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An Evaluation of Enhancement Techniques for Footwear Impressions made on Fabric

by

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A thesis presented in fulfilment of the requirements for the degree of Doctor of Philosophy

2011

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Signed: _____

Date: _____

To

All my family (here and gone)

"We will be known forever by the tracks we leave."

Native Indian Proverb

Abstract

Robust comparisons of enhancement techniques may be limited due to variations, (e.g. pressure, contaminant and surface) in the preparation of test footwear impressions. The use of chemical enhancement techniques on porous substrates, such as fabrics, poses several challenges predominantly due to the occurrence of background staining and diffusion as well as visualisation difficulties. This is the first systematic study to examine both a wide range of enhancement techniques and the effect of the interaction between the fabric and the contaminant on the subsequent enhancement. The work focuses on three commonly encountered contaminants in casework namely blood, urine and mud.

A mechanical stamping rig was developed and used to control the delivery pressure of the mark. The surface topography and porosity of the fabrics studied were investigated and had an effect on the enhancement abilities of the various techniques. This appeared to be due more to the porosity of the material rather than the topographical nature of the fibre surface. This was particularly the case for the wet contaminants (blood and urine). A mechanism for grading the enhancement ability of the reagents for a given impression was investigated and refined for impressions in blood using practitioner and forensic science student volunteers. This provided a potential means of subjectively determining both the quality and quantity of enhancement provided by a given reagent or enhancement mechanism.

The results demonstrated that several chemical techniques were suitable for the enhancement of footwear impressions in blood, urine and mud on light coloured fabrics irrespective of the nature of the fabric. Fluorescent and chemiluminescent techniques provided excellent contrast on dark surfaces when enhancing footwear impressions in blood, but were less successful for the enhancement of impressions in urine and mud. Oblique lighting did, however, provide limited enhancement of mud contaminated impressions on dark fabrics. For impressions in blood and urine, the surface topography of the fabric had little effect on the enhancement abilities of the various reagents investigated, while the success of enhancement on footwear impressions in mud depended on both the soil type and fabric surface topography.

Acknowledgements

I would like to give my sincere thanks and express my gratitude to my supervisor Niamh Nic Daéid. Her guidance, assistance and dedication throughout the entire project have been essential for this dissertation to be completed. It is greatly appreciated. Shortly after my viva (23/09/2011), Niamh was awarded a professorship. Congratulations!

Many thanks go to my external supervisor Helen Bandey at CAST (formerly HOSDB) for her constant support, encouragement and willingness to help. Your enthusiasm on impression enhancement proved to be contagious. Thanks are also due to all the personnel at CAST including Valerie Bowman, Steven Bleay, Andrew Gibson and Tomasz Ciuksza. CAST is also gratefully acknowledged for their financial assistance.

Thanks also go to all the staff at the Centre of Forensic Sciences for their help and patience. Special thanks are due to Georgina Bradshaw for assistance in the lab and lengthy chats.

Funding and support from the University of Strathclyde, the Engineering and Physical Sciences Research Council (EPSRC) and the Maltese Government is duly acknowledged.

Words of thanks also go to Lorna Dawson and her colleagues at the James Hutton Institute (formerly the Macaulay Institute of Land Use Research) for their assistance and knowledge regarding soils. Thanks to James McVee at Heriot-Watt University for his assistance in using a scanning electron microscope.

I would like to thank all my PhD colleagues, of past and present (Shane, Ainsley, Hillary, Vanitha, The Chiefs and family, Ice, Four, Sara, Yuva, Lucy, Greg, Felicity, Aude, Kachi, Alaia) who have provided numerous memories throughout this journey - your friendship made it much easier.

Special thanks go to my girlfriend Anika Ludwig for her support, belief and for enduring my moans and complaints. Thanks also go to Anika's family for their support and good times.

Finally, but not least, I would like to give my sincere thanks to all my family for their continuous support, encouragement and for their belief in me.

Publications and Presentations Related to this Research

Publications:

Farrugia K.J., Nic Daéid N., Savage K.A., Bandey H.L., Chemical enhancement of footwear impressions in blood deposited on fabric – Evaluating the use of alginate casting materials followed by chemical enhancement, *Science and Justice*, 2010, 50 (4) 200-204

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Oral Presentations:

The enhancement of footwear impressions in blood on fabric, presented at the 95th IAI International Conference, Spokane, *USA*, July 2010

The enhancement of footwear impressions made in urine on fabric, presented at the 95th IAI International Conference, Spokane, *USA*, July 2010

Poster Presentations:

Farrugia K.J., Nic Daéid N., Bandey H.L., Ciuksza T., *A Comparison of Different Techniques for the Enhancement of Bloody Footwear Impressions on Cotton Fabric*, 5th European Academy of Forensic Science Conference, UK, September 2009

Farrugia K.J., Nic Daéid N., Riches P., Bandey H.L., *Controlling the Variable of Pressure in the Production of Test Footwear Impressions*, 5th European Academy of Forensic Science Conference, UK, September 2009

Farrugia K.J., Nic Daéid N., Savage K.A., Bandey H.L., Ciuksza T., *The Enhancement of Shoeprints in Blood on Fabric with Peroxidase Reagents*, University of Strathclyde Research Day, UK, June 2010

Farrugia K.J., Nic Daéid N., Savage K.A., Bandey H.L., Ciuksza T., *The Enhancement of Shoeprints in Blood on Fabric with Peroxidase Reagents*, 95th IAI International Conference, Spokane, *USA*, July 2010

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List of Abbreviations

AB1 – Acid Black

ABTS – Azino-di-Benzthioazoline Sulphonic Acid

ACPO – Association of Chief Police Officers

AFP – Australian Federal Police

ANOVA – Analysis of Variance

APHA - American Public Health Association

AV – Acid Violet

AWRE – Atomic and Weapons Research Establishment

AY – Acid Yellow

BPB – Bromophenol Blue

BR – Basic Red

BSC – Biological Stain Commission

BY 40 – Basic Yellow 40

CAST – Centre for Applied Science and Technology

CFC – Chloro Fluoro Carbons

CSC – Conclusions Scale Committee

DAB – Diaminobenzidine

DFO – 1,8-Diazofluoren-9-one

DNA – Deoxyribonucleic Acid

ENFSI – European Network of Forensic Science Institutes

ESDA – Electro Static Detection Apparatus

ESLA – Electro Static Lifting Apparatus

EWG – Expert Working Group

FBI – Federal Bureau of Investigation

FSS – Forensic Science Service

HFC – Hydro Fluoro Carbons

HFE7100 – 1-Methoxynonafluorobutane

HFE71DE – Mixture of 1-Methoxynonafluorobutane and *trans*-1,2-Dichloroethylene

HOSDB – Home Office Scientific Development Branch

IND - Indanedione

LCV – Leuco Crystal Violet

LMG – Leucomalachite Green

MoFDT – Manual of Fingerprint Development Techniques

MPFSL – Metropolitan Police Forensic Science Laboratory

MTN – Methyl Thioninhydrin

NPIA – National Police Improvement Agency

OPD – *ortho*-Phenylenediamine

ORO – Oil Red O

PACE – Police and Criminal Evidence Act

PD – Physical Developer

PPD – *para*-Phenylenediamine

PSDB – Police Scientific Development Branch

PVS – Polyvinyl Siloxane

RCMP – Royal Canadian Mounted Police

RSD – Relative Standard Deviation

SEM – Scanning Electron Microscope

SICAR – Shoe Impression Comparison and Retrieval

SOCAP - Serious Organised Crime & Police Act

SOCO – Scenes of Crime Officer

SRDB – Scientific and Research Development Branch

SSKIB - Scottish Soils Knowledge and Information Base

STR – Short Tandem Repeat

SWGTREAD - Scientific Working Group for Shoeprint and Tire Tread Evidence

TEC - Europium tris(thenoyltrifluoroacetone)

UK – United Kingdom

USSS – United States Secret Service

UV – Ultraviolet

WEAA – Water/Ethanol/Acetic Acid

Chapter 1: Introduction

1.1 Thesis Overview

Chapter 1 of the thesis introduces the evidence of footwear impressions and the associated legislation. It also discusses its evidential value and different methodologies for the recovery of such evidence. Also presented is a brief introduction to the fabrics used in the study and the interaction of these fabric surfaces with the chosen contaminants.

Chapter 2 describes previous methods of preparing test footwear impressions. Footwear impression lifting and enhancement techniques may be affected by several variables introduced during the production of test footwear impressions, thus limiting the usefulness of enhancement technique comparisons and the results obtained. One such variable is the force applied when the impressed mark is being made. Data obtained from volunteers was used to design and build a mechanical device which could be calibrated to consistently deliver footwear impressions with the same force onto a receiving surface. Test impressions were prepared with this device for the remainder of this research

Chapter 3 describes in detail the literature and evolvement of blood enhancement over time. This chapter details the enhancement of footwear impressions on nine different types and colours of fabric and provides the backbone for subsequent experiments using contaminants other than blood. Different enhancement techniques such as protein stains, peroxidases and amino acid reagents are discussed and compared in addition to alternative lighting and photography.

Chapters 4 and 5 follow on to describe the enhancement of footwear impressions in urine and mud. The concepts of fluorescence and calibration of computer monitors and cameras is also discussed. A case scenario for the enhancement of impressions in urine on fabric is also presented. Four soils with different chemical properties were collected from around Scotland, as advised by the James Hutton Institute. Further to enhancement of soil-based footwear impressions, the soils were analysed for pH and inorganic content.

Chapter 6 describes the use and refinement of a mechanism for grading both the quality of enhancement and the level of quantity of an enhanced impression. The grading was carried out on impressions made in blood only and is presented as a potential subjective measure of enhancement quality which facilitates the selection of an optimum enhancement methodology.

Chapter 7 provides a general conclusion to the study and sequential enhancement flowcharts together with limitations of sequential enhancement. This chapter also discusses recommendations for future work.

1.2 Introduction and Legislation

It has been suggested that footwear impressions can offer reliable evidence to demonstrate or exclude the presence of an item of footwear at a crime scene [1, 2]. The Forensic Science Service (FSS) and the National Policing Improvement Agency (NPIA) state that footwear impressions are the most frequently encountered evidence at crime scenes after DNA and fingerprints [1, 2]. Footwear can acquire a number of scratches, cuts, nicks and normal wear and tear during its lifetime, resulting in a high degree of individuality. As well as brand and type of shoe, it has been suggested that a footwear impression can provide information about the approximate height and weight of the wearer [3]. Despite this, footwear impression evidence only accounted for a small percentage of evidence recovered from crime scenes in the UK during the early 2000s [4]. Footwear impression recovery from crime scenes in England and Wales was low, mainly because scene of crime officers were not actively searching for this kind of evidence or if recovered, the evidence was not processed [4]. During a 12 month period in 2002/3, data from 36 police forces in England and Wales revealed that footwear impressions were recovered from an average of only 9% of crime scenes attended by a crime scene examiner compared to a 32.7% recovery rate for fingerprints. The highest performing police force within the study recovered impressions from 19.1% of all examined crime scenes, whilst the lowest performer recovered impressions from only 1.8% of scenes [4]. During the 12 month periods of 2006/7 and 2007/8, the average recovery of footwear impressions from crime scenes

attended increased from 9% [2002/3] to 11% [5]. Footwear evidence processing was generally treated as an ‘occasional examination’ and the responsibility of analysing footwear evidence fell to fingerprint, document or toolmark examiners [6]. Since the early 2000s more resources have been dedicated for research in footwear impression evidence including the establishment of a dedicated footwear examination team by some UK police forensic laboratories [7]. Although persons involved in criminal activity are generally thought to be more forensically aware, it is uncommon for them to take precautions against leaving footwear impression evidence and such evidence can be used in investigations where no DNA or fingerprint evidence is available [8, 9]. In contrast, gloves and non-shedding clothing hinder the detection of fingerprints and trace evidence.

Footwear impression evidence became a priority for the Association of Chief Police Officers (ACPO) following legislation changes in England and Wales to the Police and Criminal Evidence (PACE) Act 1984 on 1st January 2006 [10]. The new legislation regarding footwear followed fingerprint and DNA legislation by empowering police officers to take footwear impressions or seize footwear from apprehended suspects. Footwear impression evidence is discussed in Code D of the PACE Act and as outlined under sub-section 1.3A, “Identification using footwear impressions applies when a person’s footwear impressions are taken to compare with impressions found at the scene of a crime” [10]. In accordance with the Serious Organised Crime and Police Act (SOCAP 2005) [11], police are permitted to “take, retain, speculatively search and share footwear impressions”. Furthermore, the Criminal Justice Act [2003] emphasises the importance of identification data, and increases police powers to enable them to take samples from individuals remanded in custody following arrest for a recordable offence [12]. In the UK, footwear impression evidence is predominantly used for intelligence in linking crime scenes [13].

1.3 Footwear Impression Evidence

The Centre for Applied Science and Technology (CAST), formerly the Home Office Scientific Development Branch (HOSDB), led the initiative on footwear impression evidence research in the UK between 2007 and 2010 where the main priority was to assist police forces understanding the basics of footwear impression evidence. Two areas were identified for research: 1) setting standards for the imaging of footwear impressions at scenes of crime or in custody suites and 2) development of techniques and retrieval methods for the recovery of footwear impressions [14]. Research and operational requirements are communicated to CAST through the UK National Footwear Board's Research and Development sub group [14]. Results from these projects are used to advise and provide recommendations to police forces on how to obtain evidential value from footwear impressions [14, 15].

In 2007, the NPIA [1] published a footwear impressions recovery manual for police forces with advice from CAST and Lancashire Constabulary. The manual covers the basics of impression formation, photography, casting of 3D impressions, lifting of 2D impressions and the use of fingerprint powders to develop footwear impressions. However, the manual lacks information on the chemical enhancement of footwear impressions and how to process these impressions formed from different contaminants on varying substrates.

CAST have published the Manual of Fingerprint Development Techniques [16] (MoFDT) and the Fingerprint Development Handbook [17], which describe different enhancement techniques together with health and safety guidelines and are distributed to police forces in the UK free of charge. International police forces have also purchased these publications. The handbook is used by scenes of crime officers (SOCOs) for quick reference whereas the manual provides all the necessary details for the preparation of stock and work solutions utilised in the enhancement processes in the laboratory. At a crime scene, footwear impressions can be deposited on several surfaces (e.g. carpet, wood, concrete,) and/or produced from different contaminants (e.g. blood, dust).

Figure 1.1 demonstrates examples of footwear impressions that can be found at a crime scene [18].

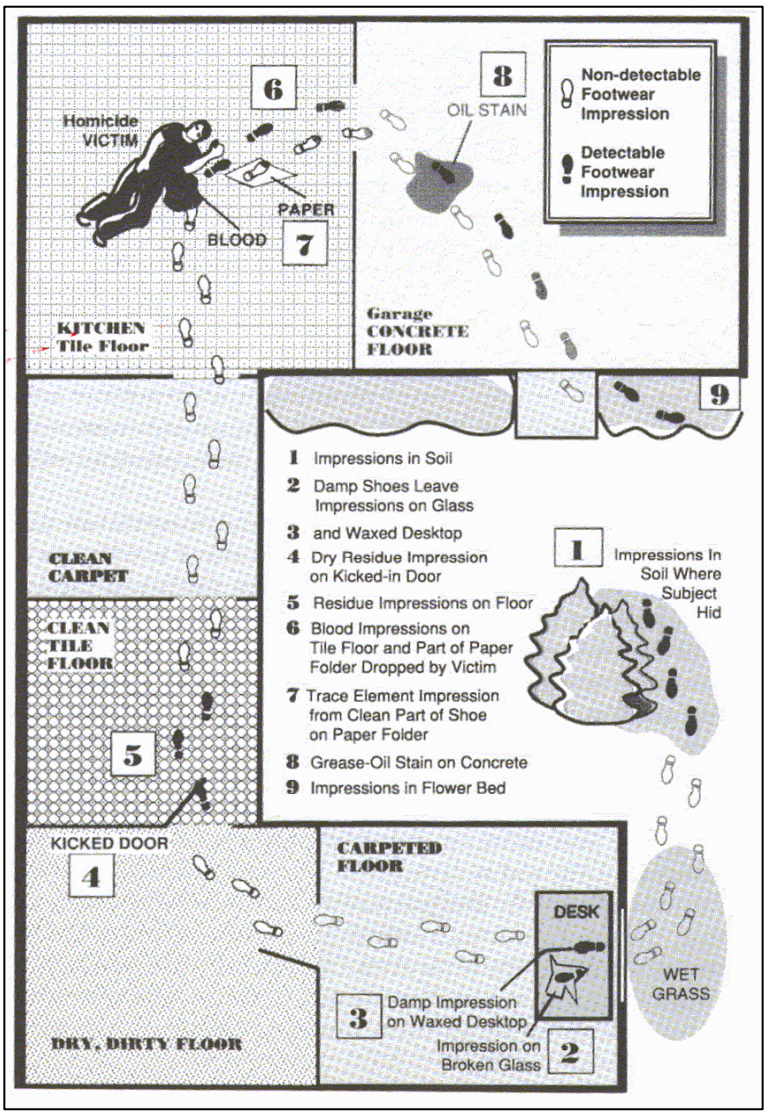

Figure 1.1 - Examples of footwear impressions at a crime scene (reproduced from [18])

Many kinds of impression evidence can be observed in forensic laboratories including footwear impressions, tire impressions as well as a wide variety of toolmark impressions and firing pin impressions. Impression evidence is where 'two or more objects are pressed or stamped against one another allowing these objects to transfer and retain characteristics from one another' [18]. There are three ways in which footwear impressions are formed: the creation of static charge, the

deformation of a surface and the transfer of a residue or contaminant. The process of walking creates a static charge on the outer sole of the shoe which is transferred when stepping onto a hard, flat surface [18]. The residual static charge is short lived and is also affected by high humidity. Deformation of the receiving surface can occur temporarily or permanently to retain the footwear details. Soil and sand can either deform temporarily or permanently, resulting in a three-dimensional impression. Other surfaces such as carpet, fabric and skin usually exhibit a three-dimensional temporary deformation, depending on factors such as residue transfer or bruising of the skin. Transfer of a contaminant generally results in a two-dimensional impression which can be classified as either positive or negative. A positive impression is created when a sole that came into contact with a residue is stepped onto a surface. The impression will depict the areas of the sole that had come in contact with the residue. Conversely, a negative impression is created when the sole makes contact with a contaminant to remove the contaminant and leaves an impression in the contaminant. The remaining impression exhibits areas which did not come in contact with the shoe sole.

An understanding of the processes involved in the manufacture of footwear can help in the identification or elimination of particular brands of footwear. Bodziak [18] extensively discusses the processes involved and benefits of such knowledge. The specific processes involved in the manufacture of athletic shoe outer soles and their significance in the examination of footwear impression evidence are also discussed [19]. Furthermore, the same author also evaluated the presence of air bubbles in polyurethane shoe outer soles which became popular for the manufacture of athletic footwear outer soles in the late 1980s [20].

Two-dimensional impressions can also be of dry or wet origin. For an impression to be of dry origin, both shoe and receiving surface must be dry (e.g. dust) whilst for an impression to be of wet origin, either or both must be wet (e.g. after walking on wet grass). Impressions can also be made in any residue or contaminant; many of which can usually be seen with the naked eye, depending on factors such as the surface type on which the impression is made and the amount of contaminant. Following photography, a wide range of enhancement techniques can be utilised to obtain better contrast and visualisation.

1.3.1 Footwear Characteristics and Location

Footwear outer soles, similar to all mass manufactured items, gain a number of characteristics during the manufacture process. Repetitive characteristics in a number of shoes, during the manufacture process, are defined as class characteristics [19]. If a number of these shoes develop extra characteristics during a deviation in the manufacturing process, then this group of shoes would attain sub-group characteristics [19]. Individual characteristics are attained due to normal wear and tear and the gait characteristics of the individual wearer. This means each footwear item could possess identifiable characteristics which differentiates them from other footwear. Individual characteristics are of great importance to footwear examination because they can potentially provide a connection to a particular pair of shoes when recovered as part of an investigation. The quality of an enhanced and recovered impression will also depend on the nature of the substrate and the type of enhancement.

1.4 Treatment of Footwear Impressions

Three-dimensional footwear impressions are most commonly found outdoors in substrates such as soil, sand, mud or snow. As a result, the impression usually cannot be taken to the laboratory and has to be treated at the crime scene. These impressions are defined as having depth in addition to length and width. Two-dimensional footwear impressions, either visible or latent, can be found indoors or outdoors and can be composed of any residue that the shoe has come in contact with. It is generally recommended that impressions on removable objects be treated at the laboratory [1].

1.4.1 Recovery of Three-Dimensional Footwear Impressions

When synthetic footwear outer soles leave a three-dimensional impression which retains sufficient detail, the chances of identification of the originating footwear can be high as detail may be retained in the substrate (e.g. mud, sand and snow) [21, 22]. Dental stone has been used to cast three-dimensional footwear impressions at crime scenes for many years and it is the first and obvious choice for scenes of crime officers (SOCOs) [23]. Dental stone is obtained in bulk from dental suppliers and has various features which make it a good casting material including the ability to set quickly, record fine detail, readily available and cost effective. It is also safe, easy to use and forms a robust cast to transfer safely to the laboratory. Plaster of Paris, a type of gypsum, was utilised to cast impressions prior to dental stone [24]. However, this medium does not offer a robust cast and most importantly, it does not record the fine detail necessary for positive identification [25]. It is generally accepted that, in most cases, a cast will allow for better observation than photographs of the same impression [25]. The importance of dental stone has been recognised by the Federal Bureau of Investigation (FBI) since 1986 when the FBI Law Enforcement Bulletin stated that “It is now recommended that only dental stone be used for casting impressions” [25]. Since then a number of other materials have been evaluated but dental stone still remains one of the most suitable casting materials used for three dimensional footwear impression recovery. A recent study has successfully captured fine detail from a 2-D footwear impression on a polystyrene cup using dental stone [26].

Pre-weighed dental stone can be stored in a zip-lock bag to which a known volume of water can be added. This facilitates the procedure at the crime scene. Recently, other pre-weighed products such as Traxtone™ and Crime-Cast™ have been introduced to the market. These two products have been evaluated and compared to the traditional bulk dental stone at different temperatures in Florida and Alaska by Bodziak and Hammer [21]. Although Traxtone™ and Crime-Cast™ were successful at producing detailed casts, the traditional bulk dental stone offered better flexibility regarding the size and the amount of material needed at the crime scene. It was also noted that setting took longer for all casting materials at lower temperatures [21].

The authors also noted that Traxtone™ had conflicting instructions on the bag and on the manual. Furthermore, this product has a long setting time, which is more pronounced at lower temperatures. Conversely, Hilderbrand [27] had indicated Traxtone™ as a desirable product arguing that it produced additional detail reproduction and durability. Other highlighted advantages include its short setting time (in direct contradiction to Bodziak and Hammer [21]) and the fact that it is ready to use at crime scenes. Hilderbrand also points out that besides inconvenience, every action of weighing bulk dental stone allows air transfer to the final mixture which can reduce the quality of the cast.

1.4.2 Recovery of Two-Dimensional Footwear Impressions

Unlike three-dimensional impressions, which are always visible, two-dimensional impressions can be either visible or latent. A greater variation of surfaces and residues exist for two-dimensional impressions. This number of different combinations translates to a considerable number of different lifting techniques and chemical enhancement options. In general, the preference is to recover the receiving surface from the scene and complete the chemical enhancement of the impression within the laboratory environment and the specific chemical enhancement reagents are discussed in subsequent chapters. In some cases this is not possible and a lifting or recovery method will be used at the scene.

1.4.2.1 Electrostatic Lifting Techniques

The Electrostatic Lifting Apparatus (ESLA) was developed in Japan in the 1970 [28]. This non-destructive instrument is highly effective in the electrostatic lifting of dry residue footwear impressions, mainly dusty impressions. In the formation of an impression, an electrostatic charge may be created on the surface, which assists dust and other residual particles to adhere to the surface [18]. A mylar film, black side facing down and metallic side facing up, is placed over the impression. A charge is then applied to the mylar film which attracts the dust residue to it. This instrument is commonly used for speculative searching of dusty impressions. Later versions were

developed to be smaller, battery operated and easier to operate with separate pieces of lifting film. If an impression is heavily coated in a residue, the first lift will usually remove the excess residue and subsequent lifting will show better detail [1].

There is still uncertainty about how best to store the resulting lift of a dusty footwear impression for transportation and storage purposes. The mylar film retains a residual charge which may attract additional dust that can damage or obliterate the lift. In any case, the first priority should always be to photograph the lift. The ESLA lift is commonly stored uncovered in a box or rolled up in a tube. Recent research at CAST [14] has established that, after early photography, for transportation purposes, electrostatic lifts should be stored flat in a non-shedding breathable container (not plastic) for subsequent photography. If this is not possible due to the length of the film, then it should be rolled up and stored in a suitable non-plastic container. However, rolling causes lines to appear on the lift and causes the detail to become faint and diffuse. This effect is more pronounced when the lift is stored in a plastic tube, possibly due to the generation of static charge on the tube when rubbed against the surfaces [14]. Cardboard boxes act as insulators and are thus protected from external charges. Furthermore, since humidity accelerates the rate of degradation, silica gel packs can be taped around the container [14]. Electrostatic lifts should also not be stored or covered in plastic since there is the possibility of a partial transfer of the impression from the lift to the plastic [18].

Document analysts utilise a non-destructive instrument called Electrostatic Detection Apparatus (ESDA) to recover indentations left on a document as a result of writing on another document which at the time of writing was overlaying the first one [29]. This instrument was developed at the London College of Printing in collaboration with the Metropolitan Police Forensic Science Laboratory (MPFSL) in 1978 with support and funding from the Home Office [30]. The ESDA has since been developed as a commercial product by Foster and Freeman Ltd. Besides document analysis, ESDA has been utilised successfully to recover footwear impressions from paper and cardboard. The ESDA process is believed to detect the areas where the paper fibres have been deformed. A pump keeps the paper to be analysed against the flat surface of the instrument. A length of clear mylar film is used to cover the paper while a high voltage is applied by means of a corona bar from a distance of about

1cm. A voltage potential (difference in voltage) is created at the indentations which attracts the toner once passed over the mylar sheet. A clear acetate sheet can then be placed over the film for safe keeping.

Although ESDA has been utilised for over thirty years, its mechanisms are not fully understood. Several theories have been put forward but none has won universal acceptance [31]. The mylar film can also be utilised in a similar way to the ESLA to detect dusty footwear impressions on paper. The dust from the impression is lifted by charging the film which can then be placed over a light box or a dark background to visualise the impression [32]. A recent study compared ESLA and the ESDA to determine if both processes could be used to develop footwear impressions of similar quality and in what sequence they should be used to develop the highest quality impression [33]. The study determined that on average, 72.4% of the individual characteristics from known impressions were identified when the ESLA was used first compared to 38.9% when the ESDA was used first. Therefore, ESLA should be carried out before ESDA. A sensitivity test for both methods was also carried out. The sensitivity test for ESLA was carried out by processing ten sheets of paper on which the known shoe was stepped upon in succession to act as a fading series. The quality of the ESLA lifts increased with the decreasing amount of dust on the paper surface. The ESDA sensitivity test consisted of processing ten sheets which had been stepped on as a stack. Only the top paper developed a high quality footwear impression [33].

1.4.2.2 Gelatin and Adhesive Lifting Techniques

Gelatin and adhesive lifters are commonly utilised by footwear impression examiners, however, this procedure can be destructive. Adhesive lifters are generally used to lift powdered impressions whereas gelatine lifters have a more versatile use. Some examiners utilise only gelatine lifts due to its advantages over adhesive lifts [18]. Gelatin lifters are thicker than adhesive lifts and consist of a vinyl backing where notes and observations can be recorded. Bodziak [18] describes the gelatin lifters as ‘covered with a thick layer of glycerol, gelatine and components with a clear cover sheet’. BVDA [34] describes gelatine lifters as being comprised of three

layers. The first layer is the carrier, which holds the second layer of gelatin in a pliable and flexible format. The third layer is the protective cover sheet. The cover sheet is a clear polyester film which is removed prior to lifting, and may be replaced once the lift is completed. These lifters lift a complete print without disturbing the surface on which the impression is found since the low tack prevents any air bubbles from being trapped between the lifter and the surface. Black gelatine lifters have also a high light absorption to enable photograph of even very faint impressions. Gelatin lifters are useful to lift impressions of wet origin where ESLA fails to recover any impressions. These lifters are available in a white colour or even transparent to compensate for any contrast problems. White lifters are mostly used for the lifting of impressions in blood which have been stained with protein dyes and muddy footwear impressions which have been treated with for example safranin O [35]. Once lifted, the impression could be photographed and analysed under better conditions in the laboratory.

In the UK there was some confusion as to the best method for storing a gelatin lifted impression. Some police forces replace the original acetate cover and others store the lift uncovered in a box. CAST [14] carried out trials to determine the best storage method and the study indicated that the best method for storage is to keep the lift uncovered in a breathable box. Low grade cardboard should be avoided since it might shed fibres onto the lift and a breathable box should be used to avoid potential condensation forming on the impression, which could contaminate or alter the recovered impression. Once the lift is transported to the laboratory from the crime scene, photography should be done as soon as possible and then stored by placing an acetate cover over the lifted impression. Removal of the acetate cover, when used at a crime scene was shown to degrade the impression and repeated removal of the acetate degraded the impression even further. In case of heavy powdering, however, repeated removal of the acetate can help to remove excess powder and increase clarity [14].

In 1997, Carlsson [36] reported that electrostatic lifting is better or equivalent to gelatin lifting techniques, except for impressions of wet origin. More recently, Wiesner *et al.* [37] reported that gelatin lifting appeared superior to electrostatic lifting on most substrates tested, especially on non-smooth, porous and fibrous

substrates. Furthermore, 'the superiority of the gel method was revealed only after a cleaning procedure was applied' [37]. In case of doubt or sequential analysis, the authors [37] recommended the use of electrostatic lifting followed by gelatin lifting.

Adhesive lifters are not recommended for lifting original impressions since their high tackiness can damage the impression. These lifters are particularly useful, however, in lifting impressions enhanced with fingerprint powder, although gelatin lifters are also suitable. A clear protective cover is usually supplied to cover the impression once lifted.

Casting materials are not limited to only three-dimensional impressions. Products such as Mikrosil® and liquid silicone have been utilised to lift powdered impressions from different surfaces successfully. Mikrosil, in particular, has been successful in obtaining fingerprint and palm print impressions from decomposed bodies or burn victims [38]. Mikrosil has also been reported in successfully lifting fingerprint detail and footwear impressions from human skin [39-41]. Alginates, primarily used for dental casts [42], have been utilised to successfully lift footwear impressions in blood from substrates such as fabric and concrete [43, 44].

1.5 Enhancement of Footwear Impressions

CAST has been evaluating and assessing the possibility of utilising fingerprint enhancement techniques listed in the MoFDT for the enhancement of footwear impressions with successful preliminary results for certain techniques, especially on non-porous surfaces [45-47].

There are many differences between the enhancement of fingerprint and footwear evidence. Fingerprint enhancement techniques will target a small number of compounds comprising of lipids, amino acids, sweat and sebaceous material. On the other hand, footwear impressions can be created from many different contaminants such as engine oil, greases, vegetable matter, blood, condiment sauces, detergent, mud and dust, which will react in different ways to the enhancement chemicals and techniques. Most research has focused on the enhancement of footwear impressions in blood and mud as these contaminants are encountered to a greater extent at crime

scenes [48-50]. Recent research by CAST [14] assessed the suitability of fingerprint development techniques for enhancing footwear impressions made in a wide range of contaminants on different non-porous substrates commonly encountered at crime scenes. This research focused on contaminants such as different soils as well as alternative ones including WD40, milk, beer, baby oil and soft drinks. Powder suspension worked for more than 50% of all contaminants tested. The major drawback for powder suspension is the excessive rinsing required post application, potentially making the technique unsuitable for porous surfaces and large, fixed, horizontal surfaces [14]. A number of techniques have also been developed specifically for footwear impression evidence such as ammonium thiocyanate and bromophenol blue for the enhancement of muddy and dusty impressions [49, 51-58].

Chemical enhancement techniques will be discussed in the following chapters related to enhancement.

1.6 Fabric Topography and Analysis

This section introduces some of the specific properties of the fabrics used in the study. Included are scanning electron microscope (SEM) images of the fabrics used as well as images of surface contamination of the fabrics with blood and mud.

1.6.1 Fabrics

Commonly available textile materials have a wide compositional range that includes naturally occurring materials, such as wool and cotton, through to fully synthetic products such as nylon and polyester. The properties of fabrics, such as porosity and surface topography, derived from these materials are highly variable and as a consequence, fabric is considered to be a difficult surface for the chemical enhancement of impressions such as fingerprints and footwear. In general, fibres can be divided into four groups: fibres occurring in nature, man-made fibres manufactured from either natural or synthetic polymers, synthetic fibres and regenerated fibres [59].

1.6.2 Textiles

Textile fabrics can be described ‘as an assembly of fibres or yarns that has a substantial surface area in relation to its thickness and such assembly must have a useful mechanical strength’ [60]. There are three basic types of textile fabrics: fabrics composed from yarns (e.g. woven and knitted fabrics), fabrics made directly from fibres (e.g. felts, non-woven and wads) and combined bonded fabrics for speciality fabrics (e.g. fire-retardant fabrics).

The yarns in woven fabrics interlace at right angles to one another where the warp threads are those oriented lengthways in the fabrics, and the weft threads are those introduced widthwise [61-63]. The number of warp and weft threads per centimetre or inch can be counted in a particular weave. The pattern of interlacing warp and weft threads is described as the weave of the fabrics. There are three main types of weaving, each one with its own subgroups: plain, twill and satin [60] and these are illustrated in figure 1.2.

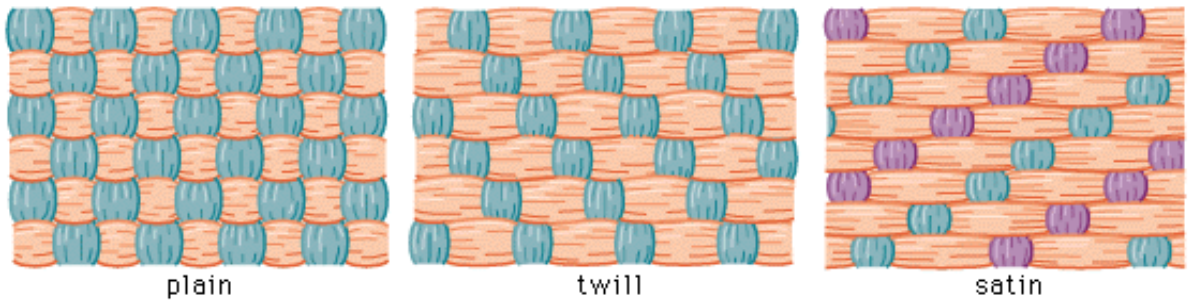

Figure 1.2 - Basic weaves in woven fabrics (reproduced from [64])

In comparison to woven fabrics, knitted fabrics are more elastic and are commonly used for clothing that requires fitting or stretching. Knitting is described ‘as a process which forms a fabric by intermeshing of loops of yarn’ [60]. There are two common and different processes in the production of knitted fabrics known as weft knitting and warp knitting as illustrated in figure 1.3 [60]. Weft knitting is considered as normal knitting and the loops made by each weft thread runs perpendicular to the coarse of the yarn whereas in warp knitting, the threads run parallel to the coarse of the yarn.

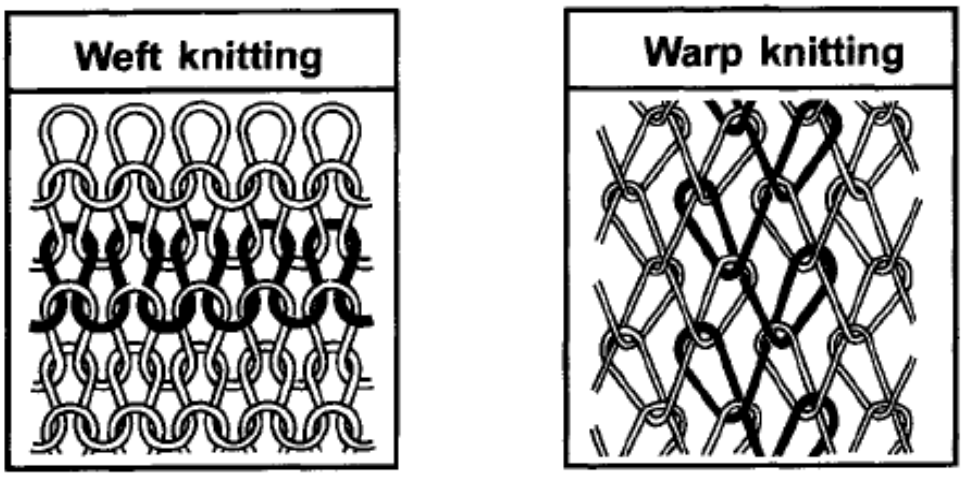

Figure 1.3– Weft and warp knitting processes in knitted fabrics.

1.6.3 Colouration of Fabric

Fibres can also be broadly classified as cellulosic (e.g. cotton, linen), proteins (e.g. wool, silk,) and synthetic polymer fibres (e.g. polyester) where the latter is hydrophobic (repel water) and the former two are hydrophilic (attract water) [65]. Solubility of a powder dye in a suitable solvent is necessary to penetrate the intermolecular micro-pores of the fibre in order to impart colour. Ideally dyes should have small and highly soluble molecules in solution, to penetrate the fabric pores and large and insoluble molecules once inside the pores, to remain attached to the fabric.

Cellulosic fibres tend to be dyed with long planar dye molecules with one or more anionic groups, most commonly $–SO_3Na$ such as acid black 1. Most synthetic fibres are hydrophilic and require dyes which will bind to such materials such as acid dyes. The nature of synthetic fibres may also be exploited in enhancement mechanisms. Given that dyes with ionic groups do not adhere to the fibres, such dyes might offer better destaining properties in the enhancement of impressions in blood for example.

1.6.4 Impressions on Fabric

Previous research on the recovery and enhancement of impressions on fabric is limited to a few publications relating to experiences in casework [66-68]. The enhancement of latent impressions has been reported with fabrics prepared from materials with a 'smooth finish and fine weave' but it was also possible to successfully enhance impressions on other types of fabric [66]. It has also been suggested that the chemical enhancement of impressions on fabric may cause background staining [69], thus reducing the effectiveness of the methods, however, Zauner [66] suggested that this may not always be the case. Initial research in the early 1970s by the UK Home Office investigating the recovery of latent fingerprints on paper and fabrics used radioactive sulphur dioxide gas [70] with limited success, and the enhanced impressions deteriorated over time. Spedding [71] however, suggested that the radioactive sulphur reacted with lipids in the fingerprint, making it potentially suitable for articles that had been immersed in water in a manner similar to physical developer [72-78] and oil red O techniques [79-85].

The Israeli Police have carried out extensive research on the recovery of footwear impressions on a variety of substrates, including cloth. The use of gelatin lifters and a hydraulic press improved the quality of lifted dust prints providing better clarity, especially when working with weak footwear impressions [86]. The dusty footwear impressions recovered with gelatin lifters might also lift loose fibres from the cloth, potentially concealing important detail. Shor *et al.* [87] illustrated how these loose fibres could be removed by using an adhesive lifter, greatly improving the detail. This 'cleaning' process could be applied a number of times depending on the substrate since the small dust particles comprising the impression remained attached to the sticky side of the gelatin lifter. The use of bromophenol blue for the enhancement of dusty footwear impressions has been successful in enhancing dusty impressions on cloth [55, 57].

When a pressure or force is applied on resilient materials, such as carpets and cushions, they are deformed elastically but return to their normal shape once the pressure is removed. The impression formed on such materials normally occurs when the fibres of the material are crushed or displaced [31]. This type of impression can

only be recovered through photography unless it is suspected that there is a latent dry dust impression, in which case an electrostatic lifter can be used [18]. Keith [68] reported a case where such an impression was recovered from a cushion. The author observed that better photographs were obtained when viewed from an angle and that "the crushed fibres reflected the ambient light differently than the undisturbed fibres and the tighter weave of the fabric".

A case reported in 1992 by Hamer and Price [88] described how the authors were successful in recovering a footwear impression from the inside surface of a T-shirt from a female murder victim. The authors believed that the impression must have occurred by the transfer of skin material and sweat to the T-shirt. Several enhancement methods were employed for the enhancement of this impression, however only 1,8-diazafluoren-9-one (DFO) followed by ninhydrin were successful. This reflected the authors' belief about the transfer being due to skin and sweat. Both skin and sweat contain amino acids which react with DFO and ninhydrin. A similar case scenario was reported by Hamm [89] where a tyre impression was recovered from the inside surface of a trousers from a hit-and-run victim. This impression did not require any chemical enhancement and was visible with the naked eye.

De Wael *et al.* [90] developed an examination protocol for the detection of blood particles on garments. Tape liftings from clothing used in murder cases involving blood were examined under low power microscopy to check for the presence of minute traces of blood followed by further examination using microspectrophotometry. The visible spectrum of haemoglobin is characteristic of blood and can be differentiated from other body fluids. This method can only confirm the presence of blood [90].

1.6.5 Fabrics Used in This Study

This project used a selection of fibre types (natural, man-made and synthetic) and included cotton, denim, polyester, 82%nylon/18% lycra as well as leather and leatherette. These fabrics, listed in table 1.1, were selected to have fabrics of varying porosity, composition and topography. Each of these will be briefly discussed.

Table 1.1 – List of fabrics

Fabric	Supplier
White Cotton [CD13] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Black Cotton [CD13D] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Patterned Cotton [SF2360/B] Twill weave; 19 warp threads/cm; 19 weft threads/cm	WBL Whaleys Bradford Ltd.
White Polyester Taffeta [SF25] Plain weave; 14 warp threads/cm; 8 weft threads/cm	WBL Whaleys Bradford Ltd.
Black Polyester Taffeta [SF25A] Plain weave; 14 warp threads/cm; 8 weft threads/cm	WBL Whaleys Bradford Ltd.
White Nylon (82%) / Lycra (18%) [SF28] Weft knitted	WBL Whaleys Bradford Ltd.
Black Nylon (82%) / Lycra (18%) [SF27] Weft knitted	WBL Whaleys Bradford Ltd.
Blue Denim [Rialto Indigo] Twill weave; 25 warp threads/cm; 19 weft threads/cm	Mandors, Glasgow, UK
Brown Bovine Leather	The Clyde Leather Co., Glasgow, UK
Plain Dyed Brown Leatherette KBT259 (C2708) (68) (F10) Synthetic fabric covered with a soft PVC layer	www.fabricuk.com

Cotton and denim

Cotton is a natural fibre containing mineral matter such as chlorides and carbonates [91]. Microscopically, cotton fibres can be identified from the extensive twists or convolutions within its structure. The material is easily dyed due to the polarity of cotton polymers which are capable of attracting any polar dye molecules into the polymer system [62]. Any dye molecule which is dispersed in water can be absorbed by the cotton polymer system.

Kadolph *et al.[63]* define denim as “a cotton or cotton/polyester blend, twill-weave, yarn dyed fabric where usually the warp is coloured and the filling is white. It is usually a left-hand twill that is commonly available with a blue (indigo) warp and white filling for use in apparel.” Denim fabric is extensively used throughout the world due to its fit, ease of use and durability.

Polyester

Polyester is derived from the condensation reaction between an acid and an alcohol. Polyester is hydrophobic and the dye molecules are unable to easily penetrate the extensive crystalline polymer system of polyester fibres [61]. Disperse dyes are commonly used for dyeing polyester since acidic dyes, commonly utilised for dyeing cotton fabrics are not suitable [62].

Nylon

Nylon fibres are formed from the condensation reaction of a diamine and dicarboxylic acid. Nylon fibres will absorb more water than polyester as nylon possesses some hydrophilic qualities. The inability of nylon to repel water completely causes the fabric to swell, weakening the molecular structure of the fabric [63]. Both acidic and disperse dyes are suitable for dyeing nylon, however, the application of heat is required to fix the dye to the fabric [92].

Lycra

Lycra, also known as elastane in Europe and spandex in North America, is extremely elastic and resilient. Lycra is prepared by four different techniques: melt extrusion, reaction spinning, solution dry spinning and solution wet spinning [63]. A prepolymer is produced which is then reacted with diamine. Although the lycra fibres appear single and continuous, the fibres are actually a bundle of tiny filaments [63]. Its main features are that it is lightweight, soft, smooth, abrasion-free and comfortable. Furthermore, the material is easily laundered and dries quickly. Lycra is usually present as a small percentage to provide stretch to the main fibres. Dyeing a mixture of nylon/lycra is similar to nylon however careful control is required since

lycra is heat sensitive [63]. This is usually not a major issue as lycra is present in small percentages.

Leather

Leather is manufactured in a three-step process involving preparation, tanning and crusting [93]. The first step removes unwanted components from the animal skin in preparation for tanning and crusting [91]. The tanning process is necessary to stabilise the animal hide and to prevent putrefaction. There are numerous tanning processes, each one dependent on the end use of the leather. Chromium is the most common tanning material utilised in this process [94]. The tanning process is achieved by 'creating stable chemical bonds to the chemically active sites on the collagen fibre' [91]. The third process involves the thinning, lubrication and other processes on the leather to provide the required characteristics such as colour and softness.

Leatherette

Leatherette, or artificial leather, is usually prepared by covering a fabric base with a pyroxylin coated sheeting of various weights and leather-like textures. It does not have the flexibility or same characteristics of genuine leather. Microscopically, in general, leatherette is rather smooth and lacks distinguishable features. Leatherette was introduced for soil-based impressions when bovine leather was not readily available.

1.6.6 Porosity and Air Permeability of the Test Fabrics

One of the defining factors for the use of chemical enhancement reagents, certainly in relation to fingerprint enhancement, is the porosity of the receiving material. Porosity of fabrics can be difficult to determine, however, it has been demonstrated that there is a complex relationship between porosity and air permeability (which is easier to determine) for fabrics [95].

The American Society for Testing and Materials (ASTM) standard D4850-08 [96] defines porosity as the "ratio of the volume of air or void contained within the boundaries of a material to the total volume (solid material plus air or void) expressed as a percentage" and the ASTM standard D737-04 [97] defines air permeability as "the rate of air flow under a differential pressure through a material".

The air permeability of the fabrics used it the study was measured by Gurley Precision Instruments, NY, USA (www.gurley.com) using an air permeometer (model number 4301N) where the results are presented in table 1.2. This technique is non-destructive and only requires a small sample for measurement (127 mm^2). The smaller values of air permeability exhibited by denim and leather (table 1.2) indicate that these fabrics have tight pores. By contrast, the particular synthetic fabrics used in this study (polyester and nylon/lycra) have high values of air permeability suggesting larger voids between the pores of the fabric weave. The less porous synthetic fabrics appear to have a higher permeability in comparison to the more porous natural fabrics such as cotton. The air permeability of fabrics can potentially have an effect on the enhancement techniques' capabilities such as background staining. Leatherette was introduced later in the study after permeability data had already been obtained.

Table 1.2 – Permeability data of fabrics

Fabric	Permeability (CFM)*	Permeability (LSM)*
White Cotton	14.9	4540.0
Black Cotton	12.3	3747.8
Patterned Cotton	19.8	6033.1
White Polyester	88.8	27057.4
Black Polyester	60.3	18373.4
White 82%Nylon / 18%Lycra	162.4	49483.3
Black 82%Nylon / 18%Lycra	178.2	54297.5
Denim	9.6	2925.1
Leather	0.7	213.3

*Cubic feet of air per square foot per minute (CFM) which can accurately be converted into SI units of litres per square metre per minute (LSM) by multiplying with 304.7. This conversion factor is obtained by multiplying 10.76 for area (square feet to square metres) and 28.32 for volume (cubic feet per minute to litres per minute).

1.6.7 Scanning Electron Microscope (SEM) Analysis of the Test Fabrics

Scanning electron microscope (SEM) analysis of the test fabrics was performed using a Hitachi S-4300 cold field emission SEM and an example of both low and high magnification are presented in figures 1.3 and 1.4. The extensive twist and convolutions of cotton are shown in figure 1.4(a) together with polyester (b) and nylon/lycra (c). Polyester, however, appeared to have a higher number of specks of delustring agent along the fibre in comparison to nylon/lycra. In figure 1.5 the fibres in denim (d) appeared to have been damaged in the manufacturing process possibly during the application of dyes and chemicals. The SEM images of bovine leather (e) revealed a complex network of fibres where proteins might also be present whereas the leatherette (f) used in this study appeared to be a smooth, synthetic surface with no identifiable features, even at higher magnifications apart from some apparent small ‘craters’ on the surface.

Information relating to the various composition of fabrics (e.g. 82% nylon/18% lycra and 100% cotton) was obtained from the manufacturer.

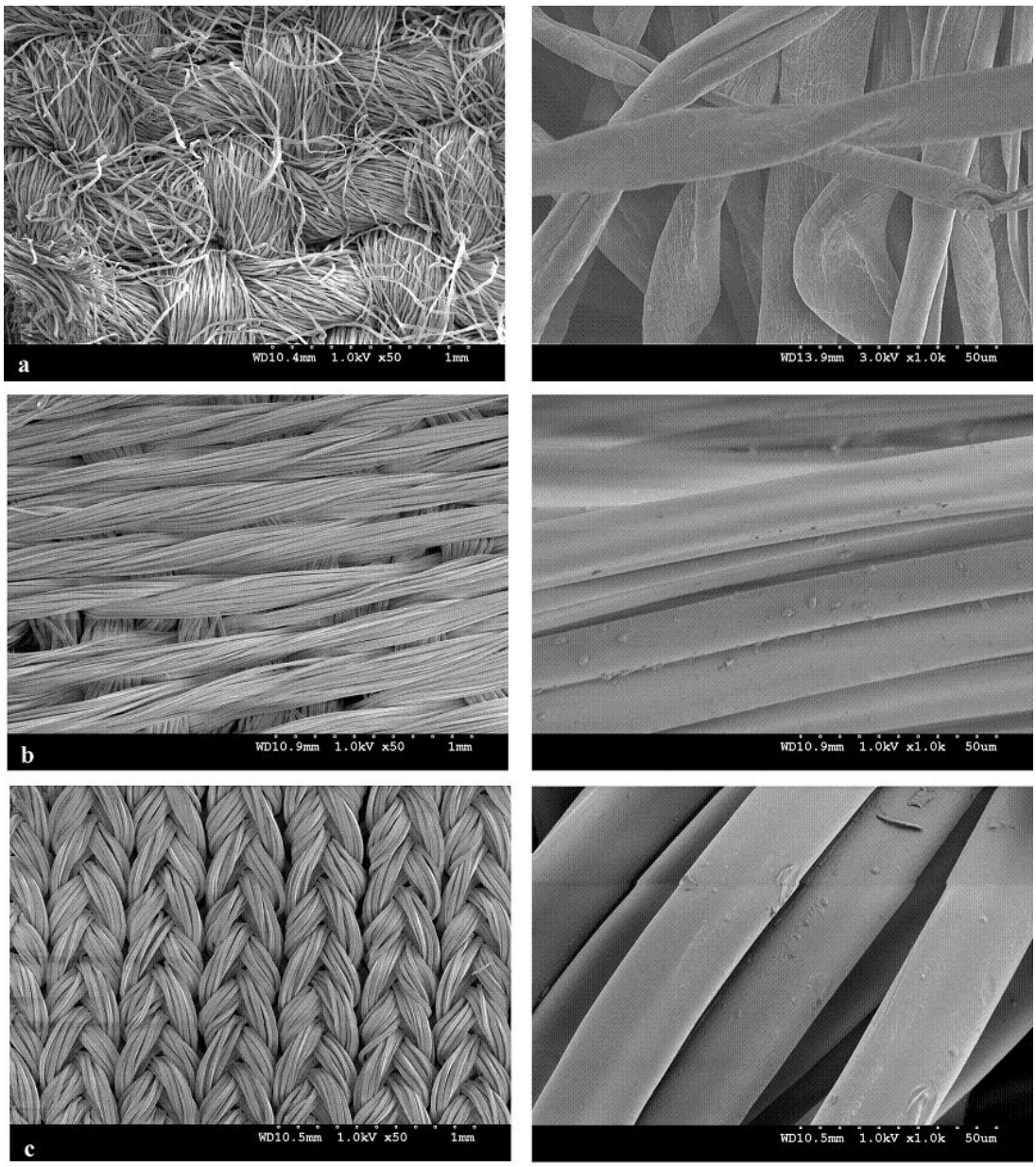

Figure 1.4 - SEM analysis of fabrics at low and high magnification: (a) 100% cotton; (b) polyester and (c) 82% nylon / 18% lycra

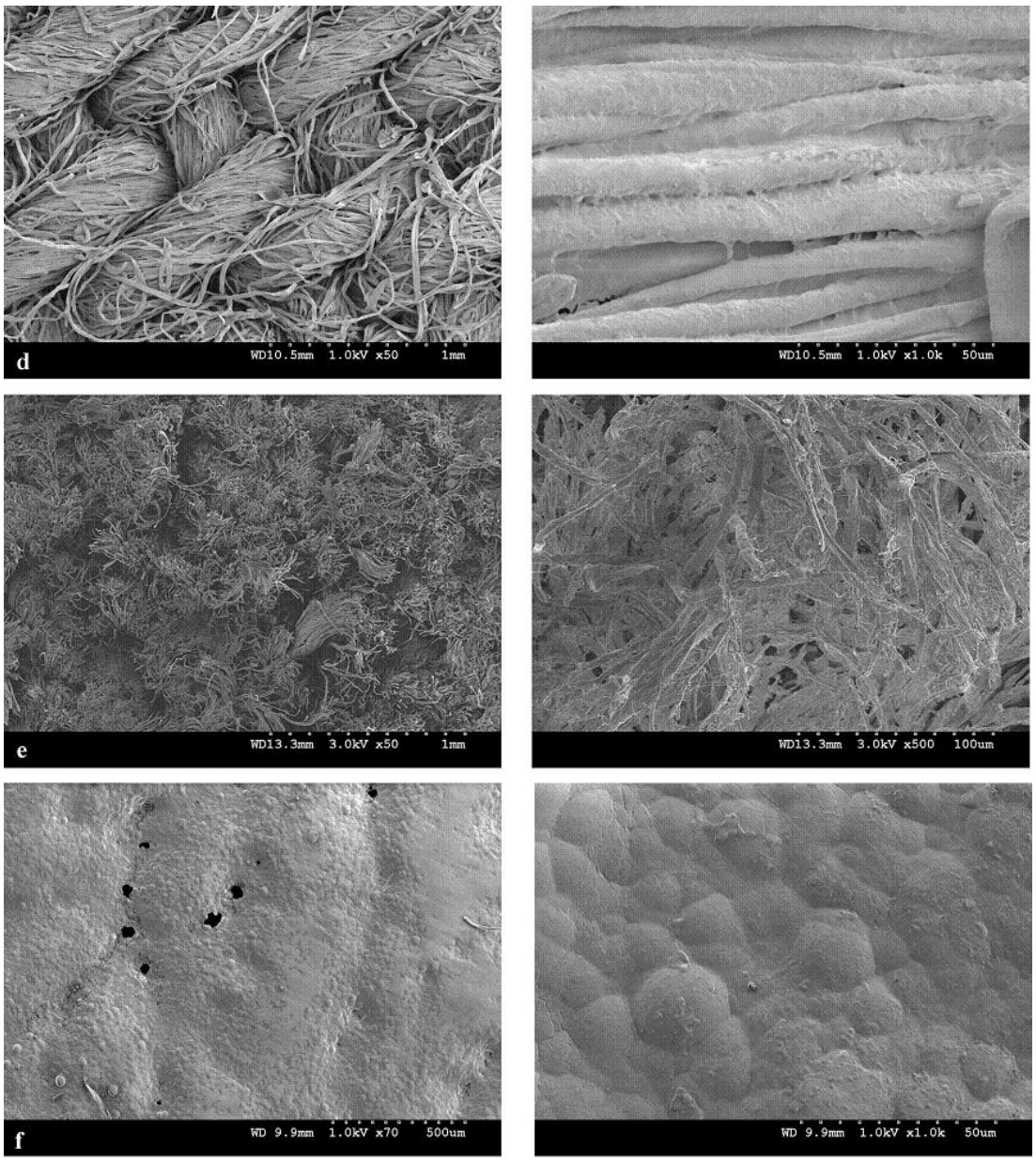

Figure 1.5 – SEM analysis of fabrics at low and high magnification: (d) denim; (e) bovine leather and (f) leatherette

1.6.8 Contaminant Distribution on the Test Fabrics

The interaction between the receiving fabric surface and the contaminant which may be present on the footwear may play an important role in the successful enhancement of the resultant impression. A footwear impression made in each contaminant (blood, urine and mud) on each of the test fabric and aged for a week were also examined using SEM and are presented in figures 1.5 and 1.6.

