); isomerization of CBD and THC under positive ESI conditions (105); an investigation into the hypothesis of transgenic (genetically modified) marijuana (106); a PCR assay for the relative quantification of THCA synthase gene (107); analysis of DNA by CE for geo-sourcing (108); differentiation between very young drug- and hemp-type cannabis seedlings and cuttings by determination of select cannabinoids by HPLC-DAD (109); classification of cultivars based on analysis of cannabinoids and terpenoids (110); preliminary analysis of genetic diversity of hemp cultivars based on ISSR molecular markers (111); use of delta13C isotope ratios for differentiation of samples (112); a study of the effects of electrical lighting power and irradiance on indoor-grown marijuana potency and yield (113); by LC/API-MS and LC/API-MS/MS (114); determination of THC, CBD, and CBN in marijuana grown in northern Thailand, by GC/FID (115); a study of the long-term storage and stability of hash oil (methods not listed in the abstract) (116); a study of the long-term

storage and stability of “cannabis resin” (methods not listed in the abstract) (117); identification and characterization of hybrid and/or high potency marijuana (methods not specified in the abstract) (118); a survey of the potency of marijuana seized in Japan in 2010 (methods not listed in the abstract) (119); use of ultrasound for improved extraction of cannabinoids for HPLC analysis (120); evaluation of the uncertainty of THC determined by HPLC (121); **2013** by HPLC-UV following cloud point extraction (122); by DNA analysis (123); by laser-ablation inductively-coupled plasma MS (LA-ICP-MS) – a review, covering many other applications (124); a study of marijuana potency from the 1970s to the 2000s (125); characterization of seeds by DNA analysis (126);

Mephedrone (4-Methylmethcathinone): 2010 by color testing, GC/MS, and FTIR (127); by LC (128); **2011** by GC/MS following derivatization with 2,2,2-trichloroethyl chloroformate (129); characterization of 2-, 3- and 4-methylmethcathinone (i.e., mephedrone and its two positional isomers) by GC/MS, NMR, and IR (130); synthesis and characterization (synthetic route and analytical methods not specified in the abstract) (131); an overview and literature review (132); **2012** determination of isotopic fractionation to link precursor to product in the synthesis of (±)-mephedrone (133); a literature review (134); a study of the degradation in alkaline solutions (135); **2013** by SERS with a portable Raman (136);

Mescaline/Peyote: 2013 analysis of “peyote tea” by GC/MS and GC/MS/MS in PCI mode (137);

Methamphetamine: 2010 enantio-discrimination of methamphetamine by circular dichroism using a porphyrin tweezer (138); an overview of law enforcement efforts against methamphetamine production in New Zealand (139); isotope fractionation during precipitation (140); recovery and identification of trace methamphetamine and pseudoephedrine on impermeable surfaces in clandestine laboratories (141); identification of three byproducts found in methamphetamine synthesized by the Emde route (142); identification of iodine and red phosphorus using AccuTOF-DART (143); use of phosphorous acid flakes in the reduction of (pseudo)ephedrine to methamphetamine (144); screening of methamphetamine/methyl sulfone exhibits using Raman spectroscopy (145); **2011** analysis by UFLC (Ultra-Fast-LC) (146); an (unsuccessful) attempted synthesis by electrolytic reduction of pseudoephedrine (147); enantioseparation and identification of methamphetamine and the ephedrines using using trifluoroacetic anhydride derivatization and chiral GC/MS (148); analysis using by highly fluorescent polyfluorenes with NH2-terminated side chains (149); chiral analysis by CE with added cyclodextrins (150); a urea – based “one-pot” methamphetamine synthesis (151); chiral separation with CE using dynamically coated capillaries (includes “related compounds”) (152); chiral analysis of the enantiomers of ephedrine, pseudoephedrine, chlorinated intermediates, and methamphetamine by derivatization with fluorinated acid anhydrides followed by GC on a cyclodextrin stationary phase, for impurity profiling of methamphetamine synthesized by the Emde method (153); a study of the efficacy of wipe sampling to determine contamination at clandestine

laboratories (with analyses by LC/MS or GC/MS) (154); **2012** comparative analysis of impurity profiles from GC/FID (155); the environmental fate of clandestine laboratory waste (156); impurity profiling of Iranian seizures using GC/MS and LC/MS (157); an overview of abuse, treatment, and U.S. law (158); identification of (1S,2S)-1-methylamino-1-phenyl-2-chloropropane as a route specific marker impurity for methamphetamine synthesized from ephedrine via chloroephedrine (159); impurity profiling of methamphetamine synthesised by the Birch method (160); impurity profiling of methamphetamine synthesized using the Nagai method (161); critical evaluation of LLE and SPME methods for impurity profiling (162); detection of trace ephedrine and pseudoephedrine in high-purity methamphetamine by HPLC (163); degradation of 1-(1',4'-cyclohexadienyl)-2-methylaminopropane in soils (164); degradation of methamphetamine production precursors and byproducts in soils (165); chiral analysis of chlorinated intermediates of methamphetamine (from the Emde synthesis) by 1D- and 2D-NMR and GC/MS (166); analysis of a sample cut with diphenylmethane, by GC/MS (167); a study of the effects of synthetic conditions on the d13C, d15N, and d2H isotope ratios of the final product (168); determination of synthetic route via impurity profiling using GC/MS (169); preparation and certification of reference quality material (170); **2013** detection of pharmaceutical impurities in methamphetamine by GC/FID and GC/MS (171); impurity profiling of methamphetamine by CE using a highly sulfated gamma-cyclodextrin as a chiral selector (includes methamphetamine, amphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine) (172); screening of methamphetamine, pseudoephedrine, and ephedrine by a portable lab-on-a-chip instrument (173); evaluation of the use of IMS in remediation of clandestine laboratories (174); influence of precursor solvent extraction on stable isotope signatures of methamphetamine prepared from OTC pharmaceuticals using the Moscow and hypophosphorous syntheses (175); impurity profiling of methamphetamine synthesized from P2P prepared from phenylacetic acid (or its esters) (176);

Methiopropamine: 2011 characterization by IR, MS, and 1H- and 13C-NMR (177); (see also Reference # 250);

Methorphan: 2012 chiral analysis by GC/MS following derivatization with (-)-menthyl chloroformate (includes MS and NMR analyses of the derivatives) (178);

Methoxetamine: 2012 by NMR, MS, and IR (with comparisons with ketamine) (179);

2-(5-Methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (5-MeO-BFE) (and its N-ethyl analog): 2012 characterization by MS, NMR, and IR (180);

4-Methoxyphencyclidine: 2011 characterization by MS, IR, and NMR (181);

4'-Methoxyphenyl–2–propanone: 2012 clandestine synthesis and characterization (182);

alpha–Methyl–3,4–methylenedioxyphenylpropionamide (MMDPPA): 2013 identified in Australia as an intermediate from helional to MDA (183; see also 184);

Methylenedioxyamphetamine (MDA): 2013 from helional (185); (see also alpha–methyl–3,4–methylenedioxyphenylpropionamide);

3,4-Methylenedioxy-N-benzyl cathinone (BMDP): 2013 characterization by LC/high res QTOF-MS, EI-MS, IR, and 1D- and 2D- 1H- and 13C-NMR (186);

Methylenedioxymethamphetamine (MDMA): 2010 use of stable isotope ratios to differentiate MDMA according to synthetic route (187); identification of some tertiary amines related to MDMA by GC- IRD (188); determination of synthetic route by ICP-MS (189); impurity profiles of MDMA prepared by four different methods (190); **2011** use of impurity profiling, stable isotope analyses, and pattern recognition techniques for characterization and sourcing (191); a historical overview (192); determination of volatile components of MDMA tablets with LC/MS and HS-SPME-GC/MS, for development of canine training aids (193); determination of volatiles by HS-SPME followed by GCxGC and GCxGC-TOFMS (194); by SERS using modified Silver nanoparticles (195); **2012** impurity profiling of MDMA prepared from piperine versus vanillin (196); isolation of MDMA using a specialized SPME cartridge with analysis by GC/MS (197); comparative analysis by GC×GC-TOF-MS (198); **2013** enantiomeric purification by batch chromatography with a cyclodextrin chiral selector (199); impurity profiling of sassafras oils by GC×GC-TOF-MS (200);

Methylenedioxypyrovalerone (MDPV): 2010 characterization by GC/MS, NMR, FTIR, and UV (201);

4-Methylethcathinone (4-MEC): 2013 by GC/MS, HPLC-DAD, and LC-MS/MS (202);

N-Methylphthalimide: 2011 characterization by GC/MS, FTIR, and NMR (203);

4'-Methyl-alpha-pyrrolidinohexanophenone (MPHP): 2011 analysis by GC/MS, HPLC/DAD, and GC/FID (toxicological focus) (204);

3,4-Methylenedioxyphenylacetone (MDP2P): 2010 differentiation of methoxy methyl phenylacetones related to MDP2P by GC/IRD (205);

3,4-Methylenedioxypyrrolidinobutyrophenone (MDPBP): 2011 characterization by IR, MS, and 1D- and 2D- 1H- and 13C- NMR (206);

4-Methylthioamphetamine (4-MTA): 2012 impurity profiling of 4-MTA produced by the nitropropene route (207); identification of by-products produced by the Leuckart method, using MS, 1H- and 13C-NMR, IR, and crystallography (208);

Morphine: 2012 analysis by FTIR and Raman, with density functional theory (DFT) calculations (209); extraction from poppy seeds, with analysis by GC/MS and GC/FID (210); quantitation in a Chinese traditional medication, by HPLC (211); analysis by cyclic voltammetry, chronoamperometry, and differential pulse voltammetry (212);

Naphyrone (naphthylpyrovalerone, 1-naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one): 2010 isomer determination by GC- ion trap-EI/CI-MS and 1D/2D NMR spectroscopy (213); **2012** an overview and literature review (214);

Oxycodone: 2010 analysis of pyrolysis products by GC and GC/MS (215);

Phencyclidine (PCP): 2013 false-positive immunoassay caused by MDPV (216);

Psilocybe Mushrooms: 2010 comparative analysis of hallucinogenic mushrooms using ATR and transflection IR (217); **2011** by DNA analysis (a review, also including some non-hallucinogenic, poisonous mushrooms) (218);

alpha-Pyrrolidinopentiophenone: 2012 by MS, NMR, and IR (219);

Salvia divinorum: 2010 thermal degradation products from Salvia divinorum smoke (220); **2012** differentiation from other Salvia species by GC/MS with principal components analysis (221); analysis of "spiked" plant materials by GC/MS (222); **2013** identification of Salvinorin A in Salvia divinorum (but not in 612 related Salvia species) by GC/MS (223); differentiation from marijuana and tobacco by DNA analysis (224);

Sibutramine: 2012 by TLC and TLC-densitometry (225); **2013** detection of illicit adulteration of botanical food supplements, by color tests, TLC, HPLC-DAD, MS, and NMR (226);

Zolpidem: 2012 by HPLC and MS (includes a degradation study) (227);

Miscellaneous Drugs: 2011 characterization of RTI-126 (228).

1.C – Common Groups or Classes of Compounds or Substances

Amphetamine-Type Stimulants (ATSs) and Related Phenethylamines (PEAs): 2010 analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMMA, and MBDB by GC/MS and GC/IRD (229); methamphetamine, 4-fluoro-, 4-chloro-, 4-bromo-, 4-iodo-, and 4-nitromethamphetamine – analysis by GC/MS following trifluoroacetyl derivatization (230); differentiation of regioisomeric ring-substituted fluorophenethylamines by product ion spectrometry (231); "Fly" and "Dragonfly" Compounds – synthesis and charaterization by GC/MS, LC/MS, and LC-MS/MS (232); **2011** GC/MS and GC/IRD studies on the ring

isomers of N-methyl-2-methoxyphenyl-3-butanamines (MPBA) related to 3,4-MDMA (233); 4-methylthioamphetamine, 4-fluoroamphetamine, 4-methylamphetamine, 3-trifluoromethylamphetamine, MDA, 2,5-dimethoxyamphetamine, and 2,4,5- and 3,4,5-trimethoxyamphetamines – mass spectrometric properties and identification of some N,N-di-(beta-arylisopropyl)formamides (synthetic impurities) (234); 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran – characterization by MS, IR, and NMR (235); amphetamine and methamphetamine – detection by digital image-based colorimetric tests (236); identification of (unspecified) ATSs by GC/MS and GC/FTIR (237); general classification of amphetamines versus non-amphetamines based on GC/FTIR and GC/MS with Principal Component Analysis coupled with Artificial Neural Networks (238); amphetamine, methamphetamine, pseudoephedrine, and five "amphetamine analogs" (not specified in the abstract) – field analysis using the Agilent Bioanalyzer (239); novel syntheses of ATS precursors (240); a review of methods for the chiral determination of ATSs (241); aminoindanes – a review (242); **2012** 4- and 5-iodo-2-aminoindan – by MS, NMR, and IR (243); 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine – analysis by GC/MS, acetylation, and GC/IRD (244); "amphetamine-type illicit drugs" by a miniaturized gas sensor system using surface ionization (245); DOB and positional isomers – differentiation of various perfluoroacylated derivatives by GC/MS and GC/IRD (246); amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine – enantioseparation by CE with contactless conductivity detection (247); a review of the chiral analysis of amphetamine "and related compounds" by CE and NMR (248); 25D-NBOMe, 25E-NBOMe, and 25G-NBOMe – characterization by GC-EI-MS (with and without derivatization with trifluoroacetic anhydride), LC-ESI-QTOF-MS (and MS/MS), FTIR, and NMR (249); **2013** methiopropamine and its 3-thienyl isomer – synthesis and analysis/differentiation by GC (250); o-, m-, p-chloro- and o-, m-, p-fluoro-amphetamine – by CE-LIF, following derivatization with fluorescein isothiocyanate (includes comparisons against CZE-UV, sweeping-MEKC-UV, and LC-Q-TOF-MS) (251); diethylpropion, fenproporex, and sibutramine – in counterfeit tablets, by ATR/FTIR (252); unspecified amphetamines and precursors – by a portable instrument combining miniaturized GC and IR Absorption Spectroscopy (253); 2-, 3-, and 4-methylamphetamine – synthesis and characterization by GC/MS, HR-ESI-MS, NMR, and IR (254); methamphetamine, MDMA, and other unspecified ATSs – by GC/MS after derivatization with iso-Bu chloroformate and SPME (toxicological focus) (255); methamphetamine, MDMA, amphetamine, DMA, and PMA – a review of impurity profiling and syntheses (256);

Anions: **2010** identification via complexation with meso-octamethylcalix(4)pyrrole and detection using EI-MS (257); **2011** by CE (258,259);

Barbiturates: **2010** mephobarbital, pentobarbital, and secobarbital – by MEKC-MS (toxicological focus) (260); **2011** spectrophotometric determination of barbituric acid in pharmaceuticals (261);

Benzodiazepines: **2011** determination of pK values by potentiometric titration (262); diazepam, estazolam, chlordiazepoxide, and triazolam – analysis by RP-HPLC (263); **2012** clotiazepam, clozapine, and pinazepam – analysis by micellar liquid chromatography (toxicological focus) (264);

Cathinones: **2010** mephedrone, butylone, 4-methyl-N-ethylcathinone, flephedrone, MDPV, and naphyrone – by GC-ion trap-MS (both EI and CI) and NMR (265); mephedrone, methylone, and bk-MBDB – characterization by FTIR, FT-Raman, 1H NMR, 13C NMR, GC/MS, and EI-HRMS (266); **2011** 4-fluoromethcathinone, pentylone, MDPBP, MDPV, and MPPP – by GC-(EI/CI)-MS and NMR (267); 4'-methylethcathinone (4-MEC) and 6 other methcathinone analogs (not specified in the abstract) by LC-MS/MS (268); analysis of isomeric byproducts and related impurities in mephedrone and ethylcathinone (269); synthesis and analysis of various methylenedioxycathinones, including bk-DMBDB (270); by Raman (271); methylone, bk-MBDB, and bk-MDEA – a review, including analyses by GC/MS, LC/MS, and LC-MS/MS (toxicological focus) (272); **2012** 10 homologous and regioisomeric aminoketones related to MDPV – analysis by GC-EI-MS (273); 3,5-difluoromethcathinone and 3,5-dichloromethcathinone – synthesis and characterization by GC/MS, NMR, IR, and GC/IRD (274); the 2,3-isomers of MDPV, butylone, and methylone – synthesis and characterization by GC, IR, GC/MS, and 1H and 13C NMR (275); 4'-methyl-N-ethylcathinone (4-MEC) and 4'-methyl-N-benzylcathinone (4-MBC) – characterization (methods not specified in the abstract (276); buphedrone and pentedrone – synthesis and characterization by FTIR, Raman, 1H- and 13C-NMR, GC/MS, and ESI-HRMS (277); mephedrone, methedrone, and 17 others not specified in the abstract – chiral separation by cyclodextrin-modified CZE (278); methcathinone and 17 other cathinones (not specified in the abstract) – chiral analysis by GC/MS following derivatization with trifluoroacetyl-L-prolyl chloride (279); 22 cathinones (not specified in the abstract) – by positive ESI MS with in-source CID (280); cathinone, methcathinone, 4-methylmethcathinone, dimethylcathinone, and 4-methoxymethcathinone – by color testing (281); screening identification of methcathinone and 5 other cathinones by portable ATR/FTIR (282); 4-methylmethcathinone, three positional isomers of fluoromethcathinones, 4-methoxymethcathinone, N-ethylcathinone, N,N-dimethylcathinone, buphedrone, and pentedrone – by GC/MS (283); "synthetic cathinones" – detection and screening using a portable ion trap DESI-MS (284); differentiation of isomeric N-alkylated fluorocathinones by GC-MS/MS (285); pentedrone and pentylone – characterization by MS, 1D- and 2D-, 1H- and 13C-NMR, and IR (286); **2013** mephedrone, methylone and MDPV – by ambient ionization MS using arrays of low-temperature plasma probes, and also following injection of trifluoroacetic anhydride directly into the plasma stream for online derivatization (287);

Ephedrines: **2010** N–acetylpseudoephedrine and N–acetylephedrine – synthesis and characterization by GC–MS, NMR, FTIR, LC–MS, and UPLC–MS (288); **2012** phenylpropanolamine, cathine, ephedrine, pseudoephedrine, and methylephedrine – analysis by HILIC, with comparison versus RPLC (289); chiral separation of enantiomers of ephedrine and pseudoephedrine in

ATSs using achiral modifiers in the gas phase (290); synthesis of alpha-aminoalcohols via the Akabori–Momotani reaction (291); **2013** comparison of RP-UHPLC and HILIC for quantitation, with medium-resolution accurate MS (292);

Erectile Dysfunction Drugs – Cialis (tadalafil), Levitra (vardenafil), and Viagra (sildenafil): **2010** detection of counterfeits by FTIR, NIR, and Raman (293); identification of (-)-trans-tadalafil, tadalafil, and sildenafil in counterfeit Cialis (294); **2011** development of "classification trees" based on infrared spectroscopic data to discriminate between genuine and counterfeit medicines (295); identification of counterfeits by impurity profiling (296); detection of counterfeits by Raman (297); **2012** differentiation of legitimate and counterfeit medications by chemometrics and chromatography (298); detection of counterfeits by image processing and statistical analysis (299); analysis of counterfeit Cialis tablets using Raman microscopy and multivariate curve resolution (300); fingerprinting of sildenafil citrate and tadalafil tablets by XRF (301); identification of sildenafil and/or vardenafil using ESI-LC/MS (302); detection of adulteration of capsule shells (a novel and unusual "smuggling" technique) by HPLC-DAD, HPLC/MS, microscopy, and Raman (303); **2013** differentiation between counterfeit and authentic Cialis and Viagra by ATR/FTIR with PCA (304); analysis and profiling by UPLC/MS (305);

Ergot Alkaloids (see also LSD): **2012** quantitative analysis using electronic absorption, fluorescence, IR, Raman, CD, ESI-MS, and MALDI-MS (specific compounds not listed in the abstract) (306);

Fentanyl Derivatives: **2012** identification of trace level fentanyl derivatives with nonaqueous CE-ESI-MS/MS (307);

***gamma*-Hydroxybutyric acid (GHB) and *gamma*-Butyrolactone (GBL):** **2010** use of IRMS to discriminate between seizures of GBL and for source determination (308); detection of GHB in solutions using a colorimetric sensor array (309); **2011** a study of the spontaneous formation of GHB from GBL in tap water (310); screening for gamma-hydroxybutyrate by ion chromatography (with comparison versus GC/MS) (311); detection of GHB and GBL in adulterated beverages, using 1H-NMR (312); **2012** sodium, potassium, magnesium and calcium salts of gamma-hydroxybutyrate – synthesis and characterization by FTIR, elemental analysis, X-ray powder diffraction analysis, color testing, and microcrystal testing (313); field testing for GHB with a rapid enzymic test (also includes commentary on MDMA, flunitrazepam, and ketamine) (314); **2013** a comprehensive study of the worldwide distribution of GBL using internet monitoring, comparison of packaging, and carbon isotopic measurements (315); in dietary supplements and foods, by GC/MS (using isotopologues for quantitation) (316);

Methylenedioxyphenethylamines and Related Compounds (note that methylenedioxysubstituted cathinones are categorized under "Cathinones")**:** **2010** identification of side chain regioisomers related to MDEA, MDMMA, and MBDB (317); **2011** methylenedioxy-2-aminoindans – synthesis and analysis of the 4,5 and 5,6 isomers by GC/MS, ATR/FTIR, and 1H- and 13C-NMR

(318); **2012** MDA, alpha-methyl-3,4-methylenedioxyphenylpropionamide (and 2-chloro-4,5-methylenedioxyamphetamine) – characterization by GC/MS, GC/IRD, ATR/FTIR, and NMR (319);

Papaver and Opium: **2010** by cyclodextrin-modified CE following ultrasound-assisted extraction of Papaver (320); identification of opium poppies using 10 genetic markers (321); **2011** differentiation of P. somniferum, P. rhoeas, and P. setigerum by GC/MS and multivariate statistical analyses (322); identification of expressed sequence tag (EST) and simple sequence repeat (SSR) markers (323); determination and analysis of opium alkaloids and crude heroin in complex mixtures by surface-ionization MS (324); **2012** Papaver setigerum by genetic and chemical components analysis (325); opium – determination of 14N and 15N isotopes by proton induced gamma-ray emission (326);

Piperazines: **2010** differentiation of methylenedioxybenzylpiperazines by GC/IRD and GC/MS (327); BZP, mCPP, MeBP, MeOPP, MePP, and TFMPP – detection in "Legal Highs" by GC/MS and HPLC-DAD (328); **2011** differentiation of methylenedioxybenzylpiperazines and methoxymethylbenzylpiperazines by GC/IRD and GC/MS (329); BZP and TFMPP – analysis by ATR/FTIR and GC/MS (330); **2012** methoxybenzoylpiperazines (OMeBzPs) and methylenedioxybenzylpiperazines (MDBPs) – differentiation using GC/MS, GC-TOF-MS, and GC/IRD (both underivatized and as perfluoroacylated derivatives (331); **2013** BZP – a review (social focus, but includes "analytical methodologies for the identification of BZP in forensic settings") (332);

Plant Materials: **2010** a review of poisonous plants (includes drugs) (333); **2011** use of cellulose d18O as an index of leaf-to-air vapor pressure difference in tropical plants (334); **2012** analysis of alkaloids from psychoactive plants by nonaqueous CE/MS (specific plants not listed in the abstract) (335); plant DNA fingerprinting – listed applications include "investigation of trade in illicit drugs" (336); **2013** identification of plant materials used as supporting matrices for pharmaceuticals, nutritional supplements, and illicit drugs, by DAD, evaporative light scattering detection, and MS (337); analysis of the plant materials used as support matrices, by DNA analysis, GC/MS, and LC/MS (338); (see also Reference Number 352);

Steroids: **2010** correlation of the product ion profiles from ESI MS/MS with molecular structures (339); analysis by GC- microchip-AP-photoionization-MS (toxicological focus) (340); identification of anabolic steroids and derivatives using bioassay-guided fractionation and UHPLC/TOFMS analysis (341); **2011** testosterone – IRMS of various black-market products collected in Austria (342); a review of the literature from 2004-2010 (343); analysis by GC/MS using hydrogen as the carrier gas (toxicological focus) (344); **2012** prediction of GC relative retention times of trimethylsilylated derivatives (345); identification of methyltestosterone in counterfeit 4-chlorodehydromethyltestosterone products, by RP-HPLC-ESI-MS (346); elucidation of the m/z 97 ion from androst-4-en-3-one-based steroids by ESI-CID and IRMPD (347); **2013** (primarily) stanozolol, testosterone and

nandrolone – a study of authentic and counterfeit products seized in Brazil from 2006 to 2011 (348);

Synthetic Cannabinoids and Cannabimimetics: [Notes: To aid searching for specific compounds, all compounds in this section are listed in alphabetical order within their individual citation (but not within the section). In addition, compounds are listed either by their acronym or full name as was specified in their respective abstract – no effort was made to transcribe acronyms to full chemical names or vice versa. Articles that include both synthetic cannabinoids and/or cannabimimetics with other drugs are detailed in the next section.] **2010** JWH–018 and JWH–073 – by color testing, TLC, GC/MS, and FTIR (349); a survey of synthetic cannabinoids and/or cannabimimetics containing products obtained from June 2008 to September 2009 in Germany/Europe (350); analysis of "Spice Gold" with GC/MS and solid probe MS (351); identification of the plants used as the base materials for products containing synthetic cannabinoids and cannabimimetics (352); JWH-018 – detection by TLC and GC/MS (353); analysis and identification of cannabicyclohexanol, CP-47,497, JWH-018, JWH-073, and oleamide in herbal products by GC/MS and LC/MS (354); an overview of synthetic cannabinoids and cannabimimetics (355); **2011** JWH-203 – characterization by LC/MS, GC/MS, LC with UV detection, NMR, and high-res MS (356); JWH–018, JWH–073, and 9 other unspecified synthetic cannabinoids – a survey of 33 smoking blend products, with analysis by GC/MS (357); JWH-015, JWH-018, JWH-019, JWH-020 JWH-073, JWH-081, JWH 200, JWH-250, WIN 55,212-2 and methanandamide – by LC-MS/MS (toxicological focus) (358); JWH-122 – characterization by NMR, "spectroscopy," and MS (359); JWH–201, JWH–250, and JWH–302 – differentiation by GC/MS fragment ion ratio comparisons (360); an overview and review of synthetic cannabinoids and cannabimimetics, including some GC/MS and LC-MS/MS data (361); (unspecified) analog of a CP 47,497-C8 type compound – by off-line LC-DAD-NMR (362); AM-694, AM-2201, JWH-122, RCS-4, and (2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (a positional isomer of RCS-4) – analysis by LC/MS, GC/MS, and NMR (363); AM-694, JWH-019, JWH-122, JWH-210, and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone – analysis by LC/MS, GC/MS MS, and NMR (364); JWH-250 – identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (365); 1-pentyl-3-(1-naphthoyl)indole, 1-butyl-3-(1-naphthoyl)indole, 1-hexyl-3-(1-naphthoyl)indole, and 3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol – by "chromatography-mass spectrometry" (chromatographic method(s) not specified in the abstract) (366); JWH-018 and JWH-073 – detection by GC/MS (367); JWH–018, JWH–018 N–(2–methylbutyl) isomer, JWH–018 N–(3–methylbutyl) isomer, JWH–201, JWH–250, JWH–302 – isomer differentiation by GC/MS retention times (368); cannabipiperidiethanone – identification and characterization by GC/MS, LC/MS, high-res MS, and NMR (369); JWH- 015, JWH-073, JWH-081, JWH-200, JWH-250, JWH-251 – identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (370); JWH-018 and JWH-073 – detection by GC/MS (371); cannabicyclohexanol (CP-47,497-C8-homolog), JWH-018, JWH-073 – determination by GC/MS (372); **2012** AM2201, JWH-018, and JWH-022 – JWH- 018 and JWH-022 identified as combustion products of AM2201, as

determined by GC/MS and Accu-TOF-DART (373); JWH-018 – by DART-TOF-MS (374); JWH-307 – characterization by NMR, GC-HRMS, ESI-MS/MS, UV, and IR (375); JWH-018 and JWH-073 – purity levels of materials from three different on-line suppliers, as determined by HPLC-UV (376); "synthetic cannabinoids" (specific compounds not listed in the abstract) – analysis by MEKC-DAD (377); AM-694, JWH-018, JWH-019, JWH-073, JWH-081, JWH-210, and JWH-250 – analysis by GC/MS and MALDI-TOF MS (378); AM-679 and 1-pentyl-3-(1-adamantoyl)indole – by LC-UV-MS/MS, LC-TOF-MS, GC/MS, and NMR (379); AM-2201, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-307, and RCS-4 – analysis by LC-ESI-MS/MS (toxicological focus) (380); AM-694, AM-2201, JWH-018, JWH-019, JWH-081, JWH-122, JWH-203, JWH-210, JWH-250, JWH-307, MAM-2201, and RCS-4 – by LC/ESI-MS/MS (toxicological focus) (381); AM-1220 and (N-methylazepan-3-yl)-3-(1-naphthoyl)indole – by TLC, GC/MS, high-res MS, LC-HR-MS/MS, and NMR (382); 3-(1-adamantoyl)-1-pentylindole – identification by GC/MS, TLC, NMR, high-res MS, and GC-MS/MS (383); AM-694, AM-2201, CP 47,497 (C=8) (cannabicyclohexanol), JWH-018, JWH-019, JWH-073, JWH-081, JWH-200, JWH-210, JWH-250, RCS-4, and RCS-8 – analysis by TLC, GC/MS, HPLC, and LC-TOF-MS (384); 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone and JWH-412 – separation by flash chromatography and analysis by GC/MS and NMR (385); "synthetic cannabinoids" (five compounds not specified in the abstract) by DART-MS with collision-induced dissociation (386); AM-251 and JWH-015 – analysis by DART-MS (387); color testing for 24 (unspecified) indole-based cannabimimetics (388); an overview (389); naphthoylindoles – by ESI-QTOFMS (390); N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA), N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), AM-1220, AM-1241, AM-1248, AM-2233, and CB-13 (CRA-13) – analysis by LC/MS, GC/MS, high-res MS, and NMR (391); 1-butyl-3-(1-(4-methyl)naphtoyl)indole – synthesis and characterization with GC/FID, 1H- and 13C-NMR, DSC, GC/MS, and elemental analysis (392); an overview and review (393); JWH-073 and its 4-methylnaphthoyl analogue – by TLC, NMR, GC/MS, and LC/MS (394); JWH-018, JWH-081, and 10 other (unspecified) "synthetic cannabinoids" – by GC/MS (395); JWH-018 – by GC/MS (396); **2013** JWH-018, JWH-019, JWH-073, and JWH-250 – by GC/MS (397); 5F-UR-144 and UR-144 – by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (398); AM-2201, JWH-203, JWH-210 and RCS-4 – by LC, high-res MS, LC-QTOF-MS, and NMR (399); 28 (unspecified) "synthetic cannabinoids" – by LC/ESI-MS/MS (toxicological focus) (400); cis- and trans- CP-47,497-C8 (and others not specified in the abstract) – extraction from plant materials by flash chromatography (401); azepane isomers of AM-1220 and AM-2233, AM-2233, and URB-597 – by LC/MS, GC/MS, "accurate MS," and NMR (402); unspecified "cannabimimetics" bearing 2,2,3,3-tetramethylcyclopropanecarbonyl moieties – by GC/MS, LC/MS, and NMR (403); JWH-213 – by LC-PDA-MS, GC/MS, high-res MS, and NMR (404); N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FUBINACA) – by LC/MS, GC/MS, high-res MS, and NMR (405); cannabicyclohexanol, JWH-018, JWH-073, JWH-081, JWH-

122, JWH-210, JWH-250, and RCS-4 – by GC/MS, LC-QTOF-MS, and HPLC (406);

Synthetic Cannabinoids and Cannabimimetics with Other Drugs: 2012 1-butyl-3-(4-methoxybenzoyl)indole, JWH-018, JWH-073, JWH-122, JWH-250, 1-pentyl-3-(4-methoxybenzoyl)indole, and phenazepam – detection in plant materials (analytical methods not specified in the abstract) (407); 12 "synthetic cannabinoids and cannabimimetics" (not specified in the abstract) and THC – by nano-LC/MS and nano-LC-MS/MS (408); AM-2201, AM-2202, JWH-019, JWH-203, JWH-210, mitragynine (Kratom), (1-(4-pentenyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone – analysis by LC/MS, GC/MS, high-res MS, and NMR (409); **2013** AB-001, AM-2232, APINACA, N,5-dimethyl-N-(1-oxo-1-(p-tolyl)butan-2-yl)-2-(N'-(p-tolyl)ureido)benzamide, (4-ethylnaphtyl)-AM-2201 (EAM-2201), 5-fluoropentyl-3-pyridinoylindole, 5FUR-144 (synonym: XLR11), 4-hydroxy-diethyltryptamine (4-OH-DET), JWH-213, JWH-307, JWH-030, 4-methylbuphedrone, (4-methylnaphtyl)-AM-2201 (MAM-2201), (4-methylnaphtyl)-JWH-022 [synonym: N-(5-fluoropentyl)-JWH-122], N-(4-pentenyl)-JWH-122, UR-144, and URB-754 – detection on plant materials (methods not specified in the abstract) (410); (see also References Numbers 424, 432, 441, 467, 469, and 470);

Tryptamines (see also Psilocybe Mushrooms): 2010 a review of the analyses of psychoactive N,N-dialkylated tryptamines (411); characterization of the byproducts from the synthesis of DMT by reductive amination, using GC- ion trap-MS (412); profiling psychoactive tryptamine-drug syntheses by MS (to identify route specific impurities) (413); **2011** preparation and analytical characterization of twelve 5-ethoxy-N,N-dialkyl-tryptamines and their deuterated analogues (414); **2012** 5-methoxy-2-methyl-N,N-dialkylated tryptamines – synthesis and characterization by 1H and 13C NMR, GC-EI-IT-MS, and CI-IT-MS/MS (415); quantitation of substituted N,N-dimethyl-tryptamines in the presence of natural type XII alkaloids by HPLC, ESI-MS, MS/MS, MALDI-MS, and Raman (416); **2013** AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole) – characterization using 1H- and 13C-NMR, GC-EI/CI-ion trap-MS, U/HPLC-DAD, and HPLC/MS (417).

1.D – Polydrug A: Mixed or Unrelated Named Compounds or Substances

2010 amphetamines, cocaine, codeine, heroin, and morphine – by CEC-ESI ion trap MS (418); 4-methylmethcathinone, 2-fluoromethamphetamine, alpha-phthalimidopropiophenone, and N-ethylcathinone by GC/MS, NMR, FTIR, and GC/IRD (419); 1,4-benzodiazepines and amfepramone – determination as adulterants in phytotherapeutic formulations by adsorptive cathodic stripping voltammetry (420); separation and detection of seven amphetamines, amphetamine, dextroamphetamine, methamphetamine, and MDMA by CZE with capacitively coupled contactless conductivity detection (421); hallucinogenic mushrooms and khat by cation exchange LC (422); morphine, morphine HCl, cocaine HCl, codeine phosphate, papaverine HCl, pethidine

HCl, and thebaine – differentiation with THz time domain spectroscopy (423); piperazines, phenethylamines (2Cs and FLYs), 4-substituted amphetamines, beta-keto-amphetamines (cathinones), 2,5-dimethoxyamphetamines, pyrrolidinophenones, and synthetic cannabinoids – a review of their analyses (toxicological focus) (424); MDMA, MDA, and methamphetamine in Ecstasy tablets by GC/FID (425); marijuana, cocaine, heroin, MDMA, amphetamine, methamphetamine (and other unspecified drugs) – detection using spectral fluorescence signatures (426); **2011** diazepam, flunitrazepam, and methadone – by FT-NIR (427); cocaine and MDMA – detection on textiles using micro-Raman (428); evaluation of the fragmentation pathways of various drugs of abuse (cannabinoids, ketamine, amphetamine, ATSs, cocaine, and opioids) by LC-QTOF MS/MS and MSE accurate-mass spectra (429); sibutramine, modafinil, ephedrine, norephedrine, metformin, theophylline, caffeine, diethylpropion, and orlistat – identification and quantification in diet aids by UHPLC-DAD (430); cocaine and heroin – an evaluation of impurity profiling for comparative analysis (431); herbal products [khat, Psilocybe mushrooms, opium, and “Spice”], designer drugs in tablet and powder form [e.g., mCPP, 3-fluoromethamphetamine (3-FMA), MDPV, and methylone], and anabolic steroids in oil and tablets – by DAPPI-MS (432); MDMA, ketamine, phenmetrazine, ephedrine, pseudoephedrine, caffeine, tramadol (possibly others not listed in the abstract) – analysis of Ecstasy tablets seized in Iran from 2007 to 2008, by physical characteristization, color testing, TLC, anion testing, residual solvent analysis, GC/MS, and LC/MS (433); methamphetamine, amphetamine, MDMA, MDEA, MBDB, MDA, and BDB – by GC/MS following derivatization with trifluoroacetic anhydride (434); heroin, dl-methamphetamine, dl-MDMA, and dl-ketamine – application of dispersive liquid-liquid microextraction and CE with UV detection for chiral separation and determination (toxicological focus) (435); cocaine and heroin – analysis of “crack” cocaine in Iran by TLC and GC/MS (proving that most such samples actually contained heroin) (436); benzodiazepines, beta-blockers, angiotensin-converting enzyme inhibitors, phenothiazines, dihydropyridine calcium channel blockers, diuretics, local anesthetics, vasodilators, anti-diabetic, antidepressant, analgesic, and antihistaminic drugs – by LC-MS/MS (toxicological focus) (437); methamphetamine, MDMA, pseudoephedrine, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene – a study of their degradation in soil (438); analysis of “Happy Water” (containing methamphetamine, caffeine, ketamine, and other components) – by GC/MS and GC/FID (439); morphine, codeine, and hydrocodone – by SERS (440); p-fluoroamphetamine, mephedrone, flephedrone, PPP (alpha-pyrrolidinopropiophenone), MDPV, bk-MBDB, pFBT (3-(p-fluorobenzoyl)-tropane), JWH-073, methylone (3,4-methylenedioxymethcathinone), and N-ethylcathinone – by GC/MS, UPLC-QTOF-MS, and NMR (441); m-CPP and MDMA tablets, cocaine, and LSD – by easy ambient sonic-spray ionization MS (442); Ecstasy Tablets – MDMA, methamphetamine, MDEA, MDA, amphetamine, caffeine, and lidocaine – by TLC and EASI-MS (443); methamphetamine, methamphetamine analogs, and MDMA – a theoretical study of the energetics of the synthesis of various ATS and MDMA (including reactants, products and by-products) (444); cocaine and heroin – a survey of seizures in Luxembourg from 2005 to 2010 (445); bunitrolol, caffeine, cocaine, codeine, diazepam, doxepin, haloperidol, 3,4-methylendioxyamphetamine,

morphine, nicotine, and zolpidem – impact of solvent choice on the analysis of basic drugs by micro-LC/MS (toxicological focus) (446); methamphetamine, MDA, MDMA, and ketamine – detection by 2D THz signatures and spectral dynamics analysis (447); **2012** methandrostenolone, sildenafil, tamoxifen, quinine, clomiphene, dehydroepiandrosterone, anastrazole, clenbuterol, stanozolol, oxandrolone, liothyronine, finasteride, and melatonin in counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders – RPLC-DAD and GC/MS (448); antidepressant drugs (sertraline, paroxetine, citalopram, venlafaxine, and fluoxetine) – determination by spectrofluorometry (449); mephedrone, BZP, MDAI, and TFMPP – by microcrystal testing, FTIR, and GC/MS (450); MDA, MDMA, methadone, cocaine, morphine, codeine and 6-monoacetylmorphine – analysis with CZE-TOF-MS (451); MBDB, MMDA-2, and D2PM (and possibly others not specified in the abstract) – enantiomeric separation after derivatization with (R)-(-)-DBD-Py-NCS by UHPLC, with fluorescence and MS detection (452); lidocaine and benzocaine – detection by HPLC with amperometric detection (453); MDMA, ketamine, cocaine, diazepam, phenobarbital, and barbital – analysis using a deep UV/Vis reflected optical fiber sensor (454); cocaine, codeine, nicotine, methadone, phenmetrazine, pentylenetetrazole, niketamide, fencamfamine, and caffeine – by GC/high-res-TOF-MS with a soft ionization source (455); atenolol, salbutamol and cocaine – detection of drug vapors using an ion funnel interface for secondary ESI-MS (456); acetaminophen, phenylephrine, glucose, and caffeine – noninvasive, quantitative analysis of simulated drug mixtures using SORS and multivariate statistical analysis (457); constituents of “legal highs” – MPDV, caffeine, butylone, TFMPP, lidocaine, 4-MEC, mephedrone, pFPP, BZP, and MDPBP – by GC/MS, LC-QTOF-MS, HPLC, and NMR (458); **2013** flunitrazepam, ketamine, and MDMA – detection by IMS (toxicological focus) (459); methoxetamine, 3-methoxyeticyclidine and 3-methoxyphencyclidine – characterization by GC- and CI- MS, NMR, and HPLC-DAD-ESI-MS/MS (toxicological focus) (460); 1,4-benzobenzodiazepines (clonazepam, flurazepam, alprazolam, midazolam, bromazepam, chlordiazepoxide, lorazepam, and diazepam) and antidepressants (bupropion, sertraline, paroxetine, and fluoxetine) – identification as adulterants in phytotherapeutic dieting formulations by voltammetry (461); anorexics (amfepramone, fenproporex, sibutramine), benzozodiazepinic anxiolytics (clonazepam, flurazepam, alprazolam, midazolam, medazepam, chlordiazepoxide, diazepam), antidepressants (bupropione, fluoxetine, sertraline, paroxetine), diuretics (hydrochlorothiazide, furosemide, chlortalidone, amiloride, spironolactone), and hypoglycemics (glimepiride, chlorpropamide, glibenclamide) – differentiation by a solid state electrochemical method (462); mephedrone, 5,6-methylenedioxy-2-aminoindane (MDAI), and MDMA – by SERS on copper coins coated with deposited silver (463); Psilocybe mushrooms, 5MeO-DIPT, tryptamine, MDMA and related compounds, and synthetic cannabinoids and cannabimimetics – an overview (464).

2. Instrument Focus

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

2.A – Polydrug B: Mixed or Unrelated Groups of Compounds or Substances

Named Groups of Compounds: 2011 opioids, tranquilizers, stimulants, and hallucinogens – analysis by flow-analysis methods with chemiluminescence or electrochemiluminescence detection (465); a review of the analytical methodologies used to determine adulterants in slimming phytotherapeutic formulations (466); designer cathinones, tryptamines, phenethylamines, and synthetic cannabinoids and cannabimimetics – an overview and review (467); phenethylamine, amphetamine, and tryptamine imine by-products – characterization by GC/MSD, IR, and NMR (468); **2012** (unspecified) synthetic cannabinoids, cannabimimetics, and cathinones – by DART-TOF-MS (469); cathinones, pyrrolidinophenones, tryptamines, and synthetic cannabinoids and cannabimimetics – a review of analytical methods (toxicological focus) (470); 24 phenylethylamines (including 8 cathinones), 3 piperazines, and 3 tryptamines (only MDA, MDMA, ethylamphetamine, and AMT were listed in the abstract) – cross- reactivity in immunosorbent assays (471); phenethylamines, tryptamines, piperazines and cathinones – a review of analyses by GC-EI/MS, LC-ESI/QTOF-MS, and (in some cases) by NMR and FTIR (472); **2013** cathinones, phenethylamines, tryptamines, and piperazines – by LC-QQQ-MS/MS in the MRM mode (toxicological focus) (473);

"Ecstasy Tablets": 2010 impurity profiling of tablets seized in Vietnam using GC and GC/MS (474); **2011** variation in likelihood ratios for same- and different-batch comparisons (specific compounds and analytical methods not specified in the abstract) (475); microwave-assisted extraction of tablets for improved impurity profiling (476); chemical profiling by analysis and identification of residual solvents by static headspace (477); **2012** detection of amines in Ecstasy tablets using a fluorogenic probe (478);

Abused Drugs and Pharmaceuticals in Municipal Wastewater Streams: 2010 by isotopic-dilution direct injection RP-LC-MS/MS (location not specified in the abstract) (479); from a wastewater treatment plant located in "the mid-Atlantic U.S.," by solid phase extraction and GC/MS (480); an overview and review of current methodologies (481); in Paris, France using HPLC-MS/MS after SPE extraction (482); in three Canadian cities (method not specified in the abstract) (483); in Zagreb, Croatia using LC-MS/MS (484); **2011** by SPE

and LC/MS, including a critical evaluation and verification of methodologies (485); a historical review (486); in Australia (methodologies not specified in the abstract) (487); a sampling strategy for sport villages to monitor doping (488); refining the estimation of illicit drug consumptions from wastewater analysis (489); for estimating total drug consumption in small, semi-enclosed population (methodologies not listed in the abstract) (490); **2012** by Mixed-Mode SPE and LC-QTOF-MS (491); for estimating cocaine consumption in the Brazilian Federal District (492); **2013** a study of the uncertainty associated with the estimation of community illicit drug consumption via analysis of sewage (493); by online-SPE-LC/MS (494);

"Illicit Drugs" – Including "Controlled Substances," "Drugs of Abuse," "Illicit Drugs," "Narcotics," "Seized Drugs" (and similar generic terms): **2010** a sensor for "drugs of abuse" (495); screening for "drugs of abuse" by LC-DAD (496); detection of "drugs" using neutron computerized tomography and artificial intelligence techniques (497); detection of "narcotics" using IMS (498); rapid analyses of "illicit drugs" by FTIR and GC/MS (499); rapid field air sampling and analysis of "illicit drugs" using dynamic planar SPME-IMS (500); determination of "illicit drugs" by UHPLC/MS (501); "illicit drug salt forms" by LC/MS (502); qualitative analysis of "narcotics" using Raman and chemometrics (503); identification of "illicit drugs" by teraHertz spectroscopy (504); detection of "illicit drugs" using a tagged neutron inspection system (505); QSAR study on GC/MS Retention Times of "illicit drugs" (506); **2011** "drugs of abuse" and pharmaceuticals – identification of active ingredients by AP glow discharge MS (507); a review and overview of adulterants in "illicit drugs" and their effects (508); acquiring LC/MS or GC/MS analyses following dissolution of microcrystalline test products from "drugs of abuse" (509); detection of "illicit drugs" on surfaces using DART-TOF-MS (510); detection of drugs by proton exchange reaction MS (511); analysis of "narcotics" by Raman (512); detection of "controlled substances" in tablets by ATR/FTIR (and LC-ESIMS) (513); analysis of "seized drugs" by HILIC (514); analysis of banknotes (Euros) from the Canary Islands for "illicit drugs" by LC and MS (515); analysis of "illicit drugs" by GCxGC (516); detection of packaged or concealed "illicit drugs" by spatially offset Raman (517); detection and identification of "illicit drugs" using neutron based techniques (518); detection of "street drugs" by 3-dimensional Spectral Fluorescent Signatures (519); analysis of "multicomponent illicit drugs" by IMS (520); recovery of "illicit drugs" from surfaces using electrostatic lifting and nanomanipulation, with analysis by nanospray ionization mass spectrometry (521); a review of analysis of "drugs of abuse" by Raman (522); screening for "illicit drugs" on banknotes by LC-MS/MS (523); **2012** a review of hyphenated LC techniques (listed applications include "drugs of abuse in alternative matrixes") (524); use of gold-plated Mylar lift films for Raman of "drug residues" (525); 18 (unspecified) "illegal adulterants" in herbal medicines and health foods for male sexual potency – by LC-EI-MS/MS (526); screening of "narcotic drugs" using MECC on a microfluidic device (527); fabrication and use of silver nanoneedles array for SERS and their application in rapid detection of "narcotics" (stated to be especially sensitive for ketamine) (528); **2013** "forensic drug analysis" by microfluidic devices – an overview (529); an evaluation of the results of impurity profiling of "illicit drugs" from different

analytical methods and/or from different laboratories (530); analysis of “seized drugs” by LC-ESI/MS/MS and AP-MALDI-MS/MS, with comparisons of the two techniques (531); an overview of advanced analytical instrumentation and methods for “drugs of abuse” (toxicological focus) (532);

Pharmaceuticals/Counterfeits (with a focus on differentiation of legitimate versus counterfeit products, or for monitoring quality control for legitimate pharmaceutics)**: 2010** use of portable Raman for identification of tablets and capsules (533); detection of counterfeits using hand-held Raman, infrared, and NIR spectrometers (534); an overview of the analysis of multi-component formulations by spectrophotometric methods (535); imaging pharmaceutical tablets and screening counterfeit drugs by infrared laser ablation metastable-induced chemical ionization (IR-LAMICI) (536); analysis by NIR chemical imaging (537); a review of the use of NIR imaging for pharmaceutical production and counterfeit detection (538); an overview and review of detection of counterfeits using portable NIR and Raman spectrometers (539); a review of the use of FTIR and ATR/FTIR imaging in pharmaceutical production (540); NIR hyperspectral unmixing for chemometric characterization of counterfeit tablets (541); an overview of the detection of counterfeit drugs using LC, CE, and NIR (542); overview and review of the detection of counterfeit drugs, using artemisinin derivatives to illustrate advances in the field (543); analysis by CE (544); identification by NIR (545); detection of counterfeits by NIR (546); an overview of the use of Raman in the pharmaceutical industry (547); application of 2D and 3D optical microscopy in the examination of suspect counterfeit tablets (548); identification by NIR and NIR chemical imaging (549); detection by NIR (550); identification of tablets by Raman and chemometrics (551); a review of the determination of drugs by TLC (552); tracing the origin of complex pharmaceutical preparations using surface desorption AP-CI-MS (553); detection of counterfeits by NIR (554); **2011** detection of counterfeit drugs by NIR (555); comparison of laboratory and handheld Raman for the identification of counterfeits (556); detection and identification of counterfeits by NIR (557); discrimination between legitimate and counterfeit products using NIR, Raman, GC/MS, and FTIR, with application of supervised classifiers (k-Nearest Neighbors, Partial Least Squares Discriminant Analysis, Probabilistic Neural Networks, and Counterpropagation Artificial Neural Networks) (558); a review of non-invasive analyses of turbid samples using deep Raman (559); isotopic finger-printing of active pharmaceutical ingredients by 13C-NMR (560); by portable Raman (561); use of DART-MS to screen tableted pharmaceuticals and detect counterfeits (562); detection and profiling of counterfeits by Raman and chemometrics (563); use of isotope-labeled excipients to identify legitimate and counterfeit products (564); an overview of “poor quality” drugs (565); detection by DOSY-NMR (566); detection of counterfeits by NIR diffuse reflectance spectroscopy (567); detection of counterfeits by quantitative NMR and DOSY NMR (568); analysis by TLC with AccuTOF-DART MS (569); overview of detection using a portable NIR spectrometer (570); detection and analysis of counterfeit pharmaceutical tablet cores by ATR/FTIR and micro-ATR/FTIR imaging (571); discrimination of illicit tablets by surface granularity (572); identification of the components in drugs by near-infrared hyperspectral unmixing of tablets (573); an overview of counterfeit drugs (574); a review of

rapid, noninvasive characterization of pharmaceuticals and counterfeits in packaging or containers using Raman (575,576); determination of the elemental distributions in tablets by confocal micro-XRF (577); invisible labeling of pharmaceuticals for identification and verification of authenticity (578); a review of chiral analyses of drugs (579); detection of counterfeits by vibrational spectroscopy (580); a review of methods used to detect counterfeits or confirm authenticity (581); overview and review of Raman for analysis of pharmaceuticals (582); an overview and review of counterfeiting (583); analysis of pharmaceuticals with hyperspectral Raman imaging and various chemometric methods (584); analysis of pharmaceuticals by DART-AccuTOF-MS following TLC separation (585); **2012** comparison of handheld to benchtop Raman instruments for the identification of authentic versus counterfeit tablets (586); detection of counterfeit tablets by transmission Raman (587); quality control screening and counterfeit detection using portable Raman (588); evaluation of differently manufactured pharmaceutical tablets (including illicit drugs and counterfeits) Raman hyperspectral images (589); use of laser-induced breakdown spectroscopy and support vector machines for classification of pharmaceuticals and counterfeits (590); by DART-MS – an overview (listed applications include "screening of counterfeit drugs") (591); analysis of "soft" pharmaceuticals and counterfeits (suppositories, etc.) by DART-MS (592); analysis of tablet packaging by Raman microscopy and 2D-correlation spectroscopy (593); monitoring and detection using NIR (594); analysis of residual solvents in counterfeits by GC/MS (595); differentiation of legitimate versus counterfeit drugs by NIR and chemometrics (596); 14 unspecified "sedative-hypnotic drugs" – detection in health foods and traditional Chinese medicines by GC/MS (597); **2013** a review of a paper-based test for screening for counterfeits (598); an overview of chromatographic and spectroscopic detection methods (599); by Raman (600); a review, focusing on HPLC and MS, but also discussing color testing, TLC, GC, Raman, NIR, FTIR, and NMR, using antimalarial drugs and sildenafil (Viagra) as illustrative examples (601); an overview of the use of GC/MS for "forensic substance identification" (602).

2.B – New and/or Improved Instrumental Techniques

Atomic Absorption Spectroscopy: **2012** a review, focusing on pharmaceuticals (listed applications include "forensic") (603);

Capillary Electrophoresis (and Related Techniques, including Tandem Techniques): **2011** CE – a review of the literature from 2006-2010 (focus is "natural products; " listed applications include pharmaceuticals and "toxicological compounds of interest to forensics") (604); **2012** evaluation and optimization of CZE for common drugs of forensic interest in aqueous matrices (605); CE – a review of the literature from 2009 to 2011 (listed focus includes illicit and abused drugs, ions, and small molecules of forensic interest) (606); **2013** a review of recent advances in electrodriven enantioseparations (listed applications include "pharmaceutical" and "forensic") (607);

Gas Chromatography: 2012 a review (listed applications include "bulk drugs") (608);

Infrared Spectroscopy: 2012 ATR/FTIR – a review (includes select chemical, pharmaceutical, and forensic applications) (609); IR of solid–dosage drug substances – an overview (610);

Infrared and Raman Spectroscopy: 2012 in Forensic Science (Reference Text) (611);

Ion Spectroscopy: 2012 IMS with an orthogonal acceleration sector TOF mass analyzer (designed for "forensic applications") (612);

Mass Spectrometry: 2010 identification of active compounds in tablets by flow-injection data-dependent tandem mass spectrometry combined with library searching (613); differentiation of structural isomers of "drug substances" using LC/Q-TOFMS and fragmentation prediction (614); **2011** ESI-MS – use of wooden toothpicks for facile loading and ionization of samples (615); ambient ionization mass spectrometry – an overview and review, including discussions of counterfeit and illicit drugs (616); DART-MS – a review (listed applications include pharmaceuticals and forensics) (617); a review of the applications of DESI-MS (includes "drugs," pharmaceuticals, and "forensics") (618); **2012** ambient desorption/ionization MS (ADI-MS) – an overview and review (listed applications include "forensics") (619); identification of unknowns utilizing accurate MS data and ChemSpider (620); an overview of recent advances (621); identification of unknowns using an API MS/MS library (622); **2013** ambient mass spectrometry – a review, including DESI, DART, and extractive ESI (listed applications include "forensic identification") (623); DESI-MS (listed applications include "illicit drugs") (624);

Microscopy: 2010 an overview (625);

Nuclear Magnetic Resonance Spectroscopy: 2012 high-precision 1H-qNMR – for determination of the purity of standards (626);

Raman: 2010 non-contact, in-the-field analysis of "hazardous materials" by portable Raman operating in various modes (627); **2011** a review (includes forensic science applications) (628); **2012** multi-wavelength excitation Raman spectrometers and microscopes (listed applications include "narcotics identification") (629);

Solvent-Microextraction: 2013 a review (listed applications include forensic and pharmaceutical) (630);

Stable Isotope Analyses: 2010 recent advances (includes drugs) (631); position specific 13C analysis for determination of source and the natural attenuation of contaminants (632); a review of the use of stable isotopes in forensic science (633); **2011** an overview of the use of IRMS, proposing a 6-step methodological approach for application to specific forensic issues (634); a general review of the use of stable isotopes to determine source (635); **2012**

an overview of the signature value of isotope deltas (636); **2013** a review of inter-laboratory comparability (637); tracking authentic pharmaceuticals by 2H- and 13C-NMR (638);

Thin Layer Chromatography: **2011** a review of TLC/MS (639); **2012** quantitative HPTLC-densitometry – converting TLC screening for counterfeit pharmaceuticals to HPTLC (640);

X-Ray Techniques: **2012** wavelength-dispersive XRF – for analysis of very small samples (listed applications include "forensic analysis") (641).

3. Miscellaneous Topics

Clandestine Laboratories – Appraisals and Safety: **2012** comparison of first responder decontamination procedures (642); testing of fire resistant fabrics after the application of flammable solvents (643); therapeutic detoxification of law enforcement personnel suffering from chronic occupational exposure to methamphetamine (644);

Education: **2011** analysis of a simulated drug sample by GC/MS and FTIR (645); analysis of a simulated drug sample by TLC and GC/MS (646); **2013** use of forensic science to teach method development in undergraduate analytical laboratories (647);

Legal Issues: **2010** legal issues (648); **2011** legal issues (649); **2012** brief news release concerning counterfeits (650); reference text (651);

Packaging: **2011** identification of plastic packaging used by body packers, by IR (652); **2012** a review of the use of SEM/EDS and FTIR to identify counterfeit pharmaceutical packaging (653); analysis of polyethylene cling film (commonly used for packaging illicit drugs) by ATR/FTIR (654);

Quality Assurance: **2010** measurement uncertainty in forensic/analytical testing (655); the uncertainty in measurement of the total mass of a substance packaged in numerous containers (656); **2011** comparison of the stability of stock solutions of drugs at freezer, refrigerator, and ambient temperatures (657); measurement uncertainty in sampling and analysis of illicit drugs (658); **2013** use of a software tool ("Drugs WorkBook") for the quantification of illicit drugs (659);

Sampling Plans: **2010** an Excel based sampling calculator (660); a probability-based sampling approach for the analysis of multiple containers of cocaine, heroin, or marijuana (661);

Soil: **2011** determination of source by XRF (662); **2012** analysis by Raman following oxidative sample preparation (663); an overview of forensic analysis for determining geographical source (664);

Other: **2010** an informal classification scheme for "designer drugs" in Israel (665); **2011** an overview of drug production and use in New Zealand (666);

synthetic chemist David Nichols discusses his research on psychedelic compounds, commenting on how his products have been abused (667); **2012** Laboratory Information Management System (LIMS) – an overview and review (668).

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Toxicology

Review 2010 - 2013

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TABLE OF CONTENTS

1 Abstract

The rapid development of forensic toxicology in recent years is evidenced by the proliferation of professional societies, growth in the awareness for the need of accreditation and quality assurance, and publication of a large number of research articles encompassing a variety of toxicology disciplines. Undeniably, advancement of forensic toxicology has been largely driven by the development of highly sophisticated instruments and improved methodologies. These breakthroughs lead to a remarkable enhancement in sensitivity and specificity of detection that render the detection of drugs and poisons at very trace level and in a wide range of biological specimens possible. Nevertheless, the continual appearance of new designer drugs has posed serious challenges to both analytical and interpretative abilities of forensic toxicologists since the chemical structures of these drugs are continuously modified in order to obscure their detection and evade legislative control.

The purpose of this paper is to review the scientific literature from 2010 to 2013. This review is divided into two parts, namely “Current Toxicological Issues” and “Advances in Toxicological Analysis” capturing the significant progress and development in the field of toxicology over the past three years by making reference to hundreds of articles and papers published in the international journals and symposiums.

2 Introduction

Toxicology is not a science that simply studies the toxic and harmful effects of chemicals, drugs and poisons; it has to draw upon knowledge, theories and techniques from diverse scientific fields such as biochemistry, chemistry, epidemiology, pharmacology and pathology in order to deal with the ever increasing complexity in this discipline. Forensic toxicologists are tasked with the challenges in detecting and identifying alcohol, drugs and poisons in bodily fluids, tissue samples and related items, and, whenever necessary, offering professional opinion to aid the medico-legal investigation of death, poisoning and drug-facilitated criminal offences in the interest of justice.

3 Current Toxicological Issues

3.1 Driving Under the Influence

Undoubtedly impaired driving caused by the influence of alcohol or drugs has led to a very large number of accidents and casualties every year worldwide since the intake of alcohol and drugs directly impairs the driving abilities, response time and judgment of the drivers as well as affects their coordination of cognitive and psychomotor functions during driving. In a clinical research using the technique of functional magnetic resonance imaging (fMRI), impaired driving behavior is associated with disruptions in functional network connectivity (1).

3.1.1 Driving under the influence of alcohol (DUIA)

Various bodily specimens may be considered for measuring the concentration of alcohol in an individual. The two most popular specimens for alcohol testing are blood and breath. Since blood alcohol analysis is invasive, expensive and time-consuming, breathalysers, which are non-invasive, become the most prevalent devices worldwide to assist the law enforcement nowadays.

That blood and breath analyses are interchangeable is based on the presumption that there is a stable relationship between the blood and breath alcohol levels. Grubb D *et al* have studied their relationship during the absorption, distribution and elimination phases of alcohol metabolism with particular emphasis on the absorption phase (2). Even though sampled blood is stored in the presence of preservative and anticoagulant, it is imperative that blood alcohol analysis should be performed as soon as possible because studies have shown that blood alcohol concentrations decreased over long term storage both under refrigeration and at room temperature (3). Besides measurements using conventional devices, recent studies have been undertaken to develop a novel non-invasive biological sensor for detecting individuals driving under the influence of alcohol by measuring biosignals (4).

As a defence argument to evade justice, drivers may allege that consumption of alcohol took place after driving by a tactic commonly known as the hip-flask defence. A research was undertaken by Jones AW on human pharmacokinetics with a major focus on elimination rate of blood alcohol (5). The study facilitated back calculation for cases in which the courts of law want

to know the defendants' blood alcohol concentration at some earlier time, such as the time of driving.

Apart from enforcement, public education and publicity are of equal importance to raise the awareness of the legal implications as well as the dangers of driving while intoxicated. High concentrations of blood alcohol (≧0.8g/L) significantly increase the risk of severe injuries while driving (6). It has been reported that educational programmes in Brazil should be targeted at specific groups in order to increase their awareness about the legal blood alcohol concentration limit and its consequence (7). A study has analyzed local drink-driving patterns by a cluster analysis approach to model the spatial-temporal variation of drink-driving distribution in Hong Kong (8). The results indicated that drivers in rural areas tend to consume more alcohol than those in urban areas. Another study (9) investigated the trend of drink driving in Hong Kong after the implementations of random breath testing and alcohol tax reductions. It was concluded that the problem of drink drinking could be combated by strategies such as random breath testing, awareness-raising campaigns and increased penalties.

It is well understood that combined consumption of alcohol and illicit drugs can have detrimental effects on driving beyond those of alcohol alone. Studies have shown that the effect of alcohol and cannabis taken simultaneously is indeed additive leading to increased risk of traffic accidents (10,11,12). It was found in reference (13) that blood concentrations of tetrahydrocannabinol (THC), the principal psychoactive ingredient of cannabis, would be higher when THC is consumed with alcohol. According to this study, this explains why drivers were more impaired in cannabis and alcohol combined conditions.

3.1.2 Driving under the influence of drugs (DUID)

It is known that use of drugs can impair driving. However the extent of impairment can be difficult to measure, predict or quantify. Furthermore, DUID is often under-reported or unrecognized. Effort has been made to investigate what types of drugs and their associated limits in blood that should be specified in DUID (14) and a consultation in this regard has been launched in the UK (15). In the USA, a national survey on drug use and health revealed that 9.4 million persons or 3.7% of the population aged 12 or older had driven under the influence of illicit drugs in 2011 (16).

Toxicological investigations of drivers killed in road traffic accidents in Norway during 2006-2008 showed that 17.9% (of 196 cases analyzed) of the fatally injured drivers had drugs or alcohol/drug concentrations above the proposed legal limit in the blood. The extent of impairment was comparable to a blood alcohol concentration (BAC) of 0.02% (17). Similar inference was found from a study in the Netherlands, which indicated patients who have been exposed to psychoactive medications, especially anxiolytics or selective serotonin re-uptake inhibitors (SSRIs), are more likely to be involved in traffic accidents (18).

Stimulants, depressants, hallucinogens and sedatives are among the frequently encountered drugs in drug-impaired drivers. Many of the drugs that affect central nervous system (CNS) produce characteristic effects. Depressants tend to slow reactions and reduce concentration. Drivers under the influence of marijuana may find complex driving situations more difficult to negotiate. Stimulants might make drivers over-confident and aggressive, while those under the influence of hallucinogens might react erratically to imaginary obstacles or sounds. In Switzerland, results from a nationwide study on DUID indicated that cannabinoids and cocaine were the most prevalent classes of drugs (besides alcohol) among DUID offenders in 2005 (48%, N = 2,291 and 25%, N = 1,184 respectively) (19). Prevalence studies conducted in different countries have demonstrated that drug-impaired driving (20,21,22,23,24), cannabinoids in particular (25,26), is a serious problem worldwide.

3.1.3 Detection of DUID

Identification of DUID drivers is generally based on two approaches, namely the impairment approach and the drug presence approach.

3.1.3.1 Impairment approach

Impairment approach utilizes standardized tests, based on a variety of observable signs and symptoms, and divided attention tests, to identify driving impairment associated with the consumption of drug. However, the relationship between the use of psychoactive drugs and degree of impairment is an extremely complex subject.

Several studies have been carried out to establish the impairment effect of amphetamine type stimulants (ATS) drugs on driving (27,28,29). In one of the studies (28), 8,709 cases of DUID plasma samples were analysed and 1,857

of them were positive to ATS, with cannabinoids being the most common (59.7%) co-consumption drugs. For those cases positive to ATS only, there is no correlation between impairment symptoms and plasma ATS concentration.

In another investigation (29), a dose of 0.42 mg/kg *d,l*-methamphetamine (MA) was taken orally by 20 healthy recreational illicit stimulant users. The mean levels *d,l*-MA detected in blood and saliva were found to be 95 ng/mL and 475 ng/mL respectively after 3 hours of drug administration. Participant's simulated driving performance was evaluated at 150 minutes post consumption. These drug levels were found not to significantly impair or improve the driving performance in the simulated test.

Standardized Field Sobriety Tests (SFSTs) are physical tests designed to assess psychomotor and cognitive functioning and divided attention component. These are believed to be accurate indicators of driving behavior following the consumption of alcohol or drugs. Evaluation of the effectiveness of SFSTs in identifying impaired driver who consumed *d*-MA and *d,l*-3,4-methylenedioxymethamphetamine (MDMA) was the main subject of the study (30).

In order to facilitate judicial process, impairment based legislative limits for DUID was suggested in Norway (31). Legislative limits for six classes of drugs (benzodiazepines, cannabis, CNS stimulants, γ-hydroxybutyric acid, hallucinogens, and opioids), which would cause degrees of impairment comparable to BAC of 0.02% and to BACs of 0.05% and 0.12 %, were proposed as impairment limits and as limits for graded sanctions respectively.

3.1.3.2 Drug presence approach

Even though collection of blood sample is considered invasive and requires the assistance of qualified healthcare professionals, who may not be available at roadside, blood remains the preferred specimen because it provides a direct evidence of the presence of drug(s) in the body and information about the respective drug(s) concentration in blood. Specific and efficient screening can be achieved by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (32,33), which has the potential to analyse large drug panels. A fully automated method was developed which is capable of quantifying 31 illicit and medicinal drugs and metabolites, including commonly abused drugs such as amphetamines and cocaine, in whole blood (32). The

published method (33), developed in a bid to both facilitate high-throughput screening and replace immunoassay, simultaneously detected 28 drugs/metabolites in 0.5 mL of whole blood in 9 minutes. Alternatively, the use of time-of-flight mass spectrometry (TOF-MS) was able to resolve isobaric compounds and identify unknown analytes by accurate mass measurement (34).

A study (35) in Sweden examined 1,000 blood samples from drivers suspected of DUID. Blood concentrations of diazepam and nordiazepam were assessed against the upper therapeutic limit of diazepam (0.83 mg/L in blood). 9% (N=90) of the cases had blood concentrations of diazepam above 0.83 mg/L, where 27% (N=267) of them were above that limit if the combined concentrations of diazepam and nordiazepam were considered. According to a study aiming at quantifying the concentrations of drugs in blood collected from suspected drugged drivers in England and Wales (36), diazepam was the second most common drug of abuse.

To combat drug driving, the use of oral fluid (OF) as an alternative specimen to detect the presence of drugs has accelerated in recent years and effort has been made to compare drug concentrations in blood and OF (37,38). Drugs concentration ratios between OF and blood (OF/B) varied considerably from drugs to drugs and patients to patients. The median OF/B ratios found for zopiclone, amphetamine, THC, MDMA, codeine, and MA were 3.8, 7.1, 4.7, 4.6, 5.4 and 2.9 respectively. On the other hand, benzodiazepines included in the study had low OF/B ratios (mean <0.5) and this can be explained by their high protein binding ability. The considerable variation in drug concentration ratios between OF and blood indicated it might not be possible to estimate drug concentration in blood precisely from that in OF. Nonetheless, OF/B ratios have been used to estimate the prevalence of drug concentrations in blood above specified limits (39).