Blood (figure 1.6)

James [98] performed experiments to observe the diffusion activity of fresh blood on different fabric types, mainly 100% cotton, 100% polyester and 50% cotton / 50% polyester. Strips of fabrics were suspended over glass dishes so that the lower end was immersed in fresh blood at a temperature of 22^{o}C and 55% relative humidity. The migration of blood through fabric occurs through the ability of blood to travel by capillary action on a porous surface. The fastest and furthest migration occurred within the fabric 50% cotton / 50% polyester for both horizontal and vertical positions [98]. These results suggest that increasing the proportion of synthetic fabrics in natural fabrics assists blood to migrate faster.

The SEM analysis of the impressions in blood illustrate that in general, the blood appeared to dry over the surface of the fibres rather than penetrating into the fibre matrix. There seems to be an exception for nylon/lycra fibres where the blood appeared to penetrate between the fibres. The blood was observed to dry faster on synthetic fabrics. Blood on denim, leather and leatherette appeared to dry in a similar manner to cotton. The rates and processes of absorption and adsorption may vary if the impression in blood is aged for less or even more than 7 days. Adsorption refers to the adhesion of blood molecules and its components to fabric whereas absorption refers to the permeation of blood through the fabric.

Urine

Impressions in urine were not visible using SEM. There was no observable difference on the fabrics before and after the deposition of urine.

Soil (figure 1.7)

When impressions made with soil as a contaminant were examined using SEM the penetration of the smaller particles of soil into the weave of the fabric for cotton and denim is clearly observed, however, larger soil particles were observed on the surface of the fabric. A similar effect was observed for the synthetic fabrics polyester and nylon/lycra, however for polyester most of the particles penetrated into the fabric. The interaction of soil with denim, leather and leatherette was similar to that observed for cotton.

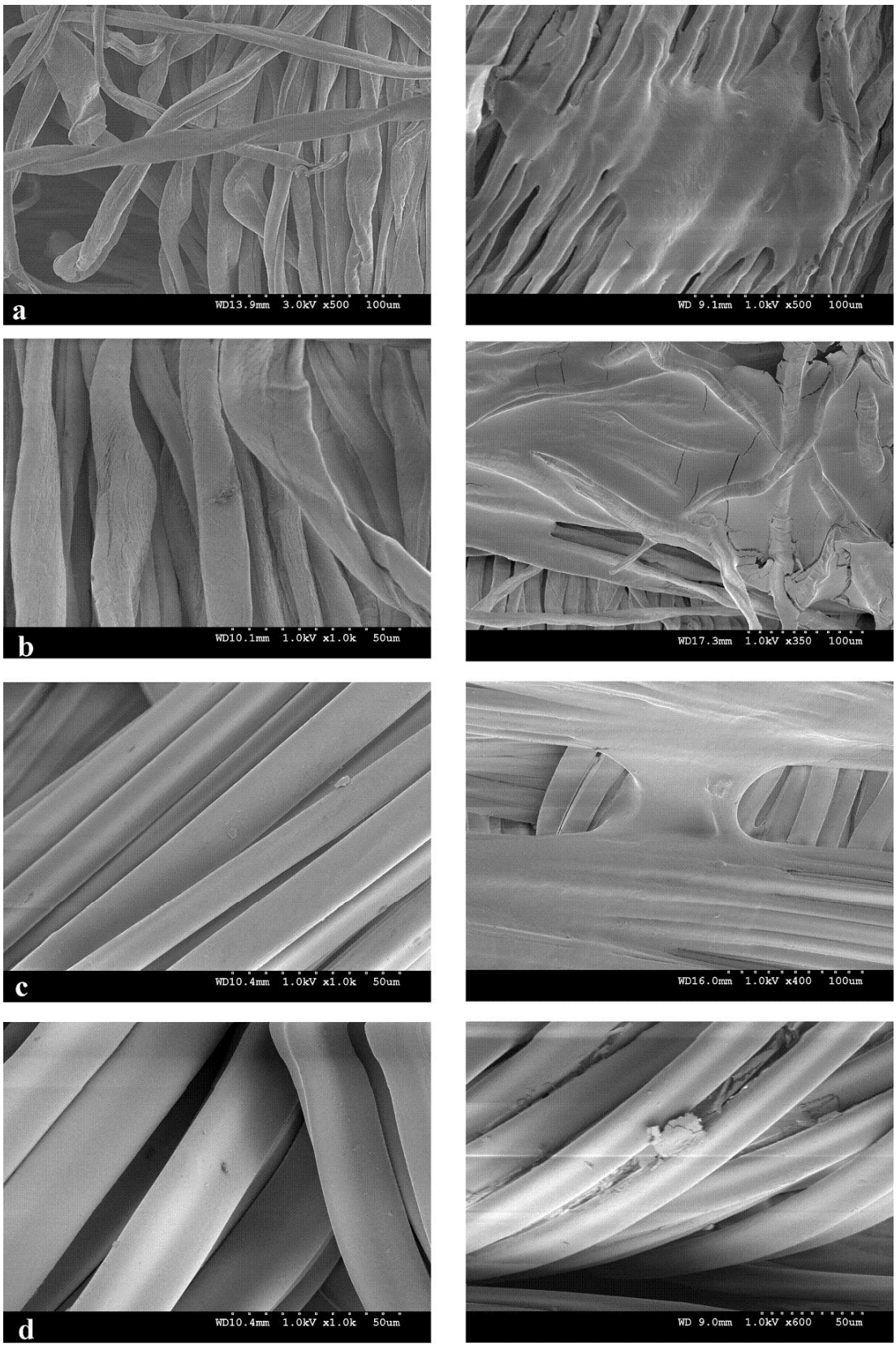

Figure 1.6 - SEM analysis of fabrics before (left) and after (right) the deposition of blood: (a) white cotton; (b) patterned cotton; (c) white polyester; (d) white nylon/lycra

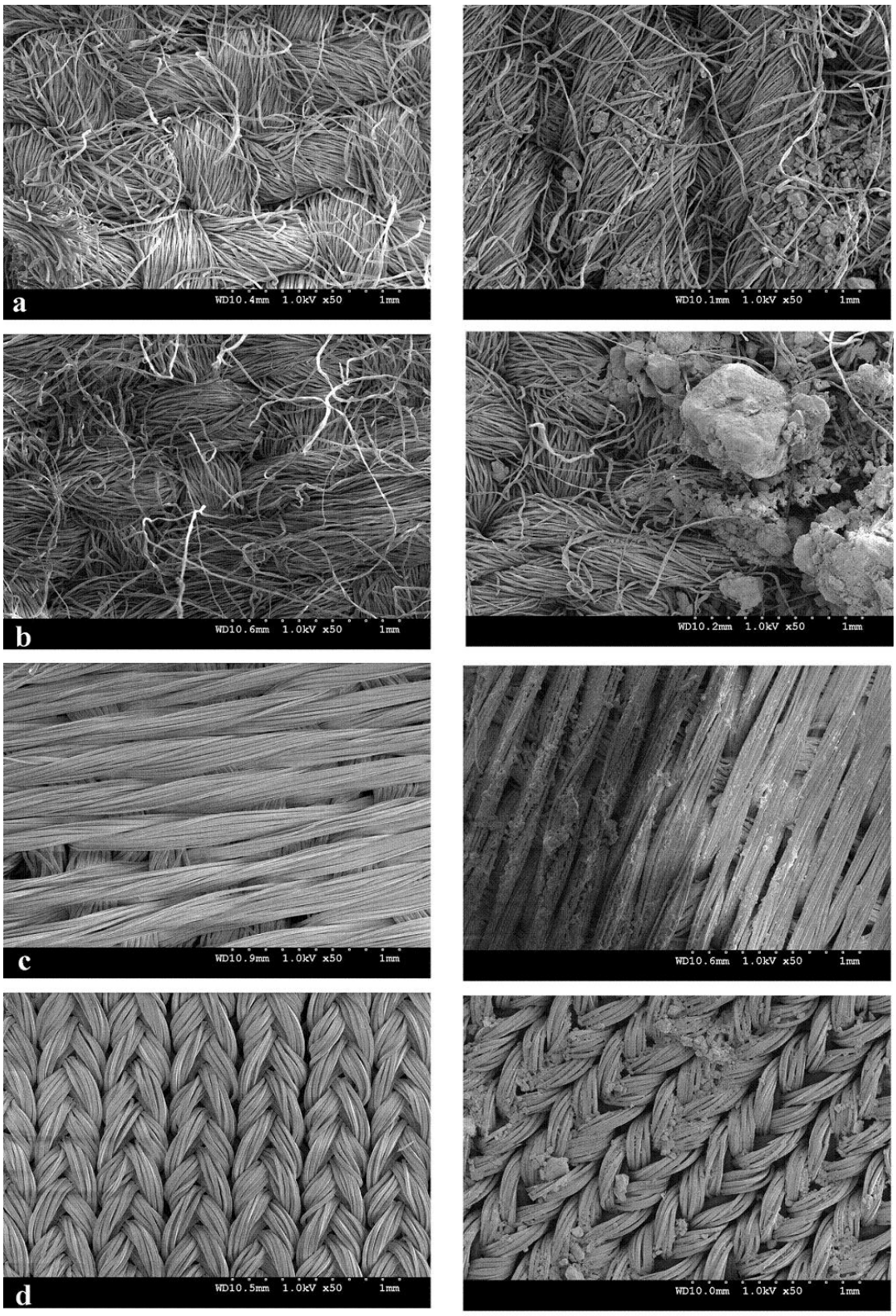

Figure 1.7 - SEM analysis of fabrics before (left) and after (right) the deposition of mud: (a) white cotton; (b) patterned cotton; (c) black polyester; (d) black nylon/lycra

1.7 Scope of Study

Several fingerprint enhancement techniques work well with footwear impression enhancement, most commonly on non-porous surfaces. However, little research has been dedicated to the development of new methods for lifting and enhancing such impressions deposited on fabric. These surfaces are considered to be difficult substrates, with arguments asserting that the infrequent occurrence of this type of evidence does not warrant time and money being diverted into this area of footwear impression research. Others arguments suggest that footwear impressions, such as those in dust, may not be retained by the fabric.

The purpose of this research is to robustly evaluate and establish the appropriate enhancement techniques and mechanisms for the development of footwear impressions made with different operationally relevant contaminants on a large range of fabrics. This includes establishing the topographical nature of the fabric and influences that this may have on the subsequent enhancement of the footwear impression. Secondly, this work addresses the notion of the evaluation of enhanced impressions in terms of both the quality and quantity of the enhancement delivered by the technique and the assessment of the overall quality of the resultant impression.

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Chapter 2: The Development of the Footwear Impression Testing Rig

2.1 Introduction

Variables introduced during the creation and visualisation of footwear impressions on fabric surfaces include the force with which the mark is made, the amount and composition of any contaminant (e.g. blood, mud, dust, etc.) which might be present on the footwear sole prior to transfer to the receiving surface and the amount of contaminant that is transferred to the footwear sole. Other variables are the nature of the receiving surface (e.g. porosity, texture and absorption/adsorption rates) and the colour perception of an enhanced impression (for example the human eye is relatively insensitive to dim red light and as a result, weakly fluorescing impressions may be undetected unless the eyes are dark adapted for a period of about 30 minutes prior to observation) [1, 2].

Controlling the amount of contaminant on a footwear sole and the subsequent amount transferred to a receiving surface can be challenging, though some methodologies have been suggested to limit these variables. This includes the preparation of soil and distilled water mixtures to study muddy impressions [3, 4] and the use of tissue with a few millimetres of blood applied to it for the study of impressions in blood [5]. In these studies, determination of the best enhancement techniques was based on several criteria such as the nature of the substrate and the safety of the reagents, however, these studies fail to mention if the impressions produced were reproducible or repeatable and the effect of force did not appear to be considered. In addition, impressions were prepared using part of a shoe sole measuring 4 x 4 cm rather than a whole shoe [3-5]. Other studies have mainly focused on chemical mechanisms underlying specific techniques or enhancement efficiency with little or no regard to potential variables in the preparation of test footwear impressions [6-16].

This research is focused on determining the efficacy of the enhancement techniques and the quality of the impressions produced by these techniques under controlled conditions, rather than exactly mimicking casework samples. In order to achieve

these aims, the application of the mark onto the receiving surface needed to be controlled as much as possible. As such a standardised delivery method was devised.

2.2 Experimental - Pressure Variation

A mechanical test rig was developed with the aim of replicating the average force delivered by stamping onto a flat surface. While it can be argued that it is impossible to exactly replicate each test footwear impression, measures can be taken to try and limit the influence on the effect of the variables encountered in producing such impressions. Within this project, the receiving surface, amount and nature of contaminant and pressure with which the mark was made were all standardised in order to minimise their effects on mark variation.

2.2.1 Initial Stamping Experiments

Stamping experiments were conducted using pressure plates (Kistler 9281C, Amherst, NY) coupled to a computer. The generated data was processed using a Vicon Workstation (Version 5.2.4) capable of measuring force in the x, y and z direction. After receiving ethical approval for the study (Appendix 1), twenty-seven volunteers (12 male and 15 female) were asked to stamp onto a block of material. The laboratory setup is illustrated in figure 2.1.

Two recipient surfaces were used: high density foam and a block of wood. In both cases, the substrates were placed over pressure plates connected to a computer which was set to produce a reading of the applied force in Newtons (N) at 1/3000 second intervals (3000 Hz). High density foam was utilised to mimic the elastic action of stamping on a victim's torso and wood was used for comparison of the same action onto an unyielding surface. Each volunteer stamped 10 times on each surface using their preferred leg giving a total of 270 sets of data per surface. The volunteers were instructed to stamp once the start button on the software was pressed by the investigator. There was a short time delay from when the software started to record the data to when the volunteer actually stamped and this was reflected in the output

generated. T-tests were performed on the generated data using SPSS (version 18) to check for statistically significant differences between genders and surfaces.

Figure 2.1 – The biomechanics laboratory and test set up

Data generated from these experiments was used to design a stamping rig which was used in subsequent preparations of test footwear impressions. The ability of the test rig to recreate the appropriate average stamping forces demonstrated by the volunteers was validated by recording data from 50 repeat tests of the developed equipment where the 'foot' was dropped from the appropriate height onto high density foam covering the pressure plate.

Finally, different contaminants (e.g. blood, mud, etc.) were then used to check the performance of the stamping rig where fabric was utilised as the receiving substrate for the test footwear impressions. The fabric was positioned on top of high density foam to provide some degree of elasticity which would be expected when stamping on a body.

2.3 Results and Discussion

2.3.1 Initial Experiments to Determine Stamping Force

Exemplars of the data obtained from the volunteers are presented in figure 2.2 and figure 2.3 which illustrate the force profiles for volunteer F1 (female 1) when stamping on foam and wood respectively, where Z1 - Z10 represent the number of repetitions. The profiles are similar in shape, though foam shows a dampening effect at 0.1s, presumably due to elasticity of the material and its ability to readily deform on application of the force, absorbing some of its magnitude. This resilience can be defined as the elasticity of a material that enables it to resume its original shape after being bent, stretched or compressed [17]. Examples of resilient materials include carpets, grass and skin. All force profiles obtained from volunteers were similar in shape to those revealed in figure 2.2 and figure 2.3. The average force from ten stamps from each volunteer was calculated and is shown in table 2.1 and table 2.2.

The data obtained was analysed using SPSS statistical software (version 18.0). The Kolmogorov-Smirnov test for normal distribution was applied to the force delivered onto the receiving surface by each volunteer [18]. The results of the Kolmogorov-Smirnov test on the force obtained by stamping on foam are presented in table 2.3. These results demonstrate that the forces produced by all twenty-seven volunteers have a normal distribution at the 0.05 (95%) level of significance. Figure 2.4 and figure 2.5 show the maximum, minimum and average stamping force on foam and wood for each volunteer.

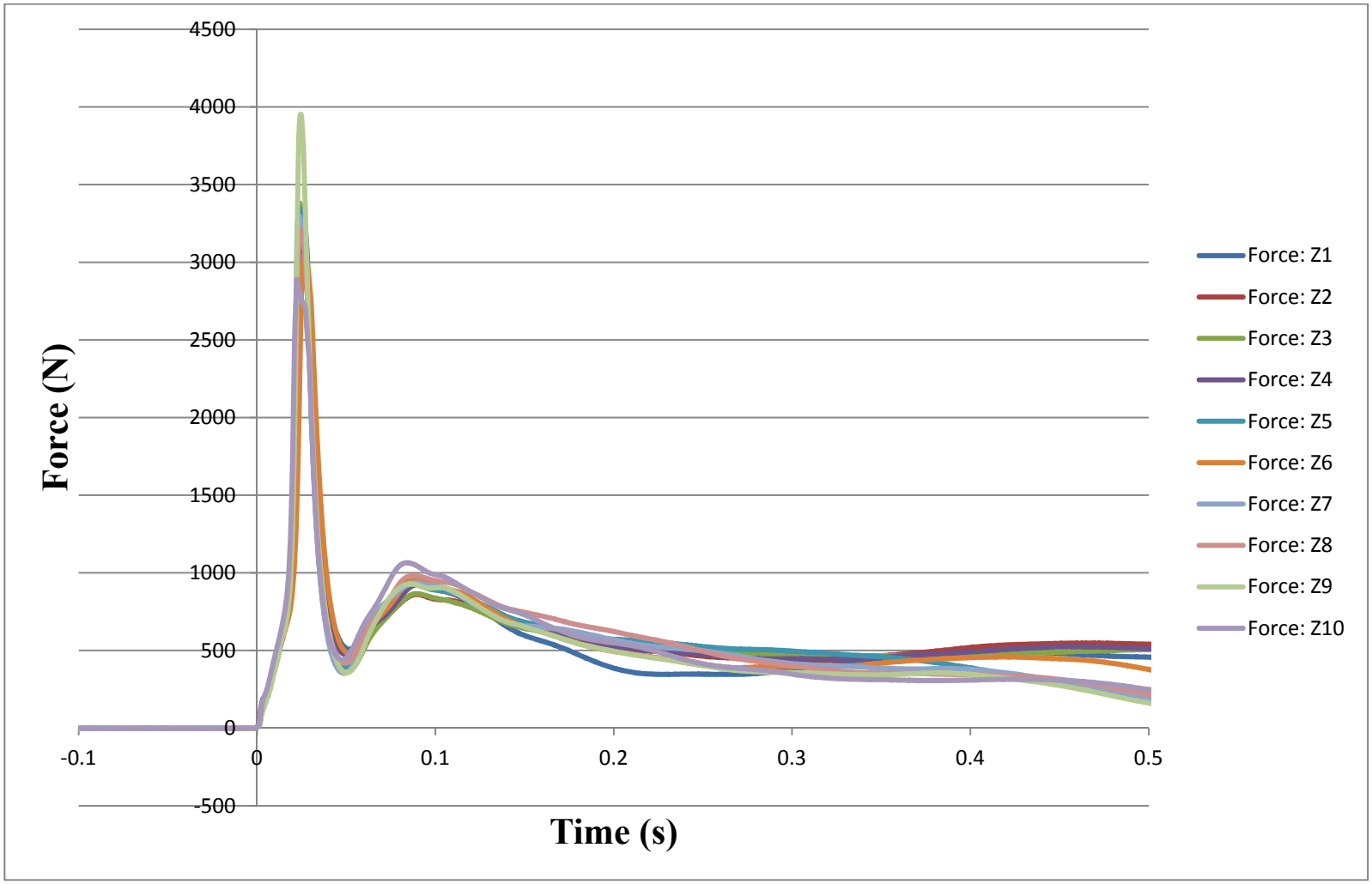

Figure 2.2 - Graph of force (N) against time (s) for volunteer F1 on foam

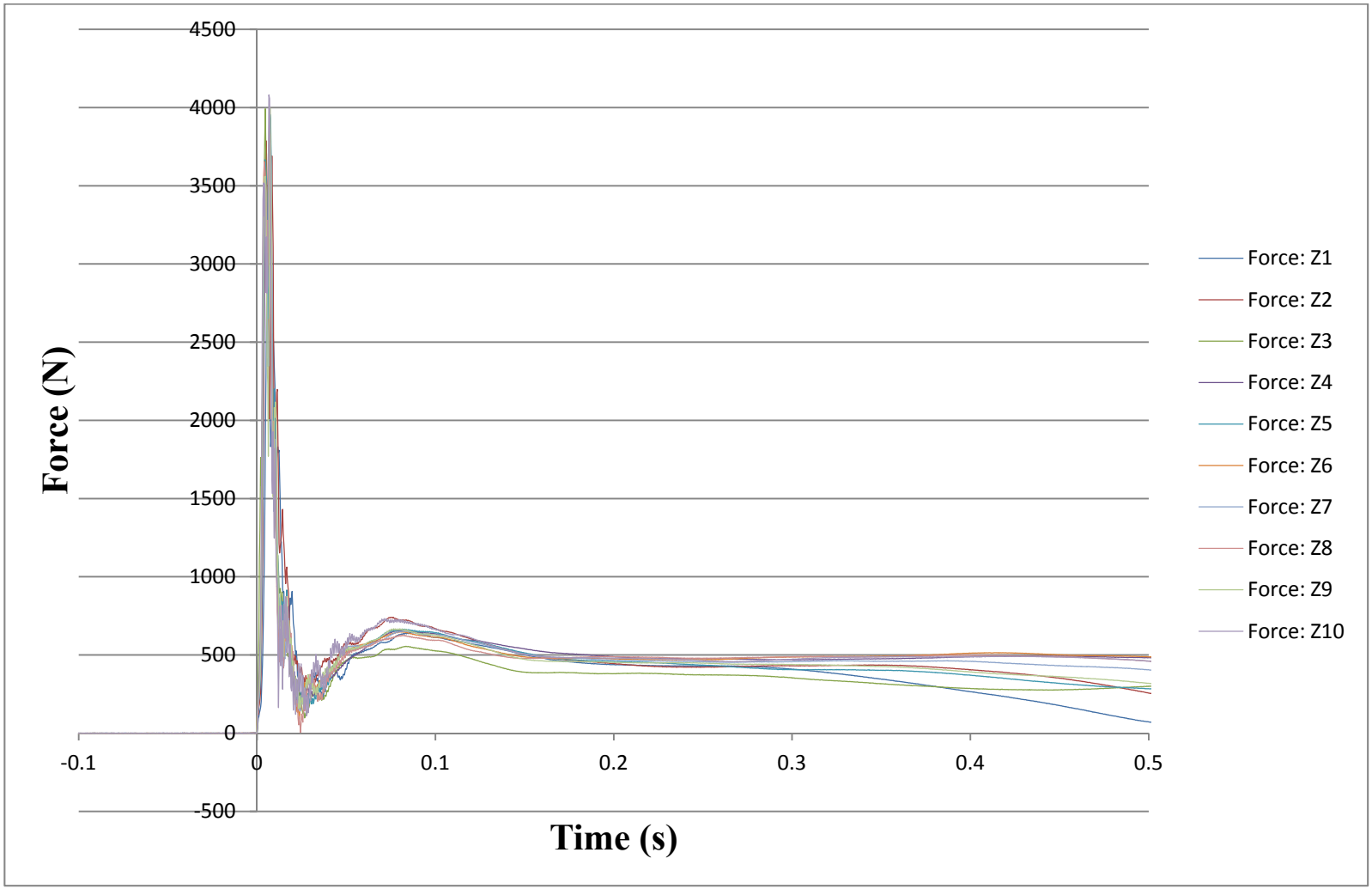

Figure 2.3 - Graph of force (N) against time (s) for volunteer F1 on wood

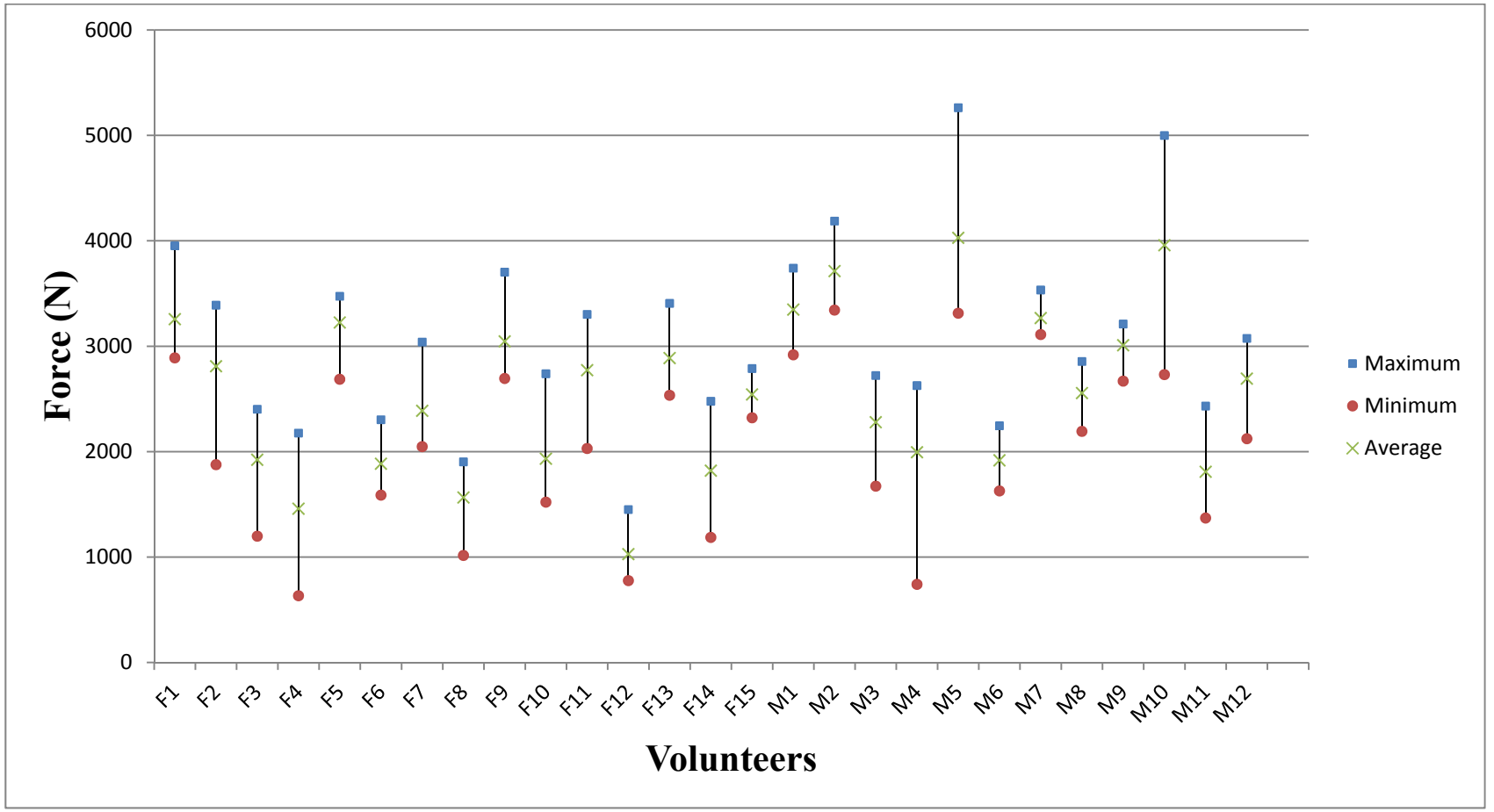

Figure 2.4 - Graph of force (N) against volunteers showing the maximum, minimum and average forces on foam

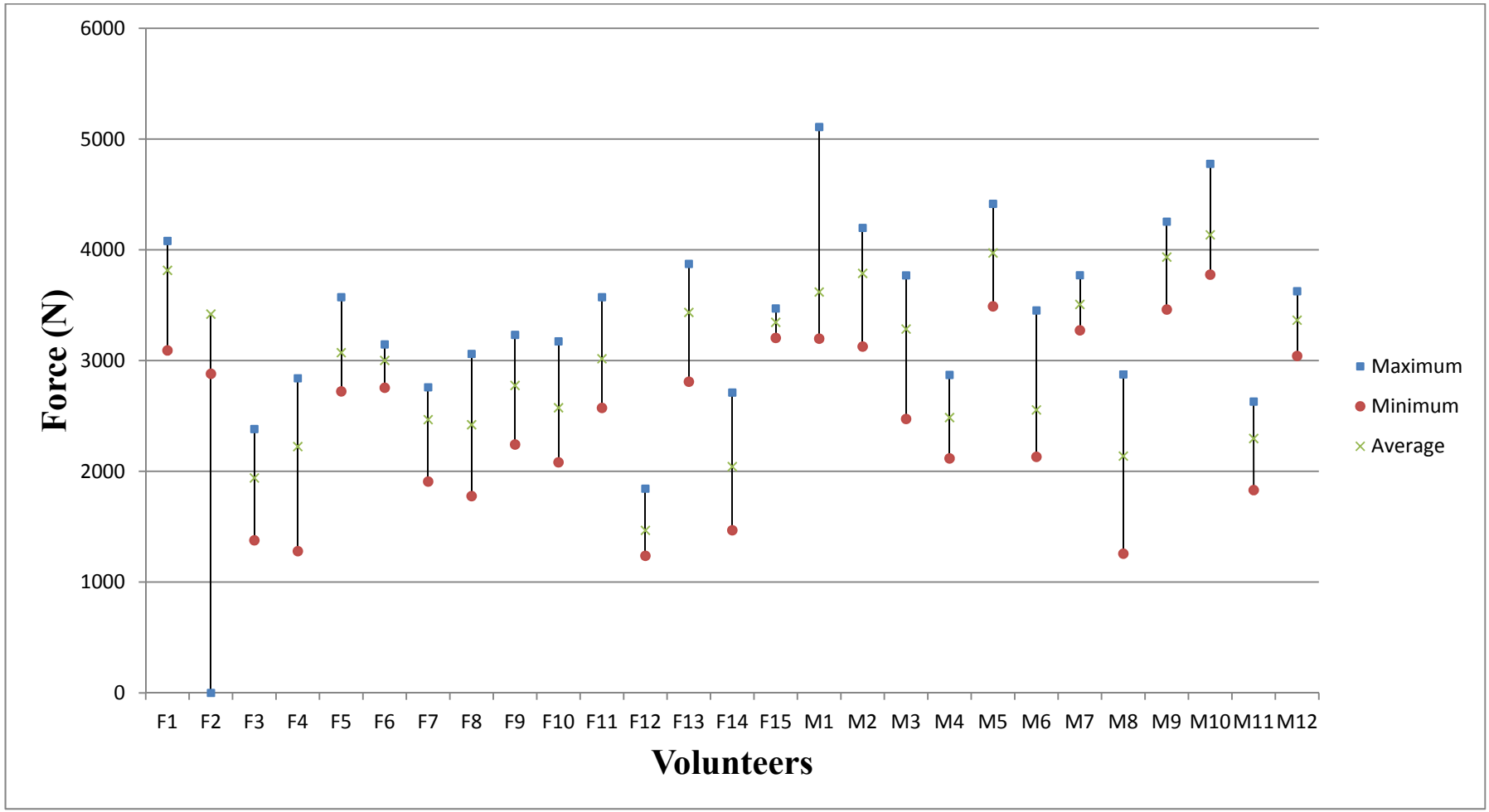

Figure 2.5 - Graph of force (N) against volunteers showing the maximum, minimum and average forces on wood

Table 2.1 – Average force from 10 stamps on foam and wood for each female volunteer

Subject	Foam			Wood		
	Average Force (N)	Standard Deviation	Rel. Standard Deviation (%)	Average Force (N)	Standard Deviation	Rel. Standard Deviation (%)
F1	3257.18	294.36	9.04	3815.25	287.59	7.54
F2	2810.42	513.79	18.28	3419.80	316.49	9.25
F3	1923.65	343.10	17.84	1940.79	305.46	15.74
F4	1459.55	538.83	36.92	2224.13	473.23	21.28
F5	2773.38	364.36	13.14	3016.97	368.74	12.22
F6	2388.04	319.83	13.39	2467.17	258.31	10.47
F7	1566.05	255.14	16.29	2421.60	369.75	15.27
F8	3046.49	296.36	9.73	2775.61	317.11	11.42
F9	1933.71	327.78	16.95	2574.69	328.37	12.75
F10	3225.66	270.97	8.40	3071.94	243.52	7.93
F11	2543.33	181.71	7.14	3345.93	83.41	2.49
F12	1028.70	210.55	20.47	1467.58	213.78	14.57
F13	2888.42	276.25	9.56	3435.09	350.27	10.20
F14	1886.08	233.47	12.38	3001.50	130.28	4.34
F15	1820.23	427.65	23.49	2042.66	357.88	17.52

Table 2.2 - Average force from 10 stamps on foam and wood for each male volunteer

Subject	Foam			Wood		
	Average Force (N)	Standard Deviation	Rel. Standard Deviation (%)	Average Force (N)	Standard Deviation	Rel. Standard Deviation (%)
M1	3348.35	310.14	9.26	3619.48	558.76	15.44
M2	3713.72	281.41	7.58	3787.67	372.65	9.84
M3	2279.81	307.16	13.47	3285.08	525.89	16.01
M4	1994.61	565.30	28.34	2486.27	199.04	8.01
M5	4028.91	643.29	15.97	3972.29	270.14	6.80
M6	1918.33	236.25	12.32	2555.01	426.64	16.70
M7	3267.97	136.41	4.17	3507.60	151.38	4.32
M8	2556.61	191.78	7.50	2138.05	493.73	23.09
M9	3010.04	196.35	6.52	3935.04	241.45	6.14
M10	3959.00	748.23	18.90	4135.76	288.19	6.97
M11	1809.90	341.80	18.89	2297.01	247.62	10.78
M12	2693.46	326.02	12.10	3365.29	214.67	6.38

Table 2.3 - The mean force, standard deviation and Kolmogorov-Smirnov (K-S) test on foam for each volunteer where F denotes female and M denotes male.

Subject	Number of stamps	Mean	Std. Deviation	K-S (Z Value)	P-Value (2-tailed)
F1	10	3257.18	294.36	0.75	0.63
F2	10	2810.42	513.79	0.52	0.95
F3	10	1923.65	343.10	0.51	0.96
F4	10	1459.55	538.83	0.41	1.00
F5	10	3225.66	270.97	0.70	0.72
F6	10	1886.08	233.47	0.82	0.51
F7	10	2388.04	319.83	0.54	0.93
F8	10	1566.05	255.14	0.58	0.90
F9	10	3046.49	296.36	0.62	0.83
F10	10	1933.71	327.78	0.72	0.67
F11	10	2773.38	364.36	0.62	0.84
F12	10	1028.70	210.55	0.71	0.69
F13	10	2888.42	276.25	0.42	0.99
F14	10	1820.23	427.65	0.35	1.00
F15	10	2543.33	181.71	0.54	0.94
M1	10	3348.35	310.14	0.64	0.81
M2	10	3713.72	281.41	0.70	0.71
M3	10	2279.81	307.16	0.57	0.90
M4	10	1994.61	565.30	0.52	0.95
M5	10	4028.91	643.29	0.78	0.57
M6	10	1918.33	236.25	0.51	0.96
M7	10	3267.97	136.41	0.46	0.98
M8	10	2556.61	191.78	0.52	0.95
M9	10	3010.04	196.35	0.72	0.68
M10	10	3959.00	748.23	0.53	0.94
M11	10	1809.90	341.80	0.51	0.96
M12	10	2693.46	326.02	0.46	0.99

T-tests were also carried out to examine statistical differences between the means of the following four sets of data: 1) female and male foam results; 2) female and male wood results; 3) female foam and wood results and 4) male foam and wood results and the results are presented in table 2.4. In all cases no significant difference was observed at the 95% confidence level.

Table 2.4 - T-test results for comparisons of the different data sets.

t-Test: Two-Sample Assuming Equal Variances

	Foam female v male		F-M Wood female v male	
	Females	*Males*	*Females*	*Males*
Mean	2303.39	2881.73	2734.71	3257.04
Variance	483322.39	629048.65	424147.53	499305.47
Observations	15	12	15	12
Pooled Variance	547441.95		457217.03	
df	25		25	
t Stat	-2.02		-1.99	
P(T<=t) 1-tail	0.03		0.03	
t Critical 1-tail	1.71		1.71	
P(T<=t) 2-tail	0.05		0.06	
t Critical 2-tail	2.06		2.06	

	Females wood v foam		Males W-F wood v foam	
	Females	*Females*	*Males*	*Males*
Mean	2734.71	2303.39	2881.73	3257.04
Variance	424147.53	483322.39	629048.65	499305.47
Observations	15	15	12	12
Pooled Variance	453734.96		564177.06	
df	28		22.00	
t Stat	1.75		-1.22	
P(T<=t) 1-tail	0.05		0.12	
t Critical 1-tail	1.70		1.72	
P(T<=t) 2-tail	0.09		0.23	
t Critical 2-tail	2.05		2.07	

In light of the variations between volunteers and the general spread of the data it was decided to use 3,500N as the target force for delivery by the developed footwear rig to simulate a hard (above average) stamping action. The objective of the footwear rig was to deliver a stamp of a constant force rather than mimic operational situations. This is because the focus of this work was to compare the enhancement ability of different chemical and other enhancement techniques across a range of surfaces using each contaminant. It was therefore essential to control the delivery of the footwear impression within a given contaminant set across the receiving surfaces.

2.3.2 Footwear Rig Development

The construction of a mechanically based stamping rig designed to deliver a constant force onto a recipient horizontal surface required the application of some basic kinematic concepts [19]. At ground level (height, h = 0), an object has no potential energy (PE) but does possess kinetic energy (KE). When the object is held at a height h, this is reversed and the object possesses potential energy and no kinetic energy as illustrated in figure 2.6. The formulae for potential and kinetic energy are provided in equations 1 and 2.

$$PE = mgh \qquad \text{[equation 1]}$$

$$KE = \frac{1}{2}mv^2 \qquad \text{[equation 2]}$$

Where:
m is mass in kilograms (kg)
g is the acceleration due to gravity in meters per second (= 9.81 m/s^2)
h is the height in meters (m)
v is the velocity in meters per second (m/s)

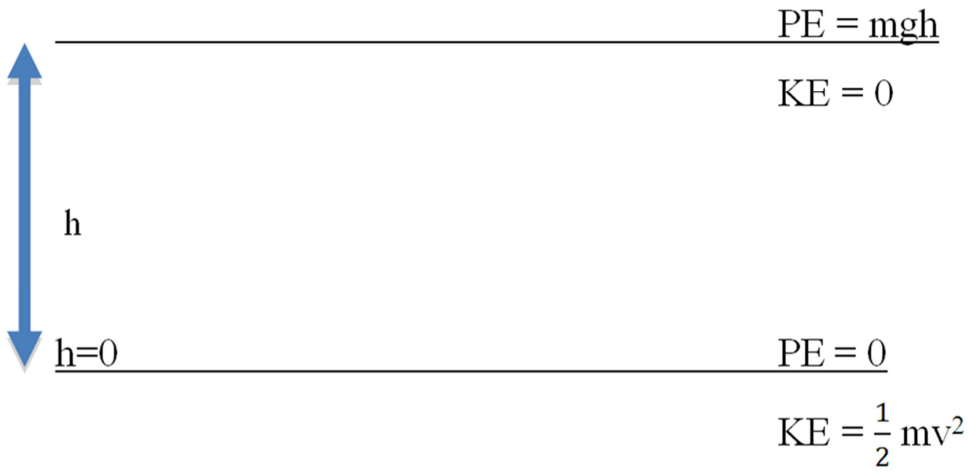

Figure 2.6 - Types of energy for an object at different heights

The principle of conservation of energy states that energy cannot be created nor destroyed, thus in its most simplistic form, potential energy and kinetic energy equate to each other. Thus equation 1 and 2 become:

$$PE = mgh = KE = \frac{1}{2}mv^2$$

$$\frac{1}{2}mv^2 = mgh$$

$$v^2 = \frac{2mgh}{m} = 2gh$$

$$v = \sqrt{2gh} \qquad \text{[equation 3]}$$

For a straight line collision or impact, the average impact force multiplied by the distance (or height) gives the change in kinetic energy [equation 4]. The distance represents the deformation distance as the shoe strikes the substrate.

$$\text{Work done} = \Delta KE = F_{av} \text{ x } d \qquad \text{[equation 4]}$$

Where:
F_{av} is the average force in Newtons (N)
d is the distance in meters (m)

Equation 4 demonstrates that if the penetration of the surface stamped upon is large, then the impact force is going to be small. For high density foam (soft surface and thus greater penetration) the impact force is expected to be smaller than for a wooden surface. Equation 4 facilitates calculation of the average impact force (F_{av}) delivered, however, the peak impact force (F_p) is required for the accurate determination of the height from which a fixed mass needs to be dropped in order to replicate the desired average force. Under theoretical conditions, if an impact force of approximately 3,500N is required on a surface that has a penetration of approximately 0.03m (the estimated visual penetration obtained from deflection of the foam upon the action of stamping), then 66.85 Joules (J) of energy will be required assuming the stamping contact follows a first approximation sinusoidal curve [20] and that air friction is negligible. It was calculated that dropping of a weight of 10.2 kilograms from a

negligible. It was calculated that dropping a weight of 10.2 kilograms from a height of 0.67m produced the desired force. The calculations are provided in Appendix 2.

A test rig was constructed which consisted of a long rod with a foot-shaped attachment fitted with a shoe at one end. A 10.2kg weight was placed on top of the 'foot'. The rig was designed to facilitate a variation in height (and therefore force) and a simple release system controlled the dropping of the weight. The desired force of 3500N was achieved using a drop height of 0.64m. This was within 5% of the calculated (theoretical) value and as such was adopted for the tests. The rig, illustrated in figure 2.7, was designed to be sturdy and was fitted with anti-sliding legs to help prevent sudden movements or sliding.

Figure 2.7 – Development of a Semi-Automated Stamping Device

2.3.3 Optimisation of the Stamping Force Rig

The rig was calibrated and validated using the pressure plates previously described to measure the force applied to the surface. Repeatability of the delivered force was assessed by undertaking 50 repeat tests of the equipment where the 'foot' was dropped from the same height (0.64m) onto high density foam covering the pressure plate. The data obtained is presented in table 2.5 and demonstrated excellent repeatability (relative standard deviation of 1.6%, 3548N ± 54, n=50). Figure 2.8 illustrates the force profile of five of the test stamps. This profile was very similar to those obtained from live volunteers, though an additional smaller peak at time = 0.5s was observed, most likely linked to a rebound effect which occurred after the initial impact of the foot on the surface.

The calibration of the rig was retested after a year and the results are presented in table 2.5 demonstrating a relative standard deviation of 4.4% (4190N ± 184). The forces and relative standard deviation recorded in the second trial were approximately 500N higher than those in the first trial. This is most likely due to wear and tear of the rig. This increase did not cause any particular difficulty for the experimental procedure or comparison of the enhanced impressions as each contaminant group (blood, urine or mud) across the test fabrics were delivered with the same force within their specific group.

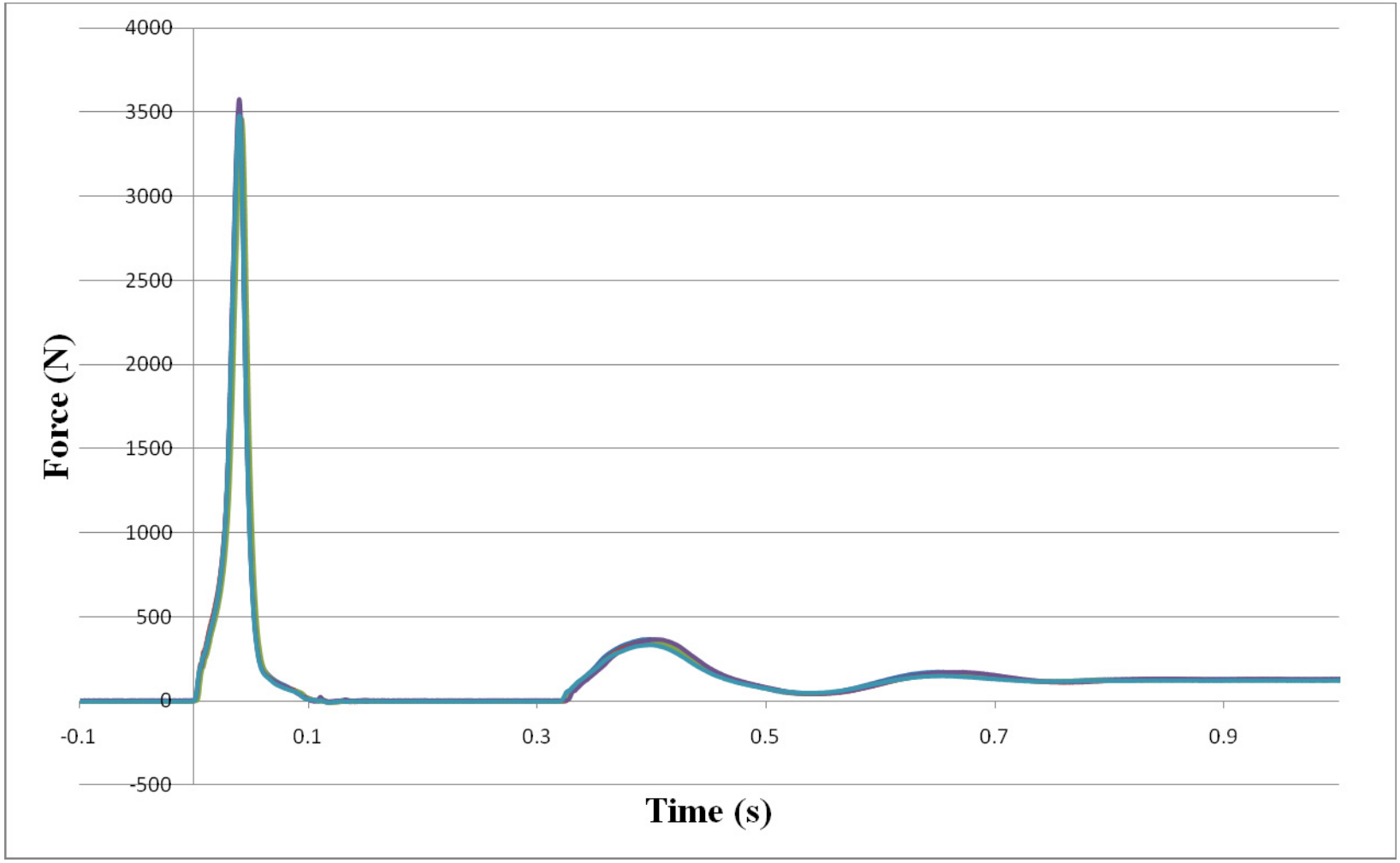

Figure 2.8 - Graph of force (N) against time (s) obtained from five randomly selected rig trials

Table 2.5 –Stamping repeatability trial

1st trial			2nd trial		
Force in Newtons (N)			Force in Newtons (N)		
3507	3460	3555	4061	4216	4149
3529	3455	3594	3893	4195	4066
3593	3512	3561	4108	4272	4152
3564	3438	3541	4256	4280	4241
3563	3457	3516	4258	4183	4064
3504	3458	3479	5252	4203	4198
3563	3500	3527	4355	4128	4096
3589	3614	3554	4310	4119	4123
3564	3675	3579	4308	3964	4227
3625	3575	3532	4320	4328	4017
3569	3612	3535	4031	4220	4075
3606	3556	3524	4103	4036	4078
3573	3529	3576	4218	4140	4051
3507	3583	3490	4277	4107	4329
3604	3536	3506	4193	4142	4218
3617	3664	3472	4256	4173	4245
3562	3595		4180	4101	

2.3.4 Footwear Impression Examples

The use of a means to control the pressure or force used to produce an impressed mark has been reported in other studies. Recent studies have demonstrated the use of a fingerprint sampler to produce consistent and reproducible fingermark depositions [21], and that pressure (as opposed to other qualities of the deposition) used for the deposition of a fingerprint directly affected the quality of enhancement achieved [22]. Another study described a method to control and calculate the amount of amino acid in a particular deposition so that comparison of fingerprint enhancement techniques is more robust [23].

Initial performance trials demonstrated the repeatability of the generated footwear impressions. Figure 2.9 represents examples of unenhanced and enhanced impressions on black cotton prepared without using the footwear rig and figure 2.10 illustrates a similar set of impressions using the footwear rig. Both sets of impressions were made using blood as the contaminant and were enhanced using Forensic Bluestar® Magnum luminol. It can be observed that the generated impressions have a greater variability in terms of consistent quality and repeatability.

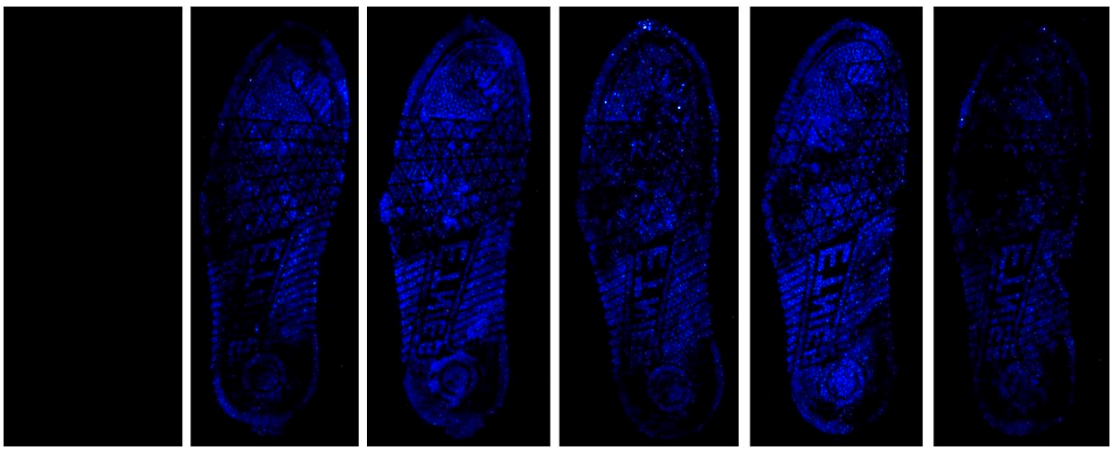

Figure 2.9 - A footwear impression in blood on black cotton before enhancement (far left) followed by 5 samples of enhanced footwear impressions in blood on same fabric using luminol (all impressions prepared without using the footwear rig)

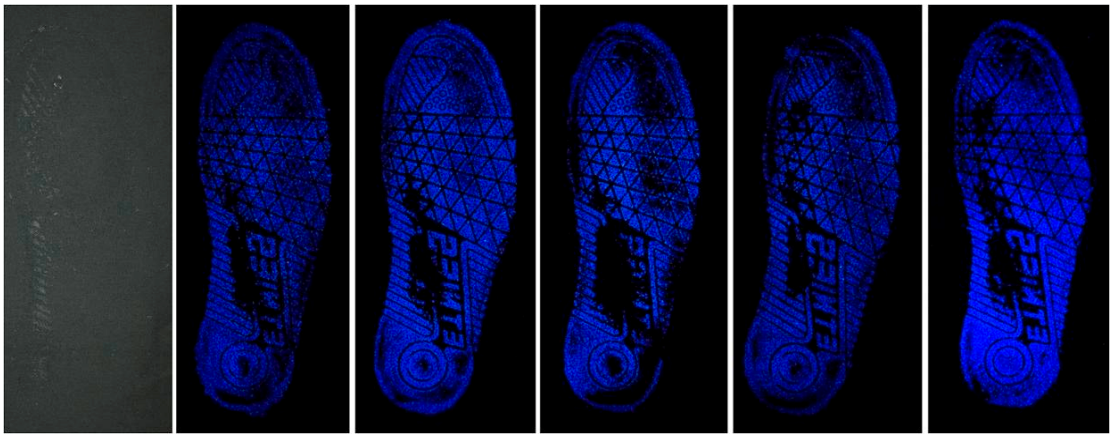

Figure 2.10 - A footwear impression in blood on black cotton before enhancement (far left) followed by 5 samples of enhanced footwear impressions in blood on same fabric using luminol (all impressions prepared using the footwear rig)

Protein stains demonstrate greater sensitivity to small changes in the concentration of blood [24, 25] and as such the impressions in blood on black cotton were also examined using the protein stain acid yellow 7 (AY7) as recommended by the Centre for Applied Science and Technology (CAST). A series of impressions in blood were prepared at three different forces using the footwear rig: 3500N (High); 2500N (Medium) and 1100N (Low). Figure 2.11 illustrates the enhancement of these impressions in blood using AY7, excited with blue light (352-509nm) using a Mason Vactron Quaser 40 and viewed with a yellow/orange band-pass filter (510nm).

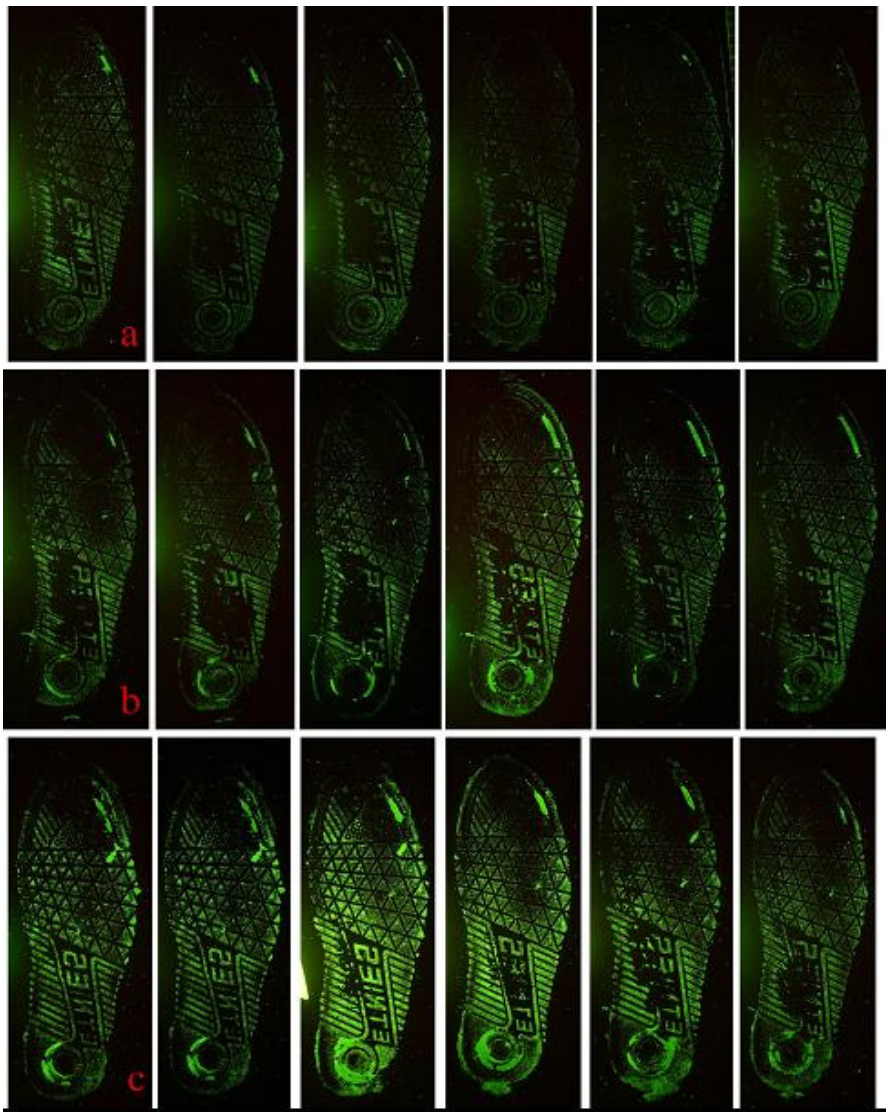

Figure 2.11 – Six repetitive impressions in blood on black cotton prepared by a mechanical rig and enhanced with AY7 fluorescence using a Mason Vactron Quaser 40 blue light (352-509nm) and viewed with a yellow/orange band-pass filter (510nm): (a) low force; (b) medium force and (c) high force

As expected, blood impressions prepared with a higher force (3500N) (figure 2.11c) and subsequent enhancement with AY7 provided stronger fluorescence than impressions in blood prepared with medium (figure 2.11b) or low force (1100N) (figure 2.11a), however all impressions were successfully enhanced. This highlights the effect of pressure when evaluating the different enhancement techniques and the importance of maintaining constant pressure as far as possible. As consequence, the variable of pressure was controlled to facilitate the direct comparisons of enhancement techniques across a given contaminant.

The effect of a depletion of the contaminant on the shoe sole as a consequence of repetitive stamping was also assessed. Two sets of depleted impressions are presented in figure 2.12, both prepared under the same conditions using the footwear rig and enhanced with AY7.

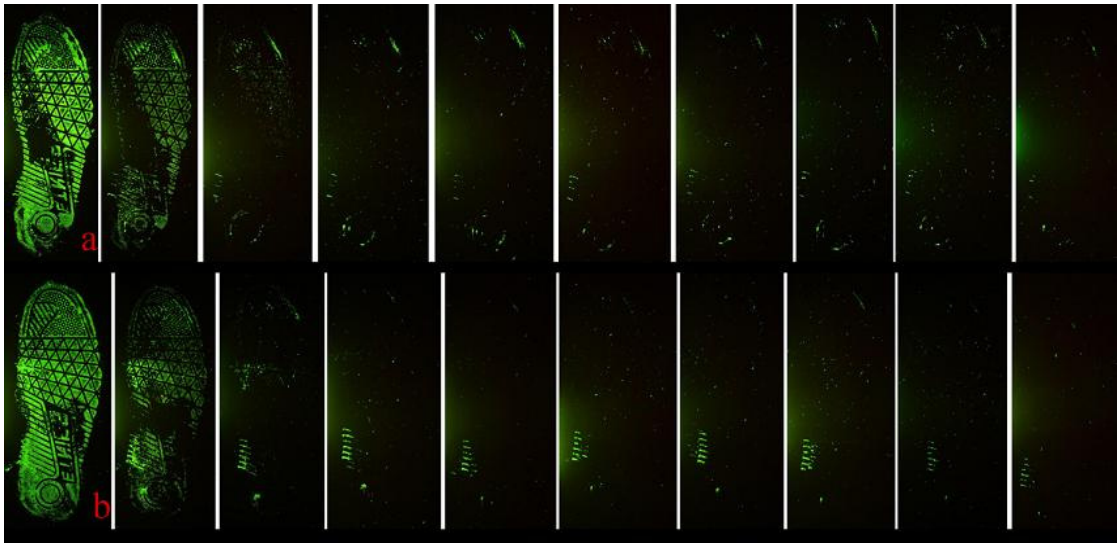

Figure 2.12 – Two diminishing series of footwear impressions in blood on black cotton prepared using a mechanical rig and enhanced with AY7 fluorescence using a Mason Vactron Quaser 40 blue light (352-509nm) and viewed with a yellow/orange band-pass filter (510nm)

2.4 Conclusion

The comparison of enhancement techniques for the visualisation of footwear impressions is hindered by uncontrolled variables such as the force applied when the impression is created. In order to compare the efficacy of enhancement techniques in laboratory trials it is essential to be able to minimise the influence of specific factors. Test rigs such as that constructed for this research are essential to this process. The footwear impressions prepared from the test rig limit some of the variables, such as the pressure and subsequent contaminant uptake and distribution, 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 all of the impressions evaluated in this work.

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Chapter 3: Chemical Enhancement of Footwear Impressions in Blood on Fabric

3.1 Introduction

Blood is a red, viscous fluid consisting of plasma and cellular components such as red blood cells, white blood cells and platelets. Other components include haemoglobin, fibrinogen, albumin, glucose and immunoglobulins [1]. In a normal human adult, the total blood volume ranges between 4.5 to 6 litres accounting for approximately 8% of the total body weight [2].

Viscosity is an internal physical property of a fluid that manifests as a resistance to flow and form whereas specific gravity is defined as the weight of a sample or fluid compared to an equal volume of water [2]. Blood is six times more viscous than water but only has a slightly higher specific gravity. This means that blood will interact with the surfaces upon which it rests in a different way to water and this may have significant consequences in the ability to enhance marks made in blood on materials such as fabrics.

It is possible that a criminal at a crime scene where blood is present will leave some footwear impressions in blood with varying pressure, ranging from heavy to light to latent [3]. There are numerous techniques described in the literature that are suitable for enhancing such impressions made in blood. Fresh blood quenches fluoresce, or fluoresces weakly, however it can be located by fluorescing the background upon which it may rest [4, 5]. Blood residues can sometimes be visualised with light at 415nm which causes the blood to appear black or by inherent short wavelength UV luminescence [4, 6]. Bodziak [3] points out that faint impressions in blood, once enhanced, can offer more detail than heavy impressions. There are different types of reagents suitable for enhancing impressions in blood such as protein stains, heme-reacting chemicals (peroxidase reagents) and amino acid staining techniques. Research by the Royal Canadian Mounted Police (RCMP) demonstrated that seven different protein stains and heme-reacting chemicals used for enhancing blood impressions did not have any detrimental effects on subsequent DNA analysis. Even

aged blood impressions (up to 14 days old) submitted to long term exposure of enhancing chemicals (up to 54 days) yielded excellent STR results using the Profiler Plus™ multiplex system [7]. Further research confirmed that several blood presumptive tests are not detrimental to subsequent DNA analysis [8]. Other chemical techniques were successful in enhancing blood spatter and impressions which had been exposed to temperatures exceeding 800°C [9].

This chapter discusses the use of chemicals for the enhancement of footwear impressions in blood on fabric. A number of protein stains and heme-reacting reagents are investigated in addition to amino acid staining, alginate casting and powder suspensions.

3.1.1 The Chemistry of Dyes

Dyes can be described as coloured organic aromatic molecules with conjugated bonds and large systems of delocalised electrons which permit molecular binding to a material [10-12]. Most dyes can be recovered and reused after staining has occurred, however, for forensic purposes, the solutions are used once per application. Many dyes have a Colour Index (C.I.) number which is a five-digit number associated with each stain available in the market developed by the Society of Dyers and Colourists (Bradford, UK) and the American Association of Textile Chemists and Colourists (Lowell, Massachusetts) [13]. This is useful to avoid ambiguity of dyes with similar names or for the same dye that might be available in different forms.

Dyes are generally separated into natural, acidic and basic groups. Natural dyes, such as haematoxylin are those which have been directly extracted from animal or plant material. Acidic and basic dyes (illustrated in figure 3.1) are in fact not acidic or basic in nature and their terminology relates to their usage as dyes in the 19th century for dyeing textiles (generally proteins within the material) under acidic or basic conditions [14, 15]. Acidic dyes (e.g. Orange G) possess coloured anions together with colourless cations whereas basic dyes (e.g. Pyronin Y) possess coloured cations together with colourless anions.

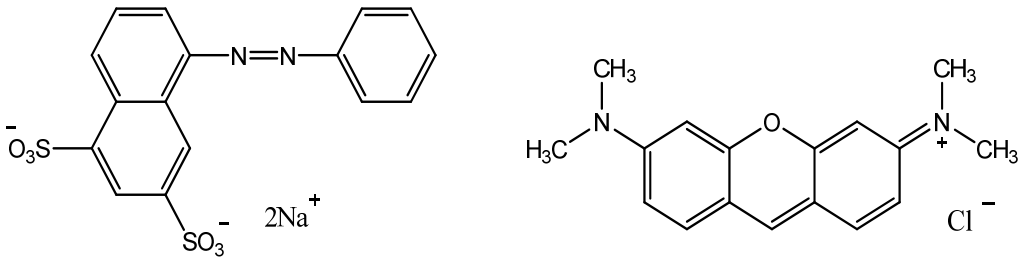


Figure 3.1 - Molecular structure of Orange G (Acidic Dye) and Pyronin Y (Basic Dye)

Under acidic conditions, the protein is dyed by an acidic dye whereas under basic conditions the protein is dyed by a basic dye as shown in figure 3.2. Stains prepared for application to proteins are often modified in order to facilitate the formation of appropriate charges of the anion and cation. For example, the Centre for Applied Science and Technology (CAST, previously HOSDB) [16] formulation of protein stains includes the addition of acetic acid to provide optimal conditions for acidic dyes.

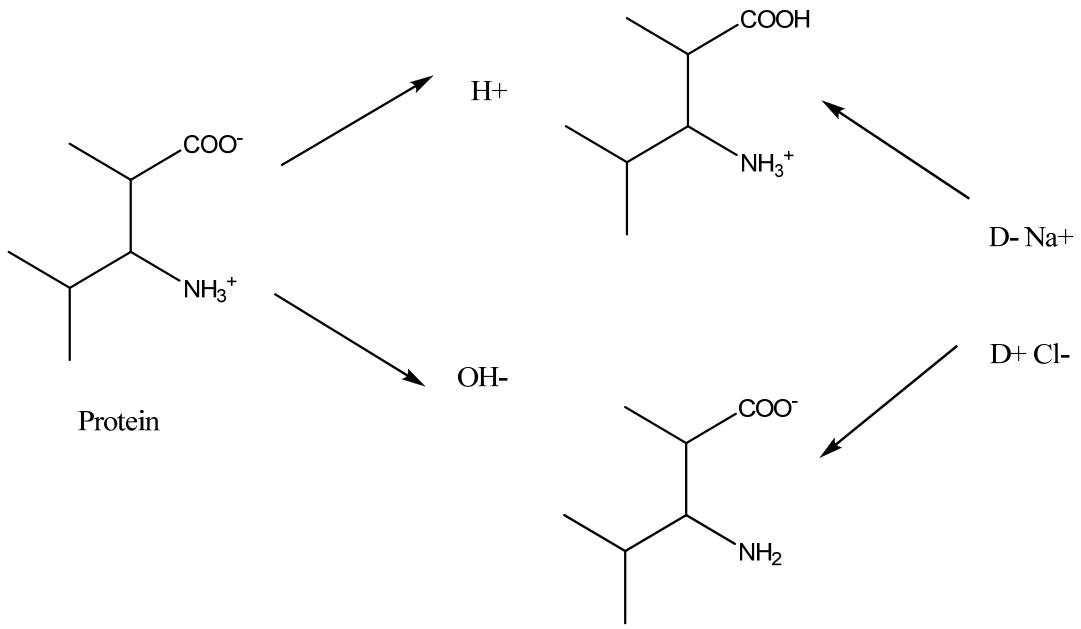


Figure 3.2 - Dye reactions under different pH conditions where D represents the dye molecule

Other factors affecting the mechanism of dyes include the affinity of a dye to a substrate, short term Van der Waal forces and hydrogen bonding, all of which are governed by thermodynamic laws. Success of the dye reacting with the substrate also depends on the solvent, pH, porosity and dye structure and stereochemistry [17].

Some dyes fluoresce which occurs when electrons are transferred to an excited state through the absorbance of energy. On returning to ground state, a photon (light) of lower energy and longer wavelength is released. Some compounds might not fluoresce if the return of the electron to a ground state occurs without emitting a photon. In other compounds, fluorescence does not take place because of problems associated with the movement and transfers of electrons between orbital sets. Fluorescence is improved by rigidity, unhindered stereochemistry and the addition of certain substituents on the conjugated aromatic dye compound. When a single substituent is present, the fluorescence is usually increased by functional groups such as –OH, $–OCH_3$ and –CN and decreased by groups such as –COOH, –Cl and $–NO_2$ [17]. Alkyl side chains in general do not have an effect on fluorescence unless they sterically hinder the planar configuration of the molecule.

3.1.2 Protein Stains

Biological stains and dyes have been widely used for a long time to impart colour to plant or animal tissues. Protein stains will not detect constituents normally present in latent fingerprints but will react with amines or other groups within all proteins present in blood and other body fluids to yield a coloured complex [18, 19]. In general, these chemicals are cheap, easy to apply and can be used for porous and non-porous substrates. However, care must be taken with porous items since background staining might be an issue. There is a large number of histological stains, some of which have been utilised in forensic science including acid yellow 7 (AY7), acid black 1 (amido black, AB1), acid violet 17 (AV17) and acid violet 19 (Hungarian Red, AV19) [20].

The application of protein stains is usually done in a three-step process involving fixing, staining and destaining. The general procedure is to fix the blood stain by

immersing or spraying for about five minutes in a fixing solution of 5-sulfosalicylic acid dihydrate. This is followed by immersing or spraying for five to ten minutes in a staining working solution. Finally the stain is immersed in two wash solutions for a further five to ten minutes each. The article is then allowed to dry before photography.

3.1.2.1 Fixation

Fixing an impression in blood before protein staining is necessary to precipitate the basic proteins thus preventing leaching or diffusion of the blood. There are a range of mechanisms by which proteins can be fixed such as cross-linking, dehydrating and precipitating [21]. Cross-linking occurs when the protein molecules join into large aggregates lowering the solubility. It is believed that the main process of fixation occurs through the disruption and change of the secondary and tertiary structure of proteins [10]. Ageing of impressions in blood is a fixing process in itself through dehydration which can be accelerated with heat or chemical treatment [3]. Hussain and Pounds [22] demonstrated that fixing impressions in blood via precipitation using 5-sulfosalicylic acid was safe, effective and convenient. 5-sulfosalicyclic acid precipitates proteins present in blood by the formation of insoluble salts or complexes and by the disruption of the protein structure [21, 23].

Naturally occurring molecules, such as proteins, have a conformation where the polar (hydrophilic, ionisable) residues are present on the surface of the molecules which then come into contact with solvents such as water. Conversely, the non-polar (lipophilic) residues are located predominantly inside the protein's core and do not normally come into contact with solvents [10]. Lipophilic residues tend to dissolve in non-polar solvents such as oils and fats rather than polar solvent such as water. A disruption of this conformation by fixative agents renders the molecules insoluble in water by exposing the lipophilic residues to the aqueous solvent as shown in figure 3.3 and figure 3.4 [10].

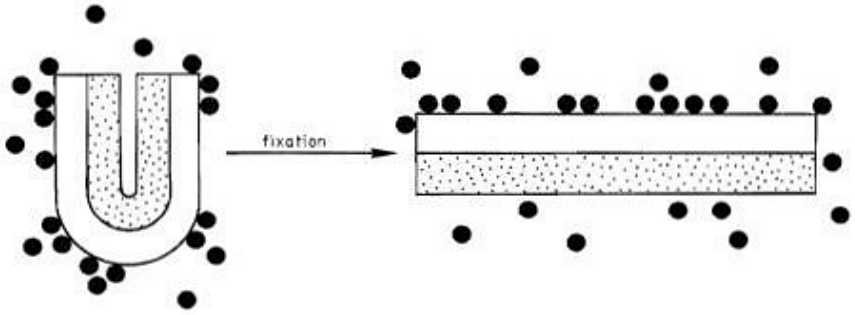


Figure 3.3- Exposure of the lipophilic residues (shaded region) during fixation: the unshaded region and bold circles represent the hydrophilic residues and the water molecules respectively (reproduced from [10])

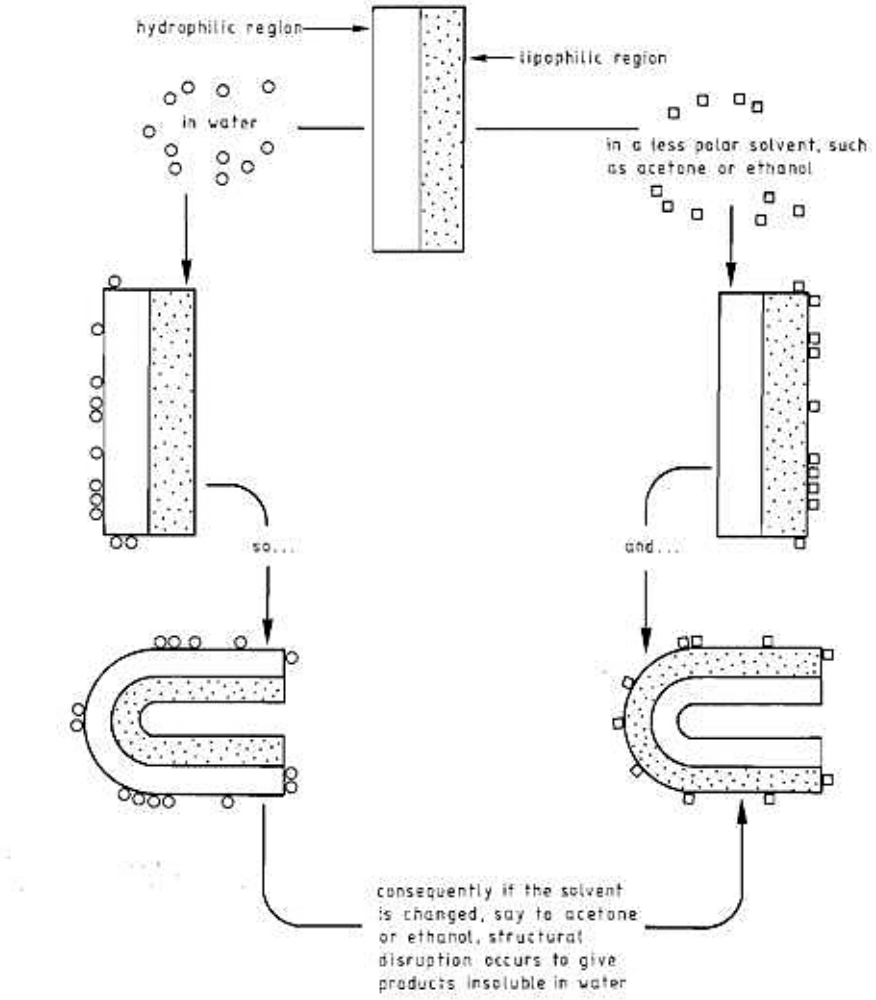


Figure 3.4 - Disruption of the secondary and tertiary structure of proteins via change in the balance of lipophilic/hydrophilic regions (reproduced from [10])

This causes the solubility of the proteins in water to fall sharply, preventing diffusion. Disruption of the protein structure can occur without any covalent bonds breaking or forming. Fixatives such as alcohols and acetone behave in such a way as to disrupt the protein structure by rearranging the hydrophilic and lipophilic residues. Since no covalent bonds are made or broken, this process can be reversible in some instances, however, in most cases the protein is trapped in the denatured state as the reversible reaction takes place at a very slow rate [10, 17].

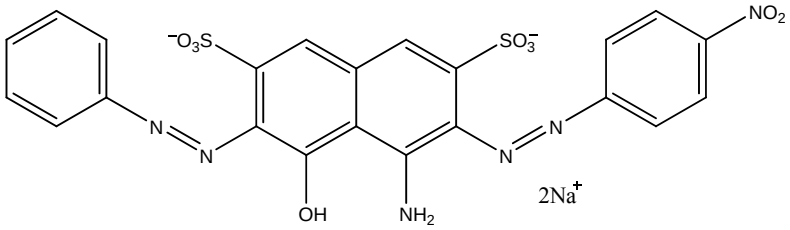
Other fixatives involve the breaking and forming of covalent bonds in proteins which will involve interference with the stabilisation of electrostatic attractions and repulsions, covalent bridges, hydrogen bonds and Van der Waals attractions. Horobin [10] stresses that the 'commonest mechanistic factor for all fixatives is the disruption of the secondary and tertiary structure of proteins via change in the balance of lipophilic/hydrophilic regions'. Besides proteins, other molecules such as nucleic acids, lipids and polysaccharides can be involved in the fixation process [24].

Besides 5-sulfosalicyclic acid, other chemicals for fixing blood have been evaluated including methanol, acetic acid, ethanol, formaldehyde, picric acid and osmium tetroxide. The last three have been disregarded for being either toxic or carcinogenic [25]. Furthermore, items fixed with osmium tetroxide did not stain well with acidic dyes as the fixation process reacts chemically with the amino and carboxyl groups of proteins [17]. Of the others, methanol proved the only effective technique. A few chemical enhancement reagents, such as LCV, contain 5-sulfosalicylic acid as part of the working solution. This might be useful for reducing the workload, however, Sears [25] reported that solutions that fixed and stained in a single step were not as stable and proved less effective than using separate fixing and staining solutions.

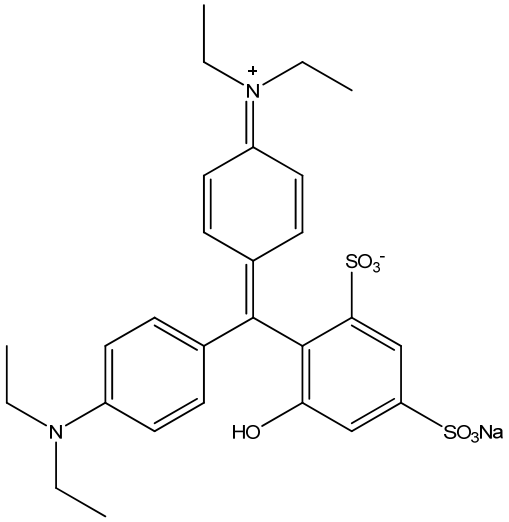
3.1.2.2 Protein Staining

Once fixed, the impression in blood is then treated with a protein stain which dyes the precipitated/fixed proteins a bright colour. Figure 3.5 presents the chemical structure of three common protein stains: acid black 1, patent blue VF (acid blue 1) and acid yellow 7. These structures are known as conjugated systems due to the presence of alternating single and double bonds which increase stability of the

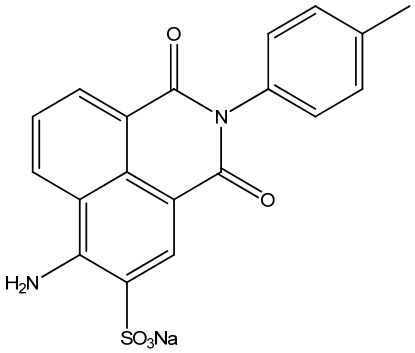
molecule as well as give rise to strong colours [26] and are exemplars of the type of molecules involved in protein stains for the enhancement of blood.



Amido Black (Acid Black 1)



Patent Blue VF (Acid Blue 1)



Brilliant Sulphoflavine (Acid Yellow 7)

Figure 3.5 - Molecular structure of some protein stains

Acid black 1 (AB1), acid violet 17 (AV17) and acid yellow 7 (AY7) are currently the protein stains recommended by CAST [16]. In 2000, CAST recommended the use of a water/ethanol/acetic acid (WEAA) based AB1 rather than the previous methanol or water based formulations [25]. Although the methanol based formulation was effective, easy and inexpensive, methanol is a toxic and flammable solvent introducing unacceptable health risks. Water based formulations which do not use toxic or flammable solvents do not always produce optimum results as their use may also lead to the diffusion of impression ridges, especially on porous surfaces and as such a balance needs to be struck in relation to the use of the staining solution operationally. Further research by Sears *et al.* [27] suggested that most protein stains behaved in a similar manner to AB1 and thus could be prepared using the WEAA solvent system with few exceptions. Comparisons between AB1, AV17 and AV19 demonstrated that AV17 outperformed AB1 and AV19 on porous surfaces. In general, AV19 was less effective than AB1 and AV17 as it provided less contrast, however a gelatin lifted impression in blood treated with AV19 will fluoresce under green light when viewed with a long pass 549 nm filter viewing filter (Quaser 40) and can thus enhance weak traces of protein containing material, even when present on dark surfaces [28, 29]. Using a weak solution of AV19 (diluted 100 times) allows for fluorescence observation of impressions in blood directly on the substrate [30]. Non-fluorescent stains have a limited or no enhancement effect on dark surfaces and various combinations of such dyes have been reported however none performed as effectively as AV17 or AB1 [27].

Benzoxanthene yellow was found to be very effective at enhancing minute traces of blood on dark, non-fluorescing, non-porous surfaces, however, the compound is no longer available [31]. A suitable replacement, brilliant sulphoflavine (acid yellow 7, AY7), is available commercially [31] and its performance improves with lighter deposits of blood. The enhancement effect is viewed as fluorescent green-yellow by exciting with blue-green light (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 510nm filter (1% cut-on point) [18, 31]. However, it has been reported that AY7 is not suitable for porous surfaces such as paper, since the dye cannot be washed off during the destaining procedure [31].

Recent research has also examined the use of different protein stains together with t-tetrabutylammonium iodide as a phase transfer catalyst for the enhancement of latent fingerprints [32-35]. The phase transfer catalyst facilitates the binding of the protein stain and the residual nucleotide/protein sweat constituent in latent fingerprints [35].

The sequential application of enhancement processes for fingerprints in blood have also been examined and CAST have produced a process flowchart (figure 3.6) showing the suggested sequence of techniques to ensure maximum enhancement of impressions in blood [18, 31]. It must be remembered that this chart is specific for fingerprints in blood and is not suitable for other constituents (sebaceous and eccrine) that such deposits may contain. The chart is useful to consider for the enhancement of footwear impressions in blood.

Using a technique such as superglue fuming before blood enhancement techniques was found to significantly reduce the quality and the contrast of the impression. Pre-treatment with superglue, however, offers other advantages mainly the enhancement of latent impressions and preservation of latent impressions that are not visualised by the staining technique [36]. If the impression in blood is important for a particular case, then the use of superglue is discouraged however techniques such as aluminium powder and vacuum metal deposition can be used before the use of protein stains [31]. AB1 treatment can follow leuco crystal violet and luminol enhancement whereas AV17 should be used after AY7. The use of powder suspensions after protein stains could also offer additional enhancement [37] though powder suspensions are not suitable for porous substrates and require excessive rinsing post application.

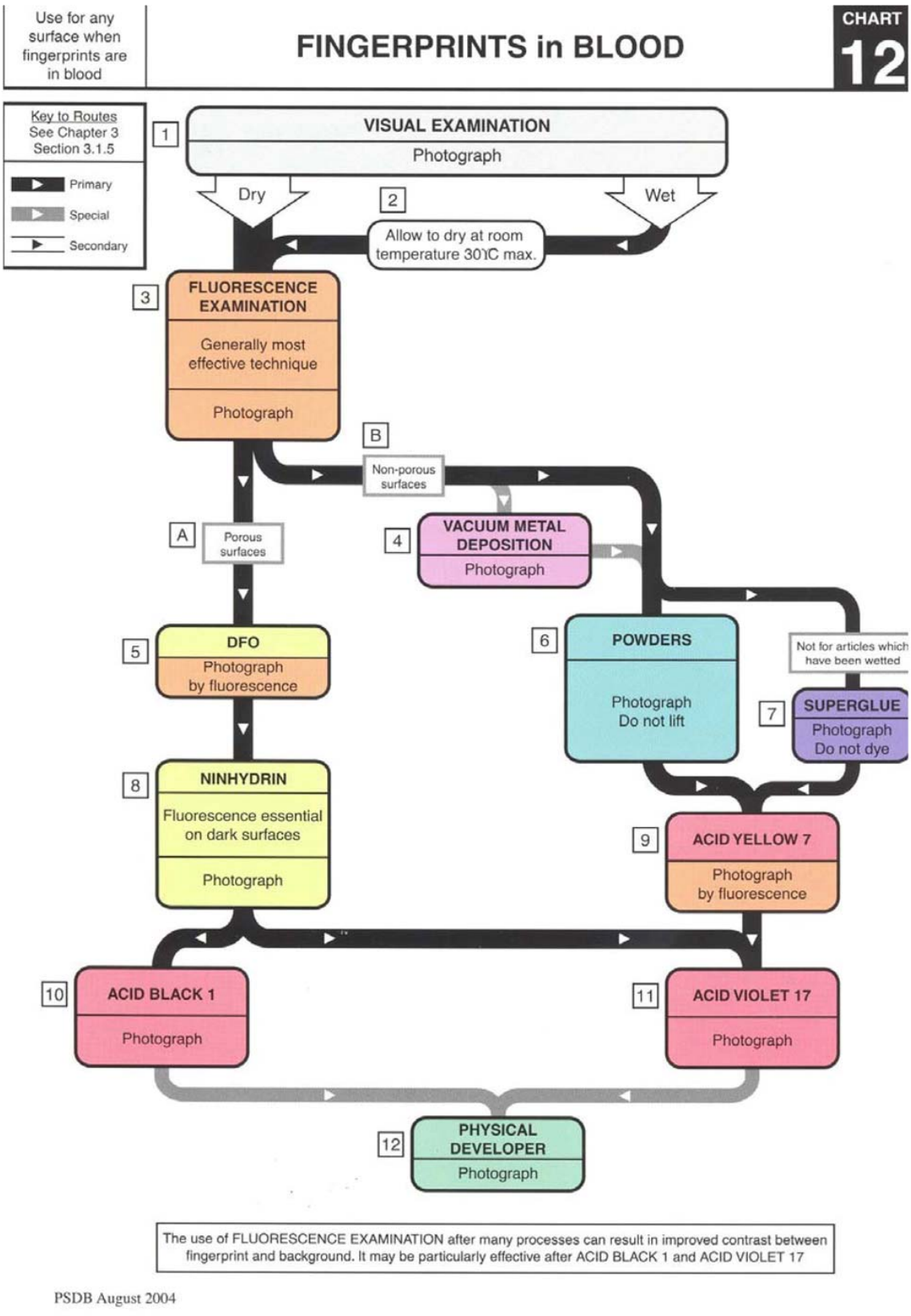


Figure 3.6 - A sequential enhancement flow chart developed by CAST for fingerprints in blood

3.1.2.3 Destaining

The enhancement process is completed by washing in a destaining solution. The washing procedure can be repeated as necessary although it might prove difficult to remove the background stain completely from porous items. The destaining solution is generally the same solvent mixture as that prepared for the protein stain solution. This helps remove the excess dye from the background but is not able to dissolve the protein stain complex with blood.

3.1.3 Peroxidase Reagents (Heme-Reacting Chemicals)

Heme-reacting chemicals react with the heme group in haemoglobin present in blood. These chemicals, also known as peroxidase reagents, are colourless dyes that are oxidised to form a coloured product [28, 38]. Compared to protein stains, these techniques do not react with body fluids apart from blood, however, trace amounts of blood in urine, saliva and other body fluids will be detected [39]. Some peroxidase reagents have been reported to react with vegetable peroxidases [40] or other substances with peroxidase activity [39]. Furthermore, background staining might occur where the background is slowly oxidised to the same colour as the blood enhanced impression. Examples of heme-reacting chemicals include leuco crystal violet (LCV), leucomalachite green (LMG), benzidine, tetramethylbenzidine (TMB), phenolphthalein, fluorescein and luminol. Some peroxidase reagents such as luminol and fluorescein do not utilise a fixing solution, whereas others such as LCV and LMG incorporate the fix in the staining solution for a one-step process.

Haemoglobin exhibits peroxidase activity by catalysing the oxidation of a number of organic compounds using peroxide to yield coloured compounds [41]. As a result, these reactions are also known as catalytic tests. The general peroxidase reaction is as follows:

$$AH_2 + ROOH \rightarrow A + ROH + H_2O$$

where AH_2 is the electron donor and ROOH is the peroxide. The simplest peroxide is hydrogen peroxide where R = H. Addition of hydrogen to the delocalised electron systems of the dyes generally interferes with the absorption of visible light [11]. Figure 3.7 illustrates the oxidation of colourless leuco crystal violet to the purple coloured crystal violet. The leuco compound is less conjugated leading to loss of colour whereas the positive charge on the dimethyl group in crystal violet is able to delocalise over the whole molecule, imparting a bright purple colour. Organic aromatic molecules such as crystal violet with conjugated bonds, and large systems of delocalised electrons can exhibit visible colour and permit molecular binding to a material [10-12].

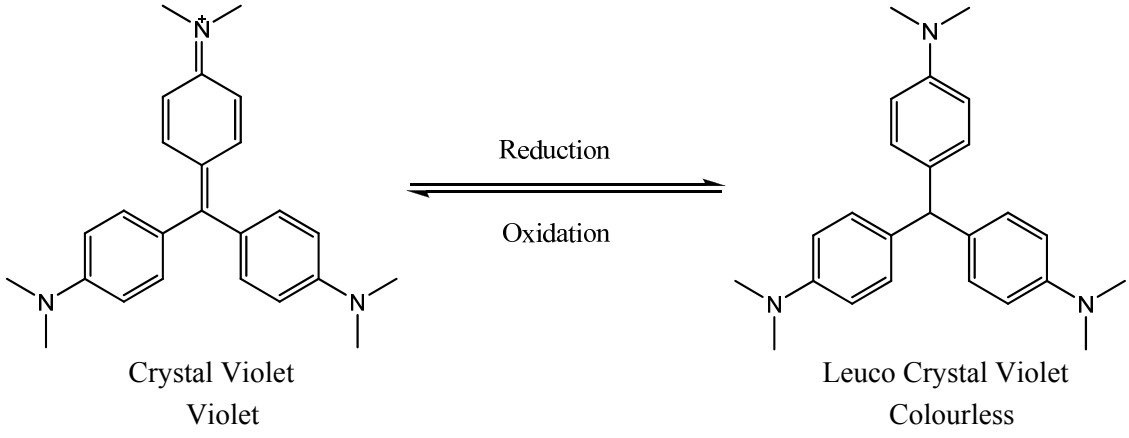


Figure 3.7 - The reduction and oxidation of crystal violet and LCV

3.1.3.1 Leuco Crystal Violet (LCV) and Leucomalachite Green (LMG)

Enhancement of impressions in blood with LCV and LMG utilise hydrogen peroxide (H_2O_2) and sodium perborate ($NaBO_3.H_2O$) respectively as oxidising agents to create an almost instantaneous colour change. Fresh solutions need to be prepared prior to use as both reagents are light and heat sensitive. Grodsky *et al.* [42] reported that the use of sodium perborate instead of hydrogen peroxide in the LMG formulation provided a great improvement in the reaction. The reaction between LMG and blood results in a green colour whereas LCV and blood results in a vivid purple colour.

Sears *et al.* [31] observed that although LCV provided better contrast than LMG, it may not be as sensitive as protein stains such as acid black 1, acid violet 17 and benzoxanthene yellow. Between these two heme reagents, LCV has almost completely replaced LMG for the enhancement of footwear impressions in blood where it can also be used for speculative searching of such impressions.

Stow [43] developed a simple, quick, one-step method for the recovery and enhancement of footwear impressions in blood. The method involves using nylon membranes, previously impregnated with LMG, as a lifting medium, producing both a lift of the impression and the enhancement simultaneously. Similar research was carried out by the Federal Bureau of Investigation (FBI) using LCV. They believed LCV is more widely used and has better enhancing qualities than LMG. The method worked well offering good lifting and enhancing detail [44].

The formulation for LCV has the advantage of incorporating the fix, allowing for fixing and enhancement of the impression in blood at the same time [3, 45]. Nevertheless, diffusion of impressions in blood is a possibility using this one-step process as the fixation process is not instantaneous. Furthermore, impressions enhanced with LCV can potentially fluoresce and luminesce as a consequence of exposing the reacted reagent to different excitation wavelengths [46]. Crystal violet, formed during the reaction of LCV with blood, fluoresces in the wavelength region 450-800nm depending on the solvent it is dissolved in [47]. Crystal violet is excited by using a green-yellow source (band pass filter 503-591nm at 1% cut-on and cut-off points respectively if using a Mason Vactron Quaser 40) and viewed with a band-pass 593nm filter (1% cut-on point).

A destaining procedure is not necessary for peroxidase reagents, but if heavy staining occurs, for example on porous surfaces, it has been suggested that the item can be rinsed with water for 2 to 3 minutes after the reagents have been applied [45]. Theeuwen *et al.* [30] have reported LCV as an excellent technique specifically for the enhancement of footwear impressions in blood. LCV, together with AB1, AV19 and Crowle's staining solution were successful in enhancing impressions in blood on both non-porous and porous items and were classified as safe to use at the crime scene, local police laboratories or specialised forensic laboratories [30].

3.1.3.2 Luminol

Luminol is a chemiluminescent test in which the peroxidase-like activity of the heme group can be used to facilitate the reaction. It has been used for many years as a presumptive test for the detection of blood and various formulations of the reagent have been reported in the literature. There are also commercial formulations now available, most notably produced by Bluestar®. Sears *et al.* [31] suggest that this method may be useful for the detection of footwear impressions in blood on dark and patterned carpets but reported that diffusion of the fine detail can occur in the enhancement of some blood-contaminated impressions such as fingerprints. Other research has highlighted the sensitivity of luminol where blood was detected through eight layers of paint [48]. Luminol requires the use of specialised photography for visualisation and different formulations of luminol have varying durations of light levels which can sometimes be disadvantageous.

The results obtained from luminol must be interpreted carefully since the reagent is known to give false positives, mainly for bleach which can be used for crime scene cleanup [49-56]. It has been suggested, however, that experienced forensic scientists should be able to distinguish between the reaction of luminol with blood and bleach [28, 50, 57, 58]. More recently it has been shown that luminol's reaction with bleach is greatly varied depending on the formulation of luminol used, the concentration and origin of the bleach and the period of time the bleach has had to dry [59]. Luminol is a useful technique for the enhancement of latent bloodstains and does not interfere with the analysis of DNA [60].

It has also been reported that luminol produces false positives in the presence of iron and copper [61, 62] and a recent study [63] has shown that false positives can be obtained in the presence of some household products (e.g. oil-based paints, alkyd varnish), food products (e.g. leek, ginger, carrot) and chemical products (e.g. $CuSO_4$, $FeSO_4$). This report also found that the ions Cu^{2+}, Fe^{2+} and Mn^{2+} catalyse the chemiluminescence reaction of luminol whereas SO_4^{2-} does not. Bluestar® luminol, when compared to other formulation of luminol (Weber and Grodsky), produced brighter and longer chemiluminescence, was easier to prepare, visualise and

photograph and was more efficient in detecting latent bloodstains after attempted cleaning by both soapy water and bleach [49, 50, 58, 64, 65].

The possibility of enhancing latent bloodstains that have been washed has been investigated by Adair and Bluestar® [66, 67]. Their studies suggested that luminol was effective at the enhancement of latent bloodstains on washed clothing. Interpretation of diluted and diffuse bloodstains should be done with caution due to the level of saturation that may take place during the washing process [66]. Cox [68] also observed a relationship between the type of fabric and the retention of the bloodstains where blood was likely to wash off regenerated and synthetic fabrics such as acetate, nylon and polyester.

3.1.3.3 Fluorescein

Fluorescein has a similar underlying chemical structure to sulfonated protein stains such as acid black 1 and acid yellow 7. The application of fluorescein to the detection of blood in forensic science was developed by Cheeseman [61, 69, 70] and it is applied as a peroxidase reagent with the additional advantage of exhibiting green fluorescence when illuminated with light at a wavelength of 450 nm. Fluorescein is soluble in alkali hydroxides and carbonates at room temperature and is reduced from fluorescein to fluorescin in alkaline solution over zinc. On coming in contact with blood, fluorescin is quickly oxidised back to fluorescein by the catalytic activity of the heme in the presence of hydrogen peroxide [28, 41]. Several studies have shown that there is no interference with the fluorescein reaction and the subsequent DNA analysis [71-74].

Some researchers suggest that fluorescein is more sensitive than luminol [61, 72, 73], however, two separate applications of fluorescein and hydrogen peroxide are normally required when using this reagent as well as an alternate light source rather than one application in the case of luminol. Fluorescein fluorescence, however, can be re-viewed without the necessity of re-application of the chemicals whereas the chemiluminescence from luminol is short lived and re-visualisation necessitates further chemical application.

Hemascein® is a commercially available fluorescein product which removes the danger of handling zinc and also allows for easy application of the reagents using a fine mist sprayer, ABAspray®. The manufacturer of the product claims that it is 2 to 5 times more sensitive than other blood detection techniques, does not interfere with DNA analysis, does not require complete darkness, is safe to handle, is stable for up to 7 years at room temperature and has a reaction duration as high as 13 minutes. It has been suggested that Hemascein® was comparable to the commercial formulation of luminol for the detection of blood-stains subjected to burning where the reaction of Hemascein® produced a constant fluorescence-based light emission for about 10 minutes surpassing the one minute chemiluminescence of luminol [75]. Other studies have shown that Hemascein® is a suitable enhancer of latent blood impressions and in some cases superior to Bluestar® luminol [76].

3.1.3.4 Other heme reagents

Diaminobenzidine (DAB) is another heme reagent suitable for the enhancement of impressions in blood. However, it is a suspected carcinogen and requires a phosphate buffer solution and a stock solution which needs to be kept at -20^0C until just prior to use [77]. Most protein stains, such as acid black 1 and acid violet 17, have been reported as providing much better performance than DAB in the enhancement of impressions in blood [30, 31]. The use of azino-di-benzthioazoline sulphonic acid (ABTS) has been suggested as a safe non-carcinogenic alternative to DAB [78]. Recent research does not recommend the use of DAB and LMG since the enhancement achieved from both dyes is poor and may not offer suitable contrast with the background matrix [38].

Recently, the use of leuco rhodamine 6G, prepared from the reduction of rhodamine 6G over zinc, has been suggested for the enhancement of latent fingerprints in blood [79]. The reagent behaves in a similar manner to fluorescein.

3.1.4 Alternative Techniques

3.1.4.1 Titanium Dioxide

If an impression in blood is present on a dark surface, enhancement by protein stains or heme-reagents may be ineffective due to problems with background contrast, unless fluorescence is utilised. In 2002, Wade [80] utilised titanium dioxide (TiO_2), also known as white powder suspension, to successfully develop latent impressions on both the non-adhesive and adhesive side of dark coloured tape. TiO_2 is a very fine, non-toxic, non-flammable, white powder with particles smaller than a micron in diameter. This enhancement gives white developed impressions providing optimal contrast on dark backgrounds. Further research by Bergeron [81] resulted in a method for developing impressions in blood on dark surfaces with TiO_2 and methanol where 1g of TiO_2 was added to 10mL of anhydrous methanol to create a suspension. No prior fixing was needed as methanol acted as a fixative agent. Minimal background staining was washed off with anhydrous methanol. Fine detail was recovered for non-porous surfaces which degraded as the porosity of the surface increased. When water was utilised instead of methanol as the solvent, the contrast obtained was reduced and the processing time increased dramatically [81]. The use of this technique at the crime scene was not recommended given the toxicity and flammability of methanol [28].

A recent study [82] recommended the use of white wet powder suspensions, alone or in conjunction with acid dyes, for the enhancement of impressions in blood on dark non-porous surfaces. However, their use on porous surfaces is not suitable due to background staining. Impressions in blood on dark surfaces have also been successfully enhanced by acid yellow 7 alone [83] and such acid dyes are a cheaper alternative to wet powder suspensions.

3.1.4.2 Ortho- and para-phenylenediamine

Caldwell *et al.* [84] optimised the use of *ortho*- and *para*-phenylenediamine (OPD and PPD) for the enhancement of impressions in blood. Both chemicals are toxic but not carcinogenic as DAB and offer the alternative enhancing colours of orange (OPD) and purple (PPD). Their use has been found to be very effective on porous surfaces. However, pH concerns and impracticality, such as solution preparation at the crime scene, makes this method inconvenient.

3.1.4.3 Ninhydrin and DFO

Ninhydrin and DFO can be utilised for the enhancement of impressions in blood in addition to latent impressions. These chemicals react with the amino acids found in blood. It is recommended that DFO is only used on porous surfaces, whereas ninhydrin can be used on both porous and non-porous surfaces [18, 19]. Ninhydrin has been reported [85] to be successful in enhancing footwear impressions in blood on paper that had been exposed to rain for over three weeks. It was also successful in enhancing minute damage features that other reagents had failed to enhance. A fuller description of ninhydrin and DFO are presented in sections 4.1.1 and 4.1.2.

3.1.4.4 Alginates

Alginate impression materials are primarily used for dental or bite mark casts because of their ease of use, low cost and good patient acceptability [86]. There are a number of different alginate materials produced by different companies with different characteristics and factors such as setting times [86-88]. The materials are generally supplied as powders which are mixed with water prior to application and setting. These powders mainly consist of sodium or potassium salts of alginic acid (11-16%), gypsum ($CaSO_4.2H_2O$) (11-17%), trisodium phosphate (1-3%) and inert fillers (65-75%) [89].

Little research has been reported in relation to the application of alginate materials on the recovery of blood impressions. Adair [90] investigated the lifting of footwear impressions made in blood both before and after enhancement with leuco crystal violet (LCV) and recovered impressions from concrete, fabric and human skin using Mikrosil®, Polyvinylsiloxane (PVS), dental stone, and alginate. Only alginates yielded successful enhancement on all of the surfaces tested. Furthermore, the alginate cast could be treated with LCV after the impression had been recovered, facilitating further enhancement of the impression. In addition, the original blood impression remained largely unchanged. The alginate casting should be photographed as soon as possible as alginates tend to shrink, resulting in the deterioration of the lifted impression [91]. Figure 3.8 shows an example of an alginate cast.

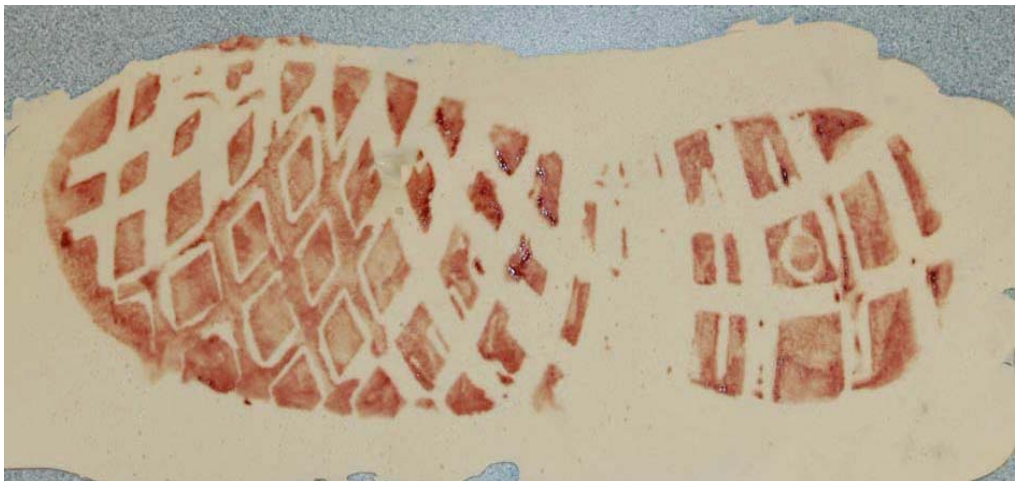


Figure 3.8 – An alginate cast of an impression in blood from human skin (reproduced from [90])

Wiesner *et al.* [92] compared a number of alginates supplied from different companies including GC, Dentsply and Heraeus Kulzer (figure 3.9). The study concluded that Aroma Dust Fine III from GC gave the best results when post-treated with acid black 1 (AB1) rather than LCV while post-treatment with 1,8-diazafluoren-9-one (DFO) did not provide any enhancement. A possible mechanism posited for the reaction of alginate with blood was that the 'water partially dissolved the blood which became biologically tied to the alginate and retained the fine detail' [92].

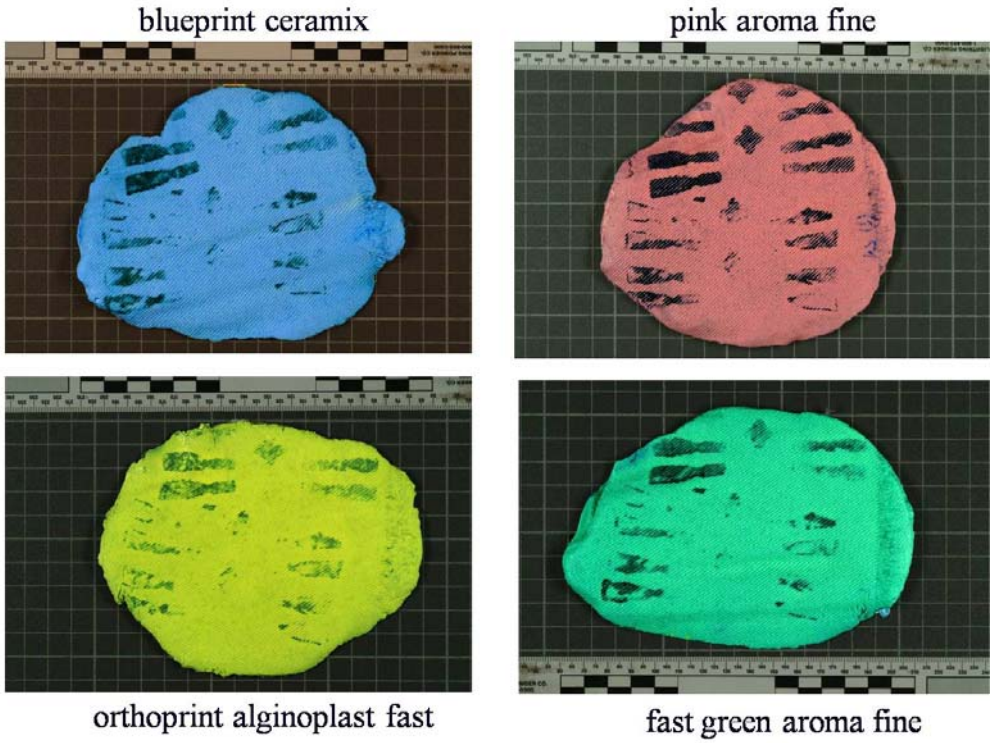


Figure 3.9 - Alginate casts of footwear impressions in blood lifted from denim using alginates from different suppliers (reproduced from [92])

3.1.4.5 Quantum Dots

A new reagent based on the use of highly fluorescent cadmium telluride quantum dots in aqueous solution was demonstrated to offer equally efficient or sometimes superior enhancement of weak fingerprints in blood on non-porous surfaces when compared to acid yellow 7 [93]. Initial research also showed that these quantum dots can enhance latent fingerprints. Further work is underway to assess its toxicity and its use at crime scenes [93].

3.2 Materials and Methods

3.2.1 Deposition of Footwear Impressions in Blood

The objective of this work was the comparison of the ability of various chemical techniques to enhance the footwear impression in blood, rather than directly mimic operational conditions normally encountered. Only when repeatability of the quality of the footwear impression produced is controlled could a direct comparison of the various stains be robustly and reliably achieved. In this work the pressure applied to the receiving surface by the blood contaminated footwear was precisely controlled using a stamping rig as described in Chapter 2.

The application of blood to the footwear sole and the subsequent transfer of blood to a substrate are challenging to control during experimental trials. Stepping into a pool of blood followed by stepping onto the fabric resulted in a heavy blood-stained and overloaded footwear impression. The following method however yielded reasonably weak and reproducible impressions in blood from mark to mark. A tray measuring 0.33 x 0.23 x 0.06 m was lined with two Kimberley® blue double ply tissues covering the whole base. 50 mL of swine blood was poured over the tissues. The tray was then pushed against the sole of the footwear attached to the rig in a walking motion. The same motion was repeated twice on clean tissues to remove excess blood before releasing the foot onto the fabric.

3.2.2 Control samples

Before the application of blood to the footwear sole, dry and wet blanks were carried out for each reagent and receiving surface. A dry blank included stamping on the fabric without any blood on the footwear sole, ensuring the footwear sole was dry. A wet blank included stepping on a distilled water-soaked tissue before stamping on the fabric. The blanks ensured that the staining observed was not due to additives, such as plasticisers, in the footwear sole and impurities in the water or substrate.

3.2.3 Photography

Six individual repeat footwear impressions were prepared as described for each enhancement technique undertaken. All impressions were allowed to age for seven days before chemical enhancement. Photography of all impressions was performed, using a Canon EOS 300D [CMOS (complementary metal-oxide semiconductor) sensor size 22.7 x 15.1 mm (3.42 cm^2)], immediately after the impression was prepared, after seven days, after chemical treatment and during fluorescence examination if required.

The conditions for general photography were determined automatically by the camera. This could have affected some of the observations obtained. Furthermore, the pictures were taken were not in RAW format which could allow for minimal data processing. The use of JPG format might also have affected the observations due to compressing and loss of resolution and detail of the original photographs.

3.2.3.1 Fluorescence Photography

Fluorescent enhancement of footwear impressions was photographed with the camera setting on Program mode (P), where the shutter speed and aperture (depth of field) where determined automatically by the camera. The exposure time varied due to the nature of the substrate. For example, a very short exposure time (fast shutter speed and small aperture) was needed for white fabrics due to the bright background fluorescence from the fabric. A fast shutter speed is open for a short period of time and allows less light to reach the camera sensor. In contrast, dark fabrics required longer exposure times thus having a slow shutter speed to allow more light to reach the camera sensor. A general camera setting could not be adopted for fluorescent photography as the background fluorescence varied from fabric to fabric. Photographing a dark image in Program mode will increase the shutter speed and the aperture (i.e. depth of field decreases) which may increase the occurrence of blurring in the picture. The use of P mode in this study provided sharp photographs that were similar or more sensitive, to what was observed with the human eye using the appropriate viewing filters.

The use of P setting averages the settings of the camera without allowing control to the user, thus in effect introducing another variable. The use of Aperture Priority setting (A) would have provided more user control of the light reaching the sensor.

3.2.3.2 Computer Monitor and Colour Calibration

Computer monitor and colour calibration were carried out according to literature methods using Adobe® Photoshop CS3/CS4 software [94-97]. The calibration was important to facilitate the direct comparison of the images using a computer monitor. The monitor calibration was performed using Adobe® Gamma within Photoshop and following the software instructions. Colour calibration was performed by photographing a Gretag Macbeth Colour Checker and then running a calibrator script (obtained from http://fors.net/chromoholics) through Photoshop software as explained by Evening [94, 95]. The Colour Checker is a checkerboard array (approximately A4 size) of 24 reference colour squares. Once the calibration script has finished running through Photoshop (approximately 80 minutes), the calibration settings are saved and named. Figure 3.10 shows a picture of the Colour Checker before (left) and after (right) calibration. Minor changes can be observed and a true representation of what the camera sensor sees is obtained via calibration. The calibration settings are only valid for the camera utilised to photograph the Colour Checker. Once the calibration settings are saved, they can be post applied to multiple images using the Adobe® Photoshop software [94, 95].

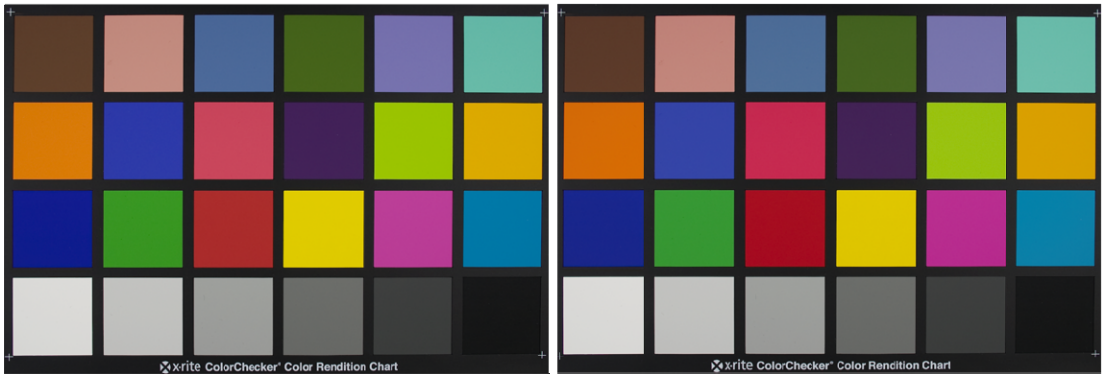


Figure 3.10 – A Colour Checker Chart before (left) and after (right) colour calibration

3.2.4 Characterisation of Protein Stains/Dyes

The various stains and dyes used in this work were characterised using UV-VIS spectrophotometry and FTIR.

The UV-Visible analysis was carried out using SI Photonics Model 420 with a fibre optic CCD array UV-Vis spectrophotometer covering a wavelength (λ) range from 350 to 700 nm and precision set as normal. The solutions for analysis were prepared as follows: the homogenised powdered stain (50mg) was weighed and transferred to a 250mL volumetric flask. The appropriate solvent (225mL) was added with thorough mixing to ensure dissolution. More solvent was then added to make a solution of 250mL with further mixing. 5mL of the solution was then diluted with 200mL of the appropriate solvent [98].

FTIR analysis using a Shimadzu 8400-S was carried out by preparing a KBr disc. A few mg of the powdered dyes was mixed with KBr, homogenised and placed in a press to produce a disc.