OF is gaining popularity as an alternative matrix for drug testing in different fields, especially in roadside drug testing because of the ease and less intrusive protocol of sample collection. The performance of various on-site OF drug testing devices was assessed (40,41,42,43), where effort has been focused on sensitivity and specificity of the devices. In one of the studies (40), eight on-site OF drug screening devices for enforcement purposes were evaluated in Belgium, Finland and the Netherlands, as a part of the European

collaborative project named “Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID)” that was carried out between October 2006 and October 2011. OF screening results were assessed against the DRUID cut-off concentrations. Overall, no device reached the 80% goal set for sensitivity, specificity and accuracy for all of the separate tests that they comprised.

Besides on-site screening devices, collection tools can have a major impact on the concentrations of drugs present in OF. Stability of two collection devices, Intercept® and StatSure Saliva Sampler™, were compared by using authentic OF samples with different substances (44). According to the study, drugs showed greater stability in StatSure than in Intercept® for storage at 4 ºC or ambient temperature for one week. Recovery of zopiclone was particularly problematic (Intercept®: 6% and StatSure: 56% after one week room temperature storage). As a result, freezing after sampling was advised. In a study focused on THC (45), recoveries of THC from on-site collectors were unsatisfactory due to the problem of drug adsorption onto the collectors.

A recent research (46) evaluated cross-reactivities of three commercial OF immunoassays: amphetamine direct enzyme-linked immunosorbent assay (ELISA) kit, MA direct ELISA kit, and Oral-View Saliva multidrug of abuse test for detection of ATS. None of the ELISA kits showed significant cross-reactivities with *d,l*-fenproporex (FEN), *d,l*-diethylpropion (DIE) and *d,l*-threo-methylphenidate (MPH) (Amphetamine ELISA: < 0.01%, < 0.006% and < 0.006% respectively; MA ELISA: All < 0.02%). Oral-View did not cross react with these drugs at 10 folds of the cutoff concentrations (50 ng/mL). It should be noted that MPH and DIE are commercialized in the United States, while FEN is used as an anorectic in Brazil and Chile.

Due to the volume of OF collected is usually relatively low, simultaneous analysis of multiple drugs in OF is expected. Recently, an ultra-high-performance liquid chromatography (UHPLC) MS/MS method was published (47) for the detection of opiates, amphetamines, cocaine, ketamine, and cannabinoids in a single 11-minute run. 466 on-site residual OF samples were collected. 250 µL of OF was spiked with deuterated internal standard and injected to UHPLC-MS/MS directly. No sample preparation was needed. Of the 466 samples, 74 samples showed the presence of cocaine and its metabolites, THC was detected in 49 samples, MDMA was detected in 11 samples and ketamine in four samples and two samples showed codeine and morphine. In

contrast, a sophisticated gas chromatography-mass spectrometry (GC-MS) method (48) was developed to simultaneously detect and quantify 50 drugs of abuse and medicinal drugs in OF, including cannabinoids, cocaine, amphetamines, opioids, benzodiazepines and other psychoactive medicines. Altogether, 4,183 OF samples were collected on-site with StatSure SalivaSampler™ device in Finland. These were analyzed with the aforesaid method as a part of the EU project DRUID. THC was found to be the most prevalent drug.

To evaluate the performance of an OF drug screening device, confirmatory results done by using liquid chromatography-MS/MS (LC-MS/MS) are needed. Evaluation of DrugWipe® benzodiazepine on-site test was carried out in Finland with whole blood specimens (49). Use of OF on-site screening tests and blood confirmatory analyses mimics the real scenario in many countries. In a total of 224 DrugWipe® OF positive cases from the Finnish police, 181 were positive for one or more benzodiazepines in the whole blood analysis. DrugWipe® OF screening device was able to report positive benzodiazepine results in OF from cases that contained only single benzodiazepine with relatively low concentration in whole blood analysis. In one of those screened-positive cases, clonazepam (therapeutic range: 20-60 ng/mL) with a concentration of 11 ng/mL in whole blood was detected.

Confirmation analysis in DUID cases has been continuously proven to be challenging in light of emerging new designer drugs and a variety of drugs affecting the CNS (50,51,52). Synthetic cannabinoids, for instance, often lead to driving impairment similar to that caused by cannabis (53) and could go undetected by routinely used drug screenings. Two suspects were arrested for DUID with amphetamine-like impairment. Target confirmatory analyses of their urine samples, which previously tested positive for amphetamines in an immunoassay screening, were found negative. A GC-MS method was thereby established for the analysis of 4-fluoroamphetamine (4-FA) in serum with a limit of detection (LOD) of 1 ng/mL. Using the new method, 4-FA was detected in serum at concentrations of 350 ng/mL and 475 ng/mL in the two subjects respectively. Another designer drug, 3,4-methylenedioxypyrovalerone (MDPV), emerged in Finland since 2008 (52). Blood samples from 3,000 drivers suspected of DUID were screened for MDPV using an LC-MS/MS method with a LOD of 3 µg/mL. 259 of them were tested positive for MDPV, accounted for 5.7% of the confirmed DUID cases in Finland from August 2009 to August

2010.

3.2 Drug-facilitated Sexual Assault (DFSA)

In recent years, there has been an increase in the number of reports involving the administration of drug(s), sometimes in conjunction with alcohol, to render a victim physically incapacitated or helpless and thus incapable of giving or withholding consent. If an individual takes advantage of such situation and has non-consensual sexual relations with the victim, it should be considered a case of DFSA. Victims may be unconscious during all or parts of the sexual assault and, upon regaining consciousness, may experience anterograde amnesia which means individuals may not recall events they experienced while under the influence of drug.

3.2.1 Detection of drugs

There has been an increase in reported DFSA cases over the last 15-20 years (54,55). In a separate report, 135 cases of DFSA in the Netherlands were studied from January 2002 until December 2006 (56). The study showed that alcohol was the most commonly found substance in DFSA cases in the Netherlands followed by non-opiate analgesics and illicit drugs (of which the most frequently encountered drugs were cocaine, MDMA, THC or their metabolites, followed by amphetamine and benzodiazepines). In the same report, it was found that blood specimens collected within 12 hours of the alleged assault were all tested positive for alcohol or drugs while those collected more than 24 hours after the alleged sexual assault were all tested negative. When urine samples were available, only 36% of the cases with collection time longer than 24 hours had negative toxicological results. It was therefore concluded that if sexual assault took place 24 hours or longer, urine rather than blood would be a more suitable specimen for collection.

Ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOF-MS) was used for the screening of 46 medicinal drugs and abused drugs (including amphetamines, cocaine, benzodiazepines and opioids) in 167 whole blood samples obtained from victims of alleged sexual assault cases in the Aarhus area, Denmark (57). The whole blood samples were extracted using a mixed mode solid phase extraction procedure and the estimated limits of quantification for the drugs ranged from 0.06 to 27 ng/g. Ethanol, barbiturates, THC and its metabolites were analyzed using other

methods. It was concluded that only a small percentage of all cases seemed to be genuine DFSA cases. It was also notable that victims tested positive of medicinal/abused drugs did not undergo a timely medical examination.

3.2.2 Drugs detected in DFSA cases

Two cases of DFSA using tetrahydrozoline (THZ), an ingredient in over-the-counter eye drops, were reported (58). THZ was detected in the urine samples by GC/MS at levels of 114 ng/mL and 150 ng/mL, respectively, in these two cases. However, THZ was not detectable in the blood for both cases. It was shown that the use of GC/MS was successful in identifying THZ in the 100 ng/mL range up to 20 hours post-exposure. Stillwell *et al.* also reported a case with THZ at a level of 1.481 ng/mL in the urine approximately 7 hours after the victim was reportedly being sexually assaulted, even though no symptoms was observable in the emergency department (59).

Gamma-hydroxybutyrate (GHB) has frequently been implicated in a number of DFSA cases. In this regard, the possibility of maintaining long term stability of GHB in both post-mortem and ante-mortem whole blood samples was investigated in reference (60). Cut-off level in the study was 10.3 mg/L and GHB concentrations were found to be stable for several years in both post-mortem and ante-mortem samples when stored at -20°C with fluoride preservation. The maximum changes in GHB concentrations were 32.4% for ante-mortem and 34.4% for post-mortem samples.

Cut-off values of exogenous GHB remained an active area in research. A study of in vitro production of GHB in blood and serum samples suggested that the 5 μg/mL cut-off for exogenous GHB could be lowered significantly if the whole blood sample is frozen immediately after collection with procedure well documented (61).

As for urine specimens, a study of urinary GHB concentrations in samples taken from 1,126 healthy female volunteers supported the use of 10 mg/L urinary GHB as the cutoff (54).

γ-butyrolactone (GBL) was known to be metabolized into GHB. Pharmacokinetics study of GHB after single uptake of a low dose of GBL showed that the GHB concentration in serum decreased below 1 μg/mL after 4-5 hour and further diminished to less than 1 μg/mL within 8 - 10 hours (62).

γ-valerolactone (GVL) is reported to be a substance that can be used as a legal substitute for GHB. But unlike GBL and 1,4-butanediol, GVL is not metabolized to GHB. Instead, the lactone ring of GVL is split to form gamma-hydroxyvaleric acid (GHV or 4-methyl-GHB) by lactonase. Andresen-Streichert *et al.* reported the detection of GVL in three cases (63). The study results indicated that GVL can be used as an alternative to GHB and its precursors, i.e. GBL and 1,4-butanediol. With one of the three cases being probably a DFSA incident, the use of GVL should be taken seriously. It was advised that GVL or GHV should be included routinely in toxicological analysis, particularly in DFSA cases.

3.2.3 Summary

When drugs are used to facilitate an assault, the victims, medical professionals and law enforcement officers are relying on the forensic toxicologist to conduct the best possible testing of the available specimens. It is imperative that adequate volumes of blood and urine samples be collected from the victims as soon as practicable. This is particularly pertinent for drugs that are eliminated quickly such as GHB and its related compounds. At the same time, forensic toxicology laboratories should properly preserve the drugs in the specimens to prevent them from deterioration, develop validated analytical procedures, and employ sophisticated instruments whenever necessary so as to improve the detection limits in their drug screening as some drugs may be present at very low levels in DFSA cases.

3.3 Workplace Drug Testing

Federal Workplace Drug Testing Programme was firstly introduced in the United States in 1988 aiming at establishing a drug free environment in workplace through a mandatory requirement for all relevant executive-level and civil-service federal employees to pass urine drug tests for drugs of abuse (64). Now, Substance Abuse & Mental Health Services Administration (SAMHSA) of the Department of Health and Human Services is authorized to promulgate scientific and technical guidelines for drug testing programme.

Meanwhile, pre-employment and workplace drug testing in the field of safety-critical and security-sensitive jobs has increased rapidly over the last decade in many European countries including Italy and Turkey

(65,66,67,68,69,70,71,72,73). Since the outcomes of testing can have serious consequences for the employees, the European Workplace Drug Testing Society (EWDTS) has formulated guidelines in order to ensure that the whole drug testing process is of high quality, accredited, and defensible, hence giving accurate and reliable information about employees' drug use profiles while respecting their privacy. Furthermore, the testing laboratories must adhere to national and international quality standards (ISO/IEC 17025) (67).

3.3.1 Urine for workplace drug testing

Urine remains the most commonly used specimen for drug testing because the technology used in urine testing is well developed and has withstood legal challenges. Drugs in urine are normally detectable several days after the last intake (74). A positive urine test result can serve as an evidence of recent use, but does not necessarily mean that an individual was impaired at the time of being tested (64).

Careful attention should be exercised at the time of collecting urine specimen from donor in order to avoid tampering by adulteration, substitution or dilution which may circumvent the purpose of drug testing. Aiming to evade detection, potassium nitrite is an effective urine adulterant due to its oxidizing potential, and has been shown to mask the presence of many drugs of abuse. A study (75) has revealed the possibility of using LC-MS to detect two stable reaction products, i.e. 2-nitro-morphine and 2-nitro-morphine-6-glucuronide in an attempt to indirectly infer morphine and morphine-6-glucuronide in urine once the specimens are suspected to be adulterated with nitrite. Since dilution of urine specimen is another deceitful tactic to avert drug detection, a study (76) has examined the effectiveness of creatinine normalization on urine drug concentrations of 5 substances (amphetamines, cocaine, marijuana, opiates, and phencyclidine) and the test results indicated that the proportion of reported positives would be affected.

Workplace urine drug testing usually adopts a two-step approach for the positive identification of drugs. This involves both a screening test and a confirmatory test. Immunoassay is commonly used as a screening tool because the method is fast, inexpensive and reasonably cost-effective. A urine specimen once presumptively screened positive by immunoassay must be subject to confirmatory testing by mass spectrometry techniques in order to eliminate false-positive results that may arise from cross reactivity in immunoassay (77,78).

Generally, immunoassay can screen for most common drugs of abuse, but fail to detect a number of emerging designer drugs. In contrast, direct analysis using LC-MS/MS offers an attractive way forward for the development of a rapid routine screen for new psychoactive substances (79). It was also reported that a multi-target screening method that allows the simultaneous detection and identification of 700 drugs and metabolites in biological fluids by using a hybrid triple-quadrupole linear ion trap mass spectrometer in a single analytical run was successfully developed. With the assistance of software program to achieve automated acquisition and library searching, the time for evaluation and interpretation of the test results could be drastically reduced (80).

THC remains one of the most frequently encountered drugs in workplace drug testing. Therefore, there is a great demand for sensitive, rapid and reliable methods for confirming the presence of this drug or its metabolite in biological samples including urine. A newly developed method employing LC-MS/MS for direct analysis and simultaneous determination of THCA and THCA-glucuronide in urine, without the need of hydrolyzing/derivatising the samples has been validated and proved to be accurate, precise and sensitive with a LOD of 5 ng/mL for both analytes. The developed method had been applied to several authentic samples of urine which were tested positive in immunoassay screening and 98% of them were confirmed (81). As a marker for detection of cannabis abuse in urine, THCA needs to be present at a concentration exceeding 15 ng/mL for a positive result to be reported. A research team presented a method (82) combining a GC-MS/MS method with a fast sample preparation procedure using microwave assisted derivatisation. This method was proven to be selective, linear over the range 5-100 ng/mL, along with excellent precision and trueness.

Another study demonstrated the use of a newly developed method employing GC-MS technique for the quantitative analysis of the new designer drug MDPV along with common stimulants including amphetamine, methamphetamine, and MDMA in urine (83).

The ongoing epidemic of prescription opioid abuse in the United States has prompted interest in semi-synthetic opioids in the federal workplace drug testing program. Cone *et al.* initiated a study characterizing the metabolism and disposition of oxycodone (OC) in human urine (84). Twelve healthy adults were administered a single oral 20 mg dose of OC in a controlled clinical

setting. Their urine specimens were collected at regular time intervals and analyzed by liquid chromatography-tandem mass spectrometry for OC and its metabolites. The data of this study provided information in facilitating the selection of appropriate test parameters for OC in urine and interpretation of test results.

3.3.2 Oral fluid for workplace drug testing

As an alternative specimen to urine for workplace drug testing, oral fluid is increasingly used because the concentrations of many drugs in oral fluid seemingly correlate well with blood/plasma concentrations. However a study indicated that cannabinoid concentrations in oral fluid cannot predict respective concurrent concentrations in plasma (85). Advancement of instrumental sensitivity makes oral fluid a suitable alternative to blood. Oral fluid is getting popular because its collection is easy, convenient and non-invasive. Furthermore, adulteration is inherently difficult (86). Even though SAMSHA is still actively seeking comments about the use of oral fluid as an alternative specimen in Federal Workplace Drug Testing Programs, EWDTS has outlined guidelines for oral fluid drug testing that suggested the maximum cut-off concentrations acceptable under the workplace drug testing programme. The recommended cut-off values may be subject to change as advances in technology or other considerations warrant identification of these substances at different concentrations (87).

As oral fluid often contains drugs in low concentrations and volumes of specimen collected are small, it is therefore necessary to have a sensitive, multi-component method for drug detection. Through a research study (44), such objective has been fulfilled by successful development of a method employing an UPLC-MS/MS with 32 drugs of abuse being determined with a cycle time of 9 minutes. Furthermore, stability of drugs in oral fluid before analysis was evaluated and test results showed that 6-acetylmorphine, cocaine and zopiclone were the least stable drugs. Therefore, samples of oral fluid should be analysed as soon as possible after collection, and the specimens should be kept frozen if immediate analysis is not possible.

Since cannabis abuse has long been a concern in workplace, many studies were undertaken to test for the abilities of different drug screening devices in detecting cannabinoids in oral fluid including the characterization of assay performance and limitations (88,89), as well as establishing the detection windows and cutoff concentrations of different cannabinoids in oral fluid (90).

In response to the concern about potentially false-positive results arising from passive exposure, Scheidweiler *et al.* proposed using 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) as a marker of cannabis intake since it is not present in cannabis smoke and was not measureable in oral fluid collected from subjects passively exposed to cannabis (91). However, THCCOOH concentrations are in the pg/mL range in oral fluid and pose considerable analytical challenges. A method employing HPLC-MS/MS triple quadrupole system was successfully developed and validated for quantifying THCCOOH with limit of quantification at the level of <15 pg/mL.

MDMA is another drug gaining popularity of being abused in workplace. However, little is known about MDMA detection window in oral fluid. Study showed that MDMA was first observed in oral fluid 0.25-1.25 hours after administration of a recreational dose and MDA was subsequently detected at 0.5-1.75 hours. In general, the windows of detection for MDMA and MDA were 47 and 29 hours, respectively, although a few specimens were positive up to 71 and 47 hours (92).

3.3.3 Hair for workplace drug testing

Hair is an excellent specimen for pre-employment drug testing because of its ability to provide historical information on drug intake of an individual from months to years through a much longer window of detection (74). In contrast to providing short-term drug abuse profile through blood and urine testing, hair drug testing provides complementary information about the long-term drug abuse history of a donor. Furthermore, sampling head hair specimen is considered non-invasive and drugs incorporated in the hair remain stable and bound for a long time leading to little concern about specimen adulteration.

In Lombardy Region, Italy, individuals undergoing hair testing for workplace drug testing can choose one of the eleven analytical laboratories accredited for forensic proposes to conduct the analyses. An inter-laboratory exercise was therefore performed to verify the level of standardization of hair testing for drugs of abuse in these accredited laboratories. Nine out of these eleven laboratories participated in this exercise. Sixteen hair strands coming from different subjects were longitudinally divided in 3-4 aliquots and distributed to participating laboratories, which were requested to apply their routine methods for testing for drugs of abuse. Results demonstrated good qualitative

performance for all participants, since no false positive results were reported by any of them (93).

Incorporation of drugs in hair varies greatly between different classes of drugs and is subject to influence of melanin affinity, lipophilicity and membrane permeability. An article has deliberated the importance of whether the analytical procedure employed for hair drug testing was sensitive enough to identify traces of drugs; this is particularly important when the urine sample(s) of the subject was positive and the hair sample(s) was negative. It was concluded that until laboratories have sensitive enough methodologies to detect a single use of drug, care should be taken to compare urine and hair findings because the negative hair findings can cast doubt on the positive urine analysis, resulting in substantial legal debate and various consequences for the subject (94).

A scientific publication reported that a simple procedure was developed and validated for qualitative and quantitative analysis of several opiates (morphine, 6-acetylmorphine, codeine, 6-acetylcodeine) and tramadol in hair by GC-MS through selected ion monitoring mode (95). Intra- and inter-day precision and trueness were in conformity with the criteria normally accepted in bioanalytical method validation. Furthermore, 6-acetylmorphine was not significantly hydrolyzed to morphine in the course of incubation.

In order to effectively monitor multiclass abused drugs in hair, a simple procedure that allows the simultaneous determination of a series of commonly abused drugs or their metabolites would be highly desirable. A method employing UPLC-MS/MS instrument for simultaneous quantitative determination of 13 drugs of abuse and their metabolites including THC, along with high sample-throughput, excellent sensitivity and selectivity was successfully developed and fully validated in a study (96). These qualities, combined with minimal sample treatment, make the cost of this screening affordable for most private and public administrations to undertake routine hair analyses for workplace drug testing.

3.4 Emergence of New Designer Drugs

The increasing popularity of new designer drugs is a growing challenge for law enforcement agencies worldwide. Emerged in early 1990’s, designer drugs

generally refer to analogues or derivatives of controlled psychoactive drugs that exert similar pharmacological effects. Their chemical structures are modified to varying degrees in order to obscure their detection and evade legislative control (97,98). However, some designer drugs identified in recent years are of entirely different chemical structures when compared to the psychoactive drugs they mimic. Though, they still affect the same receptors in the central nervous system. Normally, these drugs are designed such that they would circumvent legislative control of the existing drug ordinances.

3.4.1 Synthetic Cathinones

'Synthetic cathinones' refers to derivatives of cathinone, which is a beta-keto phenylethylamine mostly from khat plant. They are often considered "legal highs" and sold as "bath salts" or "plant food" and labeled "not for human consumption" to circumvent controlled drugs legislation. MDPV was recently classified as a Class I drug by Racing Commissioners International, indicating that it is a banned substance in equine athletes because it lacks therapeutic value in horses (98). With psychostimulant effect similar to that of amphetamines and cocaine, these recently emerged compounds have been marketed over the Internet and gained popularity among drugs abusers. Some of the synthetic cathinones, including mephedrone and naphyrone, have already entered the illicit drug market (99,100,101,102,103,104).

Detection and determination of 25 designer cathinones and their related ephedrines in blood sample using LC-MS/MS method was reported (100). The method used only 100 μL of blood and employed liquid-liquid extraction with 1 mL of 1-chlorobutane containing 10% of isopropanol. The lower limits of quantification (LLOQs) for this method were reported to be 10 ng/mL for all the compounds.

Studies of 3-bromomethcathinone and 3-fluoromethcathinone metabolism in rat urine and human liver microsomes using GC-MS and LC-HRMS found that the main metabolic steps were N-demethylation, reduction of the keto group to the corresponding alcohol, hydroxylation of the aromatic system and combinations of these steps (105).

A rapid with high sensitivity method for determining 32 cathinone derivatives and designer drugs of the phenethylamine, tryptamine and piperazine classes in serum using liquid chromatography triple quadrupole tandem mass

spectrometry (LC-QQQ-MS/MS) was reported. The limits of quantitation (LOQ) were reported to be in range of 1-10 ng/mL for each compound with LOD close to 10 pg/mL (106).

New designer drugs containing β-ketone analogues of 3,4-methylenedioxymethcathinone (βk-MDMA, 'methylone') were reported in New Zealand (107). In addition, the synthesis and analytical data for β-ketone-N,N-dimethyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (βk-DMBDB) were reported for the first time in the publication.

Fornal *et al.* also reported the use of high performance liquid chromatography-quadrupole time of flight mass spectrometry (LC-ESI-Q/TOF) for six 3,4- methylenedioxy derivatives including methylone, butylone, pentylone, MDPBP, MDPV and BMDP (108).

3.4.2 Reported fatal cases in association with the abuse of synthetic cathinones

There is a reported case of death of a 40-year-old male who injected and snorted "bath salts" containing MDPV (109). Another case of psychosis involving a 23-year-old male insufflated a bath salt product containing MDPV and 4-fluoromethcathinone (flephedrone) has been reported (110). The MDPV levels in serum and urine of the male were found to be 186 and 136 ng/mL, respectively. Flephedrone levels were reported to be 346 and 257 ng/mL in serum and urine, respectively. The bath salt product was found to contain 143 μg of MDPV and 142 μg of flephedrone per milligram of powder. Kesha *et al.* also reviewed MDPV related death cases (111).

Three fatal intoxications due to methylone, a designer cathinone were reported (112). The peripheral blood methylone concentrations in the three fatal cases were reported to be 0.84, 3.3 and 0.56 mg/L. Distribution of methylone in four post-mortem cases was also reported (113). The methylone heart blood concentrations were found to be 0.740, 0.118, 0.060 and 1.12 mg/L. The average liver-to-blood ratio was found to be 2.68.

Wyman *et al.* also reported the distribution of MDPV in a case of an exposure of a 39-year-old male to MDPV. MDPV was found uniformly distributed among multiple tissues (blood, brain, muscle, cerebrospinal fluid and lung) at concentrations of approximately 0.4 to 0.6 μg/mL. Tissue and fluids

responsible for detoxification/ excretion had higher concentrations of MDPV (kidney, liver and bile > 0.8 µg/mL). A blood concentration ≥ 0.4 µg/mL was judged sufficient to cause death (114).

3.4.3 Synthetic cannabinoids

Synthetic cannabinoids have been abused as new designer drugs since 2004 (115). They can be divided into seven major structural groups: 1) naphthoylindoles (such as JWH-018 and JWH-073); 2) naphthylmethylindoles; 3) naphthoylpyrroles, 4) naphthylmethylindenes; 5) phenylacetylindoles (such as JWH-250); 6) cyclohexylphenols (such as CP47,497); and 7) classical cannabinoids (such as HU-210) (116). Several synthetic cannabinoids, including JWH-018, JWH-073, JWH-200, CP 47-497, and CP 47-497C8 homologue, were given schedule I status by the US Drug Enforcement Administration (DEA) in early 2011 (116).

Detection and quantification of 25 synthetic cannabinoids, including WIN 48.098, AM-1241, WIN-55212-2, RCS-4 C-4 homolog, RCS-4 2-methoxy homolog, JWH-030, JWH-015m JWH-302, RCS-4, RCS-4 3methoxy homolog, JWH-250, JWH-073, JWH-251, JWH-203, JWH-018, JWH-081, JWH-007, CP 47497, JWH-019, RCS-8, CP 47,497 C-8 homolog, JWH-398, JWH-210 and HU-210 in human blood sample using LC-MS/MS were reported (115). The extraction efficiencies ranged from 30-101% and the matrix effects from 67-112%. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) was also reported (117).

Detection of JWH-018 and JWH-073 in post-mortem whole blood by UPLC-MS/MS was also reported (118). The LOD for each analyte was 0.01 ng/mL with a linear dynamic range of 0.05-50 ng/mL.

Two new types of synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA) together with five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13(CRA-13) and AM-1248 in illegal products were identified in Japan (119).

An analysis of first and second generation legal highs for synthetic

cannabinoids and synthetic stimulants by UPLC-TOFMS showed that many of the banned substances are no longer used and have been replaced by other derivatives that are federally legal in the US (120).

There are also some publications about the analysis for designer drugs and/or their metabolites in urine, for example, the analysis for CP 47,497 in human urine using LC-MS/MS (121); the detection of the urinary metabolite of 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001) (122) as well as 1-[(5-fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone (AM-694) (123) by GC-MS. The urinary metabolites of JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250 and RCS-4 were studied by LC-MS/MS. The major metabolic pathway was found to be monohydroxylation either at the N-alkyl side chain, the naphthyl moiety or the indole moiety. Moreover, metabolites with carboxylated alkyl chains were also identified for some of the compounds (124). Sixteen urinary metabolites of 3-(4-methoxybenzoyl)-1-pentylindole (RCS-4) were identified by GC-MS. The O-demethylated metabolites were found to be the most useful metabolic markers for the identification of RCS-4 ingestion (125).

In addition to LC-MS/MS, UPLC-TOFMS and GC-MS, solid-phase microextraction headspace gas chromatography-mass spectrometry (SPME-HS-GC-MS) was also used for the analysis of synthetic cannabinoids in herbal products (126).

JWH-018, JWH-073, JWH-200, CP47,497, JWH-250, HU-210 and cannabicyclohexanol (CP-47,497 C8) were determined in OF specimens collected with the Quantisal™ device using SPE and LC-MS/MS (127). The method was applied to specimens taken from two individuals and found respectively a peak concentration of JWH-018 of 35 µg/L 20 minutes after smoking “Blueberry Posh” and 5 µg/L 20 minutes after smoking “Black Mamba”. It was noted that JWH-018 was still detectable 12 hours after a single intake of “Blueberry Posh” while JWH-018 was not detectable 12 hours after intake of “Black Mamba”.

Gottard R *et al.* used LC-QTOF-MS for the screening of new psychoactive substances in hair. 435 samples were screened for the presence of 50 different synthetic cannabinoids, cathinones and phenthylamines, where 8 samples were found positive for JWH-018, JWH-073, JWH-081, JWH-250,

JWH-122, in a broad range of concentrations (0.010-1.28 ng/mg) (128).

3.4.4 Methoxetamine

Long-term use of ketamine has been reported to be associated with severe symptomatic urinary tract problems. Methoxetamine (MXE), an arylcyclohexylamine derivative of ketamine, is marketed as a “bladder safe” derivative of ketamine. It presents new healthcare threat because of its easy accessibility via the Internet, and lack of legal restrictions in many countries. A low dose of MXE is claimed to be cause for “peace and serenity”, although higher dose may act the opposite. Cases of MXE abuse by injection intramuscularly have been reported (129). A series of cases involving three individuals with acute toxicity related to the use of MXE was confirmed analytically. Their serum concentrations ranged from 0.09 to 0.2 mg/L (130). Another case of MXE abuse was also reported (131).

3.4.5 Other synthetic drugs

Direct analysis of benzylpiperazine, methylone, 5,6-methylenedioxy-2-aminoindane (MDAI), fenproporex, 4-fluoroamphetamine (4-FA), 4-methyl-N-ethylcathinone (4-MEC), 4-methylamphetamine (4-MA), methylbenzodioxolylbutanamine (MBDB), mephedrone, methylthioamphetamine (MTA), MDPV, mefenorex, nabilone, furfenorex, clobenzorex, JWH-200, AM 694, JWH-250, JWH-073, JWH-018, JWH-019, JWH-122, HU 210 and CP 47,497 in OF by liquid chromatography–electrospray ionization–tandem mass spectrometry (UHPLC-ESI-MS/MS) has also been reported (132). 250 µL OF sample was diluted with 250 µL of mobile phase and the chromatographic run time is 9 minutes. LODs of the method vary from 1 ng/mL to 20 ng/mL and the linearity ranges from the LOD to 1000 ng/L.

3.5 Survey on Trend of Common Drugs of Abuse

3.5.1 Opiates and opioids

3.5.1.1 Heroin

Heroin is the most rapidly acting opiate drug. It is highly addictive and hence is one of the most popularly abused substances. Heroin associated fatalities have been widely reported in the world because of its strong potency. A survey studying the deaths caused by illegal drugs in East Germany between 1995

and 2004 revealed that opiates, especially heroin, caused majority of the deaths, and the average age of the victims were 24 years with males accounting for 85% of all fatalities (133).

In another epidemiological study on all poisoning deaths in Epirus, Greece, in the period from 1998 to 2010 (134), a total of 126 poisoning fatalities were recorded and heroin was the most frequently detected substance.

Similarly, a study (135) on medico-legally examined fatal poisonings cases in 2007 among drug addicts in the five Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) revealed that heroin/morphine was still the main intoxicant in Norway and Sweden. However, methadone was the main intoxicant in Denmark while only a few cases were due to heroin/morphine in Iceland. Finland differed from other Nordic countries in having a high number of poisonings caused by buprenorphine and just very few caused by heroin/morphine.

Through a study on a total of 149 drug abuse deaths of teenagers aged 13-19 years from 1991 to 2006 in Maryland (136), it was reported that the increase in teenager drug abuse deaths occurred in 1999 and since then remained at a high rate. Further analysis revealed that such increase was attributable to a large degree to narcotic drugs, particularly heroin/morphine.

3.5.1.2 Methadone

Methadone has a long and successful history in the treatment of opioid addiction. However, in recent years, it has also become popular as a potent and inexpensive analgesic for patients suffering from chronic pains. Over the years, the numbers of methadone related deaths have seen a significant growth in the United States including Vermont, Western Virginia, rural southwestern Virginia, Oklahoma, Wisconsin and etc. (137,138,139,140,141,142). Such findings were also widely reported in European countries/cities and Australian state including Zurich, Montpellier of France, Ghent of Belgium, United Kingdom, Denmark, Norway and Victoria of Australia (143,144,145,146,147,148,149). The great number of reported methadone related deaths should therefore be a matter of concern especially about the source of supply such as the improper taking of the medication by

patients, diversion of the drug from the patient to someone else, or other means.

3.5.1.3 Oxycodone

A cross-sectional study analysing prescriptions for morphine and oxycodone in relation to oxycodone-related mortality data was conducted in Australia (150). The study results revealed that the prescriptions for morphine declined, while those for oxycodone increased and 465 oxycodone-related deaths were recorded during 2001-2009. Furthermore, it was concluded that in comparison to heroin, the morbidity and mortality associated with oxycodone are relatively low in Australia.

In view of the toxicity concern of oxycodone, all fatal oxycodone toxicity cases presented to the New South Wales Department of Forensic Medicine of Australia from 1999 to 2008 were retrieved with a total of 70 cases identified and studied (151). It was found that in 30% of the cases, oxycodone had not been prescribed to the decedent. Furthermore, psychoactive substances other than oxycodone were also detected, most frequently hypnosedatives (68.6%), other opioids (54.3%), antidepressants (41.4%), and alcohol (32.9%).

In the United States, unintentional poisonings were the second leading cause of injury death (after motor-vehicle crashes) with most of them caused by drug overdose. In a survey studying the drug overdose deaths in Florida from 2003 to 2009 (152), it was found that the death rate for prescription drugs increased 84.2%. The greatest increase was observed in the death rate from oxycodone (264.6%), followed by alprazolam (233.8%) and methadone (79.2%).

3.5.1.4 Fentanyl

Fentanyl is a potent, synthetic opioid analgesic and is an increasingly common drug of abuse. Fatalities in relation to fentanyl overdoses are common. A toxicology-based review of fentanyl-related deaths in New Mexico from 1986 to 2007 was undertaken (153). Amongst 154 cases identified with fentanyl present in the post-mortem samples, 96 cases were concluded as fentanyl-related drug overdoses. The number of fentanyl-related deaths has increased over the past 20 years, corresponding to both statewide increases in the medical use of fentanyl and the abuse of prescription opioids.

Similarly, a study of fentanyl in drug-related deaths in Philadelphia 2004-2006 was undertaken by reviewing data from the Philadelphia Medical Examiner's Office (154). In comparison to 2004 and 2005 data, there was a statistically significant increase in the number of drug related deaths with fentanyl tested positive in 2006. It was postulated that the change may be related to increase in the abuse of fentanyl and lack of general public awareness that fentanyl is a potent opoid.

3.5.2 Amphetamine type stimulants

Amphetamine is a major drug of abuse in Sweden. Through a study on forensic blood samples from 2001 to 2010, it was found that the mean (median) concentrations of amphetamine in blood were 1.25 (0.40) mg/L in autopsy cases and 0.61 (0.40) mg/L in users of illicit drugs (155). The major co-ingested drugs were benzodiazepines, cannabis, opiates and alcohol. In an overview of amphetamine-type stimulant mortality data in the United Kingdom from 1997 to 2007 (156), 832 amphetamine/methamphetamine and 605 ecstasy (mostly MDMA and MDA)-related deaths were respectively identified. Furthermore, it was noted that ecstasy was more typically identified in victims who were young, healthy, and less likely to be known as drug users.

Deaths involving MDMA and the concomitant use of pharmaceutical drugs in Victoria of Australia from 2002 to 2008 were investigated (157). In all, 106 fatalities were identified, of which 43 cases involved the concomitant use of MDMA with other drugs, including pharmaceuticals that were likely to result in an adverse drug reaction or varying risks.

A severe outbreak of paramethoxymethamphetamine (PMMA) and paramethoxyamphetamine (PMA) resulting in 24 fatalities in Israel was reported in a publication (158) and stimulant co-exposures may have contributed to the severity of the poisoning. The PMMA epidemic in Norway involving 12 fatal intoxications during a 6 month period (July 2010-January 2011) was also studied with evaluation on the cause of death (159).

3.5.3 Cocaine

A review of cocaine-related deaths in Bexar County, Texas was undertaken (160). The data obtained showed that cocaine was toxic over a large range

with deaths occurring at concentrations ranging from 0.01 to 78 mg/L. The analyses also indicated lethality increases when cocaine is used in combination with ethanol, heroin, opiates, and antidepressant/antipsychotic medications.

The use of cocaine in Australia has risen steadily since the late 1990s. A study was launched to identify all deaths occurring in Victoria of Australia, from 2000 to 2011. There were 49 cases of death where cocaine, benzoylecgonine, ecgonine methyl ester, methylecgonine or cocaethylene, were detected (161).

A review on the temporal and geographic shifts in urban and nonurban cocaine-related fatal overdoses in British Columbia, Canada from 2001 to 2005 was published (162). A total of 904 illicit drug overdoses were recorded, including 369 (40.8%) in nonurban areas and 532 (58.9%) related to cocaine consumption. In another publication, 21 cases of cocaine-related sudden death in south-west Spain from November 2003 to June 2006 were reported (163).

3.5.4 Gamma-hydroxybutyrate (GHB)

All death cases with GHB detected during 2000-2007 in the region of western Sweden were studied (164). Twenty-three cases were diagnosed as deaths due to GHB overdose.

Another research group in Sweden also studied the concentrations of the GHB in femoral venous blood and urine obtained at autopsy in a series of GHB-related deaths (165). Considerable poly-drug use was evident in these GHB-related deaths including ethanol, amphetamine, and various prescription medications (benzodiazepines, opiates, and antidepressants) in other cases.

3.5.5 Antidepressant and hypnotic

The contributory and incidental blood concentrations in deaths involving citalopram in New South Wales of Australia from 2001 to 2010 were investigated (166). A total of 348 cases were identified. Citalopram contributed to death in 21.0% and was incidental in 79.0%.

The toxicology and characteristics of deaths involving zolpidem in New South Wales of Australia from 2001 to 2010 were studied (167). A total of 91 cases were identified. Zolpidem was a factor contributing to death in 35 cases, of which 31 involved zolpidem toxicity.

3.6 Quality Assurance

3.6.1 Proficiency test

While forensic laboratories are required to estimate uncertainties of measurements for those quantifications reported to the end users of the information, the procedures for such estimations have been hardly discussed in the forensic literature. An article illustrated how proficiency test results provide the basis for estimating uncertainties in three instances: (i) breath alcohol analyzers, (ii) blood alcohol and (iii) toxicology. It was claimed that data from proficiency tests enable estimates of uncertainty that are empirical, simple, thorough, and applicable to a wide range of concentrations (168).

The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma was initiated by Radboud University Nijmegen Medical Center of the Netherlands in 1999, and later the Dutch Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology collaborated in the Program. The Program provides a proficiency testing program in which laboratories are alerted to potential analytical errors while performing therapeutic drug monitoring in HIV-infected patients (169).

The organization of the first international proficiency test (PT) programme on ketamine (K) and norketamine (NK) in hair samples has been discussed (170). The primary objective of the programme was to evaluate the analytical capability of participating laboratories on hair analysis for K and NK via comparison of results. Authentic samples, instead of spiked samples were used in the programme to mimic the analysis of incorporated illicit drugs in real-life situations.

The conditions of measurement required to evaluate bias in analytical results, as illustrated by the use of data from a multi-round, blind-duplicated, proficiency test, was reported (171). Results of a six-round blind-duplicated interlaboratory proficiency program for creatinine in urine showed that bias was present in each individual run with components from that batch as well as and from the laboratory over the rounds of the program. It was concluded that bias should be determined in each batch run under repeatability conditions. Measurement of laboratory bias alone is not sufficient to account for effects in each batch run.

3.6.2 Establishing the measurement uncertainty

The calculation and verification of blood alcohol measurement uncertainty for headspace gas chromatography were reported (172). The uncertainty sources, in order of decreasing magnitude, were method reproducibility, linear calibration, recovery, calibrator preparation, reference material, and sample preparation. A large set of reproducibility data was evaluated (n = 15,433) in order to encompass measurement variability across multiple conditions, operators, instruments, concentrations and timeframes. The relative, combined standard uncertainty was calculated as ±2.7%, with an expanded uncertainty of ±8.2% (99.7% level of confidence, k = 3). Bias was separately evaluated through a recovery study using standard reference material from a national metrology institute. The uncertainty estimate was verified through the use of proficiency test (PT) results.

An approach was proposed for the estimation of measurement uncertainty for analytical methods based on one-point calibration (173). The approach was applied to the estimation of measurement uncertainty for the quantitative determination of ketamine (K) and norketamine (NK) at a 100 ng/mL threshold concentration in urine. The expanded uncertainties (k = 2) were estimated to be 10 and 8 ng/mL for K and NK, respectively.

Several established and well-documented methods are available to determine and report the uncertainty in blood alcohol measurement (174). A straightforward bottom-up approach is presented that includes: 1) specifying the measurand, 2) identifying the major components of uncertainty, 3) quantifying the components, 4) statistically combining the components and 5) reporting the results. A hypothetical example is presented that employs reasonable estimates for forensic blood alcohol analysis using headspace gas chromatography.

3.6.3 Quality control materials

Quality control (QC) used in routine analysis needs to be stable and matrix-matched if practicable. However, it may be difficult to find representative and low-cost QC materials, especially for specific analytes in biological tissue. The preparation of four caprine liver pools for use as internal QC materials for trace element measurements in biological tissue was reported (175). Analytes of interest include essential and non-essential trace elements and the lanthanide series elements.

The Federal Institute for Materials Research and Testing in Germany has issued a series of large volume ethanol in water. These certified reference materials (CRMs) were primarily developed for the calibration of evidential breath alcohol analyzers in Germany. The certified parameter is the ethanol mass concentration at 20 °C. When used in a wet bath simulator, the solutions deliver gas samples that meet the requirements set by the Organization of Legal Metrology for calibration of breathalyzers (176).

An example of the use of the multivariate statistical analysis for the certification of metronidazole and captopril was demonstrated (177). The technique was quick, easy and readily provided an evaluation of the homogeneity. Through the use of statistical tools, it was possible to reduce the standard uncertainty due to between-bottle inhomogeneity and consequently the combined standard uncertainty of the certified reference materials with 95% confidence level. Metronidazole and captopril in the study are used as pharmaceutical reference materials.

Internal standards play critical roles in ensuring the accuracy of an analysis. In a publication, the use of internal standards for quantitative LC-MS bioanalysis was discussed in detail (178).

Any high-quality analytical result should include information about the associated measurement uncertainty, and the purity uncertainty of the reference is a parameter which always appears in the overall measurement uncertainty calculation of the measurand (such as the concentration or content of an analyte). A publication postulates that the purity and the uncertainty of all reference materials must be known (179).

4 Advances in Toxicological Analysis

4.1 Development of LC-MS Techniques

Over the past few years, diversified development has been found in the applications of liquid chromatography coupled with tandem mass spectrometry for the determination of drugs and their metabolites in various biological specimens. In the field of forensic toxicology, mass spectrometry (MS) has been traditionally playing a key role in the identification of drugs and their

metabolites. The development of High-resolution Mass Spectrometry (HRMS) instrumentation with improved accuracy and stability, along with new data processing techniques, has further improved the quality and productivity of metabolite identification processes.

LC-MS/MS is an increasingly important tool in therapeutic drug monitoring as it offers increased sensitivity and specificity compared to other methods (180). However, sample preparation technique, column selection, use of proper internal standard and optimization of instrumental conditions are also important issues when accurate drug measurement is to be achieved. Furthermore, technological advances such as the development of pipetting robots and online solid phase extraction greatly prompt LC-MS/MS becoming an attractive and convenient automated system for therapeutic drug monitoring in clinical laboratories.

Applications of liquid chromatography tandem mass spectrometry has proliferated at a fast pace over the past few years and several reviews have been published (128,181,182,183). In addition, drug metabolite profiling and identification by HRMS has also seen a major progress. In a review (184), HRMS-based targeted and non-targeted acquisition methods and data mining techniques (*e.g.* mass defect, product ion, and isotope pattern filters and background subtraction) that facilitate metabolite identification were examined. Methods involving multiple metabolite identification tasks with a single LC/HRMS platform and/or analysis were also presented.

Liang *et al.* have published a review on the development in liquid chromatography/mass spectrometry and emerging technologies for metabolite identification (185). In this article, the classical and practical mass spectrometry-based techniques, such as low resolution MS (quadruple, ion trap, linear ion trap, etc), high resolution MS (time-of-flight, hybrid time-of-flight instruments, Orbitrap, Fourier transform ion cyclotron resonance MS, etc) and the corresponding post acquisition data processing and mining modes (precursor ion filtering, neutral loss filtering, mass defect filter, isotope-pattern-filtering, etc) were described comprehensively.

Recent advances on metabolite identification and quantitative bioanalysis by LC-Q-TOF MS have also been studied by another team of researchers (186). The key properties of the Q-TOF MS system, including mass accuracy,

resolution, scan speed and dynamic range, were discussed. The performance and versatility of LC-Q-TOF MS were thoroughly illustrated by its applications in metabolite identification and quantitative bioanalysis. Future perspectives were also discussed in the article.

Wissenbach *et al.* have studied transferring a linear ion trap (LIT) LC-MS(n) screening approach and reference library to an LC-MS/MS system with a quadrupole-LIT hybrid mass analyzer using SmileMS, a sophisticated search algorithm (187). Modified library sets were generated to improve the detection of a compound by the used search algorithm. The data presented showed that the LIT screening approach and reference library could be used successfully on a QTRAP instrument with some limitations that could be overcome by further optimizations on settings and modifications of library.

Roman *et al.* also reported a validated liquid chromatography/time-of-flight mass spectrometry method for targeted toxicological screening of post-mortem blood samples. Separation was achieved within 12 minutes by high resolution gradient chromatography (188).

Another study has reported the successful detection and identification of 700 drugs by multi-target screening with a QTRAP LC-MS/MS system (80). Identification of the compounds in the samples was accomplished by searching the MS/MS spectra against a library developed from the electrospray ionization-MS/MS spectra of over 1,250 compounds. Data acquisition and library searching are integrated and automated by the software program.

Liu *et al.* reported the successful development of a method performing rapid screening and confirmation of drugs and toxic compounds in biological specimens using liquid chromatography/ion trap tandem mass spectrometry and automated library search in a single analytical step (189). The established method was found highly effective when applied to the analyses of post-mortem specimens (blood, urine, and hair) and external proficiency test samples provided by the College of American Pathology (CAP).

In the field of urinalysis, a published article has reported an automated determination of 21 therapeutic drugs and 21 abused drugs in human urine (190). According to the article, their analyses could simultaneously identify and

quantify the 42 drugs in human urine through an automated online solid phase extraction ultra high performance liquid chromatography method coupled with tandem mass spectrometry (SPE UHPLC-MS/MS).

Another novel analytical toxicology method has been developed for urinalysis by using a high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-MS), with 65 compounds analysed within a run time of 20 minutes (191).

Nakamura conducted a review on the procedures for multi-analyte single-stage LC-MS and LC-MS/MS using different mass analyzers for the screening, identification and/or quantification of drugs, poisons and/or their metabolites in blood, plasma, serum or urine published since 2001 (192).

d-Amphetamine is extensively used in drug research and forensic toxicology investigation. A research study on a specific and high-throughput quantitative method, with minimal sample preparation, for routine analysis of d-amphetamine in biological samples using MS^3 scan mode on a hybrid triple quadrupole-linear ion trap mass spectrometer (LC-MS/MS/MS) has been published (193). This method was successfully applied to evaluate the pharmacokinetics of d-amphetamine in rat.

Time of flight mass spectrometry provides accurate molecular mass and isotope pattern and hence determination of the molecular formula of a substance directly becomes possible. However, there are frequently a large number of possible isomers, the differentiation of which requires additional evidence. Broecker *et al.* reported their study on the combined use of LC-hybrid quadruple time-of-flight mass spectrometry (LC-QTOF-MS) and high performance liquid chromatography with photodiode array detector (HPLC-DAD) in systematic toxicological analysis (194).

LC-MS/MS has also found its application in the detection of a number of new psychoactive drugs (legal highs) (195). The method validation demonstrated limited interference from urine matrix, linear response within the measuring range (0.1 – 10 mg/mL), and acceptable imprecision in quantification (CV < 15%).

4.2 Development of Extraction Techniques

Novel extraction techniques such as on-line solid phase extraction had been introduced during the period under the present review. One of the studies reported using protein precipitation with extraction (PPE) in acetonitrile instead of the tedious liquid-liquid extraction in the quantification of 25-hydroxyvitamin D (a marker of vitamin D). Combined with a 96-well plate filtration system, the entire separation process becomes much more efficient (196). The rapid extraction was then followed by an on-line solid phase extraction (SPE) using a selective chromatographic separation. Furthermore, a trapping column was used to enhance the lifespan of the analytical column.

Savolainen *et al.* also employed an on-line solid phase extraction liquid chromatography-tandem mass spectrometry in their analysis of testosterone in serum samples (197). When compared with their previous routine LC-MS/MS method using liquid-liquid extraction with tert-butyl methyl ether for the pre-purification of the samples, the precision of the new method was notably better, especially in the lower concentration range. Therefore, the researchers concluded that the on-line SPE-pre-purification technique tested in long-term use offered a rapid and reliable technique in the LC-MS/MS analysis of serum testosterone and was a valuable tool in the improvement of efficiency in the laborious steroid analytics.

The successful application of LC-MS/MS for immunosuppressant therapeutic drug monitoring has been published (198). Authors in the article claimed that online sample clean-up with either a single analytical column or with 2D chromatography significantly reduced manual handling, minimized matrix effects and maximized specificity. It was concluded that LC-MS/MS was an attractive and versatile technique that facilitates rapid development of analytical methods.

Wang *et al.* have reported a one-step membrane extraction for the determination of 8-hydroxy-2'-deoxyguanosine in human plasma by a combination of on-line SPE and LC-MS/MS (199). Another study by Emara *et al.* also reported an on-line sample cleanup and enrichment chromatographic technique for the determination of ambroxol in human serum (200). Fernández *et al.* published a study reporting a chromatographic determination of drugs of

abuse in vitreous humor using solid-phase extraction (201).

A sensitive method using capillary electrophoresis with online large-volume sample stacking for the determination of barbiturates in biological matrix has been published (202). The technique involved injecting a large volume of sample into a capillary and removing the sample matrix plug out of the capillary by reversing the polarity. The method was satisfactorily applied to real forensic specimens.

Turbulent flow chromatography (TFC) was introduced in the mid-1990s for online sample processing in bioanalysis. It combines 'size exclusion' and traditional stationary phase column chemistry to separate macromolecules, such as proteins, from smaller molecules and analytes of interest in biological fluids. Several articles have been published relating to TFC (203,204,205). One of them is an overview of TFC in bioanalysis (203). The article aimed at reviewing the chromatographic theory of TFC and illustrating, using examples from recent literature, the application of this technique to a range of analytes in different biological matrices. Bunch *et al*. have reported a fast and simple assay for busulfan in serum or plasma by liquid chromatography-tandem mass spectrometry using turbulent flow online extraction technology (206).

Serdi *et al.* have published a paper reporting a novel low-voltage electrically-enhanced microextraction for simultaneous extraction of acidic and basic drugs from biological fluids (207). The research team termed the technique electromembrane extraction at low voltages followed by high performance liquid chromatography with ultraviolet detection. They anticipated that their techniques could have a wide application in different complicated matrices.

Testing for illicit drugs in hair has been gaining attention. Sergi *et al.* have studied on a pressurised-extraction for determination of illicit drugs in hair by LC-MS/MS (208). Their procedure, in conjunction with a decontamination step, enabled the detection of all the analytes in pg/mg level.

4.3 Analysis of Specific Drugs

4.3.1 Toxic and volatile gases

4.3.1.1 Cyanide

Cyanide is a powerful chemical asphyxiant found in some forensic cases following voluntary (suicide) or involuntary ingestion (fire, accidental exposure). A quantification method for cyanide by headspace gas chromatography coupled to mass spectrometry using a GS-GASPRO column on an HP-6890 gas chromatograph with an HP-5973N mass detector has been developed (209). Identical calibration curves were obtained when blood, gastric contents and aqueous solutions were used as the calibration standard matrix. Furthermore, this method was also successful in quantifying cyanide in gastric contents, one of most variable biological fluids.

A LC-MS/MS method using cyanide isotope $^{13}C^{15}N$ as internal standard and coupled to online extraction has been developed for cyanide determination in blood (210). The method was simple and time saving using small volume of blood sample. Hence, it is very suitable for cyanide determination in blood and could be useful in forensic toxicology.

In addition, an electrospray ionization tandem mass spectrometric (ESI-MS/MS) method has been developed for the determination of cyanide (CN^-) in blood. CN^- could be measured in the quantification range of 2.60 to 260 µg/L with the limit of detection at 0.56 µg/L in blood (211).

An analytical method utilizing chemical ionization gas chromatography-mass spectrometry has been developed for the simultaneous determination of cyanide and thiocyanate in plasma (212). Sample preparation for this analysis required essentially one step by combining the reaction of cyanide and thiocyanate with pentafluorobenzyl bromide and simultaneous extraction of the product into ethyl acetate facilitated by a phase-transfer catalyst, tetrabutylammonium sulfate. The LOD for cyanide and thiocyanate were 1 µM and 50 nM, respectively.

Cyanide concentrations vary among different types of post-mortem specimens,

and this is very important in interpreting the cause of death in post-mortem forensic toxicology. 21 cases related to cyanide intoxication by oral ingestion were studied in which heart blood, peripheral blood and gastric contents were analyzed colorimetrically for cyanide. From the difference and ratio of cyanide concentration in different types of post-mortem specimens, post-mortem redistribution of cyanide and death could be distinguished from oral ingestion (213).

Assigning a level of significance to cyanide concentrations found in the blood of fire victims is often hampered by the fact that cyanide is inherently unstable in cadavers and in stored blood samples. The effect of sodium fluoride on the stability of cyanide in post-mortem blood samples from fire victims has been studied (214). It was found that samples treated with sodium fluoride showed virtually no overall change in blood cyanide levels over a 25-30 day period whereas the unconditioned control samples showed a significant average increase of 35%. Based on the findings of this study, it is recommended that 2% sodium fluoride be added to blood samples obtained from fire victims to reduce cyanide instability due to bacteriological activity.

4.3.1.2 Carbon Monoxide (CO)

Measurement of carboxyhemoglobin (COHb) is crucial to recognizing CO as a contributor in deaths involving fires, exposure to automobile exhaust, aircraft accidents, and residential exposures. Interferences, including lipid-caused turbidity, MetHb, sulfhemoglobin, microcoagulates, putrefaction, and contamination, have called into question the accuracy of COHb measurements obtained by CO-oximetry. The reliability of post-mortem COHb measurement by CO-oximetry was discussed through a case study (215). It was concluded that CO-oximetry, with the appropriate multiwavelength technology, can be a reliable and accurate method for post-mortem COHb measurement.

An innovative headspace-gas chromatography-mass spectrometry (HS-GC-MS) method applicable for the routine determination of blood CO concentration in forensic toxicology laboratories has been developed (216). A labelled internal standard gas (^{13}CO) formed by the reaction of labelled formic acid ($H^{13}COOH$) with sulfuric acid was generated in a vial in situ. This method allows for the precise measurement of blood CO concentrations from a small

amount of blood (10 µL). It was applied to measure the CO concentration of intoxicated human blood samples from autopsies.

In a published article (217), Nowicka *et al.* reviewed various analytical methods used for the determinations of carbon monoxide in post-mortem blood. The advantages, disadvantages and the cause of errors resulting from the specificity were discussed.

4.3.1.3 Volatile organic compounds

Dynamic measurement of volatile organic compounds (VOCs) in exhaled breath under exercise conditions has been studied by a team of researchers in Austria (218). They presented an experimental setup combining breath-by-breath analyses with proton transfer reaction mass spectrometry (PTR-MS). Their data reflected the behaviour of major hemodynamic and respiratory parameters. Furthermore, a methodology for complementing continuous VOC profiles obtained by PTR-MS with simultaneous SPME/GC-MS measurements is outlined.

Rasanen *et al.* presented the successful development of a novel headspace in-tube extraction gas chromatography-mass spectrometry (ITEX-GC-MS) approach for broad-scale analysis of low molecular weight organic compounds in blood and/or urine (219). From the results of 11 representative compounds, it was demonstrated that ITEX was more sensitive than the corresponding static headspace method for analysis of volatile organic compounds.

A fast and simple screening procedure using solid-phase micro-extraction and gas chromatography-mass spectrometry (SPME-GC-MS) in full-scan mode for the determination of volatile organic compounds (VOC) was presented in a published study (220). To simulate the screening procedure, eight VOC with different chemical characteristics were chosen. The limits of detection ranged from 2.9 µg/L (xylene) to 37.1 µg/L (isoflurane) and the recoveries varied from 7.9% (chloroform) to 61.5% (benzene).

A study to investigate using the scent profile of human urine as potential source of chemical markers of human presence in collapsed buildings after

natural or man-made disasters was launched (221). The study aimed at building a library of potential biomarkers of human urine to be used for the detection of entrapped victims and to further examine their evolution profile in time. A library of potential markers of human urine was created that would be verified in further field studies using portable and sensitive instruments.

4.3.1.4 Others

It is difficult to obtain toxicological evidence inferring the cause of death being resulted from inert gas asphyxiation. Helium, due to its low atomic mass and high diffusivity, is particularly challenging in this respect. A rapid and simple gas chromatography-thermal conductivity detection method to qualitatively screen a variety of post-mortem biological specimens for the presence of helium was described in a study in which application of this developed method has been successfully demonstrated with three case examples, encompassing an array of different biological matrices (222).

A novel method was developed to measure methane in tissues (223). The method used labeled CDH_3 that was produced in-situ, resulting in reliable and precise quantification of methane content in the post-mortem samples of two victims that assisted to determine the explosion origin.

A gas chromatography-mass spectrometry (GC-MS) method for the determination of ketone bodies (β-hydroxybutyrate, acetone, and acetoacetate) in blood was presented in a study (224). The method was based on enzymatic oxidation of D-β-hydroxybutyrate to acetoacetate, followed by decarboxylation to acetone, which was then quantified by the use of headspace GC-MS using acetone-$^{13}C_3$ as an internal standard.

4.3.2 Chemical warfare agents

Organophosphorus (OP) nerve agents and sulphur or nitrogen mustard are among the most toxic organic compounds known. They are continually a threat for both military and anti-terrorist personels. Since some OP compounds can be hydrolysed, degradation products may remain and even predominate in samples acquired in the field. A team of researchers has successfully employed ESI-MS/MS in analysing non-volatile OP compounds and their degradation products (225).

An analytical method for determining OP nerve agents sarin, soman and VX adducts with tyrosine residue of albumin in rat plasma has been developed and validated using liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS). The LOD were 0.01 ng/mL for sarin and soman adducts and 0.05 ng/mL for the VX adduct with recoveries ranged from 86-111% (226).

It was known that acetylcholinesterase (AChE) enzyme activity in red blood cells (RBCs) could be used as a biomarker for monitoring the exposures to OP pesticides and chemical nerve agents. Immuno-capture /electrochemical assay of AChE activity offers an opportunity that acted as a sensitive, selective and rapid AChE activity assay for biomonitoring the exposure to OPs with a linear response obtained over standard AChE concentration ranged from 0.1 to 10 nM (227).

4.3.3 Toxic mushrooms

Many plants and animals are known to contain toxins that may be harmful to human. In recent years, a number of toxicology cases related to mushrooms poisoning have been reported in various countries (228,229,230,231,232,233,234,235). In particular, an increase of poisoning by tropical mushrooms in Japan has also been reported (236). Mushrooms poisoning can often be proved by microscopic examination of their spores in the stomach or intestinal contents. Such method has been used for detection of A. pantherina or A. muscaria poisoning (237). Two forensic toxicology reviews on mushroom toxins were published (238,239). Mushroom toxins are tabulated according to mushroom species, symptoms, toxicities and analytical methods. A method for analysing amatoxins, the most virulent mushroom toxins, by LC-TOFMS was also reported (238).

4.3.4 Chinese medicines

Aconite poisonings following the use of aconite roots are commonly encountered in Asia (240,241). Aconite roots are widely used in traditional medicines and homeopathic medicines as analgesic, anti-inflammatory and cardiotonic agents. Aconitine, mesaconitine, hypaconitine, and other Aconitum alkaloids are known cardiotoxins and neurotoxins found in all parts of the Aconitum species, especially in their roots and root tubers (aconite roots) (240,241,242,243). The Aconitum alkaloids are highly toxic and have a very

narrow safety range; they easily induce ventricular tachycardia and fibrillation even at therapeutic dose levels (244). There was a report on seven cases relating to fatal aconite poisoning in China (245). Furthermore, there were three fatal poisoning cases reported in Austria that suicide was committed through ingestion of this highly toxic herb (246).

A review on herb-induced aconite poisoning indicated that poor post-harvest processing of aconite roots, use of greater than the recommended doses and inadequate boiling of processed aconite roots during decoction preparation were important contributory factors in herb-induced aconite poisoning (247). Data on the distribution of the Aconitum alkaloids in the body in cases of aconite poisoning was reported (248). Relevant reports on percutaneous absorption of Aconitum alkaloids and aconite poisoning are reviewed (249). It was found that aconite tincture and raw aconite roots can be absorbed through the skin into systemic circulation to cause fatal and non-fatal aconite poisoning.

Strychnine and brucine, another kind of alkaloids, are the predominant active constituents present in many traditional herbal medicines such as *Strychnos nux-vomica*, which is frequently used for the treatment of nervous diseases or vomiting, as a tonic or as an aphrodisiac (250,251). Chen *et al.* has reported a simultaneous analysis of strychnine and brucine and their major metabolites in rat liver by liquid chromatography-electrospray ionization-ion trap mass spectrometry (LC-ESI-ITMS) (251). The limits of detection for strychnine and brucine were both 0.008 μg/mL. The linearity ranges of strychnine and brucine were 0.020 to 8.0 μg/mL and 0.020 to 8.5 μg/mL, respectively.

Determination of strychnine and brucine in human urine by capillary electrophoresis with field-amplified sample stacking was also reported (252). Wu *et al.* developed a method for simultaneous determination of six toxic alkaloids including aconitine, hypaconitine, gelsemine, raceanisodamine, strychnine and brucine in blood and urine using a hydrophilic interaction liquid chromatography (HILIC)-ESI-MS/MS (253). Simultaneous determination of six toxic alkaloids including brucine, strychnine, atropine sulfate, anisodamine hydrobromide, scopolamine hydrobromide and anisodine hydrobromide in human plasma and urine using capillary zone electrophoresis coupled to time-of-flight mass spectrometry was also reported in another publication (254).

4.3.5 Doping Control

Not only restricted to professional athletes, the use of doping agents has nowadays become a problem of public health since it also concerns young people and non-competing amateurs in different sports. A publication has reviewed utilizing UHPLC/MS in determining and profiling prohibited steroids in human biological matrices (255). The advantages and limitations of this technique in human sports drug testing have also been discussed in another review (256).

With a recent increasing trend of abuse of synthetic cannabinoids, a study for the use of the synthetic cannabinoids, JWH-018 and JWH-073, was conducted. 5,946 urine samples collected from U.S. athletes were tested. Metabolites of JWH-018 and/or JWH-073 were detected in 4.5% of the tested samples. It was suggested that these compounds should remain a priority for anti-doping programs (257). A detection method was developed and validated in accordance with conventional screening protocols based on enzymatic hydrolysis, liquid-liquid extraction, and liquid chromatography/electrospray tandem mass spectrometry analysis. The method was applied to approximately 7,500 urine doping control samples yielding two JWH-018 findings and demonstrated its capability for a sensitive and selective identification of JWH-018 and its metabolites in human urine (258).

A study was conducted to investigate the plasma and urine profiles ofΔ^9-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in male volunteers after they smoked cannabis (259). The author suggested that THC and THC-OH should also be used as target analytes in additional to THC-COOH for doping urine analysis.

Thevis *et al.* have published reviews for the substances banned annually between October 2009 and September 2010 (260) and between October 2010 and September 2011 (261), with the purpose to improve the quality of doping controls by reporting emerging and advancing methods that focus on detecting known and recently outlawed substances.

Since January 2009, the list of prohibited substances and methods of doping as established by the World Anti-Doping Agency (WADA) has included new

therapeutics such as the peroxisome-proliferator-activated receptor (PPAR)-delta agonist GW1516, which is categorized as a gene doping substance. A method to detect the new target GW1516 in sports drug testing samples was developed in accordance with conventional screening procedures based on enzymatic hydrolysis and liquid–liquid extraction followed by liquid chromatography, electrospray ionization, and tandem mass spectrometry (262). The authors later reported a synthetic method for GW1516 and two oxidized metabolites (263).

Clomiphene is a selective estrogen receptor modulator that is prohibited by WADA, both out-of-competition and in-competition. Lu *et al.* have identified and characterized seven unreported urinary metabolites of clomiphene arising from a new metabolic pathway (hydrogenation) by liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOFMS) (264).

A screening method based on matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF/TOF) for the qualitative determination of doping agents as well as drugs of potential abuse was reported (265). The LOD for the analysis of target doping compounds in horse samples was reported to be 100 ng/mL, while that for the analysis of cocaine and its metabolite in human urine samples was 50 ng/mL.

4.4 Alternative Specimens

Blood and urine have long been and remain the most widely used biological specimens for forensic toxicological examination as well as routine drug testing. Blood is widely used for drug testing in clinical and emergency toxicology because it offers the best correlation between drug level and pharmacological impairments to the body. On the other hand, urine testing has been playing an important role in facilitating the judicial sentencing of drugs abusers in courts and drug surveillance programmes of inmates under custodial detention.

Following the advancement of testing technology, the use of alternative specimens in the field of toxicology has gained attention along with a number of studies published. Since the application of oral fluid and hair in workplace drug testing has been discussed in detail in the previous sections, this section will focus on other alternative specimens which have attracted less attention in the past.

4.4.1 Skeletal tissue

Skeletal tissue could be useful in forensic toxicology especially for heavily decomposed sample. A review of bone marrow analysis in forensic toxicology has summarized the analytical conditions and quantification results of 45 compounds from bone marrow samples and concluded that further experimental data and validated analytical assays are required for reliable determination and quantitative interpretation (266).

Watterson *et al.* examined the effects of burial on ketamine and diazepam detection and found that fresh tissue sample may not be representative of decomposed samples in terms of skeletal tissue drug levels (267). Later in another study (268), they reported the relative distribution of ketamine and norketamine in skeletal tissue with various decomposition periods and that the decomposition time was significantly related to the drug/metabolite level ratio (DMLR).

Watterson *et al.* also examined whether different patterns of drug exposure could be discriminated through toxicological analysis of decomposed skeletal tissues. The result suggested that acute and repeated exposures to ketamine may be discriminated on the basis of the levels of ketamine and norketamine in bone as well as the ratio of ketamine level to norketamine level (269). Apart from ketamine, norketamine and diazepam, relative distribution of amitriptyline and its metabolite, nortriptyline, and that of citalopram and its metabolite, desmethylcitalopram, in skeletal tissue following outdoor decomposition were also studied (270).

4.4.2 Brain tissue

To study the persistence of drugs in brain tissue over plasma, Sampedro *et al.* developed a simultaneous screening and determination of the 17 most commonly used antipsychotic drugs using LC-MS/MS (271). The linear ranges for calibration curves prepared in the spiked brain tissue were 20-8,000 ng/g for all the drugs studied except olanzapine and the LOQ ranged between 2 ng/g and 80 ng/g.

4.4.3 Meconium

Ethanol exposure during pregnancy can have negative effect on newborns (272,273,274). Fatty acid ethyl esters (FAEEs), products of non-oxidative ethanol metabolism, have been measured in meconium and acted as reliable

markers of intrauterine exposure to ethanol (275,276,277). Roehsig *et al.* reported an optimized and validated method for the simultaneous determination of eight FAEEs by headspace solid phase microextraction (HS-SPME) and GC-MS, with synthesized deuterated d5-ethyl esters used as internal standard (278). The LOQ and LOD for each analyte were reported to be <150 and <100 ng/g, respectively.

Hutson *et al*. developed another method for the determination of FAEEs in meconium using HS-SPME/GC-MS with improved LODs ranging from 6.3-11.9 ng/g and LOQs ranging from 18.8 – 35.8 ng/g because this method was able to produce clean chromatograms (279). Although analysis for FAEEs is a validated method for identifying heavy prenatal ethanol exposure, false-positive for FAEEs result was reported for meconium sample delayed in collection. Median time to appearance of FAEE-positive samples was 59.2 hours postpartum and four of the 30 babies excreted FAEE-positive meconium in less than 24 hours postpartum (280).

Another suitable marker for the detection of recent alcohol consumption is ethyl glucuronide (EtG) and ethyl sulfate (EtS), direct metabolites of ethanol (272). Studies of EtG in hair and meconium were reported (274,281). A study of EtG and EtS in meconium and hair samples from mothers and their newborns was conducted. The result showed that neither maternal nor neonatal hair was a good predictor of gestational ethanol consumption and subsequent fetal exposure in these mother–infant dyads. The authors concluded that meconium is so far the best matrix in evaluating intrauterine exposure to ethanol, with EtG and EtS being potentially good alternative biomarkers to FAEEs (274). Bakdash *et al.* performed a study on the determination of FAEEs and EtG in meconium (282). The FAEEs were measured by HS-SPME in combination with GC-MS, while EtG was quantified by LC-MS-MS. The authors suggested that combined use of FAEE and EtG in meconium as markers for fetal alcohol exposure essentially increases the accuracy of the interpretation and helps to avoid both false-positive and false-negative results.

4.4.4 Placenta

Placenta could be an alternative to urine for drugs of abuse testing during the first trimester of gestation. Joya *et al.* reported a GC/MS method for the quantification of drugs of abuse in human placenta including amphetamine,

methamphetamine, MDMA, methadone, cocaine, benzoylecgonine, cocaethylene, morphine, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, nicotine, and cotinine with drug concentration ranges of 5–500 ng/g (283).