3.2.5 Enhancement of Footwear Impressions in Blood on Fabric

A detailed list of protein stains investigated in this work is given in table 3.1. Other techniques for blood enhancement and different types and colour of fabrics utilised in this work are listed in tables 3.2 to 3.4. Leatherette was not included in this part of the study as it was obtained later in the study when bovine leather became unavailable. In each case footwear impressions in blood were prepared as described in section 3.2.1.

Table 3.1 – List of protein stains

Name	Alternative Name	Colour Index	Supplier	Fluorescent
Acid Black 1 (AB1)	Amido Black 10B	20470	BVDA	No
Acid Violet 17 (AV17)	Coomassie Violet R-200	42650	BVDA	No
Acid Yellow 7 (AY7)	Brilliant Sulfoflavine	56205	BVDA	Yes
Acid Violet 19 (AV19)	Hungarian Red	42685	Acros	Yes
Acid Yellow 23 (AY23)	Tartrazine	19140	Sigma-Aldrich	No
Acid Blue 1 (ABlu1)	Patent Blue VF	42045	Acros	No
Acid Blue 83 (AB83)	Coomassie Brilliant Blue R	42660	BVDA	No
Acid Red 71 (AR71)	Crocein Scarlet 7B	27165	BVDA	No
Acid Green 50 (AG50)	Lissamine Green B	44090	Acros	No
Acid Red 52 (AR52)	Sulforhodamine B	45100	TCI Europe	Yes
Solvent Green 7 (SG7)	Pyranine	59040	TCI Europe	Yes
Acid Red 92 (AR92)	Phloxine B	45410	TCI Europe	No

Table 3.2 - List of peroxidase reagents

Chemical Name	Actual Chemical Name	Chemical Supplier
Leuco Crystal violet (LCV)	4,4′,4″-Methylidynetris(*N*,*N*-dimethylaniline)	Sigma Aldrich
Leucomalachite Green (LMG)	4,4′-Benzylidenebis(*N*,*N*-dimethylaniline)	Sigma Aldrich
Leuco Rhodamine 6G (LeuR6G)	Leuco Basic Red 1	Acros
Fluorescein	Acid Yellow 73	Sigma Aldrich
Hemascein®	Commercial Fluorescein	Abacus Diagnostics
Luminol	3-Aminophthalhydrazide	Bluestar Forensic Magnum

Table 3.3 – List of amino acid reagents

Chemical Name	Alternative Chemical Name	Chemical Supplier
Ninhydrin	2,2-Dihydroxy-1,3-indanedione	Sigma Aldrich
DFO	1,8-diazafluoren-9-one	BVDA
1,2-Indanedione	1*H*-Indene-1,2(3*H*)-dione	BVDA

Table 3.4 – List of fabrics

Fabric	Supplier
White Cotton [CD13] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Black Cotton [CD13D] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Patterned Cotton [SF2360/B] Twill weave; 19 warp threads/cm; 19 weft threads/cm	WBL Whaleys Bradford Ltd.
White Polyester Taffeta [SF25] Plain weave; 14 warp threads/cm; 8 weft threads/cm	WBL Whaleys Bradford Ltd.
Black Polyester Taffeta [SF25A] Plain weave; 14 warp threads/cm; 8 weft threads/cm	WBL Whaleys Bradford Ltd.
White Nylon (82%) / Lycra (18%) [SF28] Weft knitted	WBL Whaleys Bradford Ltd.
Black Nylon (82%) / Lycra (18%) [SF27] Weft knitted	WBL Whaleys Bradford Ltd.
Blue Denim [Rialto Indigo] Twill weave; 25 warp threads/cm; 19 weft threads/cm	Mandors, Glasgow, UK
Brown Bovine Leather	The Clyde Leather Co., Glasgow, UK
Plain Dyed Brown Leatherette KBT259 (C2708) (68) (F10) Synthetic fabric covered with a soft PVC layer	www.fabricuk.com

AB83 and AR71 were removed from the list of techniques in the early stages of the experimental work as their enhancement potential and colour were similar or of lesser quality to AV17 and AV19 respectively.

3.2.6 Protein Stains

All protein and amino acid stains in this study were prepared using the WEAA formulation suggested by CAST.

Fixing solution: 5-sulfosalicylic acid dihydrate (23g, Acros) was dissolved and stirred in distilled water (1L). This was used to fix the impressions in blood by immersion for a minimum period of 5 minutes.

Staining solution: The appropriate protein stain (1g) was stirred for at least 30 minutes in a solution of acetic acid (50mL, Sigma), ethanol (250mL, Sigma) and distilled water (700mL). This was used to stain the blood impressions under test by immersion for a minimum period of 5 minutes.

Destaining solution: The final destaining methodology used was to initially rinse under running tap water for several minutes to remove the excess dye (as suggested by Bodziak [3]) followed by immersion in a destaining solution of acetic acid (50mL, Sigma), ethanol (250mL, Sigma) and distilled water (700mL). The items were left to dry overnight before any photography was undertaken.

Fluorescence observations: The appropriate excitation wavelengths and viewing filters utilised for observation of the fluorescent protein stains are presented in table 3.5. Fluorescence examination was performed using a Mason Vactron Quaser 40 and a Foster and Freeman Crime-Lite® 2.

Table 3.5 - Excitation wavelength and viewing filters for fluorescent protein stains

Chemical Name	Excitation Wavelength/nm	Excitation Filters	Viewing Filter/nm	Viewing Filters
AY7	385-509	Blue	510	Yellow/Orange
AV19	473-548	Green	549	Orange
SG7	385-469	Violet/Blue	476	Yellow
AR52	503-591	Green/Yellow	593	Red

The wavelengths represented in table 3.5 show the 1% cut-on and cut-off points [99]. Other light sources may use wavelengths representing different cut-on and cut-off points.

3.2.7 Peroxidases

LCV was prepared using the formulation suggested by Bodziak [45] incorporating the fix 5-sulfosalicylic acid for a one-step process. The LMG formulation utilised in this study was prepared as suggested by the RCMP [100] using HFE 7100 and the fluorescein formulation was prepared according to the method outlined by Cheeseman [61, 69, 70]. Leuco rhodamine 6G was prepared by reduction over zinc of the commercially available rhodamine 6G as described by Yapping [79]. A commercially available formulation of luminol provided by Bluestar® was utilised in this study.

LCV formulation

5-sulphosalicyclic acid dihydrate (10g, Acros) was dissolved in 3% hydrogen peroxide (500mL, VWR). Sodium acetate (3.7g, Sigma) was added to the mixture followed by leuco crystal violet (1g, Sigma) and stirred using a magnetic stirrer until completely dissolved. The reagent was applied by spraying with an Ecospray® supplied by Bluestar® Forensic. Fluorescence observation was carried out with a Mason Vactron Quaser 40 high intensity light source using a green/yellow excitation source (band pass filter 503-591nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 593nm filter (1% cut-on point).For comparison purposes, a yellow laser (577nm) was also employed for fluorescence examination and viewed with a red viewing filter.

LMG formulation

Leucomalachite green (0.2g, BDH) was dissolved in methanol (67mL, Sigma) using a clean, dry, glass beaker. This was followed by the addition of glacial acetic acid (33mL, Sigma) and sodium perborate (0.67g, Sigma) and stirred until dissolved using a magnetic stirrer. HFE 7100 (300mL, 3M Novec) was finally added and

stirred. The resulting solution was stored in a dark coloured glass bottle and was applied by spraying with a Preval® sprayer. This formulation is unstable and should be used as soon as possible [100].

Leuco Rhodamine 6G (LeuR6G)

Stock solution: Rhodamine 6G (1g, Acros), powdered zinc (20g, Acros) and glacial acetic acid (10mL, Sigma) were added to ethanol (200mL, Sigma) and stirred with a magnetic stirrer until dissolved (with the exception of zinc). This reduction reaction was then continued for 30 minutes followed by the addition of 5-sulfosalicylic acid (0.22g, Acros) and mossy zinc (10g, Acros).

Neutralisation of zinc: Concentrated hydrochloric acid (HCl) was added to dropwise to the remaining zinc. The solution gave off bubbles (hydrogen) and heat and extra time was allowed for the bubbling and heat to dissipate before adding further acid. Dropwise addition was continued until no more bubbles were formed and no more grey powder was visible indicating the formation of soluble zinc chloride, $ZnCl_2$. The acidic solution was then neutralised by adding small amounts of sodium carbonate until no more foaming and bubbling occurred. The non-toxic and white compound, zinc carbonate, was then filtered and disposed off in the normal waste.

Working solution: LeuR6G stock solution (20mL) was added to diethyl ether (80mL, Sigma) followed by the addition of 3% hydrogen peroxide (8-10 drops, VWR) and further stirring. The solution was then stored in a dark bottle and used within the same day. The solution was applied using an Ecospray®.

Fluorescein

Solution A: A 10% NaOH solution as prepared by dissolving NaOH (10g, Sigma) in distilled water (100mL). Fluorescein (1g, Sigma) was dissolved in 10% NaOH solution (100mL). The fluorescein solution was stirred and heated gently before adding zinc powder (10g, Sigma) and brought to a gentle boil. The cooled solution was then decanted carefully to remove the zinc which was neutralised prior to disposal. A 1:20 ratio of this solution with distilled water was then prepared.

Solution B: A 10% H_2O_2 solution (VWR) was prepared by adding 30% H_2O_2 (100mL) to distilled water (200mL).

The reagents were applied by spraying solution A followed by solution B using an Ecospray® unit supplied by Bluestar® Forensic. Fluorescence observation was carried out with a Mason Vactron Quaser 40 high intensity light source using a blue excitation source (band pass filter 385-509nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 510nm filter (1% cut-on point).

Hemascein®

Stock solution: Distilled water (5mL) was added to the Hemascein® powder vial and mixed vigorously.

Working solution: The stock solution (1mL) was diluted with distilled water (100mL) in one of the ABAspray® supplied. 1-3% hydrogen peroxide was also prepared in another sprayer.

The reagents were sprayed using ABAspray® to lightly mist the target area with the working solution followed by the hydrogen peroxide solution.

Luminol

The luminol formulation utilised in this study was Bluestar® Forensic Magnum purchased from Bluestar® Forensic. It was prepared by dissolving the three tablets in liquid supplied (125mL) and then applied using an Ecospray® unit supplied by Bluestar® Forensic.

Luminol photography: The best photographic quality of the resultant chemiluminescent reactions was obtained using the following conditions: ISO400, f 5.6, exposure of 15 seconds and white balance set on tungsten, as recommended by CAST [101].

3.2.8 Amino Acid Staining Techniques

Ninhydrin

Concentrated Solution: ninhydrin (25g, Sigma) was dissolved in absolute ethanol (225mL, Sigma). Ethyl acetate (10mL, Sigma) followed by acetic acid (25mL, Sigma) was added to the slurry and stirred until a clear yellow solution was produced.

Ninhydrin Working Solution: ninhydrin concentrated solution (52mL) was measured out followed by the addition of HFE 7100 (1L, 3M Novec) whilst stirring with a magnetic stirrer.

Treatment of articles with Ninhydrin: The articles were immersed in the working solution for a maximum of five seconds. The excess solution was allowed to drain back in the tray. The fabric was allowed to dry completely before heated in a humidity oven at 80^{o}C for four minutes and a 65% relative humidity. Enhancement can occur immediately or within the next few hours/days.

DFO

Working Solution: DFO (0.25g, BVDA) was dissolved in methanol (30mL, Sigma) using a magnetic stirrer to produce a slurry. Acetic acid (20mL, Sigma) was added and stirred until a clear, yellow solution was produced followed by the addition of HFE71DE (275mL, 3M Novec) and HFE7100 (725mL, 3M Novec) with continued stirring.

Treatment of articles with DFO: The articles were immersed in the working solution for a maximum of five seconds. The excess solution was allowed to drain back in the tray. The fabric was allowed to dry completely before being heated in an oven at 100^{o}C for 20 minutes without humidifying. Fluorescence examination was carried out using a green excitation source (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 549nm filter (1% cut-on point).

1,2-Indanedione

Working Solution: 1,2-indanedione (0.25g, BVDA) was weighed and dissolved using a magnetic stirrer in ethyl acetate (90mL, Sigma), acetic acid (10mL, Sigma) and $ZnCl_2$ stock solution (0.5mL). Finally HFE-7100 (1L, 3M Novec) was added to the mixture and stirred.

$ZnCl_2$ Stock Solution: Anhydrous zinc chloride (0.2g, BDH) was dissolved in absolute alcohol (5mL, Sigma).

Treatment of articles with 1,2-Indanedione: The articles were immersed in working solution for a maximum of five seconds. The excess solution was allowed to drain back in the tray. The fabric was allowed to dry completely before heated in an oven at 100^{o}C for 10 minutes without humidifying. Fluorescence examination was carried out using a green excitation source (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 549nm filter (1% cut-on point).

3.2.9 Alginate

Alginates from three different manufacturers (GC, Dentsply and Henry Schein) were compared using a diminishing series of footwear impressions in blood on denim and 100% white cotton. A footwear sole was pressed on a blood-soaked tissue in a tray and then applied a number of times on the fabric with the first impression being the most heavily-stained. The alginate powder was mixed with water according the manufacturer's instructions and applied quickly to the impressions in blood.

The instructions on the GC Aroma Dust Fine III packaging suggested mixing one scoop of product with 20mL of water. Preliminary work demonstrated that nine scoops of product with 200mL of water provided enough material with the best consistency to cover a full footwear impression (UK size 8, EU size 42). As soon as water was added to the powder, the mixture was stirred for 30 seconds and applied quickly with a large plastic spatula on the impression in blood. A Ziploc® bag was placed over the alginate and pressure was evenly applied across its surface. After one

minute the alginate had solidified enough to remove and handle gently and was left to dry overnight.

Further examination using the alginates was carried out on white and black 82% nylon/18% lycra and cotton and blue denim with five repeats for each. Each repeat was enhanced in a different way as shown in table 3.6. Treatment of the alginate lift with AB1 and LCV was performed as described earlier.

Table 3.6 - Alginate repeats

Repeat Number	Enhancement method
1	Alginate application
2	Alginate application followed by AB1
3	AB1 followed by alginate application
4	Alginate application followed by LCV
5	LCV followed by alginate application

3.2.10 Powder Suspensions

The use of powder suspensions was attempted on light and dark fabrics to assess their suitability for the enhancement of footwear impressions in blood on fabrics. CAST discuss three formulations in their recent update to the Manual of Fingerprint Development Techniques: two black (carbon and iron (II/III) oxide) and one white (titanium dioxide). CAST recommend a formulation for the iron based working suspension but they have not, as yet, recommended formulations for the carbon or titanium based powder suspensions which can be obtained commercially from WetWop®. In this study, the CAST formulation for the iron based powder suspension (black) was utilised on light coloured fabrics together with a titanium dioxide formulation (white) suggested by Bergeron [81] on dark fabrics.

Iron (II/III) oxide powder suspension formulation

Iron (II/III) oxide (20g, Fischer I/1100/53) was weighed and poured into a 100mL plastic beaker. Stock detergent solution (20mL) was added slowly whilst stirring with a soft squirrel hair brush until no lumps remained. The stock detergent solution was prepared by measuring Triton X100 (250mL, Acros) and adding ethylene glycol (350mL, Acros) whilst stirring slowly for 10 minutes. Distilled water (400mL) was added and stirred for a further 10 minutes.

Treatment of articles

The impression was wetted with tap water prior to the application of the powder suspension with a small, animal hair brush. The working suspension was left for a few seconds and then washed under slowly running, cold tap water until all the excess powder is removed from the background. The article was allowed to dry at room temperature before examination.

Titanium Dioxide Formulation and treatment of articles

Titanium dioxide (1g, Sigma) was dissolved in methanol (10mL, Sigma) and sprayed onto the impression followed by rinsing with methanol (20mL, Sigma).

3.2.11 Preliminary Enhancement Methodology and Mini Studies

Preliminary work on the enhancement of impressions focused mainly on white cotton and polyester due to low costs and practicality. This was carried out to determine the best methodology for the enhancement technique and to assess the potential of the techniques. The experiments were conducted using a small footwear sole where the blood was applied as described in section 3.2.1 and the impression applied directly onto the fabric using normal hand pressure rather than the footwear rig device.

3.2.11.1 Ageing of Footwear Impressions in Blood on Cotton

A mini-study was devised to compare several chemical enhancement techniques for the enhancement of footwear impressions in blood on white cotton that have been aged for 1, 6, 10 and 28 days. Protein stains acid black 1 (AB1) and acid violet 19 (AV19, Hungarian Red) and heme-reacting chemicals LCV and luminol were used in this study as suitable representations for protein stains and heme-reacting chemicals. Three footwear impressions in blood were prepared for each protein stain per ageing period. The impressions in blood were prepared, as discussed previously, by contact on a blood soaked tissue in a tray, contact twice onto a clean tissue and then contact on the cotton substrate.

3.2.11.2 Evaluation of spraying mechanisms for enhancement

Footwear impressions in blood were prepared and aged for one week. A selection of spray delivery systems available from Preval®, BVDA (Netherlands), Bluestar® as well as a conventional garden sprayer were all evaluated as a means of reagent delivery for LCV, LMG and luminol.

3.2.12 Sensitivity - Diminishing Series

Test footwear impressions in blood were prepared as explained in section 3.2.1. In this case, excess blood was not removed by stepping twice on a clean tissue. A diminishing series was prepared by stepping on a blood soaked tissue (50mL of blood) and then using the footwear rig to produce ten impressions in blood for each fabric with the first one being the most blood-stained. The impressions were left to air dry for one week before being cut into four pieces and treated with the best performing enhancement techniques.

3.2.13 Sensitivity - Washing

Test footwear impressions in blood were prepared as explained in section 3.2.1. For this part of the study, four enhancement techniques (LCV, LMG, AY7 and luminol) were considered with three repeats for white and black cotton, polyester and nylon/lycra. The impressions in blood were left for 48 hours before being washed in a Hoover® washing machine with Surf® powder detergent at a temperature of 40°C on a general cycle for medium-soiled laundry. The samples were left to dry overnight before chemical treatment.

3.2.14 Sequential Chemical Enhancement

Sequential chemical enhancement was investigated using black cotton and white polyester as exemplars of dark and light, natural and synthetic fabric. Examples of a peroxidase reagent [luminol (LUM)] a protein stain [acid black 1 (AB1)], an amino acid stain [ninhydrin (NIN)] and an alginate (ALG) were used to enhance the impressions on white polyester. For black cotton, AB1 and ninhydrin were replaced with acid yellow 7 (AY7) and DFO respectively as they may provide fluorescence and hence visualisation against the dark background. Six different sequential enhancements were considered as shown in table 3.7. The application of alginates was performed at the end of the sequence since previous research with alginates revealed that their use interfered with subsequent chemical enhancement techniques [102].

Table 3.7 – Sequential enhancement for impressions in blood on black cotton

Sequence	Technique Sequence
1	AY7, LUM, DFO, ALG
2	LUM, AY7, DFO, ALG
3	DFO, AY7, LUM, ALG
4	NIN, DFO, AY7, ALG
5	LUM, DFO, AY7, ALG
6	AY7, DFO, LUM, ALG

AY7 and DFO were replaced with AB1 and ninhydrin for enhancement on white polyester

3.2.15 Chemical Enhancement of Individual Characteristics

8 individual characteristics (carvings and indentations) were introduced onto the footwear sole of a new unworn shoe using a knife and scissors (figure 3.11, table 3.8). Footwear impressions in blood were prepared on black and white cotton and 82% nylon/18% lycra (as exemplars of natural and synthetic fabrics) as described in section 3.2.1 and enhanced with the best performing reagents as determined by the study and listed in table 3.9. Each technique was performed six times on each fabric for a total of 48 impressions.

Table 3.8 – Individual characteristic description

Characteristic	Description
A	Piece of sole scratched off
B	Piercing
C	Deep scratch indentation
D	Piercing
E	Light scratch indentation
F	Deep scratch indentation
G	Deep scratch indentation
H	Light scratch indentation

Table 3.9 – Enhancement of individual characteristics

Fabric	Technique
Black cotton	AY7, luminol
White cotton	AB1, LCV
Black 82% nylon/18% lycra	AY7, luminol
White nylon/lycra	AB1, LCV

Figure 3.11 – Introduced carvings and indentation on the footwear sole

3.3 Results and Discussion

3.3.1 Characterisation of Protein Stains/Dyes

3.3.1.1 UV-VIS Spectrophotometry

The absorption maxima of dyes used in this study are recorded in table 3.10 and compared to literature values where available. Only the absorption maximum for acid yellow 7 differed from the literature but not markedly so. Exemplar UV-Vis spectra of acid violet 19 and acid blue 1 are presented in figure 3.12 and figure 3.13.

Table 3.10 – Absorption maxima of dyes

Dyes	Solvent	Absorption Maximum (nm) Observed	Absorption Maximum (nm) Literature [12, 98, 103]
Acid Black 1	H_2O	620	618
Acid Violet 17	H_2O	546	545
Acid Yellow 7	H_2O	458	425
Acid Blue 1	H_2O	411, 640	410, 635
Acid Violet 19	5mL 0.1N HCl in 200mL H_2O	545	545
Acid Yellow 23	H_2O	428	425
Coomassie Blue R-250	EtOH	589	585
Oil Red O	Toluene	532	518
Safranin	50% aq. EtOH	521	530
Bromocresol Green	MeOH	423	423
Bromophenol Blue	0.005N NaOH in MeOH	596	598
Solvent Green 7	Acidic Aq solution	403	403
Acid Red 52	0.005N NaOH in MeOH	580	585
Acid Blue 9	H_2O	410, 625	406,625
Acid Green 50	H_2O	633	633
Acid Red 92	H_2O	550	550

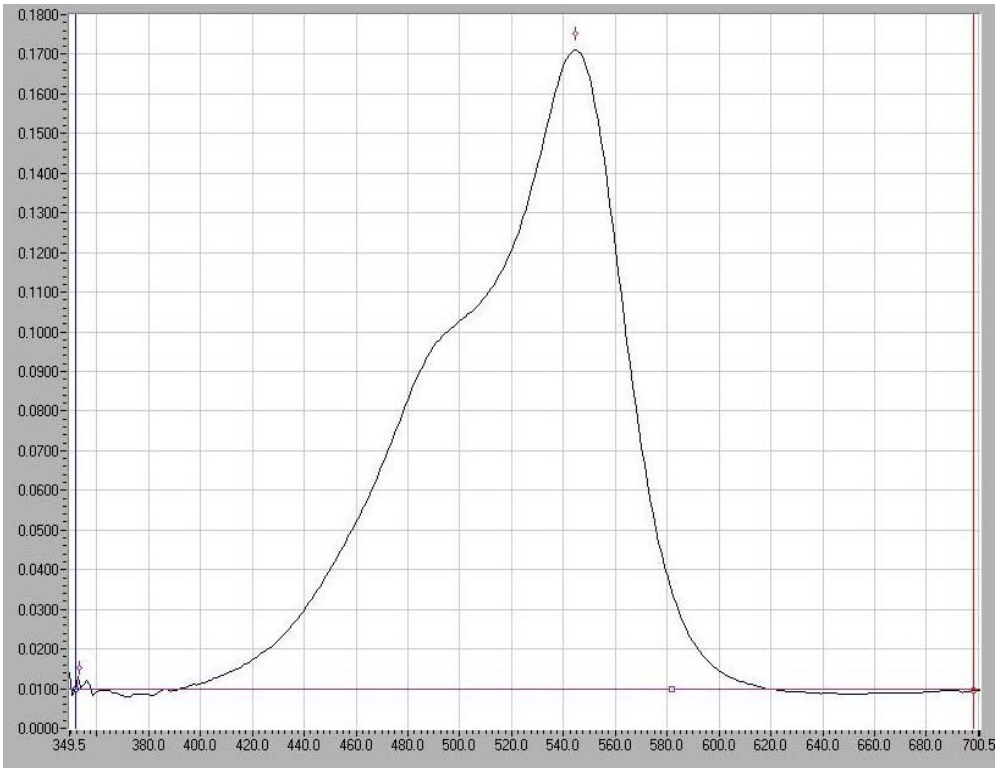


Figure 3.12 – UV-Vis spectrum of acid violet 19

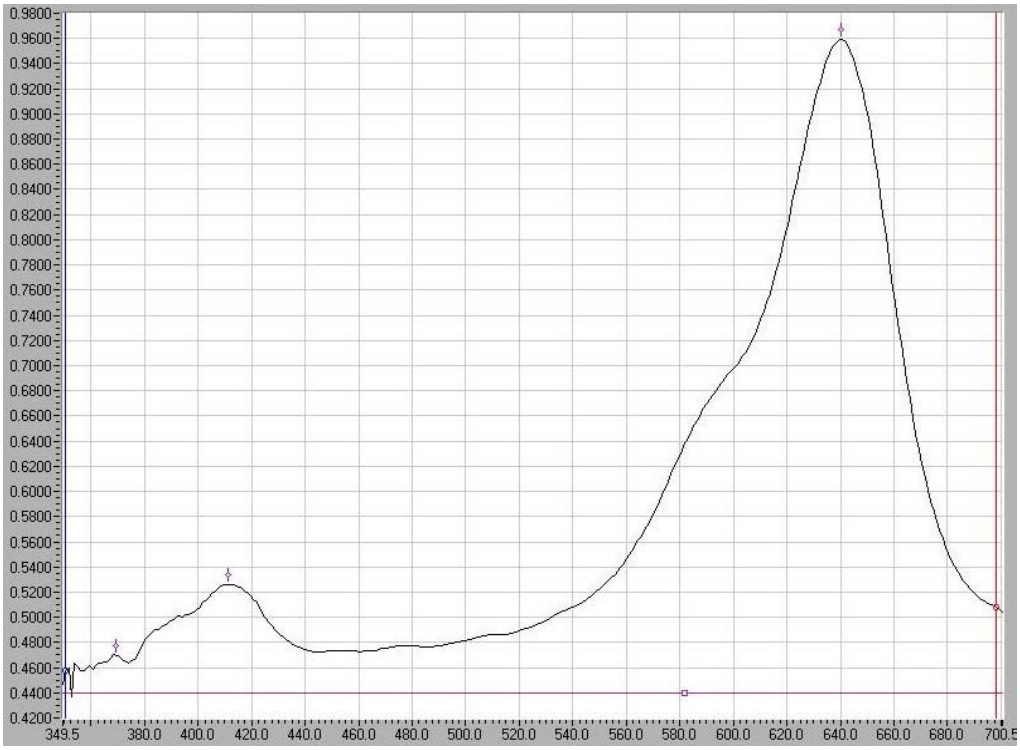


Figure 3.13 - UV-Vis spectrum of acid blue 1

3.3.1.2 FTIR

All of the dyes investigated in table 3.10 together with ninhydrin, DFO, LCV and LMG were analysed by FTIR using the KBr disc method. All the compounds analysed were aromatic showing a peak for C=C at 1500 cm^{-1}. Dyes having an –OH group exhibited a broad peak at approximately 3500 cm^{-1} and those having a carbonyl group exhibited a strong peak around 1750 cm^{-1}. Figure 3.14 and figure 3.15 show the KBr disc FTIR spectrum of DFO and bromophenol blue respectively revealing the strong C=O bond at 1750 cm^{-1} in DFO and the strong, broad O-H peak at 3500 cm^{-1} in bromophenol blue. All dyes used in this study exhibited a good IR spectrum conforming to their structure.

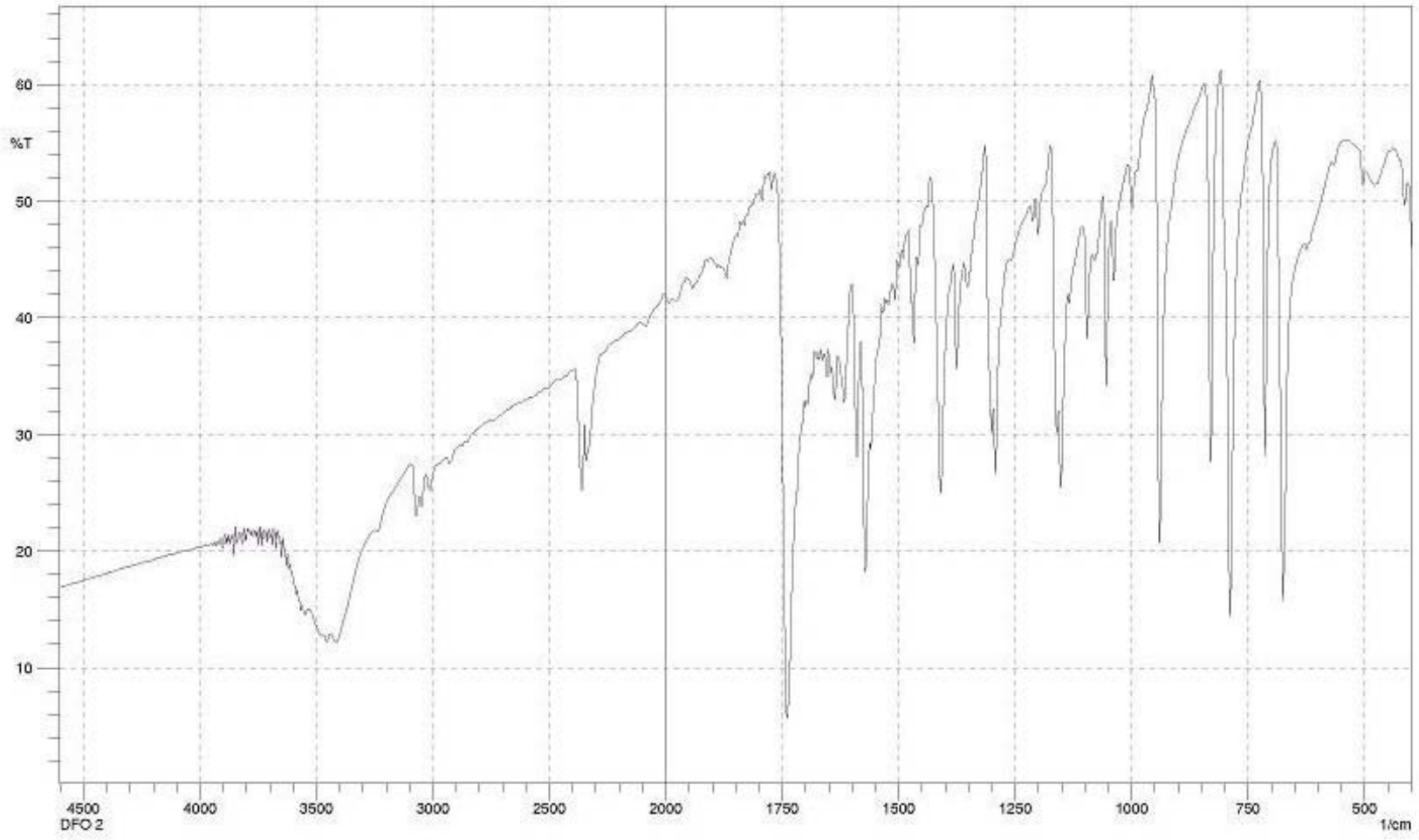


Figure 3.14 – KBr disc FTIR spectrum of DFO

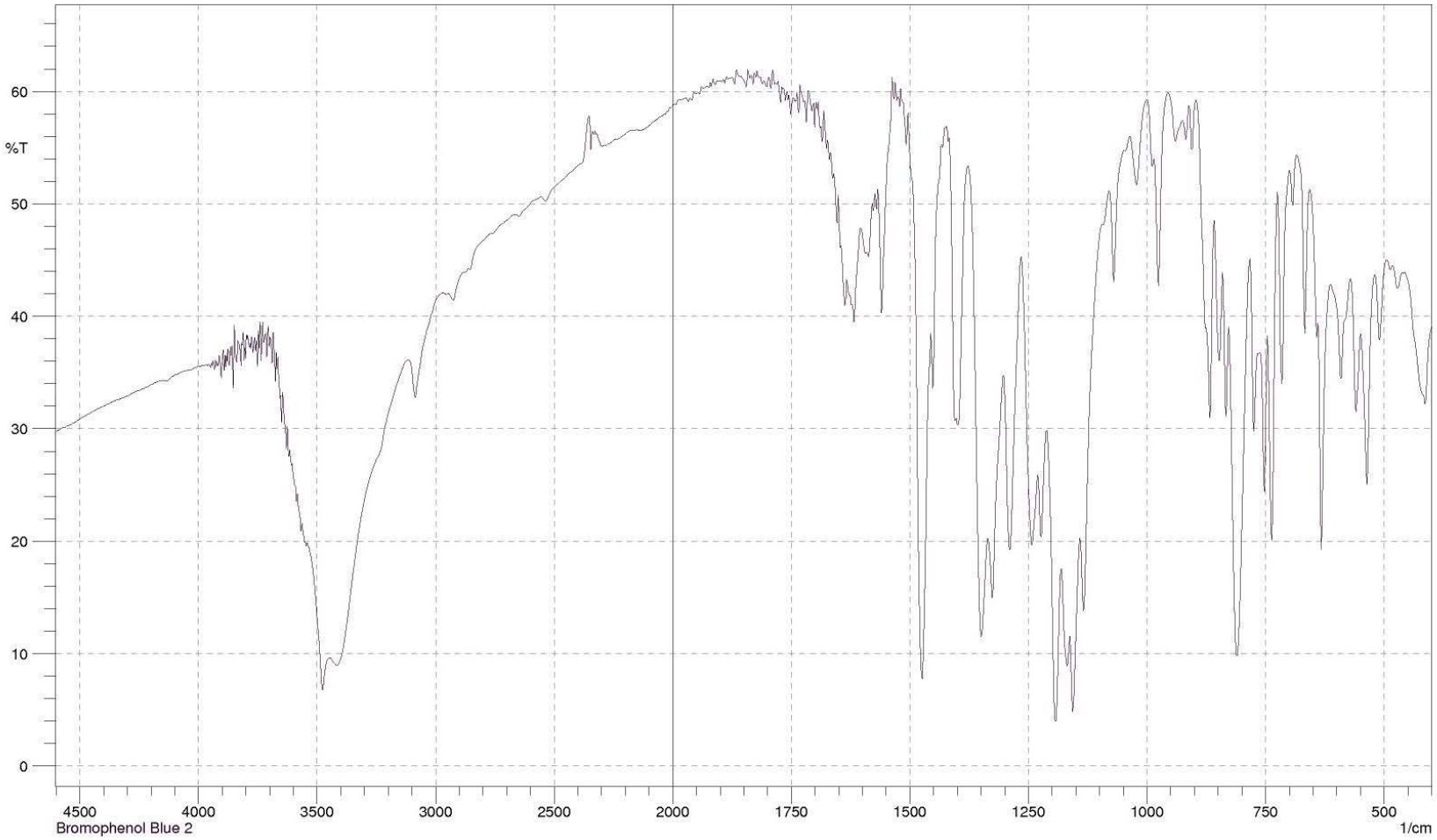


Figure 3.15 - KBr disc FTIR spectrum of bromophenol blue

3.3.2 Preliminary Enhancements

3.3.2.1 Ageing of Footwear Impressions in Blood on Cotton

Morgan-Smith *et al.* [85] suggested that footwear impressions in blood will deteriorate over time, even in indoor or sheltered environments and that although several publications have compared different chemical techniques for the enhancement of fingerprints in blood and footwear impressions, none has specifically investigated the ageing of footwear impressions in blood. Ninhydrin has been reported to be the best reagent for treating aged footwear impressions in blood on paper substrates whereas acid black 1 provided the best results on wooden and linoleum substrates [85].

All of the impressions to be treated with protein stains were fixed in 5-sulfosalicylic acid for 5 minutes, immersed in a working solution for 5-10 minutes followed by a further two wash solutions for 5-10 minutes, as indicated in the MoFDT [18]. Acid black 1 (AB1) and acid violet 19 (AV19) were used as examples of protein stains as they behaved similarly in the enhancement procedure. Diffusion of the original impression was noted for the samples aged 1 day, possibly because the impression was not dry enough and would thus require longer fixing times. There were no obvious visual changes observed for other footwear impressions aged for different periods of time. The enhancement procedure seemed to darken the portions of the impression that were already visible to the naked eye with the exception of a few additional areas. Background staining after AB1 remained strong, even after several destaining washes. In contrast, the destaining procedure for AV19 was very efficient (figure 3.16).

The use of gelatin lifters has been suggested for lifting impressions in blood after treating with protein stains [28, 29]. Impressions in blood enhanced with AV19 and lifted with a white gelatin lifter may fluoresce when illuminated with a green excitation source (515 to 560nm).

Figure 3.16 - A footwear impression in blood before and after enhancement with AV 19 (Hungarian Red)

LCV and luminol were used as examples of peroxidase stains. Diffusion of the original impressions in blood was observed for almost all impressions during all ageing periods after enhancement with LCV (by immersion and spraying), and luminol (by spraying only). Figure 3.17 shows the enhancement of a footwear impression in blood after being treated with LCV via a commercially available Ecosprayer® that eliminated diffusion. The LCV formulation incorporates 5-sulfosalicylic acid as a fix and does not require any destaining procedures. To reduce diffusion, Powell [38] investigated the use of a fixing agent before applying the LCV formulation in two different ways. The first method removed the fix from the LCV formulation and fixed separately before applying LCV. However, this resulted in the formulation not being acidic enough to fully dissolve the LCV and the amount of LCV in solution was not enough for a colour change to occur. The second method involved fixing the impression before applying the LCV formulation incorporating the fix. This procedure made the formulation too acidic and LCV was oxidised to crystal violet, a pH indicator which changes to a yellow colour in acidic condition (figure 3.18a).

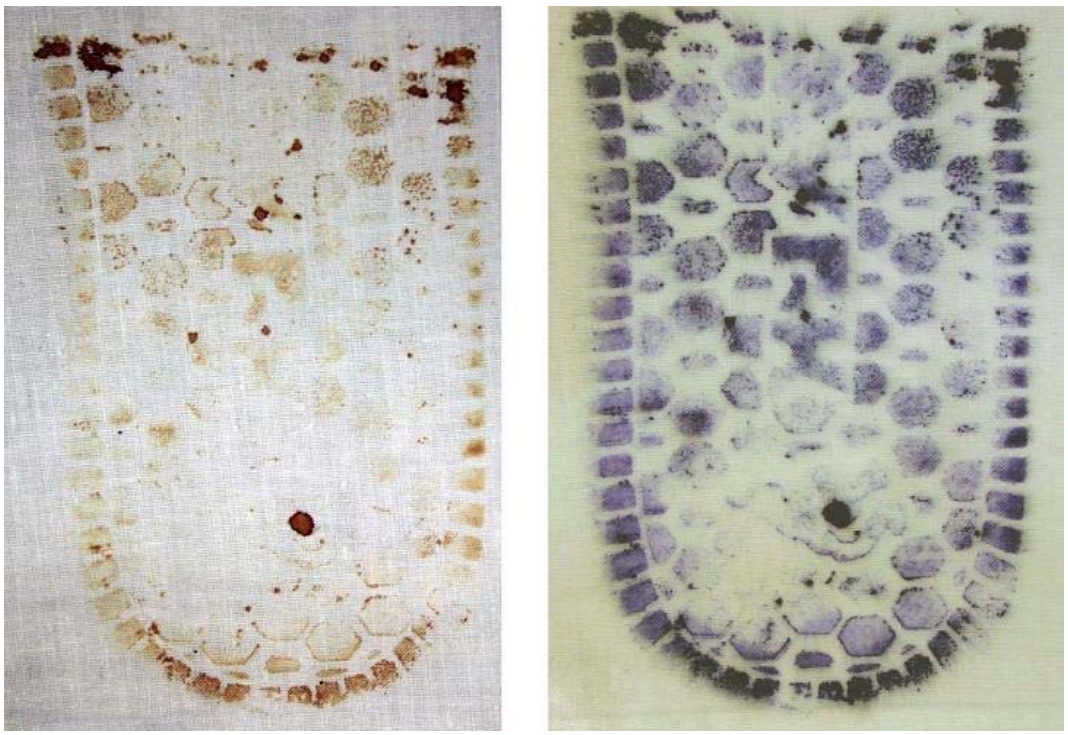
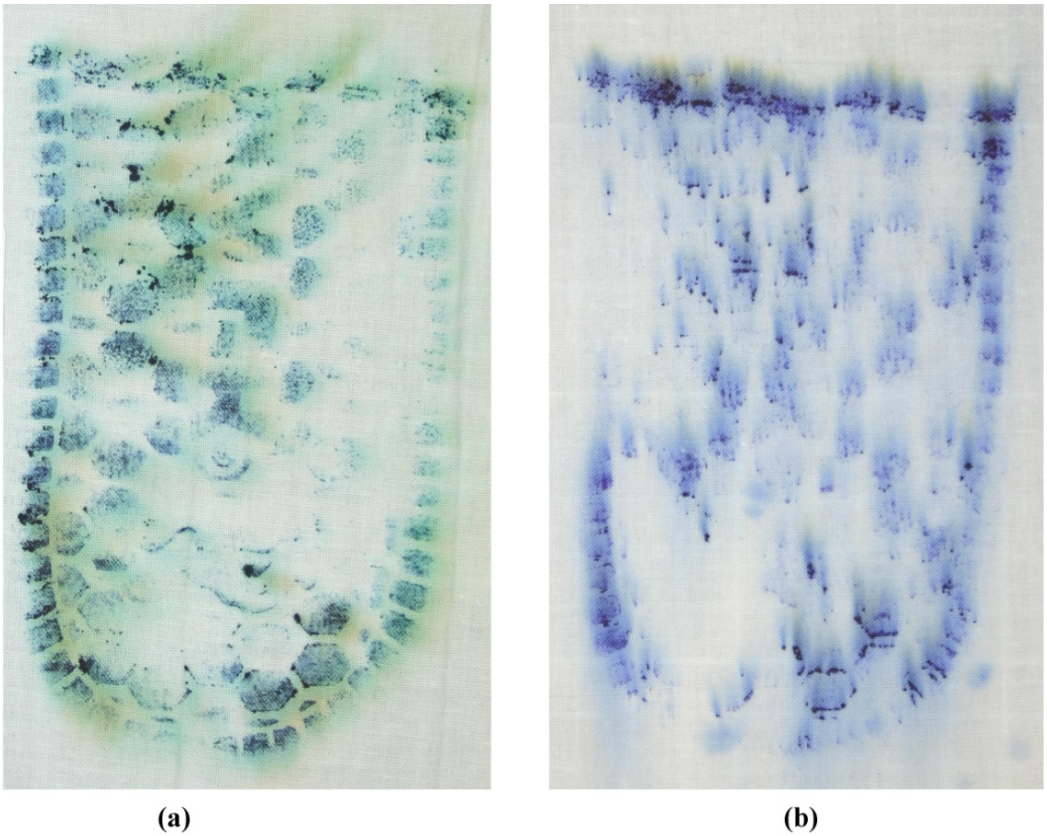


Figure 3.17 - A footwear impression in blood before and after enhancement with LCV



(a) **(b)**

Figure 3.18 - Preliminary work with LCV on footwear impressions in blood on cotton: (a) yellow tinge due to fixing the impression twice; (b) a blurry LCV enhanced impression

Fixing of the original impression is not necessary prior to the application of luminol on an impression in blood. Similar to LCV, all impressions bled or diffused after treatment with luminol. Purser [104] noted that using 5-sulfosalicylic acid as a fixative agent prior to the application of luminol was not effective as it created a layer on top of the impressions in blood, limiting the amount of luminol that reaches and reacts with the heme in the blood. Incorporating the fix within the luminol formulation was also ineffective as the fix is acidic and luminol is alkaline, thus resulting in the reduction of the intensity of chemiluminescence.

The problems of diffusion, for LCV and luminol were further investigated (section 3.3.2.2) using several different sprayers to apply the reagents.

3.3.2.2 Evaluation of Spraying Mechanisms for Enhancement of Impressions in Blood on Cotton

Diffusion of the original impressions was observed in almost all cases after application of any of the reagents using either a garden sprayer or the BVDA sprayer as illustrated in figure 3.19.

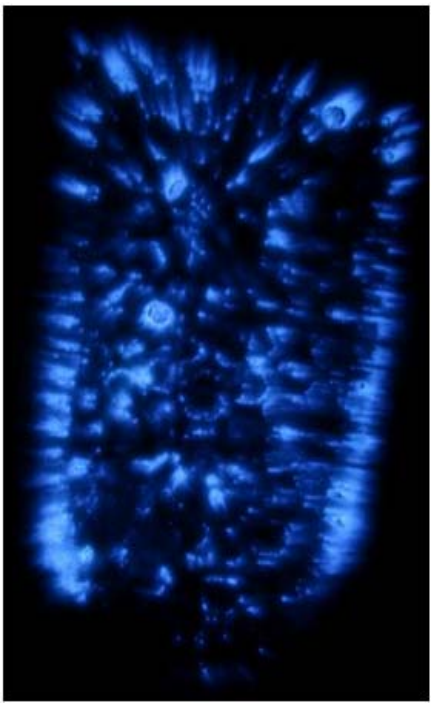


Figure 3.19 - A footwear impression in blood after enhancement with luminol using BVDA sprayer

Due to its fine mist, the Bluestar® Ecospray unit proved to be the only spray suitable for enhancing impressions in blood without diffusion and obliteration of the original impression for luminol (figure 3.19 and figure 3.20) and LCV. This agrees with recent research at CAST where the Bluestar® Ecospray unit was found to be one of the best sprayers for the application of luminol in order to avoid diffusion of the impression in blood [105]. The Ecospray® unit delivered a very fine mist and even repetitive light applications of luminol on the same impressions failed to cause diffusion.

For LMG, the Preval® sprayer provided better enhancement than the Ecospray® unit. This is most likely due to the fact that the fine mist of LMG produced by the Ecospray® unit was not sufficient to produce a vivid colour reaction with blood. It is also postulated that the fine mist delivery of HFE-7100 using Ecospray® vaporised quickly in the fume-hood and hindered the LMG enhancement.

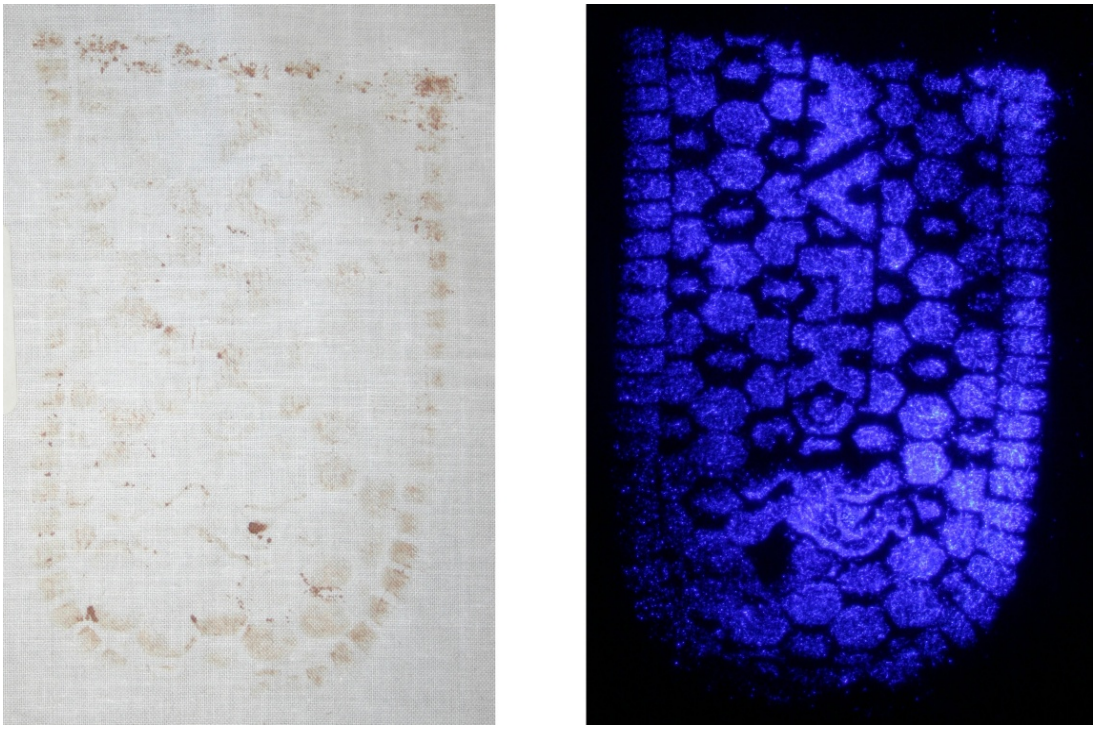


Figure 3.20 - A footwear impression in blood before and after enhancement with luminol using Ecospray®

3.3.2.3 Summary of Preliminary Experiments

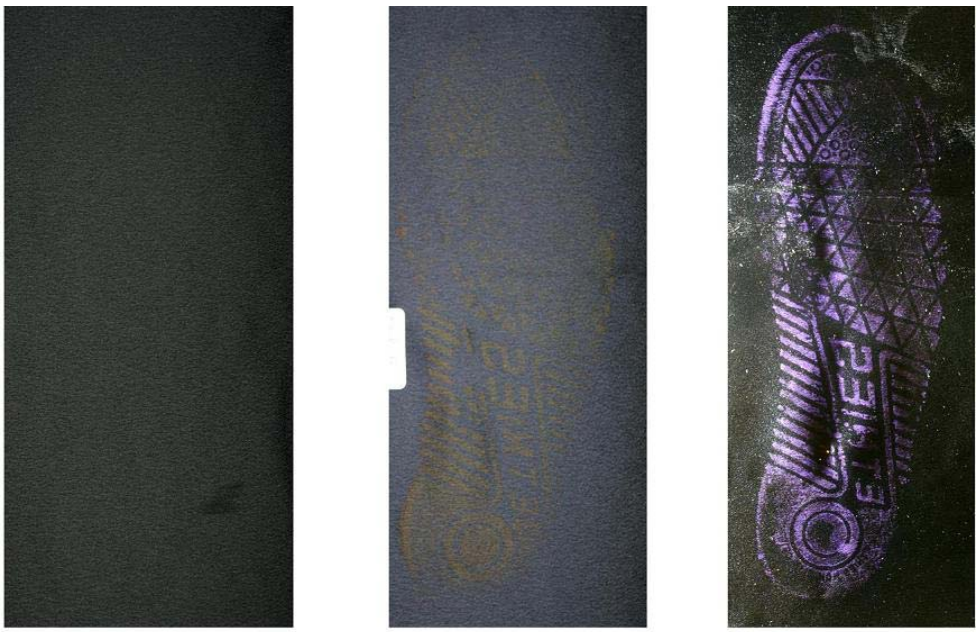
The results obtained from preliminary experiments indicated that were no obvious visual changes in the enhancement of footwear impressions in blood on cotton that were aged 1, 7, 14 and 28 days. As a result, for practical reasons, all footwear impressions in blood on fabrics were aged for 7 days.
Ecospray by Bluestar® Forensic provided the best spraying mechanism and avoided diffusion of the original footwear impression in blood. Consequently, all enhancement techniques that required spraying were applied using Ecospray® with the exception of LMG.

The use of enhancement techniques for the detection of blood do not necessarily imply the presence of blood as the techniques target components such as proteins and amino acids in blood that might be present in other solids or liquids.

3.3.3 Protein stains

All protein stains produced visual colour enhancement on light coloured fabrics. Colour enhancement was minimal on black and dark coloured fabrics due to poor contrast, however, fluorescent stains provided the added advantage of fluorescence. No enhancement was obtained on the dry or wet blanks indicating that the staining was not due to additives in the footwear sole or components in water.

The footwear impression in blood on black polyester exhibited some additional advantages when enhanced with protein stains. Under normal white lighting, the protein stain enhancement was very weak. However, with oblique lighting (Crime-Lite® 80L) the footwear impression could be visualised and is illustrated in figure 3.21. This was not observed for black nylon/lycra and cotton. The use of oblique lighting prior to treatment with protein stains did not provide visualisation as the blood appeared to absorb the lighting.



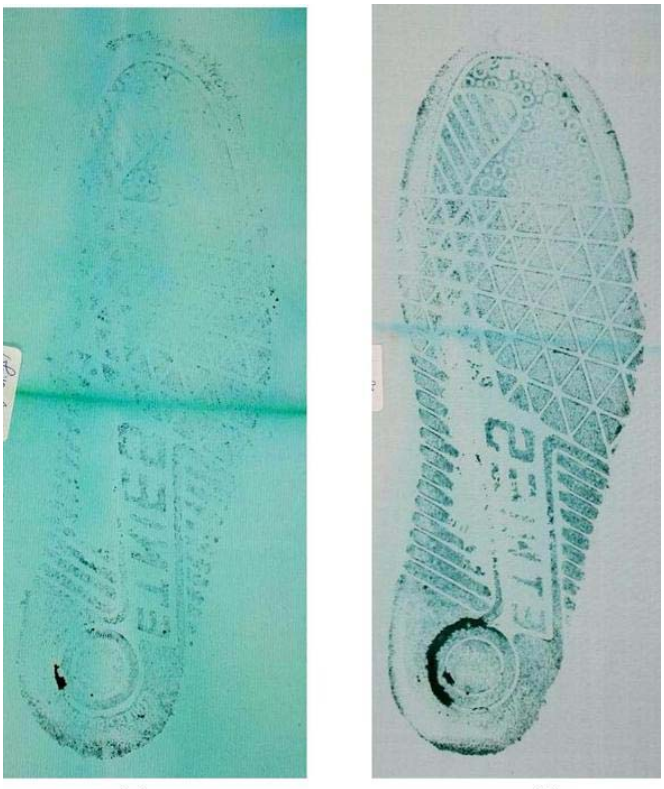
(a) (b) (c)

Figure 3.21 - Enhancement of a footwear impression in blood on black polyester with AV17: (a) impression in blood; (b) AV17 enhancement under white light; (c) AV17 enhancement observed with oblique lighting only

When the black polyester fabric was immersed in the fixing solution of 5-sulfosalicylic acid, the impression in blood could be visualised as shown in figure 3.22. The protein stains washed off easily from synthetic fabrics when compared to the natural fabrics (figure 3.23). This can be explained by the fact that some synthetic material are hydrophobic in nature and will repel acidic dyes [106]. The interaction of the blood with the fabric presumably affects the retention of the impression in blood on the materials surface, however, it does not seem to affect the enhancement technique. The success of the technique appears to be governed by the nature of the fabric itself. Section 1.4.8 may explain some of the difference observed as SEM results suggested that blood interacts differently with the various fabric surfaces. For example, blood appeared to dry over cotton surfaces but for nylon/lycra fibres the blood appeared to penetrate into the fibre matrix. This different interaction may explain the different destaining observed on these two fabrics (figure 3.23) where the procedure was more efficient on nylon/lycra. This however, does not explain the efficient destaining procedure on polyester where blood appeared to dry over the surface in a similar manner to cotton. The low porosity of nylon/lycra and polyester in this study do however reflect an efficient destaining procedure compared to the

high porosity values and inefficient destaining of cotton fibres. Processing synthetic fabrics with protein stains when folded resulted in partial transfer of the impression (figure 3.24).

Figure 3.22 - Visualisation of a footwear impression in blood on black polyester during fixation



(a) (b)

Figure 3.23 - Enhancement of a footwear impression in blood with ABlu1: (a) white cotton; (b) white nylon/lycra

Figure 3.24 - Enhancement of a footwear impression in blood on white polyester with AV19

Footwear impressions produced on leather produced a faint indentation of the footwear sole in the fabric material and the blood footwear impression was clearly visible when compared with similar impressions on the other fabrics. Enhancement of impressions in blood on leather with protein stains appeared to obliterate the impression as the stain did not wash off during the destaining procedure (figure 3.25). This may be due to the interaction of the blood with the complex protein nature of bovine leather as illustrated in figure 1.4e (pg 21). ABlu1 was the only protein stain that washed off slightly during the destaining procedure on leather, however, the staining process still obliterated the original impression.

(a) (b)

Figure 3.25 - Enhancement of a footwear impression in blood on leather with AB1: (a) before; (b) after

3.3.3.1 Fluorescent Protein Stains

In general fluorescence did not further enhance impressions on light coloured fabrics and in some circumstances the bright background fluorescence of the white fabrics, due to optical brighteners, obscured the impression [107, 108].

Fluorescence of AY7 enhanced impressions in blood on dark coloured fabrics demonstrated excellent results when examined using either a Foster and Freeman Crime-Lite® 2 with a blue excitation source or a Mason Vactron Quaser 40 with a blue excitation source (band pass filter 385-509nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 510nm filter (1% cut-on point). The Crime-Lite® had the added advantage of being small, portable and battery-powered compared to the bulky, heavy Quaser which required a connection to an electrical output. The use of a laser source with a specific excitation wavelength such as a blue laser at 460nm may create a stronger response for the fluorescence of AY7 [109]. Background staining occurred on light, coloured and natural fabrics such as white and patterned cotton after AY7 treatment, however, no background staining was observed on dark-coloured fabrics. Vivid visual yellow enhancement on impressions in blood were was obtained on light coloured fabrics in contrast to previous research by CAST [31] that had concluded that AY7 did not impart any visual colour unless dyeing times were greatly increased. The CAST [31] study had also highlighted the fact that AY7 was unsuitable for porous surfaces such as paper (fabric surfaces were not included in the CAST study) as it was impossible to wash off the dye from the background. However, the opposite was found to be true on fabrics where most of the dye washed off easily. During the destaining procedure, AY7 washed off easily from synthetic fabrics such as polyester when compared to the more porous fabrics such as 100% cotton used in this study, however, the footwear impression was still clearly visible on porous fabrics. CAST confirmed that their current research on blood impression on fabrics and AY7 agree with these results, however, the use of AY7 on paper exhibited extensive background staining. AY7 fluorescence enhancement of impressions in blood on black fabrics, irrespective of their porosity properties and interaction with the blood, provided excellent results (figure 3.26).

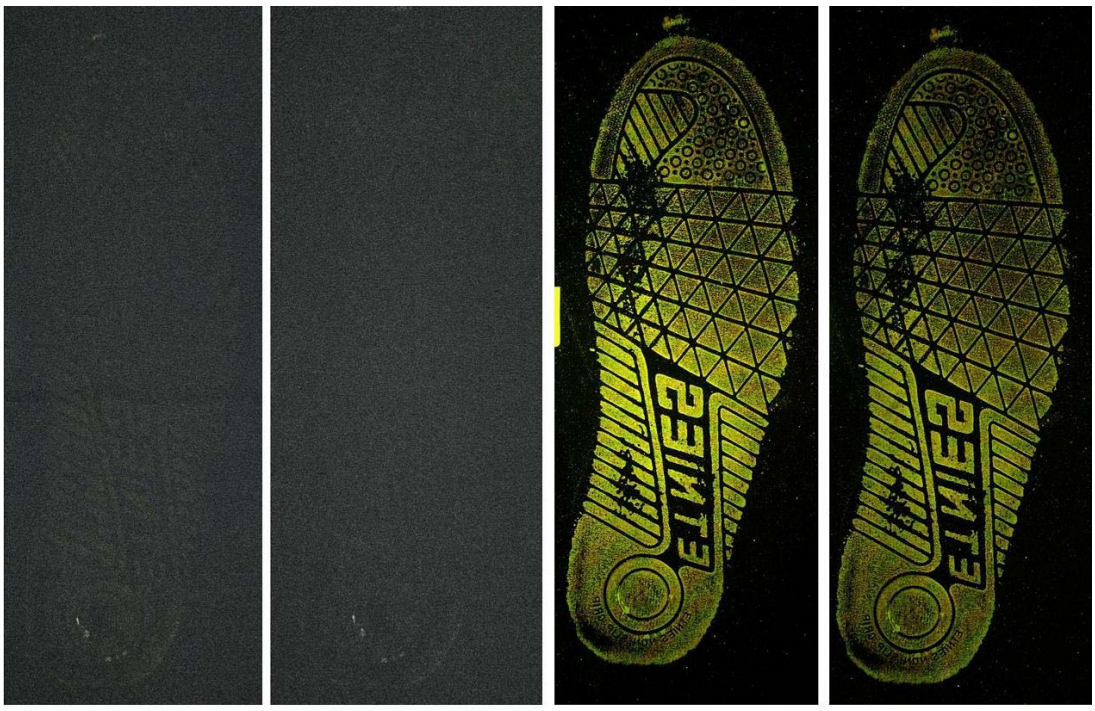


Figure 3.26 – Enhancement of a footwear impression in blood on black nylon/lycra: (a) impression in blood; (b) enhancement with AY7 under white light; (c) AY7 fluorescence using Blue Crime-Lite™; (d) AY7 fluorescence using Quaser 40

The fluorescence observed from enhancement with protein stains AV19, AR52 and SG7 behaved similar to, but not as vivid as AY7, and visual/background staining with SG7 was minimal or non-existent. The use of SG7 and AR52 has not previously been reported in the literature for the enhancement of impressions in blood. Figure 3.27 shows the fluorescence enhancement of footwear impressions in blood on black cotton with AY7, AV19, AR52 and SG7.

AY7 fluorescence on denim and leather was weak compared to the black fabrics. The problem is believed to be because of the dyes used in these fabrics. Chemical treatment with AY7 on denim was repeated using other coloured denim fabrics (blue, dark blue, red, black and grey) and fluorescence enhancement for all these fabrics was still weak suggesting the use of indigo and vat dyes commonly used in dyeing denim [110-114] might interfere with the AY7 fluorescence. Figure 3.28 shows the AY7 fluorescence enhancement of differently coloured denim.

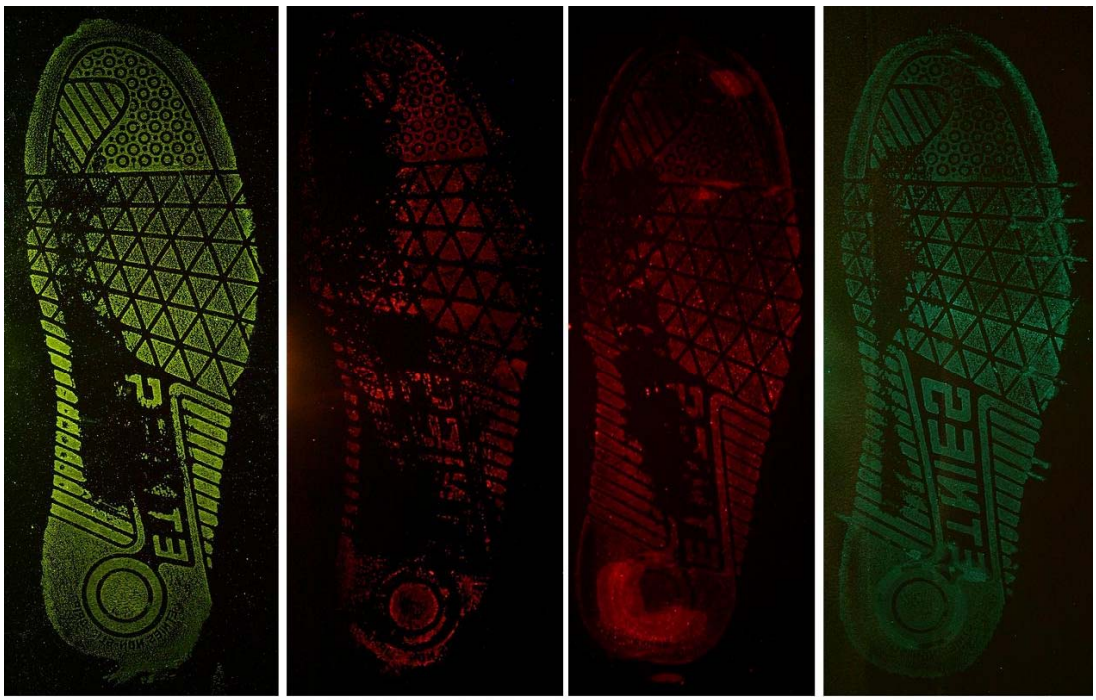


Figure 3.27 - Enhancement of a footwear impression in blood on black cotton with: (a) AY7; (b) AV19; (c) AR52 and (d) SG7

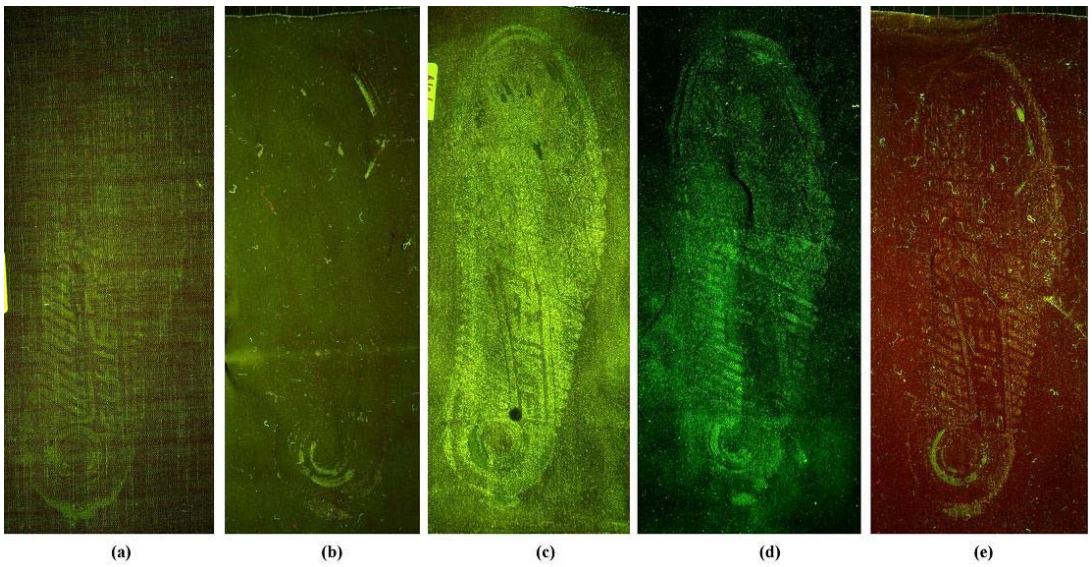
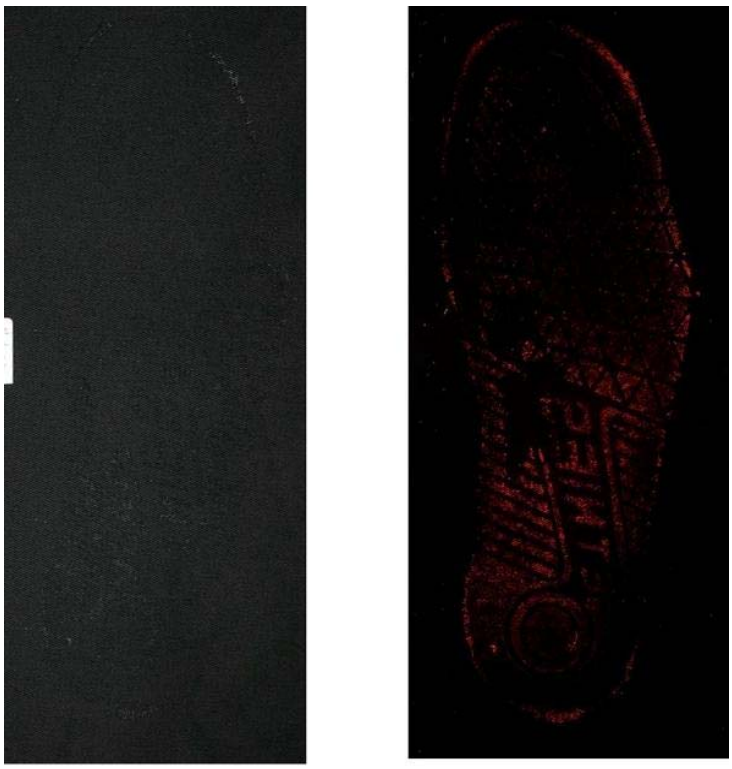


Figure 3.28 – AY7 fluorescence enhancement of footwear impressions in blood on coloured denim: (a) blue; (b) dark blue; (c) grey; (d) black; (e) red

Research carried out by the Dutch Police suggests that AV19 could also be used as a fluorescent protein stain [29, 30]. Their research indicated that lifted impressions in blood lifted with a white gelatin lifter will fluoresce when treated with AV19. Furthermore, a 1:100 dilution of the AV19 solution should provide direct fluorescence without lifting [30]. The gelatin lifter was left on the impression in blood for at least 15-30 minutes before removing [28]. Longer periods of up to two hours were tested but no further improvement in fluorescence was observed. Preliminary studies in this work revealed no enhancement by lifting with a white gelatin lifter after staining with AV19 or other protein stains on any type of fabric. The use of a BVDA gel lifter scanner (GLScan) did not provide any improvement. Diluting the AV19 stain solution by a factor of 100, however, produced a weak fluorescence through excitation with a green light (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 549nm filter (1% cut-on point) (figure 3.29).



(a) (b)

Figure 3.29 - Enhancement of a footwear impression in blood on black cotton with AV19: (a) white light; (b) green light (473-548 nm)

The use of phloxine B (acid red 92) is marketed for enhancement of impressions in blood on dark substrates since the reddish-orange development provides suitable contrast with the dark background. A recent study [115] comparing phloxine B (commercial product) and acid yellow 7 (CAST formulation) concluded that in most cases, AY7 provided better results due to fluorescence which can suppress background contrast issues. As the commercial product was not readily available, phloxine B was prepared using the recommended CAST formulation. The resultant solution was a suspension and not a clear one as with other protein stains. The formulation of phloxine B used in this study did not provide suitable enhancement on dark cotton, polyester, denim or leather. Limited enhancement was observed on light coloured fabrics, however in contrast to other protein stains, phloxine B staining washed off from white cotton but not nylon/lycra (figure 3.30).

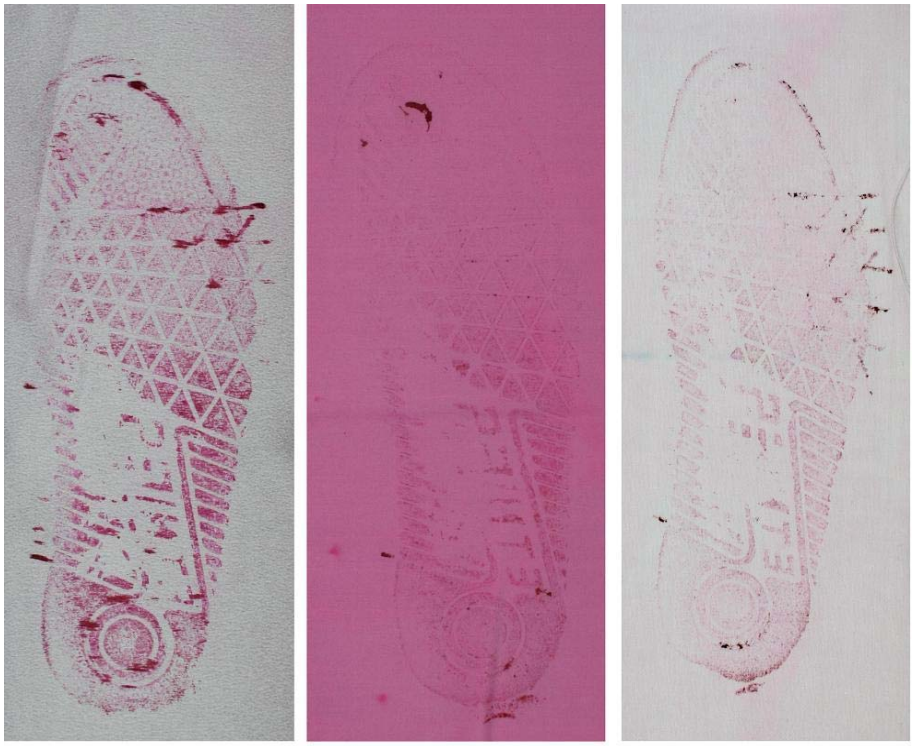


Figure 3.30 – Phloxine B enhancement of footwear impressions in blood on white fabrics: (a) polyester; (b) nylon/lycra and (c) cotton

3.3.4 Peroxidases

3.3.4.1 LCV

The purple enhancement observable when LCV interacts with blood is due to the oxidation reaction of the colourless leuco crystal violet to crystal violet via catalysation by haemoglobin in blood. When exposed to light, the oxidation reaction gradually causes the whole background surface upon which the target impression resides to turn purple [3, 28, 38, 45]. An example of background staining is illustrated in figure 3.31.

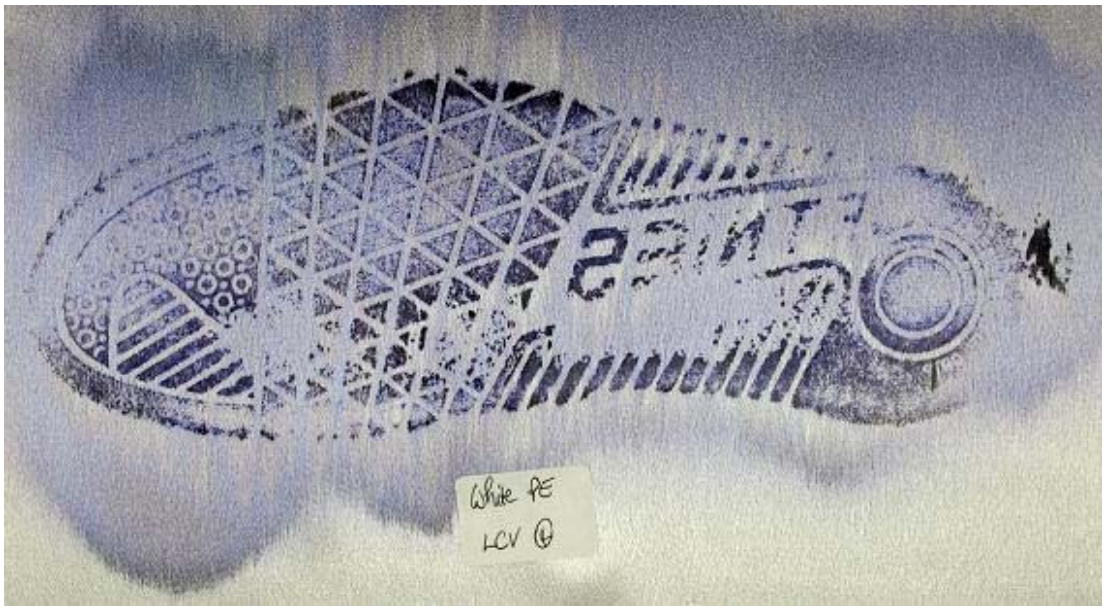


Figure 3.31 – Background staining from LCV application on white polyester after 30 minutes

Background staining can limit the operational use of LCV, however, rapid photography of the impression after the reagent application can offset this limitation. The ease of LCV application (via a spray without the requirement of fixing or destaining) makes it an attractive enhancement technique for operational use.

LCV proved to be a suitable technique for the enhancement of impressions in blood on all light coloured fabrics, including cotton (figure 3.32), polyester, nylon/lycra and leather, but failed to produce consistent enhancement for impressions on denim and dark coloured fabrics. Unsuccessful enhancement was most likely because the fabrics had a blue or dark colour similar to the blue-purple enhancement exhibited by the LCV reaction with blood, limiting the viewing contrast. Contrary to the literature [45, 46], examination of these fabrics using an alternate light source with different excitation filters failed to greatly improve the visualisation of the enhanced impressions produced, except on denim.

Figure 3.32 – Enhancement of a footwear impression in blood on patterned cotton using LCV: (a) before; (b) after

Examination of the LCV treated impressions with a Mason Vactron Quaser 40 green/yellow excitation source (band pass filter 503-591nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 593nm filter (1% cut-on point) revealed weak fluorescence on black fabrics (figure 3.33). Limited fluorescence was observed on leather substrates and fluorescence examination on light coloured fabrics did not improve on what could already be seen visually without fluorescence. The use of a yellow laser (577nm) on black fabrics and leather did not improve the fluorescence observed using the Quaser 40, however, great improvement was obtained on denim as shown in figure 3.34.

The application of oblique lighting (Crime-Lite® 80L) on black polyester did help visualise the enhanced impression slightly. Other problems associated with the use of LCV concern health and safety as crystal violet (the product formed from the reaction of blood and LCV) has recently been upgraded to a category 3 carcinogen [38].

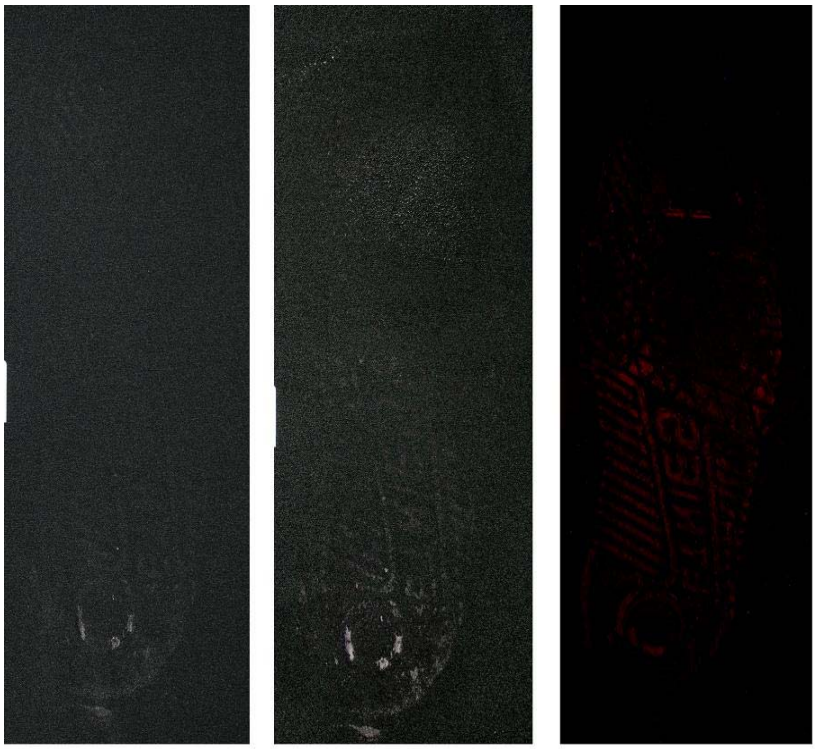


Figure 3.33 - Enhancement of a footwear impression in blood on black nylon/lycra using LCV: (a) before enhancement; (b) under white light; (c) using fluorescence

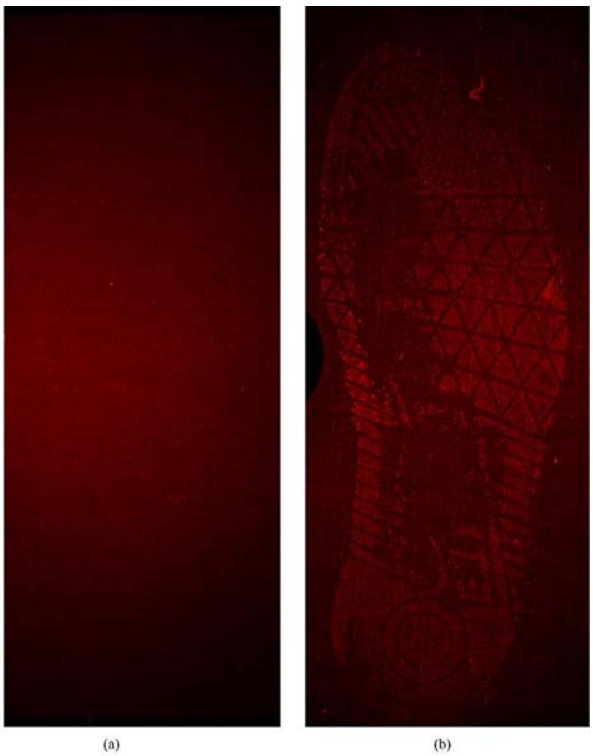


Figure 3.34 - Enhancement of a footwear impression in blood on denim and enhanced with LCV using: (a) Quaser 40 yellow/green excitation source (503-591nm) and (b) yellow laser (577nm)

The human eye is relatively insensitive to dim red light and as a result, weakly fluorescing impressions may be missed unless the eyes are dark adapted for a period of about 30 minutes prior to observation [116].

3.3.4.2 LMG

LMG enhancement of footwear impressions in blood performed similarly to LCV, however, background staining did not occur. Slight diffusion of the enhanced impression, minimised by lighter spraying, occurred on white polyester. Grodsky *et al.* [42] reported that the use of sodium perborate instead of hydrogen peroxide in the LMG formulation provided a great improvement in the reaction. The RCMP formulation [7, 100] for LMG recommends pre-fixing the impressions with methanol. Trials carried out during this study demonstrated that fixing the impression with 5-sulfosalicyclic acid or methanol/ethanol resulted in an blurred enhanced impression with less vivid green colours (figure 3.35a) and best enhancement was achieved by not using a fix (figure 3.35b).

Figure 3.35 - Enhancement of a footwear impression in blood on white cotton using LMG: (a) with fix; (b) without fix

Repeated spraying of the impression also resulted in blurred impressions. For LMG, the Preval® sprayer provided better enhancement than the Ecospray® unit, most likely because the fine mist of LMG produced by the Ecospray® unit was not sufficient to produce the colour reaction with blood. It is also postulated that the fine mist delivery of HFE-7100 using Ecospray® vaporised quickly in the fume-hood to hinder the LMG enhancement.

Enhancement on light coloured fabrics was clear and sharp with a bright vivid green colour (figure 3.36). This is contrary to previous research by CAST [38] where the use of the same LMG formulation (RCMP) was not recommended as the enhancement achieved was described as poor 'with a pale product colour causing problems with background contrast'.

Figure 3.36 - Enhancement of a footwear impression in blood on white cotton using LMG: (a) before; (b) after

The use of oblique lighting on black polyester did not aid visualisation of the LMG enhanced impression as for protein stains and LCV. Enhancement on dark coloured fabrics including denim and leather was not visible or demonstrated poor contrast. LMG enhancement on black cotton and nylon/lycra seemed to be bright at first glance but disappeared rapidly. This observation was more pronounced for denim (figure 3.37) where the green colour faded within approximately 30 seconds post application of the reagent. Repeated application of LMG failed to re-enhance the impression, even after drying.

This particular formulation for LMG must be prepared just prior to use. However, this formulation is easier to prepare compared to previous ones which involved the addition of zinc with refluxing or boiling [40, 43]. Recent research [38] evaluated the use of the acidic leuco dyes such as leuco patent blue (LBP), leucoberbelin blue (LBB) and leuco xylene cyanole (LXC). All dyes performed well on the enhancement of impressions in blood with LBP demonstrating the best sensitivity with a vibrant colour but was also the most expensive.

(a) (b)

Figure 3.37 - Enhancement of a footwear impression in blood on denim using LMG: (a) before; (b) after

3.3.4.3 Leuco Rhodamine 6G (LR6G)

Leuco rhodamine 6G (LR6G) was prepared by reduction over zinc of the commercially available rhodamine 6G as described by Yapping [79]. LR6G is oxidised to rhodamine 6G (R6G) through a reaction with the heme in haemoglobin to produce a red colour. Yapping [79] failed to mention two important issues surrounding the use of this technique. Firstly, zinc poses a flammability hazard and needs to be neutralised prior to disposal. Secondly, rhodamine 6G fluoresces when excited with the appropriate alternative lighting. Fluorescence can assist to improve the contrast of the enhanced impressions on dark substrates.

Enhancement on light coloured fabrics provided little further enhancement on the visual impression, although some extra detail could be seen (figure 3.38). Additionally, fluorescence did not greatly improve the visual enhancement. Similar to LCV, quick photography was necessary to capture the enhancement prior to overall staining of the background occurring over time.

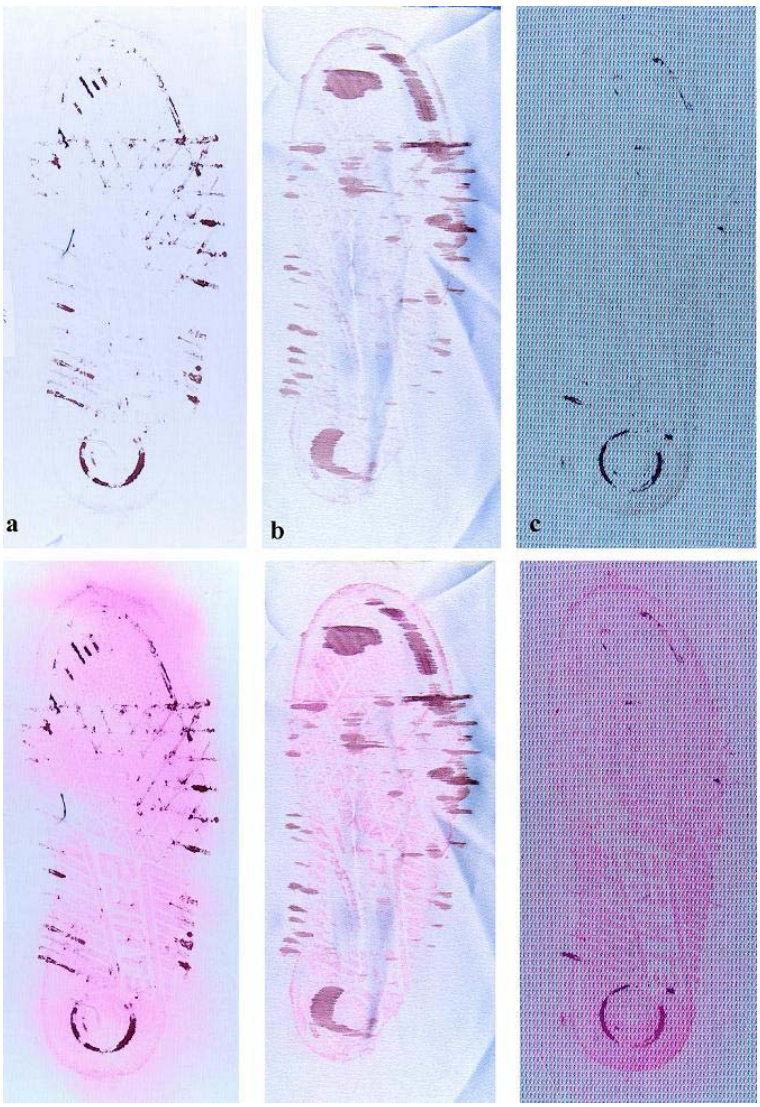


Figure 3.38 – Enhancement of a footwear impression in blood using LRG6, before (top) and after enhancement (bottom): (a) white cotton; (b) white polyester; (c) patterned cotton

Visual enhancement on dark coloured fabrics with LRG6 was limited or non-existent, however the use of a Mason Vactron 40 provided suitable fluorescence enhancement using a blue/green excitation filter (band pass filter 468-526nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 529nm filter (1% cut-on point). The fluorescent enhancement is illustrated in figure 3.39.

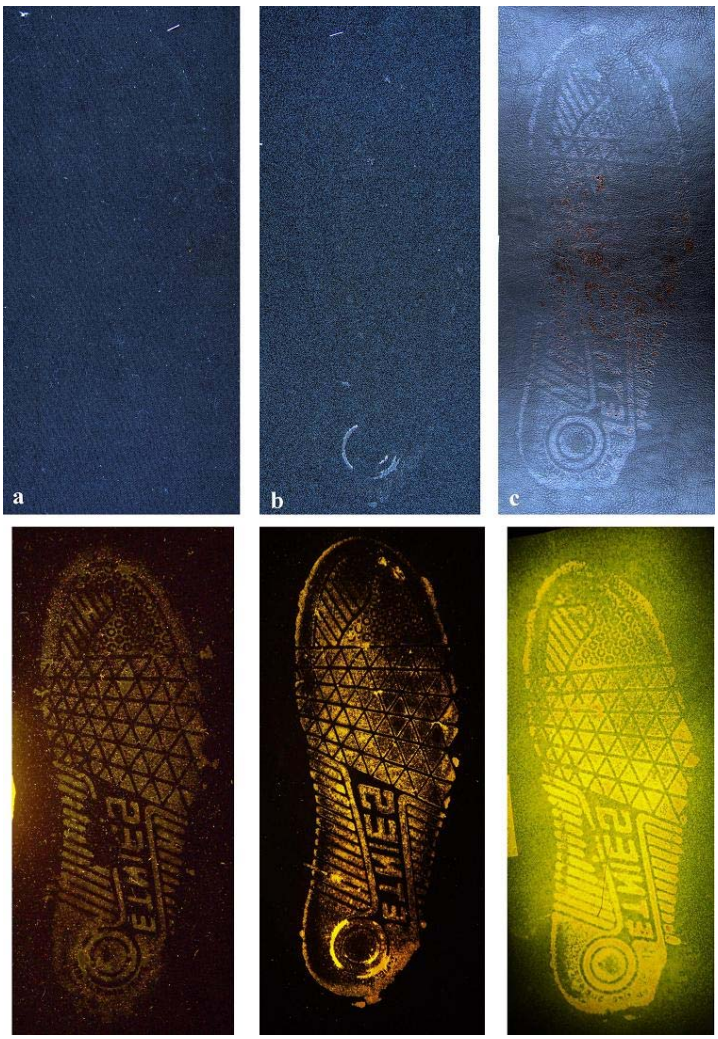
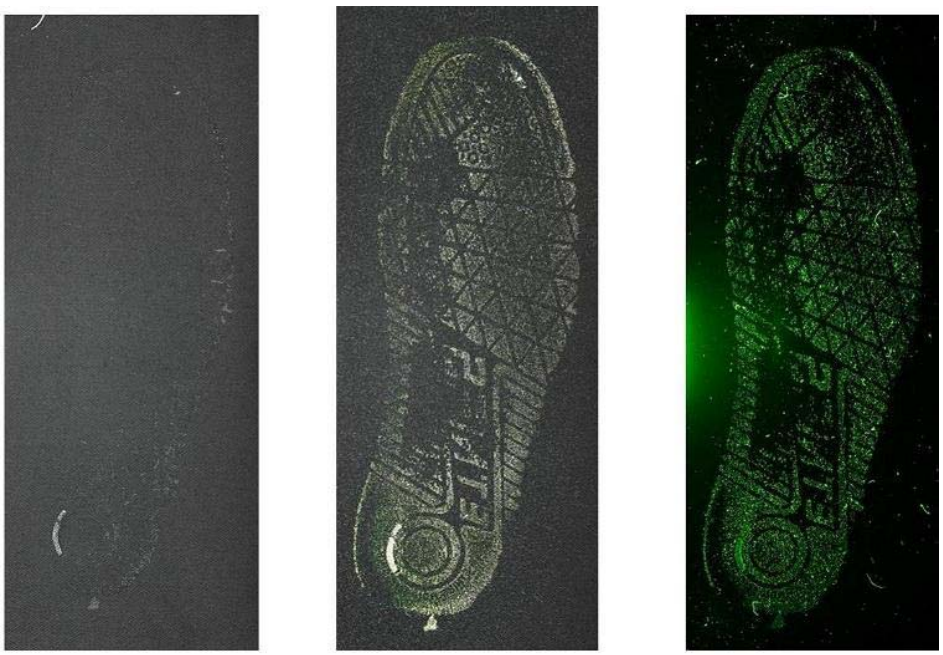


Figure 3.39 - Enhancement of a footwear impression in blood using LRG6 fluorescence, before (top) and after enhancement (bottom): (a) black cotton; (b) black nylon/lycra; (c) leatherette

3.3.4.4 Fluorescein

Fluorescein was applied via a two-step method using Ecospray®. The reagent preparation involves the reduction of fluorescein to fluorescin over zinc. Fluorescin, in turn is oxidised to the coloured fluorescein in the presence of blood where the heme and hydrogen peroxide act as catalysts.

Fluorescein enhancement was poor on light coloured fabrics and, by contrast, excellent on dark coloured fabrics. The colour reaction was visible almost instantaneously on the dark fabrics and on leather (figure 3.40), eliminating the requirement for an alternate light source.

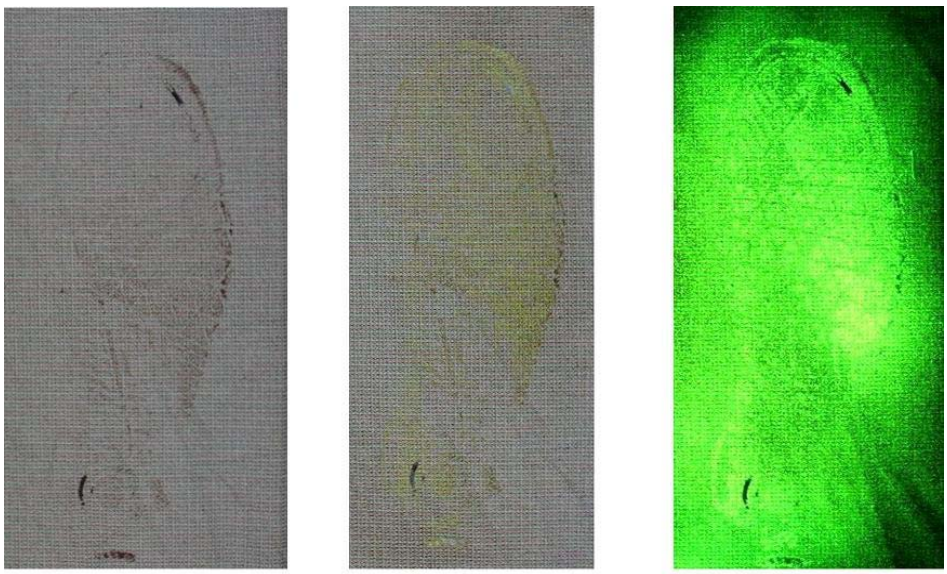


(a) (b) (c)

Figure 3.40 - Enhancement of a footwear impression in blood on black cotton using fluorescein : (a) before enhancement; (b) under white light; (c) using fluorescence

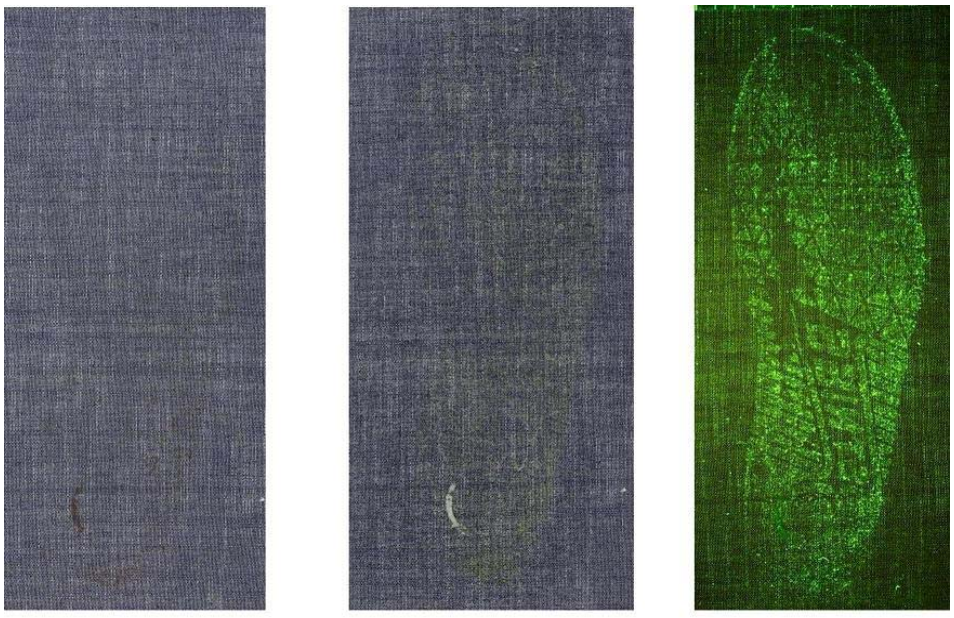
The visual enhancement on denim was poor, though fluorescence did aid visualisation of the impression on this fabric. Quick capture of the enhanced impressions using photography was essential as the bright yellow colour produced on application of the reagents began to fade after a few minutes. For example, the enhancement developed on black polyester faded almost completely after about 15 minutes.

Background staining was observed on white cotton, polyester and nylon/lycra as well as patterned cotton which interfered with the fluorescence and is illustrated in figure 3.41. A tinge of pink also developed with the background staining on white synthetic fabrics. Furthermore, the instantaneous colour reaction on light coloured fabrics was difficult to observe. Contrary to Budowle [72], no fluorescein background staining on denim was observed, either initially or over time (figure 3.42). On dark coloured fabrics the use of a Quaser 40 to observe fluorescein fluorescence provided optimal contrast for visualisation of the footwear impression.



(a) (b) (c)

Figure 3.41 - Enhancement of a footwear impression in blood on patterned cotton using fluorescein: (a) before enhancement; (b) under white light; (c) using fluorescence



(a) (b) (c)

Figure 3.42 - Enhancement of a footwear impression in blood on denim using fluorescein: (a) before enhancement; (b) under white light; (c) using fluorescence

3.3.4.5 Hemascein®

Hemascein® is a commercially available product using the fluorescein enhancement technique. It avoids the reduction reaction using flammable zinc, thus reducing the risk in the laboratory and makes the product ideal for crime scene use. The product comes supplied with two ABAsprays® for mist spraying of fluorescein and hydrogen peroxide. On application of the product with these sprayers, it was immediately noticed that the mist was less fine than the Ecosprayer supplied by Bluestar®. Diffusion was not only present on synthetic fabrics but also on natural fabrics such as cotton, however, the extent of diffusion on natural fabrics was less pronounced (figure 3.43). The fluorescence examination was carried within 10 minutes of chemical application, however, the fluorescence observed was either minimal or non-existent. The re-application of reagents provided some additional enhancement but increased the diffusion already present.

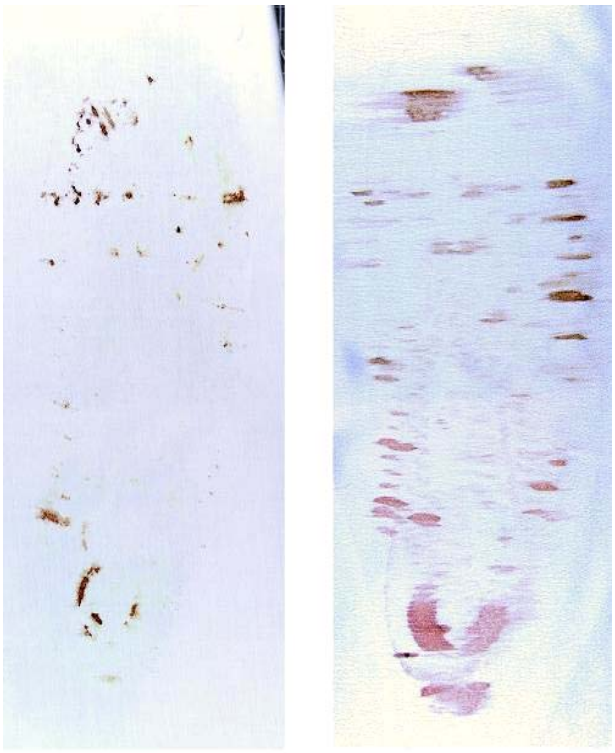


Figure 3.43 – The diffusion observed after treatment with Hemascein® using ABAsprayers®: (a) white cotton and (b) white polyester

No visual enhancement was observed on any of the types of fabrics utilised in the study. Fluorescence was weak or non-existent and re-application of the chemicals did not provide useful additional enhancement. The best fluorescent enhancement was observed on patterned cotton using the Ecosprayer (figure 3.44).

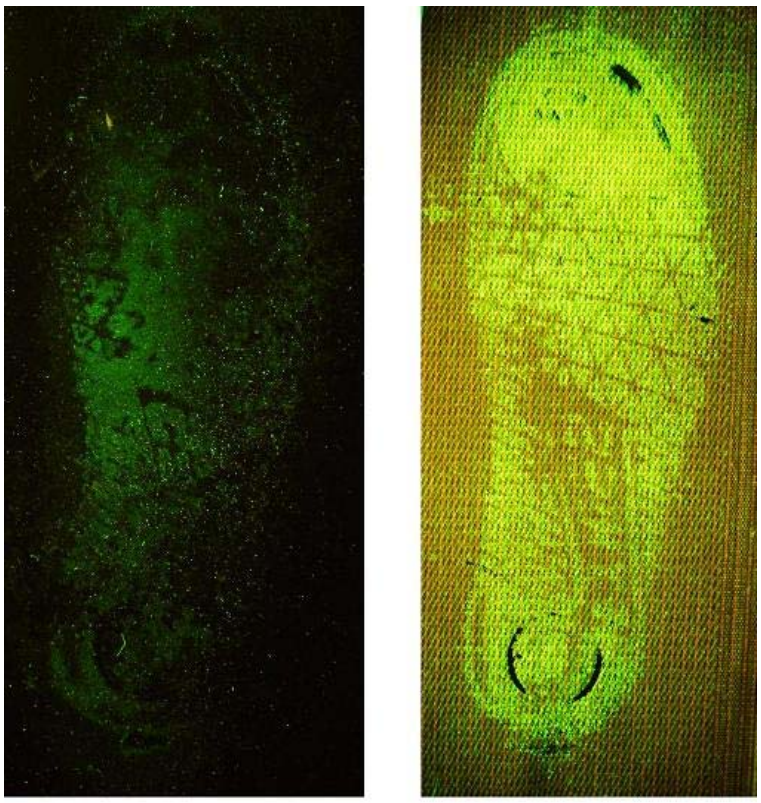


Figure 3.44 - Enhancement of a footwear impression in blood using Hemascein®: (a) black nylon/lycra; (b) patterned cotton

Although the use of Hemascein® might remove the flammable hazard involving the preparation of fluorescein, it is clearly less effective than the fluorescein reagent prepared from raw chemicals. The sprayers supplied with Hemascein® did not provide a fine mist suitable for the enhancement on impressions on fabric. The use of Ecosprayers® provided a finer mist to avoid diffusion.

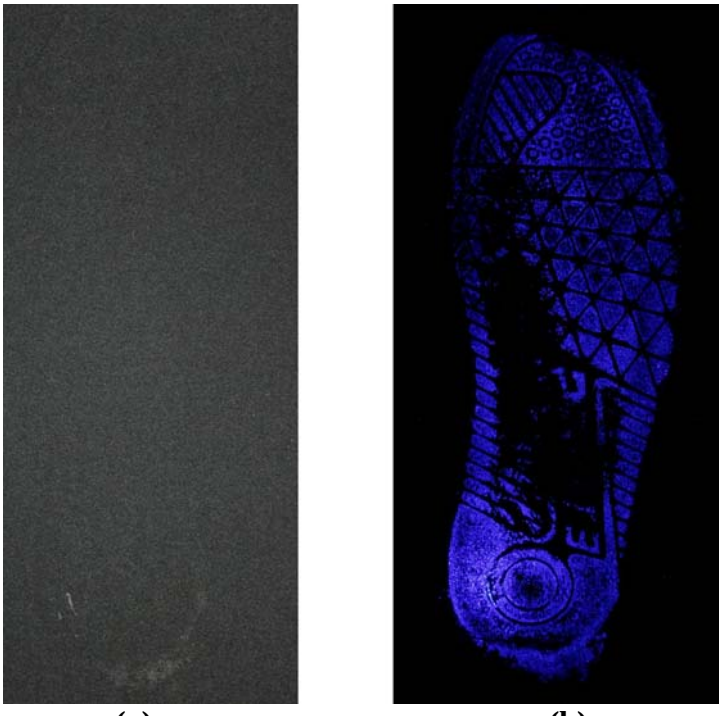
3.3.4.6 Luminol

Luminol proved to be the only technique successful in the enhancement of footwear impressions made in blood irrespective of fabric type or colour used in this study. Several studies [62, 69, 71, 73] have compared luminol and fluorescein with mixed views on which technique is the most effective. Each have their advantages and disadvantages, however, in this study luminol performed better overall with fluorescein providing slightly less diffusion on black synthetic fabrics. No background staining was observed with the application of luminol on leather samples even though the reagent is reported to react with chromium and cobalt [117-119] often used in the tanning process of leather. Previous work by CAST [31, 38] had concluded that peroxidase reagents are not suitable for the enhancement of fingerprints in blood as the minute details are not preserved, but could work very well with the enhancement of footwear impressions in blood. In 1951, Grodsky *et al*. [42] reported the sensitivity of luminol as potentially detecting blood in a dilution of 1:5,000,000. On porous surfaces, luminol sensitivity was recently reported at 1:10,000 [38] and fluorescein sensitivity at 1:100 [72]. Additional advantages of luminol over fluorescein include the ease of preparation, a one-step spraying process and the fact that no alternative light sources are required.

The strongest enhancement on denim was achieved using luminol as illustrated in figure 3.45. A commercially available luminol marketed as Forensic Bluestar® Magnum was used. The application of luminol on the synthetic fabrics polyester and nylon/lycra exhibited slight diffusion, however, lighter spraying helped minimise this effect (figure 3.46).

(a) (b)

Figure 3.45 - Enhancement of a footwear impression in blood on denim using luminol: (a) before; (b) after



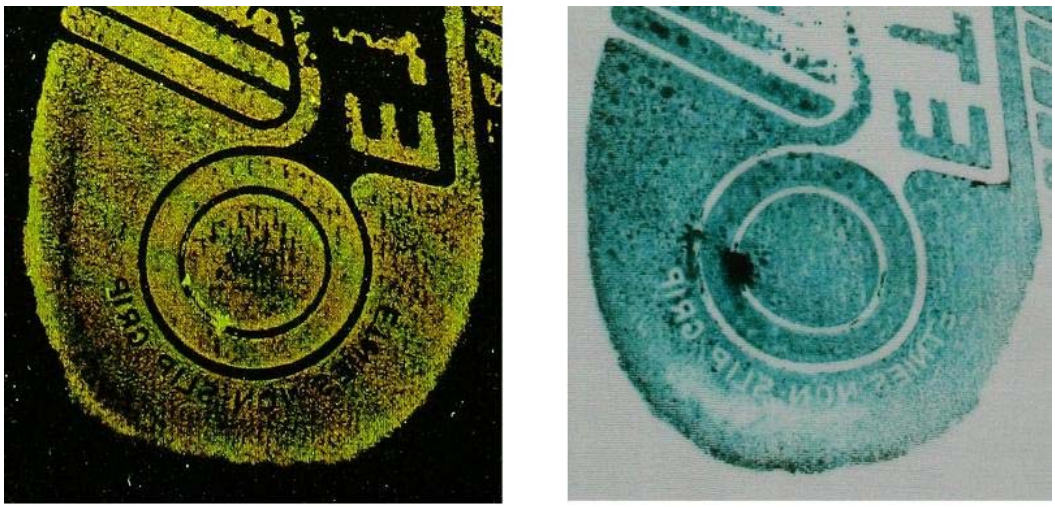
(a) (b)

Figure 3.46 - Enhancement of a footwear impression in blood on black nylon/lycra using luminol: (a) before; (b) after

The main advantage of luminol remains its sensitivity. A recent study [63] suggested that false positives can be obtained when using luminol on household products (e.g. oil-based paints, alkyd varnish), food products (e.g. leek, ginger, carrot) and chemical products (e.g. $CuSO_4$, $FeSO_4$). Nonetheless, the problem of false positive might not be relevant if the purpose of using the product is to search for footwear impressions rather than blood itself. This report also found that the ions Cu^{2+}, Fe^{2+} and Mn^{2+} catalyse the chemiluminescence reaction of luminol whereas SO_4^{2-} does not. Bluestar® luminol, when compared to other formulations of luminol, is reported to have brighter and longer chemiluminescence and is easier to prepare, visualise and photograph. Luminol can potentially be used before protein staining and other heme-reacting dyes. In general, the original impression remained visually unaffected by luminol and there was no permanent colouration.

3.3.5 Protein Stain and Peroxidases Enhancement Comparison

Due to the nature of the fabric and its interaction with blood, recovery of fine detail on the footwear sole was not always possible. This also depended on the dynamics of the impression as it was made where the blood appeared to rest on the surface on most fabrics, except for nylon/lycra. This interaction appeared to prevent individual characteristics from being recovered, however, other detail could be observed after enhancement. Figure 3.47 demonstrates exemplars of fine detail enhancement observed using a protein stain (acid yellow 7) and a peroxidase reagent (leucomalachite green). This type of fine detail enhancement was not always possible due to the nature of the fabric or the chemical technique. The fine detail on the sole read as a mirror image: “ETNIES NON-SLIP GRIP”.



(a) (b)

Figure 3.47 – Detail of the footwear sole revealed by enhancement: (a) acid yellow 7 on black cotton and (b) LMG on white nylon/lycra

3.3.6 Amino Acid Staining

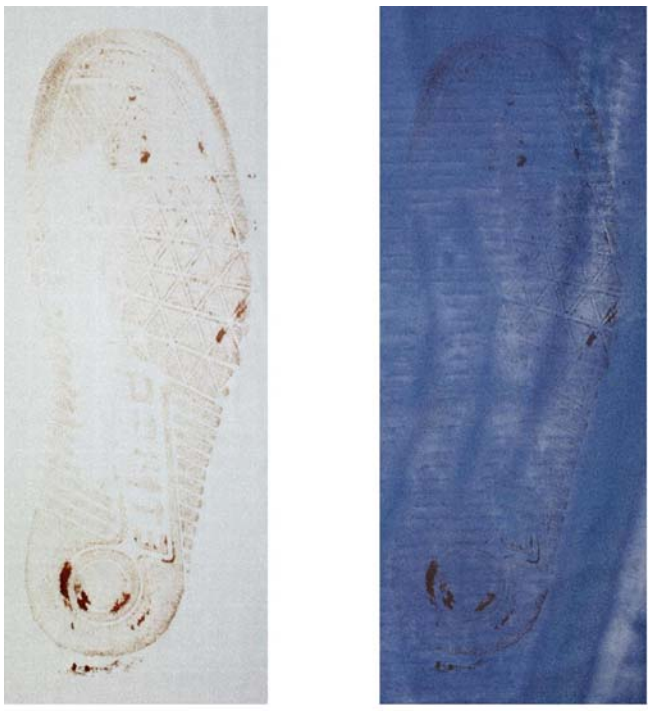
3.3.6.1 Ninhydrin

Footwear impressions in blood on dark coloured fabrics and enhanced with ninhydrin did not demonstrate particularly good contrast with the background. The enhancement achieved on light coloured fabrics, however was better and is illustrated in figure 3.48.

The background staining on nylon/lycra, illustrated in figure 3.49, covered the whole fabric, obliterating the original impression in the process. Background staining on other light-coloured fabrics such as cotton and polyester was not as pronounced as that observed on nylon/lycra. In some instances, the bright purple colour from ninhydrin enhancement appeared to be brighter after up to 24 hours. As a result, observation of articles treated with ninhydrin should be checked within 24 hours of development. The enhancement of footwear impressions in blood on denim using ninhydrin was very weak due to the low contrast between the blue colour of denim and ninhydrin's Ruhemann complex with the amino acids in blood.

(a) (b)

Figure 3.48 - Enhancement of a footwear impression in blood on white cotton using ninhydrin: (a) before; (b) after



(a) (b)

Figure 3.49 - Enhancement of a footwear impression in blood on white nylon/lycra using ninhydrin: (a) before; (b) after

The treatment of articles with ninhydrin is quick and straight-forward. Drying times are very short since HFE-7100 is very volatile. However, HFE-7100 is an expensive solvent at and the procedure requires treatment of articles in an oven that can control humidity and temperature which can be costly.

3.3.6.2 DFO and 1,2-indanedione

Very weak or no enhancement of impressions in blood on fabric was achieved using DFO as demonstrated in figure 3.50. The application of DFO was similar to ninhydrin with the use of a dry oven. DFO is more expensive than ninhydrin and required the use of two expensive solvents: HFE-7100 and HFE-71DE, however, it has the potential advantage of fluorescence. The literature suggests that DFO only reacts with minute traces of blood [4, 5]. To test this hypothesis, a diminishing series of footwear impressions in blood was prepared on white and patterned cotton. Again, no enhancement (visual or fluorescent) was achieved. Similar to DFO, no enhancement of impressions in blood on fabric was obtained with 1,2-indanedione.