Huestis *et al.* reported a study on the correlations on the placental disposition of methadone and its metabolite [2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)] of pregnant women with maternal methadone dose and neonatal outcomes. The subject women were methadone-maintained opioid-dependent and the objective was to test the ability to detect in utero exposure to illicit drugs (284). Huestis *et al.* also compared placenta and matched meconium concentrations and investigated the relationships between maternal buprenorphine dose, placenta concentrations, and neonatal outcomes following controlled administration during gestation (285).

4.4.5 Dried Blood Spots (DBS)

The introduction of LC-MS/MS instrumentation enabled the development of assays using micro quantities of blood and serum with good sensitivity and precision (286). Drug analyses using DBS have the advantages that less blood is required and the collection of sample is less invasive (287). Determination of drugs, such as rufinamide (288), gabapentin (289), fluoxetine, norfluoxetine, reboxetine, paroxetine (290), cyclosporine A and tacrolimus (291) in DBS using LC-MS/MS was reported.

Saussereau *et al.* also reported the determination of illicit drugs, including opiates (morphine and its 3- and 6-glucuronide metabolites, codeine, 6-acetylmorphine) cocainics (ecgonine methylester, benzoylecgonine, cocaine, cocaethylene) and amphetamines (amphetamine, methamphetamine, MDA, MDMA, MDEA) in DBS (287). The method required 30 μL of whole blood spotted in a Whatman card 903 and dried overnight at room temperature. LODs for the drugs ranged from 0.5 to 5.0 ng/mL.

4.4.6 Vitreous humor

A study for the determination of opiates, including free morphine, 6-acetylmorphine and codeine, in blood and vitreous humor after trimethylsilyl derivatization by GC-MS was reported (292). The average recoveries were 82% for whole blood and 100% for vitreous humor. This method was applied to a case study and the concentrations of morphine and codeine detected in the

vitreous humor samples were lower than those in the whole blood samples.

Analysis of insulin is difficult in post-mortem blood sample because of the rapid degradation of insulin by insulin-degrading enzyme. Nonetheless, Thevis *et al.* have developed a method for the determination of insulin in human vitreous humor by LC-MS/MS (293).

4.5 Interpretation of Toxicological Results

Post-mortem toxicology analyses represent one of the effective tools to facilitate forensic pathologists in determining the cause and manner of death in fatalities cases. This is accomplished by performing tests on body fluids (i.e. blood, urine and vitreous humor) and tissues samples (i.e. liver, stomach, lung and etc.) and then offering interpretation of the findings. However, reliable interpretation of the level of drugs in post-mortem specimens especially blood is difficult and complicated by a number of factors including post-mortem redistribution, simple diffusion after death from a drug depot such as the gastric content and drug stability in specimen.

4.5.1 Post-mortem redistribution

Interpretation of the analytical results constitutes one of the biggest challenges in forensic toxicology because drugs in a post-mortem blood sample may have been subjected to post-mortem changes from the time of death until samples are collected; thus, the drug concentration in post-mortem blood may not reflect the actual drug concentration in blood at the time of death. A literature review by Gisela (294) pointed out that formation of new entities as well as degradation of drugs may occur, especially in putrefied corpses. In addition, body fluids and tissues may be severely affected by autolysis and putrefaction. Therefore, specimens should be selected based on individual case history and on their availability.

Post-mortem redistribution (PMR) of drugs is one of the post-mortem changes that affects drug concentration in blood. Evaluations of PMR phenomena for commonly encountered drugs were reported (113,295,296,297,298,299,300,301,302). Post-mortem drug concentrations showed variations depending on sampling sites and characteristics of the drugs. 76 drugs found in 129 drug-related cases were studied (295). 76 drugs including psychotropic drugs, antidepressants and sedatives were

simultaneously quantified in cardiac and peripheral blood by GC-MS or LC-MS/MS.

Post-mortem redistribution of ten commonly prescribed antipsychotic drugs including 9-OH-risperidone (paliperidone), amisulpride, chlorpromazine, clozapine, haloperidol, olanzapine, promethazine, quetiapine, risperidone, and zuclopenthixol was also investigated (296). The changes in blood concentrations after admission to the mortuary can increase by 112% (for chlorpromazine and olanzapine) but might also decrease by 43% (for 9-OH-risperidone). The large standard deviations between sample pairs and substantial day-to-day unpredictable changes highlighted the difficulty in the interpretation of drug concentrations post-mortem.

A study between sertraline concentrations and postmortem redistribution was reported (297). The study involved a total of nine cases with marked post-mortem redistribution. A study involving 19 medical examiner cases (16 males and 3 females) which screened positive in cannabinoid urine immunoassay indicated that THC and its metabolites 11-OH-THC and THCA undergo only modest PMR, much less than expected based on the lipophilic nature and the high volume of redistribution (Vd) of the cannabinoids. Average central:peripheral (C:P) ratios for all analytes were less than 2.0 (299).

Andresen *et al.* conducted a comparison of the blood concentrations of fentanyl in 118 post-mortem cases with serum levels of fentanyl in 27 living persons after therapeutic administration of fentanyl patches (303). The study revealed that the post-mortem fentanyl blood concentrations were on average up to nine times higher than *in vivo* serum levels at the same dose. Gill *et al.* carried out yet another study on the post-mortem fentanyl concentrations which involved 92 decedents who had one or more fentanyl transdermal patches on their body and had fentanyl detected in their post-mortem toxicology analysis (304). Among 37 accidental fentanyl intoxication deaths, 32 involved substance abuse. The substance abuse deaths had a mean fentanyl blood concentration (26.4 ng/mL) that was over twice that of the natural group (11.8 ng/mL). The analysis also suggested a relationship between total patch dosage and mean post-mortem fentanyl concentration up to the 100-µg/h dose.

4.5.2 Drug stability in blood

4.5.2.1 Stability of zopiclone in blood

Apart from PMR, other factors such as pre-storage condition of the samples prior to examination may also affect the detected drug levels. Differences in the stability of zopiclone between spiked and authentic whole blood from subjects dosed with zopiclone were studied (305). It was found that the degradation of zopiclone in authentic blood was equal to that from spiked blood at the temperatures and times studied. The stability of zopiclone was less than 1 day at 20 °C, less than 2 weeks at 5°C but stable for 3 months at -20 °C.

4.5.2.2 Stability of GHB in blood

The stability of GHB in blood and serum samples under various storage conditions was evaluated (61). GHB was found to be stable at least for weeks in serum samples separated immediately after blood withdrawal and in whole blood samples frozen immediately after blood collection. Another study on long-term stability of GHB in post-mortem samples and samples from living persons, stored at –20 °C, using fluoride preservatives was reported (60). Re-analyses of 59 forensic whole blood samples stored several years (ranged from 0.4 to 7.2 years) at -20 °C with fluoride preservation showed that GHB concentrations did not change significantly for the interpretation of toxicological findings.

4.5.2.3 Stability of benzodiazepines in blood

Study of the stability of benzodiazepines, including lorazepam, estazolam, chlordiazepoxide and ketazolam, in post-mortem blood, bile and vitreous humor stored at different temperatures over six months has shown that benzodiazepine concentrations remained almost stable in all samples at -20°C and -80°C. Among the benzodiazepines studied, estazolam appeared to be the most stable while ketazolam being the least, totally degraded in methanolic solutions over 1 or 2 weeks at room temperature and over 8 or 12 weeks at 4 °C (306).

Karinen *et al.* conducted a study on the stability of stock solutions of a variety of illegal and medicinal drugs stored in freezer (at -20°C), refrigerators (at 4-6°C) and at ambient temperature for up to one year (307). The study indicated that lorazepam and promethazine showed significant concentration

losses after 1 month of storage at ambient temperature. Olanzapine was found to be unstable after one month of storage at ambient temperature, after three months in the refrigerator and had disappeared completely upon one year of storage. In contrast, some drugs demonstrated an increase in concentrations after one year of storage. For example, tramadol and carbamazepine concentrations increased significantly when stored in refrigerator or at ambient temperature for one year.

4.5.2.4 Stability of alcohol in blood

Ethanol analysis in biological samples is the most common test in forensic toxicology laboratories. Kelly and Mozayani published a literature review (308) to give an overview of alcohol testing and result interpretation. This review covered pharmacokinetics including absorption, distribution, and elimination of ethanol, methods for the detection of ethanol, the effect of ethanol on human performance, the role of alcohol in injuries and fatalities, and information regarding the interactions that may occur between alcohol and other drugs. An explanation on how to interpret alcohol levels as well as the extrapolation and calculation of blood alcohol levels at times prior to sample collection was also discussed. Gullberg has presented a paper in regard to the estimation of measurement uncertainty in forensic blood alcohol analysis using a simple bottom-up model (174). The coefficient of variation based on the combined uncertainty in forensic blood alcohol analysis is approximately 1-3%.

A study of blood alcohol stability in forensic ante-mortem blood samples was reported (3). 32 whole blood case samples (each with two tubes of blood) were used for this study. The blood samples were analyzed on blood alcohol concentration (BAC) before and after storage (ranging from 13 to 39 months). 25 samples demonstrated various losses in BAC in both tubes. The same blood samples were then stored at room temperature for 6 months followed by 38 °C for 7 and 28 days and analyzed for BAC at the end of each storage time period. Six months of storage at room temperature decreased BAC further for both tubes of the alcohol positive cases with a mean loss of 0.014 g/dL. Further storage at 38 °C for 7 days did not cause any significant change in BAC. Storage at 38 °C for 28 days caused some loss in BAC which was determined to be significant by statistical analysis.

4.5.3 Toxic fumes in fire-related fatalities

Carbon monoxide (CO) and hydrogen cyanide (HCN) are the most toxic fumes generated in fire-related fatalities. In February 2009, 173 persons were killed by the incident of Victorian Bushfire in Australia. Blood samples, available from 30 deceased (aged 3-80), were tested for degree of COHb saturation (309). Another study based on the data collected from deceased fire victims during 1992-2009 from two Swedish nationwide forensic databases (ToxBase and RättsBase) revealed that 17% of the victims had lethal or life-threatening blood cyanide levels (>1 μg/g), 32% had lethal COHb levels (>50% COHb) and over 31% had cyanide levels above 0.5 μg/g (310).

Since CO may be the cause of more than half of the fatal poisoning reported in many countries, an accurate and reliable analytical method to measure the COHb levels is essential for correct diagnosis. Hao *et al.* have developed a technique employing headspace-gas chromatography-mass spectrometry (HS/GC/MS) for determining CO and COHb% which are crucial to the investigation of deaths potentially related to CO exposure (311). Furthermore, Fujihara J *et al.* also evaluated the usefulness of the AVOXimeter 4000 (AVOX), a portable CO-oximeter, in measuring the HbCO% in post-mortem blood (312).

A study on the quantitative evaluation of volatile hydrocarbons in post-mortem blood of 37 fire-related deaths revealed that the concentrations of volatile hydrocarbons in post-mortem blood could be used to classify the cases into three types of fires: construction fires, gasoline- and kerosene-related fires (313). Quantitative analysis of blood revealed that the benzene and styrene concentrations were positively correlated to the COHb concentration, indicating that the deceased inhaled the hydrocarbons and carbon monoxide simultaneously.

A study on the trend in suicide by CO inhalation involving 158 cases in King County, Washington, United States during 1996-2009 was reported (314). Furthermore, carbon monoxide poisoning in Krakow during year 2002-2010 (315) and in United Arab Emirates during 2007-2009 were also shared in publications (316).

4.5.4 Intoxication by cyanide and inert gases

A suicide case involving a 48-year-old man by oral ingestion of potassium cyanide and inhalation of hydrogen cyanide was reported (317). At autopsy, hemorrhages and erosions of the mucosa of the respiratory tract, esophagus and stomach were found. Concentrations of cyanide were 0.2 mg/L in stomach contents, 0.96 mg/kg in brain tissue, 2.79 mg/kg in lungs, and 5.3 mg/L in blood.

There has been recently an increasing trend of suicide cases that involved insufflations of helium using suffocating plastic bags (318). There are two separate reports on suicide cases by asphyxiation using helium and/or argon (319,320).

4.5.5 Intoxication by drugs of abuse

The first case of fatality due to concomitant consumption of GHB and mephedrone was reported in which 43-years-old man was found dead during a drugs-based party (321). The authors aimed to bring to the attention in the emerging role of new drugs of abuse, and highlighted problems in identifying these drugs with commonly-used immunoassay screening test.

Since amphetamine is a major drug of abuse in Sweden and in other Nordic countries, a survey has studied the demographics of amphetamine abusers in Sweden and the concentrations of this stimulant in forensic blood samples, including 1,183 amphetamine-related deaths, for 10 years in the period of 2001-2010 (155). The authors found that the deaths were mostly results of the toxicity of coingested drugs or adverse drug-drug interaction.

Since abuse of illicit drugs could cause sudden cardiac death, a recent published article has conducted a review on the prevalence of major abused drugs in Europe back in 2009 (322).

A study of 385 toxicology reports related to non-natural deaths of pregnant women in Florida from 1999-2005 revealed that 54% involved prescription drugs (mostly opioids) and 46% involved illicit drugs (323). Such deaths might be intervened and prevented through more interactions with healthcare providers.

Amongst the data on poisoning deaths collected from the autopsy reports in

Estonia from 2000 to 2009, 21.5% cases were found to be poisoned by illicit drugs (324). In addition, deaths from abusing fentanyl increased sharply and remained at a high level since 2002 and the high death toll was attributed to the easy availability of illegal drugs.

5 Conclusions

Over the past three years, significant development and progress have been achieved in the field of forensic toxicology. Recent advances in analytical techniques and increased availability of state-of-the-art hyphenated mass spectrometry instruments have much enhanced the laboratories' capabilities in detecting a wider scope of drugs and/or their metabolites at very low levels in both conventional and alternative specimens. Such advancement has led to evolutionary development in driving under the influence, drug-facilitated sexual assaults and workplace drug testing.

On the other hand, the continual emergence of new drugs of abuse, particularly designer drugs, has posed challenges to toxicologists. Because of shortage of systematic pharmacological and toxicological studies on these new drugs, toxicologists would be difficult to assess their potential risks to human and evaluate their harmful effects from pharmacokinetic and pharmacodynamic perspectives. In addition, the lack of reference standards of these new drugs, in particular their metabolites, have greatly hampered the development of sensitive and effective analytical methods for their identification.

Over the past decade, forensic toxicology has been developing at fast pace with growing complexity. Professionals, experts and practitioners in this discipline should unify and work in collaboration to contend with the changes and challenges ahead through undertaking research and development studies, sharing views and experience, and promoting international cooperation. Under our united and concerted effort, it is expected that the development of forensic toxicology should be sustainable and prosperous in the coming years.

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MEDIA EVIDENCE

Forensic Audio Analysis

Review: 2010–2013

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1 Introduction

This report is a follow up to the review prepared for the 16th Interpol International Forensic Science Symposium in October 2010, and catalogues the research, advances, and application of scientific methodologies and techniques relating to the forensic examination of audio evidence. This report primarily consists of a literature review of published articles in forensic science journals and the proceedings of various working groups and forensic conferences between July 2010 and July 2013. It also contains references from other sources such as the Internet.

2 Authentication (Catalin Grigoras)

Forensic audio authentication research focused on two major topics: individual techniques to assess authenticity and a general methodology to authenticate digital audio recordings.

2.1. Individual techniques

File structure including header analysis is an important stage in digital audio authentication. Koenig & Lacey [1] presented a methodology to investigate the Olympus WMA (Windows Media Audio format) headers, showing that numerous differences between original and edited WMA files can be found for forensic purposes.

Compression history identification is another important issue in digital audio authentication. Shen et al [2] presented a method to discriminate between D-compressed AMR audio recordings and S-compressed, showing that more research is need to apply this technique on real data. Luo et al [3] proposed a method to assess the compression history of WAV files that have been previously compressed by MP3 or WMA and estimating hidden compression rates.

Other digital recording characteristics can also be used for digital audio authentication. Direct Current (DC) analysis is another technique presented by Koenig et al [4], showing its limits and the possibility to use it in forensic audio.

Malik & Miller [5] proposed a statistical framework for microphone identification explaining the effectiveness using ambient noise recordings, while in another paper, Malik and Zhao [6] propose a method to analyze acoustic reverberations. Pan et el. [7] described a fast and blind local noise level estimation method that can be employed to detect digital audio forgeries.

Chen et al [8] introduced a singularity analysis of the wavelet packet decomposition to detect speech audio forged operations in time domain.

Boss [9] presented the results of using ripple signals for digital audio analysis, showing that ripple signals are of high inter-local and very low intra-local variability.

The ENF analysis continued to garner attention and more studies have been run to extract, analyze, and compare ENF. Bao [10] proposed a new method to extract ENF using fractional Fourier Transform, while Su et al [11] explained a solution to separate the ENF components from recaptured audio recordings. Nicolalde et al [12][13] and Coetzee [14] presented detailed methods to investigate ENF amplitude and phase continuity of digital audio recordings. A correction algorithm for the effect of oscillator errors on ENF was proposed by Yuan et al [15]. Liu et al [16] presented a study of the accuracy and precision of quadratic frequency interpolation for ENF estimation. Yuan et al [17] described how simple Monte Carlo techniques and a database of grid reference data can be used to determine the operational parameters of the ENF matching process. Archer [18] analyzed the effects of lossy compression algorithms on ENF showing that hum is robust against the investigated codecs. Grigoras et al [19] presented a detailed database configuration for forensic analysis of ENF, while Jenkins & Steinhour [20] proposed developments to minimize ENF database corruption and system errors. Grigoras & Smith [21] reported advances in forensic ENF analysis including techniques to extract it, statistical tools for automatic search and analysis against a database, possible problems and proposed solutions to minimize measurement errors, database validation testing, and sample cases.

2.2. Forensic audio authentication framework

Korycki [22][23] presented different techniques for time-frequency investigations, tampering detection, and discussed the main methods used for authenticity analysis, including ENF and MP3 compression.

Gupta et al [24] explained recent developments in the audio authentication field including basic, preliminary audio analysis and advanced audio authentication techniques that exploit audio recording conditions and compressed audio features.

Grigoras et al [25] proposed an analytical framework for digital audio authentication including a neutral methodology to interpret and report the results.

Koenig & Lacey [26] presented an inconclusive digital audio authenticity examination, concluding that the recordings could not be scientifically authenticated through accepted forensic practices.

3. Forensic Speech Science (Geoffrey Stewart Morrison & Ewald Enzinger)

This section of the review focuses primarily on recent research on forensic voice comparison, but also briefly discusses recent research on disputed utterance analysis and on voice-based lie detectors. The review aims to be relatively comprehensive, but has not attempted to include all papers published in the area, and has for the most part ignored conference presentations not associated with archived proceedings papers.

3.1 Reviews and introductions

In 2010 Jessen [27] presented an overview of speaker profiling and of acoustic-phonetic approaches to forensic voice comparison, and Morrison [28] published an introduction to forensic voice comparison and evaluation of evidence intended to be accessible to a broad audience including lawyers. The latter also included a review of research on speaker identification by naïve listeners. In 2012 Amino et al [29] published a historical review of forensic voice comparison, and Perrot & Chollet [30] published a paper including a review of voice disguise techniques and their detection.

3.2 Survey of approaches and interpretive frameworks used by forensic-voice-comparison practitioners

Gold & French [31] surveyed forensic-voice-comparison practitioners as to their approaches and methodologies for evaluating the strength of evidence and the interpretive frameworks which they employed. The results were published in 2011 and included responses from 36 practitioners from 13 countries. The most striking finding, although not unexpected for those familiar with the field, was the lack of consistency across practitioners.

In terms of approach, the majority of practitioners (25 practitioners) based their evaluations on a mixture of acoustic-phonetic measurements and listening (although it was not clear how they combined the two), three used listening only (auditory approach), one used an acoustic-phonetic approach without an auditory component, and eight used an approach characterized as human-assisted automatic speaker recognition (although it was not clear what the human-assisted part consisted of and how it combined with the automatic part). Although not mentioned in Gold & French [31], the aural-spectrographic approach is also still practiced in multiple parts of the world (Morrison [32]).

In terms of interpretive framework, two practitioners made binary decisions (which logically require the imposition of a threshold on a posterior probability), and the largest group (14 practitioners) used verbal expressions from an ordinal posterior-probability scale (not necessarily the same scale across different practitioners). The second largest group (11 practitioners) used the so-called UK framework (French & Harrison [33]), and most of the remainder (7 practitioners) used the likelihood-ratio framework (4 presenting

numeric likelihood-ratio values, and 3 presenting verbal expressions). The use of posterior probabilities by forensic scientists has been criticized (e.g., Balding [34], Buckleton [35], Evett [36], Robertson & Vignaux [37]) as it logically requires consideration of prior probabilities, e.g., the trier of fact's belief as to the relative probabilities of the same-speaker versus the different-speaker hypothesis before the strength of the forensic-voice-comparison evidence is presented. The trier of fact's prior probabilities with respect to the forensic-voice-comparison evidence will usually be influenced by other evidence already presented in the trial. The forensic scientist cannot know what the trier of fact's prior probabilities will be, and they should not be exposed to other (task-irrelevant) evidence in the trial which could bias their estimate of the strength of the particular evidence which they have been asked to assess. The UK framework has also been criticized as being logically inconsistent, overly vague, and suffering from cliff-edge effects (Rose & Morrison [38], Morrison [28] [39]; see also the response in French et al [40]).

It would seem unlikely that there will be a substantial decrease in the fragmentation of the field in the short term, particularly at the practitioner level, but there are ongoing trends affecting both forensic science in general and forensic voice comparison in particular which are reflected in much of the research conducted over the last three years.

3.3 Paradigm change

One ongoing trend in the literature on the evaluation and interpretation of forensic evidence in general is the call to adopt the likelihood-ratio framework as the only logically correct framework. This has been strengthened over the last three years due to the response to the 2010 England & Wales Court of Appeal Ruling in R v T [2010 EWCA Crim 2439], e.g., Evett et al [41], Berger et al [42], Redmayne et al [43], Robertson et al [44], Morrison [45]. It was also at the core of the Royal Statistical Society's first practitioner guide for judges, lawyers, forensic scientists and expert witnesses published in 2010 (Aitken et al [46]), and a major focus of the National Institute of Standards and Technology and National Institute of Justice (NIST/NIJ) 2012 report on latent fingerprint analysis [47]. Forensic voice comparison conducted within the likelihood-ratio framework has a history going back to the mid-to-late 1990s as can be traced through earlier Interpol Forensic Science Symposium review papers: Broeders [48][49], Bijhold et al [50], Kriigel et al [51]. Morrison [39] presented a history up to 2009 of the adoption of the likelihood-ratio framework for forensic voice comparison. Researchers and practitioners in forensic speech science fully committed to the use of the likelihood-ratio framework are, however, probably still a minority of those working in the field.

Another ongoing trend affecting forensic science in general is pressure to assess the validity and reliability of analytic approaches and methodologies. Calls for this have recently been published in the 2009 National Research Council Report on Strengthening Forensic Science in the United States (NRC [52]), and in the aforementioned 2012 NIST/NIJ fingerprint report [47].

Morrison [32] reviewed calls, from the 1960s onward, for empirical testing of the validity and reliability of forensic-voice-comparison approaches and methodologies under conditions reflecting those of the case under investigation. Morrison and colleagues [28] [32] [39] [45] [53] have proposed that the field of forensic voice comparison is undergoing a paradigm shift (also affecting forensic science in general), and that the use of the likelihood-ratio framework and the empirical testing of the validity and reliability of approaches and methodologies under conditions reflecting those of the case under investigation are two essential elements of the new paradigm.

Morrison and colleagues have also proposed the use of relevant data, quantitative measurements, and statistical models as a highly preferred element of the new paradigm because such an approach is more transparent, more easily replicated, and more easily tested than an approach in which the output of the system is based directly on the subjective experience-based judgment of a human expert. This last element is presented as highly preferred rather than essential because it must be subservient to the testing element – whichever system performs the best under the conditions of the case at trial should be employed. Morrison and colleagues have described concrete procedures for collecting and selecting relevant data [53] [54] and concrete procedures and metrics for testing validity and reliability [55] [56] [57] (see also Ramos & González-Rodríguez [58]).

3.4 Empirical research on forensic voice comparison conducted within the new paradigm

Papers reviewed in this section describe empirical studies which were, to a greater or lesser extent, conducted within the new paradigm, i.e., to a greater or lesser extent likelihood ratios were calculated on the basis of data, quantitative measurements, and statistical models, and the validity and reliability of the system was tested, and the training and test data were representative of the relevant population and reflective of the recording conditions of some real or imagined forensic case.

Becker et al [59] and Solewicz et al [60] compared the performance of several automatic forensic-voice-comparison systems on a test database of recordings taken from actual forensic cases (the data suffered from a degree of heterogeneity). Systems tested were two in-house systems employed by the German Federal Criminal Police Office (BKA) (see Becker et al [61] [62] [63] for detailed descriptions of these systems, SPES and VoCS), three in-house systems employed by the Israeli National Police, and two commercial systems employed by the French Police Technique et Scientifique. The performance of the different systems was broadly similar, although relative to the other systems one system had a bias towards good performance on same-speaker trials at the cost of poor performance on different-speaker trials and another had a bias towards good performance on different-speaker trials at the cost of poor performance on same-speaker trials. The authors discussed the importance of selecting appropriate data for modeling the population, including language spoken, and/or compensation techniques to

account for mismatches between the training and test data, including mismatches in recording duration.

In 2012 Rose [64] described how likelihood ratios had been calculated from fundamental-frequency and formant-frequency measurements made on the word “yes” and the phrase “not too bad” in an actual forensic case for which the analysis was conducted in 2007. The offender recording was from a telephone call and the suspect recordings from police interviews. Rose also commented on advances made in forensic-voice-comparison research since that time.

Enzinger [65] published a preliminary report on a study based on the conditions of an actual forensic case. The case was somewhat atypical: There were two speakers on a single mobile-to-landline telephone recording. The identity of the speaker of a two-second-long utterance within the recording was in question, but it had to be one of the two aforementioned speakers. In most of the training data, one speaker was relatively far from the microphone and one relatively close, but the questioned utterance was close. The paper illustrated procedures for calculating a likelihood ratio under the conditions of this case using relevant data, quantitative measurements (cepstral coefficients in this case), and statistical models, and procedures for testing the validity and reliability of the forensic-voice-comparison system under the conditions of this case, i.e., it provided an example of how to implement the new paradigm under actual casework conditions.

A number of forensic-voice-comparison studies have investigated the effectiveness of extracting acoustic information by fitting parametric curves to the formant trajectories (and for tonal languages fundamental-frequency trajectories) of tokens of selected vowel phonemes (e.g., Chen & Rose [66], Enzinger [67], Hughes [68], Jialin & Rose [69], Li & Rose [70], Morrison [71] [72], Pingai et al [73], Rhodes [74]) and assessing whether adding these features to a baseline system (e.g., mel frequency cepstral coefficients, MFCCs, fitted to the entire speech-active sections of the recordings) leads to improvement in performance over the baseline system (e.g., Zhang et al [75] [76] [77] [78]). Initial results using high-quality voice recordings were promising, but studies using various combinations of landline- and mobile-telephone-transmitted voice recordings found little or no meaningful improvement in performance over a much cheaper baseline system, especially when mobile telephones were involved (Zhang et al [77] [78]). The latter is an important finding given the popularity of the use of formant measurements by acoustic-phonetic forensic-voice-comparison practitioners and the propensity for forensic casework to involve telephone-transmitted (especially mobile-telephone-transmitted) speech.

A number of studies investigated the effectiveness for forensic voice comparison of extracting information from glottal features. Kinoshita & Ishihara [79] and Zheng & Rose [80] tested the use of features based on the distribution of fundamental-frequency measurements made across all voiced speech in recordings, but neither compared their system’s performance with that of a baseline system. As mentioned above, several studies (Chen & Rose

[66], Jialin & Rose [69], Li & Rose [70], Zhang & Enzinger [78]) tested the use of fundamental-frequency trajectories for selected vowels in tone languages (Cantonese and Mandarin, see also Wang & Rose [81]). Enzinger et al [82] tested a number of glottal-source measurements (jitter, shimmer, and many more) extracted using commercial software, but did not obtain substantial improvement over a baseline system.

Kavanagh [83] [84] and Yim & Rose [85] investigated the effectiveness for forensic voice comparison of extracting acoustic information from the spectra of selected nasal phonemes. They did not compare the performance of their systems with a baseline system. Rose [86] [87] [88] investigated the effectiveness for forensic voice comparison of using cepstral coefficients to measure the spectra of tokens of a selected fricative phoneme and tokens of selected vowel phonemes. The data were read speech recorded over landline telephone systems. The last of these studies found an improvement in performance over a baseline system based on the entire speech-active portion of the recordings when the fricative-spectra system was fused with the baseline.

The use of long-term-formant (LTF) distributions for forensic voice comparison has been discussed in previous Interpol Forensic Science Symposium reviews (Bijhold et al [50], Kriigel et al [51]). Gold et al [89] tested the performance of an LTF forensic-voice-comparison system but did not compare the results with the performance of a baseline system. Becker [63] did not find substantial improvement over an MFCC baseline system when an LTF system was fused with the baseline system.

Rose & Winter [90], Morrison [71], and Zhang et al [75] tested the effectiveness of the Gaussian Mixture Model - Universal Background Model procedure (GMM-UBM, e.g., Reynolds et al [91]) versus the Multivariate Kernel Density procedure (MVKD, Aitken & Lucy [92]) for calculating likelihood ratios based on formant measurements. Which of the two procedures works best appears to depend on bias-variance tradeoffs related to the number of variables and number of data points used to train the models.

Rhodes [74] investigated the effect of large time differences between suspect and offender recordings on the performance of formant (including formant-trajectory) based forensic-voice-comparison systems and on the performance of a commercial forensic-voice-comparison system. Testing was conducted on recordings of eight speakers made at seven-year intervals between age 21 and 49 (a total of five time points per speaker). Performance for both systems decreased with increased time span.

A number of the studies reported above did not calibrate the forensic-voice-comparison systems employed. Calibration can ameliorate what would otherwise be very misleading results, and in some circumstances it is essential if one wishes to interpret system output as likelihood ratios. Morrison [93] published a tutorial on logistic-regression calibration and fusion including examples taken from forensic voice comparison as well as fingerprint

comparison. A number of the studies reported above tested on contemporaneous data, i.e., same-speaker test pairs were created by dividing a single recording. Apart for exceptional cases (such as in Enzinger [65]) if the recordings of known and questioned origin are in fact from the same speaker, they are non-contemporaneous recordings of that speaker. Enzinger & Morrison [94] reported on a study which empirically illustrated that testing on contemporaneous data gives an overly optimistic impression of system performance compared to testing on non-contemporaneous data.

3.5 Empirical research on forensic voice comparison not conducted within the new paradigm

Papers reviewed in this section describe empirical studies which were not conducted within the new paradigm. A number of the studies mentioned in the previous section included multiple analyses some of which were more or less compatible with the new paradigm and some of which were clearly incompatible with the new paradigm, those studies are not re-reviewed in this section.

Schwartz et al [95] described the United States Secret Service - Massachusetts Institute of Technology Lincoln Laboratory (USSS-MITLL) forensic-voice-comparison system applied to the National Institute of Standards and Technology's Human Assisted Speaker Recognition Evaluation (NIST HASR). An auditory-acoustic-phonetic system whose ultimate output was based on a human expert's judgment was fused with an automatic system. The relative weighting of the two systems in the fusion was also subjectively decided. The HASR Evaluation required that the system provide a same-speaker or different-speaker decision.

Mendes & Ferreira [96] obtained improvement in correct-identification rate when they fused a baseline MFCC system with a system based on normalized relative delays of source harmonics from selected vowels. High-quality audio recordings were used.

Thaitechawat & Foulkes [97] investigated the effectiveness for forensic voice comparison of extracting acoustic information from formant and fundamental-frequency trajectories in a tone language (Thai). Classifications were performed using discriminant analysis.

Künzel [98] tested the performance of a commercial forensic-voice-comparison system on cross-language compared to same-language test pairs. Transmission conditions tested were landline telephone, mobile telephone, and voice over Internet protocol. Cohorts of recordings in the same language and same transmission condition as the suspect recording were used to normalize system scores (Z-norm). False-alarm rates for different-speaker trials were only slightly worse for the cross-language trials than for the same-language trials.

3.6 Disputed utterance analysis

Three papers looked at issues related to the disputed utterance in the 2009 New Zealand Supreme Court case Bain v R [2009 NZSC 16]. This was a very high profile case in New Zealand. Innes [99] discussed the background to the case and the expert opinions with respect to the disputed utterance. The prosecution contended that the words spoken were “I shot the prick”, an admission of guilt, whereas the defense contended that these were not the words spoken. Some of the experts consulted thought they heard the words “I can’t breathe” (and this was actually what Bain claimed to have said, although this was not revealed at the time). Most of the experts (French, Harrison, Cawley, Foulkes, Innes) based their opinions on what they heard and some opined that it was even uncertain as to whether the disputed utterance was speech or simply breathing. None came down in support of the “I shot the prick” hypothesis. The Supreme Court ruled that the jury in the trial proper should not be allowed to hear the disputed utterance, or any reference to the prosecution hypothesis, or expert testimony relating to the disputed utterance.

Fraser et al [100] experimented on what jury members might have heard had they been asked to listen to the disputed utterance. A total of 190 listeners were tested in two conditions. On initial listening the most common response from the listeners as to what they heard was “I can’t breathe” (from 60 of the 190 listeners). Only three heard “I shot the prick” (one of these had previous knowledge of the case and the other two were police officers). After one group heard mock expert testimony in support of the hypothesis that the words spoken were “I shot the prick” the number of listeners reporting this as being what they believed the words to be raised from 1 to 32 (of 96), and then after hearing mock expert testimony to the contrary that dropped to 26. For listeners in a control group who heard mock expert testimony that the words spoken were “he shot them all” the number reporting that they believed the words to be “I shot the prick” rose from 2 to 3 (of 94). Finally, both groups were told that the words spoken were definitely not “I shot the prick” at which point the number of listeners reporting this as being what they believed the words to be dropped to 17 for the first group, but rose to 11 for the control group. This demonstrated that although very few listeners heard the words “I shot the prick” without being prompted, a substantial proportion could be induced to hear these words if they were suggested to them, and, more disturbingly, even if the suggestion came in the form of being told that these were not the words.

One expert (Rose, whose evidence the defense held back for potential presentation in the trial proper rather than in the Supreme Court hearing) opined in his report that what anybody heard was irrelevant, what mattered was what Bain said, that the best way to assess this was via acoustic analysis rather than auditory perception, and that the proper way to evaluate the strength of the evidence was via a likelihood ratio, i.e., what are the relative likelihoods of getting the acoustic properties of the disputed utterance had the speaker said “I shot the prick” versus had he said “I can’t breathe”. As a research project Morrison & Hoy [101] conducted a preliminary version of such an analysis using telephone recordings of a speaker mimicking the

speaking style of the disputed analysis. The speaker produced about 40 tokens of each phrase. A form of cepstral analysis was conducted to extract acoustic information from the first speech sound in the known tokens of “shot” [ʃ] and “can’t” [ç] and from the speech sound in the equivalent position in the disputed utterance. Statistical models were trained and tested. The measured acoustic properties of the disputed utterance were found to be approximately 31 000 times more likely under the “can’t” hypothesis than under the “shot” hypothesis.

3.7 Voice-based lie detectors

Not mentioned in previous Interpol Forensic Science Symposium reviews, there was some controversy around a paper on voice-based lie detectors (formally voice stress analyzers) published by Eriksson & Lacerda [102]. The paper included criticism of a particular commercial product, and the manufacturer of that product threatened to sue the journal publisher. The publisher withdrew the paper from their website. Other recent papers published on the topic include Hollien et al [103], Harnsberger et al [104], Harnsberger [105], Horvath et al [106], and Lacerda [107]. These papers reported on theoretical and empirical assessments of commercial systems whose explicit or implied function is lie detection via acoustic analysis of voice signals. There may be a placebo effect whereby speakers who believe an effective lie-detection system is in use are less likely to lie, and human listeners may be able to perceive that speakers are lying at levels slightly above chance, but beyond that none of the studies found any substantial evidence in support of the hypothesis that any of the systems performed at levels above chance.

4. Audio Enhancement (Jeff M. Smith)

4.1 Introduction

The enhancement, or clarification, of forensic audio is a common task related to the processing and analysis of audio evidence. This is because recordings made by law enforcement, intelligence, or the general public, which end up as forensic evidence are commonly made in non-ideal environments with non-ideal equipment leading to degraded quality and a poor ratio of signal to noise (SNR). The general goals for the enhancement of forensic audio include: to increase intelligibility of speech present in a recording which may increase the accuracy of transcription and number of words present in a transcript, to decrease listener fatigue due to recorded interferences, and to decrease the SNR in the preprocessing of recorded material for automatic speech and speaker recognition systems.

Early innovations in this area still impact the set of current solutions including spectral subtractive algorithms [108] and statistical model based algorithms [109] that are applicable to monaural recordings. Where multiple microphone sources are available, spatial filtering by means of beamforming [110] and

Independent Component Analysis (ICA) [111] can be effectively applied. Some more recent advances in this area will be described below including a discussion of research into new algorithms for speech enhancement. Additionally, special attention will be given to recent developments in the evaluation of speech intelligibility, which has recently and naturally evolved within this mature field.

Since speech enhancement research is well established and research contributions in this area are very frequent, the impact of innovative research is hard to evaluate soon after initial publication. This literature review therefore will focus on a few novel and relevant publications in the main areas related to forensic audio enhancement: monaural and binaural approaches, deconvolution, speech intelligibility evaluation, and the new areas of Compressive Sensing (CS) and Computational Auditory Scene Analysis (CASA).

4.2 Reference works

There are two recent reference publications related to this field. The 2nd Edition of the Encyclopedia of Forensic Sciences featured a chapter on Forensic Audio Enhancement and Authentication [112] by Grigoras & Smith. In this chapter the authors present a basic procedure for the handling and processing of forensic audio for both enhancement and authentication. Additionally, references to best practices are provided.

Loizou's 2nd Edition of Speech Enhancement: Theory and Practice [113] was published which continues to be a valuable reference in the area of speech enhancement. The new addition pays special attention of speech intelligibility including two new chapters on the subject.

The Scientific Working Group on Digital Evidence (SWGDE) publishes guidelines and best practices related to computer and mobile phone forensics as well as forensic audio. The Audio Committee made up of law enforcement and academia released the "Core Competencies for Forensic Audio v1.0" in September of 2011, which complements the previously drafted "Best Practices for Forensic Audio v1.0" from 2008. These documents are valuable resources for the drafting of laboratory practices and Standard Operating Procedures (SOPs) respecting consensus driven best practices for forensic audio processing and enhancement.

4.3 Enhancement of Monaural and Binaural Recordings and Future Areas of Research

Two interesting papers related to tone removal from recordings were presented at the AES 46th International Conference on Audio Forensics. Haddad & Noga [114] present a novel method for removing tone interferences by utilizing a super resolution spectrum analysis technique to remove the poles of the unwanted signal. In testing, this method showed better results than the traditional notch filter when preprocessing material for speaker

recognition tasks. Nordlund & McElveen [115] present a solution for removing non-stationary tonal noises by whitening the signal's noise floor and identifying tonal peaks for subtraction. This achieves higher-resolution subtraction reducing error and distortion.

Another useful approach in forensic audio enhancement is the separation of signals in a monaural recording by using commercially available material present in the recording (music, TV broadcast, etc.) to synthesize binaural reference cancellation. The problem with application of this method in forensics is in time domain alignment and drift of the often low-quality source to the commercial reference material. Ding & Havelock [116] propose a drift-compensated adaptive filter (DCAF) to achieve better cancellation while Alexander et al [117] apply landmark-based acoustic fingerprinting, similar to what is used in Shazam and other music identification services, to automatically align material.

Recent research by Paliwal et al [118] into processing noisy audio signals in the modulation domain has shown an improvement over traditional acoustic spectral subtraction. Another exploration of processing in the modulation domain by Zhang & Zhao [119] achieves binaural blind source separation.

In another growing area of research, computational auditory scene analysis (CASA), researchers seek to emulate with a machine the human ability to overcome the so-called "cocktail party effect". It has been shown by Wang [120] that the main concern of CASA is use of the ideal time-frequency binary mask (IBM). Recent papers on IBM estimation in speech enhancement include May et al [121] and Jensen & Hendriks [122].

Another new area of research that has had profound effect in many areas is compressed sensing or CS introduced by Candès et al and Donoho [123] [124]. This technique can help acquire and reconstruct a signal from a sparse or underrepresented dataset allowing the entire signal to be determined from relatively few measurements; fewer than those set forth by the Nyquist theorem (which requires twice the highest sampled frequency). D. Wu et al [125] have explored application of CS based speech enhancement finding that compressed speech and noise via discrete cosine transform (DCT) achieves proper signal sparsity for compressed sensing. Low et al [126] provide a good overview of compressive sensing and speech enhancement. P. Wu et al have also used CS in multichannel dereverberation, or deconvolution, of audio signals [127].

4.4 Speech Quality vs. Speech Intelligibility

As discussed earlier, one crucial aim in the enhancement of forensic audio recordings is to increase intelligibility of speech material in order to increase accuracy and words present in a transcript. Recently, it has been found that the classical methods of enhancement are effective at increasing the signal quality (increase in SNR) but do not increase intelligibility AND may actually make speech less intelligible. Subjective listening tests by Hu and Loizou

[128] using the NOIZUS database shed light on this. Hilkhuysen et al [129] recently found congruent results in testing three algorithms (spectral subtraction, MMSE, and subspace) with difficult noise types (car and talker babble).

Thus, recent changes have taken place in the research and development of speech enhancement algorithms focused on speech intelligibility. Loizou & Kim [130] discuss this further and add additional findings of interest like that fact that in testing subspace algorithms perform worst in overall quality but perform well in terms of preserving speech intelligibility. They urge researchers focusing on intelligibility to maximize greater than 0 dB the segmental SNR in the frequency domain. A predictive measure for determining speech intelligibility has been proposed Taal et al [131] with a short-time objective intelligibility measure (STOI) as a reliable means for obtaining evaluation data while avoiding costly listening experiments.

Researchers at the Center for Law Enforcement Audio Research (clear-labs.com) in the UK investigate this area with special attention to processing forensic audio by examiners. Hilkhuysen et al [132] investigate improvement of intelligibility based on parameter settings of commercial equipment chosen by experts attempting to increase intelligibility. Findings were that while parameter settings varied greatly, experts attempting to enhance noisy speech propose parameter settings which generally deteriorate intelligibility. In another interesting paper, Hilkhuysen et al [133] investigate whether repeated listening to audio material (replay) improved intelligibility or understanding of utterances. This is important because experts and those preparing transcriptions commonly replay audio material. The study found that after replaying 5 times, listener performance saturated while listeners themselves underestimated their performance believing it improved after replaying 5 times. The authors conclude that replay can improve intelligibility performance but may lead to overconfidence.

5. Organizations

Forensic audio analysis is a growing community that has members in several international working groups:
- AAFS - American Academy of Forensic Science: Within the American Academy of Forensic Science is the newly formed Digital and Multimedia Sciences section that includes forensic audio and speech analysis. http://aafs.org/digital-multimedia-sciences
- AES - Audio Engineering Society: The Audio Engineering Society is devoted exclusively to audio technology. Founded in the United States in 1948, the AES has grown to become an international organization that unites audio engineers, creative artists, scientists and students worldwide by promoting advances in audio and disseminating new knowledge and research. http://www.aes.org/
- ENFSI FSAAWG – European Network of Forensic Science Institutes Forensic Speech and Audio Analysis Working Group: a European group that

is focused on all aspects of forensic audio and speech analysis, including linguistics. “Membership of FSAAWG is open to representatives from all ENFSI member institutes. Members have to be active in the areas of forensic speech and audio analysis.” “Representatives from non-ENFSI members who are active in the field of forensic speech and audio analysis examinations can apply for associate membership.” http://www.enfsi.eu/page.php?uid=63

- The Forensic Acoustics Subcommittee (FAS) of the Acoustical Society of America (ASA) was established in 2010 and organizes a special session at an ASA meeting approximately once per year. “Membership of the ASA Forensic Acoustics Subcommittee is open to current members of the ASA.” Website: http://asa.forensic-acoustics.net/
- The Forensic Speech Science Committee (FSSC) of the Australasian Speech Science and Technology Association (ASSTA) was established in 1996. Membership of the committee is by invitation. Website: http://www.assta.org/?q=assta-forensic-speech-science-committee
- IAFPA - The International Association for Forensic Acoustics and Phonetics was established in 1991 and holds an annual conference. “Full membership is available to established phoneticians and acousticians with operational and/or academic interests in forensic applications of phonetics or acoustics.” Website: http://www.iafpa.net/
- NCMF - National Center for Media Forensics: an American center that is part of the University of Colorado which has a strong forensic audio program in addition to research and education in video and image forensics. http://www.ucdenver.edu/academics/colleges/CAM/Centers/ncmf/Pages/ncmf.aspx
- SWGDE - Scientific Working Group on Digital Evidence: an American group that includes a forensic audio committee that has produced best practices manuals and is promoting research on forensic audio. http://www.swgde.org/
- SWG-Speaker - Scientific Working Group on Speaker recognition: a new created American group to support and promote the scientific foundations and practice of speaker recognition, voice data collection, measurement, transmission, and retrieval. http://swg-speaker.org/

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Forensic Video Analysis

Review 2010-2013

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TABLE OF CONTENTS

1 Introduction

This review focuses on Forensic Video Analysis and the advances in technology during the past three years. The principal topics covered are divided into five sections: Authentication, Analytics, Video Enhancement and Analysis, Related Fields of Video Evidence, and Working Groups and Organizations. For information regarding video file repair, file carving, formats and codecs, image manipulation, and steganography, please see the review on imaging by Zeno Geradts and Arnout Ruifrok.

The selection of specific sections to be reviewed was based on a broad literature review of approximately 100 references which was further refined. For most of the references, the author reviewed the entire articles; however, only the abstracts were reviewed for those materials not accessible by the author.

Over the past few years, several aspects regarding the use of video technology for forensic purposes have been addressed through research and publication. With the proliferation of video surveillance around the world, new techniques must be developed to authenticate, analyze, enhance, and utilize the recorded data.

2 Authentication

The authenticity of videos used for forensic analysis is always vital to an investigation. If a video is deemed unreliable, its evidentiary value is nullified. Therefore, in instances where authentication is necessary, it is important to have enough information to conduct the examination appropriately.

With the growing number of surveillance systems and video data available in today's society, the value of this information to law enforcement has never been higher. "However multimedia editing tools can be used to efficiently and seamlessly alter the content of digital data, thus compromising the credibility of information" Upadhyay and Singh note [1]. In their overview of video authentication, they discuss some of the more commonly used video authentication techniques such as digital signatures, watermarking, intelligent techniques, as well as more techniques like motion trajectories and cryptography. The chart they used to display these techniques could be a useful tool to anyone working with video systems to understand the possibilities for video authentication.

In a second paper, Upadhyay and Singh explore the issues associated with developing a video authentication system [2]. According to the authors, "These issues include the classification of tampering attacks, levels of tampering attack and robustness." Their work also describes many of the shortcomings of current authentication techniques. By evaluating the current state of the art, and developing strategies to overcome current authentication weaknesses, Upadhyay and Singh strive to improve the task of video

authentication.

Another area of interest is the use of anti-forensics to aid in the identification of altered videos. In their abstract, Stamm and Liu [3] indicate “very little research exists into anti-forensic operations designed to make digital forgeries undetectable by forensic techniques.” Various searches for these terms validate these claims and may lead to further opportunities for study in the future. Unlike most forensic disciplines, digital media can be manipulated for malicious purposes. While it may be difficult or impossible to forge or alter a latent fingerprint, digital media such as video may be changed in ways that are undetectable to the untrained eye.

In another article, Stamm, Lin, and Liu [4] contend “many anti-forensic operations leave behind their own forensically detectable traces.” These traces can often lead a skilled video analyst to the portion(s) of a video or image that may have been altered, thereby undermining the malicious intent. In order to detect these alterations, an understanding of how they are created must exist. Stamm, Lin and Liu developed “a new set of techniques for evaluating the performance of anti-forensic operations” and developed a “theoretic framework for analyzing the interplay between a forensic investigator and a forger.” Studying this type of relationship could lead to better training of forensic personnel to identify digital forgeries.

Dong, Yang, and Zhu [5] propose a method to authenticate video by evaluating the data for frame-based tampering. This type of tampering “usually suffers from double MPEG compression.” To identify instances of frame manipulation, “a motion-compensated edge artifact (MCEA) based passive forensics scheme is proposed for detecting frame-based video manipulation.” According to the authors, “Experimental results show that the proposed approach is effective for frame-based tampering, such as adding/deleting frames and GOP structure change, and can predict the GOP structure of original video.” Many times a single frame, or group of frames, may be the entirety of the evidence needed in a forensic examination. The possibility to determine whether the video submitted as evidence was complete, would undoubtedly prove useful in such circumstances.

Finally, to show the importance of video authentication in a real-world scenario, Lacey and Koenig provide a case report where video recordings were examined for continuity and authenticity [6]. In the study, a Lawmate PV-500 Digital Video-Audio Recorder was submitted with evidence containing several videos. The authors were then asked whether the recordings were originals, continuous, altered, and consistent with being produced by one of the submitted recorders. By evaluating metadata, individual frames, raw video data, and test recordings, the authors were able to determine that the submitted recorder did create the video files in question. In addition, they concluded that the apparent identical frames “were not introduced by a transcoding process, but rather by a characteristic of the original video encoding algorithm.”

3 Analytics

As the volume of video data continues to grow, the task of utilizing the enormous amount of data available to law enforcement becomes paramount. Many times, an investigator or video analyst will need to view hours of surveillance video to locate the incident or suspect in question. In other cases, a CCTV operator may need to view several cameras at one time, elevating the potential to miss important activities due to the amount of detail on the screen. These processes can take valuable time away from solving a crime or locating a person or object of interest. Much of the research done during the past three years has focused on automating certain aspects of CCTV surveillance. This includes, but is not limited to license plates, objects, and behavior. Automated video analysis of any type is commonly referred to as video analytics.

Park, Lim, and Han [7] discuss a video analytic retrieval system for CCTV surveillance that relies on "the dominant colors of objects and applies the similarity measurement method of absolute (or fixed) range or relative (or variable) range." Their system would allow automated metadata generation, multiple video searches, and evidentiary video output. The ability to quickly and easily search videos is of great value when time-sensitive situations arise. Therefore, a system that would automatically provide the metadata and output the resulting video once it was located would be advantageous.

Another challenge commonly facing the implementation of CCTV systems is the ability to monitor each camera for activity. Jodoin, Konrad, and Saligrama [8] state "One of the most important – and difficult – goals of video analytics is to detect abnormalities or events that differ from what is considered usual, such as an abandoned package, a car traveling against traffic, or a fallen elderly person." Current technology already exists to detect abnormalities using motion detection in restricted areas. The alarm function on DVRs is widely used and may not require an operator to monitor the cameras. However, this process does not work in complex scenes where many objects are in motion at the same time. The authors suggest teaching the system what normal activity is using a training video. Then, the system could "identify abnormal patterns based on object dynamics, shape, or color." By utilizing the static nature of many surveillance cameras, the authors' technique applies background subtraction to obtain values for each pixel in each frame. In addition, the authors discuss how a "behavior image" concept is used to aggregate information for all pixels over any number of frames to create a 2D array, thereby reducing the memory requirements necessary for a real-time application. To accomplish their goal, a "background-behavior image is computed from a training video with normal behavior" and compared to "observed-behavior images from streaming video" to find abnormalities. This process is called "behavior subtraction." The result is a "frame abnormality map" that may show a car driving in the wrong direction, a pedestrian travelling in an uncommon way, or an item that has been left behind on a busy street. These automated processes could assist in the monitoring and

reviewing of robust surveillance systems where time-critical tasks are common.

Wiliem, et al. [9] also believe that the current state of surveillance video data is not being utilized to its fullest extent for crime prevention. They contend that current systems "rely heavily on human observers and are therefore limited by factors such as fatigue and monitoring capabilities over long periods of time." Focusing on suspicious behavior detection, the authors explore three main components to an automated process for utilizing contextual data: "a context space model, a data stream clustering algorithm, and an inference algorithm." This information can be used by the system to make more accurate detections of suspicious behavior thus aiding in the task of crime prevention.

Another event detection application is presented by Whiten, et al. from the University of Ottawa [10]. They state "When deployed, CCTV systems are used in either of two modes of operation: a) Live mode (or real-time monitoring), and b) Archival mode (or post-event analysis through recordings)." In their evaluation, they found that the current systems are not efficient at either task due to events going undetected in real-time and the difficulties of storing and managing archived videos. To attempt to alleviate these challenges, the authors first identified "an important dual computer-human" relationship that relies on both components for success. By utilizing computing power to sort through large amounts of video data, and then fine-tuning that data with the human user, event detection can be accomplished more efficiently. However, the authors also warn that event detection will inherently include false positives to avoid missing critical events in the videos. These false positives would be evaluated by the human utilizing the system and disregarded based on investigative needs. Certainly, a number of false positive inclusions would be preferred to any number of exclusions in this instance.

Angadi, Naik, and Kumar [11] state "Visual information is the most appealing and intuitive mode of conveying information. Further, the amount of information that video carries is significantly greater than that carried by any other media." However, as previously stated, one major challenge to utilizing this information is the time it takes to locate the data. The authors propose combining new techniques with conventional shot-detection methods to automate the process of locating activity-based portions of video into specific segments for review.

Like many of the previous authors, Kho, et al. [12] realize that the sheer volume of data captured by surveillance systems can be daunting during real-time investigations. The amount of available video data is useful "However, the data of CCTV will not even be processed or looked because it requires intensive labors for monitoring purpose. Therefore, the development of real time tracking systems on the contour shape like dangerous weapons or suspected motions for crime prevention is necessary in order to reduce the crime events that keep increasing nowadays." Their study focused on a solution to alleviate the time constraints caused by massive amounts of video data. As a result, the system "proved that it was performing well in

recognizing the dangerous weapon and suspected person's motion." However, the system was limited by slower performance when larger numbers of training sets and higher resolution images were introduced.

Many of the other papers in the review that dealt with video analytics focused on real-time events or the future of surveillance systems [13]. Coetzer, Merwe, and Josephs relate that video analytics is simply part of a larger system that will continue to become more useful as automation and event detection improve. The authors believe that combining information management and video surveillance will ultimately lead to intelligent video surveillance in the future.

Some of the other advances in current technology include automated license plate detection to both identify license plates and track driver movements [14-15]. In certain situations, this information can be extremely useful to law enforcement personnel when an event has just occurred and persons of interest must be located immediately. Other uses could include locating suspects' vehicles after a previously undetected crime is discovered.

In most circumstances, forensic evidence is needed to identify a specific person of interest. Calderara, Prati, and Cucchiara understand that both online uses (real-time) of video surveillance and offline (forensic) scenarios exist [16]. The authors state "Solutions for people detection, action and activity analysis, movement recording, behaviour recognition, other people-related events and anomalous situation assessment for security reasons are similar." In their paper, they "propose an integrated tool for both online analysis and offline mining for forensics, related to people moving in scene acquired by security cameras." Since many surveillance cameras are set to record data from stationary scenes such as parking lots, building entrances, etc.; it would be extremely beneficial for end users to have a tool that could automatically find scenes containing people and then track those individuals within the available data.

4 Video Enhancement and Analysis

Once a segment of video has been identified as potentially relevant to an investigation, it is often necessary to enhance and analyze the data. Enhancements can be as simple as adjusting the contrast or as complex as applying multiple filters to visualize a distorted license plate number. Video analysis may include feature analysis, vehicle identification, gait analysis, photogrammetry, or any other number of possibilities. The following articles have been published over the past three years relating to the areas of both enhancement and analysis.

4.1 Video Enhancement

Shahraki, et al. [17] conducted a survey of current video forensic tools. They state "Video forensics tools are developed as a part of digital forensics tools to

analyze digital evidences and clear vague points of them for presenting in the courts." In their paper, they introduce some commonly used forensic video tools, discuss their capabilities, compare them to one another, and finally propose an alternative framework utilizing the strengths of each system to produce a more robust solution. Included in the survey are the following products: Ocean System Dtective, Motion DSP's Ikena, Cognitech's Video Investigator, TREC – Video Forensic, FOREVID, and Kinesense. Each system's capabilities are outlined in detail and then compared in a table, highlighting both strengths and weaknesses. This information could be very useful to organizations in need of forensic video solutions.

According to Rao and Chen [18], "Video enhancement is one of the most important and difficult component (sic) of video security surveillance system (sic)." In their survey of current video enhancement techniques, they divide video enhancement into two basic categories: "self-enhancement" and "frame-based fusion enhancement." The authors use of "self-enhancement" refers to enhancing the video without using any extra data. The "frame-based fusion" method utilizes videos and images from other sources, where illumination may be significantly different. They show how using a high resolution image taken under good lighting conditions can be merged with a low quality video image to produce an enhanced frame. While this technique appears to be similar to High Dynamic Range (HDR) images, frame-based fusion utilizes different sources for the lighting differences rather than taking frames at either different exposures or during different times of the day.

Many times, areas of interest in video occur at night. These videos can be very difficult to enhance, since digital video generally has a low dynamic range. As referenced above, fusing images together may provide some enhancement under these circumstances. Yunbo, Weiyao, and Leiting [19] state "In order to efficiently enhance the dark nighttime videos, the high-quality daytime information of the same scene is often introduced to help the enhancement. However, due to camera motion, the introduced daytime may not have exactly the same scene of the nighttime videos. Thus, the final fused moving objects may not produce reasonable results." The authors believe that global motion estimation can be used to overcome such limitations, and feel their results show the effectiveness of their algorithm.

Another challenge facing forensic video experts is the noise generated by cameras in poorly lighted scenes. When a camera is stationary, an averaging process can be used to eliminate most video noise. However, in instances where pan/tilt/zoom (PTZ) cameras are used, the noise can dramatically affect the overall video quality. Maggioni, et al. [20] "propose a powerful video filtering algorithm that exploits temporal and spatial redundancy characterizing natural video sequences." By utilizing these new techniques "Experimental results prove the effectiveness of our method in terms of both subjective and objective visual quality, and show that it outperforms the state of the art in video denoising." Since noise can be extremely detrimental to night-time surveillance video, this process could be utilized to enhance many videos that may have been previously unusable.

In addition to surveillance video, the increasing accessibility to video equipment such as smartphones, tablets, and other mobile devices has led to greater amounts of amateur videos being examined for forensic purposes. Ejaz, et al. [21] agree stating, “The omnipresence of handheld video devices has led to a drastic increase in the amount of videos created by non-professional users.” Many times, these videos are handheld, which introduces additional motion atypical of surveillance videos. The authors attempt to correct the motion in these videos by estimating “camera motion parameters using optical flow features.” These parameters can be used to distinguish between intentional and accidental camera motion “by detecting sharp changes in collective motion estimate curve.” By stabilizing videos in such a manner, they are not only easier to view, but may also lead investigators to previously unseen information.

Perhaps one of the biggest challenges facing forensic video experts is low resolution images that are further compressed on a Digital Video Recorder (DVR). These images often lack the vital details needed to determine even basic information from the scene, and are generally unusable for anything other than class characteristics. Ghazali, et al. [22] propose a method to improve the resolution: “Using super resolution methods, high resolution image is obtained from a set of low resolution images, after it had undergone two main processes; image registration process based on Keren algorithm and image reconstruction process based on Projection onto Convex Set (POCS) on frequency domain.” Another study by Zamani, et al. [23] “present a multiple-frames Super-Resolution technique by combining a sequence of video frames of a subject in order to create a super-resolved frame of the subject with increased resolution and clarities (sic).” These two papers share some common authors, but the goal to improve resolution from CCTV video is critical to many investigations.

4.2 Video Analysis

Once a video has been enhanced or found suitable for analysis, it is critical to use scientifically valid techniques to produce a reliable opinion. One common analysis request is to determine the height of a subject located in a video. Many times, the subject is moving and potentially not at full height. Ramstrand, et al. [24] state “While errors associated with image distortion have been addressed in the literature, the relative effects of other sources of systematic error are largely unaddressed in the literature.” To alleviate this knowledge gap, the authors utilized forty-six adult participants who “were recorded using a 3D motion analysis system while performing eight different tasks. Height measurements captured using the 3D motion analysis system were compared to static height measurements in order to determine relative differences.” The information contained in this research paper should be reviewed by anyone currently conducting subject height analysis from videos. The variations in height due to the completion of various tasks indicate that these must be accounted for when reaching a final conclusion.

Bouchrika, et al. [25] attempt to utilize gait information from surveillance cameras as a forensic tool. The authors state “Given the continuing advances in gait biometrics, it appears prudent to investigate the translation of these

techniques for forensic use." In many instances of pre-meditated crimes, the subject will attempt to conceal their identity from possible surveillance cameras. Many disguises can defeat even the most robust biometric systems such as facial and iris recognition. If the suspects also wear gloves, thus concealing their fingerprints, and DNA is not found at the scene, the only clue as to the individual's identity may be their gait. This study shows the viability of using someone's gait as an identifiable characteristic.

5 Related Fields of Video Evidence

As has been shown by many of the publications outlined in this review, CCTV surveillance systems generate a great amount of data. Since the shift from analog to digital technology, there is a greater need to ensure that evidentiary videos be preserved. Therefore, both the party installing the system and the user who receives the resulting videos must consider data management. Zhang, et al. [26] describe a system using high definition Internet Protocol (IP) cameras that connect to a local server. The servers allow remote access to the data and also contain on-board analytics for real-time applications.

Another way to manage the data accumulated through surveillance is proposed by Kumar, Roy, and Mittal [27]. The authors' paper "presents OS-Guard (On-Site Guard), a novel on-site signature based framework for multimedia surveillance data management." The goal of their system is to cull through the data and separate "informative data" from "non-informative data" thereby alleviating the massive amount of storage typically necessary video systems. Their system utilizes both audio and video clues to determine what may be "informative" and saves them as a binary feature. According to the authors "Initial experiments for a Bank ATM monitoring scenario demonstrates promising results."

Regardless of the data collected by CCTV cameras, without the assurance that the information is preserved, the video could be rendered useless. Lim, Park, and Han [28] discuss "Evidential Video Management (EVM)" that takes information assurance to CCTV. By establishing a reliable chain of custody and ensuring an archival format that prevents deletion or over-writing, the authors attempt to alleviate any concerns with data security.

While much of the work presented in this review deals with automated processes for event detection, many systems still rely on operators to monitor surveillance images in real time. Improvements as to how control rooms are designed for optimal viewing are presented by Stedmon, Harris, and Wilson [29]. In their study, they simulated multiplexed video and conducted experiments designed to evaluate techniques aimed at improving the effectiveness of operators monitoring a scene. They state, "The findings suggested that manipulating the layout of images improved task efficiency and provided novel insights into strategies and behaviours that participants adopted." Obviously, improving the performance of individuals monitoring CCTV data is critical in real-time scenarios that rely on accuracy and attention

to detail.

In addition to creating spaces conducive to video monitoring, it is important to understand what operators are actually seeing when they view surveillance footage. Howard, et al. [30] studied the gaze of operators watching moving scenes to determine how they were able to track suspicious activity. They found "that when multiple areas of a display compete for attention, gaze is allocated according to relative levels of reported suspiciousness." In their study, they used four different urban scenes playing simultaneously to gauge participants' gaze patterns. By examining this process, a better understanding of operators' attention to "suspicious" activities was gleaned. This information could lead to better training of operators in the future by providing task-oriented simulations for evaluation.

Finally, as mentioned earlier, there is a growing proliferation of amateur videos as a result of the abundant accessibility to video recording equipment. Timan and Oudshoorn [31] have identified this as well, and state "Since the introduction of personal media devices, including mobile phones equipped with cameras and pocketsize photo and film cameras, public spaces are invaded by technologies that bear the potential to act as surveillance technologies." They go on to classify such video as "Open Circuit TV (OCTV)." The authors' research elaborates, "Despite the growing role of OCTV in surveillance, most Surveillance Studies still focus primarily on CCTV and other top-down technologies." Their paper then focuses on "this gap by exploring how nightscape visitors relate to OCTV cameras." In other words, the authors attempted to determine how those being recorded perceived OCTV cameras and how that feeling compared to traditional CCTV cameras. They found that respondents were generally more open to being recorded by CCTV cameras, but were more wary of OCTV cameras. In fact, the authors indicated that those surveyed felt safer due to the CCTV cameras and did not feel as though their privacy had been violated. However, the exact opposite was true for OCTV cameras. The authors were careful to mention that OCTV cameras may be accepted some places and not others, which will affect how those being recorded, perceive them. The authors conclude "By conceptualizing OCTV and CCTV as hybrid collectives that may take different shapes in different places, we may improve our understanding of the current changes in the surveillance landscape." Such a combination of available video can prove to be invaluable to law enforcement as evidenced by the recent bombing at the Boston Marathon.

6 Working Groups and Organizations

Forensic video is a growing community that has members in several international working groups:

AAFS- American Academy of Forensic Science: Within the American Academy of Forensic Science is the newly formed Digital and Multimedia

Sciences section that includes forensic video analysis. http://aafs.org/digital-multimedia-sciences

SWGIT- Scientific Working Group on Imaging Technology: an American group that has produced best practices manuals and guidelines for forensic video. http://www.swgit.org/

LEVA- Law Enforcement and Emergency Services Video Association: an American group focused on video processing and training. http://www.leva.org

ENFSIDIWG- European Network of Forensic Science Institutes Digital Imaging Working Group: A European group that focuses on methods, techniques, education and training. http://www.forensic.to/webhome/enfsidiwg

VQIPS- Video Quality in Public Safety Working Group: an American group of public safety (fire, police, medical) practitioners, Federal partners, manufacturers, and representatives for standards making bodies working to improve the way in which video technologies serve the public. http://www.pscr.gov/projects/video_quality/vqips/vqips.php

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Imaging

Review 2010-2013

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Abstract

In this review, the most important developments are presented for three general fields of expertise: (1) digital image technology, (2) facial image comparison, and (3) photogrammetry, crime scene recording and 3d-modeling.

Processing and analysis of large amounts of images has become a big problem, while development of new methods and technology progresses slowly. A lot of new methods have been reported on detection of image manipulation and the identification of cameras.

Facial image comparison has come under scrutiny as a new field of forensic expertise. In response to that methods and technology are being developed. A new scientific working group has been established for further development of this field of expertise. The introduction of new 3d- acquisition methods for face models has resulted in a number of new fields for research and development

Photogrammetry, crime scene recording and 3d-modeling. The introduction of software that can handle large point cloud data sets is expected to reduce the workload of the modelling process considerably. New hand-held scanners will change the procedures for crime scene recording. Data fusion will stimulate further developments in this field.

1 Introduction

In this review, the most important developments are presented for three general fields of expertise: (1) digital image and video technology, (2) facial image comparison, and (3) photogrammetry, crime scene recording and 3d-modeling.

This review is based on information from an extensive search in literature databases and participation in meetings organized by the AAFS, ENFSI and IAFSM, and contacts with the working groups SWGIT and ENFSIDIWG. Therefore, this review starts with an overview of the relevant organizations and their work in forensic visual evidence analysis. This review is certainly not complete for two reasons: most of the information used is obtained from European and American sources, and the scope of the review is limited to the fields of expertise that the authors have been working in or with.

Due to the amount of publications in certain fields, the authors have not retrieved and read all articles completely for making this overview. However, for most of the articles, abstracts provided by the literature database could be read and used.

2 Working groups and organizations

The development of forensic image analysis has several international working groups:

- **SWGIT**: an American group that has produced a lot of guidelines and best practice manuals. http://www.swigit.org
- **ENFSIDIWG**: The ENFSI Digital Imaging Working Group that is focused on methods, techniques, education and training. http://www.forensic.to/webhome/enfsidiwg
- **LEVA** : an American group focused on video processing and training: http://www.leva.org
- **EESAG**: an Australian-New Zealand group that proficiency tests for video and audio processing: http://www.nifs.com.au/eesag/about.html
- **AGIB**, A working group in Germany that is focused on facial image comparison: http://www.foto-identifikation.de/ .
- **IAFSM**, the international association for forensic and security metrology: http://www.iafsm.com
- **Forensic3D** an international group working on forensic applications of computer modeling: http://groups.yahoo.com/group/forensic3d/
- **FISWG**, A new American group since 2009 that is focused on facial image comparison: http://www.fiswg.org
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American Academy of Forensic Science

Within the American Academy of Forensic Science the Digital and Multimedia Sciences Section works in this field.

Since 2003 each year a workshop was organized on Forensic Image and Video processing was organized with the handouts on the methods for face comparison, video restoration, 3D reconstruction, length measurement, photogrammetry and image processing. Also each year a scientific session was organized on this field. More information is available on: http://www.aafs.org

ENFSI Forensic IT Working Group

The forensic IT working group of ENFSI handles with digital evidence as such. There exist some overlap with the Digital Imaging working group, and for that reason joint events are organized.

Since most CCTV-systems are digital nowadays, often the question of handling the CCTV system itself is a question of digital evidence. Hard drives and other digital media should be handled in a secure way with proper forensic imaging software. The working group organizes training conferences each year. More information is available from http://www.enfsi.eu/

Also many international conferences brought digital imaging as a subject. The ICMedia conference in Brazil from 18-21 September 2012 had the whole focus on this topic. Whereas many other conferences had a session in this

field, such as the BIT's Annual World Congress of Forensics in China, the Forensic Europe Expo in London in 2013, and the Euroforensics conference in Turkey.

3 Digital Image technology

3.1 Detection of image manipulation

Image and video files are changed for numerous reasons with and without a criminal intent. Images are scaled, cropped, rotated and compressed to make them fit for a document or a website. Contrast or colors are changed to enhance the visibility of details. This processing is often referred to as manipulation. However, manipulation could also refer to modification of an image with a criminal intent. One type of modification is a change of the visual content by hiding or inserting visual information in the original image. The other modification is non-visual addition of information, like a text message in an image that is published on a website as a means of communication between persons. This modification is referred to as steganography.

A number of clues can be used for detection of manipulation by visual inspection, like discrepancies in lighting, brightness levels, color distributions, edges, noise patterns and compression artifacts in the transitions between the tampered and original parts of the questioned image.. A lot of research was focused on automated detection of regions in an image that might have been tampered with [1-4,8,11,14,31-33, 43 47, 49, 57, 59-61, 66-68,94]. However, most of the methods that have been published do only produce indications of regions in an image that require inspection by an examiner.

A special type of detection is based on the clue of 'resampling' [18,25,26,34,36,55,98-102]. When a part of an image is pasted into another image, it is often necessary to apply rotation and resizing to make them visually fit. This resizing causes a special relationship between color values in the resized region that could be detected.

Double compression detection in JPEGS is also widely researched, as well as using the Photo Response Non Uniformity (PRNU) for detection [6, 24, 30, 51, 63, 71, 72, 79, 86, 89, 91, 93, 97, 105,109,110].

Another type of image tampering is referred to as 'copy-paste' forgery [29, 44, 48, 56, 61, 64, 69, 85, 96, 106, 108]. Objects or persons that are visible against a background with a specific texture, like blue air, green grass, trees, etc. are hidden by pasting a copy of a region in the image with the same texture over them. Detection of this type of tampering looks like a simple straight forward process. All regions in an image have to be compared to each other in order to find regions that are copies. However, the challenge is to limit the number of comparisons and to find a computational efficient method. This is a requirement when a large amount of images has to be checked. A relatively large number of methods have been proposed in the literature for this task.

Some research was found on methods that are based on the assumption that tampered images should have measurable characteristics that differ statistically from natural images [16, 23, 37, 40, 42, 50, 65, 70, 87, 88, 103, 104]. Also motion blur is used as a method for detecting tampered images [42].

All the methods mentioned in the previous chapters could produce evidence that an image has been tampered with, but they do not produce evidence that an image has not been tampered with.

In order to ensure the integrity of images and video files in forensic investigations, it is a good practice to compute hash codes [46, 95] for these files and use these codes as certificates of authenticity. If someone, e.g. the court, wants to verify the integrity he can compute this code and compare the result with the code in the certificate.

A related problem is the detection of illegal copies of image and video files. One technique for protection of original image and video files is the use of watermarking. A watermark is in most cases a hidden mark in the image that will get lost in most common copy processes. Although watermarking is already an old technique there is still some research going on [22].

The number of papers published on these topics show that the problem of detecting image tampering has not been solved yet. There are now a limited number of software packages available that offer a number of methods that can be tried on a questioned image.

Also anti forensics methods are described based on methods that are published, to prevent tampering from being detected. [75-77, 81-83]

Finally, some methods have been published that can be applied in very special cases: the detection of recaptured images and computer generated images [19].

3.2 Camera and source identification

In criminal investigations of child porn production and distribution, identification of the source of a digital image has become very important, because a specific camera, (or a cell phone camera, a webcam, or a flatbed scanner) could be linked to a suspect using other types of evidence. Identification of images that might have a common source can also be helpful in these investigations. One of the methods that is described in the literature is PRNU (Photo Response Non Uniformity).

In, but not limited to, criminal investigations of child porn production and distribution, identification of the source of a digital image has become very important, because a specific camera (or a cell phone camera, a webcam, or a flatbed scanner) could be linked to a suspect using other types of evidence. Identification of images that might have a common source can also be helpful

in these investigations. One of the methods that is described in the literature is PRNU (Photo Response Non Uniformity) noise.

Within ENFSI a second proficiency test of camera identification has been organized. In the last three years many validation and overview papers have been written, where several focus on improving the algorithms [1,3-5,9-10,11-15,18-22,24,26-27,30-36,37,41-42,44-51]. Also a paper on social network analysis has been published [49]. PRNU patterns can be found in document scanners as well [2, 37]. Manipulation detection with PRNU noise is possible too and is described several times [6, 16, 17]. This can be a useful way to detect image manipulation if the camera is available.

Another application of PRNU noise is determining the model of the camera according to two papers [8, 39]. The method can be made suitable for large databases [7, 25, 28]. In the meantime on sourceforge PRNU decompare has been published by students to attack the pattern. Several solutions are given to this attack [29,40]. Also camera modules have been exchanged between mobile phones, and the PRNU appeared to be the same for the camera module [58].

3.3 Image processing / Image Search

Within the field of image processing no major break troughs have been reported which can be used directly in casework without validation[1-5,7-11]. Super resolution of faces is in development, however can not be used in practice in most cases [6]. Image Search made some further enhancements in techniques[12-14], and is also implemented in commercial products for searching in similar images and searching faces in images.

3.4 Video file repair, File carving, Formats and Codec's

This review officially does not include video, however one exception is made for video file repair. One of the problems that video analysts share with digital evidence analysts is the problem of recognizing types of data and finding software that can handle the data. Video analysts look specifically for image and video data and players for viewing the data. The collaborative work that was done in the previous periods has not been stopped and progress is still being made by sharing web-based databases that provide information about CCTV systems, file formats, codecs and video players for law enforcement agencies. There seems to be a growing awareness within the law enforcement community that digital data carriers often contain partly overwritten files that could be useful in criminal investigations. E.g. a memory card or a flash memory chip in a cell phone could still contain images or movie files that are not accessible anymore for the cell phone user but that might not have been over written completely with new data. Parts of a deleted video file could be retrieved in data blocks that have to be collected and to be converted

into video files by adding appropriate header and control data. This process is referred to as file carving. Also images can be carved, in literature we see also publications on carving of JPEG-formats. [1-9]

Software for detection, retrieval and repair of the most common video files can be found on the website of the open source project *Defraser*. http://defraser.sourceforge.com

4 Facial Image Comparison

Within the context of person identification (individualization), different processes can be defined. Within different areas of science, different terminologies are used for the same process, and sometimes the same terminologies are used for different processes. Therefore, a clear definition of the different terms as used in this text is important and made explicit here.

Recall is here defined as the process of retrieving descriptive information of a person from long term memory in the absence of the person, his/her photograph or other image. Recall requires observation, retention and reproduction of a person's features. Recall is essential for the production of composite images, as produced by a police artist for investigational purposes. However, these images can only be used as investigative tools, and can never be used as proof of identity.

Recognition can be defined as the process of identifying or matching a person, his/her photograph or image with a mental image that one has previously stored in long term memory. Recognition requires observation and retention of a person's features and the process of comparison of the retained information with an external image whether it be the life person, a photograph or composite image. Recognition is important for investigation as well as witness statements. Recognition is within the forensic community also used for the automated searching of a facial image in a biometric database (one-to-many), typically resulting in a group of facial images ranked by computer-evaluated similarity.

Identification is the most contentious term because this most often used term can mean several things in different context, like the automated searching of a facial image in a biometric database (one-to-many) in biometrics, the examination of two facial images or a live subject and a facial image (one-to-one) for the purpose of determining if they represent the same person in forensics, or the assignment of class or family name in biology and chemistry. Therefore, the authors of this paper prefer not to use the term identification unless the meaning is unambiguous within the context.

Facial image comparison is defined as the visual examination of the differences and similarities between two facial images or a live subject and a facial image (one-to-one) for the purpose of determining if they represent the same person. In biometrics the one-to-one comparison is termed verification. The Facial Imaging Scientific Working (FISWG) group also uses the term Facial Identification for the same process. However, the authors of this review

prefer to use the term facial image comparison, because that exactly describes the process, and cannot be confused with the use of the word identification as used in other contexts.

Facial Reconstruction is used in two different meanings:

1) The process of reconstructing three-dimensional facial (computer) models of individuals from their 2D photographic images or video sequences.
2) The process of recreating the face of an individual (whose identity is often not known) from their skeletal remains through an amalgamation of artistry, forensic science, anthropology, osteology, and anatomy.

These two different uses of facial reconstruction may meet when three-dimensional computer models are used to recreate the face of an individual based on skeletal remains.

Facial composite is a graphical representation of an eyewitness's memory of a face, as recorded by a composite artist, also sometimes termed **facial sketch**.

Biometrics is the automatic identification or recognition of people based on behavioral or physiological characteristics.

4.1 Composite facial images from recall

In most of the criminal investigations of a crime, one of the first steps is to interview eyewitnesses. In these interviews the witnesses are asked to provide a description of the perpetrators. For investigational purposes this description may be made into an image by a (police) sketch artist. The sketch artist can also help the witness to recall the face of the perpetrator by showing multiples examples of facial features. Instead of sketches, it is also possible to create photocompositions using examples from databases with facial images. However, the authors of this review have no background in psychology and do not now in which way the memory of an eyewitness can be influenced by this procedure. .

The use of databases is a common interest for scientists that work on the production of composite images from recall and scientists that work on biometric recognition systems. However, rules on preserving privacy prevent openly sharing databases with forensic facial image data from real casework.

4.2 Facial image recognition

Biometric systems that can search databases with facial images, using automatically extracted facial features, are still being developed further. Although the performance of such systems, certainly when CCTV material is used, is generally disappointing, there is still interest from police and border

control agencies for these automated systems. One of the complicating issues is that the images of the unknown person often differ from the target images in the database with respect to the orientation of the head, the distance to the camera, the illumination and the image resolution. New approaches focus on better acquisition techniques in order to get better images, from which as many facial features as possible can be extracted for comparison to images in the database. Studies have been reported on the use of facial features like skin, asymmetry of the face and salient features like facial lines from different facial expressions [9, 10, 24, 27, 31, 32, 33, 37]

4.3 Facial image comparison

The result of facial image recognition is often the selection of 1 or more target facial images that could be matched with the image of the unknown person. In practice, however, this often leads to hit lists with multiple possible matches to the query image, and the correct target not necessarily on top of the hit list. In such cases, the decision has to be made by a forensic anthropologists or forensic image analysts. Since the previous review, more studies and proficiency tests have been reported on the performance of facial image comparison by lay people and experts, showing that there is a reason for concern, and that better methods and technology are needed. A number of institutes have published documents that describe their procedures for performing facial image comparison. These procedures show that measures are being taken to limit the influence of subjective judgments and that there is a need for quantitative statistical data. The FBI has started a working group in 2009 for facial image comparison that is expected to stimulate the development of better methods and technology (FISWG).

People doing facial image comparison can be found in four different kinds of professions: forensic photographers, forensic anthropologists, video investigators and imaging scientists. Knowledge of anatomy and physiology of the face is needed to get a good interpretation of differences and similarities in facial features. Similarities or differences in such images can often be explained by differences in the imaging conditions, pointing to the importance of knowledge about optics. Small facial details can be distorted, and artifacts looking like small details introduced due to noise, pixel sampling and compression, requiring knowledge about image processing for the proper interpretation of observations. Changes in image quality, pose and position, lighting and facial expression greatly influence the comparison process. Therefore, it is strongly recommended that one acquire reference images of the suspect and a number of other people with the same video camera in the same situation under similar lighting conditions. While guidelines and procedures have been developed for forensic comparison of facial photographs from surveillance video, it also has become apparent that these methods for identification have to be used extremely carefully [10, 19].

4.4 3-dimensional face comparison

The most promising approach to the complicating issues of pose and illumination is the use of 3 dimensional models for pose an illumination correction. Since the previous review, there has been an increase in reports on development of methods that are based on the use of 3-dimensional computer models of faces. A number of 3d-aquision systems are now available for the acquisition of these models. Most 3d-cameras work with a configuration of 1 or more normal digital photo cameras, a flash and the projection of a pattern on the face. These models can be used in two ways. A 3d-facial model of a suspect can be compared to a 3d-model of an unknown person, or the 3d-model of a suspect is used to compute an image that can be compared to an image of an unknown person. Since there are many sources of images and video in practice, a number of studies are focused on the (partial) reconstruction of 3d-models from 1 or more images or video streams. [2, 7, 28, 30]

4.5 Other biometrics

Biometrics is regularly announced in news items as a panacea against terrorism, security problems, fraud, illegal migration, etcetera. Biometrics, which can be defined as the (automatic) identification or recognition of people based on physiological or behavioral characteristics, is not a single method or technique, but consists of a number of techniques, with each their own advantages and drawbacks. None of the available biometric modalities combines the properties of an ideal biometrics system. We have to acknowledge that biometrics never can be 100% accurate. However, if requirements and applications are carefully considered, biometric systems can provide an important contribution to investigation, authentication and safety.

On top of the list of preferred, and in most travel documents required, biometric modalities is the face. The face has always been the most important personal feature on travel documents. The most important change the last decade is that the face is now also stored digitally in passport, and is optimized for automatic facial recognition. However, even with ISO/IEC 19794-5 compliant images, automated facial recognition is far from perfect. Even the best systems still show a verification Equal Error Rate (EER) of about 5%, and a False Reject Rate of around 10% at a False Accept Rate (FAR) of 1% if ISO/IEC 19794-5 compliant images are used (Phillips 2003 a,b, Phillips 2007). The automated systems are still very sensitive to ageing of the person depicted [8, 36]; the FRR may increase to around 20% at an FAR of 1% if the picture is more than 3 years old. The latest test results indicate that higher resolution and well controlled images may result in a 10-fold better performance.

Two papers on ear comparison have been published [6, 26] and another field of interest is face recognition in a virtual world, recognizing avatar faces [33, 34].

5 Photogrammetry, Crime scene recording and 3d-modeling

5.1 Photogrammetry

During the period of the previous review, a number of methods have been developed for measuring distances in images. An application is the estimation of the body height of perpetrators that are visible in surveillance video images [5,10, 15]. Recently, a study has been published on the use of distance measurements in the estimation of the speed of vehicles that are visible in at least two images of surveillance video [6,8]. A major challenge in this application appears to be the estimation of the time interval between the recordings of these images by a CCTV system. The use of this type of evidence has raised questions about the accuracy of the methods. Application of the proposed method requires the acquisition of reference images with a number of persons with different body heights or vehicles that drive by with different speeds, using the surveillance video system that has recorded the questioned images, and under similar lighting circumstances. . For identification purposes the evidence from body height measurements is not very strong, there is still demand for new and better methods.

5.2 Crime scene recording

Crime scene recording is performed for two different purposes. One is to get visual and spatial data that allows an investigator to go back to the crime scene for further examinations after the crime scene has been cleaned up and changed. This is referred to as crime scene recording. The other purpose is to get visual and spatial data for documentation and illustration purposes. Crime scene recording should be as objective and complete as possible. Panoramic image and laser scanning allow for such registrations at the cost of high volumes of data. For crime scene documentation, a map and a number of overview and close-up photographs can be sufficient, but do limit the possibilities of future re-examinations. In practice, it can be difficult to decide what techniques should be used, and decisions have to be based on assumptions about what could have happened at the crime scene. A panoramic image scanner will capture a lot of visual information but might miss important traces under a chair, while it can be very difficult to relate close up photographs of the bottom of this chair to the position and orientation of that chair in the room.

One of the new developments is data fusion, the combination of laser scan

data and photographs, including panoramic images. Another development is the handheld 3d-camera. A number of companies have demonstrated 3d-cameras that work with stereo vision or projection of light patterns in combination with special software that can find and compute coordinates of points on objects in the crime scene. The operator moves the camera around objects and the victim on the scene while the camera regularly acquires images from which new points are found and computed. This process is referred to as manual scanning. Some cameras can compute these points during the manual scan [1, 2, 9, 12]

New applications of data fusion will show up with the introduction of thermal imaging [16] and spectral imaging cameras on the crime scene. For instance, detections of small droplets of fluid with a thermal imaging camera could be directly related to spatial data from a handheld scanner.

Crime scene registration with lasers scanners in combination with virtual autopsy has proven to be a powerful combination of information for the purpose of reconstructing traffic accidents and bullet trajectories in shooting incidents. In several countries virtopsies are used additional to the regular autopsy. It is expected this will stimulate further developments in crime scene registration [18].

Geographic Information Systems in combination with geodimeters, also referred to as total stations, have proven to be valuable in the registration and documentation of large crime scenes like airplane crash sites. Coordinates of landmarks are measured relative to a base station. These landmarks correspond to places where exhibits have been found, or photographs have been taken, or laser scans have been performed. GIS-software also allows for: (1) setting up a search strategy using spatial data like maps, travel distances, soil and water characteristics, (2) planning a large scale search of exhibits and (3) documentation of the progress of an investigation. The latter application could be referred to as crime scene recording.

Video recording of a crime scene could become a competitive technique for scanners with new tools for browsing and searching in the recording and for relating the contents of the footage to 3d-reconstructions from the same footage. Speech annotations and video frames can be searched for by simply pointing in the partial 3d-reconstructoins that are made from this video using semi-interactive tools [17].

5.3 Crime scene modeling

Crime scene modeling is often performed for a reconstruction and visualization of the crime scene with positions of the perpetrators and victims on important moments as can be reconstructed from all evidence. On meetings of the IAFSM and the ENFSIDIWG, such visualizations have frequently been presented and discussed. Topics of discussion have been: the use of animations, the level of detail that is required, the shape and color of human models that represent the persons involved, the use of

photorealism, and the possibilities for interactive viewing and testing different possibilities for positions and actions (scenario testing). What is the influence of crime scene visualization on the observers in court? What is their interpretation of the visualization? In the period of this review no publications have been found on standards and best practices yet, but studies on these topics have been announced.

Software is available that allows for streaming point cloud in a way as Google Earth streams image data. This means that the viewer receives firstly data that presents a general overview of the shape of the point cloud. More details of the shape are filled in while the viewer does not move or change his field of view. The process of fitting a plane that e.g. represents a wall, to the point cloud data can be skipped. This not only reduces the workload for the modeler, but also eliminates an interpretation step that might not be necessary. This interpretation becomes necessary when e.g. the shape of a bullet hole has to be estimated from the point cloud data, or the shape of facial features in a 3d-scan of a face or a skull [14]l.

Special applications of state of the art crime scene recording, modeling and visualization are being developed within the Netherlands project CSI The Hague. http://www.csithehague.com In this project a high tech test and training facility is developed in which crime scene models are also used for training and education of crime scene investigators (serious gaming).

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Image Manipulation

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Digital Evidence

Review: 2010-2013

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TABLE OF CONTENTS

1 Introduction

This review has drawn on information from many sources. The field is characterised as moving very quickly and, in many ways, too quickly for the publication cycle of refereed journals. There are rapid developments in consumer technology that are quickly exploited by those with criminal intent. In addition, criminals continue to show themselves to be adept at adopting and adapting sophisticated technology, some of it Government funded, such as anonymous networks for criminal gain.

Due to the accessibility of the technology to consumers and enthusiasts and the high degree of specialisation within information technology/computer science, much of the very useful information for this review came from sources such as news bulletins, enthusiast magazines (mostly online) as well as technology magazines, again, mostly online and Government resourced reference materials.

It was not possible to produce an accessible review that covered every issue that arose over the review period. Selective examples have been chosen, some explored in depth, to provide the reader with a broad sense of the challenges of the field and to provide some guidance to the issues.

2 2010 Future Trends Reviewed

2.1 Virtualisation

Virtualisation is the simulation of a hardware platform, operating system, storage device or network services, that is, they are accessed from a remote source as needed. It is now commonly referred to as cloud computing.

It was predicted that virtualisation would become more common placing an additional burden on the forensic examiner. There are issues of data integrity, ie is the data retrived from remote storage traceable to the suspect data loaded there and can it be demonstrated that its integrity is maintained. Further, the location of the storage is often in a jurisdiction other than the location of the victim(s) and/or the suspects.

There is no doubt that virtualisation has continued to grow. In October 2011, Apple® launched iCloud® promoting its ability to work seamlessly with its consumer products.[1]

Dropbox promotes the ability to sync work across all devices where files are backed up, the user can return to older versions or restore deleted files. Further, and of particular issue for the examiner, attachments do not need to be included in emails as they are accessibly to the collaborator from the (remote) storage system.[2]

Google Drive[3] and Microsoft SkyDrive[4] promote similar services. All are easily available at nil or moderate cost.

There has been little investigation of data integrity for these services. Quick and Choo recently found that there was no changes to contents of files stored in three cloud services and some of the time stamp information remained the same.[5]

2.2 Investigative Management

The previous review discussed *ISO/IEC JTC 1/SC 27 Information Technology – Security Techniques, Guidelines of identification, collection and/or acquisition and preservation of digital evidence*. It is not possible to evaluate the impact of the promulgation of this standard on forensic practice. Anecdotally, it does not appear to have impacted the practice of digital evidence in the public sector. A literature search was not able to identify any proceedings that cited the standard in any way.[6]

2.3 Evidentiary Problems with Emerging Technologies

The efforts by law enforcement agencies to continue to improve the collection and seizure of electronic evidence are noted with several organisations issuing first responder guides or updates to earlier guides.[7891011]

Despite these efforts, there continue to be some, albeit limited reports of electronic evidence being compromised such as an inability to authenticate the evidence[12], and allowing the device owner to delete material that was germane to the investigation.[13]

2.4 Hand Held Devices

As described elsewhere in this review, hand held devices continue to grow in popularity, complexity and sophistication. Additional applications (apps) and software technologies are easily available to users. The challenge for the computer forensic examiner is to understand the operation of these apps in the context of alleged criminal activity.

2.5 Practitioner Welfare

The continually increasing demand for digital evidence assistance to child abuse investigations is acknowledged with capacity and capability needs being addressed.[14]There is a silence on matters of welfare for the digital evidence practitioner. Recognition of the issue is increasing but practicable initiatives are not yet prominent as the norm remains of ridiculing signs of apparent weakness or affectation.[151617] There is little else published in the past three years on this issue.

3 Statistics

Digital forensics is defined as the computer science and investigative methodology on digital evidence. Proper digital forensics is within bounds of

legal guidance, utilizing sound methods of chain of custody, tool validation, repeatable processes, notes, and presentation of evidence[18]. Digital evidence can be on laptops, desktops, mobile devices, networks, virtual and cloud environments. It can also be found within or as images, videos, audio, global positioning systems (GPS), cameras, information and entertainment systems in cars, and social media. Typically, digital evidence just needs to qualify as an area in which electronic data can be stored and accessed[19]. The most common areas for storing data are mobile devices, which is why Google collected data on the soaring usage of smart phones around the globe.

Google collected data on mobile device users during the first quarter of 2013 and published data on mobile device users during May 2013[20]. Table 1 refers. These statistics were calculated within 48 countries. The figure below shows a basic data chart from these results. Highlights include an increase in mobile usage between 2011 and 2013 by 8% in Australia, 9% in China, 11% in Israel, and 14% in Finland. Cellebrite[21], a mobile forensics tool company, provided an overview of the prevalence of mobile apps around the world. Between May 2012 and April 2013, Snapchat "snaps" increased from less than a million to 150 million per day. Between 2010 and 2013, photo sharing increased more than double among Facebook, Snapchat, and Instagram users. More users are going mobile with banking, despite the risks. With mobile apps for PayPal, Amazon, EBay, Intuit, Google Wallet, and most banks, ease can often outweigh the potential for damage. In fact, mobile devices have become almost a necessity. Users have used their mobile devices to lose weight, track sleep, and monitor heart rate. There's even apps for controlling glucose levels and taking Ultrasounds. With the dependence on technology growing as it is, one can safely state that these devices are not going away anytime soon.

Over the next four years, sales of desk based and notebook devices are forecast to decline, but more than compensated by significant increase in sales of ultramobile, tablet and mobile phone sales. In addition, sales of all major operating systems, except RIM (Blackberry) are forecast to increase.[22] Tables 1 and 2 refer.

Device Type	2012	2013	2014	2017
PC (Desk-Based and Notebook)	341,263	315,229	302,315	271,612
Ultramobile	9,822	23,582	38,687	96,350
Tablet	116,113	197,202	265,731	467,951
Mobile Phone	1,746,176	1,875,	1,949,722	2,128,871
Total	2,213,373	2,411,796	2,556,455	2,964,783

Table 1: Worldwide Devices Shipments by Segments (Thousands of Units)[23]

Operating System	2012	2013	2014	2017
Android	497,082	860,937	1,069,503	1,468,619
Windows	346,457	354,410	397,533	570,937
iOS/MacOS	212,899	293,428	359,483	504,147
RIM	34,722	31,253	27,150	24,121
Others	1,122,213	871,718	702,786	396,959
Total	2,213,373	2,411,796	2,556,455	2,964,783

Table 2: Worldwide Devices Shipments by Operating Systems (Thousands of Units)[24]

4 Silk Road (Drugs)

In the interval since the last year, the Silk Road (marketplace) launched in February 2011 and was reported in the media in June (Australia[25], United States[26], Global[27]). It is described as the Amazon of the illicit drug world.[28] As of March 2013, the Silk Road had 10,000 products of which 70% were drugs and the remainder including erotica, books and fake identities. Weapons and child exploiative material are not available. Sales have doubled in six months during 2012.[29] Sales are estimated to total $1US1.9 million per month with the Silk Road operators collecting $US143,000 per month in commissions.[30]

It is only accessible through Tor and by payment using Bitcoins (described elsewhere in this review). Anti-Forensics commentators give further as advice as to how users can avoid detection by conventional investigational and computer forensic techniques pleading with users to use full disc encryption.[31]

Although the transactions with Silk Road present difficulties for forensic examination, many users (clients, redistributers, onsellers etc) will experience a 'spill over' of their secure information to less protected locations. For example, the users, and those importing for distribution, will need to take delivery of the contraband in the real world and, if distributing to others, communicate in a less protected environment to off-load the contraband. Conventional digital evidence examination techniques can be applied.[323334]

There is a range of intervention strategies to by which Silk Road can be investigated.[35] It is not feasible nor desirable to attack the Tor network as it provides a social good and is receives funding by Government. Vulnerabilities in the financial structure, ie the payment system via Bitcoins. Bitcoins are redeemed for cash in the real world. Similarly, packages are delivered to the real world.

It appears that progress is being made against Silk Road. In a world first, the US Drug Enforcement Agency seized Bitcoins from an individual. Although documentation made no mention of Silk Road, Bitcoin bloggers were able to match the quantity of Bitcoins seized with a single transaction on Silk Road. This breach serves to shake the confidence of Silk Road users who had believed they operated within a fortress.[36]

5 Identity Theft and Stalking

Identity theft is not a new crime and is used for many purposes. It has become more sinister in recent years when used in combination with social media as a weapon. There is little, if any, authentication of identity on social media sites. Increasingly, people are this weakness to attack others, often jilted ex-lovers and partners.

Recent cases include a man who had physically assaulted and stalked his former wife. Posing as her, he posted photos of her and her children online soliciting sex on their behalf. He created advertisements online including one titled 'Rape me and my children' offering up her and her three children for sex and included their photos. Encouraged by such a title, some men tried to break into her house. Her daughter was approached by one of the strangers, but disturbed before anything happened. In her own investigations, she found false profiles in her name on Facebook and the pornography aggregator XTube. In effect, the woman and her children were threatened by death and sexual assault by innumerable strangers. There are numerous cases similar to this. Investigations include examining the social media sites for IP addresses to locate the device(s) used in the harassment, and forensic examination of the device(s) for artifacts related to the conduct of the offences.[373839]

Another avenue of identity theft is via the mobile phone. A security flaw in the SIM card running an older encryption technology can allow a third person to take control of the phone. A virus is sent to the SIM card through a text message disguised as being sent from the carrier. The network and phone verify their identities by comparing digital signatures. By sending a false signature for the network, some phones, in recognizing the false signature, sent an error message back to the hacker that included its own encrypted digital signature. This provides the hacker with enough information to derive the SIM card's digital key. Calls can then be eavesdropped, purchases can be made through mobile payments systems and the phone's owner can be impersonated. Taking over the phone can be completed in about two minutes.[40]

There is now a proliferation of spyware or phone tracking software that can be installed on all phones and tablet computers without the owner's knowledge with guarantees of being untraceable and undetectable. The software will read, track and monitor any activity of the phone.[4142434445] It is inexpensive and vendors variously advertise capabilities such as:

- Monitor children, life partners, business partners and employees
- Tracking Facebook communication
- Extract text messages
- Call logs
- Listen to live calls
- Track location
- View photos
- View live videos
- Monitors, records and logs all emails sent and received
- Records web sites visited
- 'Bug' the room in which the phone is located
- Works even if password is locked or password protected

6 Online Banking Fraud

Cybercriminals increasingly use online banking fraud automation techniques of which there are a variety of different strategies including:

- Proxy Trojans
- Man-in-the-middle
- Boy-in-the-browser
- Clickjacking

To facilitate these attacks, there is a range of agents that are employed with two of the better known ones being Zeus and SpyEye. Zeus was first identified in 2007, but has since been used by a major international network to steal approximately $70m. More than 90 suspects were arrested in the US plus people were also arrested in the UK and Ukraine.[46] Zeus can be fine tuned to target the information that the criminal is interested in, such as, log on credentials for online social networks, email accounts, online banking and other online financial services.

SpyEye is a tweak of Zeus. Instead of intercepting or diverting email messages, it hides bogus transactions even after users have logged out and then logged back into their accounts. It hides the fraudulent transaction and masks the amount of the transaction. A false balance is put forward to ensure victims are unaware that anything is wrong.[47]

Although online banking fraud is a primarily security issue for banks, on occasions, digital evidence experts will be called upon to assist with the investigation, and collection and analysis of evidence.

7 Credit Card Fraud

Five men were accused of conspiring in a worldwide hacking and data breach scheme that targeted major corporate networks, stole more than 160 million credit card numbers and resulted in hundreds of millions of dollars in losses. Financial institutions, credit card companies and consumers suffered hundreds of millions in losses, including more than $300 million in losses reported by just three of the corporate victims and immeasurable losses to the identity theft victims in costs associated with stolen identities and false charges.

The defendants allegedly sought out corporate victims engaged in financial transactions, retailers that received and transmitted financial data and other institutions with information they could exploit for profit. The defendants are charged with attacks on NASDAQ, 7-Eleven, Carrefour, JCP, Hannaford, Heartland, Wet Seal, Commidea, Dexia, JetBlue, Dow Jones, Euronet, Visa Jordan, Global Payment, Diners Singapore and Ingenicard. It is not alleged that the NASDAQ hack affected its trading platform.

The five men each served particular roles in the scheme. Two allegedly specialized in penetrating network security and gaining access to the corporate victims' systems. One allegedly specialized in mining the compromised networks; one provided the anonymous web-hosting services; and one allegedly sold the stolen information and distributed the proceeds of the scheme to the participants.

The conspiracy served to penetrate the computer networks of several of the largest payment processing companies, retailers and financial institutions, stealing the personal identifying information of individuals. They allegedly took usernames and passwords, means of identification, credit and debit card numbers and other corresponding personal identification information of cardholders.

The initial entry was often gained using a SQL (Structured Query Language) injection attack. SQL is used to manage data held in particular types of databases. Vulnerabilities in SQL databases were exploited to infiltrate a computer network. Once accessed, the defendants allegedly placed malware on the system. This malware created a back door leaving the system vulnerable and helping the defendants maintain access to the network.

The defendants are alleged to have used their access to the networks to install sniffers designed to identify, collect and steal data from the victims' computer networks. The defendants then allegedly used an array of computers located around the world to store the stolen data and ultimately sell it to others.

The card numbers and associated data were sold to resellers around the world. The buyers then allegedly sold the dumps through online forums or directly to individuals and organizations. The data was sold only to trusted

identity theft wholesalers. The end users encoded each dump onto the magnetic strip of a blank plastic card and cashed out the value of the dump by either withdrawing money from ATMs or making purchases with the cards.

Unlike traditional Internet service providers, the anonymous web hosting service did not retain records of their online activities. The group communicated through private and encrypted communications channels to avoid detection.

To protect against detection by the victim companies, the defendants allegedly altered the settings on victim company networks to disable security mechanisms from logging their actions. The defendants also worked to evade existing protections by security software. [48]

8 Car Jacking

Now, security researchers are turning their attention to the computers in cars, which typically contain as many as 50 distinct ECUs—short for electronic control units—that are all networked together. ECUs control or finely tune a wide array of critical functions, including steering, acceleration, braking, and dashboard displays. Accessing the control functions of the vehicles can be achieved through physical access by plugging hardware into a specific port underneath the dash, or through remote access via Bluetooth or cellular radio.

Among the attacks: suddenly engaging the brakes yanking the steering wheel, or causing it to accelerate, disabling the brakes. The cars' inner workings and all the code needed to make the attacks work have been documented.

The controller area network has no mechanism for positively identifying the ECU sending a request or using an authentication passcode to ensure a message sent to a controller is coming from a trusted source. False messages can be sent to ECUs to take an action such as turning the steering wheel or disengaging the brakes. The cars were commanded to jerk the steering wheel via the park assist system, even when moving at high speeds. Control was also exercised over acceleration, braking, and other critical functions, as well as ways to change readings displayed by speedometers, odometers, and other dashboard features.[495051]

9 Child Exploitation

Child exploitation and abuse, sexual slavery and trafficking continue to challenge Government agencies around the world. There are many cases that have been revealed and resolved since the last review. The Internet is fundamental to the business model as a means of generating and storing material, communicating and transmitting illicit product. Many child abuse networks have demonstrated a high degree of sophistication and organisation,

and technological knowledge to continue to operate, generate material, procure victims and abuse material, and communicate with fellow network members.

The technological approaches are described elsewhere in this review. Following is a sample case involving extensive cooperation between international law enforcement agencies that has been resolved in the past three years.

Boylovers Network[525354]

The largest child sex abuse case in history has been wrapped up after three years of investigation into the website boylover.net. One hundred eighty-four people have been arrested in Australia, Canada, New Zealand, and Europe, with 230 child victims rescued.

The investigation began back in 2007, when boylover.net came to the attention of the UK's Child Exploitation and Online Protection (CEOP) Centre. CEOP soon learned that the Australian Federal Police had independently identified and begun investigating boylover.net; the two agencies joined forces. By the time they were through, the case would also involve US Immigrations and Customs Enforcement, the New Zealand Police, Europol, and the Royal Canadian Mounted Police, with additional arrests carried out by police departments in Belgium, Greece, Iceland, Italy, the Netherlands, Poland, Romania, and Spain.

The boylover.net tried to stay legal by hosting only discussions about its members' sexual desires. But members used the site to make contact with one another, then move to private channels to exchange and share images and films of children being abused.

The boylover.net was heavily regulated and stringently policed internally. It had its own rank structure – new kid, kid, kid brother, brother, older brother, elder brother, moderator, director and owner.

Investigators infiltrated the site finding several members were involved in offline offending. While investigators were posing as site members, police also tracked down the boylover.net server to a physical location in the Netherlands. At that point, both the local Zaanstreek-Waterland Police and Europol were brought into the case, got access to the server and made a copy of its hard drive.

Europol analysts helped complete the case. In January 2010, they used a copy of the server's hard drive to rebuild the boylover.net forums offline, they then forensically examined the server for IP addresses of members. Europol then sent out 4,202 intelligence reports to police in 33 countries.

The challenge was to arrest suspects during an ongoing investigation without compromising the larger operation. Suspects came from many backgrounds with a significant number being IT professionals. One such member had

cleaned any images from his laptop, but forensic examination revealed child abuse images including those of his eight year old half brother who he was currently abusing.

Four Australian children were rescued from members of the network. One of the young boys was procured as an adoptee for $8000 as a five day old baby from another country. He began to be abused by his adoptive parents when he was 22 months old. His parents regularly travelled overseas with him so that he could be abused by other members of the network. Being IT professionals, his parents were sophisticated users of technology and travelled frequently. The lead came when a New Zealand fan of their work and fellow sex offender was arrested and his computer found to have legal happy snaps of the boy and his parents among images of child exploitation. Examination of the arrestee's chat logs and hard drive enabled a search warrant to be issued and, from the ensuing search, found a large volume of encrypted material. By this stage, the parents were in the and all material was sent to the US.

In New Zealand, six suspected paedophiles were arrested and three children were rescued. In Canada, two arrests were made. In the UK, Child Exploitation and Online Protection Centre identified 240 suspects and has been working with police to arrest them.

The boylover.net server, located in The Netherlands was examined over several months by Dutch police with the assistance of Europol and found to contain the details of 70,000 members.

Boylover.net has been closed. However, dark nets such as Tor live on. Tor users can "access and exchange child exploitation material and child pornography.[55]" Tor users abuse encryption mechanisms in order to hide from law enforcement. Silent Circle, a company that adds encryption to phone calls, is open to all clients who sign up. The owner will not comply will law enforcement to give up access to user accounts and content. This type of circumvention may discourage law enforcement, but new technologies can be used to fight back. Encryption benefits bad behavior as much as it does law enforcement. The same can be said about the Tor network. Law enforcement agencies around the world have been using Tor to gather reconnaissance on websites in order to get enough evidence for probable cause or about the skills that they may be up against.

10 Tor (formerly The Onion Router project)

Tor is free software and an open network that enables online anonymity to "…defend against a form of network surveillance that threatens personal freedom and privacy, confidential business activities and relationships…"[56]. It directs Internet traffic through a world wide network volunteer network comprising over 3000 relays to conceal a user's location or usage.

It can be used by family and friends to protect themselves; businesses to research competitors and maintain confidentiality of business strategy; activists to report abuses from danger zones; whistleblowers to report on corruption; media to protect their research and sources; and military and law enforcement to protect communications, investigations and intelligence gathering.[57]

It was first released in 2002 and originally sponsored by the US Naval Research Laboratory and its continued development has been sponsored by a range of organisations including government agencies such as the Naval Research Laboratory and community groups such as Human Rights Watch. During the review period, it was awarded for its social benefits due to its critical role in dissident movements[58] and providing a safe mechanism for whistleblowers to release information.[59] Edward Snowden used the Tor Network to send information about PRISM to The Washington Post and The Guardian in June 2013.[60]

Tor is based originally on The Onion Routing project with 'the onion' being a reference to the layers of encryption used. The original data, including its destination, are encrypted and re-encrypted multiple times and sent through a series of random selected relays. As it passes each relay, a layer of encryption is decrypted that only reveals the next relay in the series to which the remaining encrypted data will pass. The final relay decrypts the last layer of encryption sending the original data without revealing the sender to the destination.[61] When viewed from the destination, the traffic appears to originate at the Tor exit node.

Applications that are commonly anonymised on Tor include Internet Relay Chat (IRC), instant messaging, and World Wide Web browsing. It can also be used to provide anonymity to websites and other servers. A server that can receive inbound connections only through Tor is referred to as a hidden service as the IP address is not revealed, but is only known by its onion address. Only the Tor network can understand this address, even when hosted behind a firewall. As it is decentralised, there is no directly readable list of all hidden services.[62]

10.1 The Forensic Strategy

Rather than describe in technical detail how a Tor based hidden service, transaction or other activity may be subjected to forensic examination, following is a brief discussion of potential forensic strategies that might be employed.

A range of vulnerabilities to hidden services have been published before the review period. For example,

- services that are accessible through both Tor hidden services and public Internet are susceptible to correlation attack
- misconfigured services, uptime and downtime statistics and human error can all expose the service[63]

- Tor can protect against traffic analysis but cannot protect against traffic confirmation
- interception of usernames and passwords by operating and monitoring Tor exit nodes
- management of exit nodes is costly due to bandwidth and maintenance costs

The Institute National de Recherche en Informatique et en Automatique, INRIA (France's National Institute for Research in Computer Science and Control) were able to reveal the IP addresses of BitTorrent users on the Tor network.[64] It refers to a bad apple attack that exploits Tor's design and takes advantage of insecure application use to associate the simultaneous use of a secure application with the Tor address of the Tor in question. An insecure application is exploited to reveal the source IP address of a Tor user; and Tor is exploited to associate the use of a secure application with the IP address of a user revealed by the insecure application. This is significant as BitTorrent is estimated to use up to 40% of all traffic on Tor.

Further claims of compromise have been made[65] but are disputed[66].

Clearly the intent of the project is as a value to society. As will all technologies, it can be exploited for nefarious activities as described elsewhere in this paper. It has been commonly referred to as 'the dark web' or 'the deep web' and is used for activities that are illegal, varying by jurisdiction, or nefarious. For example, the Silk Road business model, referred elsewhere in this review, is reliant on the anonymity that Tor provides as does Bitcoin, again referred elsewhere. It is used to access censored information; organise political parties; criticise Heads of State; defamation; leaks of sensitive information; copyright infringement; child abuse; trade in controlled substances; money laundering; fraud; and identity theft. These activities are discussed elsewhere in this review.

11 BitTorrent

BitTorrent supports peer to peer file sharing used to transfer large files. It is responsible for 3% of total band width consumption despite efforts to control it.[67] It is estimated that there are more than one quarter of a billion BitTorrent users.

The BitTorrent protocol uses several basic computers that can replace large servers to efficiently distribute files to many recipients.[68]

To upload a file, a user creates descriptor file that they distribute conventionally. The file itself is made available through a BitTorrent node acting as a seed. Those with the torrent descriptor file can give it to their own BitTorrent nodes which, acting as peers, download it by connecting to the seed and/or other peers.

The file is divided into segments and, as each peer receives a new piece of the file it becomes a source (of that piece) for other peers, relieving the original seed from having to send that piece to every computer or user wishing a copy.

With BitTorrent, the task of distributing the file is shared by those who want it; it is entirely possible for the seed to send only a single copy of the file itself and eventually distribute to an unlimited number of peers.

Each piece is protected by a cryptographic hash so that each node can verify the authenticity of the entire file it receives.

BitTorrent is used for a range of legitimate purposes including sharing of film, video and music, broadcasting, personal material and software. Governments have used to communicate to with citizens and universities to distribute large data sets.

Pieces are typically downloaded non-sequentially and are rearranged into the correct order by the BitTorrent Client. With the files broken into pieces and reaching their destination thorugh a variety of pathways without passing through a central server, they are difficult to identify. It is therefore a useful tool for those seeking to distribute and view child abuse material without being detected.

Oak Ridge National Laboratory have developed software that looks for IP addresses associated with torrent files and the computers on which they are stored. The tool then prioritises IP addresses to be investigated based on data traffic patterns.

Rutgaizer et al[69] analysed activity measurements in the BitTorrent network and examined child sex abuse activity through a popular BitTorrent portal. They were able to identify certain characteristics such as search terms and correlate them with downloads.

Studies by Pung and Woodward[70] found that none of the six packet analysis programs tested were not able to fully reconstruct a file and most were not able to detect traffic related to BitTorrent usage. The conclusion being that computer forensic examiners must continue to rely on artifacts created by BitTorrent clients themselves in order to locate the evidence.

12 Virtual Currencies (Bitcoin)

There are a number of virtual or digital currencies including Linden Dollars (Second Life), QQ Coins (Tencent), Credits (Facebook), Liberty Reserve and Perfect Money.[7172] The one most relevant to the forensic examiner is Bitcoin because of use in illegal trade.

Bitcoin is a cryptocurrency devised in 2009, ie it is a mathematical currency that is not recognised by any Government as legal tender. Its value lies in the implied value of the exchange between the transacting parties. It can be transferred through a computer or smart phone without the involvement of any financial institution.

Bitcoin uses peer-to-peer technology to operate with no central authority or banks; managing transactions and the issuing of bitcoins is carried out collectively by the network. Bitcoin is open-source; its design is public, nobody owns or controls Bitcoin and everyone can take part. Through many of its unique properties, Bitcoin allows exciting uses that could not be covered by any previous payment system.[73] Noting the position of Bitcoin, it is a challenge to the traditional order of financial regulation with the potential to operate outside regulatory control.

12.1 Context and Regulatory issues

The United states Department of Treasury declared centralized and decentralized virtual currencies and their legal status within money service industries regulations, ie they are money service businesses and are therefore subject to regulation.[74] In effect, Bitcoin and other digital payment systems were declared virtual currencies as they are not legal tender under any sovereign jurisdiction. Further, Treasury determined that American entities who generate virtual currency such as bitcoins are money transmitters or money service businesses if they sell their generated currency for national currency.

Treasury's decision, consistent with traditional financial institutions compels money services businesses to disclose large transactions and suspicious activity, to comply with money laundering regulations, and to collect information about their customers.[75] Further, Treasury extended its anti-money laundering regulations to processors of bitcoin transactions.[76]

The US District Court for the eastern district of Texas, USA recently ruled that Bitcoins can be used as money as they can be used to buy goods and services, and exchanged for traditional currencies.[77]

The proponents of Bitcoins appear to ignore the volatility associated with the currency, a rise from $15 to $250 in four months and back to $100 in a period of hours. Yet, 10% of it has been hacked and stolen over the past two years.[78798081828384858687888990919293949596] One exchange handles most Bitcoin transactions and is therefore subject to manipulation.[97]

Cryptocurrencies, including Bitcoin, are taxable as they derive a realizable benefit therefore presenting the opportunity to defraud or evade taxation.[98]

The purpose of including Bitcoin in this review as a number of black markets or dealings in illicit goods use Bitcoin as payment to ensure anonymity. It is extremely difficult, although not impossible, to trace Bitcoin transactions to real people. For example, Silk Road (discussed elsewhere in this review)

uses Bitcoin as the sole means of transaction. It was estimated that Bitcoin transactions on Silk Road are worth $US1.9m per month.[99] Several internet sites selling weapons use Bitcoin as the sole currency promoting protection of the purchaser's identity through the entire sale process. Some will include erasure of serial numbers with refinishing, and unsuspicious and untraceable paperwork. They will also not conduct background checks on purchasers.[100]

Any investigation into the distribution and acquisition of illicit substances or illegal merchandise, fraud or tax evasion, money laundering and other manipulation of financial instruments will necessarily involve an examination of very complex digital evidence.

Following the recent raiding of Liberty Reserve (May 2013) activity on many virtual currency forums initially slowed and then picked up again. Many hackers saying they would accept Perfect Money. Liberty Reserve were accused of laundering $US6 billion over seven years.[101]

13 Operating Systems and Browsers

Every new item on the market is transient and can create issues for digital forensic examiners. Examiners need to learn these new technologies quickly while still tracking older technologies that remain in use. Most businesses are still utilizing Internet Explorer (IE) 8, but Microsoft has already advanced to version 10. Chrome, Firefox, Safari, and Opera update releases almost monthly. Windows 7 is still widely used in business, but 8 released last year. Macintosh is moving on from their cat series to surfing location themes based on their fall 2013 release of 'Mavericks'.

Examiners are able to find the commonalities among which evidence still exists. The fundamentals still apply, for example, even after deletion, Internet history and web pages may be recovered[102]. The same holds true for mobile devices. Quite often, mostly in Android, Google Chrome sign in may be enabled. This allows for bookmarks and web history to be shared between the Chrome browser on the Android phone as well as the home computer. Additionally, even if the history is deleted, remnants still exist in Java and Flash cache areas. Malware from browsing sessions may hide in AppData outside of Temporary Internet Files on Windows or Java cache on Macs. These are limited examples of how deletion does not permanently delete, even as technology changes.

Browsers work differently in how they save data from browsing sessions. One example is with Google Chrome. Not only can Google save browsing history for their own records under an individual's Gmail account, but they can send a detailed report out for your own records. Google tracks the time its users sign in and connects the user's YouTube history along with all other connected applications if the user desires. Second, as a user streams videos in Safari, they may be prompted to save small amounts (2kb) of data to their drive in the form of Flash Cache. YouTube history, Google searches, Registry typed

URLS, and other flash data may be helpful when an investigation involves browsing history deletion on a business computer. If flash data is still in tact, evidence of wrongful behaviour may exist (e.g. pornography videos viewed while at work). Of particular use in this case is

1. C:\Users\<username>\AppData\Local\Microsoft\Windows\Explorer\ to find thumbcache on a Windows 7 machine

2. C:\Users\<username>\AppData\Roaming\Microsoft\Windows\Cookies\ Low (IE)

3. %userprofile%\AppData\Roaming\Mozilla\Firefox\Profiles\<random text>.default\cookies.sqlite (Firefox).

Browser search terms may be found:

1. %userprofile%\AppData\Local\Microsoft\Windows\History\Low\History.IE5 (IE)

2. %userprofile%\AppData\Roaming\Mozilla\Firefox\Profiles\<random text>.default\places.sqlite (Firefox)[103].

These last two locations will have evidence deletion when the user clears his or her browsing history, but data can sometimes be recovered.

Lastly, Flash and Super Cookies that illustrate websites visited, user accounts used to visit the site, and last accessed time may be found on Windows 7 in these locations:

1. %APPDATA%\Roaming\Macromedia\Flash Player\

2. %APPDATA%\Roaming\Macromedia\Flash Player\#SharedObjects\<random profile id>

3. %APPDATA%\Roaming\Macromedia\FlashPlayer\macromedia.com\support\flashplayer\sys

EXAMINERs may also find use in Shell Bags to determine what folder locations were last touched on a Windows 7 machine:

1. NTUSER.DAT\Software\Microsoft\Windows\Shell\Bags useful to determine.

Additional investigation points for malware or suspicious behavior include Windows 7 registry areas, not limited to[103]:

1. C:\Windows\Prefetch

2. SYSTEM\CurrentControlSet\Control\Session Manager\AppCompatCache

3. NTUSER.DAT\Software\Microsoft\Windows\CurrentVersion\Explorer\ComDlg32\LastVisitedPidlMRU

To reiterate, deleting Internet cache will not destroy all evidence on hard drives. This is a common theme for Windows and Mac. Most items can be recovered with popular tools such as Encase[104] or FTK[105] file carving, NetAnalysis[106], or HstEx[107].

13.1 Windows 8 and Internet Explorer 10

The biggest change from Windows 7 to 8 is that the latter is intricately connected to a Windows account[108]. All of the applications are tied to this identification including: People, Mail, Calendar, and Messaging. There is no longer a disconnected user experience, even with local-only data. This is very similar to how the Windows Phone was introduced in 2010. Centralized cloud-based storage is the future of operating systems. Photos are now saved locally in the Pictures library, using SkyDrive cloud services, the Camera App, Photos App, by signing into Facebook or photography applications. Windows 8 now comes with Windows Defender heuristic detection[109]. To prove its reliability a researcher from Forensic Focus decided to test it. Ryan Fahey created a remote access Trojan (RAT). In his own words, "as soon as I sent the server file to the Windows 8 OS with an external drive, Windows Defender deleted it." This may not be the end to Windows malware, but it definitely helps the whack-a-mole approach to security that Windows has been fighting for years.

A helpful tool for examiners is the use of File History in Windows 8. It is not enabled by default, but when it is turned on, it will cache backups of everything (Library, Favorites, Contacts, etc.) to the system disk. An issue for examiners may be that Windows 8 comes with two options for encryption: the more outdated EFS and more modern BitLocker[108]. Internet Explorer 10 comes standard in Windows 8[110]. It allows for tracking protection in two different environments: the Desktop and UI. Roaming data has also changed. All favorites, history, and typed URLs are synced because of the requirement to sign into Microsoft accounts. The maximum number of values within the Typed URLs subkey has increased to 50 from 25[111]. This key is not recreated immediately after deletion of the Internet Explorer history. These deleted registry keys may be recovered using X-Ways Forensics regslack tool.

Local searching on Windows 8 has also changed. The 'Charms' menu allows for searching all files regardless of storage location. The user-agent string did change in Internet Explorer 10, but there is no difference in the string when using the desktop or UI versions. Additional security includes Enabled Protected Mode to restrict personal information gathering unless user specifically permit access. Also extremely important to note is that Internet Explorer 10 beat Chrome, Safari, and Firefox in a recent malware prevention test by NSS Labs. This test used 754 samples of malware. Internet Explorer 10 blocked malware 99.96% of the time, 83.16% for Chrome 25, 10% for Safari 5 and Firefox 19, and 1.87 % for Opera 12[112]. This is particularly

important for businesses and examiners who are in the field of malware forensics.

13.2 Windows 7 and Internet Explorer 9

Internet Explorer 9 enables InPrivate Browsing, but only per window and the tabs within that window[113]. Internet Explorer 9 still stores cookies and temporary Internet file during this browsing session, but these items are discarded after the browser is closed.

Web page history, form data and passwords, autocomplete, address bar, and super cookies are not stored during InPrivate Browsing. For now, the web traffic can be recovered from network traffic, but recoverable evidence off of a hard drive is an area that needs to be explored further. InPrivate Browsing sessions that crash will not be restored. Research involving InPrivate Browsing includes a test by Lance at Magnet Forensics. Lance used InPrivate Browsing then closed Internet Explorer. His cached files were deleted, but not wiped and were still in memory. Lance used Internet Evidence Finder, which recovered files from pagefile and unallocated. He noted that the time period that passes from time of browsing and evidence collection will affect data collection due to the nature of unallocated space. Also, the pagefile will also be affected the longer that time passes[114].

Internet Explorer 9 still allows for password management evidence[115]. Credentials may be edited or deleted. The password manager will obscure these saved passwords, but Nirsoft's WebBrowserPassView can collect and produce these as human readable text[116]. However, once the history file is cleared, this tool will not be able to decrypt the passwords. Nirsoft works with Internet Explorer, Opera, Chrome, and Firefox.

13.3 Chrome Incognito Mode

Chrome Incognito Mode works like Internet Explorer's 'In-Private' mode[117]. Files and web browsing are not saved in Chrome's history. Lance from Magnet Forensics was able to again recover data with Internet Evidence Finder. He recovered "Chrome browser artifacts, webmail, as well as the social networking artifacts" mostly within the pagefile.sys. Internet Explorer saved most of the data within memory and unallocated space, but Chrome uses a SQLite database, which means not as many evidentiary files will be found in unallocated space. Items were also found in RAM.

13.4 Mac OS X 10.7, 10.8, and 10.9

Currently, Mac OS X is at 10.7 and 10.8 at home and at businesses. The user caches are within: ~/Library/Caches/. This area contains browsing information, but also items pertaining to apps that are still installed or may have been deleted. It is not recommended to delete all of the caches because this could hinder performance. Items need to be manually deleted from this area, such as Spotify cache from streaming music[118]. Other areas of interest include Safari cache: ~/Library/Caches/Safari and Firefox:

~/Users/"USERNAME"/Library/Caches/Firefox/Profiles/"COMPUTERCODE.default"/Cache[119]. It is important to note that Mac operating systems can be infected. Flashback and Janicab are just the beginning. Traditional memory forensics and hard drive forensics will become more frequent and will need to change with the advancement of the new Mac operating system, Mavericks.

Mac OS X 10.9 Mavericks no longer utilizes a swap file to hold inactive memory[120].

MacBook Air moved to using flash storage instead of hard drives. Now, Mavericks is able to compress the memory of inactive applications, freeing up RAM without the use of swap files. This may create issues for forensic examiners who rely on inactive memory for additional evidence, such as grepping plain text and encryption keys (Cold Boot attack anyone?).

OS X 10.9 Mavericks will allow users to save Wi-Fi passwords across devices[121]. The Apple iCloud Keychain can already save passwords and credit card numbers, but will now be revamped for Wi-Fi network and password storage for a different Mac, iPhone, or iPad device under an Apple ID. The new operating system also allows for syncing calendar with maps support built in, along with location suggestions. Mac Mountain Lion 10.8 built in social interactions with Flickr, Vimeo, YouTube, Facebook, and Twitter. Mavericks will now also support a connected LinkedIn account. It is clear that the disconnected devices and apps are a thing of the past. This connected arena allows for additional data to be explored by EXAMINERs.

13.5 Google ChromeBook

Of particular interest is the Google ChromeBook. This netbook operating system has claimed to be invulnerable to infection. No downloads, installs, or executions can take place[122]. However, users can install Chrome extensions. If JavaScript is running, users will be vulnerable to cross-site scripting (XSS). This information means that the attacker could exploit any web site within the current browser. This would allow for a hijacked session. Another nuance with ChromeBook is that encryption is enabled by default. This feature can be turned off and can affect network traffic analysis. Similar to Incognito mode, ChromeBook has a guest browsing feature that 'erases' browsing history[117]. ChromeBook may not have the ability to execute programs, but that does not keep users from finding a way to do it. JSTorrent is a Chrome app that allows for streaming and downloading BitTorrent files. It can be used offline and saved to a local drive or Google Drive[123]. Lastly, ChromeBook now has VPN enabled, which may become a lure for Tor network users.

13.6 Rasberry Pi

Rasberry Pi is not a browser or an operating system, but a computer that can be used to connect to a command and control center to spy on others[124]. For $25, any person can buy this credit-card sized computer and connect them to sensors, like Wi-Fi adapters. This would allow for monitoring wireless traffic by any device nearby. If devices are not connected to a Wi-Fi network, they can

still be tracked through pings, in the same that an iPhone pings the iMessage server. Most of the information collected could be unencrypted and reveal items such as device type, applications used, and websites browsed. Traffic data such as photos and email headers may also be leaked.

The researcher claimed that anyone could spy on a neighbour, ex-spouse, a child, the Government, and more. These devices are so small that they could be placed anywhere and go unnoticed. Such acts are protected under law of most countries, but that may not stop a determined individual. Many security professionals who explore these technologies are attempting to increase awareness, but have been prosecuted because of it.

14 Mobile Devices: Security and Evidence Recovery

Apple iPhone 5 is still the latest release, but 5S is arriving September 10th with a possible fingerprint sensor for heightened security. IOS 7 should be released shortly after. Jelly Bean is the newest operating system for Android SmartPhones, allowing for 'Beaming' and restricting app usage. Android smartphones increased market share from 22.7% to 38.5% between 2010 and 2011, while tablet adoption doubled growth[125]. All of these new operating platforms come with new hurdles and risks. This chapter will explore some of these new challenges. The reader should leave this chapter understanding that the mode of data transport will change quickly. The most important thing investigators can do is research nuances in data recovery, test and validate tools for the task at hand. As always, examiners should have a range of tools in their toolkit.

14.1 Law Enforcement

Law enforcement has benefited from Dell's Mobile Digital Forensics hardware with SPEKTOR Forensic Intelligence software by Evidence Talks. This touchscreen mini-hard drive collector can be used while on scene and then analyzed on a Dell laptop where evidence is viewed, not downloaded. Each collector is forensically wiped of data prior to use. Investigators can read the collector logs to find out if other personnel wiped a collector. The tool was designed for finding evidence of sexually explicit images of children on electronic media. Typically, holders of this data will rename file extensions to mask the videos and images. This tool, like many other brands, can perform a file signature analysis. Police in Plant City, Florida collected iPhone evidence using this tool to reveal this type of incriminating evidence. This tool was also used by the Child Exploitation Online Protection (CEOP) Agency at five airports in the U.K. CEOP scanned passengers devices for illicit images of children.

14.2 Memory Collection and Encryption

Motivation for research on Windows Mobile LiveSD forensics includes a lack of data acquisition tools that can perform logical data acquisition of the

EEPROM and RAM of mobile devices[126]. Windows mobile devices utilize RAM for running processes. The operating system utilizes virtual memory to allocate resources within RAM. The researchers used an open source tool (HaRET) to replace the Windows Mobile operating system with the use of Linux Kernel. They dumped RAM, began using HaRET, booted Linux, then dumped EEPROM flash memory to find the encryption key in kernel address space (device.exe). On the Fly Encryption generates this encryption key when EEPROM is mounted. During this live forensic acquisition, it is important to take notes of any alterations made and keep the footprint as small as possible. Table 1 illustrates how LiveSD forensics leaves the smallest footprint out of 3 other tools.

Table 1
Comparison of LiveSD Forensics with other known forensic methodologies that acquire evidence from WMDs.

	MIAT-WM5	Itsutils	Paraben	LiveSD
RAM acquisition	×	✓	×	✓
EEPROM acquisition	Logical	Physical	Physical	Physical
On-device acquisition	✓	×	×	✓
Control over running processes	×	×	×	✓
Memory footprint (no. of 4 KB pages)	40	171	×	20

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Four other researchers used Linux volatile memory analysis on Android devices. They captured memory and performed volatile memory analysis. It must be noted that this type of analysis requires root privileges. The researchers reviewed the kernel's iomem_resource structure, performed physical to virtual address translation, then wrote each memory page to a file onto the device's SD card. Their success also included the use of Volatility to obtain a task list of processed and associated PIDs[127]. Another encryption hack includes that of German cryptographer Karsten Nohl who hacked a SIM card for the first time and presented his research at Black Hat. He was able to configure a hidden SMS that circumvents encryption methods. This allows the attacker to snoop or record telephone conversations or make phony purchases[128].

SafeSlinger by Carnegie Mellon University may not prevent a SIM card hack or adware, but it could exchange encrypted information during browsing, hiding data from service providers and preventing data from being written to the smartphone. This man-in-the-middle prevention would help prevent malware infections and secure communications with only trusted sources.

14.3 Applications

Most people do not have a mobile device without a messenger application. Research on such applications include the extraction of data from Viber and What's Up on Android. The researchers used Cellebrite UFED (Universal

Forensic Extraction Device) Classic Ultimate (V 1.8.0.0) on 5 Android phones with 3 different operating systems. The results are below[129]:

Table 9 – WhatsApp information for Physical Analyzer

	Artifacts Found	Artifacts Not Found
"WhatsApp" Artifacts Related Information In Physical Analyzer	Sent chats messages to every user	Contact list
	Received chat messages from every user	Profile picture of the User (If Any)
	Time Stamps of every chat session	Profile pictures of users with whom Chat Sessions were done
		Location of downloaded images or videos via WhatsApp

Researchers performed both extraction using UFED Physical Analyzer and a manual review of extracted UFED evidence. The UFED Physical Analyzer found that the What's Up App had chat message artifacts, timestamps and names of files sent and received. A manual review without the tool uncovered folder called "Avatars" in the 'com.WhatsApp\files\' folder contained the profile pictures from chat sessions.

UFED Physical Analyzer found absolutely no evidence from Viber. A manual examination found message logs and call history with timestamps.

Georgia Tech Information Security Center (GTISC) recently attended Black Hat to discuss their success in publishing a malicious iOS app called Mactans. It could secretly post tweets, take photos, send emails and SMS, and attack other apps. The researchers create the app to run off of an iPhone/iPad charger. The device would not need to be jailbroken and the app would not need to be downloaded[130].

RetailNext sells a product that allows retailers to view patterns of human behaviour using a mobile device's Wi-Fi networks setting. Even without being connected to a network, stores can track each shopper in the store. They can track repeat customers throughout the device's unique identifier.

Table-11 Artifacts found in the application

	Artifacts Found in file "Viber_data"	Artifacts Found in file "Viber_messages"
"Viber" Application Artifacts	Viber Numbers	1. Messages to Viber Users in Plain Text
	Total number of calls done by user	2. Phone No.s to whom messages were sent
	Phone No.s at which calls were made	3.Phone No.s from whom messages were received
	Duration of Calls to each Phone no.	4. Date of sent & Received messages
	Date of Call	5. Phone No. with whom conversation took place
		6. Total number of messages sent to a particular number

Lastly, RetailNext maps out the likelihood that a person will look at a display or turn right at a corner[131].

This attack is a lure using ads on apps to install malware on user devices then charge premium SMS text messages[132]. Twitter has 50,000 handles used for this alone. The mobile antivirus security company Lookout found that it uses obfuscation and encryption to hide tracks.

14.4 Applications Used for Hiding Tracks

With the increase in mobile devices comes the increase in app usage, particularly those that delete evidence. Mobile device availability varies by country. European cell phone storage capacity and camera features are about six months ahead of America, while South Korea, Hong Kong, and Japan are a year advanced[133]. With this poses problems for digital forensic examiners. There is a range of tools available from many countries that should be considered when establishing and maintaining a mobile forensic capability.

14.5 Burner

The first app of interest is 'Burner,' an app that allows the purchase of phone numbers for short-term use such as selling items on Craigslist[134]. The purchases are tracked with the provider and some data may be recoverable on iPhone devices. A python script named 'Oven Mitt' was able to find data

from the var\mobile\Applications directory files. Additional data can be found by searching for files with 'burner' in the name, such as the SQLite file (Burner.sqlite). Examiners may find configuration data under the plist file (com.adhoclabs.burner.plist)var\mobile\Applications\<varies>\Library\Preferences directory. Oven Mitt parses the Burner.sqlite file to uncover available Burner numbers in the 'ZBURNER' table. When temporary numbers expire, they are removed from this table. The creator of Oven Mitt noted that these other items of interest in this table include:

1. ZFRIENDLYNUMBER: the ZNUMBER field in a more human readable format
2. ZABOUT: The user provided nickname for the temporary burner number
3. ZCALLITEM: call records and SMS text content from expired numbers that were manually burned
4. ZDATE: The date/time of the activity
5. ZTYPE: The activity type. Options include sms, outbound_sms, outbound and call
6. ZCALLITEMTOINBOUNDNUMBER: Foreign key to the ZINBOUNDNUMBER table. This is the table that stores the phone number that calls or SMS messages were placed to or received from and is obviously a key field.
7. ZBODY: For SMS messages this field contains the content from the message. For missed calls where a voicemail is left this field contains a link to the voicemail message. I'll discuss voicemail more below.
8. ZCONNECTED: This field is '1' for sms messages or calls which connected. If a call was missed then this field is a '0'
9. ZCALLITEMTOBURNER: If no entry exists, this field will be blank. Timestamps are stored as UTC and Mac absolute time. Oven Mitt converts this into a readable format.

Oven Mitt was able to present outgoing calls placed and tracked these with a date and time, but these were not connected with the utilized phone number. Traditional iOS forensics can be used to find that temporary number in call logs. The researcher performed this analysis successfully.

Mobile Spy is a tool from Decipher Forensics[135] that can track online activity of mobile devices, text messages, GPS locations, emails, photos, and calls. According to the vendor, it can be used to track children, employees, spouses, and more. All of this is can be bought for a price per year comparable to most monthly phone rates. Peek Tab, from the same company, performs similar tasks on tablets. No research has been conducted to determine if antivirus or forensic tools would detect this spying.

14.6 SnapChat, Facebook Poke, and Wickr

The same company conducted research on SnapChat on Samsung Galaxy Note 2 and Galaxy S3[136]. They were able to recover images when connecting the phone to a computer. Any of the files with a .NOMEDIA extension were recoverable and attributed to the sender using AccessData's Forensic Toolkit.

A folder path under 'received_image_snaps' included viewed and expired images. SnapChat promises to allow users to send photos with a self-destruct timeframe of up to 10 seconds. This research was conducted on a sender's devices to recover their own images. Future research should focus on recovering deleted images on receiver devices. However, just about anyone can take a screenshot of an image using their phone, so receivers can save pictures regardless. Users of BlinkMe should be aware of the same pitfall.

Stroz Friedberg studied SnapChat, Facebook Poke, and Wickr on iPhone and Android then presented their results at Def Con 2013[137]. SnapChat for Android saved images to the phone, while iOS images were not retrieved. However, on iPhone 4 (with iOS 5 and 6), the researchers found that the 'user.plist' file contained metadata of the images, including timestamps and transcription information Samsung Galaxy S3 and a rooted S3 Mini contained a.XML file with the same information. Stroz Friedberg was able to find metadata and text content, but not images in Facebook Poke. The researchers could not uncover any evidence from Wickr and neither could researchers from Forensics Focus[138]. Forbes[139] claims that Wickr utilizes AES and RSA encryption that hides data even from Wickr's servers. Messages are not just deleted from phones, they are overwritten with characters to hide data from recovery tools such as Encase.

14.7 Tiger Text

TigerText is marketed towards businesses, such as hospitals so that doctors can send patient data encrypted through text. The site claims that the app is HIPAA compliant. However, May 2011, ViaForensics Inc. found that iPhone version 2.3.13 stored usernames, email addresses, financial data, and more. The full appSecure results were not posted for non-Developers. Fox News in Austin posted that Tiger Text can be timed to destruct between 1 minute to 30 days[140]. According to Roy Pollack, U.S. courts are allowing evidence from these phones to be presented in court for infidelity cases. This technology can also benefit business executives who don't want their text conversations to go public or aid the original intent of transferring business data securely. Burn Note is a similar app on the market.

14.8 Tinder

Tinder is an app that allows users to find people close by who they find attractive[141]. It is used for rating others as a no or a yes and can allow the users to be matched up will potential for meeting in person. However, it has serious security flaws that could leak Facebook ID, birth date, and exact coordinates of a user. The stalking possibilities are endless and quite similar to the 'Girls Around Me' app that created uproar in 2012, but was pulled from operation. The website is still active[142]. Currently, no forensic research about either app exists.

15 Network Forensics

Network investigations are becoming an increasingly important part of the work fo the digital forensic examiner. Anton Chuvakin[143] from Gartner defines network forensics as the ability to preserve and analyze evidence through full packet capture, headers, and contents. These three areas allow better views of malware delivery and intent, aiding reverse engineering techniques. They can also aid in other investigations, such as data loss or compliance violations.

Although there are many command line utilities for network forensics, this paper will only focus on an overview of industry and open source tools available for large projects and practical use for managers in the field. Additionally, caution should always be present when evaluating a tool for use. When extracting malicious files, it is imperative that the network be isolated, such as in a virtual machine with a host-only network adaptor. Additional details regarding settings and safety not presented in this paper should be explored prior to handling any raw data.

15.1 Tools

Wireshark [144] allows for packet capture (PCAP) and analysis on most operating systems. It utilizes three different views for a list of packets, details, and bytes. The bytes view would be most familiar to computer forensic professionals because it uses a hexdump style to view each frame by bytes and manual review of headers. Packet reassembling is enabled by default in Wireshark because file transfer over a network involves the transport of large chunks of data that may be segmented. File transfers are often limited to network packet size, which is affected by the window size.