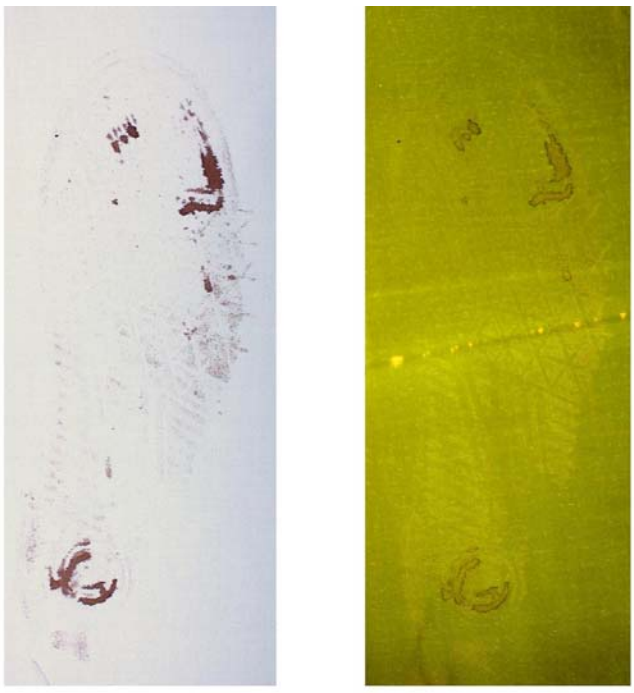


Figure 3.50 - Enhancement of a footwear impression in blood on white cotton using DFO: (a) before; (b) after

3.3.7 Alginates

The alginate from GC, *Aroma Dust Fine III Regular Set*, gave the best overall results and revealed the best contrast for the lifted impression. It also produced an impression with the greatest detail and consistency and the least amount of damage to the original impression. This is similar to the findings of the Israeli Police [92]. *Blueprint Cremix* from Dentsply provided good impression lifting, however, destroyed the original impression. The Henry Schein alginate gave poor results when compared to the other alginates. During the tests, some seepage of water occurred into the fabric damaging the impression. One solution to this problem was to mix the water with alginate for a longer period of time, however, because of the fast setting time of alginates this was not always possible. A preliminary comparison of the three alginates for the recovery of footwear impressions in blood on white cotton is illustrated in figure 3.51. The results obtained suggested that the GC alginate might be a suitable method for the lifting and post-enhancement of footwear impressions in blood on dark surfaces (figure 3.52).

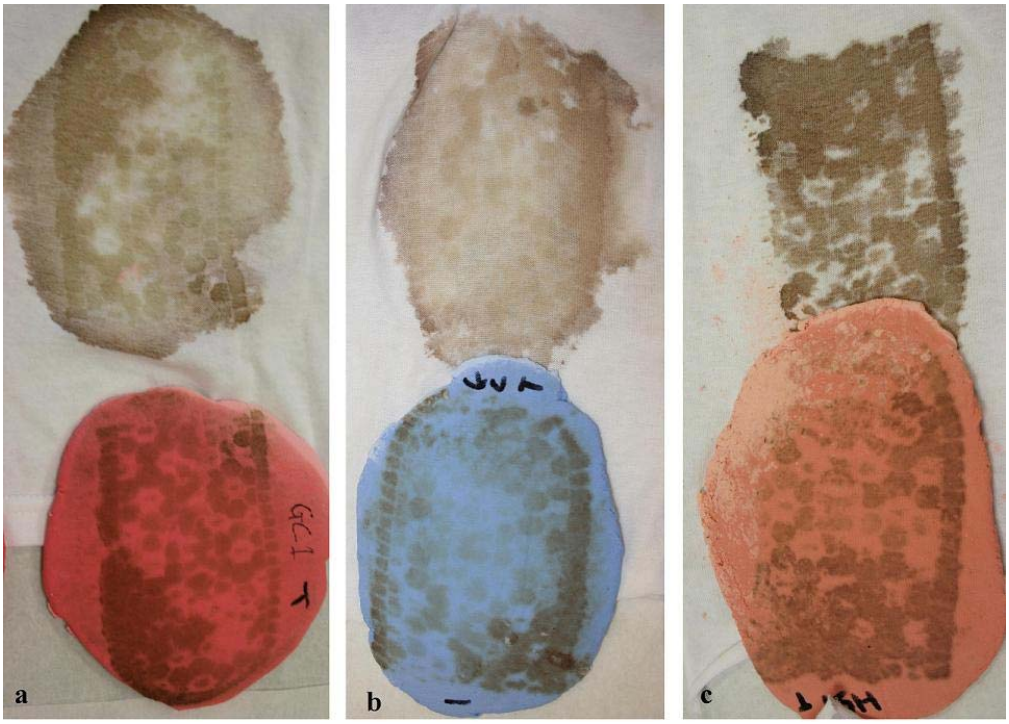


Figure 3.51 – A comparison of three alginates for the recovery of footwear impressions in blood: (a) GC Aroma Dust Fine III; (b) Blueprint Cremix; (c) Henry Schein

Figure 3.52 - Lifting of impressions in blood with GC alginate on denim

Although the use of alginates for lifting blood impressions on clothing was investigated by Adair [90] and Wiesner [92], neither study mentioned the fast shrinking rate of alginates. The footwear impression detail on the alginate could be compromised due to shrinkage of the alginate as the water was lost during the drying process. Photography was carried out right after lifting the alginate and after drying overnight (16 hours) where it was observed that the effect of shrinkage overnight was negligible. In general the alginate lift decreased by about a third after 72 hours. It is important to note that the lifted impression is a mirror image of the original impression. Figure 3.53 reveals loss of detail from an alginate cast after three weeks.

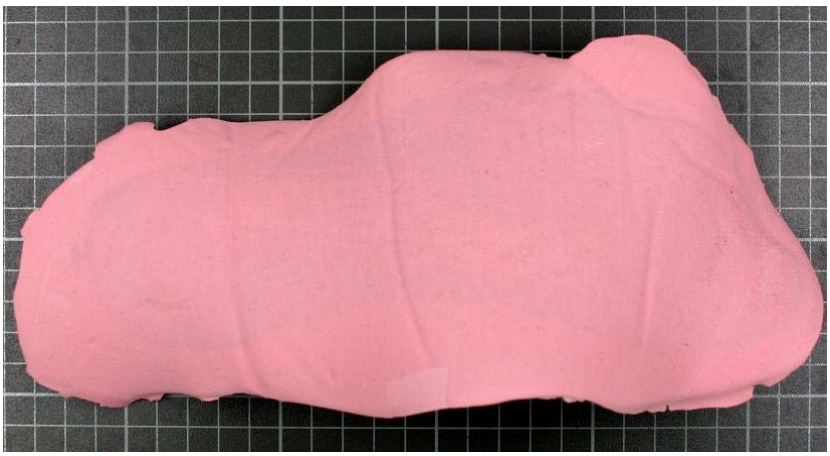


Figure 3.53 – Loss of footwear impression detail during alginate shrinkage

Although, the alginate cast has been reported to leave the impression in blood visually unchanged [90, 92], the water present in the paste seemed to affect the porous fabric. Some seepage of water occurred from the alginate mix into the fabric causing damage to the impression and the fabric seemed to remain 'permanently wet' after the alginate lift, although this effect was also observed on the less porous fabric nylon/lycra.

The trials discussed in table 3.6 were performed using GC alginate as the best performing alginate. The best enhancement results obtained by post-enhancement occurred with acid black 1 (AB1) (figure 3.54 and figure 3.55). Post enhancement with leuco crystal violet (LCV) provided good enhancement of the impression recovered by the alginate (figure 3.56), however, the sharpness and colour brightness achieved seemed to be inferior to that obtained using AB1. This difference can be explained by the fact that treatment with AB1 involves the fixation process separately before staining whereas LCV treatment uses the fix and staining in a one-step process.

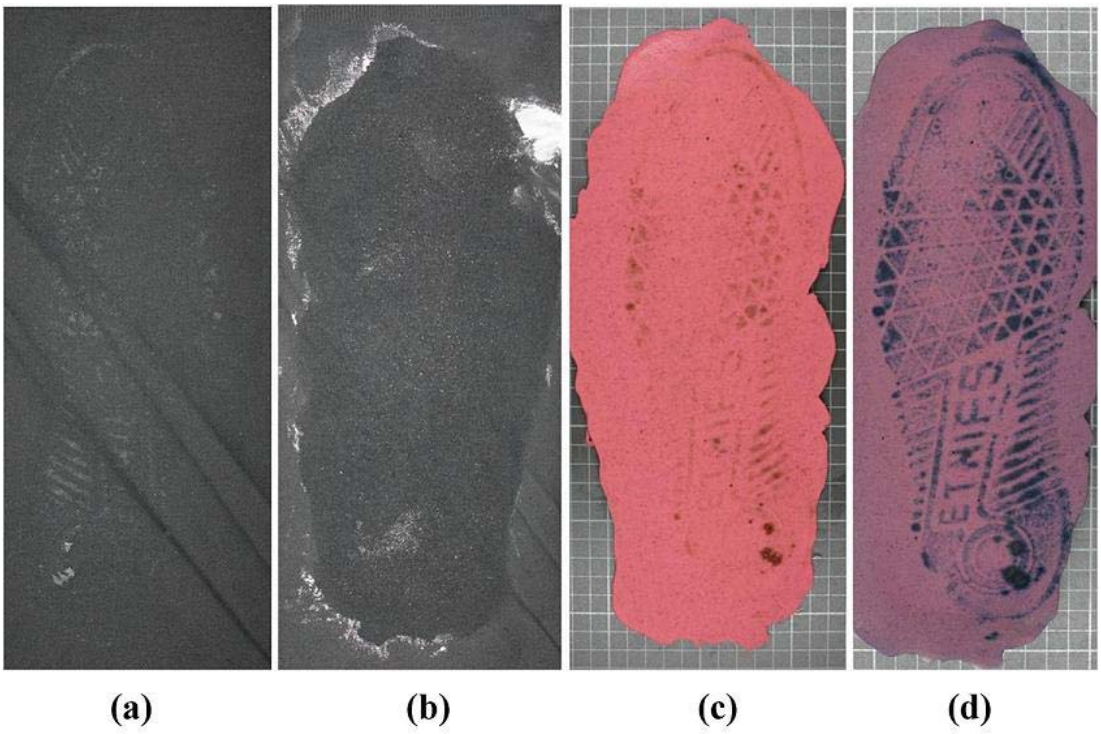


Figure 3.54 – Enhancement of a footwear impression in blood on black cotton using alginate and AB1: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with AB1

Figure 3.55 – Enhancement of a footwear impression in blood on denim using alginate and AB1: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with AB1

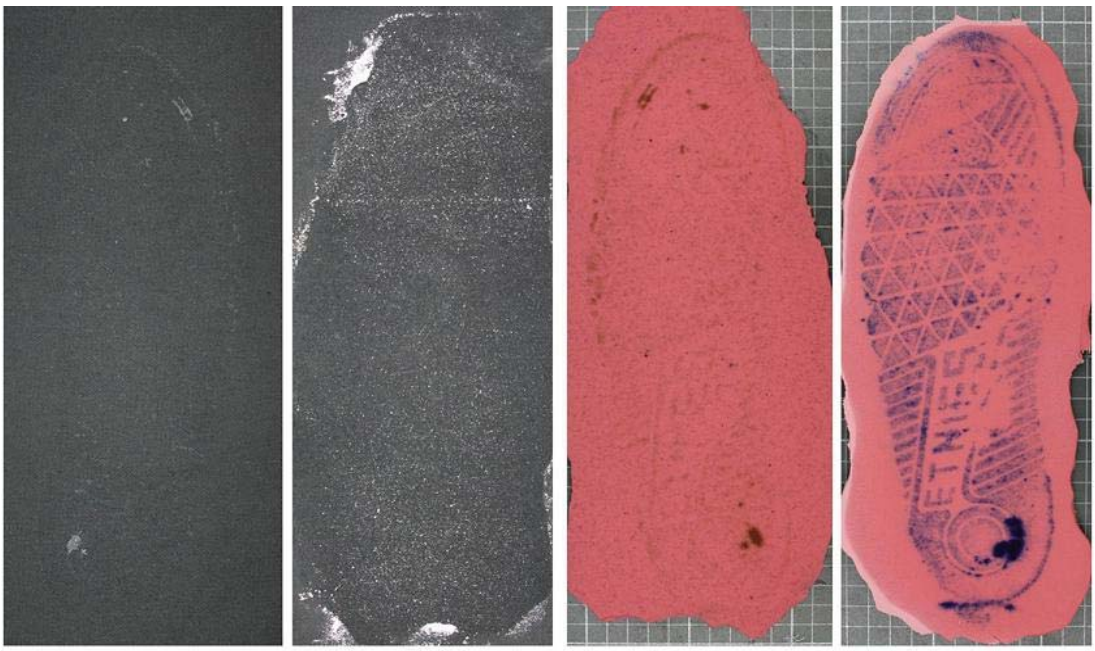


Figure 3.56 – Enhancement of a footwear impression in blood on black cotton using alginate and LCV: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with LCV

The use of alginate lifting followed by chemical enhancement provided an excellent technique for the enhancement of impressions on dark fabrics and denim due to an improved contrast with the background.

After alginate lifting, the fabrics were treated with a protein stain (acid yellow 7), a heme-reagent (luminol) and an amino acid staining technique (ninhydrin). None of these techniques enhanced the residual blood impression remaining on the fabric surface, indicating the interference of alginate with the blood impression. Luminol gave a weak and rapidly fading chemiluminescence.

3.3.8 Powder Suspensions

Powder suspensions are not normally recommended for the enhancement of impressions on porous substrates due to background staining [37]. This assertion was tested using both black and white fabrics (cotton, polyester and nylon/lycra).

3.3.8.1 White Powder Suspension

The formulation of white powder suspension used in this study was that suggested by Bergeron [81]. Methanol was replaced by ethanol (less toxic and flammable than methanol) for the preparation of titanium dioxide suspension with poor results. A methanol formulation was thus used followed by rinsing of the impression with methanol. Slight enhancement was observed on black cotton, however, in most instances the enhancement was poor. The best enhancement was observed on black polyester and no enhancement was achieved on black nylon/lycra due to background staining as illustrated in figure 3.57.

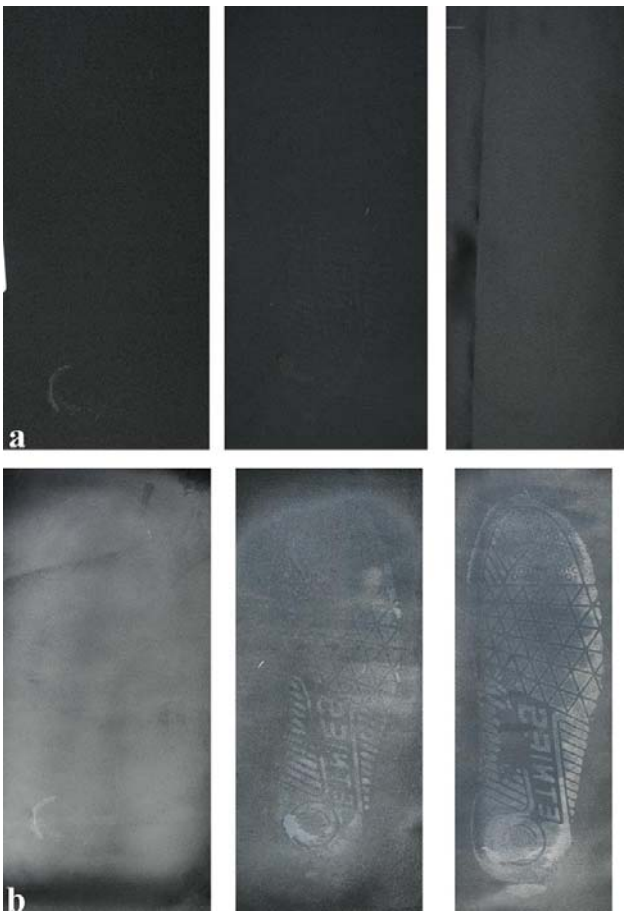


Figure 3.57 – The enhancement of footwear impressions in blood with white powder suspension on black nylon/lycra, cotton and polyester: (a) before enhancement; (b) after enhancement

3.3.8.2 Black Powder Suspension

The black powder suspension formulation, prepared as recommended by CAST, was utilised on white fabrics. The formulation proved difficult to rinse out from the background on cotton but rinsed almost completely from polyester and nylon/lycra. The subsequent background staining on white cotton obliterated the original footwear impression whereas the enhancement on both white polyester and nylon/lycra were easy to observe as illustrated in figure 3.58.

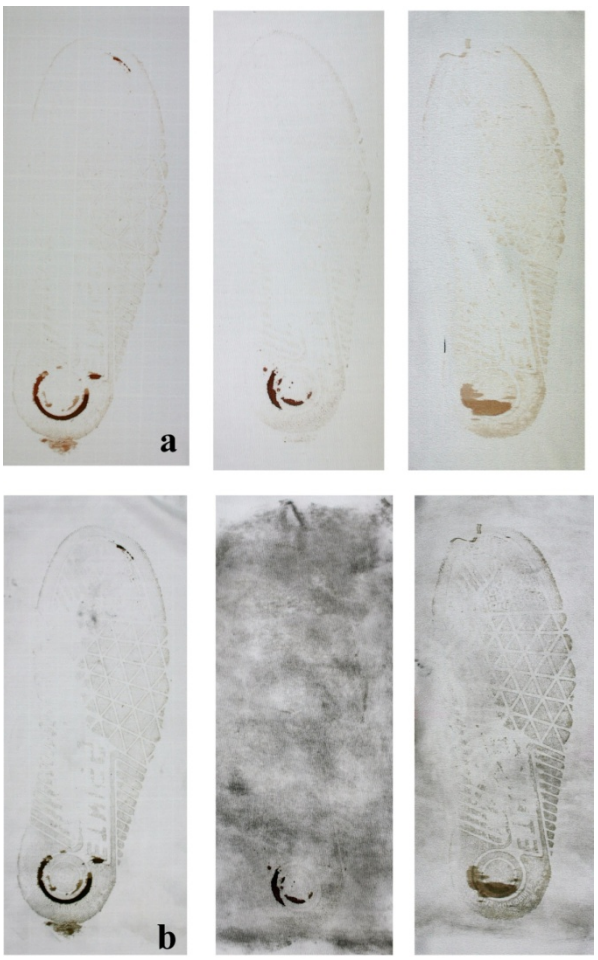


Figure 3.58 - The enhancement of footwear impressions in blood with black powder suspension on white nylon/lycra, cotton and polyester: (a) before enhancement; (b) after enhancement

The use of powder suspensions on fabrics appears to be limited from this preliminary work. Furthermore, there are a number of other techniques, such as acid yellow 7 and LCV, which might give superior enhancement results.

3.3.9 Diminishing Series

A diminishing series for impressions in blood was prepared as explained in section 3.2.12 and left to air dry for a week before being treated with acid yellow 7, luminol, LCV and LMG as the best performing techniques across the fabrics investigated. The enhancement provided by each technique was directly compared and is illustrated in figure 3.59.

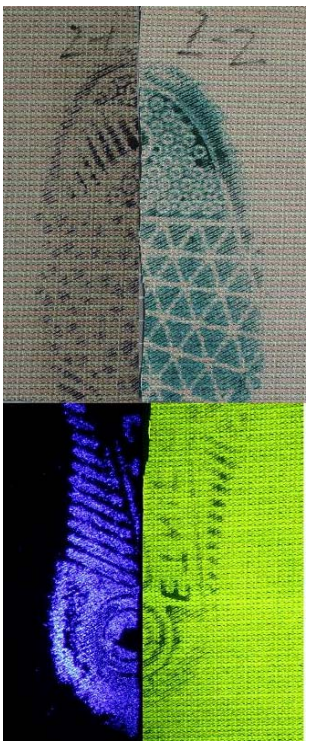


Figure 3.59 – 2nd impression of a diminishing series on patterned cotton treated with 4 different techniques (from top left going clockwise: LCV, LMG, acid yellow 7 and luminol)

3.3.9.1 Acid Yellow 7

AY7 enhancement for the diminishing series in blood was not possible beyond the third impression. Fluorescence improved contrast on black coloured fabrics and slightly weakened the contrast on light coloured fabrics. The AY7 fluorescence on white cotton caused the blood to appear as black and the background as yellow when the usual expectation for AY7 is for the blood to appear as bright yellow and the background dark. This is most probably due to optical brighteners used to impart a bright white colour and introduced during the manufacture of white fabrics. As discussed previously, bright AY7 fluorescence on white fabrics appeared to obscure the impression. An interesting observation was that the first impression on denim and leather fluoresced strongly, compared to the second impression and the rest of the series. This suggests that fluorescence was weak on denim and leather in previous studies because of the absence of blood in the impression rather than an effect of the surface and that previous results were obtained as a result of the poor ability of these fabrics to retain blood.

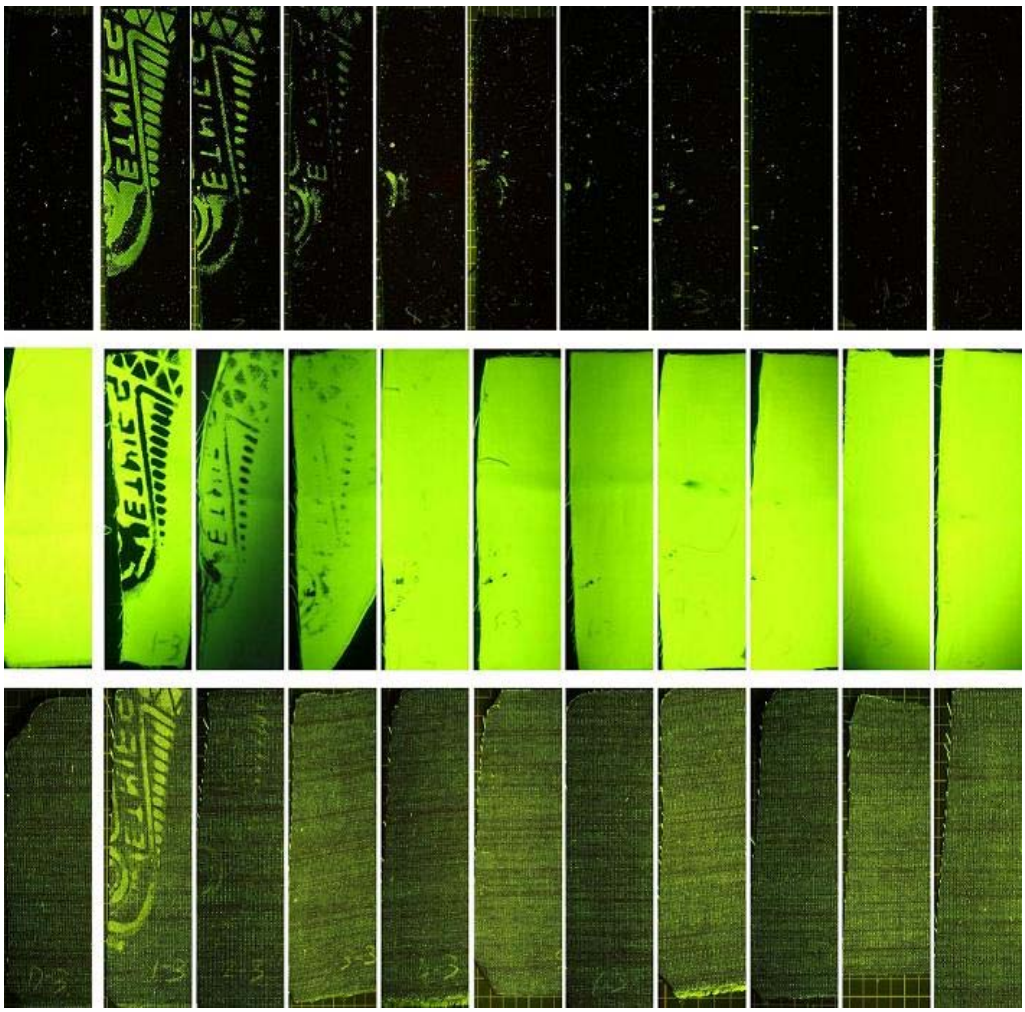


Figure 3.60 – AY7 fluorescence enhancement for a diminishing series in blood for: black cotton, white cotton and denim

3.3.9.2 Luminol

Luminol has been widely reported for its sensitivity in the enhancement of blood stains [2,8] and it was hypothesised that luminol enhancement of the diminishing footwear impression series would be successful up to the tenth and last impression of the diminishing series. However the results demonstrated that, although luminol detected blood up to the tenth impression on most fabrics, the entire footwear sole could only be visualised up to the third or fourth impression at best. These results suggest that the footwear sole loses a lot of the accumulated blood after the first few impressions. The first impressions in the series appeared to be very bright indicating that luminol works better with minute traces of blood.

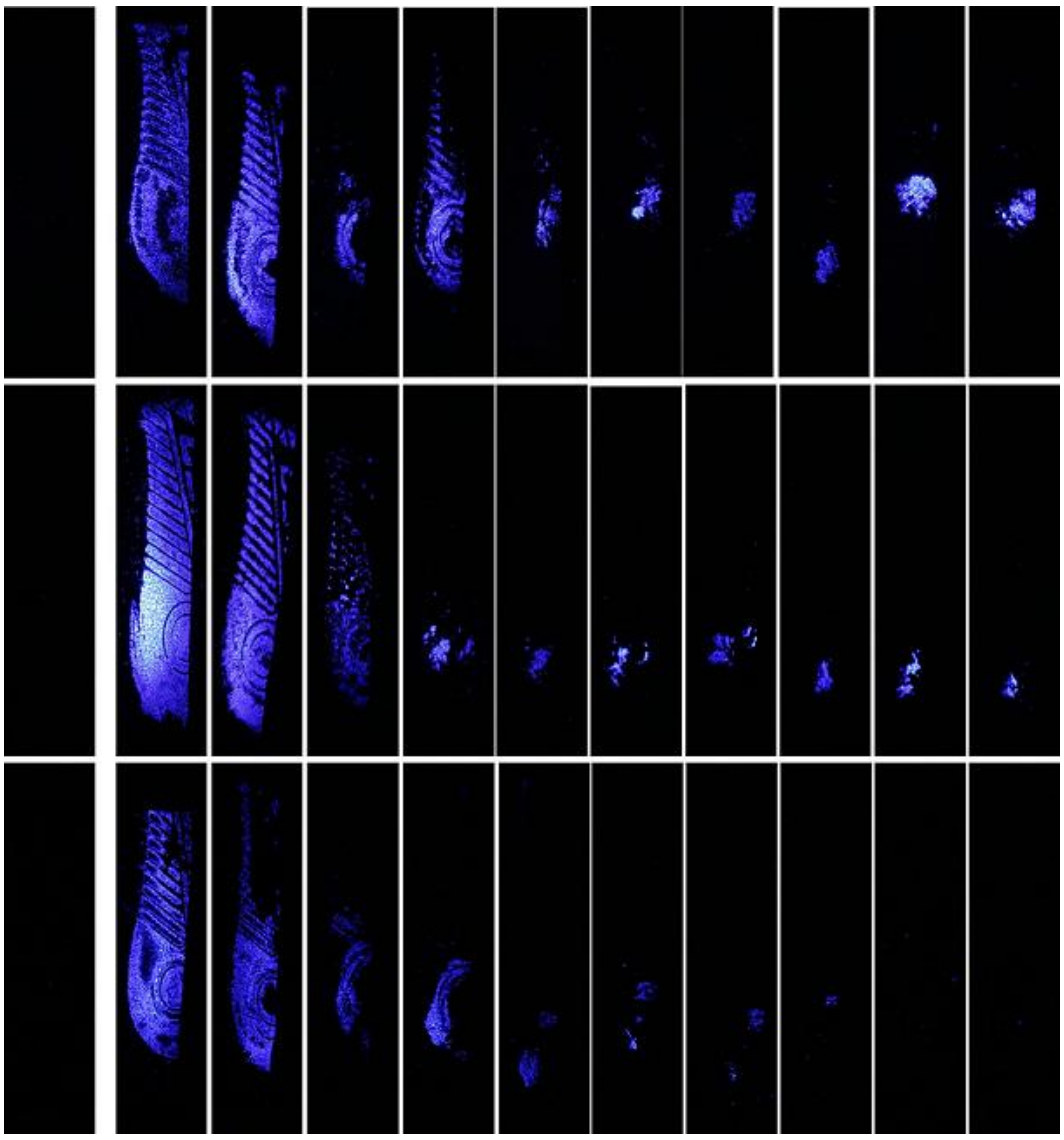


Figure 3.61 - Luminol enhancement for a diminishing series in blood for: patterned cotton, black nylon/lycra and leather

3.3.9.3 LMG and LCV

LMG and LCV enhancement on dark coloured fabrics did not provide good contrast. Nonetheless, on all fabrics the first two impressions in blood on dark fabrics were enhanced due to heavy blood staining except for black polyester where no enhancement was observed with LMG or LCV. Impressions in blood on light coloured fabric were enhanced with LMG and LCV up to the fourth or fifth impression. Diffused enhancement was observed on white polyester for both of these peroxidase reagents.

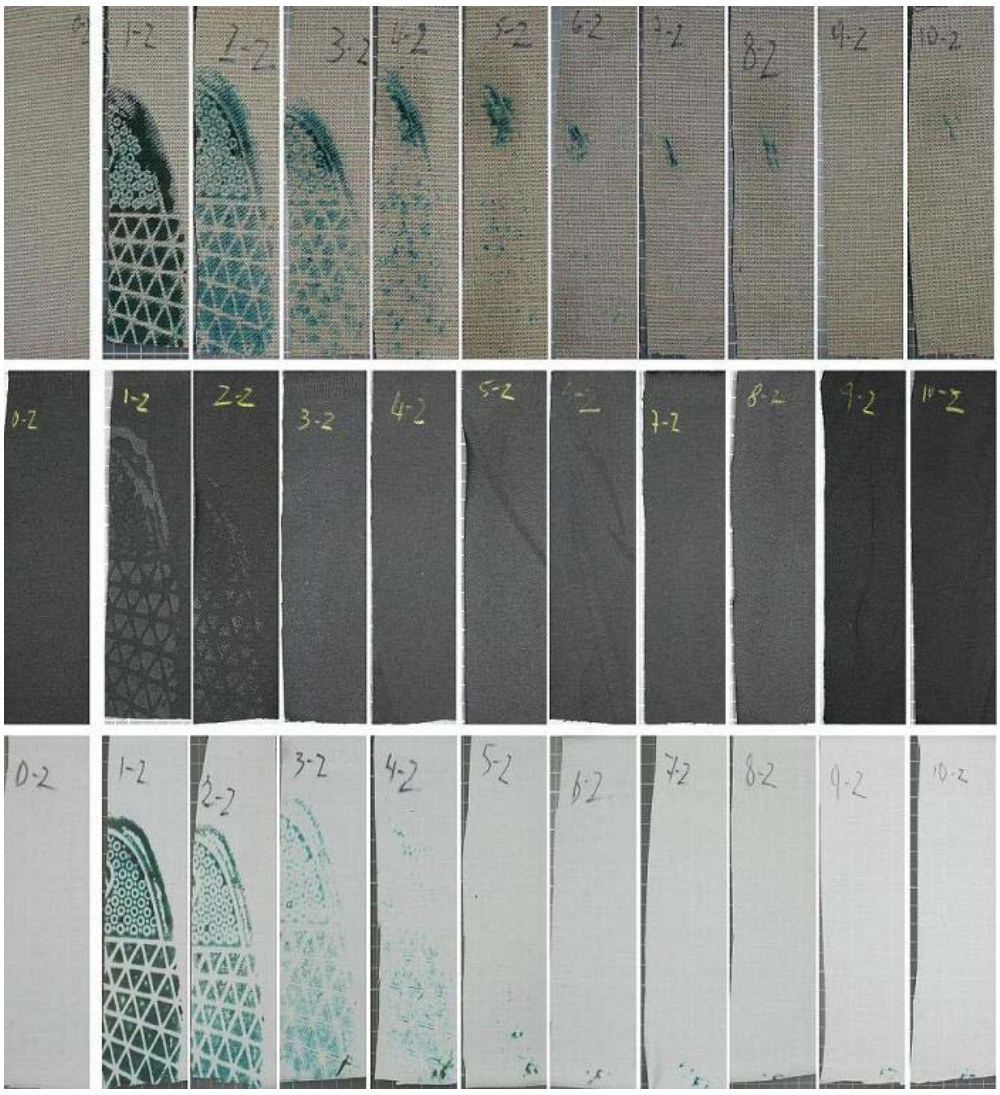


Figure 3.62 - LMG enhancement for a diminishing series in blood for: patterned cotton, black cotton and white nylon/lycra

All four techniques failed to enhance the complete footwear sole beyond the fourth impression. In a few instances, blood was detected up to the tenth impression where the heel part of the sole was enhanced. AY7 fared better at developing impressions in blood on black fabrics and struggled to enhance past the first impression on denim and leather. LMG and LCV provided good enhancement on light coloured fabrics but failed to enhance impressions past the second impression on dark surfaces due to poor contrast. These two peroxidases also failed to enhance the first heavy stained impression on black polyester. Luminol detected blood up to the tenth impression for most fabrics, however, footwear detail could only be observed up to the fourth or fifth impression.

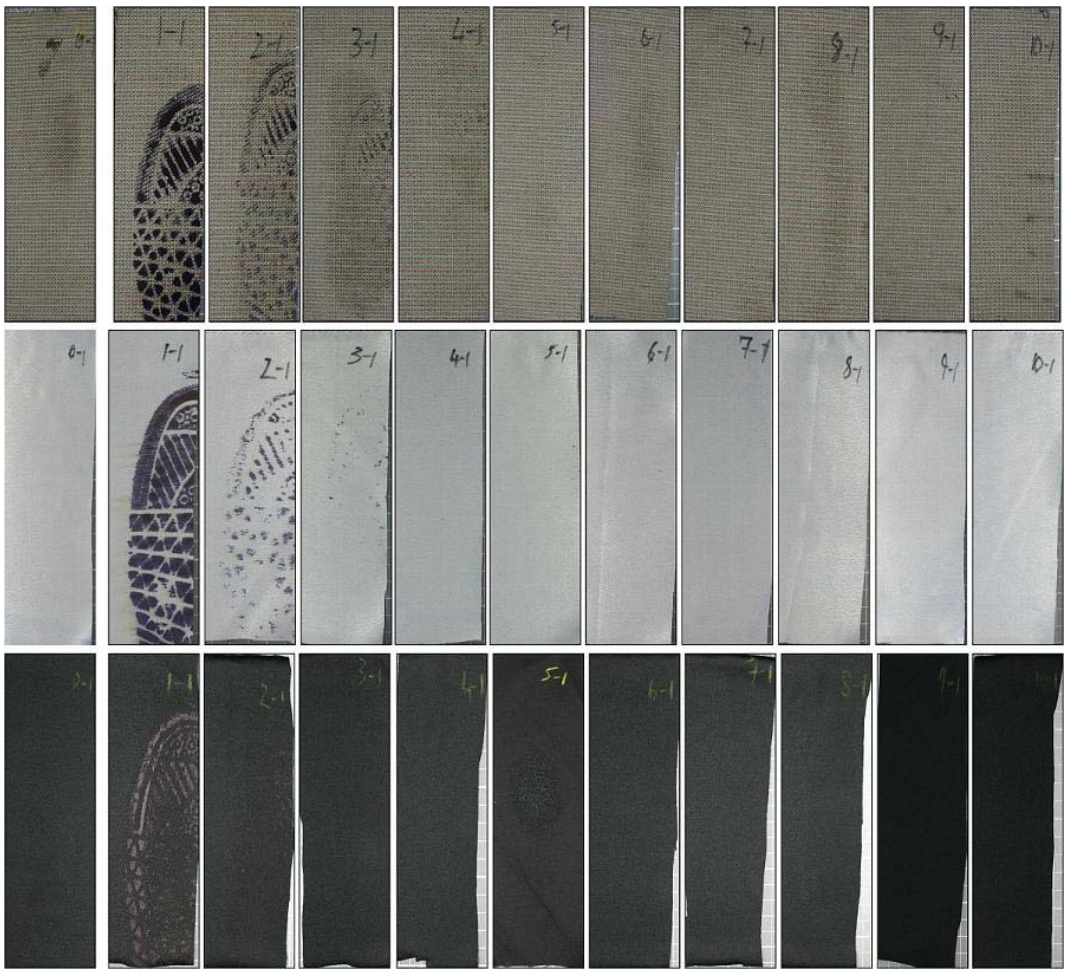


Figure 3.63 - LCV enhancement for a diminishing series in blood for: patterned cotton, white polyester and black nylon/lycra

3.3.10 Sensitivity Series

Four enhancement techniques (LCV, LMG, AY7 and luminol) were considered with three repeats for white and black cotton, polyester and nylon/lycra. No enhancement of the footwear impressions was observed after washing and air drying for any of the reagents studied, although luminol showed slight scattered dots of chemiluminescence, indicating the potential presence of blood. This is in contrast to previous research [66, 68] where blood was detected using luminol and other techniques after washing. This difference can possibly be explained by the fact that in this study, weak latent blood impressions were prepared whereas in other studies heavier bloodstained impressions were used.

Cox [68] also observed a relationship between the type of fabric and the retention of the bloodstains where blood was likely to wash off synthetic fabrics such as acetate, nylon and polyester. This relationship is partly in line with the SEM analysis of footwear impressions in blood on fabric (figure 1.5). As the blood appeared to dry on polyester without penetrating the fabric, there was potentially a higher chance for the impression in blood to be washed off. The SEM analysis however did not explain why the impression in blood washed off nylon/lycra although the blood appeared to penetrate deeply into the fabric. Nonetheless, the high permeability values (table 1.1) of these fabrics suggest that both polyester and nylon/lycra have large pores. These values suggested that although blood might have penetrated into the fabric, it was not trapped due to the large pores of these fabrics.

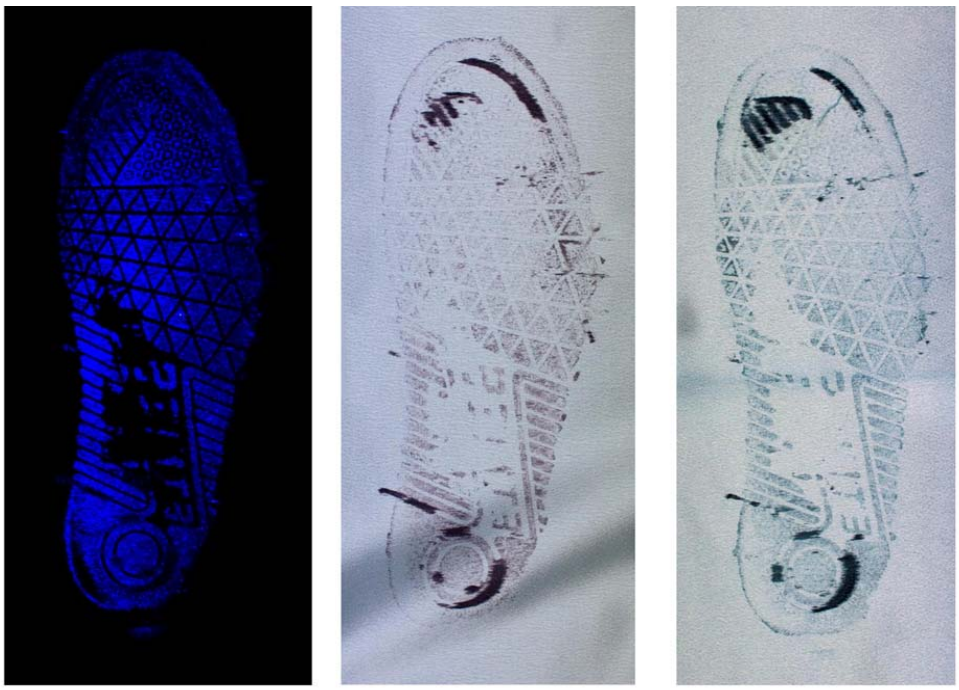
3.3.11 Sequential Chemical Enhancement

The sequential chemical enhancement of footwear impressions in blood on fabric was attempted using techniques that target different components in blood such as proteins (acid black 1 and acid yellow 7) and iron/haemoglobin (luminol). Table 3.7 illustrates the different sequences attempted on white polyester and black cotton. These two fabrics were selected as exemplars of light and dark as well as synthetic and natural fibres. Previous work had shown that treating the impression in blood with chemical techniques after alginate lifting did not yield any enhancement. As a consequence, alginate lifting was attempted at the end of the sequence.

The main observations during these sequences were that luminol gave no or very weak chemiluminescence when applied after ninhydrin or DFO. Luminol enhancement was also poor after the use of protein stains, however, the effect was not as pronounced as that obtained when luminol was used after ninhydrin and DFO. It also appeared that one technique would give suitable results without the requirement of sequential techniques: for example AY7 fluorescence gave suitable results when utilised on its own. The use of protein stains and ninhydrin after luminol did not demonstrate any deterioration of the impression. On most occasions, the use

of alginates at the end of the sequence did not lift the footwear impressions, however, in some instances AB1 enhancement on the alginate helped visualise the impression.

Figure 3.64 illustrates the sequential chemical enhancement of a footwear impression in blood on polyester using luminol followed by ninhydrin and AB1.



(a) (b) (c)

Figure 3.64 – Sequential chemical enhancement of a footwear impression in blood on white polyester: (a) luminol; (b) ninhydrin and (c) acid black 1 (AB1)

Diffusion and obliteration of the original footwear impression is a possibility when using one technique, so extra caution is necessary when using sequential treatment. The use of one technique might provide suitable enhancement, however, if sequential treatment is attempted, the use of luminol as first technique is recommended.

3.3.12 Chemical Enhancement of Individual Characteristics

None of the 8 individual characteristics shown in figure 3.11 were enhanced after chemical treatment with AY7 or luminol. These two chemicals were selected as suitable exemplars of a protein stain and a peroxidase reagent respectively. It was firstly hypothesised that this effect is due to the nature of the fabric and contaminant where the blood seeps into the fabric limiting the enhancement of fine detail. However, for most fabrics with the exception of nylon/lycra, SEM analysis demonstrated that the blood appeared to dry over the fabric surface.

3.4 Conclusion

The results clearly demonstrated that there were minimal variations in the six repetitions of test footwear impression for each fabric-technique combination. AY7 was the most suitable protein stain for footwear impressions made in blood and deposited onto dark fabrics, however, limited fluorescence was observed for similar impressions on denim and leather using this enhancement reagent. Comparable but weaker results were obtained with other fluorescent protein stains on the dark fabrics. Other protein stains, particularly AB1 and AV17, performed better on light coloured fabrics than AY7.

Of the four peroxidase reagents studied, Bluestar® Forensic Magnum luminol was the best performing enhancement technique overall, enhancing impressions on all surfaces and was the only technique to provide a clear enhancement of the impressions on denim. LCV and LMG provided good enhancement on patterned cotton and light coloured fabrics but were poor enhancers of impressions on darker fabrics whereas fluorescein and luminol provided excellent enhancement results due to optimal contrast with the background. None of the peroxidase reagents successfully enhanced impressions in blood that had been subjected to washing.

In general luminol was the most effective reagent for weaker impressions and provided good footwear detail up to the fifth impressed impression in a diminishing series. Similarly luminol detected blood up to the tenth impression for most fabrics where as the other reagents tested provided little enhancement past the second or third impression in the series. Luminol provided excellent results on denim and leather where all other techniques performed poorly. However, acid yellow 7 and fluorescein appear to offer better results and less diffusion than luminol for the enhancement of footwear impressions in blood on black cotton, polyester and nylon/lycra. Occasionally, it was possible to detect the fine detail of the footwear sole impression.

The use of alginates is an available technique for the *in situ* recovery of blood impressions. It can easily be prepared and applied at a crime scene if the article cannot be moved to the laboratory. Lifting with alginate followed by enhancement with AB1 provided good definition for all impressions on the test fabrics.

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Chapter 4: Chemical Enhancement of Footwear Impressions in Urine on Fabric

4.1 Introduction

The identification of urine is generally based on the detection of inorganic anions such as phosphate and sulphate as well as organic compounds such as urea, ammonia and creatine [1]. Urine also contains a variety of other compounds including uric acid, creatinine, chlorine and Tamm-Horsfall glycoprotein [1-3]. Different compounds present in body fluids, such as nucleic acids, proteins and lipids, dried semen, metabolite breakdown products in urine and heme in blood have the ability to exhibit fluorescence [2]. Furthermore, body fluids have characteristic emission spectra which can be used to distinguish them. Some compounds are present in more than one body fluid and the difference in their relative concentrations has been used as a means to attempt to differentiate the body fluids [2]. Fluorescence of urine tends to be much weaker than other body fluids as it is more dilute [3].

Since amino acids are expected to be present in urine, enhancement techniques that target amino acids such as ninhydrin and its analogues may be suitable for the enhancement of footwear impressions in urine. The use of protein stains and 4-dimethlyaminocinnamaldehyde (DMAC) may also be suitable for the detection of proteins and urea.

There are a range of enhancement techniques which have been reported in the literature as having potential to enhance urine stains. This chapter examines the possibility of enhancing footwear impressions in urine on fabric with protein stains, amino acid targeting reagents and DMAC. The objective of this work was to compare the ability of various chemical reagents to enhance footwear impressions in urine which had been deposited on various fabrics under laboratory conditions.

4.1.1 Ninhydrin

Ninhydrin is a non-specific amino acid reagent useful for the development of latent fingerprints [4-6]. Enhancement of footwear impressions using ninhydrin is not common practice, however, Bodziak [7] reports that ninhydrin has been utilised to enhance footwear impressions in blood on porous and non-porous surfaces, where heat and steam accelerate the process. The reaction of ninhydrin results in an intermediate colour change, varying from orange to purple depending on the fingerprint's deposition and development conditions [8, 9]. The final product is a purple-coloured compound called 'Ruhemanns's Purple'.

1,1,2-trichlorotrifluoroethane (CFC 113) has been utilised for many years as a solvent for the application of ninhydrin, and more recently 1,8-diazafluoren-9-one (DFO), a ninhydrin analogue. The ozone depleting solvent CFC 113 has now been withdrawn in the European Union and in many other countries and thus a replacement became necessary. Research into the use of heptane as an alternative to CFC 113 demonstrated that heptane based ninhydrin formulations developed as many latent fingerprints as the CFC 113 based formulation, however, heptane introduced an added fire and explosive risk to the laboratory environment or crime scene [10-12]. Further research from CAST and the National Research Institute of Police Science in Tokyo revealed that two liquid hydrofluorocarbons, HFC-4310 and HFE-7100, were efficient and safe substitute solvents to CFC113 for ninhydrin application [11, 13]. These formulations, however, did not work as well with 1,-8-diazofluoren-9-one (DFO) and a mixture of HFE-7100 and HFE-71DE is recommended as the best current substitute to CFC113. [8, 9, 11].

The MoFDT [8] indicates that ninhydrin can be used as part of a sequential process and if used after DFO in sequential treatment of the same fingerprint impression, additional detail could be developed. In contrast, little or no further development occurs if DFO is used after ninhydrin. Under optimum conditions, DFO will develop more latent fingerprint impressions than ninhydrin, however the latter develops visible prints whereas DFO may also require an additional fluorescent step for visualisation [8, 9]. Physical developer can potentially further enhance fingerprint impressions if applied in sequence after DFO and ninhydrin [4, 14].

4.1.2 1,8-Diazafluorene-9-one (DFO)

1,8-Diazafluorenone-9-one (DFO) is a ninhydrin analogue developed by Grigg *et al* [15] during a search for new ninhydrin analogues. This technique is used for latent fingerprint development on porous substrates, most commonly paper, where it reacts with amino acids and possibly with other fingerprint components such as sweat [4, 8, 9]. The reaction of DFO with amino acids gives a magenta colour which is visually less intense than the purple colour obtained with ninhydrin. The main advantage of DFO is that it exhibits enhancement producing both colour and fluorescence. Latent fingerprints treated with DFO show a faint red colour and luminesce brightly under green light (absorption maximum at about 470 nm and emission maximum at about 570 nm) [16]. However, the enhanced image may be obscured if a porous substrate itself displays fluorescent behaviour.

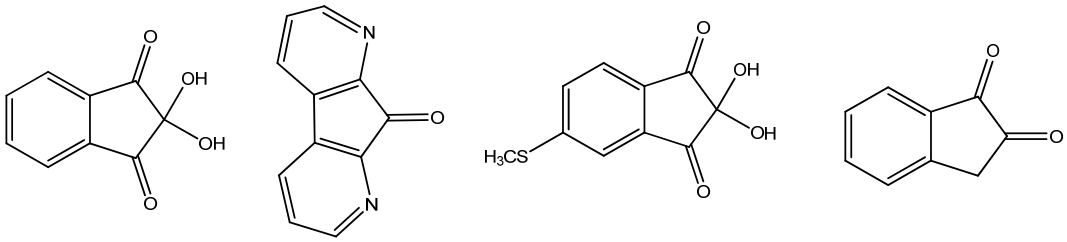
A modified dry version of DFO enhancement was reported by Bratton and Juhala [17]. The technique involves DFO enhancement from DFO soaked filter papers, subsequently processed with a steam iron filled with a 5% acetic acid solution before heating in a mounting press at 100^{o}C for ten minutes [17].

4.1.3 Other Ninhydrin Analogues

Ninhydrin analogues developed in the 1980s exhibited similar fingerprint enhancement results to ninhydrin [18, 19] and are illustrated in figure 4.1. Small changes in the chemical structure facilitated an improved enhancement of impressions through, for example, the introduction of fluorescence.

5-methylthioninhydrin (5-MTN) and 5-methoxyninhydrin, developed by Heffner and Joullie [20], are reagents utilised for processing latent impression evidence in a similar but alternative mechanism to ninhydrin. They reported that treated latent impressions produced a bright purple colour that was stronger than that produced with ninhydrin. Furthermore, post-enhancement treatment with zinc chloride produced a red-purple colour which fluoresced under green light. The developed prints were reported to produce stronger fluorescence than those developed with

DFO [21, 22]. Recent research also demonstrated that 5-methoxyninhydrin and 5-methyltioninhydrin act as dual fingerprint reagents with enhanced sensitivity producing impressions that are both coloured and fluorescent [23].



Ninhydrin DFO 5-MTN 1,2-IND

Figure 4.1 - The chemical structures of DFO, Ninhydrin, 5-MTN and 1,2-IND

1,2-indanedione (1,2-IND), another ninhydrin analogue developed by the University of Pennsylvania and the United States Secret Service (USSS) [24, 25], has received more attention than 5-MTN and 5-methoxyninhydrin and is commonly compared to DFO. 1,2-IND and its analogues produce pink colours that also fluoresce when used to enhance latent fingerprints. Several agencies, such as CAST, USSS, Israeli police, the Royal Canadian Mountain Police (RCMP) and the Australian Federal Police (AFP), have researched the optimum conditions for the application of 1,2-IND and its effectiveness compared to DFO for the enhancement of latent fingerprints on porous surfaces [26-35].

It has been reported [34] that variable climatic conditions between different countries (and even within) can have an effect on the performance of 1,2-IND and DFO. Research in countries such as Israel, USA and Australia has shown that 1,2-IND is superior to DFO but the opposite was found to be true in the UK and Canada [34, 35]. A recent study by CAST [35] compared different formulations of 1,2-IND with the addition of zinc salts where the most effective formulation was then compared to DFO. The study confirmed that the addition of zinc salts greatly improves the fluorescence intensity, as shown in previous research [30], but also that it can be incorporated in the formulation for a one-step process rather than after enhancement [36]. Nonetheless, the study illustrated that DFO marginally outperformed 1,2-IND

for the enhancement of latent fingerprints on porous surfaces, at least in the UK [35]. Finally, sequential enhancement using 1,2-IND and ninhydrin were shown to not be very effective [26, 27, 37].

4.1.4 Dimethylaminocinnamaldehyde (DMAC)

4-dimethlyaminocinnamaldehyde (DMAC) is a yellow powder which has several reported formulations and application methods [38-42]. DMAC reacts with urea and amino acids to give a magenta-coloured product offering also the advantage of yellow-green fluorescence using an excitation filter for the wavelength range 400-519 nm [42, 43]. Figure 4.2 shows the reaction between DMAC and urea.

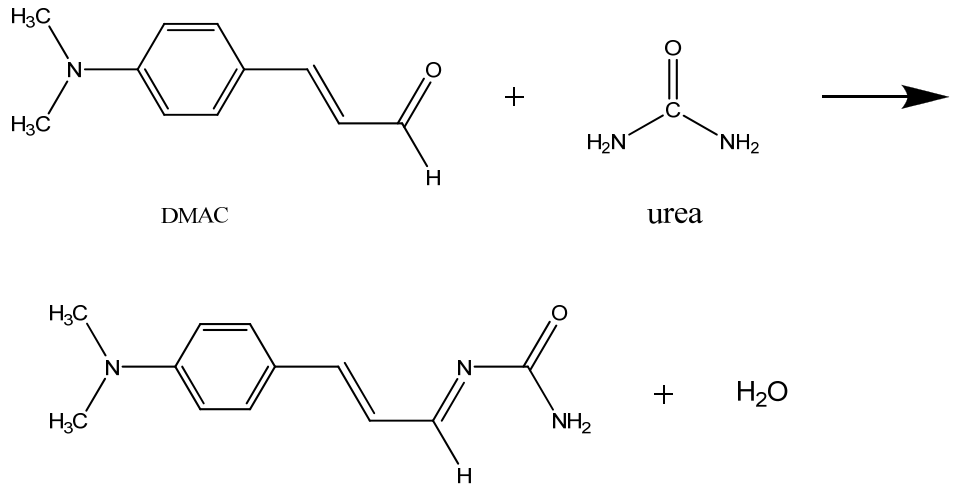


Figure 4.2 - The reaction between DMAC and urea

During the development of the DMAC process, Sasson and Almog [44] reported that DMAC was superior to ninhydrin on latent fingerprints up to 72 hours old. Several successful formulations and applications (dipping, spraying or contact-transfer) of DMAC have been proposed [14, 42, 45]. Trials by CAST [42] demonstrated that methanol could be replaced with ethanol as a safer substitute without any detriment to the process. Brennan *et al.* [38, 39] reported the use of DMAC as a fuming agent with potential success for sequential treatment before the ninhydrin-DFO-physical developer sequence. Ramotowski [46] and Flynn [45] both reported that latent

fingerprints were enhanced when papers soaked with DMAC were put in contact with the print. The speed of the reaction was increased when a steaming iron was used on the paper.

Other research studies [42] revealed that the contact transfer technique did not appear to react primarily with urea but with the amino acid component of fingerprints. This is in contrast to the solution-dipping DMAC mechanism [38, 39, 44]. Although DFO can be considered as the most effective process for developing latent impressions on porous items, the use of DMAC before DFO and ninhydrin has been demonstrated to develop additional impressions [42].

4.1.5 Other Techniques

There are a range of tests that can be utilised for the detection of urine such as the Nessler's reagent [3], the Jaffe test [47] and a spray reagent containing urease and bromothymol blue [48]. However, most of these tests are test-tube based and have been developed for the detection of urine rather than for spraying on footwear impressions suspected to contain urine. Urea is found in high concentrations in urine and tests which detect urea depend on the ability of the enzyme urease to break down urea into ammonia and carbon dioxide. Ammonia is then detected by reagents such as Nessler's reagent (mercuric iodide in potassium iodide) or 4-dimethlyaminocinnamaldehyde (DMAC) [3]. Creatinine is also found in high concentrations in urine and its presence can be detected by the Jaffe, Salkowski and Sagakuchi tests. The Jaffe test involves the reaction of picric acid and sodium hydroxide with creatinine to yield a red product [1, 3], while the Salkowski test uses sodium nitroprusside to react with creatinine in the presence of heat and potassium ferrocyanide as an oxidising agent to yield a Prussian blue product [1, 2]. The Sakaguchi test, which is less sensitive than the Jaffe test, uses o-nitrobenzaldeyhde to develop a red colour upon reacting with creatinine [49].

4.2 Materials and Methods

4.2.1 Excitation and Emission Spectra of Urine

Excitation and emission spectra were recorded using a Shimadzu RF5301 spectrofluorophotometer. Undiluted urine samples from three volunteers and urine stains produced by these samples on different fabrics were examined. The instrument allowed for measurement of liquid samples (in special quartz cells) as well as direct measurement of stains on fabrics. A number of different excitation and emission wavelengths were utilised.

4.2.2 Preparation of Footwear Impressions in Urine on Fabric

Human urine (25mL), obtained with ethical consent, was poured over two Kimberley® double ply tissues in a tray measuring 0.33m x 0.23m x 0.06m. The tray was put in contact with the sole of the footwear attached to the stamping rig (as previously described) using a stepping motion and the footwear then released onto the fabric. The fabric used was the same as that detailed in table 3.4 (page 84). Six repetitions were carried out for each technique and fabric utilised in the study. Each impression was allowed to age for seven days prior to enhancement. Sensitivity tests of the techniques using a diminishing series, sequential series and an ageing study were carried out on the best performing fabric and enhancement reagents.