Other tools may be used in conjunction with Wireshark. One includes ASCII character decoding of mail messages bodies. The SMTP protocol used in some types of mail may utilize Base64 or MIME encoding, which can be decrypted and opened utilizing the content type native application. For instance, if a company suspected that an employee was sending proprietary documents to another company and then deleting the emails, the company would want to recover the evidence. In this case, evidence preservation may not be possible through forensic imaging of the suspect's drive. Instead, investigators may want to either preserve server data or network traffic. Most business email providers, including Microsoft Exchange servers that provide email to the user application Outlook, use SMTP. This type of analysis would allow for preservation of email headers, subject, and message content.

Wireshark also allows for viewing various other network protocols that may be useful as backup when other forensic preservations methods are not available. This includes viewing HTTP traffic from Gmail, Yahoo, and Hotmail accounts. SSL traffic will need to be decrypted after filtering, utilizing a private key saved on the system. That private key may be unlocked utilizing a

passphrase to create an unprotected key file. Investigators will need to configure Wireshark to use this key.

If Wireshark cannot provide the timely response a business strives for, then Sourcefire may be the solution. Sourcefire [145] sells an SSL-Inspection appliance that can send traffic to business security teams investigating possible malicious behavior. Traffic, such as outbound Gmail messages, may be intercepted and analyzed when such behavior warrants an investigation.

NetworkMiner[146] may be used on Windows, Linux, Mac OS X, and FreeBSD. It is a passive network sniffer and PCAP tool. Its main functions include PCAP capture and analysis, file regeneration (e.g.: audio, video, executable, etc.), operating system and network detection (e.g.: hostnames, open ports, sessions), and web certificate collection. This may be used to parse Wireshark PCAPs as well. The digital forensic examiner would find the host-centric view useful as most network forensic tools utilize manual filtering rather than isolation of packet views by machine. Information is grouped by host and can be useful to disseminate information into neat collections.

Project:DINO [147] (Drop In Network Observer) from Carnegie Mellon's Computer Emergency Response Team (CERT) is used to analyze PCAP files from Wireshark or any other tcpdump. DINO extracts files using tcpxtract then creates a summary for users. Additionally, DINO uses netflow data and Google maps to depict IP origins (Figure below). The Network Situational Awareness (NetSA) group at CERT manages all updates to this tool.

16 Cloud Computing, Virtualisation and Data Remnants

Cloud computing is widely discussed from software-as-a-service to storage issues to privacy and encryption. This particular section will only delve into how cloud data may be of use to digital forensic examiners and law enforcement. A great number of businesses have leaped forward to expand storage capacity through cloud environments. So, it should not surprise anyone that criminal activity would gravitate towards these mechanisms as a way to stealthily gain persistence and steal data.

Cloud computing is the move from local maintained servers towards remote data centers with hundreds of entry and exit points. This modern shift from local data centers to cloud computing has become a way for businesses to get the storage capacity and speed they cannot handle locally. This is not without pitfalls for businesses and for digital forensics. Cloud forensics is essentially a type of network forensics because the area of compromise is one in which an examiner cannot physically examine and is typically data in transit. Cloud computing also impacts the responsibilities law enforcement officers have over evidence seizure. Remote access over the Web can create breeding grounds for misuse or theft[148]. It adds complexity to laws, such as, but not limited to: the U.S. Computer Fraud and Abuse Act, Council of Europe

Convention on Cybercrime Treaty, and Mauritius' Computer Misuse and Cybercrime Act 2003. There are additional legal and compliance issues that will be addressed later in this report. However, it should be mentioned that any type of remote hosting of data involves a loss of control and necessitates reconsideration of the corporate approach to the risk.

Without a perimeter, computer examiners have to extend their approach to collecting evidence, authenticating that evidence, attributing ownership, establishing timeframes, and securing content at a point in time. Currently, standardization of best practices is immature because the intricacies vary per case and cloud system. For instance, examiners cannot solely focus on data in transit or stored between data centers and businesses. Network forensics for cloud computing systems can involve examining all components of the cloud architecture. "Data in the cloud environment can be replicated to any data center in the world that is owned and operated by the cloud provider. **Error! Bookmark not defined.**" Additionally, cloud companies may not have access to incident data. The following cases provide insight into a handful of the types of criminal activities and problem areas with the use of cloud services.

The Forensic Research and Development Task Force in China posted a Dropbox cloud services study by Darren Quick and Kim-Kwang Raymond Choo[149]. Dropbox is a file hosting service that connects users to files remotely and at home on user profiles. Quick and Choo created twenty-eight virtual environments (VMs) running Windows 7. They analyzed the differences among four browsers with the use of Dropbox: Google Chrome, Internet Explorer, Apple Safari, and Mozilla Firefox. Each VM included a tool for erasing data while some of the environments only accessed or uploaded data without saving to the local user profile. Four VMs were used to upload, four for accessing, four for downloading, four using the Windows 7 control panel uninstall application feature, four using CCleaner, four using Eraser, and four as the controls. The researchers hashed and forensically imaged each memory file (vmem).

Quick and Choo found that the web account for Dropbox retained host data such as the owner of each computer associated with a profile, timestamps, and IP addresses. The application installs to the local machine under: C:\Users\[username]\AppData\Roaming\' folder. Merely installing Dropbox updated the registry, prefetch, and event logs. Dropbox updates the registry within the RecentDocs key within NTUSER.dat. Prefetch illustrated the execution of the Dropbox application. The firewall event logs illustrated connections from Dropbox. Group policy logs updated when Internet Explorer was used to download Dropbox data.

Viewing the VMEM data in Hex viewed offered a display of usernames for Dropbox accounts, display name or computer name, and login email if a web browser was used for logging in. Memory files were also useful for finding shortcuts, typed URLs in Registry, and browser history. RAM captures recovered additional data such as full files, usernames, and passwords. The researchers also discovered that CCleaner and Eraser did not get rid of all

Dropbox data and thus, items were recovered after deletion. A summary of the file paths and relevant artifacts may be found on Choo and Quick's Google site.

This data aids the digital forensic community when connections must be made between Dropbox accounts and users. Such a connection is helpful, such as in a case reported on NBC news on July15, 2013[150]. A group of hackers called Comment Crew utilized public shared Dropbox folders to target users and spread malware. The spear-phishing attack by Comment Crew exploited an Adobe Flash Player vulnerability, embedded within a Microsoft Word document. This document targeted users involved in commercial relations among the U.S. and some of the Southeast Asian Nations. The embedded PDF document within the Word document was an executable that copied itself onto the user's system and beaconed out to a WordPress site. The site contained instructions for encrypted communications to the next compromised site. Further research on this Trojan shows that it installs itself as a Program File and thus, updates the SOFTWARE registry key. Previous advanced persistent threats (APTs) by this hacking group include intellectual property (IP) theft and spyware.

Research regarding how cloud service providers save user data can be quite useful for investigators and law enforcement. It saves time to know where to look and what questions to ask service providers when a crime is committed. It also saves time for digital forensic examiners when they need to find evidence of malware on a victim's system. Typical malware can be detected by businesses' security operation centers (SOC). But, these same businesses may miss cloud-based malware passing through the firewall. User connections to a blog will not appear suspicious and encrypted data connections can hide future outbound data transfers.

Quick and Choo also performed a study of SkyDrive during August 2013[151]. Again, they tried to determine data remnants left behind from a cloud application and utilized anti-forensics tools to delete data usage. They accessed SkyDrive as software and from a browser. They attempted to identify, preserve, collect, and analyze all data as a typical investigation of a hard drive would allow. Quick and Choo identified and preserved virtual hard drives (VMDK files) and memory. They preserved each VMDK using AccessData FTK Imager 2.9. They were able to find usernames from cookies, memory captures, and the pagefile. Unencrypted passwords were recovered from memory. Evidence of accessing SkyDrive was found in Cookies, web history,

FavIcons, FileSlack, and unallocated space. Firefox stored usernames in the 'formhistory.sqlite'database. Chrome stored usernames in the Autofill 'Web Data' file. Full text of data files accessed were stored in System Volume Information and in memory. It is especially important for investigators to know that CCleaner and Eraser did not delete this evidence.

Jason Hale[152], U.S. computer forensics examiner from One Source Discovery, dedicated a blog to the digital forensics field. Recently, he stepped through an

analysis of Amazon Cloud Drive. Amazon Cloud Drive leaves behind artifacts that are essential to an investigation. There are no current tools on the market that can delve into Drive's artifacts, but Hale's blog goes through a brief analysis. First, he steps through how a user may utilize Amazon Cloud Drive.

A user may currently interact with an Amazon Cloud Drive in one of three ways: (1) a Desktop app on Windows and Mac OS X, (2) web browser, and (3) mobile app for iPhone and Android. Each of these locations store artifacts differently. Hale only focused on the first two areas.

Hale's analysis of the Desktop app begins after a user enters credentials. The user can then upload files to the app. Of interest is the 'ADriveNativeClientService.log' file found within Users\<user>\AppData\Local\Amazon\CloudDrive, if analyzing on a Windows 7 machine. The log tracks completed file transfers along with file metadata and origin. A database file is saved under the same AppData location and will show files in queue to be uploaded. Lastly, the investigator will be able to find data stored in unallocated space.

Hale[153] continues with his analysis by focusing on web application data saved from Amazon Cloud Drive. First, the index.dat file will be updated to reference Amazon's Cloud Drive website and will include the action completed, customer ID number and file name. The browser cache will show user actions such as upload or deletion of a file. Amazon Cloud provides both a temporary trash deletion function and a permanent deletion function. Parsing this data can be cumbersome, but provides a plethora of options based on the needs of an investigation. Investigators may harvest: "file name, object ID, amazon customer ID, file creation date, file last updated date, cloud path, file size, the file's MD5, and the type of operation (upload, recycle, or permanent deletion)." Investigators will need to decode timestamps for file creation and last updated.

Evernote is another widely used and targeted cloud product. Evernote can be used as a mobile app or web application. It is used to take text and voice notes, make to-do lists, clip articles from the web, take photos, and even distribute malware. ThreatPost[154] provided a synopsis of how Evernote was used as a command and control (C&C) server to feed instructions to a Trojan. Fortunately, this attempt was not successful. If it were, it could have dropped malicious files to obtain operating system details, user's name, computer name, timezone, and business affiliation. Because of the malware's stealth, such compromises are difficult to detect. An analysis of the user's system may provide details from cache that would recognize an additional IP and files modified or accessed which would aid in the event of such a compromise.

Another popular cloud application is Google Docs. This tool is used for saving and creating documents within the Google cloud environment. Google Docs[155] has also been used to spread malware by using the cloud service to hide C&C traffic. The malware looks specifically for Windows 8 and Windows Server 2012 and installs a keylogger to steal login credentials, copy files, and possibly use all of it for blackmail. All data transfer is encrypted by HTTPS

while sent over the network. Future research needs to focus on how cloud providers modify their security and privacy settings to avoid mapping cloud data to their local IP and machine. Cloud analysis is still a new territory for digital forensics and procedures have not yet been standardized for all environments. The increase in storage capacity that comes from switching to cloud environments also poses a significant challenge. The use of cryptography and the ability to permanently delete data also presents a challenge, therefore, tools and methods should be explored further to increase the chances of an examiner recovering useful information.

Recent research out of Austria described some major disadvantages regarding digital forensics in cloud environments[156]: large-scale storage, encryption of data prior to cloud transfer, jurisdiction and legal issues, a lack of regulatory guidance and highly dynamic and fleeting data. The major advantages mentioned in this research include successful password recovery or cracking hash of passwords within distributed cloud environments and forensically sound hypervisor investigations. Hypervisor forensics allows access to resources without altering system state unlike most live forensic acquisitions. Hypervisors aka Virtual Machine Manager is the virtual operating system that dictates resources among running processes such that data cannot get in contact with physical devices such as NICs or CPU until these resources are managed. Therefore, as long as there is access to the Hypervisor, it is probable to gather network data. This would not be possible in cases where infrastructure components are remote or if the cloud instances are turned off. Before any instances are shutdown, non-volatile data would need to be stored offline[157].

A second tactic explored in this research is the use of python scripts to obtain live memory off of virtual machines. The researchers claim that Xen is one of the most widely used hypervisors and the open source tool XenAccess can aid in memory forensics in that environment. A third point made by these researchers is that correlation of evidence across various environments is possible. Virtual Machine Introspection can be used among different hypervisors to create a common insight of these distributed systems.

Other researchers [158] attempted to gather forensic evidence from infrastructure-as-a-service cloud computing services. These researchers were successful using Encase, FTK, FTK Imager (disk and memory), Fastdump, Memoryze, dd copies, agent injection, and AWS export. They were able to acquire full memory remotely with accurate timestamps. It was suggested that digital examiners might want to also concentrate within company boundaries, such as desktops or systems that access cloud resources. Additional investigation of the cloud environment will need to be coordinated with the service provider and is usually a task for law enforcement.

It should kept in mind that data will be difficult to track once it has escaped the boundary into external processes. “Once the data has gone into the Internet or onto the cloud, network forensics becomes part of computer and systems forensics to determine which systems were connected to each other and at what time.” The investigation will require the use of firewall logs, system logs,

and network traffic. Pulling servers offline is not typically feasible and network traffic is limited to the forensics tool run at the server level. Due to the way hypervisor and virtualization systems work, investigators will not be able to collect all evidence off of the local network or isolate all compromised computers.

Digital forensic examiners will need to be familiar with virtualization with regards to how the hypervisor allocated resources such as RAM and NIC. The dynamic environment limits the amount and credibility of collected evidence. Recently, Amazon announced the use of ElastiCache, a faster in-memory cache system than of their other EC2 product. This type of cache speeds up dynamic web applications while throwing out old data when out of memory or reusing memory when needed. Investigators could be successful and can also be limited by memory forensics and network-based tools. The reliability of tools for collecting evidence in this area still needs to be explored. Parsing logs may be a better solution, but also, quite time consuming.

Cloud environments may create difficulties, but items of interest can still be found. Some cloud products cache credentials, which appear in any part of the machine a user has access to. When browser cache is left in tact, investigators may find file fragments and remnants. Some applications, like Dropbox and Mozy, have a private cache on local machines. The cache is refreshed every 3 days. This would provide the investigator with details within a 3 day period, symbolic links between local folders and online drives, and user information. Mklink is used similarly to Dropbox and Mozy. It can be used on Windows systems to create symbolic links to transfer files over HTTP to escape detection within a network. Overall, these and other cloud computing applications may leak data to other systems. It is important to note that these types of connections can be made by users and by malware. Firewall logs may aid in determining attribution of activity. Overall, cloud forensics needs to be streamlined and explored in more detail to ensure full evidence capture and admissibility of data in court. The practices mentioned here are useful for issues arriving after an incident, but prevention techniques have been explored as well.

There is at least one company focusing on the utilization of cloud as well as end-user malware prevention. Invincea[159] is providing threat data through a cloud service. Invincea's Threat Intelligence Appliance gathers data from the Web and email as a user continues working, even through an infection. Essentially, the user is segregated from saving files to his or her local drive and thus, malware behavior is viewed on the Threat Data Server. This behavioral data is used to gather information regarding infections, such as where the malware intends to install itself and where it tried to go next. The data also tracks: the infection source URL and file type, timeline, registry changes, and all connections. This type analysis is a new frontier for network forensics and investigators in general, especially in the rise of infections from legitimate websites compromised by persistent enemies.

17 Tools, Validation and Standards

There are a range of tools available to the forensic examiner with more becoming available all the time. The most appropriate tool for the job should be chosen.

Tools should be validated prior to use whether they are commercial off the shelf products, free ware obtained from the Internet or tools written within one's own laboratory. Accreditation to ISO17025 or equivalent standard requires this to be undertaken, that the tools being used are reliable when used within that organisation.

During the review period, most digital evidence tools in wide use were tested by the National Institute of Standards and Technology on behalf of the National Institutes of Justice. Around 90 tools have been tested for which reports of the testing is available and can be found at the National Criminal Justice Reference Service web site.[160] The central coordination of the extensive program of testing is an important resource for law enforcement agencies, significantly reducing the burden on each agency to validate the tools that it uses.

18 Legal Issues

18.1 International Convention

Apart from the technological challenges of digital evidence that will impact the majority of investigations encountered in all states, the other complexity results from the multi-jurisdictional nature of cybercrime. The benefits of harmonisation of laws between jurisdictions are clear with, for example, dual criminality being a precondition of both mutual assistance and extradition. Complete harmonisation is not achievable, however, for many reasons including the current priorities of Governments, development and consistency with existing laws and social norms of the jurisdiction.

The Council of Europe Convention on Cybercrime is the only multinational instrument addressing cybercrime. A number of non-European member countries were involved in the drafting and have also singed. It was opened for signature in November 2001 and came into force on 1 July 2004. As of the date of this review, the Convention entered into force in an additional 10 European states (now totalling 35) and an additional three non-European states (now totalling four). A further 12 European states, and 17 non-European states that participated in its elaboration, are yet to either sign and/or ratify the Convention.[161]

Importantly, for digital evidence practitioners and investigators, the Convention provides the expedited preservation of stored computer data as well as the real-time collection of traffic and content data. It commits signatories to adopt domestic legislative and other measures to establish

criminal offences for computer related forgery, fraud, child abuse (referred to as 'child pornography') and copyright infringement.[162][163]

It has been proposed that the United Nations consider an independent Criminal Court or Tribunal for Cyberspace to enable the global justice community to take measures on global cyberattacks against critical government and private industry information infrastructures.[164][165]

A number of states have enacted or are in the process of reviewing legislation to deal with emerging and evolving criminal activity related to computer crime or cyber crime. Some examples include India[166], Malaysia[167], and Philippines[168].

18.2 Cloud Computing

Previous reviews have briefly mentioned current issues in law and digital evidence. This review will examine issues in law and digital evidence in more depth and therefore reach back to decisions and learned papers that precede the review period. The laws concerning digital evidence in most jurisdictions has stricter requirements than those concerning other forensic sciences, eg the requirements to seize, return or destruction of exhibits following examination, legal professional privilege, liability for damage, divulging passwords, to name a few. Naturally there exist significant differences between jurisdictions and, at times, the laws of different jurisdictions are in conflict.

The growth of cloud computing is based on a business model that seeks greater efficiency of scale and better value for money than alternatives. This business driver has consequences for admissibility of evidence within the judicial environment where established standards of evidence collection, custody and preservation may have been compromised. The business of Internet and cloud computing are not restricted by jurisdiction. In addition, the forensic practitioner has a personal liability to ensure that his/her actions are compliant with the laws of the jurisdiction.

The material of this section is primarily sourced from the US and therefore primarily concerns US law. This is, in most, as most rulings on digital evidence have taken place within US jurisdictions. Where available, rulings from other jurisdictions have also been included.

Failure by digital evidence practitioners to address the legal implications of cloud and Internet forensics will have dire consequences.[169]

A forensic analysis conducted in the cloud cannot be demonstrated to be correct and repeatable when it is unclear what operating system and software was used for the analysis.[170]

Network forensics, the capture of live data in transit from one computer to another, has become well established in practice. With Internet forensics however, the investigator has control over only one end of the network and

can therefore only capture a point in time. These difficulties become greater as there are no tools that can capture data as it moves through networks apart from 'freeware' and 'shareware'. Such tools do not comply with precedent case law (Lorraine v Markel American Insurance Co) where the seized data was ruled as inadmissible as it had not been authenticated using hashing, meta-data, and the collection of data in its native format.[171]

Internet evidence is subject to the hearsay rule, but be admitted if it meets an exception of which there are many. In the United States, a court needs only to be able to infer that a document is genuine to find it to be authentic.[172] This changes in the case of an email string where each email is considered to be a separate communication and therefore subject to separate authentication and admissibility requirements.

While hearsay exceptions can be straightforward for Internet evidence, it does not follow for cloud forensics. The software and data are stored on third party servers often in another jurisdiction to the user and the investigator. Neither party has control over the data or system. This lack of control makes collection the problem with cloud based evidence as the examiner has no access to the physical hard drive nor control over the network. So, the examiner can, at best, have access to the data through the end user's web browser or through a computer connected to the same network's access.[173]

Watson, as cited by Lilliard[174], offers the following caveats concerning collection of evidence from the cloud:

1. You cannot guarantee that your forensic computer is not compromised after you have accessed the cloud and downloaded some of its contents. Forensic best practice currently sees no forensic workstation connected to the Internet for this reason.
2. You cannot trust that the view in your browser reflects the correct state of the cloud information, especially since the reply to your Web request may pass through dozens of machines before it gets to you. For example, some contemporary banking malware will steal money from your bank account but show a modified version of your statement online so that you don't spot the theft.
3. There is no guarantee that data is displayable, so you will be forced to download some data as you cannot record it from the computer display, with the attendant problems of compromised machines, difficulties with large data sets, and so on.
4. The cloud servers may give you a different view of the data from the suspect browser (for example, Amazon shows different welcome pages to different users) due to differing location, different adverts with potential malware in them, and so on.

The US Supreme Court in Melendez-Diaz v Massachusetts (2008/2009) found that notarised forensic analysts' reports without live testimony violate the right to confront a witness. Therefore the admissibility of a communication that exists only in the cloud may be subject to secure declaration, deposition

testimony or even live testimony of the author(s), the recipients(s), the data custodian , and/or the cloud provider itself.[175]

The burden of proof in most legal systems lies with the prosecution to prove beyond reasonable doubt that the accused is guilty as charged. If data has been stored in the cloud, the court must be satisfied that the data has not been contaminated. Further, a cloud service provider could theoretically store a user's data over several data centres worldwide. It is very difficult to ensure that the retrieved data presented as evidence is complete, accurate, and verifiable beyond reasonable doubt.[176]

The discovery process is impacted by the significant differences between jurisdiction in law governing access to personal data. In common law jurisdictions, the ability to obtain and the obligation to provide information are paramount. The UK Civil Procedures Rules makes forensic experts cross-examinable on their knowledge of the rules as they are now responsible and accountable for e-disclosure.[177]

Civil law jurisdictions have more restrictive approaches to e-disclosure. Some civil law jurisdictions have blocking statutes to restrict discovery by foreign jurisdictions. For example, France prohibits disclosure of certain types of information intended as evidence for foreign judicial or administrative procedures. A person who discloses such information may be criminally and civilly liable.[178]

The Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 has a comprehensive privacy framework provided by the EU Data Protection. However, within the framework, each member state has its own unique law implementing this directive. The EU prohibits the transfer of personal information of the EU residents out of the EU to the United States and the vast majority of countries around the world. The 2009 Review of the European Data Protection Directive is highly critical of the lack of international accord on data protection and the failure of rules to address ubiquitous computing environments. This scenario presents a nightmare for cloud forensics where activities might involve the transfer of data from one jurisdiction to another for data concerning personal information of EU residents, perhaps an e-mail address or employment information. All stakeholders, including investigators, should consider the kind of data they are likely to encounter in the cloud, where subjects reside, where and how data will be stored, where servers are located, the likelihood of the data being transferred, the possibility of restricting it to certain geographical areas, and the presence of an effective compliance plan.[179]

Under section 55 of the UK Data Protection Act (1998) it is now an offence to obtain personal data from data controllers without consent. Digital forensic practitioners will also be liable for prosecution under the Act.[180]

18.3 Admissibility of Evidence

In dealing with the aforementioned issues, each jurisdiction will need to develop robust best practice guides. These guides will continue to evolve over time as new information and communications technologies emerge, case law precedents are established as courts hear more matters, and laws within jurisdictions and conventions between jurisdictions are established. Some examples of guides already developed include:

- The United States Department of Justice has provided guidance on obtaining and admitting electronic evidence to attorneys. The guidance is comprehensive covering all aspects of admissibility to court officials.[181]
- The Association of Chief Police Officers has determined that the use of open source tools "…may need to be validated through limited in-house testing" and that units undertaking computer examination can create a quality management system.[182]
- The Crown Prosecution Service Digital Guidance.[183]

19 BitTorrent

Between 2010 and 2012, 200,000 BitTorrent users have been sued in mass file-sharing lawsuits. It is believed that users are offed the opportunity to settle the case for between $1500 to $3000.[184] Tellingly, in 2011, 18.8% of North American Internet traffic was used by peer-to-peer networks which equates to 132 billion music file transfers and 11 billion movie file transfers on the BitTorrent network.[185]

BitTorrent metafiles do not store file contents, therefore it is debatable as to whether publishers of BitTorrent metafiles violate copyrights by linking to copyright material without the authorisation of copyright holders. BitTorrent trackers are servers that assist in the communication between peers using the BitTorrent protocol. Several jurisdictions have taken successful legal action against websites that host BitTorrent trackers and the British High Court ordered five Internet service providers to block BitTorrent search engine The Pirate Bay.[186]

20 Future Trends

20.1 Cloud Computing and Virtualisation

Cloud computing, virtualization and shared working space for remote working will continue to grow. The business model is being promoted strongly by several service providers and customers are becoming increasingly confident. Last year, the US Department of Defense released a Cloud Computing Strategy and the US Navy is exploring the use of cloud computing for unclassified and non-mission critical information.[187][188] It can reasonably be

anticipated that business and other areas of Government will follow the same path.

The challenges are consistent with the discussion in the Legal Issues section of this document. There are significant gaps in knowledge concerning the efficacy of stored data over which the individual or business has no control, and the legal rights of access to that data in the event of an investigation.

20.2 Anonymous Networks

The battle between those engaging in criminal activity wishing to remain anonymous and law enforcement to expose them will continue. There has been some success by law enforcement to expose some very sophisticated anonymous networks, success achieved through complementarity of good police work, the application of technology and international cooperation.

20.3 Emerging Technologies

New technologies will continue to emerge particularly with the advent of more powerful and cheaper processor products. For example, Adapteva has produced a low-cost parallel chip board for Linux supercomputing.[189] While this presents additional power for those who would do evil, it also provides an opportunity for digital evidence practitioners to access additional computing power for those situations where brute might be an appropriate method. This will present challenges for the management of digital evidence units as using emerging technologies to their best advantage requires additional skills that are not in high abundance in digital evidence facilities.

20.4 Hand-held Devices

As the forecasts by Gartner [190]demonstrated, there will be a strong movement to hand held devices at the expense of the desktop and notebook devices. It can be reasonably expected that the number of apps for these devices will continue grow. The forensic examiner will need to be able to quickly grasp how the apps behave, what artifacts can be exploited for investigative and forensic purposes, and then present that evidence in court. User preferences are for faster more immediate applications and will feed that demand.

Security features are to become a standard feature of the operating systems for devices rather than the top tier models as is currently the case.

21 Conclusion

Once again, there have been extraordinary developments in the field of digital evidence. The technology companies continue to produce products that consumers desire and can exploit. The consumer is intimately involved in the development process as evidenced by the number of apps that are available.

The field constantly challenges practitioners and this is expected to continue. Continual investment in the knowledge of digital evidence practitioners must be maintained otherwise they will fall behind.

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IDENTIFICATION SCIENCES

Fingermarks and other Impressions

Review 2010 – 2013

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Introduction

The purpose of this paper is to provide an overview of the papers dealing with fingerprints and other impressions that have been published between August 2010 and June 2013. We tried to offer an extensive coverage of the published sources (mainly in English), but remain conscious that exhaustiveness is not possible. The reader will realise that the area is very active and counts with more than 470 publications. We cover here both matters in relation to the detection of marks (mainly fingermarks) and matters associated with the forensic identification process.

In introduction, we would like to highlight some important books and reports that bring an important contribution to the field and can be used as key references:

- The Scientific Working Group on Friction Ridge Analysis, Study and Technology (SWGFAST) has published in 2011 a fingerprint sourcebook available online[1] (1). This book covers many subjects in the fingerprint individualisation process. There are chapters on history; anatomy and physiology; morphogenesis; the recording of exemplars; classification systems; automated identification systems; fingerprint detection, preservation, examination methodology; documentation; equipment; quality assurance; the interaction with the law; research on individualization; abilities and vulnerabilities in this area.

- The Home Office Centre for Applied Science and Technology (CAST) published in 2012 a comprehensive source book dealing with the full range of fingermark detection techniques (2). It is an essential reference for any laboratory, especially with the prospect of the generalised introduction of accreditation under ISO 17025. It is also available online[2].

- The *Advances in Fingerprint Technology* is now in its third edition (3). Ramotowski edited a rich and exhaustive volume covering the most advanced topics from the full spectrum of detection techniques to statistical modelling and digital imaging.

- A extensive report has been written by a group of experts convened under the NIST (National Institute of Standard and Technology) and addresses human factors in latent print analysis (4). The report is also available online[3]. It not only analyses current practices and their contribution to errors, but also investigates how to reduce error and how to implement these solutions practically. Report writing and documentation is as much part of this report as pre-trial communications, working conditions (lighting, workstations, for example), education and training, and finally the role of management.

- After years of dispute in Scotland, the report of the Fingerprint Inquiry – a judicial inquiry devoted to the mis-identification of both Shirley McKie and David Asbury – has been published (5), also available online[4]. The report

[1] http://nij.gov/pubs-sum/225320.htm

[2] https://www.gov.uk/government/publications/fingerprint-source-book

[3] http://www.nist.gov/manuscript-publication-search.cfm?pub_id=910745

[4] http://www.thefingerprintinquiryscotland.org.uk/inquiry/21.html

gives a set of recommendations (86 in total) that should be considered carefully by all laboratories. Among them, we note selectively the following:

> *Recommendation 1: Fingerprint evidence should be recognised as opinion evidence, not fact, and those involved in the criminal justice system need to assess it as such on its merits.*
>
> *Recommendation 3: Examiners should discontinue reporting conclusions on identification or exclusion with a claim to 100% certainty or on any other basis suggesting that fingerprint evidence is infallible.*
>
> *Recommendation 9: Features on which examiners rely should be demonstrable to a lay person with normal eye sight as observable in the mark.*
>
> *Recommendation 53: Subject to any requirement under ISO 17025 and recommendations 50 and 51, note-taking as to the detail found on analysis and the process of comparison, though not mandatory, should become the general practice for all fingerprint comparison work.*
>
> *Recommendation 66: Before a finding of 'unable to exclude' is led in evidence, careful consideration will require to be given to (a) the types of mark for which such a finding is meaningful and (b) the proper interpretation of the finding. An examiner led in evidence to support such a finding will require to give a careful explanation of its limitations.*

In our last report (6), we reported on the increased scrutiny both by the courts and by commentators or scholars on the way fingerprint evidence was admitted and presented. During this reviewing period, we noticed a decrease of the number of challenges in court (e.g *Daubert* of *Frye* hearings). However, a few cases raised important issues both in England and Wales and in the United States of America.

In *R. v. Smith* (7), the court of Appeal of England and Wales quashed a conviction and made general observations regarding the provision of fingerprint evidence. The court was astonished by the absence of contemporaneous notes taken during the examination process, stating that “No competent forensic scientist in other areas of forensic science these days would conduct an examination without keeping detailed notes of his examination and the reasons for his conclusions.” In relation to the reports produced, the court’s decision stressed that: “The quality of the reports provided by the Nottinghamshire Fingerprint Bureau for the trial reflected standards that existed in other areas of forensic science some years ago and not the vastly improved standards expected in contemporary forensic science.” This case is echoing the issues raised in the Fingerprint Inquiry in Scotland and has triggered the UK forensic science regulator to re-think quality standard in this area (8).

In *State of Minnesota v. Terrell Matthew Dixon* (9), the court after hearing highly recognized experts from both parties ruled that the State met its burden of demonstrating that the ACE-V method of friction-ridge-print analysis is widely accepted as reliable by experts in the field and that a fingerprint expert may be allowed to testify that she framed her identification opinion “to a reasonable scientific certainty.”

In *United States of America v. Clacy Watson Herrera* (10), the court went as far as suggesting that fingerprint expert offering opinion regarding sources is akin to an art expert or similar to eyewitness testimony. The court went on saying "Matching evidence of the kinds that we've just described, including fingerprint evidence, is less rigorous than the kind of scientific matching involved in DNA evidence;" and recognized that "evidence doesn't have to be infallible to be probative" and hence declared to be admissible.

In *United States of America v. John Charles McCluskey* (11), the court concluded "that the fingerprint identification testimony, while perhaps not "scientific," is sufficiently reliable to be admitted into evidence at trial", but the expert "will not be permitted to testify that any individual is the source of a particular print "to the exclusion of all others," or that she is "100% certain" about an identification, or any variant thereof. There simply is no evidence in the record to support such a conclusion. To the contrary, the National Research Council, the FBI, and SWGFAST have all recognized the lack of scientific basis for such testimony and have advised against permitting examiners to express opinions to this level of certainty. Such a conclusion lacks a reliable scientific basis."

These few US cases testify to the developing attitude from courts to refrain from accepting fingerprint evidence as facts that could be expressed with 100% certainty or suggesting that the evidence alone is enabling the exclusion of all others in the world except the concerned individual. Legal scholars also rightly call for more humble conclusions in the area (12-14).

The 2009 report of the US National Academy of Sciences triggered some additional comments during our reviewing period. One paper attempted to find an appropriate consensus and foster a culture of research in all identification areas, including fingerprints (15). That paper was well received and judged very balanced by Bono (16). As noted by Margot in response, the research culture has to start with an appropriate academic culture with forensic science being recognized as an academic discipline (17).

Finally, we would like to draw attention to two articles that address several debates that also concern the impressions domain (18, 19). Also in a larger forensic perspective is the presentation of a project to be carried out in the Australian justice system, aiming at assessing the effectiveness of forensic science (20).

Fingermarks

1.1 Friction ridge skin individualization process

Biedermann and colleagues (21) make a strong case for the use of probabilistic statements in the forensic identification disciplines, rather than stating blunt certainties. They rightly insisted on the probabilistic nature of the endeavour. The inferential and probabilistic principles involved in matters of individualisation have been explained and updated in a short entry of the Encyclopedia of Forensic Sciences (22), stressing on the decision theory that underpins that type of conclusion. An updated overview of the standards of proofs used in various countries

has also been published (23). Broadly speaking the practice divides between countries applying a numerical standard (a fixed number of minutiae in agreement are required to declare an identification) and countries applying a holistic approach (the assessment is left to the examiner's judgement based on the whole range of available features). A call to move from the numerical standard approach towards a holistic approach when the case allows has been made in Czech Republic (24).

Two short articles covering history, current practice and the usefulness of probability models (25, 26) also highlight the usefulness of such models.

Cole (27) discusses individualization, and data supporting it, in a reaction to the report of the National Academy of Sciences (28). A reaction of the European Network of Forensic Science Institutes has also been published (29), stating the different initiatives of the EFPWG (European Fingerprint Working Group) as well as Interpol that address some of the points made in the report. Kaye discusses uniqueness, and why even a large number of pairwise comparisons do not allow proving it (14). He then suggests alternative ways of expressing conclusions, rather than individualisation. A discussion of the arguments supporting uniqueness, and of the reasons why uniqueness is unproven but also irrelevant not only to forensic identification sciences but also to the legal system has been published (30). Cole exposes the difficulties associated with new ways of testifying that experts have recently explored (31), but what is clear from the recent literature is that the days where invoking "uniqueness" as the main (if not the only) supporting argument for an individualization conclusions are over.

We cannot overemphasize the need for training and continuous education in the area, especially in the light of the recent changes that occurred and to come. The paper by Mustonen and Himberg (32) describing a novel approach developed in Finland to educate fingerprint experts is inspirational.

1.1.1 Fingerprint features

Irmak (33) suggests a link between friction ridges and Merkel cells. In a study by Kücken and Champod (34), the formation of friction ridges is modelled, linking the distribution of Merkel cells to the ridge pattern on the surface of the skin. In particular, a very small perturbation of the Merkel cell arrangement (one cell) leads to differences at the level of minutiae. This study therefore provides an explanation of the variability of friction ridge patterns due to morphological events.

A study carried out on identical twins using two matching algorithms showed that both can distinguish fingerprints from identical twins. Accuracy is lowered however when including twins rather than random non-corresponding sources. Furthermore, the probability of observing the same pattern on the prints from the analogous fingers from twins does not vary between the four fingers studied (left and right index and middle fingers) (35).

A MSc thesis analysed distortion in fingerprints (36). In particular, an objective measure of the difference between elements extracted from marks from the same source is given; this is also contrasted with observations carried out on impressions from different sources. A different type of within-finger variability, variation due to growth, has also been analysed statistically (37). First, it is shown that fingerprints

grow isotropically, and second, a model, linking fingerprint growth to overall body growth is developed. The difference observed between the modelled fingerprint and the fingerprint at the oldest recorded age are of the same order of magnitude as between a control and a rolled impression taken at the same age. Schneider reported also on the algorithmic modification required to enable matching fingerprints taken at different periods of growth (38).

Level 1 features

General pattern frequencies in the palm have been studied (39) on the basis of 499 individuals' palmprints. The frequencies with which loops, whorls, deltas and vestiges are observed are shown for the different areas of both palms. A comparison between palms of men and women didn't show any significant differences.

Several studies have been carried out on the subject of ridge density, in different populations and different papillary areas (fingers, palms), always showing that women have higher ridge density than men in a given population (40-46). Jowaheer and colleagues use different analytical methods to analyse these gender differences (47); a correct prediction rate of 90% or above is obtained depending on the analytical method used. Eshak and colleagues show in addition that women have a smaller finger width and surface, and a larger ridge count. Additional information concerning the distribution of ridge densities over the palm is given by Gutiérrez-Redomero and Alonso-Rodrìguez (43). Other studies use ridge density measures to distinguish samples of males originating from different populations (40, 44, 48).

Sangam (49) has studied the distribution of general patterns on the fingers in the general population. Also, a comparison between male and female individuals has been carried out, and statistically significant differences in ridge counts, where males had higher counts than females, have been found. Saleem and colleagues (50) published general pattern frequencies in a population from Pakistan.

General patterns in polydactyly and syndactyly cases have been described and illustrated (51). In several cases, no general pattern was observed on the supernumerary finger in cases of incomplete radial polysyndactyly (as well as on the main finger in one case). In complete radial polydactyly, many radial loops were observed on the supernumerary fingers (4 out of 7 fingers, from 2 out of 4 subjects).

The frequencies of general patterns in 100 fingerprints of poliomyelitis patients have been reported (52). The frequencies of general patterns have been analysed for the different blood groups, and significant differences in these frequencies obtained (53). A person displaying absence of papillary ridges has been described (54), as well as an overview of possible causes of such an absence. The study of the family of the initial patient with this disorder shows that a skin-specific isoform of the SMARCAD1 gene is implicated in the regulation of dermatoglyph development (55).

An anthropomorphic discussion of pattern force and its potential role in the formation of particular characteristics in the centre of whorls is proposed by Viellieux and Thornton (56).

Level 2 features

Based on a sample of 2000 fingerprints from 200 individuals from Spain, Gutiérrez-Redomero and colleagues analyse frequency data on minutiae (57). Twenty different minutia types are analysed on different areas of the finger surface, and interesting relationships between minutia type and placement on the finger, general pattern and finger number are presented. In a further study, a similar analysis is presented for two Argentinian samples, and a difference in minutiae frequencies between the Argentinian samples and the Spanish sample are demonstrated (58); differences persist when conditioning the comparison by pattern type. Therefore the difference between the populations is a difference in minutiae type frequency, not only one of pattern type. Interestingly, no differences in minutiae type frequencies were observed between the sexes in the Spanish sample, whereas such a difference was significant in the Argentinian samples (58). Taylor and colleagues reported on the spatial analysis of minutiae taken from more 1200 fingerprints (59). Using GIS (Geographic Information Systems)-based spatial characterisation, they computed density of minutiae, ratio between ridge endings and bifurcations and confirmed higher minutiae densities associated with pattern with higher degrees of line curvature and around focal points (core and deltas).

Level 3 features

Pore area reproducibility has been studied on microscopic images of fingerprints as well as on 500 dpi livescan images (60). While pore area was reproducible when several images were taken within one hour, this reproducibility was not observed between images taken on different days. Furthermore, on images taken at 500dpi, the pore area could not be measured, due to lack of resolution (60). Anthonioz and colleagues have also studied reproducibility of level 3 features, noting that the most reproducible features are pore position along the ridge and particular shapes of minutiae, while ridge edge shapes are subject to artefacts from the development methods (61). The stability over a long time (up to 48 years in the sample studied) of pores and ridge edge features, as well as the value they can add to a comparison, are discussed by Oklevski (62, 63). The relationship between minutiae and pores as well as their joint use is described in more detail in a following study (64). This second study also investigates the persistence of characteristics of the flexion creases of the palm. The finer details of the distal transverse crease are not stable over many years (but over up to 5, most characteristics did reproduce), while greater stability is observed in the proximal transverse crease and thenar crease (64).

Sex differences in the frequency as well as type and shape of pores in a South Indian population have been investigated (65). As would be expected from research on ridge density, women have a higher frequency of pores than men, while there was no significant sex difference in type and shape of pores. Similar results are presented in (66); pore type, position across the ridge, and size show no difference between genders, while pore frequency is higher for females. In the same study (66), an increase in overall size of pores with age was observed.

1.1.2 Probability models

Neumann and colleagues present a very advanced model to compute likelihood ratios (weight of evidence associated with a comparison between a mark and a print), integrating variations in annotations and due to distortion (67). Data concerning the

validation of that model is also presented. To date, this work presents the most extensive validation exercise towards a probabilistic system that can be implemented in casework. Then, two extensions to the model have been proposed: the comparison to the complete ten fingerprints of a suspect (68) as well as the possibility of taking into account general pattern (69).

Taylor and colleagues computed false-match probabilities using Monte Carlo simulations (59) on a dataset of 1200 fingerprints. As expected the probability of a false match decreases as fingerprint attributes (e.g., minutiae number, minutiae type) were added to the model and also depends on the location on the fingerprint (core, delta or periphery). Srihari and Su (70, 71) reported on the use of graphical models to represent the spatial distributions of minutiae and their dependencies. The model is used to compute the probability of random correspondence and then compute a likelihood ratio. The model has been applied to the marks and prints associated to the Brandon Mayfield case and the NIST27 dataset. Murch and colleagues (72) developed a hierarchical representation of relations among minutia and friction ridges, allowing searching rare features. They also developed a model to synthesize fingerprints.

A different model to compute likelihood ratios, based on morphometric and spatial analyses of minutiae configurations has been presented (73, 74). This model is based on the differentiation between impressions from a common source and between close non-matches, such as the impressions in a candidate list from an AFIS.

The operational use of probability models has also been studied (75) using the model developed by Neumann and colleagues (67). The marks considered were those not recovered initially (due to low quality), recovered but considered of insufficient quality for identification in the analysis stage, or marks that were compared to a fingerprint and where the conclusion was inconclusive, all in the normal course of casework. A few additional associations were found by examining a large amount of marks. While a generalized application of the model to all marks does not seem cost-efficient, some contexts where the use of such marks with a probability model is cost-efficient are highlighted. An analytical approach to the selection of marks to evaluate using a decision-theoretic framework is the subject of another study (76).

A very interesting paper details how to measure the validity and reliability of likelihood ratios, and why it is important to measure these elements (77).

1.1.3 ACE-V methodology, bias and performance

A PhD thesis (78) investigates the ACE-V methodology through different experiments, step by step, as well as addressing critical decision points of experts and testing tools to aid expert endeavour in fingerprint analysis. The mere possibility of validating the ACE-V methodology is questioned by Speckels (79).

Doak (80) stresses the importance of carrying out an analysis on the reference print as well as the mark, with particular insistence on the specific problematic of livescans. This analysis of the reference is recommended at least in the area in common with the mark.

Consistency in the analysis between- and within experts has been tested, paying also attention to the role played by the presence of a comparison print (81). The presence of the true source comparison print significantly decreases the number of characteristics annotated. Furthermore, both within- and between experts, differences (which were sometimes large) in the numbers of minutiae that were annotated have been observed. Hicklin and colleagues (82) study the process of analysis including local and overall assessment of clarity. This endeavour is based on a survey of examiners, where the participants were asked to assess the local quality of 70 marks (83). The standardisation of the process is proposed (82), and an interface presented. This interface allows to annotate local quality manually or automatically. Colour-coding different quality areas (84) or features (85) during analysis (and comparison) has been proposed. Such standardised annotations can be used operationally but also facilitate communication. The final decision in analysis, which is whether the mark is suitable for comparison or for identification, has been studied concerning biasing influences (86, 87). The presence of a matching or a non-matching comparison print influences the suitability decision. Also, the (biasing) knowledge of a previous determination may, in some situations, influence the decision made. In particular, more decisions of “unsuitable” were made when the cue given was “unsuitable”. The effects were weaker for examiners with IAI certification. Also, how these value judgements are carried out has been studied (the so-called “white-box” study) using the quantity of features annotated as well as the quality map and how these elements link to the value judgement (88). Results show that minutiae count is the best predictor of final value judgement.

A discussion of confirmation biases, first from a psychological perspective, and then in the forensic domain has been proposed by Kassin and colleagues; reforms to counter these biases are also proposed (89). This paper is followed by a rich series of discussions. The whole bundle provides a very up-to-date synthesis of the current debate on bias in forensic science and in fingerprint comparison in particular (90-100).

The impact of the candidate list proposed by AFIS, and in particular the rank of the true source, on the examiner has been studied (101). While false exclusions and false decisions of ‘inconclusive’ are not linked to the true source rank in the list, false identifications were carried out with candidates at the top of the list; in these cases, the true source was lower in the list than the erroneously identified candidate.

The eye movements of novices and experts when comparing a mark to a print have been tracked, and the similarities within each group as well as the differences between the groups highlighted (102, 103). The initial results showed larger variation in the locations visited by the eyes for experts; however, once time was constrained to 20 seconds for experts and novices, the expert group showed higher consistency.

During the analysis and the evaluation stages, tools could inform the judgment of the specialists, for example an automated quality tool, a likelihood ratio, as well as an expert consensus (104). The expert consensus and quality information positively influenced decisions, while this wasn’t the case for the likelihood ratio.

The subject of verification of all decisions is discussed by Black (105), based on the answers of different agencies to a questionnaire. Most responding agencies carried out verification for all identification decisions. However, a non-negligible percentage

of agencies also carried out verifications of exclusions (55%), inconclusive decisions (52%), as well as 'no value' decisions (36%), and some carry out reviews of the original evidence items.

The accuracy, reliability, repeatability and reproducibility of fingerprint decisions have been tested in the so-called "black box" study (106, 107). Very interesting results on the accuracy and reliability of decisions are reported in (106), detailing the percentages of decisions of identification and exclusion that indeed correspond to ground truth. A very low rate of false positives was observed (0.1%). Among the marks determined as of value for ID, examiners are unanimous on 48% of mated pairs and on 33% of non-mated pairs (on average, a pair was examined by 23 examiners). This demonstrates a certain lack of consensus. This (lack of) reproducibility was then compared to the repeatability (intra-examiner). Here, 89.1% of individualization decisions and 90.1% of exclusion decisions were repeated. Most changes of opinion were towards inconclusive decisions. Interestingly, none of the four false positive errors included in this study were repeated (107). The observed lack of repeatability and reproducibility increases with the difficulty (as judged by the examiners). Another research group in Australia (108-110) demonstrated (if that was ever questioned) that fingerprint experts outperformed novice participants in comparison tasks and in the reliability of their associated conclusions. They showed the benefits of training and experience in the area.

1.1.4 Automated fingerprint identification systems

Our review is here restricted to a small part of the papers dealing with fingerprint/palm biometric systems with an emphasis on the forensic considerations. A methodology of comparing AFIS algorithms, as well as some factors impacting AFIS performances are presented by DeJongh & Rodriguez (111). Manual minutiae annotation is superior to automated annotation, but the difference is small for fingerprints (as opposed to marks). When comparing the regions above and below the core, better performance is obtained above the core. As distortion increases, performance decreases, and while a difference in orientation of 15° (with respect to the optimum) does not degrade performance, a difference in orientation of 45° does, and with a difference of 90° the true donor was no longer in a candidate list of 50. Puertas and colleagues (112) have obtained similar results; manual minutiae extraction yielded better results than automatic for marks; however, the manual annotation for only 12 minutiae (rather than all visible minutiae) yielded results that were inferior to automatic extraction. A case study explains the reason for a miss (no hit declared when the donor was in fact on the database) on an AFIS search (113).

Different articles address the need for image databases for research. A method for creating large numbers of simulated marks in order to test AFIS or to develop probabilistic models has thus been described (114). Park and colleagues propose a digital library for testing algorithms, including services to experiment and analyse, in particular, fingerprint matching algorithms (115). An open-source biometric repository, containing, amongst others, simulated crime-scene fingermarks with a known source is described by Tear and colleagues (116).

A case report on the possibility to increase the size of fingerprints by 20% for an automated search of a child's fingerprints against a database has been published (117). In the case report, a hit has been obtained in this way between the increased

marks and a set of inked prints taken at adulthood. Another case report concerns the AFIS search of charred prints (118). On a charred body, prints of the funnel area of the palms and the right thumb could be recovered, casted with Mikrosil and submitted for searching in an automated system. No hit was obtained. After identification through other means, it was observed that the prints obtained from the corpse were much smaller (2/3 of the size) than the previously obtained reference material due to tissue shrinking.

Dominick and colleagues (119) investigated distortion due to heating of the substrate (uPVC) and its impact on AFIS searches. Searches were carried out before and after heating and the ratio between the first (true source) and the second score in the list was used for evaluation. Out of 50 unheated fingermarks, the true source was the first candidate in the list for 32. Out of the remaining 18, none matched its donor in first rank in the subsequent, post-heating search; no improvement was therefore obtained with the heating. After heating, when the original score ratio (between the first and second candidate on the list) was above 2.2, the post-heat mark almost always hit in the first rank as well, which was not the case otherwise. Horizontal distortion was more deleterious to AFIS results than vertical distortion. Overall the heat-distorted fingermarks were matched by the automated system correctly in 64% of cases.
Improvement of matching by the use of simultaneous impressions through the integration of the match score of the individual marks of a simultaneous impression into a single result has been tested successfully (120).

Matcher improvement is also the subject of a study on overlapping impressions (121). Additional information from an examiner concerning one of the overlapping impression, in particular the region of interest, singular points as well as pointers (“orientation cues”) help to construct an orientation field for the mark in question. This in turn aids better separation, leading to improved matching performance.

Jain (122) treats the integration of an extended feature set into a search algorithm. The recommendations include to integrate level 1 and 2 extended features (ridge quality map, ridge flow map at level 1, and ridge skeleton at level 2), and to improve the quality of reference prints in order to be able to use level 3 extended features. An improvement of search results when including manually marked features in addition to the image has been reported by NIST (National Institute of Standards and Technology) (123, 124). Improvements were also reported when integrating pore features into final match scores (125); however, at least in some conditions, this improvement is very small (126). Different matchers are assessed concerning their usefulness for mark-to print matching, for example on a crime-scene, and the NFIQ algorithm is tested in order to verify whether it can be used for marks. While the matchers were fast enough for use on a crime scene, error rates were too high. Also, the NFIQ algorithm is not appropriate for the assessment of latent mark quality (127).
Several articles specifically address the question of the matching of latent fingermarks (128, 129) and of latent palm-marks (130, 131). Matching under distortion conditions (132) and matching when bad quality areas are present (133) have also been presented. Ground truth labelling, in particular of the matched minutiae, has been carried out in the NIST SD27 database, and performances of two matchers on this database are reported (134). One study investigating the performances obtained when matching latent to latent marks shows that there is room for improvement in this task (135).

1.1.5 Quality assurance and integration into the legal process

Bertram and colleagues (136) establish a link between form blindness and underperformance in fingerprint comparison, even after controlling for various other variables. This link indicates that form blindness testing could be used in the recruitment of future fingerprint trainees. Concerning the education of forensic scientists, Houck and Boyle carry out a content analysis of 9 books on fingerprints. This allows defining a certain number of subjects as well as the order in which they are treated, and use these recurring subjects as a basis for the establishment of a fingerprint curriculum (137). A standard for fingerprint individualisation (without a numerical standard) has been advocated, and one such standard has been articulated (138).

The question about how well non-specialists, e.g. the jurors in the U.S. legal systems, would understand testimony on fingerprints given in a probabilistic form has been addressed (139). A mock trial has been set up at a meeting of the International Association for Identification, and mock jurors (not fingerprint specialists, but people from the general public) assisted to testimony given on a fingerprint comparison where the result was expressed in the form of a likelihood ratio. Jurors understood the testimony rather well, and integrated the result into their reasoning. Some jurors, however, felt they did not understand the testimony, or that it was particularly useful. Also on the subject of testimony, Eldridge (140) discusses a Daubert hearing where issues mentioned in the NAS report (28) were cited by the defence. She details questions asked as well as the answers given.

Daubert challenges of forensic identification evidence types have been counted and classified; 176 such challenges (almost a third of all challenges to forensic identification evidence) concerned fingerprints, and in 12 of those cases the evidence was excluded or limited (141). The reasons behind exclusions or limitation of forensic identification evidence are exposed in a second contribution (142). These reasons are reliability issues (57% in fingerprint testimony) which include the lack of a demonstration of reliability in the case at hand, insufficient documentation, existence of observer bias, unrealistic proficiency testing and implausible error rates. Swofford (143) exposes the legal validity of fingerprint individualisations, as well as their scientific reliability in a rebuttal to some challenges.

On the issue of communication, Found and Edmund detail the contents of a report in the pattern evidence domains (144). The different parts such a report should contain are stated, and an indication of the expected content is given. How to report inconclusive results is discussed by Maceo (145), in particular the fact that the reason for the inconclusive result should be clearly stated.

The CSI effect has been investigated with two surveys; this effect, as commonly understood, is not found in the results, although in certain cases, jurors expected and put weight on forensic evidence (146).

1.1.6 Fingerprint forgery and alteration

Several publications about fingerprint fabrication but also on fabricated marks are available for this period of review. Their focus ranges from spoofing using ‘gummy

fingers' of biometric readers to printed marks (using amino acids) left on crime scenes.

The scores obtained from an automated system using a livescan device and fake fingers as well as genuine ones from 12 subjects show that there is a lower score obtained for fake fingerprints. However, these lower scores are detected most easily in relation with the score obtained for the real finger. Finally, fakes obtained by a cast moulded directly on the finger (rather than from a latent mark) yield higher scores (147). Factors explaining successful spoofing attacks on a multispectral livescan sensor providing liveness detection have also been evaluated (148). Fakes created from direct moulds yielded higher success rates; other factors were the impostor, in particular the correspondence in general pattern between the impostor and the genuine finger; also, the number of uses of the mould was limited, and the ridge thickness of the genuine subject also played a role in the number of successful spoofing attempts. The direct impression of amino acids using an inkjet printer, and how to automatically detect false fingermarks created in this way as well as describing their visual properties, is the subject of several studies (149-152). For quality assurance purposes, the reproducibility of such printed impressions is also assessed (153).

Whether marks can actually be transferred is explored in a series of two articles (154, 155). Marks are powdered, lifted, the lift is applied to a clean surface and the mark powdered again. Transfer is possible, however, marks show halos due to the residue of the lifter surrounding the transferred mark. The transferred marks nevertheless show a large amount of features.
The detection of whether a finger presented to a livescan device is alive or not has been carried out using spectral analysis between 400 and 1650nm (156). Differences in spectra are observed between a) live and dead fingers, in particular dynamically as the live fingers show a 'blanching' effect when pressure is applied, and b) live and fake fingers. The fake finger surfaces used were not transparent.

The deliberate alteration of a subject's fingerprints, so as not to be detected, is the subject of a study by Yoon and colleagues (157). Case studies are carried out and a classification of alterations into 3 classes is proposed. Then, the impacts on matchers of these alterations as well as a way to detect them, going further than a standard quality detection algorithm, are described. A case study on the question of alteration has also been published (158), where a person had altered fingerprints, due to the application of a chemical; this chemical remains unknown, however.

1.2 Composition, aging and persistence of fingermarks

Two reviews about the topic of composition and aging of fingermarks were recently published (159, 160). Some studies specifically focused on the composition of fingermarks in terms of amino acids (161), lipid composition (162-164), age of the donor (165), as well as exposition to environmental conditions (166) or vacuum (167). A fingermark sampler (168, 169) and a modified dispensing device (170) were proposed to reproducibly leave marks or spot tests on substrates. Many studies tried to link the composition of the secretions (and their evolution with time) with the efficiency of some detection techniques (163, 165, 171-174), or with

the age of the marks (175-182). All other articles using the composition of fingermarks for imaging purposes (or to detect contaminants) are described in "2.4.2. Chemical Imaging".

Used acronyms: CE-MS (capillary electrophoresis – mass spectrometry), CWL (chromatic white light), GC-MS (gas chromatography – mass spectrometry), NIN (ninhydrin), PVC (polyvinyl chloride), UV (ultraviolet), VMD (vacuum metal deposition).

Reviews - An updated overview of the composition of the secretion residue encompassed qualitative and quantitative data about fresh and aged marks, as well as the influence of numerous factors (i.e. donor, deposition conditions, substrate, environmental exposition and detection techniques) (159). In another publication, a thorough intercomparison between numerous analytical techniques illustrated how such techniques could be used to analyse and characterize secretion residue (160).

Amino acids - The identification and quantification of amino acids in latent fingermarks has been performed using CE-MS analysis (161). Twelve amino acids were identified in the analysed secretion residue, among which nine were quantified; the resulting relative abundances being consistent with previous studies in the field (e.g. serine and glycine as the most abundant amino acids). The authors also discussed the advantages and limitations of CE-MS compared with GC-MS. The relationship between palmar moisture and "quality" of the donor in terms of latent fingermarks has been explored (172). If most of the donors are considered as "average", there are always people being known as "poor" donors or "excellent" ones, when considering a detection technique (NIN in this case). Almog *et al.* showed that the palmar moisture level was not the main factor influencing the donorship for amino acid reagents (for example, some "excellent" donors had dry hands whereas some "weak" donors had moist palms). The authors hypothesized that the main factors influencing the quality of the marks were most likely the amino acid concentration in sweat, the density of pores, and the contact pressure. When studying the origin of the auto-fluorescence of fingermarks, which may be observed under UV for some marks, Lambrechts *et al.* concluded that tryptophan (if included in a protein sequence), its metabolites (e.g. kynurenine), and pheophorbide A (a decomposition product of chlorophyll) could play a role in the phenomenon (171). Further research is recommended, though.

Lipids - The "surface lipids" present in the external layer of fingermarks were analysed, especially the triacylglycerols, to study the influence of the gender or the use of cosmetics (162). No gender specificity has been emphasized, as confirmed by another study which concluded that the lipid composition does not vary significantly as a function of the age or gender of the donor (163). In the same study, Fritz *et al.* observed that the greatest loss of material appears during the first 3 months after deposition. Beyond this point, no significant variation in lipid composition was detected over a 9-month period. This could constitute an element in favour of the detection techniques targeting lipids. In a third study, it has been shown that the ratios of several fatty acids (and their corresponding methyl esters) were found to vary significantly between individuals of different race and gender (164). However, the authors recognized the limits of their study, especially too small a sample size.

Children - A study aimed at determining the fingermark composition of children (ranging from 2-year-old to 11-year-old) and showed that the carboxylic acid salts fraction was more stable than the esters one (165). The study also confirmed that the composition of children's marks differs from adult's ones in terms of relative ratio between the main components (i.e. carboxylic acid salts, esters, and proteins). The authors recommend adapting the detection techniques to target such components, so that children marks could be efficiently detected.

Aging - A study focused on the determination of specific patterns of degradations over time when marks are exposed to various environmental conditions (e.g. temperature, relative humidity, air currents, composition of secretions, exposure to daylight, type of substrate) (166). Titanium dioxide powdering was chosen to assess the quality of the "altered" marks. Some conclusions were expected, such as a better preservation on glass rather than on plastic, as well as a greater resistance of sebum-rich marks compared to sweaty ones. The authors somewhat observed that marks exposed to direct sunlight (indoors) degrade similarly to those kept in the dark, where environmental conditions are more constant. This last observation is contrary to the commonly accepted evolution pattern. The exposition of fingermarks to vacuum conditions was also studied (167). It was shown that fingermarks lose ca. 26% of their mass when exposed for one hour to vacuum conditions, which is equivalent to ca. 5 weeks of ageing under ambient conditions. If the exposition to vacuum persists, a significant loss of lipids (e.g. tetradecanoic and pentadecanoic acids) is observed, which is not the case when ageing occurs under ambient conditions. This could have an influence on the efficiency of detection techniques relying on the lipidic fraction of the secretions, especially if applied after a technique requiring vacuum conditions (e.g. VMD).

Age determination - In an attempt to assess the age of a mark based on its composition, Koenig *et al.* analysed the wax esters contained in secretion residue and identified seven for being present in most of the studied samples (175). The authors defined ratios (including wax esters, squalene and cholesterol) to try limiting the variability for a same individual and introducing a new method to determine the age of a fingermark (176). Another strategy aiming at determining the age of a fingermark is based on the use of a contactless CWL sensor (177-182). The main factors of influence were determined to be: sweat composition, temperature, humidity, wind, UV-radiation, surface type, contamination of the fingertip with water-containing substances, scan resolution and measured area size. Contact time, contact pressure and smearing of the mark were determined to be of minor importance.

Persistence - Sebaceous marks left on PVC shutters and white-painted aluminium frames were shown to resist smearing and scraping attempts, and could still be further detected by powdering (173). The authors hypothesized that the coating process (e.g. hydrophobization agents or plasticizers) could be the reason of this enhanced resistance of the marks. The same authors also showed that such marks could survive the use of cleaning agents, since only 2 brands (among 6 tested) were able to remove the marks in presence (174).

Sampling - A fingermark "sampler" has been proposed as a way to maximize the deposition of comparable marks, by controlling the applied force, the angle and area of contact, as well as the time of contact (168, 169). This way of doing may improve

the reproducibility and consistency of marks left when developing a new detection technique or comparing its efficiency with existing methods. Another strategy consists in using a micro-dispensing device to "print" viscous material (such as artificial sebum) and prepare artificial fingermarks (or spot tests) on various substrates in a repeatable manner (170). It should be noted that the artificial sebum used in this study encompass 10 components, among which olive oil (20%), jojoba oil (15%), coconut oil (15%), as well as oleic acid, paraffin wax, and palmitic acid (10% each) for the major constituents.

1.3 Fingermark detection using chemical or physico-chemical processes

This chapter is structured according to the reagents, the nature of the substrates, or the scenario (e.g. arson scenes). In addition to the detailed sections, the following contributions constitute recent reviews that can serve as good starting points for readers not accustomed to the range of methods available in the field of latent fingermark detection (183) and forensic science in general (184). Recent developments in fingermark detection received also attention in China (185).

When reading the contributions of these last three years, we observed a great versatility in the fingermark sampling protocols, if described by the authors. These information should encompass the nature of the secretions that were used (i.e. natural/non-enriched, eccrine-rich, or sebum-rich), the number of donors as well as of fingermarks per donor, the time between the deposition of the fingermarks and their processing, and if depletion series were considered. Some protocols were pretty close to realistic conditions (not necessarily casework-like), while other considered far-off realistic conditions. For example, most of the chemical imaging studies are based on highly-enriched marks obtained by touching pure contaminant powder with sebum-rich fingertips before leaving a mark. This observation emphasizes the fact that the sampling protocol is a key-element to be smartly designed since it eventually influences the possibility to compare experimental results between researchers and to operationally implement a new technique. In this context, the publication of Sears *et al.* (186) constitutes a useful guide to help standardizing the sampling protocols. However, for easiness of reading, we decided not to put the focus on the nature of the samples in this report, but rather on the conclusions made by the different authors. The readers must keep in mind that some conclusions may be obtained from idealized or non-realistic samples. Another topic of research deals with the objective assessment of fingermark quality (post-detection). In this context, a relative contrast index model is proposed, using measures carried out with a microspectrophotometer on the ridges and in the valleys (187). The integration of quality levels implemented in ULW (universal latent workstation) before and after the detection of the fingermarks represents another assessment method (188).

1.3.1 Amino acid reagents

Ninhydrin has been used as a tool to evaluate fingerprint donorship (172). On a more practical aspect, acetone applied on ink to improve ninhydrin-developed mark is described by Coughlan (189). The combination of 5-methylthioninhydrin and zinc chloride is evaluated (190). Only one occurrence of DFO has been found, regarding a transfer of DFO-treated fingermark (191). Reaction mechanisms of 1,2-indanedione with amino

acid are studied in details (192, 193), as well as the effect of zinc and europium chloride on the luminescence (194). 1,2-indanedione is also used to evaluate the effect of postal distribution process on the recovery of fingermarks (195). Other amino acid reagents like naphtoquinones (196) and p-dimethylaminobenzaldehyde (197) are studied. Finally, comparison studies between various amino acids are proposed, mostly to evaluate 1,2-indanedione or 1,2-indanedione/zinc chloride performances (198-200). Its application on thermal papers is described in "2.3.7. Substrate – Thermal papers".

Used acronyms: DFO (1,8-diaza-9-fluorenone), DMAB (p-dimethylaminobenzaldehyde), IND (1,2-indanedione), IND/Zn (1,2-indanedione/zinc chloride), 5MTN (5-methylthioninhydrin), NIN (ninhydrin).

Ninhydrin and analogues - NIN has been used to assess the relation between palmar moisture and fingerprint donorship (172). NIN application itself is not the core of this paper, but is only used as a tool. The results are detailed in section "2.2. Composition, aging and persistence of fingermarks". NIN-developed marks, obscured by pen ink, can be rendered more visible by immersing the samples into laboratory-grade acetone to fade the ink (189). This treatment is not immediately detrimental to NIN-developed fingermarks, however a fading of NIN has been observed after several months. 5MTN is evaluated by Porpiglia *et al.* (190). 5MTN can be considered as a dual reagent, leading to coloured and luminescent results. Combined with zinc chloride, this molecule was shown to effectively detect fingermarks, but an evaluation against alternative amino acid reagents shows that the performances were significantly lower than DFO or IND/Zn.

DFO - DFO-treated fingermarks have been transferred from banknotes to white paper sheets (191). The transfer occurs during the treatment under the heat press. Reversed marks initially present on the banknotes were detected on the paper. This technique can help suppressing the background of the substrate.

1,2-Indanedione - Spindler *et al.* (193) present a fundamental study about the effects of several parameters on the reaction of IND. Among other parameters, ambient humidity is shown to have a strong effect on the reaction. The increase of luminescence by the addition of catalytic amounts of zinc chloride is also studied and confirmed. Mechanism studies of the IND-amino acid reaction are further detailed in her PhD thesis (192).
Effect of zinc and europium chloride on the luminescence of an IND solution has been studied (194). Post-treatment with zinc chloride proved to enhance the luminescence whereas europium chloride did not lead to significant improvement. Finally, IND/Zn was used to investigate the effect of the postal distribution process (195). Test envelopes were used to assess the possibility to recover fingermarks. It has been shown that a great amount of marks can still be detected with sufficient quality. A relatively small number of deposits were affected by the handling of envelops during the distribution process.

Other reagents - The use of substituted naphtoquinones is described in a preliminary study (196). Naphtoquinones successfully produced purple-brown fingermarks with red luminescence. The intensity of colour and luminescence

depends on the naphtoquinone type. Further studies are required to determine the actual efficiency in comparison to current benchmark reagents. Another preliminary work shows that DMAB successfully reacts with amino acids and can be used to detect fingermarks on porous substrates (197). The obtained results are both coloured and luminescent.

Comparison studies - Envelopes aged from 1 to 21 years were used to compare the efficiency of NIN, DFO and IND (198). This study shows that the age of the fingermarks does not influence the ability of the methods. The authors conclude that IND gives superior results in comparison to NIN and DFO, even on old marks. The performances of DFO and IND/Zn were also compared in an extensive study (199). It was found that IND/Zn developed more fingermarks, with a brighter luminescence. These results led to a nationwide field trial, which is still underway. A comparison is also performed by Berdejo *et al.* (200) between four amino acid reagents (DFO, 5MTN, lawsone and IND/Zn). IND proved to be superior to all other reagents, but contrary to previous studies, it is stated that zinc chloride does not improved the fluorescence. The processing of thermal paper with IND/Zn has been described in combination with heat or destaining solutions (201), or by the use of a dry method (202). These studies are detailed in section "2.3.7. Substrate - Thermal papers".

1.3.2 Cyanoacrylate fuming

A lot of publications dealt with the well-known cyanoacrylate fuming process. Some authors focused on the role played by humidity (203) and temperature (204-207) on the polymerization mechanism. Others proposed different ways of rejuvenating old marks to improve the detection quality (208-210). New dyes were proposed (211, 212) as well as a proposition to subsequently apply VMD after cyanoacrylate fuming (213). A recent trend consists in a one-step procedure allowing to obtain marks readily fluorescent, without the need for stain post-processing (206, 214, 215). Miscellaneously, two quality control tests were proposed (216, 217), the possibility for DNA transfer was proved (218), the effect of fuming on the composition of some plastic bags studied (219), and the use of a commercial fuming device evaluated (220). All the articles dealing with the use of chemically-modified cyanoacrylate monomers for imaging purposes (221, 222) are described in section "2.4.2. Chemical Imaging".

Used acronyms: CA (cyanoacrylate or cyanoacrylate fuming), BY40 (basic yellow 40), DMAB (p-dimethylaminobenzaldehyde), GC-MS (gas chromatography – mass spectrometry), HCN (hydrogen cyanide), LDPE (low-density polyethylene), NIR (near infrared), R6G (rhodamine 6G), RAM (rhodamine – ardrox – methylene blue), RH (relative humidity), SPME (solid phase microextraction), TWA (time-weighted average), UV (ultraviolet), VMD (vacuum metal deposition).

Fumigation / Polymerization mechanism - Lowering the temperature during the CA process may influence the polymerization mechanism, in terms of initiation sites, quality of the polymer chains, as well as their morphology. A decrease in temperature could consequently promote a larger coverage of dense polymer chains over the mark (204), especially with aged fingermarks. A similar conclusion has been reached

by other researchers, who recommend forcing the condensation on items before the fuming processing (205, 206). Ideally, the temperature of the items should be decreased by ca. -4.5°C to ca. -11°C (i.e. -5 °F to -20 °F) relative to the ambient temperature, before processing them in the fuming cabinet. The authors also observed an increase of adherence of the CA dye (i.e. R6G) which is subsequently applied. In another study, it was confirmed that 80% RH constitutes the optimum level of relative humidity for the development of the most high quality marks (203). Overheating the CA monomers could generate HCN, even if none of the tested superglues generated detectable amounts of HCN when heated for 30 min at 180°C (207). Nevertheless, quantifiable amounts of HCN were generated from the thermal decomposition of CA monomers and polymers when heating at 200°C and above. Even if the released quantities are below the TWA concentration limit for workplace exposure, it is recommended to limit the heating temperatures of home-made fuming systems to values below 240°C. The relative humidity during long-term storage of items (e.g. months) before their processing seems to have no significant influence on the quality of development (223). Consequently, it is not recommended to install equipment maintaining constant environmental conditions (note: storage temperature was varying between 22 and 25°C).

Pretreatments - It is commonly accepted that the effectiveness of CA is reduced when dealing with aged or dry marks. Different protocols were consequently proposed to "rejuvenate" old marks before their processing: exposition to acetic acid or ammonia vapours for 15 minutes (208) or to vapours of a 10% w/v methylamine solution (209). Pinto *et al.* observed an increase of the ridge thickness after CA following acetic acid vapours pretreatment, compared to conventionally-processed marks. Another experiment consisted in dusting the marks with valine-containing powders prior to CA (210). The aim was to enrich the sebaceous fraction of the marks (more likely to survive upon aging) with polymerization initiators. The obtained results were mitigated in comparison with the chemical vapour enhancement.

Post-treatments / Sequence - Styryl 11 was shown to give better results than R6G when observed in the near infrared region (NIR; >700 nm) (211). Styryl 11 has a maximum absorbance at 575nm, and a strong emission at 766 nm. One advantage of visualizing marks in the NIR region is that it is unlikely to face an unwanted background luminescence (in comparison with stains emitting in the visible range), for example with aluminium soft drink cans. It is also possible to combine Styryl 11 with R6G ("STaR11") to extend the Stoke's shift. In another study, DMAB was proposed as a vapour-phase stain for CA-treated fingermarks (212). The described procedure consists in leaving the items in a close container with DMAB powder for at least 48 hours, and subsequently observing the detected marks under UV light. The authors report the obtaining of fluorescent marks on substrates not suitable for a conventional liquid-based staining process (e.g. unglazed earthenware flower pot). Finally, the sequential application of CA and VMD on plastic has been studied at a molecular level (213). The sequence "CA/BY40 – gold/zinc VMD" was proved to be adapted to the processing of LDPE substrates.

One-step fluorescent cyanoacrylate - A new generation of CA process has been tested. It consists in fuming and staining the items - simultaneously - by co-evaporating the CA monomer with a fluorescent powder (214). The biggest advantage of a one-step process is to avoid the use of organic solvents (staining post-treatment), which may be time-consuming and detrimental for some substrates.

In this study, the prototype of the Polycyano (Foster and Freeman – UK) was tested. The overall quality of the development was comparable to what is obtained using the current two-step fuming and dye stain procedure (e.g. R6G, Ardrox, RAM). One drawback, that could be noted, is that the process requires the modification of the fuming cabinet to increase the temperature of the heating plate to 230°C. This procedure may be costly and lead to the generation of HCN as stated by Fung *et al.* (207). In another research, people were interested in expanding the excitation range of a dye-stained CA by using a sublimating dye (i.e. Sublaprint Red R70011) (206). The new combination of dyes extended the excitation range from 365-505 nm to 365-530 nm (which is more compatible with the 530 nm single wavelength light source found in some agency laboratories). Finally, the synthesis of CA monomers functionalized with fluorescent groups has also been attempted (215). However, these monomers were unable to detect fingermarks when fumigated (like a conventional CA monomer). The authors proposed to solubilize the fluorescent monomers in xylene and applied them by a quick immersion of the item to be processed.

Quality controls - Two control tests were designed to assess the quality of development upon CA: (a) one based on sodium hydroxide spots, and recommended for blood cases when it is expected to use luminol subsequently on the scene (216), and (b) one based on fingermarks made of artificial sweat (217). Thiburce *et al.* showed that an optimized exposition to CA may have a positive impact on the subsequent application of luminol (or Bluestar®), with longer-lasting chemiluminescence (216). On the contrary, an excessive quantity of CA polymer may completely hinder the reaction of luminol with the underlying blood (shoe)marks. Velthuis *et al.* considered a mixture of different compounds (i.e. fatty acids, amino acids, glycerides) to mimic natural sweat, which was subsequently jellified using gelatin leaves (217). The control test consisted in marks left on plastic/glass using a silicone fingertip in contact with the jellified artificial sweat.

Miscellaneous - A study showed the possibility for DNA to accumulate both outside and inside a CA chamber, as well as for DNA to transfer from one exhibit to another during the fuming process (218). Recommendations are given by the authors to limit such unwanted contaminations (e.g. sampling for DNA before the fuming, limiting the number of evidence in the chamber, decontaminating using UV light-equipped chamber). The effect of CA on the composition of polyethylene bags (used to carry illicit drugs, for example) was studied using SPME/GC-MS analyses (219). A portable CA system (SUPERfume® from Foster and Freeman – UK) was tested, and its efficiency compared with the use of aluminium powder on crime scene (220). As a result, SUPERfume was more effective on textured and smooth plastic surfaces (and for marks stored at 37°C), whereas aluminium powder was shown to be more effective on glass, enamelled metal paint, and varnished wood (and for marks stored below 20°C). When facing a scene to be processed, the authors consequently recommend to consider each surface independently (if possible) or to dust the surfaces made of glass, enamelled metal paint, and varnished wood before using the SUPERfume equipment.

1.3.3 Lipid stains and lipid-oriented techniques

Evolutions of the Oil red O technique consisted in optimizing the formulation (224, 225), proposing a luminescent alternative (226), and

evaluating its performance in sequence with DFO and ninhydrin (227). About the physical developer, quality control procedures were proposed (228, 229), an extended shelf-life has been measured for the new formulation of physical developer (230), and its robustness has been tested (231). The interest of introducing physical developer in a sequence after amino acid reagents has also been confirmed (232). Finally, TECTOPO has been applied on paper (233) and Nile red proposed as a new lipid stain (234).

Used acronyms: DFO (1,8-diaza-9-fluorenone), EDTA (ethylenediamine-tetraacetic acid), IND (1,2-indanedione), NIN (ninhydrin), ORO (oil red O), PD (physical developer), R6G (rhodamine 6G).

Oil Red O - An alternative to the original formulation of ORO (see: (226)) is proposed, in which the methanol-based solution is replaced by propylene-glycol (224). This new formulation was proved to be as efficient as the original one, but is safer, quicker, and requires fewer reagents. It however suffers from the same limitations as the original formulation, especially on some kinds of substrates and on aged fingermarks. A sequence of treatment for porous substrates is further proposed: "IND (/HFE-7100) – ORO (/propylene-glycol) – PD" (225). The authors recommended reducing the immersion time in the IND solution to less than 5 seconds, to avoid a detrimental effect on the lipid fraction of the secretion (which could impact the subsequent ORO treatment). Another research aimed at studying the sensitivity of DFO, NIN, and ORO over time, as well as the contrast of the resulting marks, for different kinds of porous substrates (227). It has been shown that ORO could be applied subsequently to the "DFO (/HFE-7100) – NIN (/HFE-7100)" sequence, which could result in an enhancement of the already-detected marks and detection of latent-remaining ones (especially on kraft paper, cardboard and thermal paper). On white and recycled papers, no additional marks were detected by ORO when applied after DFO and NIN. Finally, a luminescent alternative to the original ORO formulation has been proposed, which could be used on wetted and dark porous substrates (226). Substrates of interest are first processed with the original ORO, and immediately followed by the spraying of a R6G staining solution. The marks obtained using this procedure appear as dark ridges on a luminescent background.

Physical developer - In order to test the reliability of a prepared PD working solution, two procedures were proposed: (a) printed standardized test strips using a modified inkjet printer (228), and (b) EDTA spot tests on Whatman #2 filter paper (229). The pattern developed by Kupferschmid *et al.* contains geometric shapes made of ascorbic acid and oleic acid aqueous solutions (used as inks by the printer). The correlation between the number of detected shapes on the test strip and the quality of fingermark development was shown to be high. The EDTA spot test procedure represents an inexpensive, reliable, stable, and rapid alternative to a printed pattern as well as to gold chloride spot tests on filter paper. The shelf-life of the last PD formulation (in which Synperonic N has been replaced by Tween 20) has been thoroughly studied (230). As a result, it has been shown that the shelf-life of the PD working solution was of 10-15 days for the Synperonic N formulation, but rises up to 2 1/2 months when using Tween 20. The robustness of the PD formulation was tested by comparing the efficiency of different working solutions, among which voluntary alterations and modifications were introduced (231). It has been shown that

(a) the order in which constituents of the redox solution are added has no influence, (b) Tween 20 is an advantageous alternative to Synperonic N, and (c) maleic acid is to be preferred to malic acid during the prewash. The sequence "DFO – NIN – PD" was tested, and the results confirmed what had already been pointed out about the usefulness of using PD in terms of detection of additional marks and quality improvement of the already detected ones (232).

TECTOPO – This lipid reagent has been used to detect marks on papers using time-resolved luminescence (233).

Nile Red - Nile Red is a newly proposed fluorescent lipid stain to be used on wetted porous substrates. When compared with PD, it appeared that PD remains the most reliable and sensitive technique. Nevertheless, Nile red can be added at the end of the treatment sequence for porous substrates, after PD (234).

1.3.4 Dry micro-/nano-sized powders and powder suspensions

Two different powder applications are described (235, 236), as well as two lifting processes (237, 238). Several new micro-sized powders like magnetic powder based on indigenous minerals (239), iron flakes (240), turmeric (241), silica gel G (242) and synthetic food and festival colours (243) are evaluated. Phosphorescent powder is also discussed (244, 245). Powder application proved to be the best method on particular substrates, such as fruits and vegetables (246). Powder was used to evaluate the potential of fingermark recovery on cell phones (247).
For the nano-sized powders, various types are discussed. Among them are aluminium oxide (248), calcium carbonate (249) and iron oxide (250). Quantum dots coated with polymers (251) or silica (252) can also be applied, as well as embedded in porous matrix (253-255). Anti-stokes nanopowders are presented (256, 257). Two papers depict the uses of silica nanoparticles (258, 259). All the other articles dealing with the use of functionalized silica nanoparticles to study the composition of secretion residue and allow the detection of exogenous elements in fingermarks using analytical methods (260-262) are described in section "2.4.2. Chemical Imaging". Finally, zinc carbonate is used as wet suspension powder (263-267).

Used acronyms: CA (cyanoacrylate fuming), CdS (cadmium sulphide), CdSe (cadmium selenide), CdTe (cadmium telluride), NIR (near infrared), NPs (nanoparticles), QDs (quantum dots), R6G (rhodamine 6G), SPR (small particle reagent).

Application method and lifting - The cotton wool powdering technique has been evaluated (235). It proved to be efficient and easy-to-use since larger surfaces can be covered. The obtained marks were judged to be of a comparable quality as those obtained with squirrel-hair brush. Powder can also be applied using an aerosol spray (236). After modifications in formulation and in aerosol technology, this technique proved to be a viable method for crime scene use. The amount of powder is controlled and the contact with the substrate is reduced. The gelatin lifting process has also been extensively evaluated (237). Gelatin lifting prior to powdering is less

effective than powdering only but it might be considered in case of contaminated surfaces or incompatibilities with powders. It proved to work well on smooth surfaces, but its effectiveness decreases with age of the mark. In the case of fingermarks made of dust or deposited on dusty surface, dust print lifters are effective tools to recover fingermarks when powder dusting cannot (238).

Dry micron-sized powder - A magnetic powder based on an indigenous mineral from Thailand is tested (239). The minerals are grinded and mixed with nickel. The powder is applied with a magnetic brush and marks of good quality are obtained. Iron flakes of different dimensions, produced with a high-energy milling device, are evaluated for fingermarks powdering (240). 50 µm flakes can sometimes develop marks of better quality than conventional black magnetic powders. Turmeric powder, normally used as an ingredient in Indian food, is used to detect fingermarks (241). This powder, cheap and non-toxic, is efficient on various non-porous substrates. Fingermark detection with silica gel G (242), synthetic food and festival colours (243) are presented. These powders are efficient on non-porous substrates. Phosphorescent (glow-in-the-dark) powder is compared to fluorescent powders (244). The authors stated that a better contrast is generally obtained with glow-in-the-dark powder. Another powder with upconversion properties is investigated by Drabarek *et al.* (245, 268). Anti-stokes phosphor pigments mixed with white powder can detect fingermarks of good quality without background staining, since an infrared illumination is used and visualization made in the visible range.
Fingermark detection on fruits and vegetables is discussed by Fergusson *et al.* (246). The most promising methods are black magnetic powder and black powder suspensions. Recovery of fingermarks on cell phones is evaluated by Lodhi *et al.* (247). Identifiable marks were obtained on 11% of the items (n = 121, 13 marks) using silk black powder.

Dry nano-sized powder – This section is also focused on powdering, but using dry NPs, nanostructured materials or materials containing NPs. Aluminium oxide NPs are covered with two dyes (R6G and styryl 11) and mixed with silver magnetic powder (248). The results can by visualised in both visible and NIR regions. On the same principle, Khokhar *et al.* (249) used nanopowders made of calcium carbonate or copper both coated with an organic compound. Iron oxide NPs coated with a silver layer have been applied under dry form on fingermarks (250).

Different QDs can also be powdered on fingermarks. CdS QDs coated with various polymers are applied on glass and aluminium foils (251). CdTe QDs were also coated with silica layers (252). When applied on fingermarks deposited on non-porous surface, the powder adheres on fingermarks leading to luminescent results. QDs can be embedded in different porous matrix used as a template for the NPs synthesis. CdS QDs (253) and CdSe QDs (254) were embedded in phosphate heterostructures. Gao *et al.* (255) used CdTe in montmorillonite to produce a nanostructured powder. After application on fingermarks, they obtained luminescent results. Anti-stokes nanopowder made of $NaYF_4$:Er,Yb (256) and YVO_4:Er,Yb (256) have been powdered. There are only two works reporting the use of silica NPs powder for fingermark detection. Liu *et al.* (258) synthesized silica NPs containing R6G. The obtained powder is mixed with magnetic iron powder and applied with a magnetic brush. The second paper is based on lanthanide-doped yttrium zirconate entrapped in silica NPs (259). The obtained powder is applied on fresh fingermarks.

These two publications are mainly focused on the optical properties rather on the efficiency to detect fingermarks. No comparison is made with traditional powders.

Other functionalized silica NPs were used as a dusting powder to allow the detection of exogenous pharmaceutical drugs (metabolites) and explosive (contamination) in fingermarks using analytical methods (260, 261), as well as to study the composition of sweat secretion (262) – see section "2.4.2. Chemical Imaging".

Wet powder suspension - New SPR based on the use of zinc carbonate have been studied. Eosin Y (263) or crystal violet (264) are added in the formulation. These methods proved to be efficient on surfaces that have been immersed into water. Zinc carbonate SPR is effective on compact disc and does not interfere with data retrieval (265). A comparison study between CA, powdering and SPR to recover marks on wet transparent foils has been performed (266). SPR proved to be the most efficient technique, even for marks exposed to water during at least one week. In similar study about the recovery of mark on glass and metal surfaces that have been wet, CA proved to give the best results compared to SPR (267).

1.3.5 Nanoparticles in solution

Uses of nano-sized powders and nanostructured powders have been discussed in the previous section (2.3.4). Only nanoparticles in solution are described here, and are classified by their composition, starting with gold nanoparticles (269-275) and followed by semi-conductor nanoparticles (quantum dots) (276-283). The other nanoparticles types, such as metal oxide (248, 249), silica nanoparticles (258, 259) and up-convertors (256, 257), are only applied as powder and were therefore described in the previous section. An extensive review on the use of nanoparticles applied for fingermark detection is proposed by Dilag et al. (284). The authors describe uses of gold nanoparticles, fluorescent dye-doped nano-powders and quantum dots. Another review on the same topic is made by Hazarika and Russell (285). An entire book chapter is also dedicated to the fingermark detection with nanoparticles (286). This contribution deals with the synthesis of different nanoparticles types (gold, quantum dots and silica nanoparticles) and their application to fingermark detection. It also makes assumptions about the interaction principles between nanoparticles and fingermark secretion. All the articles dealing with the use of nanoparticles functionalized with antibodies (287-291) are described in section "2.3.6. Immunogenic detection".

Used acronyms: CA (cyanoacrylate fuming), CdS (cadmium sulphide), CdSe (cadmium selenide), CdTe (cadmium telluride), MMD (multi-metal deposition), NPs (nanoparticles), PAMAM (poly[amido amine]), PD (physical developer), QDs (quantum dots), SiO_2 NPs (silica or silicon oxide nanoparticles), SMD (single-metal deposition), VMD (vacuum metal deposition), ZnS (zinc sulphide).

Gold nanoparticles - General uses of gold in forensic sciences are described by Mohamed (269). The review encompasses the topic of illicit drug and describes the use of silver, gold and other metals to detect fingermarks (PD, MMD, SMD, VMD).

Works on gold NPs are various. Fairley *et al.* (270 90) propose an evaluation of the effectiveness of various multi-metal deposition techniques (MMD I & II, and SMD). MMD II is considered as the most effective, but since it is more labour intensive, MMD I appears as the best compromise between practicality and effectiveness. In general, it performs better than VMD or CA on cling film and plasticised vinyl, but it is not effective on leather and masking tape. The final results obtained with SMD are not considered as sufficient, due to lack of contrast and consistency. Bécue *et al.* (271) described a new version of SMD. The synthesis of the gold NPs has been simplified, and an amino acid is also grafted onto the surface. The modifications led to a simplification of the synthetic procedure, and the new formulation shows a stronger resistance to pH variations. Results are more reliable than with the previous SMD formulation. Contrary to the two previous works for which NPs were already present in the solution, Hussain *et al.* (272) applied a solution containing gold chloride. In this case, the fingermark residues act as an initiator for the particles growth. There is an *in situ* formation of NPs onto the fingermarks.

When detecting fingermarks, a contrast is generally obtained by specifically targeting the sweat secretions, but in a recent work, gold NPs have been designed to target the cellulose of the paper instead of the marks (273, 274). The NPs functionalized with a thiolic ligand possess an affinity for the substrate and negative fingermarks have been obtained after a post-treatment using PD. Specific targeting was also possible when NPs (gold and silver) were attracted onto the metallic surfaces by electrodeposition (275). Negative marks were obtained, the secretions acting as an insulator.

Quantum dots - Among QDs applied in aqueous solution, CdTe QDs are the most common. These NPs functionalized with a carboxylic group have been applied on fresh fingermarks deposited on various surfaces (276), as well as on cellulose tape (277), but with mitigate results. The same NPs functionalized with a double carboxylic group were applied on non-porous surfaces (278, 279). The results are obtained after a very short immersion time of 1 to 10 seconds, or by spraying the surface with the solution. Gao *et al.* (280) used another functional group, leading to the formation of positively-charged NPs. According to the authors, these particles give better results than the negatively-charged ones. Contrary to toxic cadmium-based NPs, Moret *et al.* (281) describe the use of ZnS QDs to detect fingermarks in blood. Results obtained on non-porous have been compared to Acid Yellow 7. CdS (282) and CdSe (283) prepared in PAMAM dendrimers and stabilized in water were applied on adhesive tapes and tin foils. Sebaceous fingermarks aged up to one month were effectively detected.

Other nanoparticles - Antibody-functionalized silver NPs were used to specifically target sweat components, followed by chemical imaging using Raman spectroscopy (292). This article is described in details in section "2.3.6. Immunogenic detection". This section also describes the use of other types of antibody-functionalized NPs, which can target specific compounds that can be present in the secretion like drug metabolites (288), explosives (285), cotinine (290, 291) and amino acids (287).

1.3.6 Immunogenic detection (antibody/antigen)

Immunogenic-based techniques have been recently used to specifically target antigens contained in the fingermark residue. Reviews of the field

have been proposed, from the early attempts to the current research strategies (285, 293). All the work done on immunogenic techniques consisted in combining the antibodies with (magnetic) nanoparticles, so that they could target L-amino acids (287), drugs and metabolites (288, 290, 291), human immunoglobulin G (292), and body fluids (289). A new approach is also currently investigated: the use of aptamers (i.e. single-stranded nucleic acid oligonucleotides) to specifically target secretion components (293).

Enantioselective anti-L-amino acid antibodies conjugated to gold nanoparticles were used to detect marks on non-porous substrates (287). Early results were presented, and the field is still being investigated since conventional methods are shown to produce superior fingermark details on fresh samples. It should be noted that the author emphasized the role played by nutrition habits on the efficiency of the technique (phenylalanine-based sweeteners, for example).

Magnetic nanoparticles were functionalized with a series of antibodies to specifically target the following antigens: morphine (288), benzoylecgonine (288), cotinine (290, 291). All the authors emphasized the high selectivity of the techniques, as well as the possibility to observe the detection in luminescence. However, nothing is said about the introduction of such an approach in the existing detection sequences (forensic context). Despite it is not in direct link with the detection of fingermarks, it is interesting to cite the immunodetection and localization of body fluids (i.e. blood and saliva) on various substrates using magnetic nanoparticles functionalized with the corresponding antibodies (289). Finally, silver nanoparticles functionalized with antibodies able to recognize human immunoglobulins (IgG) were used to specifically target sweat components of fingermarks (292). By doing so, it was then possible to visualize the fingermarks through a Surface Enhancement Raman Spectroscopy imaging process, as described in section "2.4.2. Chemical Imaging".

1.3.7 Substrate - Thermal papers

Among the different techniques developed to detect fingermarks on thermal paper, we can cite: steam (294), controlled application of heat (295), amino acid reagents (202, 296), background darkening treatment (201, 296), as well as iodine to retrieve erased text (297).

Used acronyms: BY40 (basic yellow 40), DABCO (1,4-diazabicyclo[2.2.2]octane), DFO (1,8-diaza-9-fluorenone), IND/Zn (1,2-indanedione/zinc chloride), LED (light-emitting diode), NIN (ninhydrin), PVP (polyvinylpyrrolidone)

Steam / Heat - The use of a fabric steamer to detect fingermarks on thermal paper has been studied (294). The authors concluded that sebaceous marks are more likely to be detected, especially if they are fresh (the rate of successful detection decreasing over time). The results were somewhat inferior to the ones obtained with some conventional techniques, such as acetic acid fumes and NIN (in HFE-7100). In an effort to uniformly apply heat on thermal paper, an apparatus has been designed and tested (295). The sample is placed between two rectangular plates: a brass one

(further heated to 44°C) and a glass one (allowing the observation of the detection process). The use of a 465 nm blue LED illumination could help in the observation of the ridges development.

Iodine - The use of iodine vapour on thermal paper could help in retrieving the text that was initially present, but faded over time or erased as a result of the application of a fingermark enhancement technique (297). The recovered texts may appear as positive (dark writings on white background) or negative (white writings on dark background), and remain visible for several weeks. This technique is also efficient for texts printed using dot-matrix printers, and seems unaffected by the age of the document.

Amino acid reagents - A "dry" application of IND/Zn, with low temperature heat, is reported as a non-destructive way of detecting fingermarks on thermal papers (202). Luminescent fingermarks could be obtained without darkening the background (as it would be the case with the traditional procedure) by placing the sample in sandwich between two reagent-impregnated filter papers and heated at 60°C for 15 minutes in a Ziploc™ bag. Schwarz *et al.* propose different chemical possibilities to deal with thermal papers when applying amino acid reagents (IND, NIN, or DFO): (a) preventing the darkening of the background by including PVP (Kollidon® 12 PF) into a conventional NIN formulation (aka NinK12) (296), or (b) chemically reversing the background darkening by using a "whitening" solution (G3 or DABCO) (201, 296). It has to be noted that the G3 and DABCO solutions erase any printed text present on the document.

1.3.8 Substrate - Metal and cartridge cases

The visualization of fingermarks on metal surfaces through an electrochemical process has been further studied (298-304), as well as electrostatic deposition (305), electrolysis (306), vapours of S_2N_2 (307), and electrochromic process (308, 309). Metal sputtering was also assessed (310), as well as thermal development (311). On cartridge cases, different techniques and sequences of detection were tested (312-314). The possibility to recover fingermarks from cartridge cases submitted to arson conditions (315) is described in section "2.3.11. Scenario - Arson scenes". The use of chemical imaging to detect fingermarks on metal substrates (i.e. platinum, gold, silver, copper and stainless steel) (316) is described in section "2.4.2. Chemical Imaging". Some case reports were also described, dealing with successful fingermark detection on a cartridge case (317) and with the overall recovery rates observed in forensic laboratories (318, 319).

Used acronyms: ATF FSL (Alcohol, Tobacco, Firearms and Explosives Forensic Science Laboratory), CA (cyanoacrylate fuming), DPD (Denver Police Department), R6G (rhodamine 6G), RUVIS (reflected ultraviolet imaging systems)

Metal corrosion effect (brass) - The process leading to the visualization of fingermarks on brass cartridge cases through an electrochemical process (corrosion) has been further studied (298-302). RUVIS was shown to be inappropriate to

observe corrosion on fired brass cartridges for which white light is to be preferred (304), combined with the use of a selective colour mapping process (e.g. using Adobe Photoshop®) (303). The electrostatic deposition system causes no detrimental effect on the ballistic identification process, once the deposited powder has been washed thoroughly after fingermark detection (305). Other researchers studied the enhancement of fingermarks using electrolysis on fired brass cartridges (306). For this experiment, brass cartridges were immersed in diluted hydrochloric acid (HCl) before applying a voltage to initiate the galvanic corrosion reaction. As a result, a visible contrast appears between the ridges and the metal surface. It has to be noted that the fired group of cartridges showed better results compared to the unfired group. Vapours of S_2N_2 were used to detect marks on metal items, for which the marks have been removed (deliberately or as a consequence of an explosion) (307). S_2N_2 polymerizes around localized physical imperfections on the substrate surface, and by the same way at the level of the corrosive modifications due to the presence of sweat (even if the mark has been erased).

Metal surfaces - The electrochromic enhancement of fingermarks on metal substrates was compared with conventional methods (i.e. dry powder, wet powder and CA) (308, 309). Briefly, the electrochromic enhancement relies on the deposition of a conducting polymer on the metal substrates. The secretions inhibit the polymerization, resulting in a negative image of the mark. In this study, stainless steel samples were exposed to different scenarios (e.g. immersion in water, high temperature, washing using soap) then further processed for fingermark detection. Dry dusting and electrochromic enhancement gave the overall best results. Metal sputtering (= vacuum evaporation) of copper or gold onto stainless steel substrates allowed the detection of fingermarks (310). It was observed that sputtered gold and copper tend to concentrate in the ridges, resulting in dark ridges on metal background. Gold sputtering was favoured, especially for aged marks and for its stability against oxidation. Thermal development of fingermarks on metal surfaces was also further studied (311). Brass appeared to be the least dependent on temperature up to 600°C, aluminium gave poor visualization when heated above 280°C, and stainless steel only gave good visualization when heated above 600°C (a loss of detail starting to appear when heated a 900°C).

Cartridges cases - A comparison of different techniques to detect fingermarks on fired cartridge cases showed that the Gun blue solution, the palladium deposition, and the CA/BY40 were all successful in detecting marks, with a preference for the first two techniques in terms of mark quality (312). The authors observed that most of the ridge details were detected below the bottom third of the cases. It has to be noted that no fingermark detection was observed with the electrostatic deposition (device built by following the descriptions given in the literature). These results were confirmed by another study for which six enhancement techniques to detect fingermarks on unfired brass cartridge cases were compared (313). Two sequences provided the best results and showed no statistical difference in terms of efficiency: "CA – Gun Blue – BY40" and "CA – Palladium deposition". Powder suspension produced the poorest results. Acidified hydrogen peroxide (H_2O_2) is a technique allowing the detection of fingermarks on brass, and in this case on fired brass cartridges (314). The authors recommend to use it after CA/R6G; they also set a maximum processing time of 75 seconds to visualize ridges (average detection time of 24 seconds), emphasizing that this process could negatively interfere with firearms

examinations since noticeable effects on the cartridge characteristics may be observed after 20 seconds of treatment.

Case reports - Babin has reported the successful application of CA on a fired 9mm cartridge casing, with the detection of a partial mark consisting of a smudged core with identifiable ridges above it (317). Among the firearms processed by the ATF FSL in San Francisco over a three-year-period (Jan'07 to Dec'09), a recovery rate of 13% was obtained on firearms (n = 598, 168 identifiable marks), 7.6% on ammunition magazines (n = 423, 46 marks), and 0.12% on cartridges (n = 6,698, 8 marks) (318). Whenever possible, the authors recommend removing the grips of the firearms prior to processing, because the area underneath the grips can yield identifiable marks. A similar study conducted at the DPD over a 2-year period (May'08 to May'10) showed a recovery rate of 3.7% on firearms (n = 189, 7 identifiable marks), 10.0% on ammunition magazines (n = 110, 11 marks), 0.25% on live cartridges (n = 817, 2 marks), and 0% on spent cartridge cases (n = 200, no mark) (319). The firearm evidence items were processed with orange magnetic powder (leading to 6 marks), CA and RUVIS (leading to the successful detection of 14 marks).

1.3.9 Substrate – Tapes and adhesives

In the field of tapes, people were interested in the best way to separate duct tapes stuck together (320, 321) or on paper (322), in determining an optimized detection sequence for processing the adhesive side of tapes (323), especially if blood marks have to be found (324). The use of engineered nanoparticles to detect fingermarks on the adhesive side of tapes (277, 282) is described in section "2.3.5. Nanoparticles in solution".

Used acronyms: CA (cyanoacrylate fuming), RAY (rhodamine – Ardrox – basic yellow 40), SSP (sticky-side powder)

Manual separation was determined to be the only method to separate duct tapes stuck together while still enabling the recovery of latent fingermarks on the adhesive side (320). In another study, the use of liquid nitrogen applied with a cryogun was preferred over gradual force and adhesive neutralizer (Un-Du®) (321). In both studies, the use of an adhesive neutralizer was strongly discouraged for duct tape, since its use degraded the adhesive support and consequently the marks in presence. In another study (322), the Turkish solution (a mixture of solvents) outperformed Un-Du® to help removing adhesives from papers.

Using RAY as a post-CA dye constitutes an efficient way to process the adhesive side of tapes (323). Moreover, the following sequence has been shown to give optimal results (for the adhesive side): "CA – gentian violet – black/white powder suspension – RAY dye", with results superior to the use of the dye alone post-CA. Amido black proved to be the best method for developing blood fingermarks on the adhesive side of duct tape, when compared with Wetwop, SSP, Liqui-Nox, and gentian violet (324). Moreover, amido black does not seem to hinder the subsequent application of techniques dedicated to non-blood marks. For non-blood mark detection, Wetwop and SSP offered the best results (Wetwop offering the advantage of giving positive results with both blood and non-blood marks).

1.3.10 Substrate – Skin

An extensive review on the recovery of fingermarks on human skin is proposed by Wilkinson (325). Färber et al. (326) report the results of a large European project, and Beaudoin (327) presents a comparison between amido black and ortho-tolidine.

An extensive review on the recovery of fingermarks on human skin is proposed by Wilkinson (325). In a more practical approach, Färber *et al.* (326) report the results of a European project called “Latent Fingerprints and DNA on Human Skin”. The purpose was to conduct a systematic research on the recovery of fingermarks and DNA on skin. The marks were treated with magnetic or black powder, and were lifted with a gelatine foil or silicone casting material. The lifts were systematically swabbed and analysed to detect DNA. The authors recommend the use of magnetic powder, lifted with silicone casting material (Isomark®).

The recovery of fingermarks in blood on skin was also studied (327). Amido black and ortho-tolidine were compared and despite the toxicity of the latter, it remains the most effective technique. Furthermore, amido black cannot be adequately cleaned and may interfere with the autopsy findings.

1.3.11 Scenario - Arson scenes

The possibility to retrieve fingermarks from items exposed to extreme heat was explored (328), as well as blood marks (329) and marks on cartridge cases (315).

Used acronyms: BY40 (basic yellow 40), CA (cyanoacrylate fuming), NIN (ninhydrin), VMD (vacuum metal deposition), WPS (white powder suspension)

The possibility to recover fingermarks from glass and white ceramic tiles exposed to fire showed that ridge details are still retrievable, especially if the marks were protected from direct exposure to heat above 350°C (328). The most efficient technique was found to be CA followed by BY40 stain (except at 200°C, for which iron powder suspension gave better results). As the temperature increased, it has been observed that the effectiveness of both techniques decreased. However, if the items have been wetted, CA should be discarded and replaced by powder suspensions. It should be noted that the detergent within the powder suspension formulation may help removing the soot on some of the items. Finally, silver VMD could represent a potential alternative for items exposed to higher temperatures (>700°C).

Mock scenes - Mock scenes into which blood marks were planted on various common substrates (e.g. glass & plastic bottles, knife, envelope, magazine) were set in fire then extinguished to assess the possibility to recover marks (329). The author observed that (a) the likelihood of retrieving marks is related with the average temperature in the scene, (b) marks of various qualities (from poor to excellent) were recovered using a LASER (optical observation), acid violet or NIN, and (c) Kastle-

Meyer tests (presumptive blood test) gave sometimes negative results on known blood marks.

In another experiment, shotgun cartridges cases bearing latent fingermarks were left on mock scenes which were set in fire, then extinguished (315). In a first attempt, the following sequence was applied on the retrieved cases: "soot removal – visual observation – CA/dye – WPS", but led to no mark detection. In a second set of experiments, electrostatic deposition of charge powder (developed by Bond) was applied and led to the detection of ridges on some (undamaged) cases.

1.3.12 Scenario - Blood marks

A review of the blood fingermark detection techniques was made by Bossers et al. (330), in which the different techniques are classified according to the detection principles involved. Studies about the detection of marks in blood mostly consist in comparing different known techniques, considering various substrates (324, 327, 331, 332). Two studies are dedicated to the development of new reagents based on wet powder suspension (333) and semi-conductor nanoparticles (281). The comportment of latent fingermarks exposed to blood is the subject of two studies (334, 335). The other publications are focused on the detection of bloodstains, either chemically (336-339), or optically (340-344). The possibility to recover blood marks on common substrates submitted to arson conditions (329) is described in section "2.3.11. Scenario – Arson scenes". The use of functionalized nanoparticles conjugated to antibodies specific to blood and saliva (289) is described in section "2.3.6. Immunogenic detection".

Used acronyms: AB (amido black), AY7 (acid yellow 7), IR (infrared), LCV (leucocrystal violet), SERS (surface-enhanced Raman spectroscopy), TiO_2 (titanium dioxide), UV (ultraviolet).

Comparison studies - The formulation of AY7 has been modified (345). In the new protocol, the fixing step was merged with the staining step. The optimized formulation is easier to use and appears to be more sensitive. The pH of the solution was also modified to increase the chance of obtaining a DNA profile. Agarwal *et al.* (331) compared Phloxine B and AY7 when applied on dark-coloured substrates. AY7 appears to be more effective than Phloxine B, due the fluorescence properties of the former. A comparison between three reagents (AB 10b, TiO_2 in methanol and AY7) applied on knives with black handles was performed (332). In this study, AB was shown not to be suitable for the detection whereas the two other reagents lead to good results. TiO_2 is recommended for the detection of aged blood marks and AY7 appears to be a better option for the use on crime scenes, for fresh marks. Other techniques were evaluated on various substrates. AB proved to be the best method for developing blood fingermarks on the adhesive side of duct tape (324). Wetwop also gave positive results with both bloody and non-bloody marks. White Wetwop, containing TiO_2, was proved to enhance the quality of marks in blood on non-porous surfaces (333). It can be used alone or in conjunction with acid dyes, but it is detrimental to DNA. In another study, ortho-tolidine appears to be the best technique to detect fingermarks in blood on skin, compared to AB (327). Semi-conductor zinc sulphide nanoparticles (ZnS) doped with copper have also been used to detect blood

fingermarks (281). These nanoparticles proved to be better than AY7 on most substrates.

Blood exposition - Two papers studied the behaviour of a latent mark exposed to blood. Reitnauer (335) focused on sebaceous marks deposited on painted drywalls. It appears that a sebaceous mark exposed to heavy blood deposit will develop the furrows of the impression. Praska and Langenburg (334) did a similar study with marks deposited on glass. They found that a latent mark can be developed after exposition to dilute and whole blood, but this phenomenon did not appear consistently. These marks are distinguishable from blood marks, but may appear as genuine marks after an enhancement with AB and LCV.

Bloodstain detection (chemical) - Three different chemical enhancement techniques for latent bloodstains were evaluated (luminol, Bluestar® and Hemascein®) (338). All reagents are highly sensitive, but with a surface dependency. Luminol and Bluestar performed similarly, while Hemascein gives poor results on wood surface. It also cross-reacts with many substances, giving more false positive results than the two other techniques. The sensitivity and reliability of Hemascein was also tested (337). It is found to be reliable up to a dilution of 1:100,000 on light-coloured surfaces. Effect of Hemascein on subsequent DNA analysis has yet to be determined. Performances of luminol, fluorescein, hydrogen peroxide, as well as optical methods like UV and IR were evaluated to detect bloodstains on dark surfaces (339). Sensitivity, specificity, ability to work on various surface types and further DNA analysis were evaluated for each method. For the authors, the use of hydrogen peroxide (H_2O_2) is the most efficient method.

Bloodstain detection (optical) - IR photography has been used to detect and localize latent bloodstain evidence lying beneath a layer (or multiple layers) of paint, using a tungsten halogen lamp as source of visible and IR light (341). Blood marks have been detected beneath up to six layers of paint under reflected IR, depending on the characteristics of the paint (especially their IR transmission capability). In addition to IR, bloodstain beneath layers of paint can also be detected using an alternative light source, Bluestar, luminol and fluorescein (336). All techniques are said to be effective. It is recommended to apply the optical methods before any chemical enhancement. Among all chemicals tested, Bluestar produced the best results. IR imaging has also been tested to distinguish bloodstains on fabrics from stains of fruits and vegetables (344). This technique proved to be effective to differentiate blood from other stains. SERS was also used to detect blood (340). This technique is non-destructive and can successfully detect blood with a dilution of 1:100,000.

Hyperspectral imaging has been used to observe bloodstain patterns on black fabrics, hardly visible to the naked eye (343). In another study (342), the authors describe an evaluation of three different types of light source. These articles are described in section "2.4.1. Photography and alternative light sources".

1.3.13 Fingermark detection and DNA analysis

The effect of fingermark detection techniques on subsequent DNA analyses was extensively studied, in terms of potential detrimental effects (346-349), or contamination risks during the detection process (218, 346).

Choosing between DNA or fingermark is also addressed (350), especially when dealing with firearms (351).

Used acronyms: CA (cyanoacrylate fuming), DFO (1,8-diaza-9-fluorenone), PD (physical developer), VMD (vacuum metal deposition)

The effect of fingermark detection techniques on subsequent DNA analyses was extensively studied (346-349). As already known, most of the techniques do not affect DNA analysis (e.g. dry powders, wet powders, CA, DFO, VMD), but some were identified as deleterious (e.g. PD and silver nitrate). Bhoelai *et al.* also showed that the washing steps (e.g. during CA dye staining) reduced the amount of DNA, and that any immersion step could lead to DNA contamination between samples (346). Norlin *et al.* showed that fingermarks on adhesives (enhanced by wet powders) gave the highest DNA amount, most certainly due to cell shedding caused by the adhesive layer (348). A study showed that DNA could accumulate both inside and outside of a CA chamber, as well as to be transferred between items if processed simultaneously (218). The risks are low but could become problematic if DNA typing systems become more sensitive. The authors propose a number of recommendations to be taken in consideration.

Ferraro discussed about the choice that has sometimes to be made between "swabbing for touch DNA" or "processing the items for fingermarks" (350), as for seized firearms (351). A survey has been conducted with firearms seized within an Indianapolis police district over a two-year-period (Jul'07 to Aug'09). Touch DNA (collected using TriggerPro kits) produced a much larger volume of usable forensic evidence than fingermarks (65.0% vs. 14.3% of the cases, respectively), but identification outcomes for the two methods were equal (2.5% vs. 2.7%, respectively). Considering this, and given that touch DNA takes more time to generate results and is more costly, fingermark detection remains the most cost-effective technique.

1.3.14 CBRNE-related evidence

Only explosive-related scenario seemed to have been covered during these last three years. Post-blast mock scenes were processed to estimate the chance of recovery of planted marks (352-355). Another goal was to identify the presence of explosive contaminants in fingermarks using analytical techniques (356, 357). It should be noted that all the articles dealing with chemical imaging of explosive-contaminated fingermarks (261, 358-360) are described in section "2.4.2. Chemical Imaging".

Used acronyms: CA (cyanoacrylate fuming), CBRNE (chemical, biological, radiological, nuclear and explosive), LCV (leuco crystal violet), NIN (ninhydrin), SR-FTIR (synchrotron radiation-based Fourier transform infrared micro-imaging), PETN (pentaerythritol tetranitrate), RDX (research department explosive), RUVIS (reflected ultraviolet imaging system), SPR (small particle reagent), TNT (trinitrotoluene), VBIED (vehicle-borne improvised explosive device).

Explosive threat - The likelihood of recovering fingermarks on various materials used to build an explosive device (e.g. initiators, containers, electrical components, timing mechanisms, adhesives) was discussed (353). Batteries, switches, adhesives, tape, paper and cardboard are the surfaces from which fingermarks could most likely be recovered. Post-blast evidence can be processed by the conventional fingermark detection techniques (e.g. CA, powders, Wetwop®, LCV). The effects of blast to latent fingermarks left on items present in a VBIED (e.g. cell phone, computer hard drive) and on the vehicle surfaces were studied (354). Observation using a RUVIS, CA with dye staining, metallic dry powders (e.g. gold, copper), as well as SPR were efficient in recovering the latent marks. It should be noted that a large number of the fingermarks in presence were unaffected by the blast effect. The effects of using a water-based disrupting device on a VBIED were also studied, in terms of fingermarks and DNA recovery (355). Fingermarks were left inside and outside the vehicle as well as on objects present inside (i.e. glass and plastic bottles). The disrupting device left a sticky residue after its use (due to the gel sometimes added to the water) which then dries. Fingermarks were successfully detected using dry powder or CA (for small items). Most of the marks were left unaffected (especially outside the vehicle). Metal corrosion on post-blast copper pipe fragments allowed the detection of fingermarks through visual examination or using a selective colour mapping process (352).

SR-FTIR has been used to identify contaminants present in fingermark secretion (e.g. cream, drugs, explosives – PETN, TNT, RDX) (356). It is possible to transfer the marks from a hard-to-reach place, using a Mylar foil as a lifting medium, so that it could be subsequently analysed. The authors emphasize that the location of the latent fingermarks as well as the substances being the source of the contamination were known a priori. A wide-field Raman imaging was used to detect traces of explosives in fingermarks left on problematic Raman surfaces (e.g. plastics, painted metals), using an automated background subtraction process (357).

1.3.15 Miscellaneous detection techniques

A lot of studies dealt with low-pressure sublimation of reagents (361), materials (362-364) or metals (362, 365-367) to detect marks on various substrates. The processing of grease-contaminated marks and substrates has also been a hot topic (368-372). The detection sequence for plastic packaging films was updated (373), and two iodine-fixing reagents were proposed (374, 375). Among the remaining miscellaneous techniques, it is possible to cite: update on the use of silver nitrate (376), electrochemiluminescence to detect marks on conductive substrates (377-379), use of aqueous electrolytes to detect marks on metal (380), measure of the decay of surface charge to detect marks on plastic (381, 382), spraying of diacetylene monomers (383), chemical lifting of fingermarks from non-porous substrates (384), and electrodeposition of Prussian blue (385).

Used acronyms: BV2 (basic violet 2), BV3 (basic violet 3 = gentian violet), BY40 (basic yellow 40), CA (cyanoacrylate fuming), CAST (Home Office Centre for Applied Science and Technology), CTF (columnar thin film), ESDA (electrostatic detection apparatus), FTIR (Fourier transform infrared

spectroscopy), HDDCPU (2,4-hexadiyne-1,6-bis[p-chlorophenylurethane]), HDDPU (2,4-hexadiyne-1,6-bis[phenylurethane]), IND (1,2-indanedione), NIN (ninhydrin), NY3 (natural yellow 3 = curcumin), PET (polyethylene terephthalate), PGME (1-methoxy-2-propanol), PVC (polyvinyl chloride), R6G (rhodamine 6G), Rubpy (ruthenium[II] tris[2,20-bipyridyl]), SB (solvent black 3 = sudan black), SPR (small particle reagent), TPE (tetraphenylethene), uPVC (unplasticized PVC), VMD (vacuum metal deposition), ZnO (zinc oxide).

Low-pressure sublimation - A prototype system was proposed to allow the application of 7 common reagents (i.e. iodine, CA, CA/R6G, CA/fluorescent dye, IND, NIN, fluorescent powder) on porous and non-porous substrates, without the need of any solvent (361). The technique is based on vacuum sublimation combined with a gas injection delivery system, allowing the reagent to come in contact with the exposed surfaces. The quality of development was shown to be comparable to the traditional protocols (no statistical difference). This process also presents the advantage to have no negative effect on drug chemistry and DNA, but some reagents could have a detrimental effect on inks (forensic document). In the field of VMD, the traditional gold/zinc process was used to visualize grab impressions on fabrics (365). Ridge details could only be obtained on smooth non-porous fabrics (such as nylon), whereas other fabrics lead to the visualization of contact area, which could be further processed for DNA taping. Vacuum deposition of ZnO yielded to the detection of fingermarks on PET plastic substrates, without the need for gold seeds (366). The technique is said to give better ridge details for aged marks (e.g. 45 days). Finally, the sublimation of copper phthalocyanine can lead to deep blue-coloured ridges on light-coloured substrates (367). The technique is efficient on porous substrates (such as paper), but requires exposure times of at least 30 minutes, and was shown to be inefficient on non-porous items (such as glass, uPVC, or ceramic tiles). A specific thermal evaporation of chalcogenic glass or gold under vacuum allowed the visualization of fingermarks on non-porous substrates (e.g., glass, plastic and tape) through the deposition of a small layer of nanoscale wires (i.e. CTFs) (362). The technique is particularly seen as a way to study the topological details of the secretions and determine the sequence of deposition of overlapping marks. The same technique has been applied on untreated marks, as well as on CA-fumed and dusted ones (363), or by thermally evaporating calcium fluoride and silica (364). In this last study, the marks were further enhanced using R6G or IND, which acted as fluorescent dyes for the CTFs.

Grease-contaminated marks/substrate - The detection of fingermarks using lipid staining agents (i.e. SB, BV2 and BV3) is reported (368). The authors observed differences in the staining of sebaceous components by the three reagents, proposing to use them in sequence to increase the likelihood of detection. They also proposed a reduction of the dye stain concentrations by 25% without having a detrimental effect on the staining efficiency. Finally, they identified BV2 as a promising lipid stain (over BV3), mainly for its fluorescence properties. Another large scale study, encompassing more than 35 domestic greasy contaminants and several detection techniques, led to the proposition of recommended detection sequences (369). Two formulations of SB were compared: one made in ethanol and one made in PGME (370). The PGME-based formulation was preferred in terms of effectiveness and safety of use (lower flammability). The authors also recommend reducing the

staining time to less than 2 minutes, to avoid heavy background staining. They also emphasized that old staining solutions could be used, even if it is currently recommended not to use solutions older than 1 month. Finally, a new dye-staining reagent was tested: NY3 (371). Given its fluorescence properties, this stain could replace SB for the processing of contaminated marks (i.e. animal fats and vegetable oils) left on dark non-porous surfaces. In another context, when an item has been (voluntary) exposed to a petroleum-based contaminant (e.g. WD-40, gasoline, kerosene, oils), the use of heptane could help degreasing it before it is processed for fingermark detection (372). The procedure consists in applying heptane (CO_2-propelled, for example by using "Paslode Degreaser" or "Dynamo") to remove the contaminant, then letting the surface dry before applying a conventional detection technique. By decreasing order, SB, SPR, CA followed by powder, and powder alone allowed the observation of ridge details. A degradation of the marks was observed, especially after 2 weeks in contact with the contaminant.

Flexible plastics - The CAST conducted a study to re-assess the best sequence for processing flexible plastic packaging films (e.g. supermarket bags, trash can liners, protective product films), while voluntarily omitting PVC-based plastics such as cling film and shrink wrap because fingermark recovery rates from these materials are known to be low (373). The previously-stated most effective technique to be applied on packaging film was VMD (study from 1986), but a decrease of the efficiency of the VMD was recently observed, supposedly due to changes in the chemistry of the plastic material. The new recommended technique is CA/BY40. Powder suspensions are also recommended, as they develop as many fingermarks as CA and present the advantage of working on wetted items. VMD could still be applied, but it is recommended to introduce it after CA or powder suspension.

Iodine - Brucine has been proposed as an efficient way to fix iodine-processed marks, which are known to fade out quite quickly (374). After the fixing process, marks remain visible for one week on non-porous substrates and one month on porous ones. It should be noted that this fixing step seems to have a detrimental effect on the subsequent NIN process, since no marks (or very faint ridges) were visible after applying the amino acid reagent. α-naphthyl amine is also proposed to be used as pretreatment vapors, before iodine fuming (375). As a result, the detected marks appear as red-colored, not fading with time.

Miscellaneous - The use of silver nitrate to detect marks on porous substrates has been re-evaluated by Schwarz & Hermanowski (376). They concluded that silver nitrate could give results on modern papers, but is not recommended for use regarding the appearance of the marks and background staining, especially when compared with the conventional amino acid reagents (e.g. NIN).

Electrochemiluminescence was used to detect latent fingermarks on conductive substrates (377-379). The ridge pattern acts as an inert mask, resulting in negative images of the fingermarks in presence. The visualization is caused by the electroluminescence reaction between Rubpy and tri-n-propylamine, occurring only where the metal remains untouched by the fingertip (377, 378). In another study, rubrene was applied, according to two different application protocols: being a lipophilic compound, it can be applied to stain the sebum-rich ridges, or it can also be applied to stain the background (379).

Aqueous electrolyte solutions were used to detect fingermarks on metal (i.e. copper, aluminium, iron, brass, zinc) and non-metallic substrates (i.e. glass, plastic) (380). The technique simply consists in immersing the samples in solutions of different pH values (using sulfuric acid or sodium hydroxide) and observing the fingermarks appear.

A method based on the decay of surface charge measured by an electric potential sensor is proposed to detect marks on plastic (381, 382). This technique is different from the ESDA, which is based on the application of a large electric field. It is hypothesized by the authors that the decay of the surface charge may constitute a way to date or estimate the sequence of deposition of the marks in presence.

The spraying of two diacetylene monomers in acetone (i.e. HDDPU and HDDCPU) was shown to successfully detect fingermarks (especially sebum-rich ones) on both porous and non-porous substrates, leading to a purple-on-white contrast (383). Due to the chemical structures of the reagents, it is also possible to chemically image the marks left on an illustrated substrate using FTIR.

Chemical lifting of fingermarks from non-porous substrates using a thermoplastic polyurethane resin combined with fluorescein is reported (384). The marks appear in red, after exposing the film a few seconds to hot air (i.e. 100°C). TPE solution was shown to aggregate into sebum-rich secretion residue left on non-porous substrates (386). Given that TPE is non-luminescent in the soluble state but becomes luminescent after forming aggregates, the resulting marks become blue-luminescent under UV light.

Spatially selective electrodeposition of Prussian blue (385) was performed to visualize fingermarks on conductive substrates. The marks act as masks preventing the deposition of the dye, resulting in a blue-coloration of the substrate only.

1.4 Photography, forensic light sources, and digital/chemical imaging

1.4.1 Photography and alternative light sources

Digital imaging was shown to be useful when suppressing an unwanted background illustration or dealing with round objects (387), as well as for enhancing a coloured mark on a coloured substrate (388, 389). The smart combination of observation filters is not to be neglected given the enhancements that could be obtained (390). Some studies showed the advantages of using laser (391, 392) or LED (393) to record fingermarks. Imaging in the UV (394, 395) and in the IR range (341, 343, 396) showed their advantages. All the articles dealing with the recording of blood marks using alternative light sources (336, 342, 344) are described in section "2.3.12. Scenario – Blood marks".

Used acronyms: CCD (charge-coupled device), DEUS (digital enclosed ultraviolet imaging system), DFO (1,8-diaza-9-fluorenone), IND/Zn (1,2-indanedione/zinc chloride), IR (infrared), LED (light emitting diode), NIN (ninhydrin), NIR (near-infrared), RUVIS (reflected ultraviolet imaging system), UVC (ultraviolet C)

Photography - Two examples of how digital imaging could help in visualizing fingermarks were proposed (387). The first case consists in suppressing the contribution of an illustrated background for a DFO-processed mark on a printed document. The second case consists in overlapping sequential pictures of friction ridges on a cartridge case, then to merge them to generate a flat panoramic view of the detected mark. The use of colour channels in Adobe Photoshop® is illustrated to enhance a NIN-processed mark on a coloured substrate (388, 389). Dalrymple demonstrated that the combination of narrow bandpass filters with a barrier filter could be advantageous when capturing a fingermark in luminescence, especially when the fluorescence of the background may be problematic (390). Optimal conditions (filtering between 470-575 nm) for the recording of NIN-developed marks were investigated as a function of various substrates (397).

Laser - A study aimed at evaluating the best light source to visualize fingermarks detected using IND/Zn, as well as using two emerging amino acid reagents (i.e. genipin and lawsone) (391). The Coherent TracER lasers (460 nm, 532 nm, 577 nm) proved to be the most sensitive at detecting untreated fingermarks, and led to higher ridge clarity. Genipin and lawsone gave unsatisfactory results, and require more development before becoming competitive (formulation and detection protocols). A pulsed Nd-YAG laser and a cooled CCD camera with an image intensifier were used to visualize fingermarks on porous substrates bearing printed texts (392). For this experiment, paper sheets were black-printed using different laser and inkjet printers. The native fluorescence of the marks was observed using optical filters and a time-resolved method. The fluorescence of most printed papers is weak, because ink or toner absorbs the fluorescence of the paper. Excitation at 280 nm is preferred (over 230 nm).

LED - A LED emitting in the IR range (i.e. 940 nm) was used to non-destructively record fingermarks powdered at crime scene, before lifting them (393). By recording in the IR range (900 – 950 nm), black-powdered ridges appear black while multi-coloured background or printings disappear or appear as a single bright colour.

UV - Three fingermark imaging systems based on UVC light source were compared: (a) a DEUS system (home-made UVC-sensitive back-thinned CCD and camera), (b) a RUVIS system UVC-sensitive image intensifier, and (c) a flatbed scanner fitted with a UVC light source (394). The DEUS system gave the best results on porous and non-porous substrates, followed by the RUVIS and the flatbed scanner. It should be noted that using a digital camera with real-time output (i.e. "live" mode) increases the effectiveness of imaging fingermarks. Reflected UV to visualize or enhance latent marks has been extensively described and explained by Richards and Leintz (395). This article is more focused on bitemarks and shoemarks, but constitutes a good overview for people interested in buying the adequate equipment to record reflected UV images (to visualize fingermarks).

IR - A CONDOR Hyperspectral Imaging System was used to visualize untreated fingermarks present on various substrates (e.g. paper, adhesive, aluminum) (396). Data was collected from 400 to 720 nm and digitally processed to reduce the background interference and increase the resulting contrast. This non-destructive method could have its place when chemical treatment is not possible, for example on

delicate supports. A visible/NIR CONDOR Hyperspectral Imaging System (650 to 1100 nm) has also been used to observe bloodstain patterns on black fabrics, hardly visible to the naked eye (343). This technique combines digital imaging with conventional spectroscopy for analysis of samples. In another study, IR photography has been used to detect and localize latent bloodstain evidence lying beneath a layer (or multiple layers) of paint, using a tungsten halogen lamp as source of visible and IR light (341). Blood marks could be detected beneath up to six layers of paint under reflected IR, depending on the characteristics of the paint (especially their IR transmission capability).

1.4.2 Chemical imaging

Chemical imaging has for aim to provide additional information, more than just the morphological one (ridge pattern) (160, 285), for example by enhancing the presence of explosives or metabolites in the sweat residue. Some are non-destructive (e.g. FTIR, Raman, OC-LIBS, CWL), while others require covering the fingermark with a matrix before allowing the analysis (e.g. MALDI). From a chemical point of view, specifically modified CA monomers or reagents were synthesized to be suitable for chemical imaging (221, 222, 383). SERS was used to specifically visualize or target secretion components (292, 398) as well as exogeneous contaminants (360, 399). The use of a CWL sensor has been extensively studied to estimate the age of fingermarks (177-182), but also to separate overlapping marks (400) or localize marks on various substrates (401-407). A group of researchers proposed to use a new kind of powder to detect fingermarks and allow their analysis using a MALDI-MS(I) technique (408-410). MALDI-MS can be used in an extended range of scenario (408, 410-412), but is mainly used to visualize exogenous materials contained in the secretions metabolites (408, 410, 412-415), as well as trying to determine the sex of the donor (416). Among the miscellaneous techniques, it is possible to cite: the use of OC-LIBS to localize explosives in secretion residues (358), use of SECM to image fingermarks (359, 417, 418), ToF-SIMS to determine the chronology of events between writing and fingermark deposition (419, 420), ESDA to reach the same goal (421), and SALDI-ToF-MS to detect exogeneous material in secretion residues (260-262), and finally capillary-scale ion chromatography to detect gunshot residues (422).

Used acronyms: ATR (attenuated total reflectance), CA (cyanoacrylate or cyanoacrylate fuming), CHCA (α-cyano-4-hydroxycinnamic acid), CWL (chromatic white light), DART (direct analysis in real time), DNT (dinitrotolueme), ESDA (electrostatic deposition detection apparatus), FTIR (Fourier transform infrared spectroscopy), GC (gas chromatography), MALDI (matrix assisted laser desorption ionisation), MeV (mega electron volt), MNT (mononitrotoluene), MS (mass spectrometry), MSI (MS with imaging), NIN (ninhydrin), OC-LIBS (optical catapulting in combination with laser induced breakdown spectroscopy), SALDI (surface-assisted laser desorption ionization), SECM (scanning electrochemical microscopy), SERS (surface-enhanced Raman spectroscopy), SIMS (secondary ion mass spectrometry), TNT

(trinitrotoluene), ToF (time of flight), VMD (vacuum metal deposition), XPS (X-ray photoelectron spectroscopy).

A review about the advantages and use of chemical imaging has been proposed by Hazarika and Russell (285), and a comparison between various analytical techniques (e.g. MALDI-MS, ToF-SIMS, MS, XPS, ATR-FTIR) by Bailey *et al.* (160). In this study GC/MS was found to be the most sensitive to amino acids, MALDI to lipids and peptides, and XPS to the carbon configuration and inorganics. XPS, MeV-SIMS, ToF-SIMS, and ATR-FTIR spectroscopic imaging present the advantage of requiring no sample preparation.

FTIR - Tahtouh *et al.* synthesized modified CA monomers specifically designed to optimize their visualization through an FTIR-based chemical imaging process, while keeping their ability to be fumed on marks (221, 222). Highly interesting results were obtained with one of the monomers (1-cyanoethyl 2-cyanoacrylate) on Australian polymer banknotes, especially on the intaglio printings. De Grazia *et al.* imaged marks processed using diacetylene copolymers on both porous and non-porous substrates (383).

Raman and SERS - SERS was used to visualize fingermarks through the targeting of lipids and amino acid components (398). For the SERS effect to occur, it is necessary that metal nanoparticles are in contact with the analytes. Antibody-functionalized silver nanoparticles were also used to specifically target sweat components, followed by SERS imaging (292). To allow an optimized visualization, the nanoparticles were also functionalized with a Raman probe, i.e. 4-mercaptobenzoic acid, for which the Raman peaks were identified and easily imaged. A semi-automated Raman-based chemical imaging was used to visualize fingermarks, as well as to identify threat materials present in the secretions (e.g. drugs, explosives) (360). To gain a lot of time, only a limited number of points of interest were analysed, selected on the basis of the fingermark optical images. This method also works if the fingermark has been processed with CA. Finally, fingermarks contaminated with b-carotene and fish oil were imaged on various substrates (e.g. paper, cardboard, metal, adhesive) using a line-scanning Raman imaging system (399).

CWL sensor - The CWL sensor is a technology that makes use of the chromatic aberration of light to generate a topographic image of the sample. CWL sensors were used to separate overlapped fingermarks (400), to localize marks on various non-porous substrates (e.g. glass, varnished wood, metal, plastic) (401-403), and is seen as a key element of a contact-less acquisition device (404), which could be used on crime scene (405). A CWL sensor has also been used to estimate the age of fingermarks left on various substrates (177-182), as described in "2.2. Composition, aging and persistence of fingermarks". Another application of a CWL sensor aimed at classifying the surfaces according to texture parameters, and hopefully allowing the detection of fingermarks (406, 407).

MALDI-MS - Contrary to the other non-destructive techniques (such as FTIR or Raman), MALDI-MS requires covering the fingermark with a specific matrix before performing the analysis. A two-step matrix application method is commonly applied in this context, i.e. the “dry-wet” method, for which the matrix is first dusted with CHCA

onto the sample then solvent-sprayed (408, 409). More recently, curcumin was proposed as an efficient, natural and colored matrix for MALDI-MS analysis, in replacement of CHCA or as solvent-free matrix (410). The authors using that method emphasize the fact that the powdering step allows by the same way the visualization of the latent marks, given that the matrix absorbs UV light and fluoresces. A review of the use of MALDI-MSI to visualize fingermarks is proposed by Francese *et al.* (411). In details, MALDI-MSI was used to visualize fingermarks (or separate overlapping fingermarks) using ion signals that are characteristic of secretion endogeneous species (e.g. amino acids, lipids) (408, 410, 412), metabolites (413), and contaminating substances such as condom lubricant (414, 415), antiseptic (408), or drug (410, 412). Some authors also claimed being able to determine the sex of a fingermark donor by using MALDI-MS (success rate from 67.5 to 85%) (416). This study was based on multivariate modelling of mass spectrometric profiles of fingermark peptides and small proteins contained in the secretion.

Miscellaneous - OC-LIBS has been used to analyse explosive residues (i.e. TNT, DNT and MNT) in contaminated fingermarks left on glass (358). Discrimination between explosive and non-explosive materials is possible.
SECM has been applied on fingermarks which were artificially contaminated with an explosive (i.e. picric acid) (359) or left on glass and detected using an alternate VMD process (i.e. Al-ZnO) (417). SECM has also been applied to detect fingermarks on metal substrates (i.e. platinum, gold, silver, copper and stainless steel) (316), or various substrates (418). SECM is a technique based on the response given by a local oxydo-reduction reaction, which takes place if a target is present in the sample.

ToF-SIMS chemical mapping was used to determine whether a fingermark has been deposited before or after a text was written (419) or printed using a laser printer (420). This technique requires: (a) the presence of some endogeneous ions in sweat and not in the laser ink (e.g. Na^+, K^+ and $C_3H_5^+$ ions), and (b) the visualization of these ions only if the mark is deposited above ink (and has consequently been left after the ink was printed). However, the ink signal could sometimes be visualized from beneath the ridge, or be lower than expected even when lying on top of the fingermark (419). The ESDA was also shown to allow determining the order of deposition (fingermark or ink) when processing laser-printed documents (421). If a text is printed after a mark has been left, the ESDA will result in unbroken white lines, whereas the opposite scenario (i.e. a mark left on a printed text) will result in dark lines bearing ridge details. It should be noted that: (a) the technique can be applied after a NIN process, and (b) the sequence determination success rate drops quickly as the mark age. The order of deposition between latent fingermarks and laser printed ink has been examined using chemical mapping with secondary ion mass spectrometry (423). Blind testing on 21 samples results in correct determination for all samples.

SALDI-ToF-MS was used to detect terbinafine (i.e. a medication) as a metabolite in sweat secretions (260). To reach this goal, magnetizable carbon black-doped silica nanoparticles were used to dust the fingermarks and act as signal enhancing agents for SALDI-ToF-MS. The same particles were used to detect the presence of explosive in sweat secretion on various substrates (i.e. stainless steel, glass, paper, plastic bag, metal drinks can, wood laminate, adhesive and white ceramic tile), using SALDI-ToF-MS and DART-MS (261). Seven common explosives were used (i.e. six nitro-organic- and one peroxide-type) and were detected in the nanogram range. The

same nanoparticles and analytical technique were also used to study the composition of the secretion residue in terms of polar and non-polar constituents (i.e. amino acids and squalene / fatty acids, respectively) (262).
Capillary-scale ion chromatography was applied to detect gunshot residue or black-powder contamination in secretion residue, as well as exogeneous species in the sweat of smokers (422).

Miscellaneous marks

1.5 Earmarks and earprints

The possibilities of identification offered by the comparison between earmarks and earprints are still the subject of a few publications. The operational successes obtained in the region of Hamburg have been reported (424). The paper also provide an extensive bibliography related to the early work carried out in Germany in that area.

A method of earprint deposition has been proposed (425). An apparatus based on an ear defender headset, integrating a spring that allows controlling the force with which an ear is pressed to a substrate is presented. The ears were coloured beforehand with yellow vegetable dye. High reproducibility of the measured variables on different earprints, taken by different operators, was achieved.

A pilot study of ear identification based on photographs, aiming at the investigation of personal identification by the ear from surveillance videos has been carried out (426). The authors divide the ear into four regions (concha, helix, antihelix and lobe), and measure the relative surface (with respect to the entire ear) of these different surfaces. Good reproducibility (within and between observers) is found, and a low probability of observing the same measurements on two different ears has been computed using a parametric model.

One study (427) investigates sex differences in the external ear of the Indian population and finds that there are differences between male and female donors with respect to lobe length and breadth as well as ear length, breadth and the height at the base of the auricle.

Junod and colleagues (428) presented an automatic system allowing the matching between earmarks and earprints. The system also allows assigning weight of evidence (in the form of a likelihood ratio) to each comparison undertaken. The authors detailed the system performance including measures of the rates of misleading evidence. For mark to print comparison, the equal error rate is 2.3%. The system has been tested on a database of 1229 donors and also in cases from police forces. A review of automatic systems used from earmarks and earprints has also been published (429).

1.6 Foot morphology

The link between foot dominance and morphological characteristics as well as the link between foot and hand dominance have been investigated (430). These links

would allow, from barefoot impressions from the crime scene, to determine first the dominant foot and then the dominant hand. Foot width and two foot lengths (related to the first and second toe, respectively) were used as descriptors. Results did not show very clear relationships between these factors.

Hammer and colleagues (431) studied the possibility of carrying out comparisons between the impressions on shoe insoles with inked comparison material. Both impressions from insoles and inked materials were used for these comparisons. A number of measurements (chosen for discrimination as well as discernibility on the insoles) were carried out and compared, and overlay comparison was also used. Like-to-like (insoles to insoles) comparisons showed more similarity when indeed from the same source; it was however still possible, in this study, to attribute the impressions on shoe insoles to the right source using inked impressions as a reference. In casework involving a question about the mark on the insole of a shoe, the authors recommend using shoes known to have been worn by the putative source as comparison material.

1.7 Lipmarks

Reviews on lip prints as well as their forensic use have been carried out in the time covered by the present review (432-434). Vanishree and coauthors (435) describe detection techniques useful for the visualisation of latent lip marks. Three dyes (Sudan Black, vermilion and indigo) have been compared for the visualisation of lipmarks left with classic or long – lasting lipstick on china as well as cotton and satin fabric (436). Their performance has been found to be similar.

Several studies assess the frequencies of different lip patterns in populations (437-445); with the exception of (441) and (444) these studies also find differences in the frequencies of patterns between the genders. Verghese and Mestri describe frequencies (446) and furthermore exclude a link between lip patterns and blood group. The relationship between sex and lip patterns (447) and between age, sex, and lip patterns (448) has been investigated in more detail; sex differences have been found (447), but in (448) they depended on the age class of subjects. Ludwig and Page (440) also investigate the comparison between photographs of lips and lip impressions using more intricate detail than just the classification results, and present such comparisons in detail. One study aims at establishing uniqueness of lip prints based on a sample of 200 individuals, including five pairs of twins (449). The authors also show some similarity of patterns between parents and children on the basis of five families (consisting of mother, father and 2 children). Finally, the lip prints of 20 individuals were recorded at a 3-month interval to show permanence (449). A similar study investigating individuality and permanence (over the time of one year) has been carried out (450). Three other studies investigated the question of uniqueness on a sample of 100 individuals (451), one of 200 individuals (452) and on one of 124 individuals (453). Choraś (454) proposes a method for automated feature extraction from lips.

1.8 Identification of deceased individuals

Campbell (455) describes the retrieval of a fingerprint from the underside of the epidermis of a body whose outer epidermis was too decomposed to obtain a good

image. Subsequently, a hit in the AFIS was obtained. A revivification method of the epidermis used in Germany has also been published (456).