4.2.3 Fluorescence

A Mason Vactron Quaser 40 was utilised for fluorescence observations. Initial fluorescence examination of the urine impressions on fabric was carried out prior to any chemical enhancement. A wavelength range of 400-700nm was used as recommended for the visualisation of body fluids [47]. The appropriate excitation and viewing wavelengths/filters which were utilised for observation of fluorescent chemical reagents are presented in table 4.1.

Table 4.1 - Excitation wavelength and viewing filters for a Mason Vactron Quaser 40

Chemical Name	Excitation Wavelength/nm	Excitation Filters	Viewing Filter/nm	Viewing Filter
AY7	385-509	Blue	510	Yellow/Orange
DFO	473-548	Green	549	Orange
1,2-IND	473-548	Green	549	Orange
DMAC	473-548	Green	549	Orange

The wavelengths represented in this table show the 1% cut-on and cut-off points [50]. Computer monitor, colour calibration and fluorescence photography were performed as described previously in section 3.2.3 and in the literature [51].

4.2.4 Enhancement Reagents

Preliminary work for the enhancement of footwear impressions in urine on the various fabrics was undertaken using the chemical reagents listed in table 4.2. The best performing techniques were then examined further.

Table 4.2 - List of chemical reagents for the detection of urine impressions

Chemical Name	Alternative Chemical Name	Supplier
Acid Black 1	Amido Black 10B (C.I. 20470)	BVDA
Acid Violet 17	Coomassie Violet R200 (C.I. 42650)	BVDA
Acid Yellow 7	Brilliant Sulfoflavine (C.I. 56205)	BVDA
Ninhydrin	2,2-Dihydroxy-1,3-indanedione	Sigma Aldrich
DFO	1,8-Diazafluorenone-9-one	BVDA
1,2-IND	1,2-indanedione	BVDA
DMAC	4-dimethly-aminocinnam-aldehyde	Acros
HFE-7100	Nonafluorobutyl methyl ether	3M Novec
HFE-71DE	Trans-1,2-dichloroethylene	3M Novec

Techniques such as the Jaffe test were omitted from the study due to added health and safety implications, rendering them as implausible reagents for operational use. All chemical reagents were prepared as recommended by CAST [35, 42, 52].

4.2.5 Protein Stains

Protein stains were applied as described previously in section 3.2.6.

4.2.6 Ninhydrin, DFO and 1,2-IND

These techniques were applied in the same way as described in section 3.2.8. However, in this instance, a hair dryer was used for heating samples treated with ninhydrin.

4.2.7 DMAC

Pre-impregnated DMAC sheets: DMAC (Acros, 0.25g) was weighed and dissolved in ethanol (Sigma, 100mL) with stirring. The solution was poured into a dipping tray and white A4 sheets of copier paper were soaked in the solution. The sheets were left to dry in a fume hood, sealed in a Ziploc bag and stored in a refrigerator until needed.

Treatment of articles: Articles to be treated were placed between two sheets of paper pre-impregnated with DMAC such that a pre-impregnated DMAC sheet was placed between each article and then finally wrapped in two sheets of aluminium foil. The layers were either heated for about 30 seconds via an iron or left in a press overnight. DMAC enhanced impressions emit light in the green, yellow and orange region of the spectrum with a maximum at approximately 530nm. The impressions were viewed using blue (385-509nm), blue/green (468nm) and green (473-548nm) Quaser bands. Best results were obtained using a green excitation source (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 549nm filter (1% cut-on point).

4.2.8 Sensitivity of Techniques and Ageing of Impressions

The best performing combinations of enhancement technique and fabric were examined further by creating a diminishing series of footwear impressions where impressions were produced one after the other without re-loading the footwear sole with urine in between impressions. The diminishing series was prepared up to the fifth impression and allowed to age for one week. Each impression was then cut in half and chemically treated to facilitate the following comparisons: NIN-DFO, DFO-DMAC and NIN-DMAC. Samples were also prepared as previously described and aged for two months prior to enhancement.

4.2.9 Chemical Enhancement of Individual Characteristics

8 individual characteristics (carvings and indentations) were introduced onto the new footwear sole by means of a knife and scissors as previously described in section 3.2.15. Footwear impressions in urine were prepared as previously described and enhanced with the best performing reagent and fabric combinations. The ability to enhance the individual characteristics was assessed.

4.3 Results and Discussion

4.3.1 Excitation and Emission Spectra of Urine

The main fluorophores in human urine that fluoresce under UV light are tryptophan and its metabolites indoxyl sulphate and 5-hydroxyindole-3-acetate [53-55]. Undiluted human urine weakly fluoresces under short wave UV excitation (250-300nm) [53] and this fluorescence can be reduced by high concentrations of indoxyl sulphate 'that contribute to the concentration quenching of fluorophores by ammonium' [55]. This can explain the difference in excitation and emission spectra of urine from different donors illustrated in figures 4.3 - 4.6.

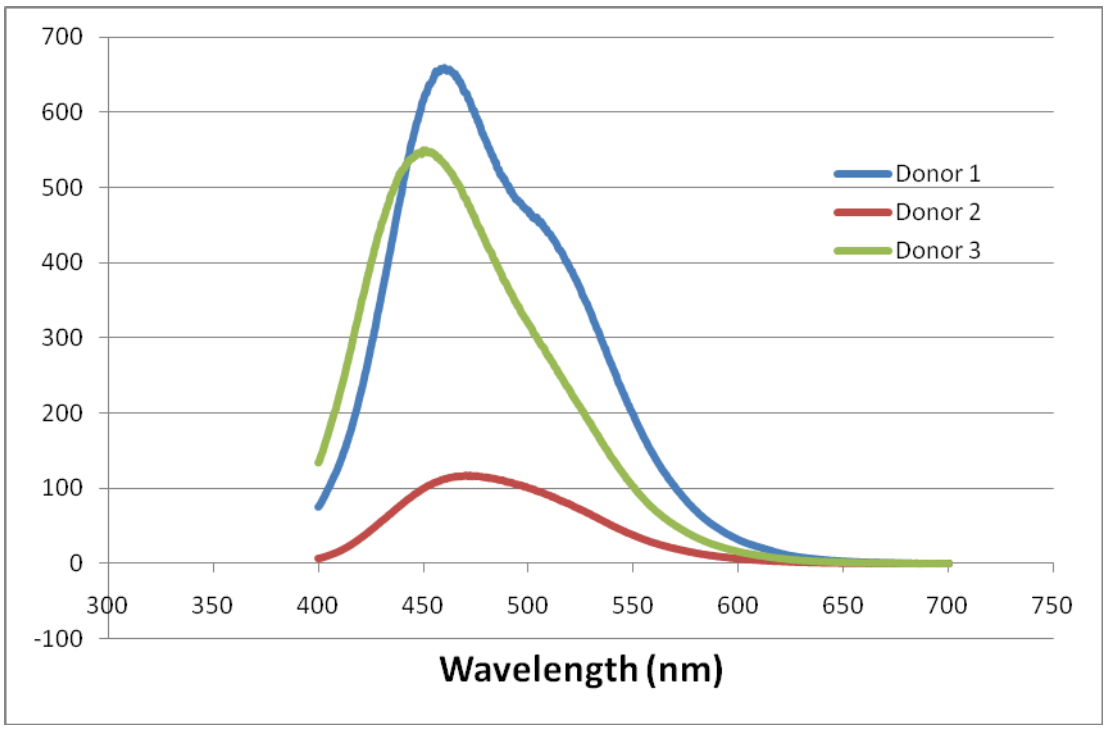


Figure 4.3 - Emission spectrum (400-700nm) of undiluted human urine from three different donors (excitation at 380nm)

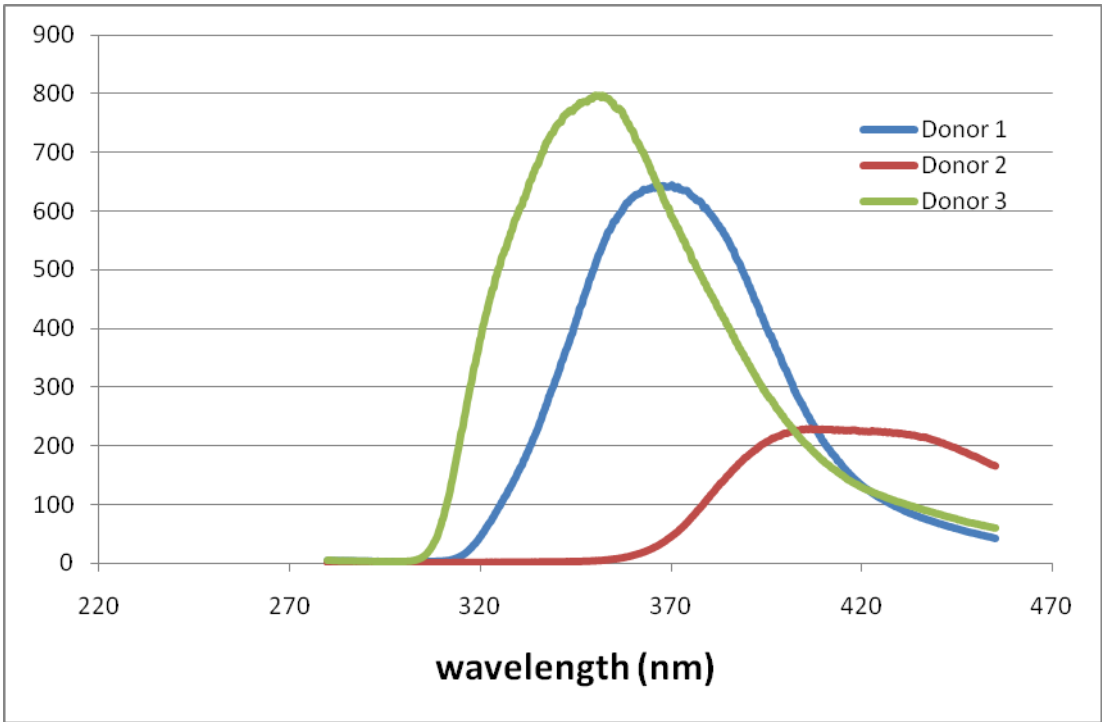


Figure 4.4 - Excitation spectrum (280-455nm) of undiluted human urine from three different donors (emission at 475nm)

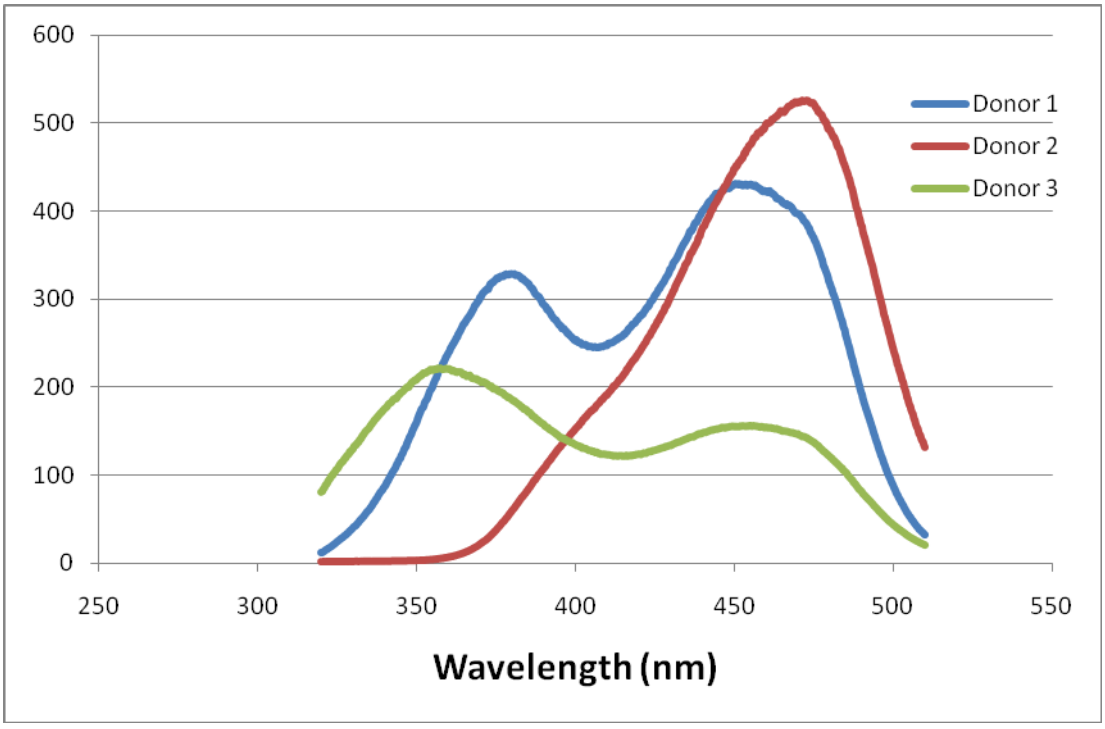


Figure 4.5 - Excitation spectrum (320-510nm) of undiluted human urine from three different donors (emission at 530nm)

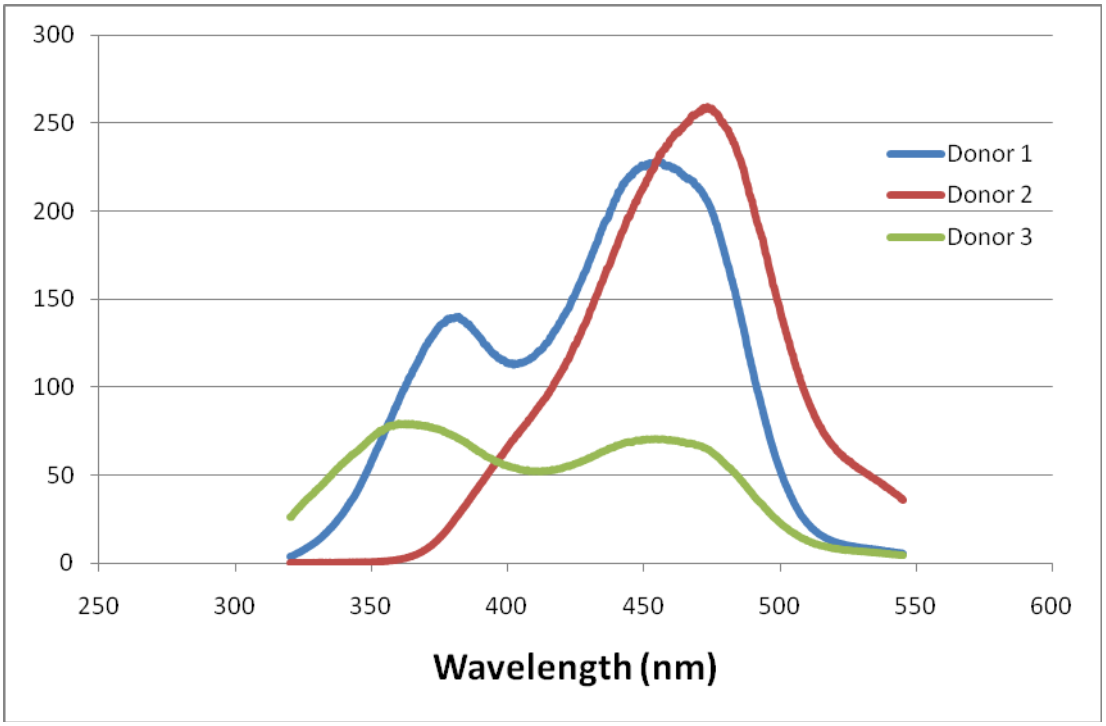


Figure 4.6 - Excitation spectrum (320-545nm) of undiluted human urine from three different donors (emission at 560nm)

An emission spectrum is produced when different wavelengths of fluorescent light emitted by a particular sample are measured by the instrument with a fixed excitation wavelength. Similarly, an excitation spectrum is produced when an emission wavelength is held constant and the excitation wavelength scans a predetermined range of wavelengths. The emission spectrum 400-700nm with an excitation at 380nm in figure 4.3 produced the most consistent results between the three donors. This emission spectrum reveals a maximum emission at approximately 470nm, suggesting that a strong fluorescence of urine would be observed by using a violet/blue excitation source (band pass filter 350-469nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 476nm filter (1% cut-on point) if using a Mason Vactron Quaser 40. This observation differs slightly to previous research where the strongest emission was observed around 440nm [56]. The excitation spectra (figures 4.4-4.6) at different wavelengths vary between donors indicating possible differences in fluorophore concentrations. Measurements of the same excitation and emission spectra, as those utilised for undiluted human urine, on one-week old urine stains on different fabrics and the appropriate fabric blanks (no urine present) were indistinguishable.

4.3.2 Preliminary Enhancement Results

Urine samples from a male, a female and a vegetarian volunteer were used to prepare the impressions. It was suspected that a vegetarian's diet might have an effect on the enhancement obtained. No appreciable difference was observed in the quality of the enhancement. Consequently, male urine was utilised for the remainder of the experiments and the urine sample was obtained from the same person for all tests.

The initial unenhanced urine impression was visible as soon as it was prepared on the fabric but the visibility decreased as the stain dried with the exception of stains on polyester where the impression remained visible for at least a week. The urine impressions on polyester were diffused and blurred presumably due to the hydrophobic nature of the fabric. This reduced the visibility on any fine detail in the impression on this surface as illustrated in figure 4.7b.

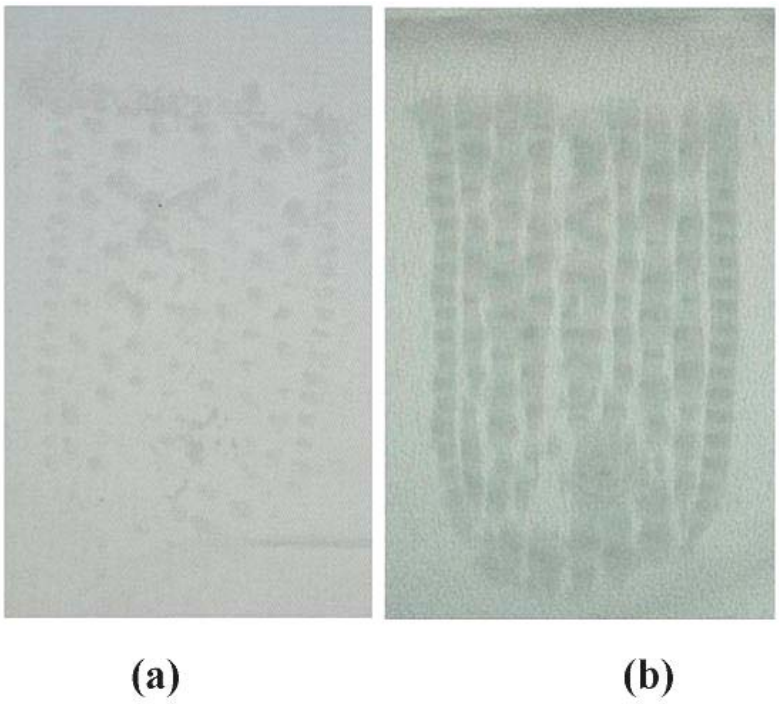


Figure 4.7 - Urine impressions photographed immediately after application: (a) on white cotton and (b) on white polyester

4.3.2.1 Fluorescence

The emission spectrum in figure 4.3 revealed that a strong fluorescence of urine would be observed by using a violet/blue excitation source (band pass filter 350-469nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 476nm filter (1% cut-on point) if using a Mason Vactron Quaser 40. Initial fluorescence prior to enhancement was undertaken using a Quaser (Quaser 40, Mason Vactron UK) and the appropriate excitation and viewing filters [50]. Different excitation filters, including violet/blue (385-469), blue (385-519), green (473-548) and red (503-591), offered good visualisation of the impressions as illustrated in figure 4.8. No impressions were visualised using fluorescence on black fabrics, denim or leather.

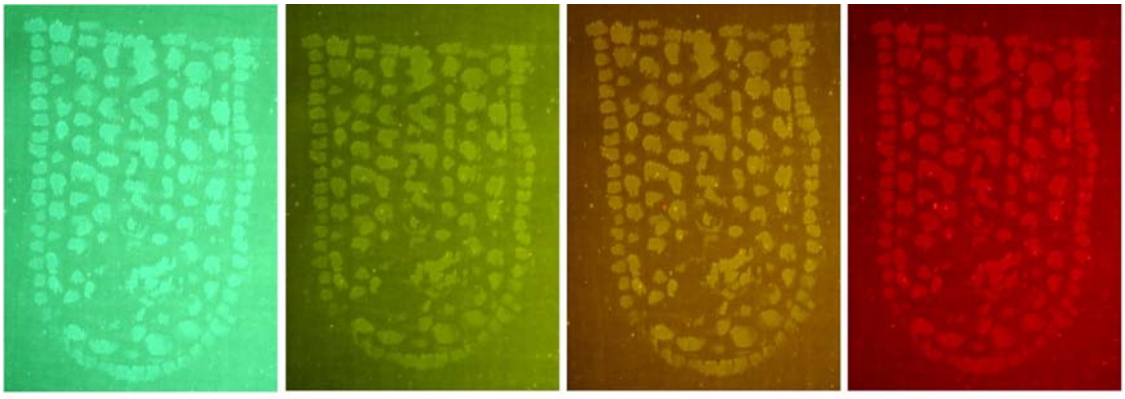


Figure 4.8 - Preliminary work on the fluorescence of footwear impressions in urine on white cottonwith different Mason Vactron Quaser 40 excitation sources: (a) violet/blue [350-469nm]; (b) blue [385-509nm]; (c) green [473-548nm]; (d) green/yellow [503-591nm]

4.3.2.2 Protein Stains

The protein stains AB1, AV17 and AY7 were initially tested for the enhancement of urine on white cotton and white polyester. AY7 enhancement was also attempted on black cotton and black polyester as enhancement of impressions in blood using this reagent on dark substrates had previously been successful [51, 57, 58]. No enhancement, either visual or fluorescent, was observed for any fabric, presumably due to the fact that the proteins present in urine were greatly diluted in comparison to

the protein levels found in blood. All protein stains failed to produce suitable enhancement results and as such were not investigated further.

4.3.2.3 Ninhydrin and DFO

Ninhydrin and DFO provided good enhancement results for impressions made in urine on white fabrics, however, no enhancement (visual or fluorescent) was achieved on dark coloured fabrics.

A humidity oven was not available and as a consequence ninhydrin was applied as per crime scene recommendations [52]. The reagent was applied with a brush and the resultant impression heated using a hair dryer. However, better results were achieved by dipping the article to be treated into ninhydrin and using a hair dryer for drying. A slight tinge of lilac was observed after about two minutes of heat from the hair dryer. The articles were observed again after about an hour and maximum contrast was obtained after 24 hours as shown in figure 4.9.

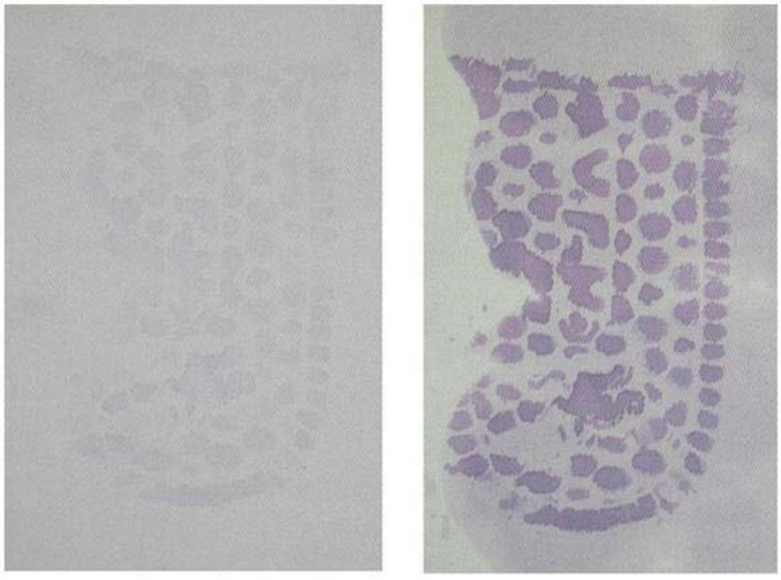


Figure 4.9 – Preliminary ninhydrin enhancement of a footwear impression in urine on white cotton fabric: (a) after 5 minutes and (b) after 1 hour

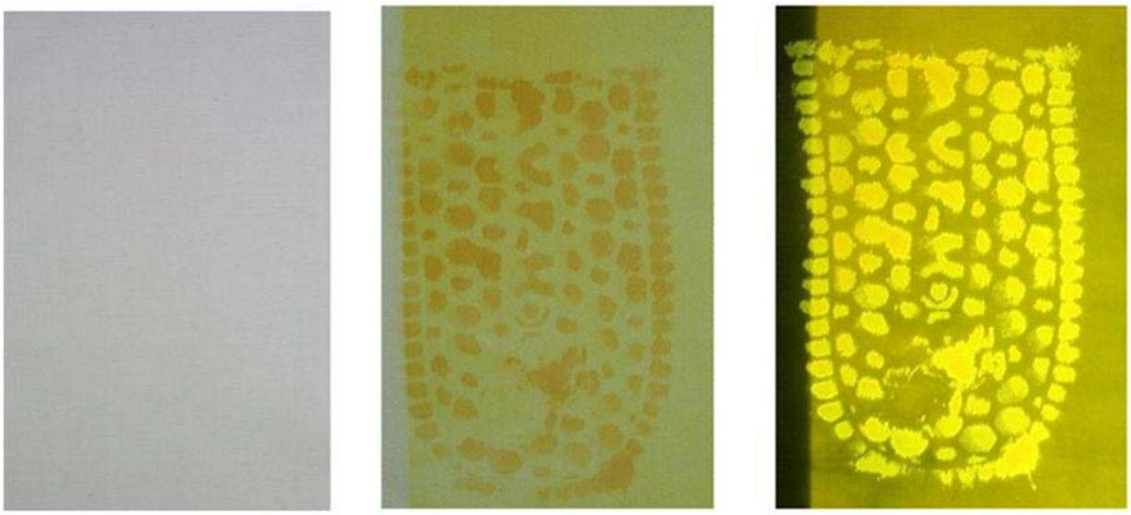
Initial experiment with DFO produced a pink/magenta colour as illustrated in figure 4.10. Fluorescence examination on light coloured fabrics improved the contrast and brightness of the impression allowing more detail of the footwear sole to be observed.

Figure 4.10 - Preliminary DFO enhancement of a footwear impression in urine on white cotton fabric

4.3.2.4 DMAC

Similar to DFO, enhancement achieved with DMAC on impressions made in urine was successful on light coloured fabrics but failed to produce any results on dark coloured fabrics. Visual observation and enhancement under white light was sometimes achievable, however, best results were obtained during fluorescence examination under green light using a wavelength of 473-548nm.

Two methods of DMAC enhancement were performed as described in section 4.2.7. Both methods produced good enhancement results. For practical reasons, the pressing method was deemed to be a better method even though the articles to be analysed had to be left in a press overnight, however, a bigger number of articles could be analysed simultaneously.



(a) (b) (c)

Figure 4.11 – Preliminary DMAC enhancement of a footwear impression in urine on white cotton fabric: (a) before enhancement; (b) enhancement under white light and (c) enhancement under green light

4.3.3 In Depth Studies of Amino Acid Stains

The use of a stamping rig to create footwear impressions in urine allowed for robust comparisons of enhancement techniques. Furthermore, there was minimal variation within the six repetitions for each fabric-technique combination as illustrated in figure 4.12.

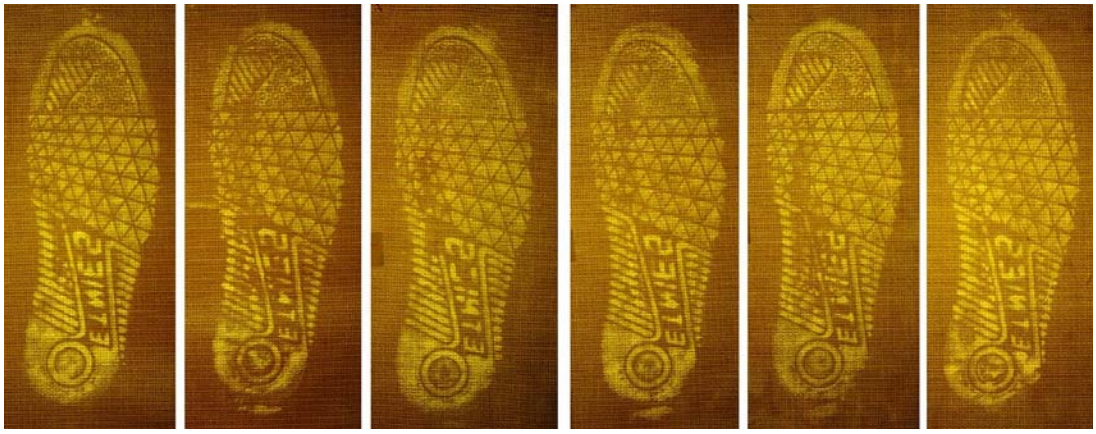
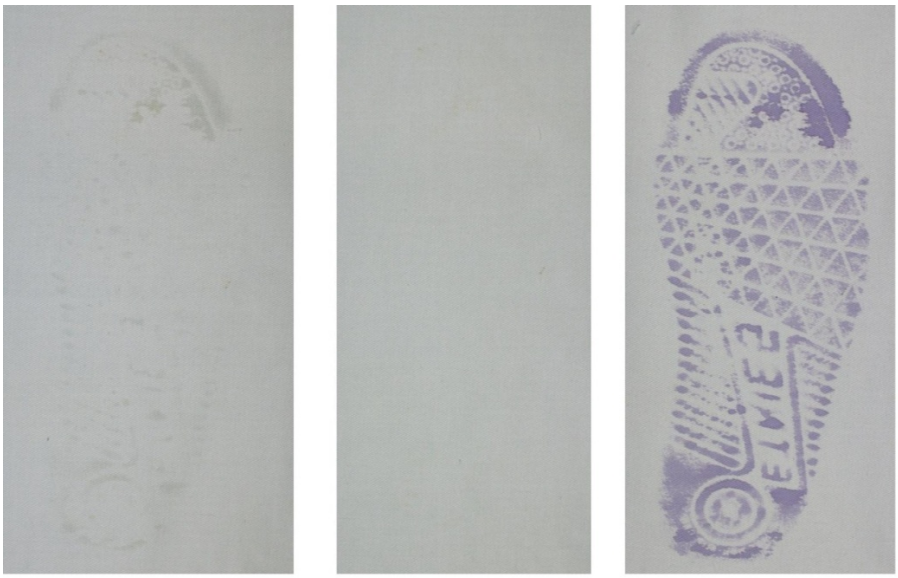


Figure 4.12 - Enhancement of six repetitive footwear impressions in urine on patterned cotton with DFO fluorescence using a Quaser 40 green excitation source

4.3.3.1 Ninhydrin

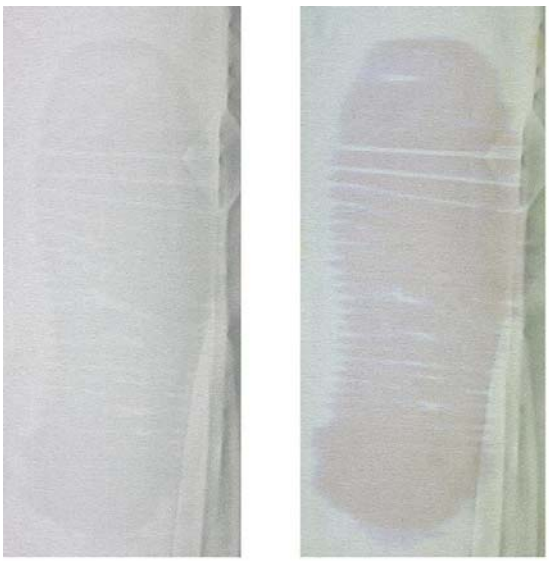
The use of ninhydrin for the enhancement of urine impressions on light coloured cotton fabric provided excellent results. The purple enhancement colour initially visible after application of heat became progressively darker over time reaching a maximum after about 24 hours. The purple colour was only visible on light coloured fabrics as presented in figure 4.13, and this was because of the poor colour contrast with the background on all dark coloured fabrics, denim and leather.



(a) (b) (c)

Figure 4.13 - Enhancement of a footwear impression in urine on white cotton: (a) fresh urine impression; (b) 1 week old urine impression; (c) ninhydrin enhancement

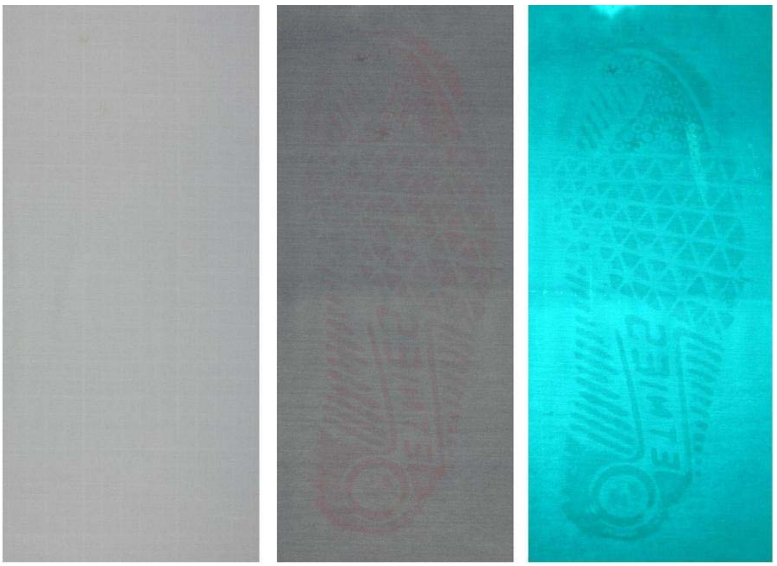
Enhancement of impressions on polyester produced poor quality impressions presumably because of the nature of the interaction between the fabric surface and the urine sample. This is presented in figure 4.14.



(a) (b)

Figure 4.14 - Enhancement of a footwear impression in urine on white polyester : (a) 1 week old urine impression; (b) ninhydrin enhancement

Ninhydrin enhancement on nylon/lycra spread to stain the whole background. A similar effect was observed in the enhancement of impressions in blood on this fabric. However, quick photography facilitated the capture of the impressions and the use of a blue excitation filter improved the contrast when background staining occurred as illustrated in figure 4.15.

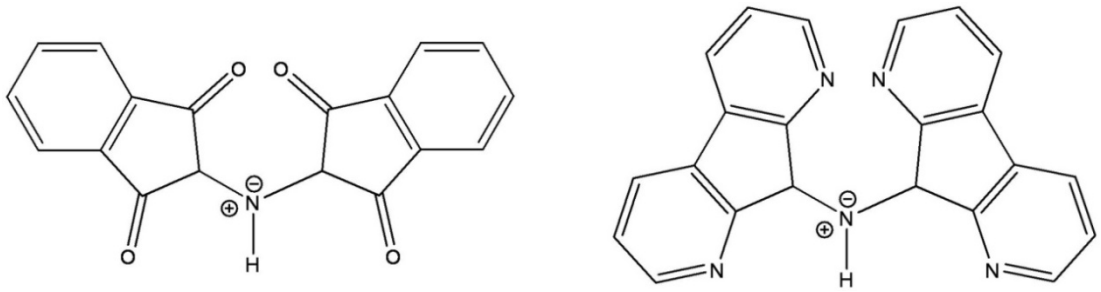


(a) (b) (c)

Figure 4.15 - Enhancement of a footwear impression in urine on white nylon/lycra: (a) 1 week old urine impression; (b) ninhydrin enhancement under white light; (c) ninhydrin enhancement using a Quaser 40 blue excitation source

4.3.3.2 DFO

DFO is reported to react with traces of amino acids [59, 60] and was successful in the enhancement of impressions in urine made on all of the light coloured fabrics. This reinforces the hypothesis that DFO reacts with minute traces of amino acids as would be expected for urine impressions. Grigg *et al.* [15] proposed a possible reaction mechanism for the reaction of DFO with amino acids. The DFO magenta complex (figure 4.16), analogue to the ninhydrin's Ruhemann purple complex, was later confirmed by X-ray crystallography [61].



(a) (b)

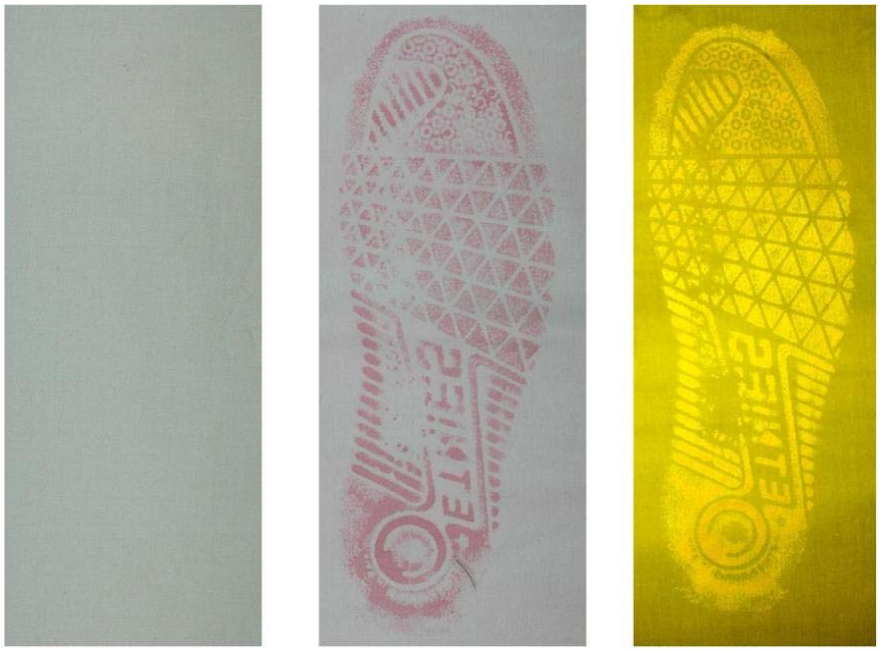
Figure 4.16 – Ninhydrin Ruhemann's purple complex (a) and DFO red complex (b)

Stoilovic [60] proposed that exposure of DFO treated samples for 20 to 30 seconds at 160^{o}C or 10 seconds at 180^{o}C produced superior development than the conventional method of dipping in DFO solution, drying and heating at 100^{o}C for 20 minutes. Although Stoilovic [60] demonstrated that the proposed method provided at least twice the luminescence with less background development, the comparison was undertaken with a DFO solution using 1,1,2-trichlorotrifluoroethane (Fluorisol) rather than the formulation suggested by CAST [52] using HFE-7100 and HFE-71DE. The use of methanol in the DFO formulation is an integral part of the DFO reaction, as it has been shown that the solvent promotes the formation of an unstable hemiketal [61]. A hemiketal is produced from the addition of an alcohol to a carbonyl group in a ketone. Under weakly acidic conditions and with the loss of water, this hemiketal changes to a reactive intermediate which is more susceptible to attack by the nitrogen on the amino acids.

Following treatment in the oven at 100^{o}C for 20 minutes, the footwear impressions produced an image which was bright magenta on light coloured fabrics and was further enhanced using fluorescence.

On white cotton, no fluorescence enhancement was necessary as the impression was easily visualised as illustrated in figure 4.17. However, visualisation of the impressions on patterned cotton demonstrated poor contrast and could only be observed with fluorescence as shown in figure 4.18.

No colour was observed on dark coloured fabrics, denim and leather due to poor contrast. Fluorescence was also not observed on these fabrics. Only diffused enhancement was achieved on polyester which is due to the urine impression seeping into the fabric rather than failure of the enhancement technique itself.



(a) (b) (c)

Figure 4.17 - Enhancement of a footwear impression in urine on white cotton: (a) 1 week old urine impression; (b) DFO enhancement under white light; (c) DFO enhancement using a Quaser 40 green excitation source

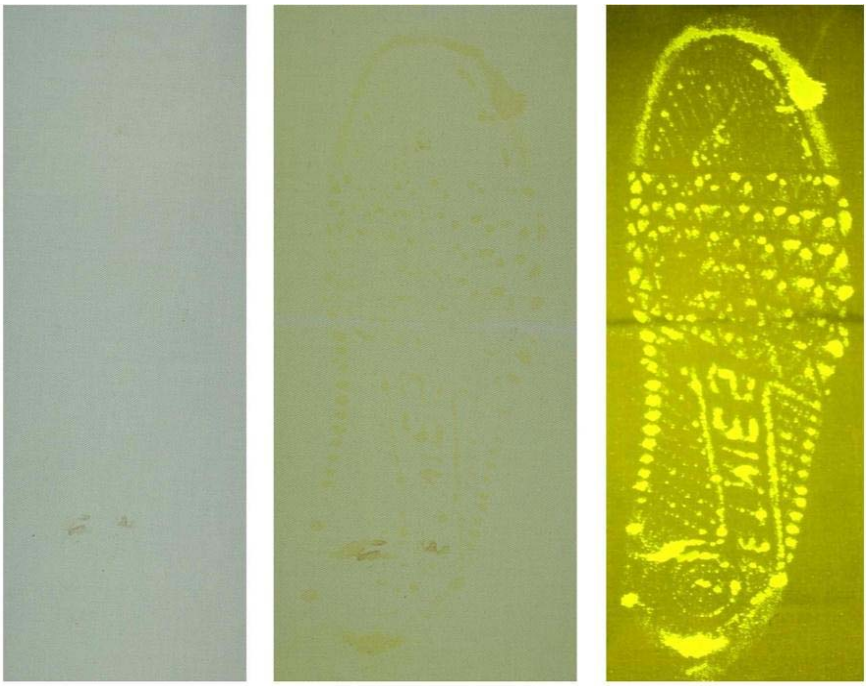
(a) (b) (c)

Figure 4.18 - Enhancement of a footwear impression in urine on patterned cotton: (a) 1 week old urine impression; (b) DFO enhancement under white light; (c) DFO enhancement using a Quaser 40 green excitation source

4.3.3.3 DMAC

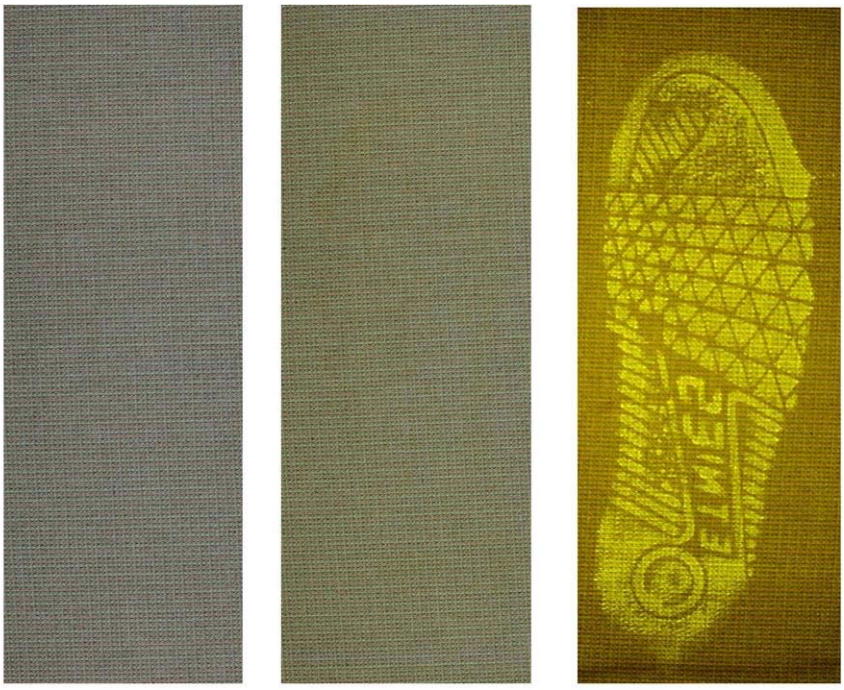
The application of this technique involved the use of DMAC pre-impregnated paper as suggested by Ramotowski [46]. DMAC is believed to react with amino acids rather than urea [42] and DMAC enhancement of impressions in urine was similar to that obtained from DFO.

Successful enhancement was achieved again on all light coloured fabrics. Visual observation after DMAC enhancement on white cotton was possible (although weaker than DFO) and fluorescence examination further enhanced the impression (figure 4.19). The impression in urine on patterned cotton required the use of fluorescence as the visual colouration was weak or non-existent (figure 4.20). The enhancement of the impressions prepared on nylon/lycra by DMAC was limited however some enhancement could be observed using fluorescence (figure 4.21). As with DFO, however, no visual or fluorescent enhancement was achieved on dark coloured fabrics, denim or leather.



(a) (b) (c)

Figure 4.19 - Enhancement of a footwear impression in urine on white cotton: (a) 1 week old urine impression; (b) DMAC enhancement under white light; (c) DMAC enhancement using a Quaser 40 green excitation source



(a) (b) (c)

Figure 4.20 - Enhancement of a footwear impression in urine on patterned cotton: (a) 1 week old urine impression; (b) DMAC enhancement under white light; (c) DMAC enhancement using a Quaser 40 green excitation source

Figure 4.21 - Enhancement of a footwear impression in urine on white nylon/lycra: (a) 1 week old urine impression; (b) DMAC enhancement under white light; (c) DMAC enhancement using a Quaser 40 green excitation source

4.3.3.4 1,2-IND

1,2-IND produced similar fluorescent enhancement results to DFO as illustrated in figure 4.22, however visual enhancement on light coloured fabric was considerably weaker.

Similar to DFO and DMAC, no visual or fluorescent enhancement was achieved on black fabrics, denim or leather. The use of lasers at different wavelengths did not improve the fluorescence achieved by the Quaser 40 and the lack of success with DFO, DMAC and 1,2-IND in enhancing impressions on dark fabrics, denim and leather may possibly be due to interference from the dyes utilised in the manufacturing processes. DFO was found to marginally outperform 1,2-IND in other studies for the enhancement of latent fingerprints on porous surfaces [35].

Figure 4.22 - Enhancement of a footwear impression in urine on patterned cotton: (a) urine impression after 1 week; (b) enhancement with fluorescence only using blue excitation filter [385-509nm]; (c) 1,2-IND visual enhancement; (d) 1,2-IND enhancement using a Quaser 40 green excitation source

The use of enhancement techniques for the detection of urine do not necessarily imply the presence of urine as the techniques target components such as amino acids that might be present in other solids or liquids.

4.3.4 Enhancement of Fine Detail

It was not always possible to recover the fine detail of the footwear impression due to the nature of the fabric and the interaction between the contaminant and the receiving surface. The impression in urine on polyester was for example very poor with little or no detail evident as illustrated in figure 4.14. However, on surfaces where good quality enhancement was achieved (for example white and patterned cotton), considerable fine detail was visible using ninhydrin, DFO, DMAC and 1,2-IND as illustrated in figure 4.23.

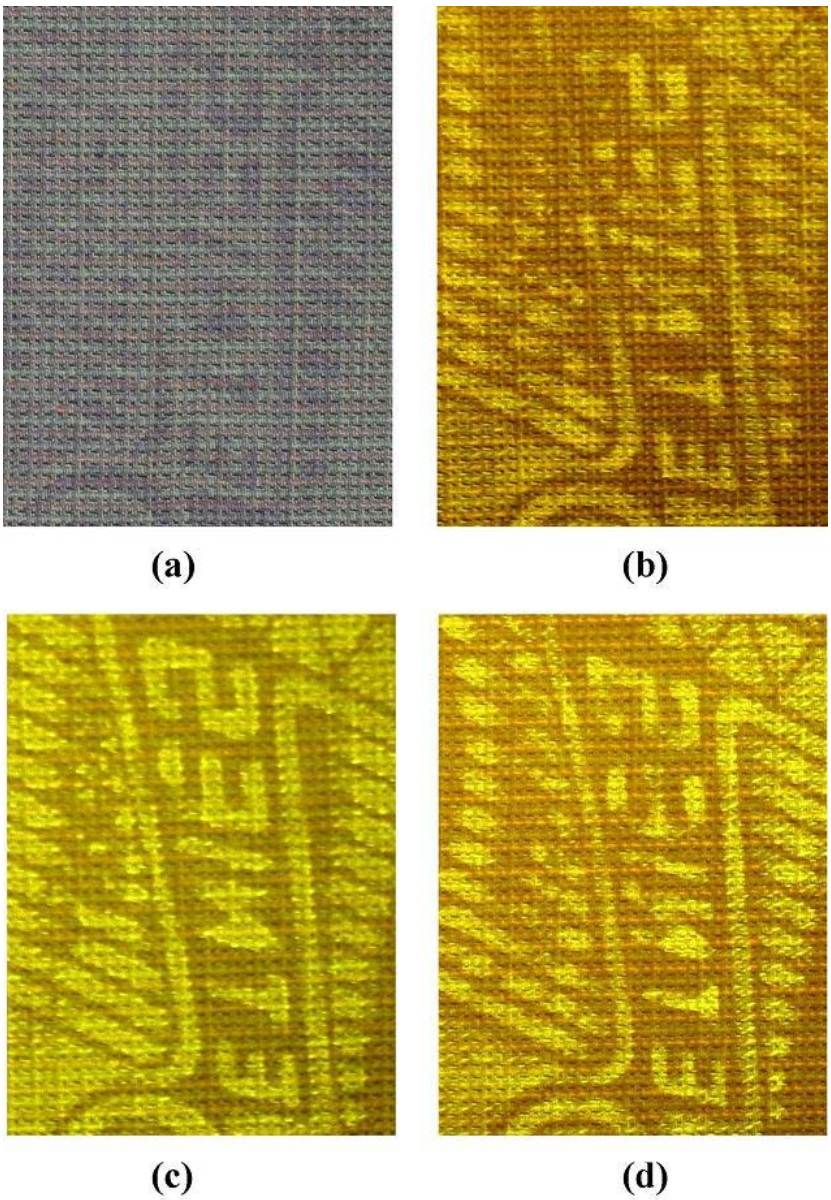


Figure 4.23 - Close-up detail of the enhancement of footwear impressions in urine on patterned cotton with: (a) ninhydrin; (b) DFO; (c) DMAC and (d) 1,2-IND

4.3.5 Diminishing Series

A diminishing series of footwear impressions in urine was prepared for the light coloured fabrics only, that is: white cotton, white nylon/lycra and patterned cotton. Polyester was excluded because of the diffusion observed and dark fabrics, denim and leather were excluded as no enhancement (visual or fluorescent) was obtained.

The diminishing series of impressions were prepared as previously described. The impressions were enhanced with DFO, DMAC and ninhydrin only as the best performing techniques. After one week, each impression was cut in half and chemically treated in order that direct comparisons could be made between: NIN-DFO, DFO-DMAC and NIN-DMAC. DFO enhancement outperformed both DMAC and ninhydrin enhancement on white cotton as illustrated in figure 4.24.

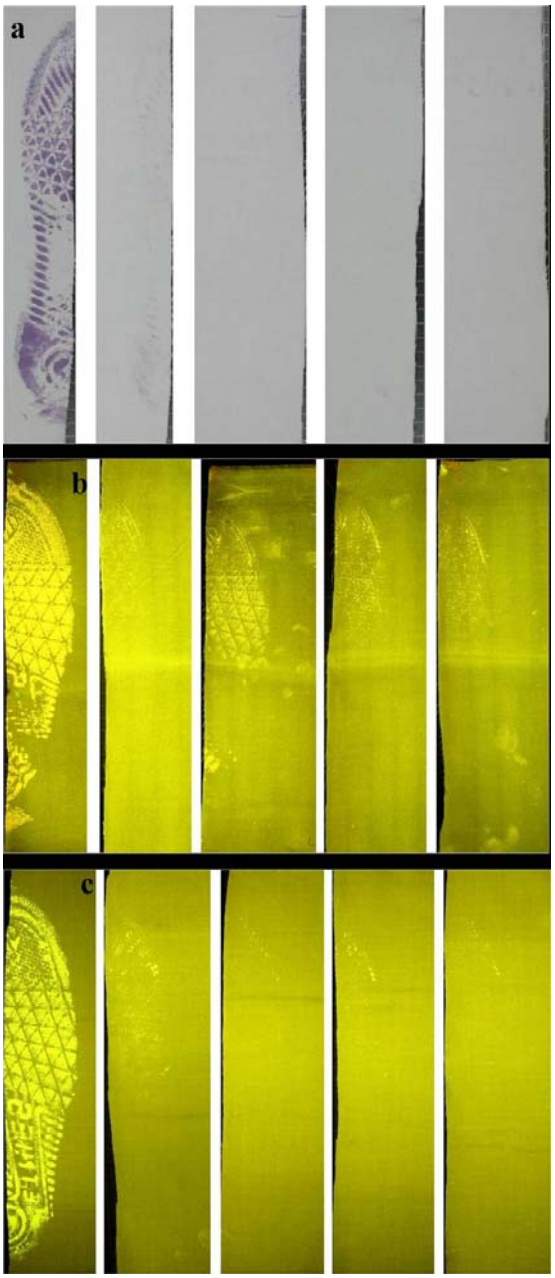


Figure 4.24 – Diminishing series (from left to right) of footwear impressions in urine on white cotton and enhanced with (a) ninhydrin, (b) DFO fluorescence and (c) DMAC fluorescence

Similar results were observed for patterned cotton and white nylon/lycra although background staining on nylon/lycra interfered with DMAC fluorescence. The enhancement of urine impressions with DFO proved to be the most sensitive (figure 4.24b), although the fourth and fifth impressions were weak. General fluorescence examination only provided enhancement up to the second impression in the series.

Figure 4.25 illustrates the comparison of ninhydrin, DFO and DMAC on the three different fabrics. The first impression in urine on patterned cotton demonstrated that DFO and DMAC (figure 4.25 (b)) provided similar enhancement results, however, DFO outperformed DMAC as the urine impressions weakened over the diminishing series. DMAC enhancement was superior to ninhydrin on white and patterned cotton when fluorescence was used, however, ninhydrin was superior to DMAC on white nylon/lycra as the extensive background staining hindered the enhancement using DMAC (figure 4.25 (c)).

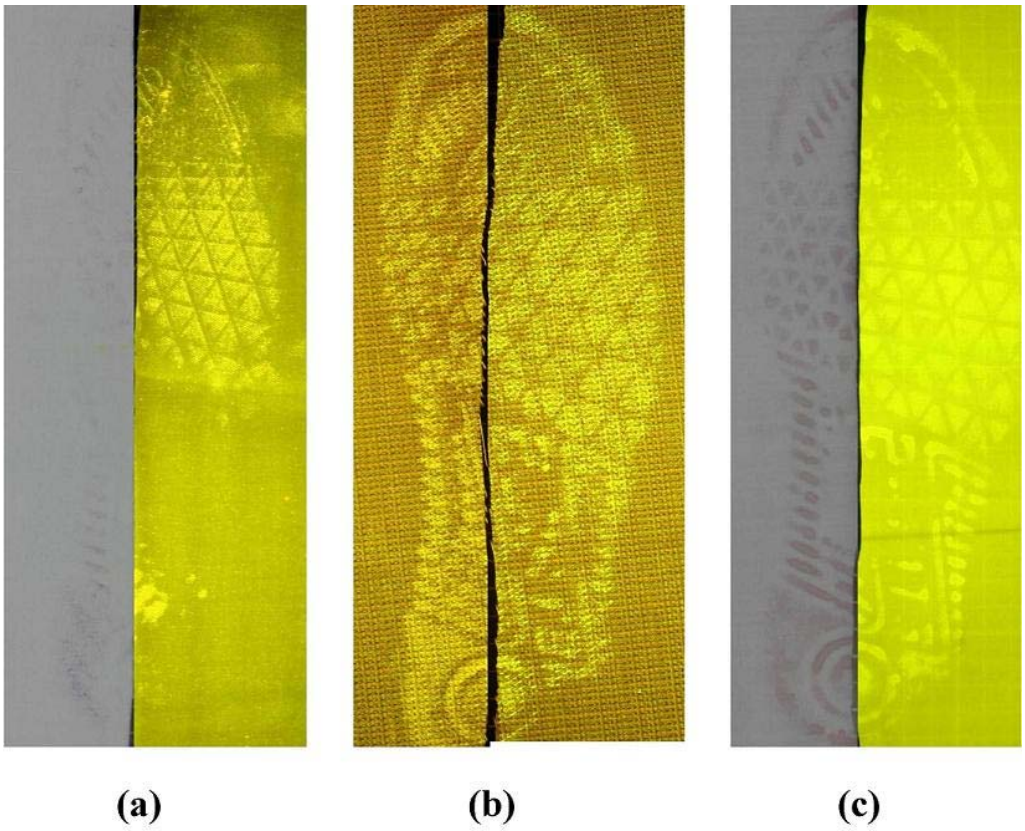


Figure 4.25 – Diminishing series of footwear impressions in urine: (a) NIN-DFO on white cotton 2nd impression; (b) DFO-DMAC on patterned cotton 1st impression; (c) NIN-DMAC on white nylon/lycra 1st impression

4.3.6 Ageing Study and Sequential Treatment

The ageing study and sequential treatment investigation were carried out together. Footwear impressions in urine were prepared as described previously and left to age for two months. Three repeat impressions on white cotton, patterned cotton and white nylon/lycra respectively were treated with ninhydrin, DFO and DMAC. Sequential treatments considered were as follows: DFO-NIN, DFO-NIN-DMAC, NIN-DFO and DMAC-DFO-NIN. Sequential treatment of DFO and ninhydrin is well established for fingerprints where the use of ninhydrin after DFO has been reported to improve enhancement. Consequently the sequence DMAC-NIN-DFO was not considered.