An analysis of the identification methods used on 134 bodies of unknown identity shows that 10 were identified by their fingerprints. Such identification was only carried out when a pre-mortem set of prints of the suspected identity was present in the national database (457). The admissibility of fingerprint evidence, in particular in the U.S. and Canada, is mentioned in an article detailing different means of identifying deceased individuals (458). In order to properly identify deceased individuals in an institute of legal medicine, livescans of two fingers were taken from bodies upon entry, and the identity verified when the bodies were released (459). This was used as an additional insurance of the proper identity. Identification of deceased individuals when identity theft has occurred is the object of another article (460); several case reports including cases where identification through fingerprints is problematic due to identity theft are presented (460). The fingerprints of 109, up to then unidentified human remains, have been sent to larger fingerprint databases (Department of Homeland Security Biometric Support Center and the FBI Criminal Justice Information Services Special Processing Center rather than the local database). This allowed the identification of 51 of these cold cases (461). The special case of a 2650 year old body has been reported during the period of review (462); using photography and the image enhancement tools of an automated fingerprint identification system, the general patterns of the fingers of the right hand were still visible, and there were enough minutiae on the image of the right thumb for an individualisation.
The practice of retrieving latent impressions at the residence of a presumed identity of a deceased individual in order to identify this individual is described and defended using Occam's razor (463). The same argument is also applied then to fake fingerprints, stating the fact that in general, the most simple explanation of the presence of a mark on a scene is touch by the finger rather than planting.

1.9 Various subjects

In order to detect / avoid tampering with raw fingerprint images in biometric systems, a watermarking method is proposed by Li (464).

The marks left while wearing gloves have been studied (465). The authors indicate how impressions from friction ridge skin may be left even when gloves are worn, when the material constituting the glove is very thin and flexible.

Crime scenes and case reports

Used acronyms: CA (cyanoacrylate fuming), DFO (1,8-diaza-9-fluorenone), ORO (oil red O), RAM (rhodamine – Ardrox – methylene blue)

Beaudoin has reported the use of ORO on a 21-year-old cold case involving the processing of papers (used to start a fire) (466). DFO was applied first, giving negative results, and was followed by ORO, which led to the detection of two fingermarks. In this article, the recipe and application protocol are described.

The use of a 532 nm TracER laser led to the observation of an additional fingermark on a duct tape processed with superglue followed by TapeGlo (fluorescent stain) (467). This mark was barely visible using a conventional alternate light source (Omniprint 1000B).

Successful recovery of latent marks on an *Agave Americana* (six-feet-plant with thick green leaves) was reported in the context of a home robbery (468). Black (magnetic) powder followed by lifting, and CA followed by RAM dye staining were chosen.

The inside of interior door handles should not be neglected when processing a (stolen) car, given that very good quality marks may be detected (469).

The case reports related with cartridge casings (317-319) are described in section "2.3.8. Substrate – Metal and cartridge cases".

Rubber-like casting materials (i.e. Accutrans and Reprorubber) were chosen to allow the fingerprinting of an Egyptian mummy without causing damage to it (470).

Wendt *et al.* (471) presents the setting of the new fingerprint detection laboratory in Kiel (Germany).

The management issues associated with a fingerprint unit are covered by Tomaszycki (472).

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Body Fluid Identification and DNA Typing in Forensic Biology

Review 2010 – 2013

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1. Introduction

This review focuses on some of the most notable developments that occurred during the years 2010-2013 in forensic biology, and which are likely (at least in the view of the author) to become major trends in the near future. The selected topics include the evolution of autosomal STRs, extended minimal typing sets and new generation multiplex, as well as challenges brought on by ever increasingly sensitive typing techniques; the development of automated DNA typing tabletop instruments destined to be field-operated by non-scientists; the evolution of Y-STRs and the characterization of rapidly mutating and highly discriminating new Y-STR markers; small biallelic markers such as SNPs and the emerging indels; phenotypic profiling with markers for visible traits; emerging technologies for body fluid identification such as messenger RNAs, microRNAs and DNA differential methylation patterns analysis; and potential future multipurpose analytical platforms. Other topics have been extensively covered in previous reviews, or could be covered in future reviews, including expert systems for automated DNA analysis, X-chromosome markers, mitochondrial DNA analysis, and protocols for treatment of biological evidence in mass disasters or terrorist events.

2. Evolution of autosomal STRs

2.1. Extended minimal typing sets and recent multiplexes.

Over the last few years, there has been international acknowledgment of the need to expand the obligatory, minimal sets of STR loci for routine typing in forensic laboratories. Major reasons included the need to increase international data compatibility and sharing, and also the need to increase discrimination power in order to aid in kinship analysis and missing person cases as well as to reduce likelihood of adventitious matches as database sizes continue to increase. In Europe, there was also a will to improve sensitivity and success rate with degraded DNA – i.e. shorter amplification products - and overall robustness.

In 2008-2009, following the Prüm treaty and recommendations from the European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) [1, 2], the European community added 5 new STR loci (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) to the already existing 7-loci set, to establish the 12-loci extended European Standard Set (extended ESS).

In 2012, the CODIS Core Loci Working Group recommended that the CODIS core be expanded by the addition of D2S1338 and D19S433, already commonly used worldwide as well as in US, and of four of the five new European loci (D1S1656, D2S441, D10S1248, D12S391). The Y-STR DYS391 is also added to the new CODIS core to confirm gender when amelogenin null alleles are observed [3, 4]. TPOX is removed from the core and put on the list of three loci recommended for optional additional inclusion by the manufacturers (in ranking order of preference: TPOX, D22S1045 and SE33), which brings the new CODIS core to 18 autosomal STR loci (20 core loci with the Y-STR and amelogenin). Therefore, with the exception

of D22S1045, the extended ESS is included in the new CODIS core, bringing to 11 autosomal STR loci the overlap between CODIS and European cores.

In response to these recommendations, manufacturers have made available a number of new multiplexes [5]. Several multiplexes were developed that add the 5 new European loci to the 10 SGM Plus® loci, all of which are now available in versions that also include the highly polymorphic SE33 loci: NGM™ and NGM SElect™ [6] (5-dye, Life Technologies), the set of PowerPlex® ESI 16/17 [7, 8] and ESX 16/17 systems [9] (5-dye, Promega), and the Investigator ESSplex and ESSplex SE (5-dye, Qiagen, cannot be sold in US due to patent restrictions). Life Technologies and Promega buffer systems have been improved to increase robustness, and all kits address the issue of shorter amplicon size and higher performance with degraded material. In the Life Technologies kits, 10 of the 15/16 STR loci are less than 250 bp in length, including 3 of the new European loci measuring under 125 bp in length. In the Qiagen kits 4 of the 5 new European loci are less than 200 bp in length. Promega ESI systems focus on miniaturization of loci from the 'conventional' ESS (amplicon size less than 230 bp), while the ESX systems deliver the 5 new European loci as mini STRs (3 loci less than 125 bp, and 2 loci from 125 to 185 bp). The evaluation of all these kits and concordance studies were recently performed by the ENFSI, which found all kits fit-for-purpose [10].

To match both the new CODIS core and the extended ESS and provide maximal discrimination power, other more recently developed multiplexes include up to 24 loci. In addition, these new generation multiplexes integrate in the same amplification reaction both "standard" and mini-STRs. GlobalFiler™, a 6-dye system from Life Technologies to be released in 2013, integrates the new CODIS core loci (18 autosomal STRs, 1 Y-STR, amelogenin) with the 3 optional loci recommended by the CODIS Core Loci Working Group (TPOX, D22S1045, SE33, for a total of 21 autosomal STRs), plus one Y-indel. The Y-indel is smaller than amelogenin (80 bp range). The kit includes 10 autosomal mini-STRs of less than 220 bp in length. The probability of identity value is less than 10^{-11} for the 10 mini-STRs alone, and less than 10^{-26} for the 21 autosomal STRs. PowerPlex Fusion, a 5-dye system from Promega launched in September 2012, includes the same loci as GlobalFiler except for SE33 and the Y-indel, but with the addition of Penta D and Penta E (for a total of 22 autosomal STRs). Eight autosomal loci of this system are less than 220 bp in length, and the probability of identity value is less than 10^{-27} for the 22 autosomal STRs. It is worth noting that if adding the CS-7 Custom system from Promega (a complementary system recently designed for kinship testing that includes LPL, F13B, FESFPS, F13A01, Penta C, Penta D and Penta E), the number of autosomal STR loci now commercially available for typing reaches 26 (FESFPS and Penta E are linked and require the use of haplotype frequency tables).

2.2. Increased sensitivity and low template DNA (LT DNA).

Since the introduction of multiplex PCR and STR analysis, the field of forensic DNA typing has witnessed a continuous improvement in technological performance, not only in kits discrimination power but also in overall analysis sensitivity. Improved sensitivity and robustness of the kits, plus the switch to capillary electrophoresis and the development of more sensitive instruments lead to the detection of ever smaller amounts of genetic material. However, in addition to its obvious benefits, the extreme sensitivity of DNA analysis techniques has brought new and more complex

challenges. Now more than ever, routine casework DNA typing requires stringent procedures, not only at the laboratory, but also before a sample reaches the laboratory. Police standard operating procedures must be upgraded to minimize exhibit contamination risks. Laboratory plasticware and other disposable materials must be improved to meet forensic standards. Recently a common position statement from ENFSI, SWGDAM and BSAG [11] proposed to manufacturers the introduction of a new “DNA free” product grade for forensic applications. The statement also recommended that forensic laboratories maintain elimination databases including DNA profiles from all categories of personnel or visitors and also from investigators and crime scene technicians, as well as unexplained profiles observed in negative controls. Higher sensitivity also impacts casework interpretation and assessment of plausible scenarios to explain the obtained results. Secondary DNA transfer has been demonstrated under a variety of conditions [12, 13, 14], and a lot of work still needs to be done to better understand the relation between a given amount of DNA and the manner it got there, whether it is through direct contact (primary transfer), or secondary or even tertiary transfer [15].

But the consequences of higher sensitivity are not limited to increased vigilance for contamination risks or for the likelihood of secondary DNA transfers. The number of partial and/or bona fide mixed DNA results has risen exponentially, and stochastic effects that were less of a concern in early days of STR analysis are now part of the everyday life of the forensic biologist, raising the complexity of results interpretation to a much higher level [15]. And as the number of complex results increased, so has the number of controversial judicial cases [16-18].

Initially restricted to specific amplification protocols (higher number of amplification cycles), “low copy number DNA”, now “low template DNA” or LT DNA, is now generally recognised as a much larger concept. Indeed, even with “standard” amplification protocols (optimal amount of input DNA, 28-30 amplification cycles), any mixed sample may actually include low template contributors, and raise the same interpretation and statistical issues than results obtained from smaller amounts of DNA.

The relationship between signal intensity and the amount of input DNA is not absolute. It could be said that the PCR process is inherently stochastic to some degree, as exemplified by the relative peak height of the two alleles of a heterozygous pair (allelic balance). Nevertheless DNA amplification provides reproducible, consistent results with good allelic balance when an optimal amount of DNA template is being amplified. However, as the number of DNA template molecules diminishes, increasing allelic imbalance is observed. In the most extreme form of allelic imbalance, one allele from a heterozygous pair may fall below the detection threshold resulting in a drop-out. In addition, low intensity alleles presumably originating from fragments of chromosomes normally present in the environment may appear sporadically (the so-called drop-ins) and be confused with the alleles of an authentic contributor.

Over the years two main schools of thought have developed regarding the interpretation of LT DNA, namely the “threshold approach” and the “probabilistic approach”. In the former approach, thresholds are set to establish a frontier that separates peak heights at which stochastic effects may have occurred, from peak heights at which it can be assumed they have not. Peaks below the stochastic

threshold value are not included in the statistical calculation of the weight of the evidence [19, 20]. In the probabilistic approach, the likelihood of stochastic effects at a given peak height is taken into account in a continuous manner, by integrating a probability of drop-out and a probability of drop-in in the calculation [21, 22]. The debate between the two approaches has culminated in a series of articles, followed by a number of commentaries and rebuttals, published in 2009-2010 in Forensic Science International: Genetics. This lead to an editorial in January 2011, asking for more research and banning further publication of letters to the editor unless it provides new data and insights to the problem [23].

There is no doubt that the probabilistic approach better translates the continuous increase in allelic imbalance observed when the number of template molecules diminishes. In the threshold approach, peaks right below the stochastic threshold, which obviously have a very low likelihood of drop-out (since they are so close to the threshold), are interpreted the opposite way than peaks right above the threshold, and ignored from the statistical calculation. This has been described as the “falling off the cliff” situation.

Then again, major problems with the probabilistic approach have been the fact that software and statistical tools were lagging behind the theory, coupled with the lack of guidelines on proper methods to determine drop-out and drop-in probabilities. However considerable progress was made in that regard over the last couple of years, and extensive research and biostatistical tool developments are currently ongoing. Recently, the International Society for Forensic Genetics (ISFG) DNA commission has published recommendations on using the probabilistic approach for the interpretation of mixtures that included an experimental design to determine drop-out and drop-in probabilities [22]. The ISFG also launched an initiative to develop biostatistical software, and made several open source software and tools for the interpretation of complex mixtures available on its website. In December 2012, Forensic Science International: Genetics published a special focus issue that included 10 articles on the interpretation and biostatistical analysis of complex and low template DNA samples.

With the highly sensitive STR typing systems available today, complex DNA mixtures that include low template contributors have come to represent a large proportion of DNA results obtained from casework. It becomes increasingly impossible to put them all aside as “too complex for interpretation”. The sound interpretation of such results, and particularly the attribution of a proper statistical weight, has become one of the most important challenges for forensic biologists. This will certainly remain so for the next few years. It’s one thing to have access to increasingly sensitive analysis systems, it’s another to manage the ever growing number of complex results.

2.3. Microfluidics and “Rapid DNA”.

By the end of the first decade of 2000, following the enormous technological progress accomplished over the last 15 years in genomics, microfluidics and miniaturisation of molecular biology reactions, the US government developed a project for making new instruments available to various law enforcement agencies, allowing rapid DNA analysis of reference samples by non-scientists. The project, named “Accelerated Nuclear DNA equipment” (ANDE), is jointly sponsored by the Department of Defense, the Department of Justice and the Department of Homeland Security. In

2009, NetBio was awarded exclusive funding to further develop integrated, microfluidic-based rapid STR typing and produce a prototype in a defined timeline. Subsequently NetBio partnered with GE Healthcare Life Sciences. Since then, other companies and laboratories have put efforts in developing instruments as well [24, 25], such as IntegenX, the Lockheed Martin/Safran Morpho/ZyGEM consortium, and the Center for Applied NanoBioscience & Medicine from the University of Arizona [26-28]. As of this writing, only the DNAscan™ instrument from NetBio/GE and the RapidHIT™ 200 instrument from IntegenX are available. Both are mobile small tabletop instruments designed to analyze standardized samples such as buccal swabs, and integrating all DNA typing steps, from loading sample cartridges to obtaining a DNA profile, in a single fully automated apparatus that can be run by non-scientist operators at the push of a button. DNA profiles can be obtained in less than 90 minutes. No refrigerated storage space is needed, as reagent cartridges/cassettes are stored at room temperature. Contamination risks are minimized by using self-contained cassettes and built-in traceability maintains the chain of custody. The RapidHIT™ 200 instrument can analyze 1 to 8 swabs; it uses the PowerPlex 16 system in the US and the PowerPlex ESI system in Europe. The DNAscan™ instrument can analyze up to 5 swabs, and uses the PowerPlex 16 system.

3. Evolution of Y-STRs

Analysis of STRs located on the Y-chromosome is now common in forensic casework. It is used to obtain male DNA profiles from samples containing large proportions of female DNA, or to determine the minimal number of male contributors (belonging to different paternal lineages) in a DNA mixture. However, analysts are still confronted with the main inherent limitations of Y-STR typing: low statistical weight and dependency on the size of haplotype databases, and the inability to distinguish males belonging even remotely to the same paternal lineage. In order to improve the former, international efforts have been put to build large consolidated Y-haplotype databases accessible to the forensic community, and forensic laboratories around the world are encouraged to enrich these databases with their own population data upon review and publication in forensic journals. There are now two main Y-STR databases: the Y-chromosome haplotype reference database (YHRD, Europe, as of this writing more than 100 000 haplotypes from more than 800 different populations, including over 53 000 haplotypes on 17 loci) and the US Y-STR database (as of this writing, over 25 000 haplotypes, including more than 15 000 haplotypes on 17 loci).

Another limitation of the current set of Y-STR markers is haplotype resolution across world-wide populations: very high for some population (e.g. European), but not so for others, for instance inbred populations or populations that went through a recent bottleneck (founder effect) [29]. Therefore, concurrently to the building of large haplotype reference database, research continues to further characterize Y-STR loci in order to expand the panels available and increase haplotype diversity.

Recently, much interest was raised by an extensive study performed on Y-STRs mutation rates and mutation mechanisms. The authors investigated 186 Y-STR loci in nearly 2 000 DNA-confirmed father-son pairs [30]. Although the majority of loci

were found to have a typical mutation rate in the orders of 10^{-4} – 10^{-3}, the authors identified 13 loci with much higher mutation rates, in the order of 10^{-2}, which they named “rapidly mutating” Y-STRs or RM Y-STRs. From the 2 000 father-son pairs typed on 186 loci, a total of 924 confirmed mutations were observed. Half of these mutations were covered by the 13 RM STRs, highlighting the potential of these loci to provide higher haplotype diversity and particularly to distinguish related males.

The forensic applications of these RM Y-STRs were specifically investigated in a subsequent study [31]. A set of 604 unrelated males from 51 populations in 8 geographic regions were typed with either YFiler or the 13 RM Y-STR (in 3 multiplex reactions using 1-2 ng DNA each), and the discrimination capacity of both STR sets were compared. Analysis of the 17 YFiler loci produced 511 unique haplotypes, plus 33 haplotypes shared among 85 males. In comparison, analysis of the 13 RM Y-STRs allowed observing 595 unique haplotypes, and only 3 haplotypes were shared among 8 males. In addition, pairwise comparisons between individuals showed an average of ~12 allelic differences with YFiler and ~18 allelic differences with RM Y-STRs. Thus the smaller set of RM Y-STRs showed much higher discrimination capacity and haplotype diversity. Furthermore, the authors report that the level of population substructure between geographic regions detected with RM Y-STRs is much lower than when detected with YFiler, indicating that RM Y-STRs may erase or reduce founder effects and generate a more homogeneous distribution of haplotypes across worldwide populations.

The capacity of RM Y-STRs and YFiler to distinguish male relatives was also assessed with 156 pairs of males related to various degrees. The results obtained showed that the 17 loci of YFiler could differentiate 7.7% of father-son pairs, 8% of brother pairs, and 25% of cousin pairs. In great contrast, the 13 loci of the RM Y-STRs could differentiate 48.7% of father-son pairs, 60% of brother pairs, and 75% of cousin pairs.

These exciting results highlight the need for thorough characterization of STR loci, and the need to use loci that were properly ascertained for specific uses [29]. For the same reasons they are of superior value for discriminating among males, RM Y-STRs would obviously not be appropriate for paternity and kinship testing. For those, currently used Y-STRs with low and moderate mutation rates are the most appropriate markers for the moment.

There remains the problem of estimating the statistical weight upon non-exclusion. If RM Y-STRs were to complete or replace the current Y-STR panel in the near future, the replacement of hundreds of thousands of Y-STR haplotypes to the existing Y-STR databases would be a titanic task. Moreover, since RM Y-STRs are more polymorphic, it follows that reliable frequency estimates may require even larger haplotype databases. In any event, it will be a while before the higher power of discrimination of RM Y-STRs impacts on statistical weight in casework. For the moment, the 13 RM Y-STR set can be used as an adjunct system in cases where non-exclusion is observed, enhancing the discrimination power and the chances of revealing what may be the “true” result, i.e. an exclusion.

Some RM Y-STRs have already been included in new generation Y-STR multiplexes. In 2012 Promega launched the PowerPlex® Y23 system, which allows typing of all 17 YFiler loci (that already included PowerY loci) plus 6 additional loci, including 2

RM Y-STRs: DYS570 and DYS576 [32]. These loci and D1S481 (also included in PP Y23) have been found to be some of the most effective loci for increasing haplotype resolution in several population studies [29]. Already a couple of thousands of profiles (as of this writing) in the US Y-STR database and more than 5 300 profiles (as of this writing) in the YHRD database have been typed on all 23 Promega loci. Life Technologies is also announcing the preparation of a new version of its YFiler multiplex with an expanded marker set.

4. SNPs

SNP stands for “single nucleotide polymorphism”. It designates individual genome sequence variations where a single base has been inserted, deleted, or substituted. With rare exceptions [33], SNPs are generally biallelic: insertion/no insertion, deletion/no deletion, nucleotide/alternative nucleotide. Therefore for a given SNP locus only 3 possible genotypes may be found in the population: individuals may be homozygous for one allele, homozygous for the other allele, or heterozygous. For identity testing, this low discriminatory power has to be compensated by analysing a large number of SNP loci.

However SNPs present specific advantages that make them a powerful alternative or complementary tool in situations where conventional STRs provide limited information. They allow amplification of very short fragments and therefore analysis of highly degraded material, such as in mass disasters and missing person cases. In addition, their mutation rates are orders of magnitude lower than STRs, which represents an important advantage to help resolve complex paternity cases [34, 35], provided that appropriate SNPs are selected [34], and typed in sufficient numbers [33, 36]. Their high abundance and amenability to high throughput technologies such as microarrays may be of use in specific relationship testing [33, 37]. However at this moment, their utility is mostly restricted to analysis of single source samples: in a mixture, the discriminatory power vanishes as the number of loci showing both alleles rapidly increases with the number of contributors [34]; moreover, mixture resolution is hindered by the lack of relationship between input DNA and signal intensity [33]. Indeed, although one of the most commonly used SNP analysis chemistry (single base extension, often referred to as SNaPshot), uses same technologies and instruments as STR typing (PCR and capillary electrophoresis), it is not as straightforward: it involves consecutive amplification reactions that may increase stochastic differences between alleles, and there are variations in incorporation efficiencies of the four base terminators.

Over the last few years there have been extensive developments on the use of SNPs for forensic applications, including identity/kinship testing and more particularly prediction of ancestry or visible traits [34, 38]. Progress in that area have been driven by human genomics and the development of high throughput technologies and the discovery of several million SNPs throughout the genome [39]. Concerted international scientific initiatives have been undertaken to identify and validate the best SNPs candidates for different forensic applications, such as the SNPforID consortium (identity testing) and the VisiGen consortium (prediction of visible traits).

4.1. Identity and kinship testing.

For identity and kinship testing, selected SNPs must have a high allelic diversity across populations (loci with high heterozygosity), and a low degree of population differentiation (low Fst value) [40]. A number of large SNPs panels have now been developed for identification purposes, including the 70 SNPs assay by Orchid CellMark, and a 52 SNPs multiplex developed and validated for forensic use by the SNPforID consortium in 2006/2007 [41, 42]. These 52 SNPs showed good polymorphism across 9 populations, with the highest heterozygocity in Europeans. Initially, SNaPshot-based (52-locus PCR multiplex followed by two 23- and 29-plex single base extension assays), the assay allowed obtaining SNP profiles from 0.5 ng of template DNA, with amplicons no more than 115 bp in length and a mean match probability of at least 5 X 10^{-19} [41]. Subsequently, other SNP analysis chemistries were evaluated in order to improve background and allelic balance, and 48 of the most informative SNPs from the panel were used to develop the GenPlex™ HID system, manufactured by Life Technologies and based on PCR amplification followed by an oligo ligation assay [43-45]. Although good results could be obtained with GenPlex HID from 0.25 ng of good quality DNA [44], the assay proved to be laborious and needed further optimization for analysis of degraded samples [46]. It was later discontinued. In 2010, Pakstis et al. [47] reported the identification of a large panel of 86 SNPs obtained from screening more than 500 candidate SNPs with samples from 44 populations throughout the world. The selected SNPs showed no significant linkage disequilibrium, very high average heterozygocity and low Fst values across all studied populations, making it an identity/kinship testing panel of universally excellent value. A subset of 44 of these SNPs has been multiplexed in a SNaPshot-based assay that allows obtaining SNP profiles from 0.5 ng of template DNA, with amplicons no more than 125 bp in length [48].

Recently, the enormous multiplexing capacity of a genomic technology has become a candidate to address a long-standing challenge in forensic casework, namely mixture resolution, by taking full advantage of the high abundance of SNPs. High density microarrays allow simultaneous genotyping of millions of SNPs. This technology has been put forward as a revolutionary approach to address complex DNA mixtures, and to determine whether an individual is excluded or not, even when contributing as little as 0.1% of the total DNA [49]. However, the statistical basis of this approach has been criticised by Egeland et al. [50], who provided a mathematical demonstration, as well as evidences from simulation and microarray typing experiments, that the approach proposed by Homer et al. would lead to erroneous conclusions. In the meantime the theoretical framework of a different strategy has been presented by Voskoboinik and Darvasi [51], who suggested to use a panel of SNPs specifically selected for low heterozygocity in order to take advantage of their low (0.05 – 0.1) minor allele frequency. The authors argue that if using a sufficient number of such SNPs (≥1 000), then any individual would carry a specific set of 100-200 rare alleles, and a DNA mixture will carry this particular set only if the one individual is represented in the mixture. Using SNP information from the International HapMap project, the authors performed simulation studies, RMNE calculations and LR calculations, and found that their method generally provides highly significant results.

4.2. Phenotypic profiling.

Skin tone, hair color, eye color, facial morphology are strongly determined by genetics. The high abundance of SNPs, their wide distribution throughout the genome and the progress made in mapping the human genome allow identifying SNPs that, unlike SNPs for identity testing, don't necessarily have high heterozygocity and low Fst values but rather strong genetic linkage to specific visible characteristics. The identification of SNPs located within or near genes influencing various visible traits obviously represents a major interest for forensics, as such SNPs can be used as markers to predict a perpetrator physical appearance.

Among the first classes of SNPs that were used for this type of application are the ancestry informative markers (AIMs), i.e. SNPs whose allele frequencies are very different in different ethnic groups, and that can be used as biogeographical markers. In the mid 2000, DNAPrint Genomics developed a panel of 176 such SNPs, designed to assign ancestry to different biogeographical groups in order to use it as an indirect predictor of physical appearance [52]. The results were expressed in terms of ancestry proportion or admixture, for example 85% European and 15% sub-saharan African. A set of photography representative of the general physical appearance of individuals with given ancestry proportions was provided as an aid for interpreting the results. However the typing assay was based on a chemistry now discontinued and DNAPrint Genomics ceased operations in 2009. Other AIM-SNPs multiplexes currently available for casework include a set of 24 SNPs in two SNaPshot multiplex reactions developed by Lao et al. in 2010 [53], a 34-plex (also SNaPshot-based) developed by the SNPforID consortium in 2007 [54], and recently improved by replacing an East-Asian SNP and readjusting assay conditions [55], and a 23-plex designed to complement the 34-plex and to differentiate European and South Asian ancestries (Eurasiaplex, [56]). However, although these markers can provide a fairly reliable ancestry assessment in un-admixed individuals (and therefore some inference on likely physical appearance) Fondevila et al. [55] pointed out the limitations of such small SNP sets in making accurate assessments in individuals of mixed ancestry. Enlarging the set with additional highly efficient SNPs, or combining the 34-plex with other carefully ascertained AIM markers such as STRs [57] or indels (another type of bi-allelic DNA variant, see below) [58] will be necessary to improve ancestry inference precision.

Recent progress in eye and hair color genetics have identified a number of markers with high predictive value. This led to the development of a 6 SNPs assay for prediction of blue and brown eye color, validated for forensic use, the IrisPlex system [59, 60]. This multiplex is SNaPshot-based and can generate profiles from 0.25 – 0.5 ng (sensitivity threshold around 30 pg) with amplicons less than 130 bp in length. A subsequent study tested the IrisPlex prediction model across multiple European populations on more than 3800 individuals. The authors report a prediction accuracy rate for blue and brown eye color ranging from 91% to 98% [61]. Subsequently, the assay was further expanded to hair color in the HIrisPlex assay [62] by the addition of 18 more markers, 17 SNPs and one indel, for a total of 24 predictive DNA variants. HIrisPlex is based on the same technology than IrisPlex, with a sensitivity threshold around 60 pg and amplicons less than 160 bp in length. After testing with more than 1 500 individuals from three different regions of Europe, the authors report an average prediction accuracy of 69.5% for blond, 78.5% for brown, 80% for red and 87.5% for black hair color. Although exciting and readily applicable, these assays are still relatively rough as they only allow typing of color categories, while human eye and hair color is rather a continuum. More precise and quantitative determination of

hair and eye color [63], together with identification of additional markers, should allow to further refine the accuracy of such assays.

The field of predicting physical appearance for forensic purposes, also referred to as forensic DNA phenotyping, has developed exponentially over the last few years. Efforts are ongoing in genetic and genomic research to identify new and better DNA markers for forensic purposes such as skin pigmentation, body height, age [64]. Of particular interest are the studies on DNA markers for facial morphology. Recently the VisiGen consortium has identified five loci influencing facial morphology in Europeans. The group conducted a genome-wide association study on almost 10 000 individuals, using 3D head magnetic resonance images for phenotyping facial features [65]. Significant progress is to be expected in the next few years that should add a number of new investigative tools regarding different visible traits to the already existing panel of conventional STR identification tools.

4.3. Microarrays.

With the continuous progress in identifying new categories of genetic markers, the pressure to integrate large amount of information in a single assay keeps increasing. However, the multiplexing capacity of conventional PCR-based technologies presents limitations in that respect, allowing analysis of up to a few dozen loci only. This leads to genomic technologies such as microarrays, which present multiplexing capacity of a completely different magnitude, allowing simultaneous typing of thousands to millions of SNPs. Recently, the VisiGen consortium reported the development of the first commercially available chip for forensic purposes, the Identitas Version 1 Forensic Chip [66], which interrogates simultaneously more than 200 000 SNPs. The chip, based on the Illumina Infinium technology, includes over 190 000 autosomal SNPs that were selected for kinship and biogeographic ancestry inference, as well as for appearance traits such as eye and hair color. Sex chromosomes and mitochondrial SNPs are also included. The chip's performance was assessed on more than 3 000 DNA samples. The authors report high prediction accuracy for first to third degree relatedness; 94% average prediction accuracy for ancestry, 70 – 85% for blue/brown eye color, and 48 – 72% for red/black/blond/brown hair color. This report demonstrates that although in early stages, forensic applications of genomic technologies are feasible. The performance of such tools will certainly increase as more and more markers with high predictive value are identified.

5. Indels

Small indels have been the subject of a growing interest in forensics over the last few years. They could be described as "the new SNPs". Small Indels designate a type of polymorphism similar to SNPs, but which does not involve sequence substitution. Indel stands for "insertion/deletion" and, as the name indicates, refer to genetic variation where a sequence has been inserted or deleted at a given point in the genome. The size of the variable sequence may range from 2 to 10 000 bp, but a large number of indels are typically less than 50 bp in length (for a review, see [67]). Like SNPs, indels are biallelic: the insertion (or the deletion) is either present or not.

Like SNPs, indels are also highly abundant and distributed throughout the genome, and their mutation rate is very low. Therefore they present the same advantages and disadvantages as SNPs: allowing amplification of very short fragments, utility in kinship analysis, and low discrimination power which has to be compensated by the typing of a large number of loci in identity and kinship testing. There is however one distinctive feature: typing indels is more straightforward than typing SNPs, and mimics conventional STR typing. This presents a number of advantages, including the fact that it preserves the relationship between the amount of input DNA and the profile peak heights, and therefore overall profile balance (of particular interest for mixed samples) [33].

Indel panels have been identified for ancestry assessment (AIM indels) or for identity/kinship analysis. Pereira et al. [58] developed a single-tube multiplex assay for typing 46 AIM-indels selected to measure population admixture proportions of 4 different origins (African, European, East-Asian and Native American). Although it was designed to detect or correct for population substructure in genome wide association (GWA) studies, the assay can also be of use in forensic applications. Zaumsegel et al. [68] identified 21 short indels to distinguish among 3 major population groups (European, African and Asian), and developed a multiplex both sensitive (requiring <0.5 ng of template DNA) and suitable for casework samples (amplicon size <200 bp).

For forensic identity/kinship testing there are currently two indel multiplexes available: a 38-indel multiplex developed by Pereira et al. [69], and a commercial 30-indel multiplex from Qiagen, validated for forensic use, the Investigator DIPplex® kit [70]. The 38-plex produces amplicons ranging from 57 to 158 bp in length and allows obtaining full profiles from 0.3 ng of input DNA. Qiagen DIPplex kit produces amplicons no more than 150 bp in length and uses 0.25 – 0.5 ng of DNA per reaction. The performance of the two assays has been recently studied further [71]. Both panels showed good polymorphism across major population groups, with RMP ranging from 10^{-11} – 10^{-13} for DIPplex and 10^{-14}-10^{-15} for 38-plex. Both multiplexes performed well when tested on artificially fragmented DNA, while in artificial mixture analysis, DIPplex presented better balanced profiles and therefore a better potential in detecting mixtures. In order to verify whether these kits can be used as supplementary markers and statistically combined with STRs, the authors expanded a previously constructed genetic map to add the 68 indels and 23 NIST mini-STRs to 39 STRs [71]. They found that only three indels require exclusion from calculations when combined with established STRs, due to very close linkage. Similar adjustments have to be made when combining the two indels kits, but this only reduces the total panel of indel markers to 64.

Due to their very low mutation rates, biallelic markers such SNPs and indels have been proposed as a tool to help resolve ambiguous paternity cases. When the alleged father shows only a few incompatibilities, with a high paternity index at the remaining STR loci, biallelic markers have been suggested as markers of choice to extend the set of analysed markers and help discriminate between related potential fathers. This strategy has been recently carefully assessed by Pinto et al. [36], who showed that small panels of biallelic markers may be insufficient for that purpose. The authors have determined that in 1.5% of duos and 0.3% of trios involving a 2nd degree relative, analysis of 15 STR plus 30 biallelic markers will show only one STR incompatibility and no biallelic marker incompatibility, therefore providing no further

resolution to the case. For duos, at least 100 biallelic markers with highest heterozygocity (equally frequent alleles) are required to reduce the probability of finding no incompatibilities to less than 1%. The authors conclude that although panels of supplementary biallelic markers are indeed useful to exclude false paternity, results are to be taken with caution when no additional incompatibilities are found, as compatible biallelic markers will mathematically reinforce the hypothesis of paternity, maybe erroneously.

6. Body fluid identification

Conventional methods for identifying body fluids have been used by forensic laboratories for several years without striking technical changes. For semen, identification of spermatozoa by microscope examination remains the gold standard confirmatory test. In addition to visual evidence of the presence of spermatozoa, microscope examination supplies cell morphology information that protein-based assays such as alkaline phosphatase or prostate specific antigen testing cannot provide: namely the presence of tails, from which inferences can be made regarding the time of the assault, a critical point in some sexual assault cases.

Searching spermatozoa under the microscope under phase contrast or after histological staining is a tedious and time-consuming task. It can be particularly laborious when there are only a few spermatozoa, if any, or when epithelial cells, cell debris or yeast are abundant. By the end of the first decade of 2000, Independent Forensics developed the Sperm Hy-Liter™ staining kit, which contains a fluorescently labeled human sperm head-specific mouse monoclonal antibody [72]. After staining, sperm heads are specifically and easily detected with the fluorescent Alexa 488 dye; the presence of nuclei in spermatozoa and in other cells is confirmed with a fluorescent marker for double stranded DNA (DAPI), and the cell morphology can be examined under phase contrast. Recently, De Moors et al. [73] thoroughly investigated the assay for casework applications and found it highly specific, sensitive, reliable and robust. Other groups also used Sperm Hy-Liter™ for single sperm cell isolation [74, 75]. The extent at which this assay facilitates the detection of spermatozoa certainly represents a remarkable improvement.

Other current body fluid identification methods for semen, blood and saliva remain with inherent limitations in terms of specificity, sample consumption and variation in technologies not allowing for parallel processing. In addition, there are no identification methods for other body fluids of importance in forensic biology, namely vaginal fluids and menstrual blood, nor is there any for skin cells on touched objects. Consequently, and for a number of years, significant efforts have been deployed to find new or alternative methods to identify body fluids in casework, first by analysis of cell-specific messenger RNA, and more recently by analysis of differentially expressed microRNA and differential DNA methylation patterns.

6.1. Differentially expressed messenger RNAs.

Although RNA has long been notorious for its post-mortem and in vitro instability, it is now well recognized that in some particular conditions such as in dry stains, it may

be recovered in sufficient quantity and quality for mRNA analysis [76-78]. This allowed taking advantage of messenger RNAs (mRNAs) differential patterns of expression to determine cellular origin of a forensic sample and develop novel tools for body fluid and tissue identification. In mRNA profiling, RNA is extracted and reverse transcribed. From the cDNAs obtained, the sequences of interest are amplified either by end-point PCR or by quantitative real-time PCR followed by normalization to an endogenous control. RNA approaches (as other molecular biology approaches) present a number of advantages over conventional techniques: RNA/DNA co-extraction protocols and multiplexing of several markers allow minimizing consumption of biological material, and good markers may be identified for fluids or cell types for which no conventional test is available yet, such as vaginal secretions, menstrual blood or skin cells on touched samples. However, identifying appropriate markers and developing reliable assays is no simple task [79-87]. Most often, a given mRNA is expressed in a variety of tissues although at different levels; mRNAs will also vary in stability. An additional difficulty in developing a PCR-based RNA assay is the fact that no human specific RNA quantitation system is currently available, which is of importance for fluids such as vaginal secretions or saliva that may contain significant amounts of bacterial or yeast RNA. Thus, in cases where body fluid identification rest upon differential expression of the marker in different cell types, normalization with housekeeping markers is required. Furthermore, upon multiplexing, balancing the different markers may prove to be a challenge. Level of expression of different markers may differ largely from one to another, and PCR conditions must be optimized so that the signal from one marker is not lost while the signal from another is saturated.

6.1.1. Blood, saliva and semen.

Over the last few years, increasing efforts have been put to identify the best markers and develop robust and sensitive mRNA-based assays for a number of biological fluids [79]. Recently, the EDNAP organized a series of 3 collaborative exercises for mRNA profiling, regrouping several laboratories. The purpose of these exercises was to evaluate the feasibility and value of the mRNA profiling approach for body fluid identification. In the first exercise [80], the 16 participating laboratories analysed 3 blood markers in singleplex on 7 blood stains and one dilution series. Most laboratories had no previous experience with RNA. All but one participant could successfully set up the method and detect mRNA markers in dried blood stains, although with different sensitivities. It was found that all three markers were robust and reproducible and, notably, the sensitivity of one marker (HBB) was found comparable to that of tetra methylbenzidine and Hexagon OBTI conventional tests (HBB mRNA detectable in 0.001µl of blood). In the second exercise [81] a RNA/DNA co-extraction protocol was tested on 6 blood stains and two dilution series. In addition, authentic or mock casework samples were optionally analysed by the participating laboratories. Two multiplexes were used: a highly sensitive duplex and a moderately sensitive pentaplex. All 18 participating laboratories could successfully detect mRNA markers in dried blood stains. Thirteen laboratories used the co-extraction protocol and were able to simultaneously confirm the presence of blood and obtain the DNA profile of the donor. Positive identification of blood and good quality DNA profiles were also obtained from casework samples. Finally, in the third exercise [82], the mRNA profiling approach was tested on 20 saliva and semen stains, four dilution series, and optional authentic or mock casework samples. The assays used were a saliva triplex and a semen pentaplex. The majority of participating laboratories were able to detect mRNA markers in dried stains, and

correctly identify saliva stains, semen stains, and saliva/semen mixed stains. Laboratories that performed co-extraction were able to simultaneously identify the presence of saliva or semen and to obtain the DNA profile of the donor. Altogether, it was concluded from these 3 exercises that the results obtained supported the mRNA approach as a sensitive and robust assay for blood, saliva and semen identification in dry stains.

6.1.2. Simultaneous typing for multiple body fluids.

Efforts are pursued to identify mRNA markers for other forensically important body fluids such as vaginal secretions and menstrual blood [83-86], and to develop multiplexes allowing simultaneous typing of multiple body fluids [83-85]. Recently, Lindenberg et al. [87] reported the development of a 19-plex for the simultaneous detection of several body fluids, including circulatory blood (3 markers), semen (2 markers) and menstrual blood (2 markers). Three more markers, predominantly expressed in the tongue and originally selected for the detection of saliva, showed regular cross-reactivity with skin, vaginal and menstrual samples and were therefore designated as general mucosa markers. The authors selected 2 more markers for the specific detection of saliva. Finally, 2 markers previously reported as showing high expression in skin [88] were also included, with 3 housekeeping markers. This assay showed good sensitivity with full RNA profiles being obtained from 0.05 µl of blood, semen or saliva. Good specificity was also observed when testing a variety of body fluid samples and skin swabs from different body parts, and results could be obtained from aged samples in storage from 8 months to 28 years. The authors [87] suggest a profiling strategy to make up for the lack of human specific RNA quantitation, avoid overloading of the cDNA reaction and obtain amplification signals in the proper range, and also provide some interpretation guidance on the markers expected to be observed in different fluids/tissues when using the assay.

Research is ongoing to identify better, more specific and more sensitive novel mRNA markers, and markers for vaginal secretions and for skin cells on touched samples are of particular interest. The identification of good mRNA markers able to distinguish between epithelial cells of different types (vaginal, oral, skin) is a particular challenge. Recently, Hanson et al. [89, 90] used new sequencing technologies (whole transcriptome deep sequencing or RNA-Seq) to sequence RNA both from vaginal swabs and from skin, and compared them to each other in order to identify new specific transcripts.

6.1.3. Vaginal secretions.

Sequencing of vaginal swabs RNA allowed identifying tens of thousands of vaginal mRNA transcripts, and after evaluation of more than 200 candidates, 6 promising candidates were found that showed low abundance or were almost undetected in skin samples [90]. The assays with candidate markers were found to be of moderate sensitivity, requiring 5 ng of input RNA (RiboGreen® quantification, not specific to human) for optimal detection in vaginal samples. The specificity of the candidates was further assessed using RNA from blood (5 donors), semen (5 donors), saliva (15-24 donors) and menstrual blood (5 donors). As expected, the markers were detected in some menstrual blood samples, but none of the markers could be detected in circulatory blood and semen RNA. Three markers could be detected in saliva with 5 ng of input RNA, one marker (MY0Z1) showed no cross-reactivity with 5 ng and low cross-reactivity with 10 -100 ng of saliva RNA, and one marker

(CYP2B7P1) demonstrated superior specificity and could not be detected in 100 ng of saliva RNA.

The 2 best vaginal markers were also assessed with 4 simulated casework samples. Both markers were strongly detected in a swab of male fingers following digital penetration, a penile swab after intercourse (but not on a penile swab taken immediately before intercourse), male underwear sampled 3 hours after intercourse, and on the surface of a vaginally inserted foreign object.

6.1.4. Skin.

In order to identify novel skin candidate markers, the sequencing approach was also used with human skin RNA from a commercial source, which allowed obtaining 20 candidates. Eighty-three more candidates were targeted through literature searches, for a total of 103 candidate skin markers [89]. After evaluation, 5 of these were selected since they displayed significantly lower expression in vaginal samples. Using these markers, the authors could set up sensitive assays that detected skin transcripts in 5-25 pg of total skin RNA (RiboGreen® quantification). Initial specificity testing with up to 25 ng of input RNA showed no detection in blood, semen and saliva RNA, but some markers were detected in a small number of vaginal secretions and menstrual blood samples. When using lower, optimal amounts of input RNA (25 pg, 250 pg, 5 ng, depending on the marker), cross-reactivity could no longer be detected.

However, when testing a variety of touched objects, co-expression of all five skin markers was not observed for any samples, and only 1 or 2 markers were detected in most cases, including the most sensitive one (LCE1C). The authors also report that in preliminary experiments using RNA/DNA co-extraction on touch DNA samples, in many instances, a STR profile was observed while no RNA profiling results could be obtained. The authors conclude that the identification of skin cells on touched samples requires further research to determine whether the DNA from shed skin is from cellular origin or rather “naked DNA”, in which case mRNA may not be present in sufficient quantity to be detected.

Large efforts continue to be made in order to identify the best markers and develop reliable, sensitive and robust mRNA assays for body fluid identification, and a huge amount of work lies ahead to validate the assays on casework-type samples. The inherent limitations of mRNA analysis remain, notably, the sensitivity of mRNA to environmental conditions. For instance, it is yet unknown how the semen markers will perform on semen that remained hours or days within the vaginal, anal or oral cavity, such as is the case with typical samples from a sexual assault kit.

6.2. Differentially expressed microRNAs.

Not surprisingly, attention recently turned to another class of RNA that can show tissue-specific expression, the so-called microRNAs or miRNAs. MicroRNAs are small non coding RNAs of 18-25 bases in length. Theoretically, the small size of miRNA makes it less susceptible to degradation and could represent a key advantage in body fluid identification in forensic samples.

MiRNAs regulate gene expression at the post-transcriptional level by incorporating to a RNA-induced silencing complex (RISC) and hybridizing to the 3’ end of specific

messenger RNA targets, thus repressing their translation or causing their decay (for a review see [91]). It was shown that miRNAs can be expressed in a tissue-specific manner [92, 93].

In 2009 Hanson et al. [94] published the first comprehensive study on miRNA expression in dried, forensically relevant biological fluids. The authors examined the expression of 452 miRNAs in blood, semen, saliva, vaginal secretions and menstrual blood. No strictly fluid-specific miRNA could be identified; in fact, most of the miRNAs tested were found to be expressed in multiple body fluids or not expressed at all. Nevertheless, using qPCR and normalization to a reference miRNA, 9 miRNAs could be identified that were differentially expressed, to a degree sufficient to allow the identification of the body fluid of origin, using only 50 pg of total RNA.

Subsequent studies confirmed the potential of miRNAs for body fluid identification, but highlighted as well specific technical difficulties in correctly identifying miRNA markers. Using microarray analysis (718 human miRNAs) followed by validation with qPCR analysis, Zubakov et al. [95] identified 7 miRNA markers for blood and for semen, the most sensitive of which (2 for blood, 2 for semen) could be detected in as little as 2 pg of total RNA. However, for menstrual blood, vaginal secretions and saliva, contradictory expression pattern results were obtained from microarray analysis and qPCR analysis. In addition, the body fluid specificity of markers identified by Zubakov et al. [95] and Hanson et al. [94] did not overlap. Using an approach similar to Zubakov et al. [95] but a different microarray (800 miRNAs), Courts et al. [96] characterized 3 miRNA markers for saliva and 3 markers for venous blood. Finally, also using microarray and qPCR analysis, Wang et al. [97] identified miRNA markers for venous blood (2), semen (2) and menstrual blood (1), that could be detected in as little as 10 pg of total RNA. Like Zubakov et al. [95], for saliva and vaginal secretions, their team observed a lack of concordance between expression pattern results from microarray analysis and qPCR analysis. Some of the markers they determined overlapped with some of the markers of the three other groups. Wang et al. [97] address the issue of the observed discrepancies between findings from different teams and suggest the following possible factors: differences in sets of miRNAs and screening platforms, natural variation between individual RNA donors, as well as differences between criteria used for selection from microarray expression data. Wang et al. [97] also point out that, as probe design is likely to be more difficult for microRNA arrays than for messenger RNA arrays, currently available miRNA microarray systems fail to show good inter-platform concordance. In addition to microarray screening and validation of good reference markers, the team denotes data analysis as a critical step in investigating miRNA expression [98].

Research on the use of miRNAs for body fluid identification in forensic biology is still at early stages. A lot of work remains to be done to identify specific, reliable body fluid markers. Once the inherent technical difficulties are overcome, validation studies on compromised, casework-type samples will tell if the results meet the theory, and if microRNAs are superior and more robust body fluid markers when compared to messenger RNAs.

6.3. Differential patterns of DNA methylation.

DNA methylation is a covalent modification that occurs at the 5’ position of cytosine in some CpG dinucleotides and plays a role in the long term silencing of transcription.

Sequences in the genome fall into two categories, CpG poor regions and CpG islands, the latter often found in promoter regions. It has been shown that different cell types may have different methylation patterns [99, 100].

The potential applications of DNA methylation patterns for forensic body fluid identification have been evaluated using different techniques. From literature searches, Madi et al. [101] selected potential markers for blood (1 marker), semen (1 marker), saliva (1 marker) and epithelia (1 marker). The methylation state of CpG spanning each locus was determined in the different body fluids/tissue using bisulfite treatment (converts unmethylated cytosines to uracil) followed by pyrosequencing (a sequencing-by-synthesis method that monitors nucleotide addition and sequence extension in real time). In the resulting sequence, unmethylated C remains as such, while methylated C have been converted to T. Ten to 11 samples of each fluid/tissue were collected and analyzed, and the percent methylation values at each CpG were averaged for each tissue type DNA. For 3 markers, the DNA methylation level was markedly different in the target fluid when compared to other fluids/tissue: the blood marker was hypermethylated in blood (but not in others); the saliva marker was hypermethylated in saliva (but not in others), and the semen marker was hypomethylated in semen while hypermethylated in all others. A fourth marker showed statistically different methylation levels between blood, saliva, semen and epithelial cells DNA. In this initial study undertaken to identify relevant loci and demonstrate feasibility, the authors did not use amounts of DNA below 1 ng.

Bisulfite conversion was also used by Lee et al. [102] who characterized 5 candidate loci expected, from literature data, to be good markers for blood (3 loci) and semen (2 loci). After bisulfite treatment, pooled DNA (6-16 donors) was amplified, cloned and sequenced, and the results were analysed to establish methylation maps for each locus in venous blood, saliva, semen, menstrual blood and vaginal secretions. Two loci showed all-or-none differential methylation patterns between spermatozoa-containing semen DNA (virtually unmethylated) and all other fluids DNA (hypermethylated), one locus was found hypomethylated in semen and hypermethylated to some extent in menstrual blood and vaginal secretions, and the results from a fourth locus suggested that more detailed CpG site-specific DNA methylation analyses could allow vaginal secretions identification. In a subsequent study [103], the authors investigated whether the 3 semen-specific loci were susceptible to age-related methylation changes, and looked at the DNA methylation level in semen from young (<30 years of age) or elderly (>50 years of age) men. The methylation level of the markers was found to be stable over time. In addition, the capacity of the 4 previous loci to identify spermatozoa-containing semen and menstrual blood/vaginal secretions was evaluated by comparing 2 multiplex technologies, a methylation-sensitive restriction enzyme PCR assay, and a methylation SNaPshot assay. In the former (1 ng genomic DNA), primers encompass the recognition site of a methylation-sensitive restriction enzyme (Hha I) and therefore, methylated DNA is protected from digestion and the PCR fragment amplified, while unmethylated DNA is cleaved and no amplification product can be obtained. In the latter assay, 0.5-10 ng genomic DNA is treated with bisulfite, after which the single base primer extension is performed using a primer that hybridizes immediately upstream of the CpG being interrogated. Therefore, methylated and unmethylated CpG are both detected with distinct signals. Individual DNA methylation profiles from 144 samples (blood, saliva, semen, menstrual blood and vaginal secretions) were determined using both assays. Semen could be readily

identified as no amplicon or unmethylated amplicon (depending on the assay) was produced, and menstrual blood and vaginal secretions generated methylation profiles similar to each other but quantitatively different than circulatory blood and saliva profiles.

The potential of DNA methylation patterns in forensic body fluid identification has also been investigated by Frumkin et al. [104]. Using a software program developed to search CpG islands, the team selected 205 loci that contain a Hha I recognition sequence and screened them using a methylation-sensitive restriction enzyme PCR assay. Thirty-eight loci showed significant body fluid/tissue differential amplification patterns, of which 16 were used to test body fluid identification, including a 15 loci stand-alone multiplex assay. As methylation levels are determined from peak heights, possible variations in template concentrations and PCR efficiency between samples are normalized using an algorithm that calculates peak height ratios between all pairs of co-amplified loci, and derives a likelihood score for the potential tissue source. When tested on 50 DNA samples (14 blood, 14 saliva, 11 semen and 11 skin epidermis), typical methylation profiles could be obtained for each body fluid/tissue type.

Recently, using that same approach, the team made available the first commercial body fluid identification kit based on DNA methylation: the DNA Source Identifier (DSI)-semen from Nucleix Ltd [105]. The semen-specific multiplex assay includes 8 loci: 2 loci are methylated in all forensically relevant tissues and peaks should be present in all samples (PCR positive control peaks); one locus is unmethylated in all forensically relevant tissues and no peak should be observed in all the completely digested samples (digestion control); 3 loci are methylated in semen (peaks should be present) but not in other fluids/tissues (no peaks) and 2 loci are methylated in other fluids/tissues (peaks should be present) but not in semen (no peaks). In this streamlined assay, 0.5 ng of extracted DNA undergo a single reaction in which Hha I digestion (15 minutes) is immediately followed by PCR amplification. The kit is accompanied by a proprietary analysis program, SourceIdentifier™ (Nucleix, Tel Aviv, Israël) that measures the relative peak heights. The algorithm then compares the relative intensity of tissue identification peaks against the mean amplification control peak height and assigns source identification: “semen”, “non-semen” or “inconclusive”.

The kit has been validated for forensic use [106] and was shown to be a reliable tool, sensitive down to 31 pg of neat semen DNA, and able to detect semen in buccal cells/semen mixtures at a ratio of 6:1 (upon visual examination of the electropherograms in both cases). The SourceIdentifier software was found to be too stringent, providing reliable assignations for the presence of semen but not for its absence, and the visual interpretation of data is recommended for casework. The validation also revealed that the kit is not compatible with the bead-based DNA extraction system PrepFilerPlus® (Life Technologies), possibly due to the co-purification of digestion inhibitors.

As for microRNAs, research on forensic applications of DNA methylation patterns analysis is still at early stages. Identification of additional reliable markers for various body fluids, and validation studies on casework-type samples will determine whether these assays will become part of a novel set of body fluid identification tools in the next few years.

7. Integrated forensic genetics and new genomic platforms

In forensic biology casework, the primary objective has always been to do more with less, i.e. to obtain all the needed information using as little material as possible. Extended multiplexing, integration of “standard” STRs with miniSTRs and other compatible markers such as indels, efforts to develop protocols and to identify markers allowing parallel analysis for body fluid identification and DNA typing, were all put forward in response to this imperative.

Thousands to millions of SNPs may be simultaneously interrogated on microarrays, and the forensic chip developed by the Visigen consortium (Identitas version 1), described earlier, integrates typing of more than 200 000 SNP markers for kinship, ancestry, eye and hair color, X and Y chromosome, and mitochondrial DNA [66]. However, current international databases are set up with STR genotypes and haplotypes, and microarrays are not suitable for STR typing.

Genomic platforms such as next generation sequencing (NGS) have the potential to be used as multipurpose platforms, allowing integrated typing of numerous markers from various categories in a single run: STRs, SNPs, indels, mitochondrial DNA sequences. NGS can sequence an entire genome in a matter of days, and therefore provide genetic information on a completely different scale of magnitude when compared to current genotyping technologies. However some concerns have been raised regarding NGS ability to meet forensic standards, notably because of relatively high sequencing error rates [107, 108]. Several issues currently confront analysis of forensic markers on NGS platforms. NGS requires high-quality DNA in sufficient quantity, and it is slower than current STR typing in terms of number of samples that can be processed on a weekly or monthly basis. Reduction of sample preparation labour, resolution of STR alleles (for instance with compound repeat motifs), compatibility of allele calls with existing STR databases, mixture resolution, as well as cost efficiency, remain a challenge. However, sequencing costs are continuously dropping, and large efforts are currently being deployed to develop forensic applications on NGS platforms and resolve these many issues [108-114]. If the pace of NGS technical development keeps up, one could envision in a foreseeable future that single source pristine samples could be typed for multiple markers - identity, phenotypic, body fluid - integrated on a single NGS platform. Analysis of refractory, degraded or mixed casework samples, often available in minute amounts, will certainly represent a challenge of a much higher degree of complexity.

8. Concluding remarks

STR typing has evolved with increasing discriminatory power, robustness and sensitivity. New markers such as indels are now added to the DNA analysis toolbox. At the moment, the sound interpretation of (numerous) mixtures with low template contributors and the attribution of a correct match probability are some of the most important challenges in routine casework STR typing, as shown by many workshops and meetings recently dedicated to this issue (“The hidden side of DNA profiles: artifacts, errors and uncertain evidence”, April 27-28th 2012, Rome; “Advanced

course on DNA profiling evidence with special emphasis on interpretation of complex mixtures", August 22nd 2012, 6th European Academy of Forensic Science Conference, The Hague; "Mixtures using sound statistics, interpretation and conclusions", 23rd Promega International Symposium on Human Identification, October 15th 2012, Nashville TN). Extensive research on this subject is currently ongoing and appropriate statistical tools are being developed and made available to forensic biologists. Personnel continuing education remains a key element for forensic laboratories around the world.

Rapid DNA is at our doorstep. Robust and reliable instruments that can be operated by non-scientists at the push of a button are already available from two manufacturers. It has now become a matter of funding for law enforcement stakeholders to be able to perform STR typing of reference samples, and for field-DNA typing to become a routine investigation tool.

Y-STR analysis greatly benefits from the international efforts of the past few years to build large, consolidated Y-haplotype reference databases. However, for some populations such as those confronted with a founder effect, the relevance of these population data may be questionable, and the problem of size of (appropriate) haplotype database and low statistical weight remains. The recent identification of highly discriminating, rapidly mutating Y-STRs represents a breakthrough that provides solutions to several limitations of Y-STR analysis. Not only does it help distinguishing related males, but it also improves haplotype resolution across populations, and appears to reduce or erase founder effects. Yet it is going to take a while before this set of markers can be included in existing databases and impact statistical weight. This highlights the fundamental need for in-depth basic genetic studies on forensic markers that can ensure the use of the most adequate genetic tools.

Research in phenotypic profiling is progressing, sustained by continuous advances in human genomics. For the time being and as far as can be anticipated, phenotypic profiling is going to be applicable to single source samples. Although still relatively rough, good tools can already be used for eye and hair color prediction on the same instruments now used for STR typing, and a forensic chip is now commercially available for simultaneous analysis of kinship, ancestry and eye/hair color SNP markers. In the near future, more precise and quantitative measurements of eye and hair color should lead to the identification of additional markers (SNPs or indels), and further refine the accuracy of such assays. Important progresses are also expected in the search for other markers for visible traits such as facial morphology markers. For some traits, a large number of loci may be required to make reliable inference, for example if using ancestry markers as an indirect predictor of skin tone. In the long term, as progress are made regarding various visible traits, it may no longer be adequate to analyse a number of separate sets of markers using conventional chemistries with limited multiplexing capacity, and phenotypic profiling may need to be performed on other types of platform (microarrays or others).

Substantial progress has also been made in the search for reliable alternative body fluid identification methods that could allow multiplexing of several markers, as well as analysis in parallel with STR typing, therefore minimizing sample consumption. New markers are being identified for forensically important body fluids such as vaginal secretions and menstrual blood. However, at this stage, most of the work has

been conducted on good quality samples. But more is being done in order to identify the most specific, sensitive and robust markers; and future extensive validation studies on compromised casework-type of samples will determine whether messenger RNAs, microRNAs or DNA methylation patterns can make up novel sets of body fluid identification tools. These new tools will be extremely valuable in a number of instances, for example to identify the presence of vaginal secretions on objects used to penetrate the victim of a sexual assault, to distinguish between circulatory blood and menstrual blood, or in cases where no body fluid testing is performed on a very small stain in order to preserve the entire sample for DNA typing. Multiplexing of several body fluid markers will allow establishing the composition of a stain or a sample, i.e. determining at once what it is made, and not made of, in a single assay. However molecular biology methods will not totally replace conventional screening tools, due to immediate orientation provided by current methods. For example, when searching for sperm on a bed sheet, negative stains are left aside and subsequent analysis efforts are concentrated on promising ones. It is unlikely that, in any foreseeable future, it will be more time/cost effective to collect all stains observed on a bed sheet and send them all to RNA/DNA profiling. However, once a promising stain has been identified through conventional testing and collected, it may be of critical importance to determine the exact composition of that stain, for instance identifying the presence of vaginal secretions or saliva, or confirm the presence of semen in an ambiguous stain.

In the future, it could be advantageous to obtain all needed information, body fluid, identity and phenotype, not only using the same instruments but simultaneously from the same test. Although SNaPshot, body fluid identification and STR (or indel) typing are performed on the same instruments (PCR and CE), they are not all compatible for simultaneous analysis, and the number of loci would exceed PCR multiplexing capacity. Therefore such an all-in-one tool would require a change of technology. Genomic platforms such as NGS platforms have the potential to be used as multipurpose platforms for the analysis of large numbers of markers of various categories, but would represent a very substantial technological change for forensic laboratories.

Yet, for forensic casework not all information is needed from all samples: Y-chromosome analysis is required only in a proportion of sexual assault cases; the investigation may have already targeted a suspect for identity testing, and therefore phenotype testing would not be needed; a significant proportion of casework samples are mixtures, and it is not known if and when phenotypic markers will be of any use for mixed samples. Therefore the decisive factors for future technological directions are going to be practicality, ease of use, throughput and most importantly, cost-effectiveness. If samples can be tested for all markers at once on a multipurpose platform with CODIS-compatible STR allele calls and a sufficient throughput, in a streamlined manner, and at a competitive cost, then a technological switch could be envisioned by the forensic community. Otherwise forensic laboratories will continue to proceed with sensible analytical strategies on a sample by sample basis, for example routine body fluid and STR typing, followed by typing of other markers depending on the results obtained from identity testing and the context of the case.

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Questioned Documents

Review 2010-2013

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I especially thank Dr Kevin Sullivan (PFS) for making the document readable and understandable. His corrections were invaluable to me.

TABLE OF CONTENTS

1 Introduction

This paper has the ambition of being an exhaustive review of the technical advances in the field of Document Examination including handwriting reported since the 16th Interpol Forensic Science Symposium in 2010. The review is essentially based on articles published in the major forensic or generalist science journals as well as presentations at international forensic meetings during the period 2010 – 2013.

The main goal is to gather all useful and relevant elements for the improvement or even implementation of a questioned documents forensic lab. It also aims to help laboratories in choosing a direction for new internal developments for coming years and to facilitate their technology intelligence. Whilst every effort has been made to capture all developments in this review, some omissions are possible.

Due to the high number of references found (275) and the variety of sources, it is important to underline that the scientific basis of all papers and presentations are not validated by the authors of this review. Two different kinds of publications are referenced: forensic publications and "generalist" publications. In this paper only forensic publications are commented upon, the others are included as background information only.

The fields of expertise in questioned documents are various, so we decided to sort the bibliography regarding the main topic of each paper: handwriting, ink composition, writing ink analysis, toner and inkjet ink analysis, aging, printing and/or writing sequence, paper substrate analysis, indented impressions, altered documents, security documents, quality assurance and miscellaneous.

2 Sources Of References

The review covers information from the scientific literature or publications from international meetings (excluding posters). This includes information from forensic or questioned document literature such as AAFS or ASQDE and from specific literature such as Colloids and Surface, Dyes and Pigment, and the Microchemical Journal:

- *AAFS*
- *American Society Questioned Document Examiner*
- *Applied Radiation and Isotopes*
- *Applied Surface Science*
- *Archives of Physical Medicine and Rehabilitation*
- *Colloids and Surfaces A: Physicochemical and Engineering Aspects,*
- *Developments in Handwriting and Signature Identification in the Digital Age*
- *Document Recognition and Retrieval XVIII (IS&T/SPIE International Symposium on Electronic Imaging)*
- *Dyes and Pigments*

- *Encyclopedia of forensic sciences 2013*
- *Forensic Science International*
- *Image and Vision Computing*
- *Information Fusion*
- *Infrared Physics & Technology*
- *International Journal of Mass Spectrometry*
- *Journal of Archaeological Science*
- *Journal of Chromatography A*
- *Journal of Cultural Heritage*
- *Journal of Electroanalytical Chemistry*
- *Journal of Forensic Document Examination*
- *Journal of Forensic Sciences*
- *Materials Letters*
- *Measurement*
- *Microchemical Journal*
- *Modern Approaches in Applied Intelligence: Proceedings of the Twenty-Fourth International Conference on Industrial, Engineering and Other Applications of Applied Intelligent Systems,*
- *Nanotechnology 2012: Electronics, Devices, Fabrication, MEMS, Fluidics and Computational*
- *NIP26: International Conference on Digital Printing Technologies and Digital Fabrication 2010*
- *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,*
- *Optics and Lasers in Engineering*
- *Optik - International Journal for Light and Electron Optics*
- *Pattern Recognition Letters*
- *Pattern Recognition*
- *Polymer Degradation and Stability*
- *Proceedings of the 2011 ACM Symposium on Document Engineering*
- *Proceedings of the Eleventh International Conference on Document Analysis and Recognition (ICDAR 2011)*
- *Proceedings of the Tenth IAPR International Workshop on Document Analysis Systems*
- *Proceedings of the Thirteenth International Conference on Frontiers in Handwriting Recognition (ICFHR 2012)*
- *Science & Justice*
- *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*
- *Spectrochimica Acta Part B: Atomic Spectroscopy*
- *Talanta*
- *TrAC Trends in Analytical Chemistry*
- *Twelfth International Conference on Frontiers in Handwriting Recognition, November 2010*
- *Twenty-First International Conference on Pattern Recognition (ICPR 2012)*

- *Vibrational Spectroscopy*

Finally, it is important for a document examiner to participate in specific conferences or forums. This is one of the means of being informed or trained in the latest developments of the printing or analytical techniques, and thus to gain the widest point of view of the field of document examination.

3 State Of The Art Of The Equipment

The increasing number of analytical techniques available now makes it possible to gain a good knowledge of the composition of an ink, dyes or other products belonging to the formulation (volatile solvents and other products).
Until the advent of RAMAN spectroscopy, no chemical technique allowed an analysis of the dyes without deteriorating the questioned document. Although non-destructive processes are always preferable, today some analytical techniques produce minimum damage of the sample (ink or paper).
Depending on the nature of the ink (pigment or dye) or the paper, the expert has at his disposal, a broad panel of analytical techniques able to provide additional information regarding the formulation of these products:

- Thin Layer Chromatography (TLC)
- High Performance Thin Layer Chromatography (HPTLC)
- High Performance Liquid Chromatography (HPLC)
- Fourier Transform Infra Red spectroscopy (FTIR)
- Attenuated Total Reflectance Fourier Transform Infra Red spectroscopy (ATR-FTIR)
- Ultra Violet Visible (UV-VIS)
- Near Infra Red(NIR)
- Pyrolysis Gas Chromatography / Mass Spectra (Py/GC / MS)
- Solid-Phase MicroExtraction / Gas Chromatography / Mass Spectra (SMPE/GC/MS
- Gas Chromatography / Mass Spectra (GC / MS)
- Laser Desorbtion Ionization – Mass Spectra (LDI – MS)
- Inductively coupled plasma optical emission spectrometry (ICP-OES)
- Inductively coupled plasma mass spectrometry (ICP-MS)
- Laser Ablation Inductively coupled plasma mass spectrometry (LA ICP-MS)
- Raman spectroscopy
- Surface Enhanced Resonance Raman Scattering (SERRS)
- X-ray analysis
- Micro Raman
- Micro UV-Vis Spectroscopy
- Positive and Negative Ion-Electrospray Ionisation Mass Spectrometry
- Dispersive X-ray microanalysis
- SEM (Scanning Electron Microscopy)
- Capillary Electrophoresis
- Microspectrometry

- Direct Analysis in Real Time
- Isotope Ratio Mass Spectrometry (IRMS)
- Direct analysis in real time mass spectrometry (DART-MS)
- Laser Induced Breakdown Spectroscopy (LIBS)

We have excluded from this list all the basic optical techniques used by experts in questioned documents that are well known by the forensic community.

4 Ballpoint, Gel, Markers, Pen Inks, Lipstick

The writing inks continue to be a popular topic of research for specialists in document analysis. 43 references are listed, so this is the topic most frequently covered within the bibliography: [1-43]

Classification of references is according to the analytical techniques that were used for ink analysis.

Overview [1], [2]
More and more techniques are becoming available to documents examiners, so it is not always apparent which one(s) to choose. These overviews can permit the reader to make a good strategic technical choice.
J. Siegel [1] surveyed the analytical methods used to chemically characterize and compare inks.

J. de Koeijer [2] also gave an overview of both the traditional methods and the newer trends in the forensic analysis of inks, toner, and paper.

HPTLC – TLC [3-9]
This method is one of the oldest analytical techniques available for the document examiner. Sometimes old databases exist but the user must adapt to using new plates which can raise problems of repeatability and reproducibility that endanger the relevance of the original database. *C. Neumann et al* [3] described the results of a project designed to improve ink samples' analytical and search processes. The project focused on the development of improved standardization procedures to ensure the best possible reproducibility between analyses run on different HPTLC plates. The successful implementation of this new calibration method enabled the development of mathematical algorithms and of a software package to complement the existing ink library.

LDI-MS / LDI-TOF-MS [10-13]
B. Matthews et al [10] described a technique for dye identification in ballpoint pen inks using LDI-TOFMS on single ink-bearing paper fibres and its application to a case. This sampling process caused imperceptible damage to the surface of the document. Clear mass spectrometric identification of the ink dyes was obtained.

M. Gallidabino [11] evaluated the potential for the discrimination of blue ballpoint inks by both positive and negative modes by LDI-MS. The results showed that additional information provided by anionic dyes and pigments significantly increased the discrimination power of the positive mode. In fact, it was demonstrated that

classifications obtained by the two modes were, to some extent, complementary (i.e., inks with specific cationic dyes did not necessarily contain the same anionic components). These results were consistent with those obtained by *C. Weyermann et al* [12] where it was shown that positive mode results generally yielded a lower differential power (DP) than the negative mode due to a higher intra-variability compared to the inter-variability in the mass spectra of the ink samples.

LA-ICP-MS [14], [15]
F. Alamilla et al [14] improved the discrimination power (DP) and provided objective results, achieving a complete differentiation among different brands and a partial differentiation within blue pen inks from the same brands. The designed data treatment, together with the use of multivariate statistical tools, represented an easy and useful tool for differentiating among blue ballpoint pen inks, with hardly sample destruction and without the need for methodological calibrations, being its use potentially advantageous from a forensic-practice standpoint

GC-MS [16-18]
C. Kim et al [17] defined gel pen inks by microscopy and VOCs using HSSPME GC/MS. They show that it's possible to discriminate between inks made in Japan and Korea through detecting the presence of two VOCs (Japanese inks contained 1,2-ethanediol, 52.83~95.84 %, while Korean inks contained 1,2-propanediol, 76.17~93.51 %).

Retention Time Locking
M. Ezcurra et al [19] described the use of retention time locking (RTL) for the 1st time in forensic science. The RTL tool assured reproducible retention times, and the realignment of the chromatograms assured rescaling of the time axis of the chromatogram. In order to determine the qualitative composition of dyes present in each ink, thin-layer chromatography (TLC) was used, followed by the identification of those colorants by liquid chromatography tandem mass spectrometry (LC/MS-MS).

DART-MS
R. Jones et al [20] undertook analysis of writing inks on paper using direct analysis in real time mass spectrometry and also built a library-search with a success rate of 92%.

Spectroscopy: FTIR – Raman – LIBS [21-30]
The method of classifying blue pen ink using Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy associated with linear discriminant analysis (LDA) was developed by *C. Santos Silva et al* [21]. This was able to differentiate successfully all brands of pen used on each type of paper and could be a helpful tool for detection and confirmation of counterfeits in documents of legal importance.

X. Wang et al [23] confirmed that combination of Raman and FT-IR spectroscopic methods can provide a powerful non destructive discriminating tool for identification of the ink.

HSI – Non destructive method [31-34]
D. Hammond [32] provided empirical data relating to the potential existence and frequency of inter-examiner variation in the results obtained through the non-destructive examination of writing inks.

S. Nedley et al [33] demonstrated how HSI can be utilized by document examiners in daily casework as well as ultimately show the analytical capabilities HSI has for discriminating black ballpoint pen inks.

X-Ray (archaeology or old documents) – Potentiometry – Size Exclusion Chromatography – Miscellaneous [34-43]
These various publications dealt with the possibility of applying Total Reflection X-ray Fluorescence to qualitative and quantitative differentiation of documents printed with rare earth tagged and untagged inks [35], the treatment of iron gall ink [36], and more generally ink in historical paper or pottery.

5 Increase Of Inkjet And Toner Printing

The continuing developments in the quality of impressions, combined with the ever reducing cost of inkjet and toner printers, has allowed this technology to spread and thus be used in increasing numbers of homes, for any type of document, including for a criminal aim, hence this type of impression frequently requires analysis in our laboratories.
Many publications describe the analytical methods able to give information on the composition of these inks. These techniques can vary according to whether the inks contain dyes or pigments.

5.1 Toner

Few publications deal with toner analysis. However *A. Almeida Assis et al* [44] showed that FTIR is a powerful tool for toner analysis. This method was considered to be non-destructive, where questioned documents' substrate (paper sheets) has no influence on the final result, showing high repeatability and intermediate precision. This method allowed the construction of a database with 100% positive identification to the correct group.

In this context (non destructive method), *M. Skenderović Božičević et al* [45] identified a common origin of toner printed counterfeit banknotes by micro-Raman spectroscopy. For each specimen cyan, magenta and yellow toners were analysed separately. The yellow toners displayed the most distinctive Raman spectra. The results showed that micro-Raman spectroscopy can be successfully applied as a method for the analysis of colour toner printed counterfeits, such as banknotes and documents, in order to establish links between more or less closely related specimens of counterfeits by measuring the properties of a colour toner.

V. Aginsky [46] undertook examination of paper and toner in page insertion/substitution cases using TLC, GC-MS and FT-IR microspectroscopy. These three analytical methods may allow the examiner to achieve a high level of certainty when evaluating which of two competing hypotheses is more probable.

Finally, statistical evaluation of the results through use of Bayesian networks was described by *A. Biedermann* et al [47].

5.2 Inkjet

Despite the fact that inkjet printers are very common (SOHO) and are usually used in anonymous or threatening letters and in counterfeit documents, there are a surprisingly small number of publications on their use in the questioned documents field. Nevertheless, we have referenced fourteen: [48-61]

In this field *L. Heudt et al* [48] demonstrated the capabilities of three methods: Raman spectroscopy, LDMS and MALDI-MS for the discrimination of colour inkjet inks.

M. Szafarska, et al [49], [51] used capillary electrophoresis for the examination of colour and inkjet printing inks for forensic purposes. The results obtained showed that the proposed procedure was a useful tool for ink discrimination and group identification of dyes originating from colour inkjet printing inks. Consequently, the developed method could be applied in the forensic field, including investigation of the authenticity of documents. For black ink, micellar electrokinetic capillary chromatography (MECC) was applied and a database of electrophoretic separation results of inks has been constructed for further forensic use.

R. Sharma et al [56] also built a database using Fourier transform-infrared spectroscopy in the examination of the cases related to inkjet printers. The technique is destructive in nature but it will provide much assistance to the forensic community in the examination of cases related to inkjet printer inks.

A study on the stability and the utility of satellite droplets for classification of ink jet printers was made by *L. Ning* [57]. It was observed that satellite droplets produced by one ink jet device varied in appearance with different print modes, ink, media and other factors. The structure can indicate the properties of the ink, and possibly the brand of printer. They were very useful for ascertaining certain characteristics for ink jet classification, including halftone dot, nozzle arrangement, and stepping of paper feed. They can also assist in determining print modes, without which no ink jet output can be produced. Therefore, satellites should be taken into consideration when FDEs are examining an ink jet -printed document.

S. Houlgrave et al [59] descibed a novel approach for the analysis of inkjet inks, using a time-of-flight mass spectrometer, coupled with a Direct Analysis in Real Time (DART™) ion source (AccuTOF™ DART™) to build a classification. These techniques were used to determine if inkjet inks from various manufacturers and models of printers could be reliably differentiated, characterized, and identified.

K. Herlaar et al [60] searched for discriminating features in B&W inkjet prints, not only to be able to exclude a specific printer as a possible source, but also for individualizing printers. This results obtained using the ImageXpert system were encouraging. This digital approach should be taken further.

6 Ink Aging / Dating

This field is now coming to maturity, indeed several publications deal with the general principles. A consensus is now starting to be reached, together with a willingness to improve and standardize methods even though there still exists significant controversy about the accuracy, reliability, and validity of the dynamic procedures.
The field of dating is as much about the ink as the paper and as such thirteen scientific works are referenced: [62-73]

Some publications explain the main principles and the philosophy of the field for example *W. Mazzella et al* [62], *C. Weyermann* [63], [64] and *G. LaPorte* [65]. There are two analytical approaches for determining the age of an ink on a questioned document: static and dynamic. The static approach to ink dating generally applies to methods that are based on comparisons with a standard reference collection of inks to determine the first date of production. The dynamic approach includes methods that incorporate procedures for the purpose of measuring the physical and/or chemical properties of an ink that change with time. In addition to the aforementioned methods, there is a third approach that considers the relative age of the documents and aims at reconstructing their chronology.

According to these three methods, several analytical approaches may be employed such as solvent evaporation, dye degradation or even a combination of both.
A. Cantú [66] undertook studies of the evaporation of a solvent from a solution and its application to writing ink aging. An equation was developed for the drying process that was based on a different and rather simple model. This model considered the evaporation of a solution in an opened vertical container (e.g., a beaker) consisting of a volatile, non-hygroscopic solvent with a non-volatile solute dissolved in it.

S. Senior, *et al* [67] described the characterization and dating of blue ballpoint pen inks using principal component analysis of UV–vis absorption spectra, IR spectroscopy, and HPTLC. This concluded that the PCA loadings are useful in individualization of a questioned ink from a database. The PCA of ink lines extracted at different times can be used to estimate the time at which a questioned document was written. The results proved that the UV–vis spectra are an effective tool to separate blue ballpoint pen ink in most cases rather than IR and HPTLC.

Y. Wu et al [68] described the differentiation and dating of gel pen ink entries on paper by laser desorption ionization and quadruple-time of flight mass spectrometry. The degradation processes of the dye components in the ink entries under various aging conditions were studied utilising LDI-MS. The results showed that the variations of relative intensities for the main dye components have a close relationship with aging time, and the degradation of the main dye components were significant under natural storage conditions, which can provide important evidence for dating of the ink entries on paper.

In combining solvent evaporation and dye degradation, *S. Cengiz*, *et al* [69] determined the age of ink entries from questioned documents with TD-GC/MS and HPLC methods. More precisely, the enhancement of the ink age determination methods using dynamic physicochemical properties of the ink entries on a document was shown such as the vanishing rate of phenoxy ethanol (PE) with TD-GC/MS that

is used in traditional analyses of volatile organic components, and the fading rate of the pigments Crystal Violet (CV), Methyl Violet (MV), Tetramethyl Para Rosaniline (TPR), and other changes in pigment..

C. Weyermann et al [70], made the observation that several ink dating methods based on solvents analysis using gas chromatography/mass spectrometry (GC/MS) have been developed over many years. These methods followed the drying of solvents from ballpoint pen inks on paper and seem very promising. However, several questions have arisen over the last few years among questioned documents examiners regarding the transparency and reproducibility of these techniques, for which this paper proposed some solutions.

In addition, the relative dating approach, which is less precise but equally useful, was described by *S. Brown et al* [71], utilising the analysis of anatase which is an important industrial white pigment and date-marker for artwork. Barium Sulfate, (BaSO4), was shown by Raman microscopy to be readily identifiable in early (1920s) industrially produced anatase (TiO2) and thus, if present, may act as a date-marker for early industrial anatase. Later processes (except that for producing Titanox B) did not involve usage of Barium Sulfate. This is relevant to the possible dating of certain artwork.

After ink dating, the forensic document examiner is frequently confronted with the aging of paper. *G. Hodgins et al* [72] undertook dating of documents and photographs based upon atomic-bomb derived radiocarbon content. The method indicated when paper and photographic materials were manufactured, or more precisely, when the organisms used as raw materials for them were living. It does not identify when a piece of paper was printed or written upon, or when a photographic image was produced. In the field of the artwork, *H. Oda et al* [73] described the radiocarbon dating of kohitsugire calligraphies attributed to Fujiwara Shunzei: Akihiro-gire, Oie-gire, and Ryosa-gire.

7 Paper Analysis

The analysis of paper in the field of forensics, is gradually becoming the focus of more and more articles, with 22 published in different journals since the last review: [74-95]

T. Fritz et al [74] provided a complete overview of the paper analysis field and described different pitfalls (paper is a product of mass consumption and, producers may use manufacturing processes that are similar to those of their competitors).

C. Berger [75] undertook an objective paper structure comparison: in assessing algorithms based on the Fourier power spectra of light transmission images, good results were obtained by using the 2D correlation of images derived from the power spectra as a similarity score.

Most of the articles identified in relation to the analysis of paper utilized analytical techniques such as ICP-MS: [76-79].

T. Trejos et al [76] showed that elemental analysis, using either LA-ICP-MS or LIBS, provided an effective, practical and robust technique for the discrimination of document paper and gel inks with minimum mass removal (9–15 µg) and minimum damage to the document's substrate. *E. Riddell* [77] treated the results obtained by ICP-MS by principal component analysis (PCA) and this was then used to associate or discriminate the paper samples, based on elemental profiles. Reams of the same type of paper were closely associated, while reams of different types of paper could be differentiated. Traditional spectroscopy methods are also used:

V. Causin et al [80] showed that good results were obtained by UV–VIS spectroscopy, an inexpensive technique which is readily available in most forensic laboratories.

M. Bicchieri et al [81] worked on non-destructive spectroscopic characterization of parchment documents. Experimental results demonstrated that the chosen non-destructive techniques (Raman, ATR-FTIR and SEM/EDS) provided a good differentiation between parchment manufacturing procedures, western with lime and eastern with enzymatic treatment. For instance, Raman spectroscopy appeared to be the most effective molecular technique on western parchment, whereas ATR-FTIR allowed the enzymatic de-hairing procedure to be distinguished from the chemical one.

S. Kwong et al [82] used Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) in the analysis of paper. ATR-FTIR proved not be highly discriminating among paper brands, however given the simplicity of the technique, its non-destructive nature and the increasing availability of ATR-FTIR instruments, this technique was considered to be a useful addition to the methods of paper analysis.

Some other analytical methods have also been described, for example the use of X-rays by *V. Causin et al* [83] which provided a very high level of discrimination, and direct analysis in real time mass spectrometry (DART-MS) by *J. Adams* [84] which showed that papers that contain rosin versus alkyl ketene dimer (AKD) are readily differentiated by size. The DART-MS methodology was fast and simple, and the spectra were repeatable.

Ancient papers can be studied by ToF-SIMS and XPS: *F. Benetti et al* [85] used these techniques to determine the manufacturing process, provenance and state of conservation of ancient papers.

8 Determination Of Writing Or Printing Sequence

In common with the dating of inks, determining printing or writing sequences remains a major challenge in the field of document examination.
Some optical (ESDA, microscopy or 3D profilometry) or spectroscopy (Raman, FTIR) techniques can be used but with varying degrees of success.

Introduction of the digital techniques in document examination has enabled the Forensic Document Examiners to work with better accuracy and in non-destructive ways. *R. Kaur* and al [96] examined the sequence of intersecting strokes of printers

(inkjet printer, laser printer, dot-matrix printer) and typewriters with writing instruments (gel ink pen, ballpoint pen and fountain pen) of different colours using the Docucenter Expert via PIA-6000 software utilizing extended depth of focus. The continuity of the stroke is the only characteristic which has been observed at the point of intersection.
Whilst some techniques perform well, others give poorer results, for example *R. Kaur et al* [97] assessed the application of the Video Spectral Comparator (absorption spectra) for establishing the chronological order of intersecting printed strokes and writing pen strokes, and particularly the sequence of intersecting strokes of laser printers (black, blue, red and green) and typewriter ink (black) with the strokes of gel pen ink, ballpoint pen ink and fountain pen ink (black, blue, red and green). Unfortunately the results determined by studying their absorption spectra were negative, and FDEs are advised against its use in the examination of the sequence of intersecting strokes for these specified inks.

I. Montani et al [98] examined heterogeneous crossing sequences between toner and rollerball pen strokes by digital microscopy and 3-D laser profilometry and correct opinions of the sequence were given for all case scenarios, using both techniques. The findings confirmed the potential of 3-D laser profilometry and demonstrated the efficiency of digital microscopy as a new technique for determining the sequence of line crossings involving rollerball pen ink and toner.

R. Radley et al [99] undertook a comprehensive overview of impressions/Ink intersection sequencing which assists in the determination of the execution order of visible ink lines and intersecting ESDA impressions. Critical factors, suggested procedures, interpretation and tips on conducting the work were considered and addressed in detail in this report. Consideration was also given to conflicting papers on this topic.

Y. Wang et al [100] determined the sequence of intersecting lines from laser toner and seal ink by Fourier transform infrared microspectroscopy and scanning electron microscope / energy dispersive X-ray mapping and determined that this method may be the basis for sequencing superimposed lines from other writing instruments.

A. Raza, and B. Saha [101] demonstrated that a Raman scattering tool was able to determine the sequence of heterogeneous intersection strokes involving a blue stamp pad ink and other writing instruments, such as ballpoint pen ink (red and black), pencil and laser printer toner. However, this method was unable to resolve the exact sequencing for the intersection strokes involving stamp ink used in the study and blue ballpoint ink or gel pen ink (all colours).

G.Naisbitt et al [102] studied ink analysis and line crossing to determine the suitability of different techniques (visual microscopy, FTIR, and Raman microscopy), from which they created a user's guide for the most appropriate analysis based on the evidence to be examined.

9 Document Security

A substantial increase in publications related to document security has been noted in the review period, totalling twenty two contributions. Some could be classified in other headings, including ink analysis by chemical methods or analysis by traditional

forensic tools (ESDA, VSC etc), however from a security documents perspective these can be conveniently classified under the following three categories:

Fraud and forensic intelligence*: [103-113]*
The general strategy to analyse security documentation has been reviewed by *T. Trubshoe, et al* [103]. This discussed the nature of documents that are used for deceit and assisting criminal activities, and then addressed the different strategies that are to be considered in combating forged and counterfeit documents. These strategies include security features utilized in the manufacture of high-security documents, the role of the issuing authority, and the different levels of examinations undertaken, which combine to disrupt the manufacture of fraudulent and counterfeit documents and the organized criminal activities that they enable.
K. Cox, [104] also considered the state of- the-art instrumentation utilised in the detection of altered and counterfeit travel and identity documents.

In addition, *B. Dasarathy* [105] provided an overview of information fusion in the domain of watermarking and document security.

G. Wood [106] reviewed the types of abuse that passports and other identification documents are prone to and explained why, until now, the lack of security in digital printing has had a direct impact on the way in which these documents are manufactured and issued.

C. Bayer-Broring, [107] described a novel method of interpreting evidence when first attempts fail the analysis of an electronic passport in a real case example.

M. Aloyoni et al [108] described the deciphering of 3 groups of fraudulent traveler's checks that were caught in Saudi Arabia banks during Hajj (pilgrimage). This detection of different groups of fraudulent security document could be treated according to the *S. Baechler* method [109] which dealt with security document forensic profiling and intelligence against document fraud.

New solutions to increase security levels: *[114-116]*
J. Hayward et al [116] proposed the use of botanical DNA to forensically tag and authenticate objects for security purposes.

Ink analysis by chemical methods or analysis by traditional forensic tools: *[45], [117-124]*
When optical methods are inefficient, the document examiner may utilise chemical analysis. *M. de Almeida et al* [118] described the use of Raman spectroscopy and PLS-DA incorporating an uncertainty estimation in order to discriminate authentic and counterfeit banknotes.

M. Skenderović Božičević et al [45] also used Raman spectroscopy in identifying a common origin of toner printed counterfeit banknotes. In contrast, *J. Zięba-Palus et al* [119] established the chemical composition of printing ink in official documentation (court tax marks of 50 and 200 PLN) by combining several analytical methods including infrared (IR), visible, X-ray fluorescence, and Raman spectrometry.

R. Cessna, and R. Voiles [120] described alternative methods for dry seal analysis. Reflectance transformation imaging (RTI) is a new imaging technique based upon

the combination of multiple digital images of an object illuminated from different angles. This is considered to be a good alternative to other techniques for dry seal examination.

W. Romão et al [121] analysed Brazilian vehicle documents for authenticity by Easy Ambient Sonic-Spray Ionization Mass Spectrometry. This study concluded that this analytical technique offers an effective way to characterize the counterfeiting method.

S. Sugawara et al [122] detected the falsification of security documents using a white light interferometer to measure the surface distortion of the cover film of security documents. The method was found to be useful for the authentication of genuine documents.

10 Handwriting

More than 90 items on handwriting comparison have been identified in this review exercise. It is quite difficult to make out what can be considered the most important research as the scope of writing is vast including diverse topics such as environmental influence of the writers, automatic recognition of signatures or writing, ability of one part of the population to make a forgery etc [125-215].

10.1 Skills and Knowledge

C. Neumann [127] reviewed the exact meaning, requirements and the implications underlying the terminology proposed in the ASTM standard E1658. Through the use of examples, the assignment and meaning of probabilities in statement conclusions were investigated. This paper also considered how to address questions on errors and contextual bias.

R. Morris et al [128] addressed the pertinent question, “What is the basis for a handwriting elimination?” To conclude that a known writer did not write a questioned handwriting, the Forensic Document Examiner must determine that the known writer could not and did not write the questioned writing under any circumstances, including, but not limited to, intentional or accidental distortion, more than one writing style, writing position, drugs, or other transitory or permanent factors, etc. In most instances involving signatures and short writings, the evidence in the writing is insufficient to make such a determination. The key element to eliminating a writer is for the FDE to fully understand that it is the combination of differences, taken collectively, that determines the truly significant differences that provide the basis for the elimination. The authors have noted that even minor variations in writing characteristics, qualities, and features have been deemed so significant and individualistic by some FDEs that they have maintained that these superficial differences are sufficient to eliminate a writer.

S. Mumtazah Syed Ahmad et al [129] demonstrated that among all the investigated dynamic features, pen pressure was the most distinctive and was significantly different for the two authentication groups as well as for the different perceived classifications. In addition, all the relationships investigated, namely authenticity

group versus size, graphical complexity, and legibility, were found to be positive for pen pressure.

10.2 Likelihood Ratio

F. Taroni et al [130] used data collected from female and male writers to conduct a comparative analysis of likelihood ratio based evidence assessment procedures in both evaluative and investigative proceedings. While the use of likelihood ratios in the former situation is now well established (typically, in order to discriminate between propositions of authorship of a given individual versus another, unknown individual), focus on the investigative setting requires further development. This paper sought to highlight that investigative settings, too, can represent an area of application for which the likelihood ratio may offer a logical support. As an example, the inference of gender of the writer of an incriminating handwritten text was analysed and discussed in this paper. The more general viewpoint according to which likelihood ratio analyses can be helpful for investigative proceedings was supported here through various simulations. These offered a characterisation of the robustness of the proposed likelihood ratio methodology.

R. Marquis et al [131] & [132] also worked on handwriting evidence evaluation based on the shape of characters using the application of multivariate likelihood ratios. It was concluded that this original Bayesian methodology provided a coherent and rigorous tool for the assessment of handwriting evidence, contributing to the integration of the field of handwriting examination into science.

10.3 Environmental influence

Environmental influence has been studied by several FDE. This approach was found to give some useful information about the writer. For example, *J. Anand* [133] assessed the variation in the writing of rural and urban people from their letter characteristics in roman script. This information was considered to be potentially useful in assessing the possible background of an individual, particularly a writer of an anonymous letter. The conclusions from this study were that the educational background of the individual can influence the development of handwriting characteristics.

On a related theme, *P. Zilly et al* [134] studied the use of a specialized gang alphabet and the transfer of characteristics from that alphabet into the normal daily writing habits of a gang member. Likewise *D. Nguyen* et al [135] provided additional empirical data on the frequency of signature styles based upon a relatively new objectively based system for the classification of signatures as one of three types: text based, mixed, or stylized. Results showed that female signatures were vastly different than male signatures. As for ethnicity, the data showed that Asians produced fewer text based signatures and more stylized signatures compared to non-Asians. In contrast, among all ethnic groups, African-Americans were found to produce the highest percentage of text based signatures. In addition, African-Americans were also found to produce the lowest percentage of stylized signatures compared to non-African Americans. All genders and ethnicities had about the same percentage of people who had a mixed signature. This research could be the foundation for further research regarding signature styles of genders or ethnicities.

10.4 Digital Document

The paper by *L. Holmes et al* [136] described the development and testing of a new online method for the proficiency testing of signature comparison. The study provided further validation of the existence of expertise in the area of signature identification and supported the use of online proficiency testing as an alternative to the traditional paper method as a means of demonstrating competence of FDEs in this field.

J. Masson [137] & [138], determined which features were reliably and accurately depicted in scanned images and which were not. Comparisons of the image quality obtained using various scanning parameters and transmission methods were made. In addition, examinations from originals, from first-generation photocopies, and from scanned images were undertaken.

S. Ibrahim [139] assessed the forensic value of non-original signatures on travel and identity documents. It was concluded that these documents cannot be used to forensically compare signatures with legitimate, known sample signatures for the simple reason that so many elements critical to the evaluation of the signature are not present because the poor quality of the print.

10.5 Automated comparisons, On-line / Off-line analysis

These automatic methods will always be based on a probabilistic approach in which relevant criteria have to be chosen. The aforementioned is the most difficult step since handwriting is an evolving biometric, where relevant criteria may be different from one writer to another.

C. Saunders et al [140] provided a strategy for determining the probability of observing two writers with indistinguishable writing profiles (regardless of the comparison methodology used) with a random match probability that could be estimated statistically. They illustrated this using a suitable sample of documents and an automated comparison procedure based on Pearson's chi-squared statistic applied to frequency distributions of letter shapes extracted from handwriting samples.

W. Flynn [141] demonstrated that signatures captured at a rate of 100Hz or faster contained sufficient detail and fidelity to arrive at reliable forensic conclusions as to authorship. In addition, Microsoft Excel™ was shown to produce very accurate graphical plots from the captured raw data.

S. Srihari [142] described the role of automation in the forensic examination of handwritten items and the use of the CEDAR-FOX system as an interactive tool for FDEs which assisted in performing several steps of the standard procedure.

G. Watts [143] described The Forensic Language-Independent Analysis System for Handwriting Identification (FLASH ID). This system has the potential to expedite examination of large volumes of evidence, and may some day be used for objective verification of conclusions.

11 Indented Impression

There were few publications on this topic in the review period, as the technique is already widely proven, and paper usage directly from reams of printing paper can explain this loss. However *L. Olson,* [216] described the optimisation of the development conditions to improve the quality and quantity of machine-made indentations (laser printer) on paper.

E. Wooton, and J. Brough, [217] considered marks imparted by postal service processing. This paper re-capped that Electrostatic Detection Apparatus (ESDA) examinations commonly reveal bands and/or lines on the documents being examined. Work done previously has established that the relative placement of some of those features corresponds to components in digital printing or photocopying processes (specifically, roller/feed mechanisms). However, this paper identified that sometimes these marks were artefacts of processing equipment used by the US Postal Service, since they had to have been created when the letters were inside the envelopes, and it was unlikely for there to be any other automated processes between sealing an envelope and its being sorted by the Postal Service's automated equipment in this particular case.

12 Quality Assurance

There have been only a few publications concerned with quality assurance. *T. Burkes* [218], described the work past present and future of US SWGDOC, regarding a number quality assurance matters: (1) standardizing and improving the capacity of expert document examination; (2) standardizing operating procedures, protocols, and terminology; (3) consolidating and enhancing the profession of forensic document examination; and, (4) promoting self-regulation, documentation, training, continuing education, and research in the area of forensic document examination. SWGDOC has either written and/or updated eighteen standards published through ASTM International. There are also fifteen additional draft standards that have been prepared for balloting. SWGDOC's current goals are to: (1) strengthen the content and the enforcement of published performance standards; (2) continue to write and foster the publication of performance standards for sub-discipline examinations; (3) publish and maintain the *Daubert Factors for Attorneys and Daubert Factors for Forensic Document Examiners* presentations (as they relate to forensic document examination); (4) participate in and support a Human Factors Working Group for Forensic Document Examination; and, (5) expand the participant pool to include academics, statisticians, legal professionals, and practitioners from other forensic disciplines.

M. DeKalb et al [219] produced a guide for the development of forensic document examination capacity. This guide provides practical assistance for the establishment or upgrading of forensic document examination capacities in two categories of service providers: (1) immigration and border control agencies; and (2) forensic science laboratories. This guide is intended to assist both donor and beneficiary countries in their efforts to design, build, and strengthen forensic document examination and intelligence dissemination capacities. Fraudulent identity and security documents are integral prerequisites for the smuggling of migrants,

trafficking in persons, terrorist mobility, facilitating the smuggling of drugs, weapons and other goods, and committing fraud. Fraudulent documents are the grease that eases cross-border crime of all types. The focus of the guide is on staff skill and educational requirements needed to perform forensic document examinations and to provide court testimony, intelligence alerts and training. It includes recommendations on forensic equipment, reference collections and databases as well as general guidance for designing, establishing, and maintaining a forensic document examination facility are included. This guide is not intended to be used as a simple checklist of equipment and materials, but as an aid for developing capacity in the area of document examinations.

C. Neumann et al [220], assessed the ASTM standards 1789-04 and 1422-05 for the forensic examination of ink, and reviewed these two standards in the light of developments within the field and proposed some practical improvements in terms of the standardization of analyses, the comparison of ink samples, and the interpretation of ink examination. Some of these suggestions have already been included in a DHS funded project aimed at creating a digital ink library for the United States Secret Service.

13 Miscellaneous

54 papers are list in this section that are difficult to classify in the previous categories: [222-275].

Palynology

R. Morgan et al [222] described the recovery of pollen evidence from documents and its forensic implications. Pollen grains may well be present, and their analysis has the potential to reveal not only the timing of the generation of the document, but the spatial trends revealed indicate that it may well be possible to establish the sequence of significant events for forensic reconstruction. As such forensic palynology was demonstrated to have great potential in aiding forensic investigations, and is as yet an under-utilised form of trace evidence.

Intersecting fingerprint and print

S. Fieldhouse et al [223] studied the intersecting between fingerprint and writing or printing by filtered light analysis, electrostatic detection device and Raman spectroscopy, to determine the sequence of application. The results suggested that the sequence of laser printing and latent marks could be determined via electrostatic detection device examination of undeveloped and Ninhydrin developed samples.
In the same field, *N. Attard Montalto et al* [224] determined the order of deposition of natural latent fingerprints and laser printed ink using chemical mapping with secondary ion mass spectrometry. *M. Bailey et al* [225] also analysed the depth profiling of fingerprint and written text signals by the same technique as above. The images obtained and the sputtering behaviour of the samples was found to be indicative of the sequence of ink and fingerprint deposits.

Barcodes [226-232].
7 publications explained that with the development of competence regarding barcodes, QDEs will be better able to identify evidence of alterations, counterfeiting and unauthorized production [226], or give generalist information about this technology.

Expert in court law [233-242]
These publications provided some useful advice to experts. This included for example, an understanding of how judges evaluate the admissibility of forensic document examination under the guidelines established by Daubert, Joiner, Kumho, Federal Rule of Evidence 702, and subsequent case precedents [233]. Also included was guidance on explaining why handwriting and hand printing evidence is not always conclusive when attempting to identify a writer [234].

Pdf Files
J. Parker [243] explained how PDF software tools can be used productively for questioned document examinations by forensic document examiners operating at a "software user" level. The paper also took the view that forensic document examiners only require an "elementary" level of expertise in understanding PDF technology, rather than needing a deeper level of technical understanding, such as comprehending computer programming code.

Digital printing technology [244-250]
These articles are too disparate to warrant comments.

Document analysis [251-264].
These papers described wide-ranging topics including the detection of transcribed seal impressions using 3-D pressure traces [252], and an update of the typestyle classification program (TYPE) into a Windows® Based Format (WinType) [253].

Miscellaneous
Falling within the miscellaneous category were a number of publications including different history or overview of document examination [265] [266]; neuroscience in handwriting [270]; linguistics [271]; a study of a new writing support for forensic purposes [272]; a Quantitative Hyperspectral Imaging Technique for Measuring Material Degradation Effects and Analyzing TLC Plate Traces [273] and general information about chemical analysis in forensic sciences [268], [274], [275].

14 Challenges

Science continues to provide Forensic Document Examiners with more analytical techniques (chemistry, optical processes, electronic, statistic & probabilistic or digital tools) that are of assistance. However, with these new tools comes the requirement for more skills that can be difficult to acquire. So, new techniques should be chosen in consideration of any anticipated benefits they might provide, because sometimes old techniques may give the same or even better results. For this reason it is necessary to have a rational and coherent plan for all the combined skills and techniques offered by a laboratory.

For document examiners to remain efficient and effective in their work it is becoming necessary for them to acquire new specialists skills in data and signal processing, whilst continuing to keep abreast of the developments in the technologies of printing, the fast progress of which makes the work increasingly difficult….a lot of work!

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Ballpoint, Gel, Markers, Pen Inks, Lipstick

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Forensic Science Management

Review 2010-2013

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1 Introduction

The management of forensic science is a new topic for this review but a crucial one. The fact that it is now to be included in all Interpol Forensic Science Managers Symposia—and that the name of the symposium has added the word "Managers"—is indicative of the importance of this set of papers to the proper delivery of forensic services. Although some papers appear in what are typically science journals, one of the strongest signals of the importance of management to forensic science is the creation of a new journal, *Forensic Science Policy and Management: An International Journal* published by Taylor and Francis and edited by Houck and Siegel. The journal is the official publication of the American Society of Crime Laboratory Directors (ASCLD) and publishes articles in management, leadership, quality, education, process improvement, and related topics. Many of the articles mentioned herein come from that journal.

One of the main difficulties in the writing of this review is where to draw the line—when does an article on science or medicine stop and when does it become one on management? Is ethics part of management and, if so, what part; if not, then where? Also, it is tricky to find articles on management in forensic science given the ubiquity of the word "manage" in titles that have little or nothing to do with "manage*ment*". Therefore, articles may have been overlooked, with apologies. It is anticipated that the categories for this review in the next edition will change, reflecting the shifting landscape of issues, concerns, and solutions for forensic laboratory managers. The increase in information and eagerness to report on and discuss topics of management in the forensic sciences is heartening; the science is important, yes, but so is how it's managed.

The main topics derived from the literature for this review are accreditation, crime scene management, education and research, efficiency, funding, leadership, management, quality, science and the law, and staffing. A short commentary on the closure of the UK Forensic Science Service is also included.

2 Accreditation

Accreditation is an external check on qualifications and minimum standards of a quality system. An accreditation scheme should be adaptable and flexible to assure quality in the face of changing system requirements and scientific methods. Funding, regulatory guidance, and time management are significantly affected by accreditation. Important findings were identified in the evaluation of forensic laboratory accreditation, comparing different processes and suggesting possible solutions.

Accreditation emphasizes developing procedures that can be continuously improved upon rather than adhering to traditional strict protocols (1), recognizing the inherent push for improvement in any quality system. Sharing of data, technologies, standards, policies, and protocol development through a central point of contact or group allows for a coordination of knowledge and capabilities (2). Though the concept of an external governing body "judging" a laboratory creates many concerns for staff, it was found that the accreditation of a laboratory positively

influenced leadership, communication, and preventative actions through increasing problem solving techniques (3). Analyzing problems, identifying their root cause, and taking corrective action promoted continuous improvement within the laboratories. A survey that evaluated the current perceptions of mandatory accreditation by Texas (U.S.A.) forensic laboratory managers, for example, found that after eight years of operation under accreditation, all eleven responding laboratories identified mandated accreditation as a useful aspect of forensic laboratory development (4). Most responding laboratories also demonstrated an overall improvement in case turnaround time. Accreditation provides a common language leading to internationally-shared goals of excellence:

> "On an international front, education of proposed practices in other countries will aid in cohesion within the field as a whole. Having ISO/IEC 17025 standards throughout the forensic world is crucial for all parties involved and beneficial to realizing shared goals of excellence. To accomplish this, the international forensic community needs to be willing to assist in creating and upholding a uniform ideology." (2), page 140.

While accreditation is beneficial when implemented appropriately (3, 4), Willis cautioned laboratories not to become over-reliant on accreditation as a safeguard against the miscarriages of justice because scientific quality could suffer. The interpretations of forensic tests results are not standardized between laboratories. Though the processes that constitute forensic tests are well controlled by accreditation, the quality of results were dependent on reasoning abilities and decisions that often did not address standard error rates in compliance with requirements for judicial admissibility (3, 4). Management should use, as well as teach, logical and balanced frameworks that identify the capacities and limitations of every scientific finding (2, 3).

3 Crime Scene Management

Poorly managed crime scenes were indicated as a root cause of poor evidence collection and for higher risk of wrongful convictions (11). One study, for example, found that the majority of unprocessed evidence at crime scenes was not submitted for analysis because of an absence of suspects or unfamiliarity with forensic testing (12). In contrast, Whitman and Koppl argue that unsubmitted evidence is due to crime laboratories being administratively or practically identified with law enforcement, which causes bias and consequent errors (13). They identified the problem as structural and suggested it would be eradicated by assigning a case manager and implementing policy changes which required written justification when evidence was not submitted in rape and homicide cases. A different study found that 33% of responding officers said they would collect more evidence at the request of victims; evidence was often collected based on the officer's perceptions of modern technology; and perceived benign evidence often wasn't submitted for analysis (14). Technology, typically, was seen as a central influence on scene management to increase efficiency, unbiased accuracy, and cost effectiveness (15, 16). It was argued that television shows influence crime scene personnel and, in the face of few if any formal policies concerning evidence collection, this also lead to skewed evidence submissions. As one commentator wrote,

> …the fallacy is that CSI started with familiar, common, and basic concepts - such as the mere collection of fingerprints, trace evidence, and DNA at crime scenes) - and then packaged it to sell to a television audience in the form of exaggerated technology and concrete science. This elaborate packaging is alluring, but it camouflages (or some would argue, simply ignores) the true scope of forensic science's capabilities and limitations. In CSI and its brethren programs, the line between fiction and fact becomes blurred when the comfort of certainty takes precedent over the reality of ambiguity (71, page 12).

The role of crime scene personnel and how they are characterized influenced their effectiveness (18). Seven critical skill sets were identified for the most effective personnel: knowledge, experience, professionalism, attitude, communication, cognitive abilities, and stress management (11). It was concluded that when other (non-crime scene) personnel identified the complexity and scope of the crime scene role as a combination of tasks including cognitive elements, the crime scene personnel were much more effective in investigating high-value property crimes than when working with personnel who categorized the job as solely collecting evidence.

4 Education and Research

Rapid growth in interest in forensic science and courses in higher education has led to concerns on the quality of education and preparedness of new recruits. For instance, one study suggested that the rapid expansion in forensic science education has led to two types of emerging courses: "authentic" and "inauthentic" (19). "Inauthentic" courses were found to have overlooked operational forensic science knowledge, practice, and identity, resulting in unprepared graduates. It was concluded that "authentic" courses maintained relationships with law enforcement and forensic science agencies while teaching operational forensic science objectives in the classroom and lab (19, 20). Scholars also argued that a formal education in natural sciences and research met recruitment expectations with the reality of operational practices instilled in graduates (20, 21) and that an extended, supportable educational foundation needs to be laid to create a "learned profession" (65).

Some laboratory managers feel specific forensic science degrees to be second to other natural science degrees because they believed forensic science relevancy could be easily added to a recruit's repertoire (20). It was also argued that recruits with strong natural science research backgrounds were less likely to have mismatched expectations about working in forensic laboratories than those without them (22). Internships decreased the likelihood of a new recruit leaving the field early because interns, having experienced day-to-day work in an operational laboratory, held more realistic expectations (22). Laboratories and interns, as well as educational programs, benefited by forming a symbiotic relationship through internships that involved research projects. Students receive training and mentorship while laboratories achieve validation, method development, and other operational benefits with minimal staff involvement or impacts to casework (Moorehead, 2011, Forensic Interns: Force Multipliers in the Crime Lab)(22, 23).

Use of collaborative research groups between academic institutions and forensic laboratories suggested organizational growth along with educational training (24). In accord with this theory, successes of cross-jurisdictional projects were found to be based on whether research outputs met stakeholder's expectations in both results and quality (25). The use of attrition models were also found to help with future research (26). The use of an attrition model in forensic anthropology, for example, suggested that case conversions (actions leading to closure) would improve through communication, research, and education tailored to reflect caseload (27). Successful collaborations were also found to benefit most when researchers, law enforcement, forensic laboratory managers, and scientists maintained ongoing relationships with each other (25).

Currently no sustainable source of state or federal money exists to support forensic science education or research at the graduate level (28) in the U.S. The situation is repeated in the UK, where a recent parliament committee report criticized the closure of the Forensic Science Service (116). The Committee noted was difficult for forensic researchers to obtain funding for their research; one minister concluded, "Research and development is the lifeblood of forensic science...It may take years before we realise the consequences of neglecting R&D. The Government and Research Councils should now treat forensic science research as a strategic priority."

Several areas were identified as being deficient in forensic research and education as well as funding. Network forensics, unidentified decedents, and taphonomy, for example, were suggested areas of research that would contribute and improve the field of forensic sciences (29-31). The literature recommended that awareness of the rapid growth in available forensic science courses, as well as growing technologies and changes, such as bullet deflection training, would increase transferrable skills and assist international unity (4, 32, 33). It was also recommended that educators of both science and law work together to shape new learning and teaching methods in law and forensic science, while also campaigning for increased funding to bridge divides (34, 35).

5 Funding of Forensic Sciences

With fiscal crises present and looming, governments are obliged to look for greater fiscal responsibility and the typical offered solution is budget cuts (36, 37). Simply cutting costs, however, is not a guarantee of success. As salaries decreased, for example, attraction of experienced applicants and employee retention also decreased. Personnel tend to be the majority of a laboratories' budget. A survey revealed that if given a 10% increase in annual budgets, 86% of laboratories would hire more scientists and technicians; 71% of laboratories expressed that staff members were deficient in training (39). Broader reflections of budget allocations suggested that current recessions were root causes for budget cuts aimed at forensic science laboratories and that public laboratories may have an advantage over private one because public laboratories are not burdened with the economic costs of return rates on investment for owners. Recessions prove public laboratories are not immune from economic failures, however, and may suffer furloughs or

layoffs (36, 40). Management of budgets is critical in achieving consistency and precision within forensic sciences (38).

It was suggested that laboratory managers use practical alternatives to develop cost effective methods that allow for cross-jurisdictional agreements and increase communications (36, 38). Strategic cost effectiveness that maintains efficiency was also recommended to laboratory directors as efforts to develop communication between laboratories (36, 38). For example, Ontario's Centre of Forensic Sciences (Canada) was recognized for employing strategic planning that involved monitoring metrics which coordinated with project goals (37). Analyses of DNA casework concluded that a cost to benefit ratio resulted in a U-shaped curve that showed how optimization of laboratory operations is scaled to case submissions—more is not always better and can lead to expensive inefficiencies (41).

6 Leadership in Forensic Sciences

Forensic science regularly faces scrutiny and critics argue that this is due to a significant deficiency in forensic science leadership (5). An absence of leadership has been shown to lead to inadequate implementation of standards and mistakes in forensic science have contributed to wrongful convictions that have ultimately led to questioning of the forensic science profession as a whole (6, 8). It was suggested that good decision making in forensic science was often a reflection of leadership and literature argued that effective leaders ensured standardization and addressed challenges produced from resource constraints (8). Becker, Dale, and Pavur make a number of recommendations for leadership challenges:

- "Forensic leaders must identify common laboratory outcomes, both tangible and in- tangible, in terms that are quantifiable….
- Leaders must identify common laboratory outcomes for quality that are both tangible and intangible in terms that are quantifiable…
- Leaders must benchmark metrics for productivity, efficiency, cost, and quality with similar-sized laboratories in scope of services and customer demographics…
- Leaders must collaborate with similar-sized laboratories to define best practices, comparing metrics for productivity, efficiency, and quality…
- Leaders must continually monitor these metrics at least monthly (not annually) using statistical analysis tools (e.g., histograms, control charts, Pareto charts) popularized by Deming (2000)…
- Leaders must use cost-benefit analyses and cost-effectiveness analyses as part of the decision tree to solve problems…" (8, pages 220-221).

These recommendations touch on the main themes of this review and the interested reader is directed to the original paper. The literature agreed that effective leadership was necessary at both the national and local levels in order to succeed in the forensic science environment (5, 8). Scholars agreed that it is important to practice leadership through integrity, based upon ethical values (7, 8). One Dutch study determined that leaders need to be made aware of how context can influence decisions (9). The authors came to a number of important conclusions,

- Managers are motivated to obtain more information when it is initially made

available, regardless of whether it is needed or not.

- Forensic decision makers devote more attention to emotionally charged cases, which involves the personal values of the decision maker.
- Tactical but unverified information is used to make decisions.
- Forensic decision makers tend to default to previous decision patterns in the face of tight time constraints; that is, they "go with what they know" rather than a more objective, case-based assessment.

When the participants were confronted with these results expressed "strong denial and disbelief", indicating that "insights into ''real-life'' decision making have not penetrated deeply into the forensic science community". The authors suggest adopting "devil's advocate" perspectives to consistently challenge decision-making preconceptions, and scenarios and education that include ''real-life'' decision-making courses to enhance the self-awareness of the forensic science community (9). This, and other studies, suggest that systematic and scientific changes through leadership courses would reduce the problem and help maintain case integrity (5-9).

7 Management

Management is the effective allocation of resources to achieve a stated goal. The global recession, placing greater constraints on resources, has led to magnified criticisms about management and forensic science administration (37, 43). The NAS report recommended "establishing and enforcing the best practices for forensic science professionals and laboratories" (44). Conversely, it was argued that science has been pushed aside by management (45), although this study misses the mark: Defensible, documented work is what holds scientists and agencies accountable to stakeholders and the public.

A group of researchers recommended using a "balanced scorecard" approach for management of forensic laboratories, a novel approach for that industry. The balanced scorecard is a performance measurement matrix designed to capture financial and non-financial metrics highlight critical success factors for an organization. The scorecard approach does so by aligning organization strategy to key performance objectives, that is goals with actions. The scorecard balances leaders' perspective in two ways. First, it provides a mix of performance metrics from across the organization in a holistic fashion so that no one metric or group of metrics dominates the assessment process. Second, the balanced scorecard re-focuses leaders away from short term performance pressures (common to new leaders) and toward long-term laboratory needs that contribute to future laboratory performance (10, 37, 38). The scorecard measures financial performance, learning and growth, value chains, and the customer experience (see Figure 1). In support, aspects of strategic budget management were addressed by examining the context of singular issues within individual laboratories, including leadership transitions, overall budgeting, and staffing reassignments (8, 40, 46). One study advocated a review of staffing provisions of all public sector laboratories (47):

> "The task now falls to lab directors to exercise as much due diligence in the analysis of staffing data as they do to the analysis of forensic data. Understanding the DNA of the lab will assist in better determining the appropriate actions to take in efficiently and effectively

> managing the staffing process. Moreover, that knowledge will assist in creating a working formula that better serves the lab's long–term plans to create a business case that allows for sustainability. The primary goal is to hire the right people, the right number of people, and put them in the right places." (47, page 9).

A proposed management model using design science suggested an appropriate research paradigm for forensic evidence management; however, the authors noted that their proposal was an initial effort and the "implementation of such a high-level model across [multiple] domains is fraught with difficulty" (48, page 299; also see 49).

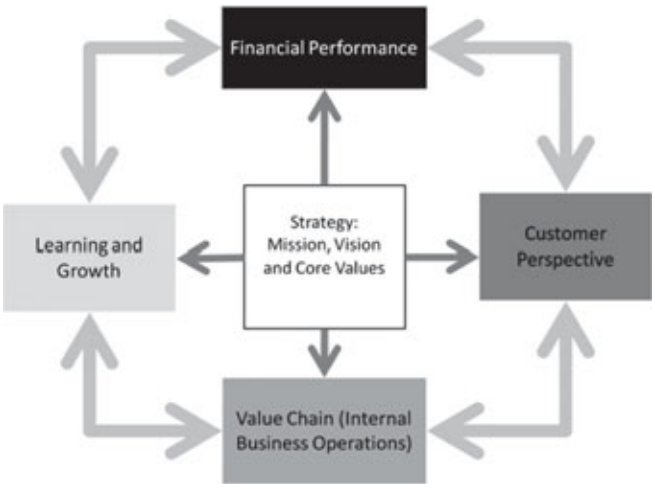

Figure 1. The four metrics of the balanced scorecard (10).

Researchers reported that sharing of performance metrics between employees, units, and laboratories offered significant improvement in performance, case management, and growth of leadership (38, 50); in one example, simply sharing standardized performance metrics (FORESIGHT process) increased productivity by 20%. A specific look at a report on human factors in latent fingerprint work focuses one chapter on the role of management in developing and maintaining a system for producing high-quality results, reviewing the components of a quality organization focused on latent print analysis. These include management personnel, accreditation, certification, proficiency testing, and a systems approach to error identification and mitigation (119).

8 Quality

Quality is the consistent production of a product or service that is sufficiently beneficial to one or more people. The term, quality management, can be thought of as the process of overseeing activities to achieve a desired level of acceptance by another; this includes establishing and implementing methods to control processes and maintain levels of assurance.

The literature offers a variety of views on quality in the forensic sciences. Christian (53) objected to the NAS report's recommendation to create a federal bureaucracy, arguing that it was considered and rejected during the formation of Clinical Laboratory Improvement Amendment of 1988 and in the U.S. Security and

Exchange Commission's (SEC) failure to detect Madoff's activities in the largest financial fraud scheme on record. He does not, however, rule out a federal role in regulation of forensic science. Christian offers the mechanisms developed through the implementation and modification of CLIA '88 as a viable model for the forensic sciences. Another paper offered a discussion on performance measures and concluded that performance measures should be based on the quality of outcomes and results rather than inputs and processes (54). For example, one laboratory that was investigated had seven quality performance measurements in place with only one focused toward an outcome of quality. The authors advocated for many current practices in forensic laboratories to count towards quality rather than strict performance metrics. Finally, the importance of tacit knowledge, known but not made explicit or formalized, should accompany standard operating procedures (SOPs) to increase overall quality management, improve contextual learning, and deepen techniques beyond the minimal steps of an SOP (55).

Another approach that avoids the creation of a US federal government entity was offered that recommended alternative models to promote self-regulation of the forensic enterprise (120). Using independent public and private sector oversight models, like the National Transportation Safety Board (NTSB) and the National Fire Prevention Association (NFPA), the authors make suggestions for a National Forensic Science Board in the US. Detailing the makeup, goals, and organization of the board, the authors provide a draft concept of operations, governance model, and enterprise architecture.

A survey in Australia investigated the formation of a quality assurance committee to review in an over-arching fashion the quality practices of a laboratory system. The review sought feedback from all staff across all teams and disciplines. The review sought to simplify the components of the quality system and achieve enhanced business outputs as well as improved customer and staff satisfaction (56). Too much emphasis on quality, however, appeared to reduce "experimentalism and active questioning of science" (45). For instance, in an assessment of an academic forensic anthropology laboratory that focused on high efficiency and quality, it was inferred that graduate students were better able to integrate new operating procedures in analyses than senior staff. Despite the emphasis on quality, senior staff often used shortcuts inconsistent with the accreditation and quality measures in place (57).

Finally, a study of hierarchical testing of postmortem samples indicated that cost savings may effect quality (58). Screening for drugs on decedents started with urine and positive results required further testing of blood for confirmation. The study evaluated the clinical and cost-effectiveness of this practice. The urine screens yielded a sensitivity of 64% and a specificity of 73%. While the cost savings were theoretically 34% (because urine screens are cheaper), the low quality of the urine tests required too much rework to be cost effective.

10 Science & Law

Forensic science and the law are strange, occasionally troublesome, but ultimately inevitable bedfellows (71). The tensions of the relationship between science and the law are multiple and varied:

> Intensive demands of judicial authorities absorb most resources of forensic laboratories around the world. Treatments of evidence are never sufficiently rapid and cheap, and requirements for accredited procedures to ensure chain of custody as well as to control technical methods focus the attention on tests and consume resources. There are too few opportunities to investigate into fundamental developments because of operational constraints. This is compounded by the general divide that exists between centralised laboratories, the police and field activities (59; page 12).

A growing number of voices support forensic science being defined as a separate, interdisciplinary science of its own and not simply an "applied science" (59-61, 65). This includes a refocusing on evidence and evidential meaning rather than being concerned with a purely legal interpretation (59).

In one of the more important papers in this review, Strom & Hickman recommended non-traditional changes within law enforcement and forensic laboratories in order to decrease backlogs (62).They note, sadly, that forensic laboratories are not "typically thought of as a decision stage within the criminal justice process", ignoring simple yet highly effective methods to smooth workflow. The authors conducted interviews with state and local police agencies, prosecutors, and forensic laboratory personnel in ten U.S. jurisdictions, focusing on controlled substances cases. The study demonstrated that the approach with the greatest potential to reduce backlogs and maximize resources was communication between laboratories, the police, and prosecutors. After the law enforcement officer at arrest, the forensic laboratory and its staff are the second major decision point in the criminal justice process. Respondents to the interviews noted that law enforcement agencies did not uniformly use or know how to use field testing kits, pushing the burden to the laboratory. Thus, poor communication from field to laboratory and from prosecution (the third major point) to the laboratory contributes to many of the issues seen in this study, including overflowing evidence rooms, unnecessarily retained or destroyed evidence, persistent demands for rushed analyses, and—most important—unnecessary laboratory requests. This last factor leads to what Strom and Hickman call "artificial backlogs", requests for analysis initially submitted that are no longer required. As the authors note,

> "As one laboratory respondent noted, although the laboratory currently has a backlog of 3,400 controlled substances cases, it is likely that only 1,500 of them represent "true" cases in need of analysis…Study participants estimated that 50% to 75% of the drug case backlog represented cases that had already been pled out or dismissed, a clear result of the lack of adequate feed- back loops necessary for rational decision making on the laboratories' part." (62, pages 65 and 66).

Other factors contributing to backlogs included the volume of case submissions, insufficient staffing, and the intense resources needed to process clandestine laboratories. Although this paper used controlled substances as a case focus, many of these findings could be applied to other areas of forensic laboratories, leading to potentially fruitful research.

An investigation of New Zealand forensic science services found a financially self-sustaining laboratory that worked under a fee for service arrangement (42). The author notes strengths of this one supplier/one customer market (monopoly/monopsony), including the political empowerment of the laboratory and non-existent backlogs; the weaknesses include the imperative of commercial drivers and the subsequent mismatching of economic value versus forensic value. Overall, for that market with its attendant limited caseload, "The focus on value for money and cost/benefit in a pseudo-commercial environment may in certain respects be a 'mixed blessing' but it has led to…improving the effectiveness of forensic science and initiatives to add value to the forensic science services delivered" (42, page 155).

Ever since the 2009 NAS report recommended that forensic laboratories be administratively separated from law enforcement, the notion of independent laboratories has been discussed with greater frequency in the literature (61). The first independent forensic laboratory post-NAS study was established in Washington, D.C. in 2012 (107); other jurisdictions are considering related efforts (108). Differing literature claims that the legal system has contributed to the growth of forensic sciences and independence of the sciences would not lead to an elimination of informational asymmetry, but rather would increase the probability that defense attorneys had influence over evidential testing (65, 66). Contradictory to the NAS report, it was suggested that forensic science redefine itself by specifying capabilities and limitations to lead investigative services in conjunction with law enforcement (65, 67). It was also argued that if scientists had full control of crime scenes and access to knowledge about cases, testing redundancy and backlogs would be minimized (65, 68). A case review identified three basic needs for a forensic scientist and criminal justice professional: transparent writing in expert reports, expert conclusions supported by sufficient data, and all statistical evidence must be understandable to juries (69). A case review of the application of liberalizing processes in England and Wales defined evidential value as how the item relates to the delivery of justice, which further connected the two fields (68). In a study where Bayesian tests were used to assess prosecution and defense hypotheses, it was concluded that the partnership with forensic science and law enforcement informed police more on forensics and decreased redundancy and backlogs; it also suggested increased tensions between law enforcement and forensic laboratories when forensic scientists were given more influence over which tests would be conducted on items in question (70).

In a newly retrospective topic, one study discussed the treatment of DNA analysis as "exceptional" (the NAS report's "gold standard" comment being one of the strongest indications of this). The author welcomed "an expansion of precision estimation (expressed through probability figures), upgraded procedural standards and practitioner credentials, protections against error, and so forth" but cautioned that science is still science and any method should not be above reproach or revision. Finally, Cowan & Koppl (66) suggested considering laboratory consolidation to increase ease of accreditation and decrease cost to taxpayers while allowing cross-jurisdictional management of crime laboratories with scientific inquiries available to prosecution and defense (64).

The push for forensic science to "re-claim" itself from its detractors grows (61) and

the call for the profession to assert its “rights” comes, interestingly, from above and below (60, 61, 65). As one commentator put it:

> Perhaps it is this reality, that forensic science is at once a powerful friend and imposing enemy, that ignites such controversy. It has so much to give and yet so much that can be taken away. To a resource-strapped university, it seems to be a source of new tuition dollars. To a law professor looking to gain notoriety, it is a topic that attracts widespread attention and fuels energetic debate. To an attorney desperate to win a case or exert political will, it becomes an available pawn. To the forensic scientists who want to do the best work they can for the criminal justice system, however, it is an increasingly intolerable situation (61, page ii).

As other studies in this review point out, forensic science has a key role to play in its own utility and future; the science, therefore, should be left to the scientists.

11 Efficiency

Forensic science laboratories, whether public or private, are held accountable by those they serve. The functions of those laboratories may not be well understood, however, by either those that manage or oversee the management of the laboratory. Therefore, performance has been based on “non-standard, ill-defined, or non-existent criteria. Successes and improvements go unrecognized and opportunities for advancing the mission and goals of the organization are squandered” (109, page XX). The terms efficiency and effectiveness have been defined for clarity (109). Effectiveness is the capability of producing an outcome, while efficiency is producing that outcome in the most economical fashion. While many in the literature decry the emphasis on efficiency over effectiveness, the fact remains that resources (time, money, people) are limited and finite. Because money is only an input in government (as it cannot generate profits like the private sector), if resources are not used to their maximum benefit, scientific quality suffers, backlogs increase, and the stakeholders are not well served (3).

Numerous papers address the efficient and effective allocation of resources, most notably the extended series of papers from the FORESIGHT project (10, 36-41, 80). Initially a pilot project funded by the US National Institute of Justice, FORESIGHT now has over 80 laboratories worldwide participating in the process. FORESIGHT is a performance benchmarking system for governmental forensic science laboratories and uses standardized definitions for metrics to evaluate work processes. The data generated links financial information to casework and subsidiary tasks, linking performance to accountability. FORESIGHT provides the information necessary to assess resource allocations, professional development of staff, drive efficiencies, and evaluate the value of services—the mission is to measure, preserve what works, and change what does not.

FORESIGHT has led to significant improvement at the individual laboratory level and at the forensic industry level. Prior to FORESIGHT, evaluation of efficiency and effectiveness of a crime laboratory was virtually impossible without a common industry language and corresponding performance benchmarks. That common

language has been created (36) and industry-specific metrics have been developed for comparison across laboratories and across time for an individual laboratory (111). FORESIGHT has led to the development of decomposition metrics, borrowing from financial management techniques in for-profit industries and adapting them for use in the public sector (112) These decomposition metrics offer participants comparisons to industry standards and a means to evaluate targeted internal strategies for change (37). FORESIGHT has led to a macro view of the provision of forensic science services, showing that individual laboratories are highly efficient in the provision of services, but rarely cost effective because of the reliance on political jurisdictions, rather than economic markets, for the provision of services (113).

Research from FORESIGHT data suggests that cross-jurisdictional cooperation would permit huge gains in efficiencies (36). Such cross-jurisdictional gains have implications beyond the justice system and offer lessons for improvement in a variety of public services. For example, one laboratory was recommended to FORESIGHT by the National Institute of Justice as one of the best performing laboratories for DNA analysis. At the tactical level, the information sharing of FORESIGHT member best practices contributes to process improvement by all members. Even with an *ex ante* exemplary performance, during the first four years in Project FORESIGHT, this laboratory was able to reduce their cost per sample for DNA analysis by 31%, increase productivity by 58%, while decreasing total staffing by 7% and increasing total costs by a mere 2%. The impacts are significant for the individual laboratory, but the social gains dwarf the gains to the individual laboratory. Using the analysis of Doleac (113), the social gains from the additional DNA analysis implies an annual societal gain of approximately $4.7 million from the productivity increase since participation in FORESIGHT. This type of analysis helps laboratories create cogent, relevant narratives for resource requests and support.
While the primary size-determining factors for public sector operations allow a political entity to exercise great control, they do not necessarily lead to a cost-effective approach to the provision of services (McAndrew 2012). Lacking the pressure from competitors to find more cost-effective solutions, the public laboratory may continue to operate at an efficient level. However, it runs the danger of forced change in an economic crisis, and this may have undesirable consequences, as seen with the FSS (Dougan 2012). To remedy the situation, laboratories need to understand the proactive alternatives that couple the efficient delivery of services with a cost-effective level of activity. That cost-effective level could involve cross-jurisdictional delivery of services through agreements on insourcing or outsourcing cases. It could also include out- sourcing some casework to the private sector or even abandonment of some services to private providers (112; Figure 2).

Efficiency is scale dependent, as well. Creating the “best” forensic laboratory system is not having the newest instrumentation or huge budgets but should also examine the optimal jurisdiction size and the optimal level of output of forensic services to be provided. The law of diminishing marginal returns (LDMR) in economics dictates that each additional benefit to a system will only add a proportional benefit as more are added; that is, you cannot hire staff infinitely and expect a linear improvement in productivity. The “right size” of a laboratory is determined by the number of cases submitted, the processing efficiency, and the demands of the stakeholder base (delivery times, etc.). With government budgets decreasing, collaborative and cooperative solutions must be sought out to improve

efficiencies from economies of scale (114, 115).

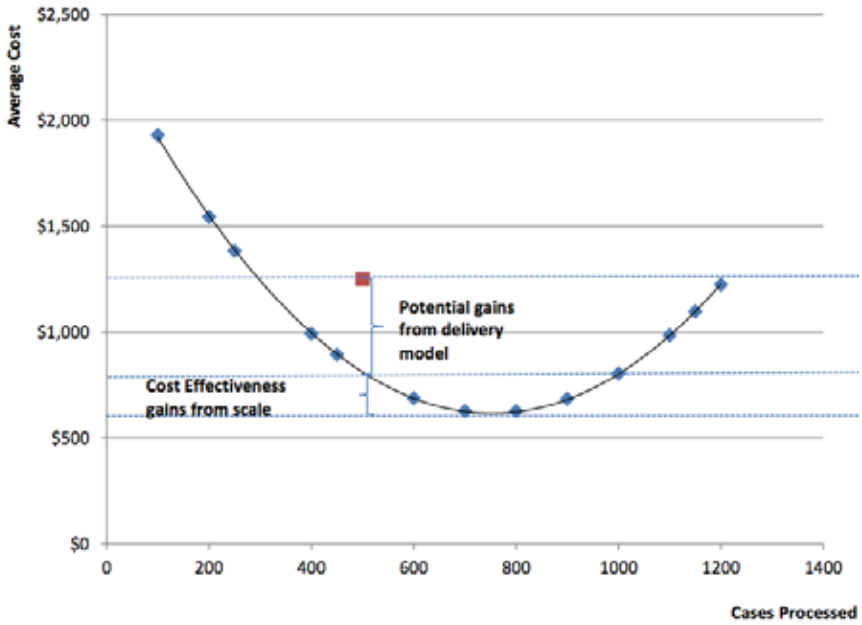

Figure 2. Two dimensional view of potential gains in forensic work (112)

Technical issues also pertain to efficiency. The use of radio frequency identification devices (RFID) to tag evidence (101), including in in “hot” zones, due to CBRN events (89), was simulated. With an accuracy rate of between 66% and 98% on people and objects, the technology needs further development and evaluation. The quest for a “paperless” forensic laboratory was discussed, noting that while electronic documents do save money on supplies, it can sometimes take longer to gain an electronic signature than a “real” one (96). Like any enterprise-wide effort, support must come from management with a true understanding and commitment from the staff.

12 Staffing

Staffing is the key resource for a forensic service provider; staff typically consume for 70% to 80% of a laboratory’s costs (see Figure 3). A central paper that offers a roadmap for staffing (47) suggests a review of both labor demand and supply are significant for a laboratory to create key staffing performance indicators and to maintain a viable workforce. Assessing how many new employees are required, the type of personnel needed, recruiting sources is essential for long-term stability and productivity. Further analysis of market trends will highlight changes in talent pools; a gap analysis then compares the areas of concern that require action on behalf of the laboratory with the potential prospects for hire over time.

	Summary Statistics		
Investigative Area	Mean	Median	Std. Dev.
Blood Alcohol	71.81%	74.28%	9.75%
Digital Evidence (computer, audio, video)	62.00%	75.55%	28.68%
DNA Casework	62.20%	60.82%	12.51%
DNA Database	46.76%	38.97%	15.12%
Document Examination (including handwriting)	78.08%	82.47%	11.14%
Drugs - Controlled Substances	74.73%	76.04%	9.95%
Explosives	72.88%	75.03%	16.98%
Fingerprint Identification	81.05%	84.03%	10.58%
Fire analysis	76.02%	75.32%	11.80%
Firearms and Ballistics	80.69%	83.08%	9.51%
Gun Shot Residue (GSR)	76.94%	79.33%	11.00%
Marks & Impressions	79.26%	81.70%	12.14%
Serology/Biology	77.04%	80.06%	11.99%
Toxicology ante mortem (excluding BAC)	66.85%	64.39%	11.34%
Toxicology post mortem (excluding BAC)	67.23%	64.17%	12.85%
Trace Evidence (includes Hairs & Fibers, Paint & Glass)	71.55%	72.11%	13.39%

Figure 3. Personnel expense as a percentage of total laboratory expense, 2010-2011. FORESIGHT data, West Virginia University.

An example of this type of planning is offered in (11), where the authors provide selection criteria that map potential success for recruiting crime scene personnel. The guidelines suggest using multi-source portfolio of information:

- Psychometric assessment measuring a number of different cognitive abilities,
- Leadership potential,
- Stress reaction/tolerance
- Targeted written selection criteria and focused standard and behavioral interview questions, and
- A thorough medical assessment.

Although the concern of choosing a new hire is important, the turnover rate of the laboratory holds urgent attention as well. Employee turnover is costly, in time and money (8). Given the proportional costs of personnel offered above, any cost effective methods that can recruit personnel who work well and stay with an organization are desirable. Therefore, delivering a workplace for long-term employee retention must be achieved by examining the root cause for employee turnover (106).

Using assessment tools developed in industrial psychology, project personnel evaluated the connections between human capital resources and their development and the performance of the laboratory (106). The authors reported that an intertwined relationship of embeddedness, strategic vision, autonomy in the laboratory, task significance, and transparent leadership increase employee retention. *Embeddedness* is a term used to describe the three factors influencing the decision to continue employment in a workplace; the three factors are link, fit, and sacrifice:

- *Link* is the relationship of the worker to other people and activities. Encouraging positive work relationships by arranging outside activities is recommended to *link* the employees.
- *Fit* is the extent of how the workers skills, abilities, and values match the demands from the workplace. Ongoing evaluations and opportunities for improvement must be implemented.
- *Sacrifice* is what the employee would give up should they leave their current workplace. Incentive mechanisms, such as creating opportunities for promotions, boost positive embeddedness.

When the director of the laboratory and/or management solicit a communal input on the laboratory's vision, they are demonstrating trust to their employees to offer feedback as well as an opportunity fully understand the mission of the laboratory. With that in mind, a worker will begin to possess a high sense of allegiance to the mission and assign significance to their tasks. Furthermore, discussion with workers of stakeholders' investment into the success of the laboratory would encourage workers to weigh significance to their duties.

Staffing is a major component that can decide the success of a forensic science laboratory. Without appropriate management of lab personnel, operations within the laboratory will disintegrate and fail to properly carry out the mission of the organization. If laboratories are unable to add to their staff to accommodate the added requirements of accreditation and proposed reform, time management becomes an even greater priority. Ultimately, without additional support, the qualitative or quantitative value of the crime laboratory could be jeopardized. (2)

13 Forensic Science Service Closure

The UK Forensic Science Service (FSS) was closed in March of 2012. The FSS was the primary forensic science agency for the UK and a world leader in the profession, pioneering DNA profiling and creating the world's first DNA database (April 1995). The FSS became an executive agency of the Home Office in April 1991 and then a trading fund in April of 1999 in attempts to create market efficiencies in the delivery of forensic services. In December of 2005, the FSS became a government-owned company, responsible for profit and losses; it was the Home Office's only government-owned company. A variety of market pressures, including the increasing and changing use of competitive contracting by various police forces resulted in the loss of market share for the FSS. Despite influxes of cash from the UK government and the closure of three regional laboratories to stem losses, in December 2010 the UK government announced that the FSS was to be closed by March 2012. Strong political and scientific criticism followed, decrying the damage to the UK criminal justice system (118), quality of forensic services, and research in the science.

In July of 2013, a UK parliamentary committee on Science and Technology issued a second scathing report (116), saying that the government was too slow to recognize the wider costs of the FSS closure. The segregation and jurisdictional fragmentation of forensic services—a common criticism in the US—led to a lack of transparency of new and ongoing police expenditures on forensic science. The Committee also

found that some of the new police forensic units were not adequately pursuing accreditation (ISO 17025), emphasizing the role of the Forensic Science Regulator (FSR). At the writing of this review, the debate continues, including a more realistic analysis of the true cost of closing the FSS, £300-350M compared with the government's figure of £95M; the additional costs come from the added costs of the police forces filling the gap of forensic services after the FSS closure (117).

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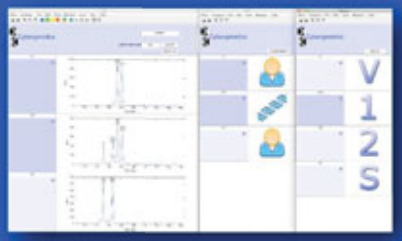

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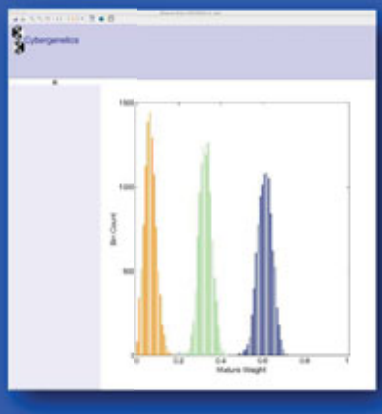

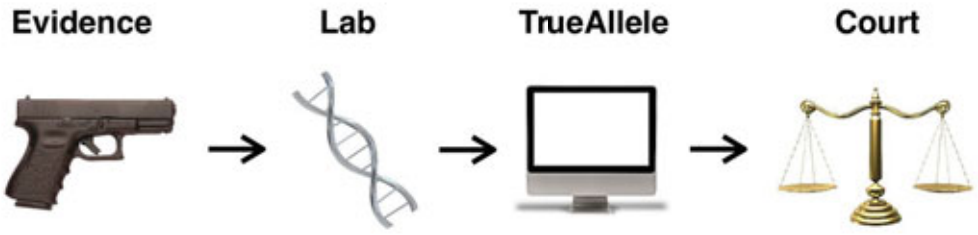

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