After ageing the urine impressions for two months, DFO provided excellent enhancement detail with a visual pink-magenta colour that was brighter than that achieved with the one-week old impressions. DFO enhancement on patterned cotton could be observed readily and is illustrated in figure 4.26. The visual enhancement obtained appeared sharper than the subsequent fluorescent enhancement. DFO enhancement of aged impressions revealed a weak pink-magenta colour (figure 4.27) which was further enhanced with fluorescence.

(a) (b) (c)

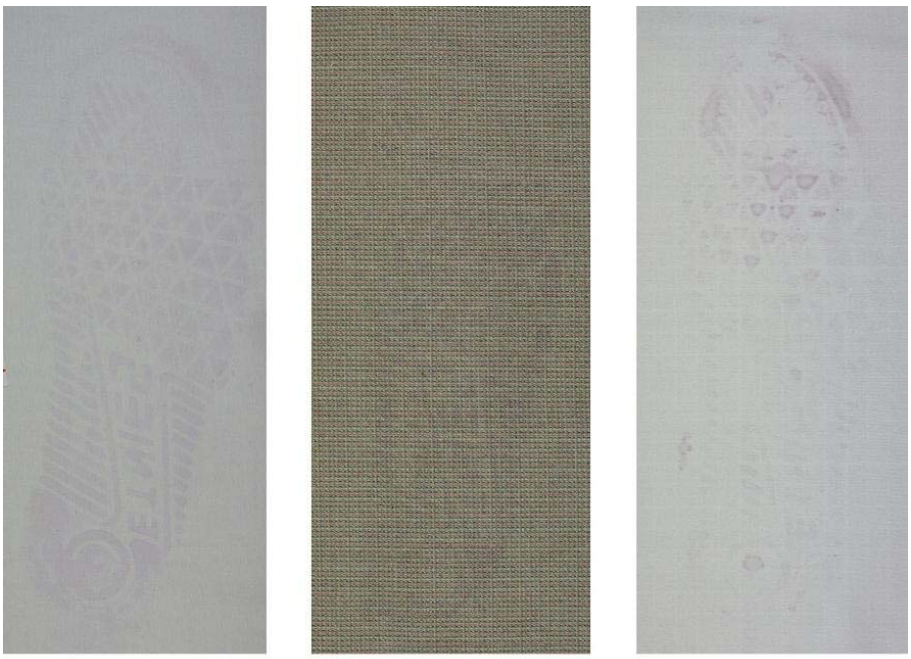
Figure 4.26 - Enhancement of a footwear impression in urine on patterned cotton: (a) two month old urine impression; (b) DFO enhancement under white light; (c) DFO enhancement using a Quaser 40 green excitation source

(a) (b) (c)

Figure 4.27 – Enhancement of two month old footwear impressions in urine with DFO: (a) white cotton; (b) patterned cotton; (c) white nylon/lycra

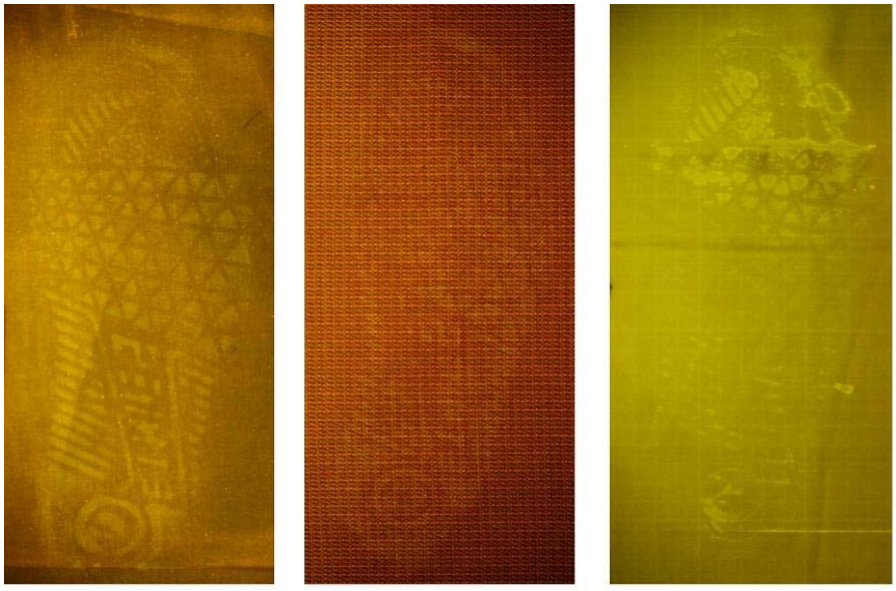
The one week old impressions required fluorescence enhancement as the visual red enhancement was weak (figure 4.18) whereas ninhydrin enhancement of the two month old impressions was weaker than one week old impressions. The impressions could be very weakly visualised on white cotton and nylon/lycra, however, the impressions on the patterned cotton provided poor contrast as shown in figure 4.28.

Similar to ninhydrin, enhancement of two month old urine impressions with DMAC was poor even when fluorescence was used. This was the case for each of the fabrics studied with the exception of white cotton (figure 4.29).



(a) (b) (c)

Figure 4.28 - Enhancement of two month old footwear impressions in urine with ninhydrin: (a) white cotton; (b) patterned cotton; (c) white nylon/lycra



(a) (b) (c)

Figure 4.29 - Enhancement of two month old footwear impressions in urine with DMAC using a Quaser 40 green excitation source: (a) white cotton; (b) patterned cotton; (c) white nylon/lycra

The best sequential treatment was observed to be DMAC-DFO-NIN. There was a major improvement in the enhancement of the impression when DFO was used after DMAC, however, the use of ninhydrin after DFO did not further enhance the urine impressions (figure 4.30).

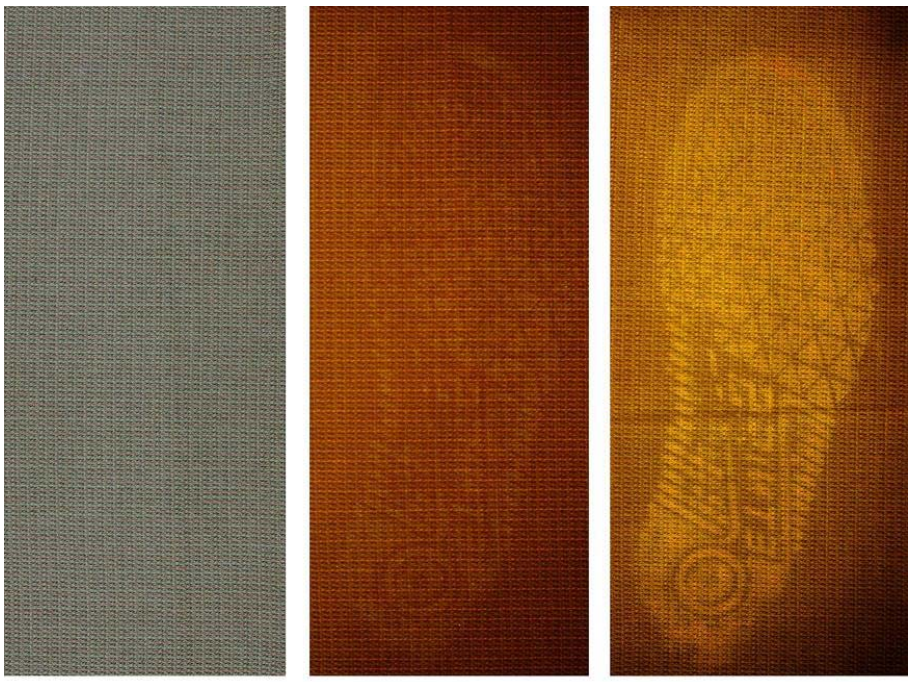
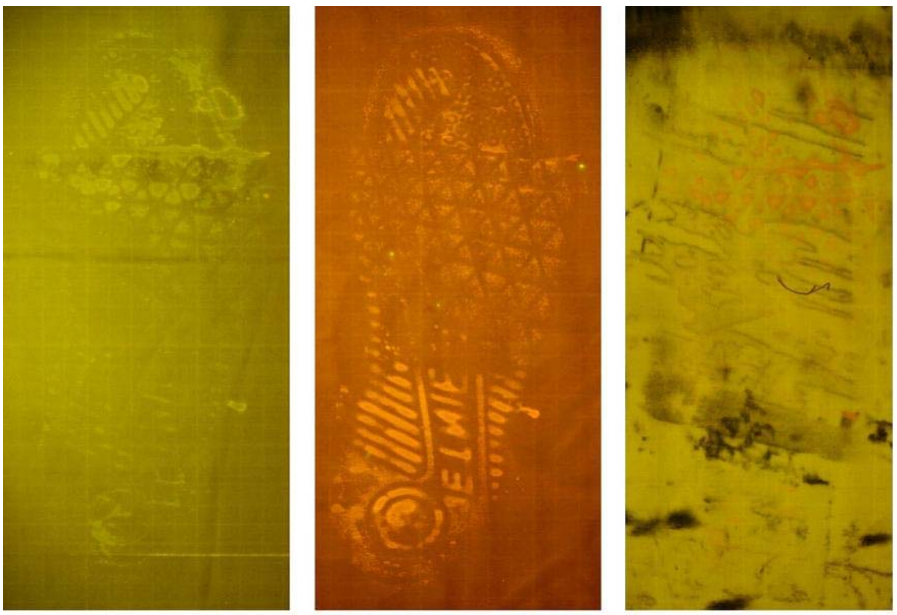


Figure 4.30 – Sequential enhancement of a footwear impression in urine on patterned cotton: (a) 2 month old urine impression; (b) DMAC enhancement under green light; (c) DFO enhancement under green light

Additionally, when DFO was used after DMAC on white nylon/lycra, background staining developed to obliterate the enhanced impression as shown in figure 4.31. It is postulated that this is due to the interaction of the chemical reagents with the polymers in the fabric. As a consequence, for nylon/lycra, the use of DFO-NIN-DMAC might be more suitable.

In general, the use of DFO alone developed the best impressions across the fabrics examined rather than when used in a sequential treatment and this is in agreement with the work of Lee *et al.* [42]. In this study, the use of DMAC after DFO and ninhydrin did not develop any additional detail (figure 4.32).



(a) (b) (c)

Figure 4.31 – Enhancement of two month old footwear impressions in urine on white nylon/lycra: (a) DMAC enhancement under green light; (b) DFO enhancement under green light; (c) DMAC enhancement followed by DFO enhancement under green light

(a) (b)

Figure 4.32 - Enhancement of two month old footwear impressions in urine: (a) DFO enhancement (b) DFO followed by DMAC

4.3.7 Chemical Enhancement of Individual Characteristics

None of the 8 individual characteristics were enhanced after chemical treatment with ninhydrin, DFO, 1,2-IND and DMAC despite having excellent fine detail in some of the impressions as previously illustrated. This may be because of the nature of the interaction between the fabric and contaminant where the urine seeped into the fabric matrix and as a consequence limited the enhancement. Nonetheless, the enhancement obtained could potentially be useful in determining the brand and the size of the shoe.

4.3.8 Mini Case Study

A mini case study to assess the potential enhancement of footwear impressions in urine in a realistic situation was also examined. The test area chosen was a male washroom within a bar on a busy evening. This area was being frequently used during the evening of the test. After walking through the test area a clean piece of white cotton material was stamped upon. This process was repeated to provide three test samples in total. The test fabric was retrieved, packaged in paper bags and stored for one week prior to analysis. After one week, a footwear impression in urine was weakly visible on the samples. Enhancement by DFO greatly improved the quality of the impression in all cases and is illustrated in figure 4.33.

(a) (b)

Figure 4.33 - Enhancement of one week old footwear impression in urine during a case scenario: (a) after one week; (b) DFO enhancement under white light

4.4 Conclusion

This part of the study has comprehensively explored the enhancement of impressions made in urine on the various test fabrics. It has reasserted the fact that urine from different individuals can exhibit different fluorescent properties but that, in general, strong fluorescence is observed by using a violet/blue excitation source (350-469nm) and viewing with a yellow filter (476nm).

It has been demonstrated that four reagents, 1,2-IND, ninhydrin, DMAC and DFO all provide some enhancement of the impressions to varying degrees and in some cases even after a period of two months post deposition. Of these DFO provided the best enhancement overall with no tangible advantage observed when the reagents are used in sequence.

Enhancement was only possible on lighter coloured fabrics with the more porous fabrics (cotton and patterned cotton) providing the best results. It is suggested that the presence of dyestuffs or other chemical products may inhibit the fluorescence of urine and the fluorescence of the chemically enhanced urine stains.

DFO, ninhydrin and 1,2-IND are versatile chemical enhancement reagents as they will also enhance impressions in blood and as such their potential as general screening reagents for a second body fluid is most advantageous. Furthermore, it has been demonstrated that non-destructive lighting techniques can provide a suitable means of enhancing impressions in urine and that the use of alternative or oblique lighting should be performed first before any chemical treatment.

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Chapter 5: Chemical Enhancement of Soil-Based Footwear Impression on Fabric

5.1 Introduction

Soil is "the collection of natural bodies in the earth's surface, in places modified or even made by man of earthy materials, containing living matter and supporting or capable of supporting plants out-of-doors" [1]. There are a wide range of soils with different chemical, physical and biological characteristics including mineralogy, elemental concentrations, pH and organic matter content. Commonly available enhancement techniques for muddy footwear impressions normally target iron or calcium ions [2-10]. It is thus hypothesised that the efficiency of the enhancement techniques will differ with the type of soil (and their associated chemical characteristics) adherent to the fabric. The presence of iron compounds in soil imparts colour and indicates drainage characteristics and weathering conditions of the soil [11]. Calcium is abundant in soils as carbonate, phosphate, silicate, fluoride and sulphate salts, however, is typically deficient in very acidic and sodium-rich alkali soils [11].

The objective of this work was to compare the ability of various enhancement reagents to enhance footwear impressions in soil made on fabric. In this way, it was postulated that the various enhancement techniques suggested in the existing literature could be linked to specific characteristics of the soil. In each case the soil used was chemically characterised so that its elemental composition was known.

5.1.1 Enhancement Techniques which React with Metal Ions

The techniques used for enhancement of footwear impressions on soil or dust are classified by their targeting compounds as presented in table 5.1. Most of the enhancement techniques rely on a reaction occurring between the reagents and metal ions present in the soil with reactions with Fe^{3+} predominating.

Table 5.1 – Potential enhancement techniques for soil-based footwear impressions

Reaction with metal ions	Reaction with other components
Ammonium Thiocyanate	Safranin
Potassium Thiocyanate	Physical Developer
Potassium Ferrocyanide	Oil Red O
Phenanthroline Hydrosulfite	Aluminium Test
Ammonium pyrrolidinedithiocarbamate	Phosphorus Test (Ascorbic Acid Method)
2,2'-Dipyridil	Bromophenol Blue
8-hydroxyquinoline	Bromocresol Green
Tetramethylbenzidine	Alizarin Red S (CI 58005)
	DFO / DFO and gelatin lifting

Someha [9] describes five chemical techniques (potassium thiocyanate, potassium ferrocyanide, phenanthroline hydrosulfite, ammonium pyrrolidinedithiocarbamate and 2,2'-dipyridil) which target iron within the soil and which demonstrated successful enhancement on muddy footwear impressions in Japan where the soil has a mean average iron content of 10%. All five techniques utilised hydrochloric acid to liberate ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions. It has been reported that these techniques offered vibrant coloured enhancement [9].

5.1.2 Ammonium Thiocyanate and Potassium Thiocyanate

Ammonium and potassium thiocyanate are known to react with ferric iron (Fe^{3+}) and these reagents are commonly used to enhance muddy footwear impressions on surfaces to improve contrast [12]. The reaction with Fe^{3+} produces a reddish brown colour. Studies evaluating these two compounds determined that they both work equally well in enhancing dusty footwear impressions on plywood and brown paper [13]. Some preference has been suggested for ammonium thiocyanate because of its ease of application (mix and spray) over potassium thiocyanate which requires an additional separation of layers [3]. Working solutions of both reagents have a shelf life of about five weeks [13]. The reaction of iron with thiocyanate ions is:

$$[Fe(H_2O)_6]^{3+} + SCN^- \rightarrow [Fe(SCN)(H_2O)_5]^{2+} + H_2O$$

5.1.3 Potassium Ferrocyanide

The reaction of potassium ferrocyanide with Fe^{3+} produces a dark blue precipitate (Prussian blue). Potassium ferrocyanide also reacts with ferrous iron (Fe^{2+}) to form a white precipitate which is then oxidised quickly in air to give a blue colour [14]. The reaction of potassium ferrocyanide with iron is:

$$K^+ + Fe^{3+} + [Fe(CN)_6]^{4-} \longrightarrow KFe[Fe(CN)_6]$$

5.1.4 Ammonium Pyrrolidinedithiocarbamate

Ammonium pyrrolidinedithiocarbamate (APD), also known as ammonium pyrrolidinecarbodithioate ($C_5H_9NS_2 \cdot NH_3$), reacts with Fe^{3+} to give a black colour which fades quickly, although improved results have been reported on non-porous articles [4]. There is no toxic or odours emitted during application as is the case with thiocyanates. The reaction of APD with iron is:

$$3C_5H_9NS_2 \cdot NH_3 + Fe^{3+} \longrightarrow Fe(C_5H_9NS_2)_3 + 3NH_3$$

5.1.5 2,2'-Dipyridil

2,2'-dipyridil is bipyridine isomer which can be used for the colourimetric analysis of iron. Ascorbic acid is added to reduce Fe^{3+} to Fe^{2+} to react with 2,2'-dipyridil resulting in a red complex as illustrated in figure 5.1.

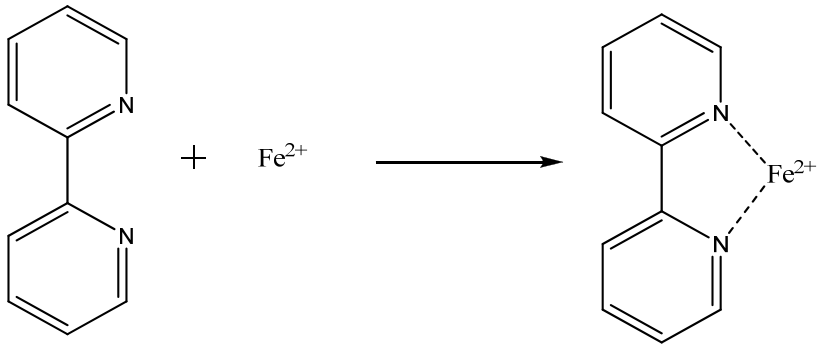


Figure 5.1 – The reaction of 2,2'-dipyridil with ferrous iron

5.1.6 Phenanthroline Hydrosulfite

Ferric iron is reduced from Fe^{3+} to Fe^{2+} by sodium hydrosulfite. The reaction of Fe^{2+} with phenanthroline, illustrated in figure 5.2, produces a red coloured complex [15].

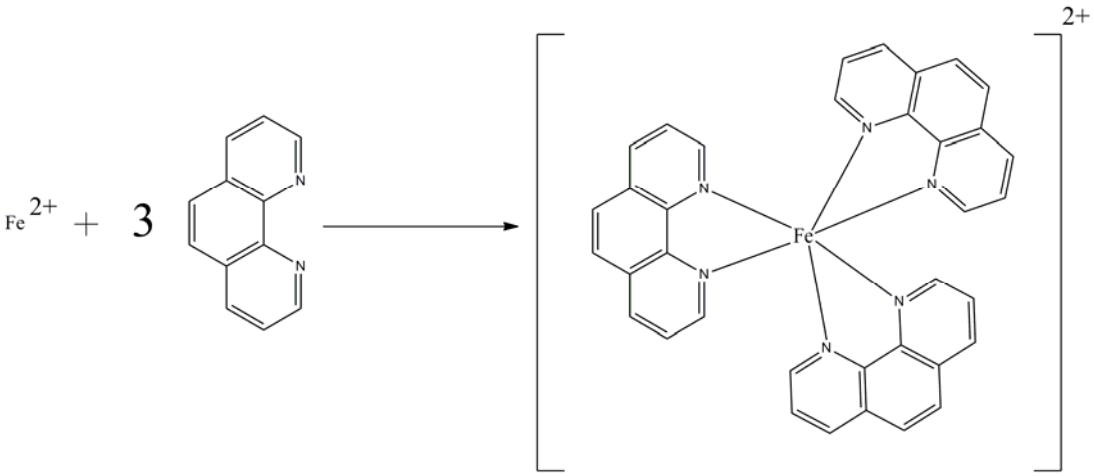


Figure 5.2 – The reaction of Fe^{2+} with 1,10 phenanthroline

5.1.7 8-Hydroxyquinoline

8-hydroxyquinoline, presented in figure 5.3, reacts with trace amounts of calcium, magnesium, iron, aluminium and other metal ions [12]. 8-Hyrdoxyquinoline is a multidentate ligand which acts as a chelating agent, combining with metal cations to form stable chelated complexes [16].

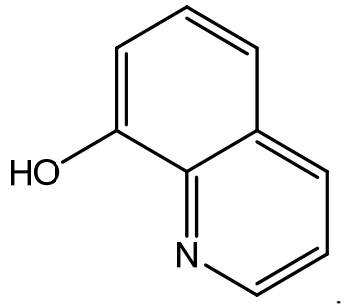


Figure 5.3 - The chemical structure of 8-hydroxyquinoline

8-hydroxyquinoline is not a common reagent for footwear impression enhancement but has been used in certain instances to enhance latent impressions on raw wood. A positive reaction results in fluorescence which is detectable in the ultraviolet (UV) light range. One report explains how 8-hydroxyquinoline was utilised to develop a footwear impression on a piece of compressed particle board when normal photographic enhancement techniques did not reveal any impression [17]. 8-hydroxyquinoline treatment revealed excellent detail when viewed under long wave UV light. Other research articles have indicated different formulations and applications and suggest that 8-hydroxyquinoline might be useful for examining large floor areas at crime scenes since it can be sprayed onto the area of interest [18].

5.1.8 Tetramethylbenzidine (TMB)

Tetramethylbenzidine, TMB, has been successfully used as a peroxidase reagent for the enhancement of footwear impressions in blood on porous surfaces [19] and as a presumptive test for blood [20]. It reacts with iron in haemoglobin in a similar way to other peroxidase reagents such as LCV, LMG and fluorescein. The same reagent formulation can be applied for the enhancement of muddy footwear impressions [10, 21]. Hemastix® is a commercially available TMB strip test which eliminates the use of dangerous TMB solutions as TMB is believed to be carcinogenic, although to a lesser degree than benzidine and o-tolidine [22, 23]. Hemastix® was recently classified as a specific test that is easy to transport and apply at crime scenes and does not interfere with subsequent DNA analysis [24]. Figure 5.4 shows the catalytic reaction of TMB in the presence of iron to give a blue-green product.

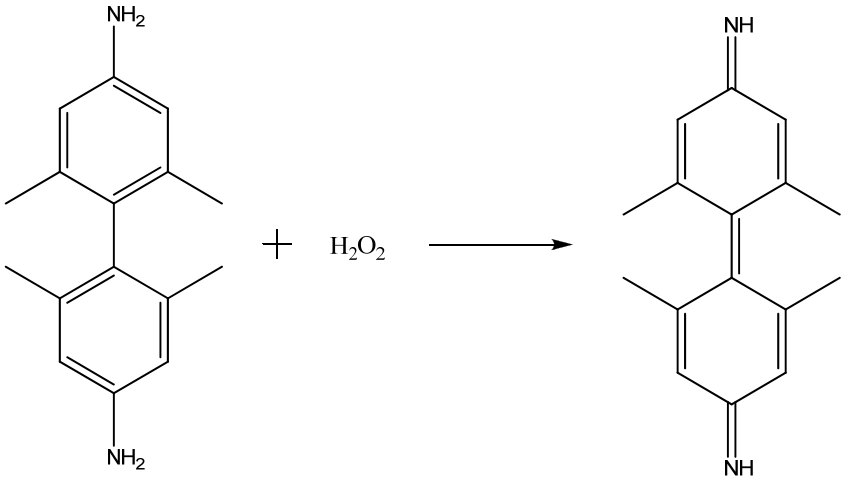


Figure 5.4 – Catalytic oxidation of TMB with hydrogen peroxide

5.2 Enhancement Techniques which React with Calcium

Various techniques are known to react with calcium ions and have been used for staining calcium deposits in human tissues [25]. Theeuwen *et al.* [10] have also classified these techniques as reacting with amino acids.

5.2.1 Alizarin Red S

Alizarin red S, presented in figure 5.5, can act as a chelating agent for several ions, including calcium. A red complex is formed from the reaction of alizarin red S with calcium [26]. Alizarin red S has been successfully used in Israel for the enhancement of footwear impressions in dust [5]. It was reported that a 1% solution of alizarin red S in distilled water resulted in complete red background staining on paper, potentially due to the calcium carbonate content in the paper. This drawback was removed by using a saturated solution of alizarin red S in ethanol [5]. Bodziak [12] highlights the facts that soil, dust and mud have different compositions in different parts of the world and some techniques might not work in every country. Israel for example has rich calcium carbonate soils.

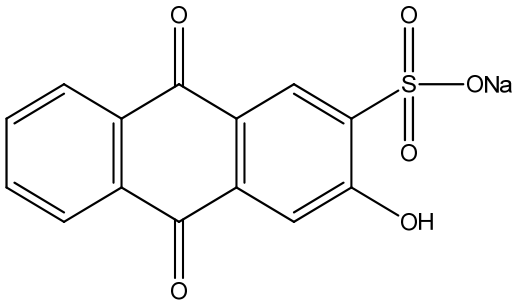


Figure 5.5 - The chemical structure of alizarin red S

5.2.2 Bromophenol Blue

Bromophenol Blue (BPB), presented in figure 5.6, is a pH indicator and was examined as a possible chemical reagent for the enhancement of dusty footwear impressions by Glattstein *et al.* [6]. BPB exhibits a colour change from yellow at a pH of about 3.0 to blue at a pH of about 4.6. Similar to the previous thiocyanate enhancement techniques, this process was specifically developed for the enhancement of footwear impressions. BPB reacts with trace amounts of calcium carbonate ($CaCO_3$), commonly found in soil, to exhibit an immediate colour change from yellow to an intense blue colour.

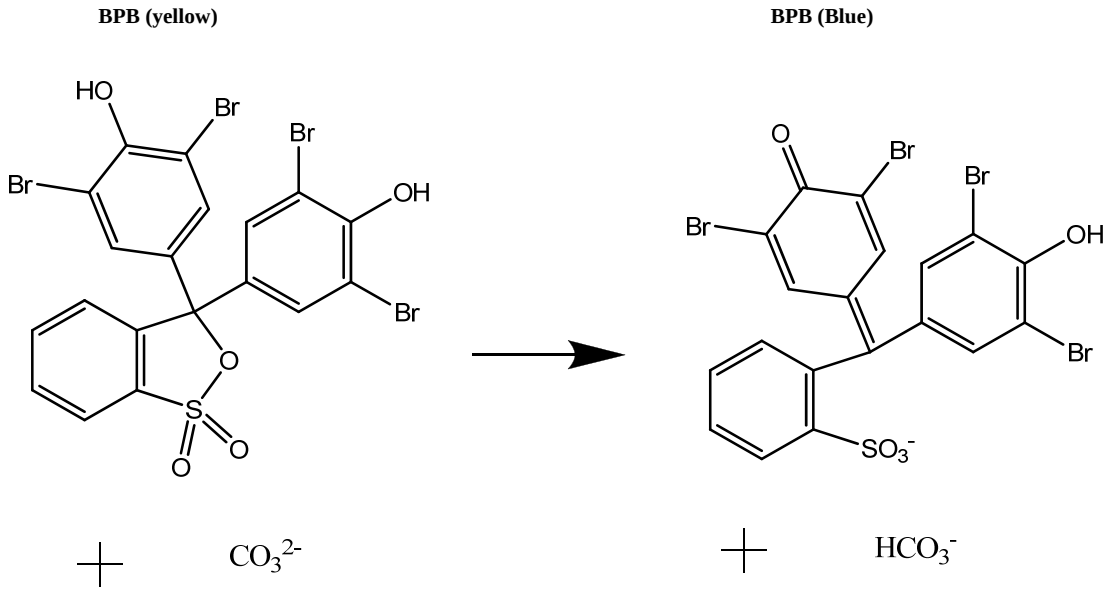


Figure 5.6 - Chemical structures of BPB before and after reaction with carbonate ions

In some cases, further enhancement may be required and the impression can be exposed to water vapour from a boiling kettle to provide a medium for achieving a quick equilibrium with respect to pH [6]. Water vapour exposure must be controlled carefully since the surface background can also change colour, masking the developed impression. Different experiments have been successful without the need of water vapour due to difference in relative humidity or the environment [12]. It has been reported that BPB is more effective reagent than ammonium thiocyanate for the enhancement of impressions in dust in various regions in Israel [6], however, the technique is limited when insufficient carbonates are present in the dust. Further research by Shor *et al.* [8] illustrated that a better contrast and enhancement can be achieved by lifting a dusty footwear impression with a white adhesive followed by subsequent treatment with BPB.

In the UK, the effectiveness of BPB was evaluated for targeting carbonate ions in chalky/alkaline soils commonly found in the south east of the country [27]. However, the results were disappointing for dusty footwear impressions prepared by different soils and treatment with BPB added no advantage over visual examination of the impression. This enhancement technique was also tested on a number of different contaminants including soft drinks, condiment sauces, milk and detergents. Slight enhancement was observed for footwear impressions made in brown sauce, milk and washing-up liquid [27].

5.3 Reaction with Other Components

1,8-diazafluoren-9-one (DFO), physical developer (PD), oil red O (ORO), superglue, iodine, safranin and vacuum metal deposition may all have the potential of enhancing muddy footwear impressions [10, 21]. These techniques target different components such as amino acids, lipids, oil deposits and as well as other inorganic ions such as chlorides. Although these techniques are commonly used for the enhancement of latent fingerprints, there is little or no information in the literature about their use for the enhancement of footwear impressions.

5.3.1 1,8-Diazafluoren-9-one

1,8-diazafluoren-9-one (DFO), illustrated in figure 5.7, has been utilised to enhance wet or muddy footwear impressions on paper as well as developing impressions in blood [28]. No enhancement has been reported when DFO is applied directly to a wet or muddy impression, however, considerable enhancement was achieved when DFO was applied after the impression had been lifted and activated with a black gelatin lifter [29]. Velders [29] suggests that the activation is probably due to the presence and transfer of amino acids from the gelatin layer to stimulate the paper fibres. This phenomenon is explained further by Theeuwen *et al.* [10] where the authors suggest that "the fibres may have been activated by a combination of water present in the mud and mechanical damage caused by grains of sand, both making the structure of the fibres more open and thus enabling amino acids to migrate" [10]. Lifting allows for the activation of both the visible and the latent parts of the muddy shoeprint, as well as removing excess dirt/mud for better contrast [29]. The substrate was treated with DFO in the same procedure as with fingerprints and heated for ten minutes at 100^{o}C [29, 30]. The fluorescing DFO treated impression, with an excitation wavelength of around 535 nm, could then be photographed. This technique offered the best enhancement for muddy footwear impressions on porous substrates compared to other techniques [10].

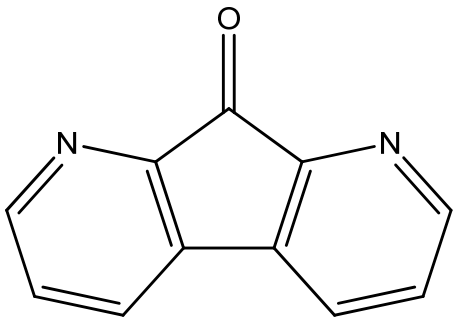


Figure 5.7 - The chemical structure of DFO

5.3.2 Physical Developer

Physical Developer (PD) is a fingerprint enhancement technique for porous surfaces developed in the UK by the Atomic Weapons Research Establishment (AWRE) in collaboration with CAST [31]. The technique was developed for use when techniques such as ninhydrin failed to develop or enhance latent impressions. This technique is currently the most accepted technique suitable for developing latent impressions on wet, porous substrates [32, 33]. Amino acids dissolve in water and as a result, treatment with amino acid targeting reagents such as ninhydrin and DFO may not yield positive results. PD is based on a photographic physical developer where the process consists of an aqueous solution of a buffer, a ferrous/ferric redox system, a cationic surfactant (detergent) and silver ions [12, 30, 32-38]. Champod *et al.* [35] describe the PD solution as 'a delicate balance of ferrous, ferric and silver ions stabilised by the presence of citric acid and a surfactant'. The surfactant prevents the premature deposition of silver metal and in the UK, liquid Symperonic-N is utilised. Unfortunately, the production of this chemical has been terminated, however, CAST has bought all of the remaining stock, and distribute it to police forces around the UK when needed [39].

The PD technique targets the water-insoluble part of the latent print residue which includes mostly lipids (fats, oils and sebaceous material) but also water resistant proteins, lipoproteins and water-soluble components, such as amino acids and salts, that get trapped in the lipids [33, 35]. PD has also been incorporated into a sequential treatment after DFO and ninhydrin processing since it complements DFO and ninhydrin [40].

The first step in the PD process is to wash the item to be examined in maleic acid to remove any trace contaminants or debris. As a consequence of immersion, metallic silver is deposited on the surface of the item, preferentially in areas containing oily and fatty deposits (for example in latent fingerprints) to develop a dark grey colour. The last step involves thorough rinsing with tap water. Bodziak [12] explains that excessive minerals potentially present in tap water from certain localities could compromise developed prints and as such distilled water is commonly used. Unserrated plastic forceps are also recommended as metal forceps can result in a

deposition of silver on the item in areas where there has been contact with the forceps. If the developed prints or impressions show poor contrast, further enhancement can be obtained by immersion in a 50% solution of household bleach (hypochlorite) for two to three minutes [37]. This process helps to remove stains from ninhydrin and darkens the PD print for better contrast through an oxidative process [41]. This technique has also been successful in enhancing latent footwear impressions on porous substrates [12, 37, 42]. Beecroft [43] also reported that enhancement of footwear impressions developed with PD can be further improved by fuming with iodine.

The main advantages of PD are that it can develop latent impressions on items that have been wet or have been subjected to high humidity. It also works well with aged impressions and has been demonstrated to successfully develop thirty-year old prints on test materials [33]. Furthermore, prior treatment with iodine, ninhydrin or potassium thiocyanate does not affect the PD enhancement process [12].

The process is, however, time consuming, delicate and expensive. Commercially available physical developer kits have worked quite well and drastically reduced the preparation time and overall expenditure. Ramotowski [44] compared four different kits to a solution of physical developer prepared from its component chemicals and also evaluated commercially available distilled white vinegar as a potential substitute for maleic acid. The results illustrated that PD kits produced comparable ridge detail on developed fingerprints to freshly prepared PD solution [33, 34, 45, 46]. Other research [47] confirmed the comparable qualities of commercial and non-commercial physical developer solutions. In addition, the U.S. Secret Service (USSS) is currently researching the development of a non-silver based physical developer to provide a fluorescent quality to the developed prints [48].

5.3.3 Oil Red O (ORO)

Until recently, physical developer has been the only technique capable of developing latent impressions on porous surfaces that have been wetted or subjected to high humidity. Oil Red O (ORO), illustrated in figure 5.8, is a lysochrome dye used in

biology for targeting soluble lipids. It is a red powder with a maximum UV absorption at 518nm [25] and recent research suggested that ORO is a technique which is much less complex and expensive than PD and provides results of impressive clarity and intensity for latent impressions on wetted surfaces [49-51]. A suitable sequential treatment has been suggested as DFO - ninhydrin – ORO - PD for optimal enhancement results [52].

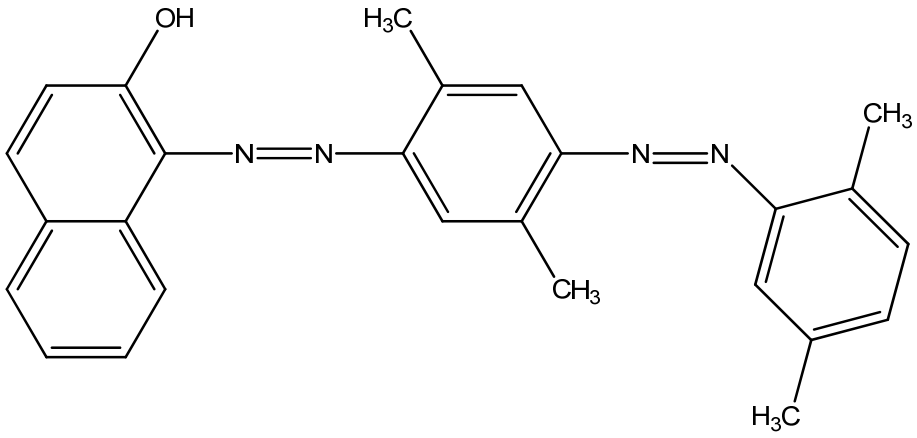


Figure 5.8 - The chemical structure of ORO

The application of ORO occurs in three stages which are colouration, neutralisation and drying. A buffer, consisting of sodium carbonate and nitric acid, is used to neutralise the alkaline colouring solution to make the treated items stable and less fragile [49].

ORO has been evaluated and compared to PD by a number of researchers including the USSS, the UK Forensic Science Service and the University of Technology, Sydney in collaboration with the Australian Federal Police (AFP) [49, 50, 53-55]. Research by the USSS demonstrated that the water insoluble part of the latent impression could be divided into two groups consisting of labile and robust components [53]. The conclusions from this research indicated that the labile group consisted of components that were detected by ORO or other lipophilic reagents and that the developed prints were removed by solvents of low dielectric constants (defined as the ratio of the permittivity of a substance to the permittivity of free space). The robust group consisted of components that were detected by PD,

remained on paper for decades and were not removed by solvents of low or high dielectric constants.

ORO is recognised as a complimentary technique to physical developer which has the advantages of being less expensive and less complicated to use. ORO can be used on dry porous items or those that have been exposed to water [49, 54] and works best with latent impressions which are fresh, as the quality declines significantly for impressions older than four weeks [55]. Figure 5.9 and figure 5.10 illustrate a comparison of ORO and PD with varying fingerprint age and the effect of varying exposure to water respectively [55].

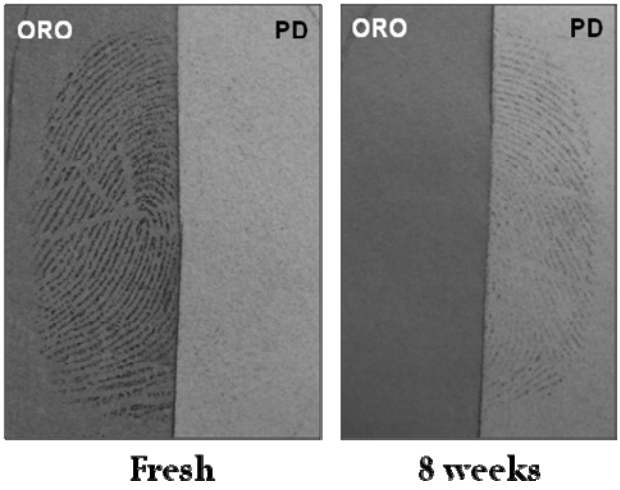


Figure 5.9 - PD vs ORO: fresh vs 8 week old fingerprint (reproduced from [55])

ORO can also be used in sequence with other development techniques where DFO and ninhydrin are followed by ORO and then by PD. However, the solvents used in DFO and ninhydrin may also remove some of the constituents targeted by ORO, making its use in sequential processing less beneficial [56]. There is no information in the literature relating to the use of ORO for the enhancement of footwear impressions.

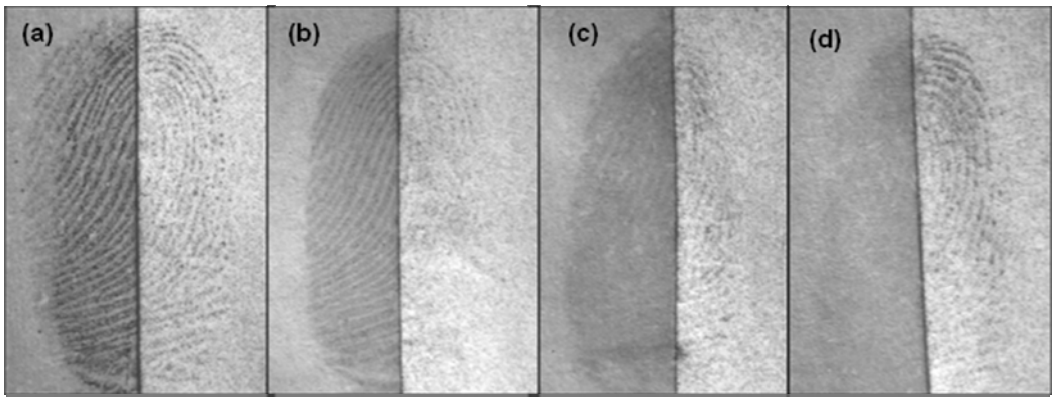
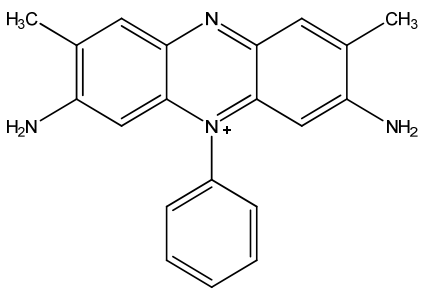


Figure 5.10 - Fresh fingerprints on Xerox white copy paper (left – ORO, right-PD) (a) No water pre-treatment (b) Dip in water (c) 1 hour in water (d) 24 hours in water (reproduced from [55])

5.3.4 Safranin O

Safranin O, (also known as Basic Red 2) $C_{20}H_{19}ClN_4$, is a biological red stain commonly used for histological purposes. Horobin and Kiernan [25] and the Biological Stain Commission [57] define Safranin O as a mixture of dimethyl Safranin and trimethyl Safranin whereas Gurr [58] defines it as just dimethyl Safranin. Figure 5.11 shows the chemical structure of dimethyl and trimethyl Safranin.

Dimethyl Safranin



Trimethyl Safranin

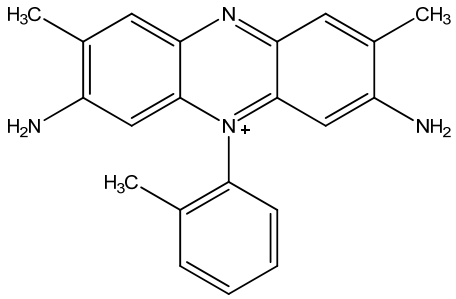


Figure 5.11 - The chemical structure of dimethyl and trimethyl Safranin

This staining method has been successfully used by Velders to enhance wet and muddy footwear impressions on glass, plastic, flooring and other smooth non-absorbing surfaces [59]. The literature does not explain what this chemical targets although it has been suggested that it may target oils and greases [18]. After photography, the procedure described by Velders involves lifting the print with a black gelatin lifter followed by the pouring of Safranin O (1g of Safranin O in 1 litre of distilled water) over the impression [59]. Safranin O is then left in contact with the impression for about two minutes before being rinsed with distilled water. An air gun is then utilised to remove any traces of water. A white gelatin lifter is then applied to the impression and left in situ for several minutes. Once removed, the gelatin lifter is photographed using a green light source 473-548nm and viewed with a Schott 549nm filter (if using a Mason Vactron Quaser 40) [59].

This technique was reported to be the most successful technique for the optimal enhancement of muddy footwear impressions on non-porous substrates [10]. This dye can be used on untreated footwear impressions or as a dye for impressions treated with cyanoacrylate (superglue). Mazzella and Lennard [60] reported that cyanoacrylate prints treated with Safranin O absorbed in the blue-green spectrum region at 495 nm while giving a strong orange-red luminescence at 585 nm. The authors highlight the fact that the developed prints showed no indication of instability over time and that this technique can offer advantages on highly luminescent surfaces that interfere with conventional stains such as Basic Yellow 40 (BY 40) and Rhodamine 6G. Other uses of Safranin O in forensic science include its use as a dye for pollen [61].

5.3.5 Ascorbic Acid

The American Public Health Association (APHA) has suggested the use of an ascrobic acid method for the detection of phosphorus in water [62]. Since phosphorus can be abundantly present in soils, this test might also be useful for the enhancement of muddy footwear impressions [11]. The detection of phosphorus requires the conversion of phosphorus to the more soluble orthophospate compound by means of

a strong acid such as sulfuric acid. There are several techniques for the detection of phosphorus. One of the most common techniques for the colormetric detection of phosphorus involves ammonium molybdate and antimonyl tartrate reacting in an acid medium with orthophospate to form an intensely coloured antimony-phospo-molybdate complex which is reduced to an intensely blue-coloured complex by ascorbic acid. The colour observed is proportional to the phosphorus concentration [62]. Elements such as barium, lead and silver can potentially interfere with the complex by forming a precipiate. The interference from silica produces a pale-blue complex. This method has not yet been reported in the literature for the enhancement of muddy footwear impressions.

5.3.6 Pyrocatechol Violet

Pyrocatechol violet (PCV), illustrated in figure 5.12, produces a blue colour when a chelating complex is formed with aluminium [63, 64] which can be present in soils and mud. The complex formed is fairly soluble in water and absorbs light at a high wavelength around 580-585 nm [65]. PCV has not been reported in the literature for the enhancement of muddy footwear impressions.

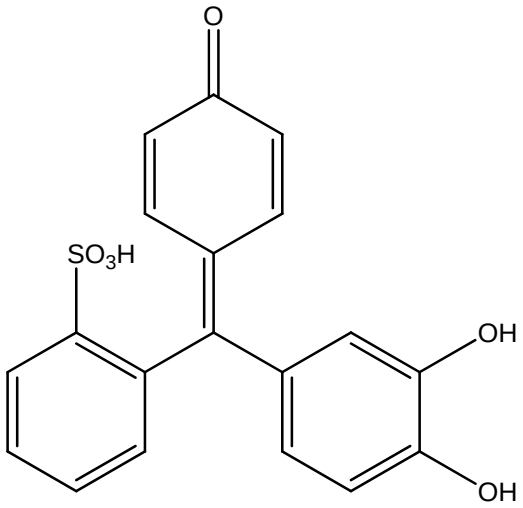


Figure 5.12 - The chemical structure of PCV

5.3.7 Other Suitable Methods

Other methods such as cyanoacrylate (superglue) fuming, iodine fuming and vacuum metal deposition might offer suitable enhancement of footwear impressions, however, reports in the literature for the enhancement of muddy footwear impressions using these techniques is limited.

In 1987, Hueske and Erfert [66] reported the enhancement of a dusty footwear impression on glass by superglue fuming followed by dusting with black fingerprint powder. It is believed that the dust particles collected oxygen and moisture necessary for the initiation of cyanoacrylate polymerisation [66, 67]. Research based on casework [68] demonstrated the successful enhancement of wet footwear impressions, such as those in blood and mud, by superglue fuming followed by the application of a contrasting colour fingerprint powder. Substrates in this study ranged from glass, wood and different types and textures of floor covering. Cushman and Simmons [69] carried out further research when they tried to enhance footwear impressions on clear plastic sheets by superglue fuming after subjects had walked in an office area or walked outside through grass. Better quality enhancement was obtained for impressions made after walking through grass. This can be explained because water can act as a catalyst for the polymerisation of cyanoacrylate [69]. Successful enhancement by superglue fumes and fingerprint powder was also reported by Petraco *et al.* [70] with soil/grass residue impressions on newsprint. In another case and related research by Forensic Science Northern Ireland (FSNI) [71], latent footwear impressions in grease and oil on plastic bags were developed by superglue fumes followed by staining with BY40. Development and enhancement was of sufficient quality for comparison and inclusion on the Shoe Impression Comparison and Retrieval (SICAR) database.

A thorough study on the use of superglue fuming for the enhancement of footwear impressions was carried out by the New South Wales Police Service in Australia [72]. Footwear impressions of wet and dry origin on clean and dust covered substrates were prepared, where the substrates included items such as glass, ceramic tiles, vinyl tiles, metal, wood and plastic. Five different types of enhancement were compared: fingerprint powders alone, superglue fuming followed by the application

of fingerprint powder or Rhodamine 6G and the latter process repeated again where the items to be examined were refrigerated for 15 minutes at 2^0C. Dry impressions yielded poor results for all types of enhancement. All techniques enhanced wet origin impressions, although Rhodamine 6G could not be used on all substrates since it stained the entire background on vinyl tiles, wood and metal. This research showed that the best enhancement method was refrigeration followed by superglue fuming and powdering. It has been suggested that condensation formed on the substrate during refrigeration aided the polymerisation process for better enhancement [73].

Iodine vapour has been used for a number of years in the enhancement of latent fingerprints. The preparation and application of this non-destructive technique is described in the MoFDT and can be used on most porous and non-porous surfaces [30, 36]. Iodine reacts with the fatty and oily residue of fingerprints to form a brown print. The technique is simple but insensitive, especially for fingerprints more than a few days old [30, 36]. Theeuwen *et al.* [10] reported that iodine is not a suitable technique for the enhancement of muddy footwear impressions on porous substrates.

Vacuum metal deposition (VMD) is one of the most sensitive techniques for the enhancement of fingerprints on non-porous surfaces, however, equipment and maintenance costs have negated its widespread use [74]. The possibility of detecting fingerprints on some surfaces by the selective condensation of metals under vacuum was reported in 1968 by Theys [75]. Kent *et al.* [76] suggested the use of gold and zinc as a safer substitute to gold and cadmium with successful results.

5.4 Materials and Methods

5.4.1 Deposition of the footwear impressions and preparation of the test marks

Muddy footwear impressions were prepared in a series of steps where several different methods were tested. Method 1 involved moving and scraping the sole of the item of footwear over dry soil in a tray. The shoe was then tapped to remove any excess solid into the tray before walking on two double ply tissues, soaked with 50mL of distilled water and then stepping onto the fabric. Each time a new impression was prepared, the sole was thoroughly cleaned with soapy water and brush and thoroughly dried with absorbent tissues. Method 2 used the procedure described by Croft *et al.* [4] to prepare a 20% weight by volume mix of soil in water (e.g. 20g of soil in 100mL of water) after sieving through 1cm and 425µm sieves. Method 3 started by sifting the soil material through a 4mm sieve into a tray (up to a depth of about 3cm) to remove any stones and twigs. Excess water was then added to the tray, mixed and allowed to settle for about two hours before decanting. The mixture was then allowed to settle for a further two hours. Method 3 gave repeatable footwear impressions and was utilised for subsequent experiments. After stepping into a slurry, a diminishing series of muddy footwear impressions was prepared from 0 - 10 (11 impressions) using the stamping rig where the 0^{th} impression was the first most substrate loaded impression and the rest of the impressions were prepared without re-loading with the substrate. The fabrics used were as previously described in section 3.2.5 where bovine leather was replaced with plain dyed brown leatherette [KBT259 (C2708) (68) (F10)] purchased from www.fabricuk.com.

Photography of all impressions was performed immediately after the impression was prepared, after 7 days and after enhancement. Photography using oblique lighting and alternative light sources was also attempted.

5.4.2 Positive and Negative Controls

Before the contact between the soil and the sole of the footwear, dry and wet negative controls were carried out for each reagent and fabric surface. A dry negative

control was performed by stamping on the fabric without any soil on the sole of the footwear, ensuring the sole was dry. A wet negative control involved stepping on a distilled water-soaked tissue before stamping on the fabric. These blanks ensured that the staining observed was not due to additives such as plasticisers in the footwear sole, impurities in the water or fabric dyes. A positive control was performed for the detection of iron (III) by testing the appropriate reagents on a known ferric chloride ($FeCl_3$) solution and observing any subsequent colour change. Positive controls for other elements were not performed.

5.4.3 Soil Sampling

The mineralogical composition of soils differs considerably across a given landscape and different soils can vary significantly in their elemental compositions [77]. In order to explore the effects that elemental composition may have on the chemical enhancement of a soil contaminated footwear impression, four soil types were chosen in consultation with soil geologists at the John Hutton Institute which represented the main different types of soil found in Scotland. In addition to these, a sample of soil recovered from the roadside (under the M8 motorway in Glasgow) was also investigated. Although this sample set is not truly representative of all the characteristics of soils found in the UK, it improves upon previous studies where only one soil or a mix of soils was considered [10].

The four soils sampled for examination were a calcareous soil [from North Berwick], an organic soil [from Cornalees], and two mineral soils [from Kilbirnie (high clay content) and Wemyss Bay (low clay content)], as illustrated in table 5.2. Using the Scottish Soil Knowledge and Information Base (SSKIB) [78], locations were chosen to represent a soil series which had the desired range of parameters. Soils were collected from the top 5mm of ground using a sterile (cleaned with acidic and basic solutions) trowel and stored in a sterile container before transportation back to the laboratory where samples were air dried, sieved and prepared for the deposition of footwear impressions.

Table 5.2 - Soil types used in the study

Soil Type	Soil Area Location	Soil Grid Position	Soil Description
Calcareous	North Berwick	NS 32495, 54282	fine, dark brown, sandy, sticky
Organic	Cornalees	NS 23473, 70754	Grey, rocky
Mineral low clay (10%)	Wemyss Bay	NT 51591 85718	Red/light brown
Mineral high clay (30%)	Kilbirnie	NS 19888, 70600	Brown/black, lumpy

5.4.4 Soil Analysis

5.4.4.1 pH Analysis

The pH (H_2O) was obtained by adding deionised water (45mL) to the appropriate soil sample (15g) before taking a pH reading using a calibrated pH meter. The pH ($CaCl_2$) was performed by adding $CaCl_2$ (5mL of 0.1M) to the previous slurry prior to taking the pH reading.

5.4.4.2 Elemental Analysis

Elemental analysis was carried out by the John Hutton Institute (Aberdeen, Scotland) using an Agilent 7500ce inductively coupled plasma – mass spectrometry (ICP-MS) and a Perkin Elmer 5300DV inductively coupled plasma optical emission spectrometer (ICP-OES). ICP-OES works better for the detection of lower atomic weight elements whereas the ICP-MS is suited for the detection of higher atomic weight elements and also provides lower detection limits of measurement.

The samples were digested by adding concentrated HCl (21mL) to the appropriate dried soil (2g) followed by HNO_3 (7mL). The sample was then refluxed for 2 hours, filtered through a 541 Whatman filter paper and diluted to 100mL.

5.4.4.3 ICP-MS Conditions

Elements analysed by ICP-MS are listed in table 5.3 with the associated tune steps represented in table 5.4.

Table 5.3 - Elements analysed by ICP-MS

Atomic Mass	Element	Tune step
78	Se	1
52	Cr	2
59	Co	2
60	Ni	2
63	Cu	2
66	Zn	3
88	Sr	3
95	Mo	3
109	Ag	3
111	Cd	3
137	Ba	3
195	Pt	3
202	Hg	3
208	Pb	3
75	As	4

Table 5.4 - ICP-MS Operating Conditions

	Tune Step 1	Tune Step 2	Tune Step 3	Tune Step 4
RF power (W)	1400	1400	1400	1400
Carrier gas (L/min)	0.87	0.87	0.87	0.87
Nebuliser pump (rps)	0.09	0.09	0.09	0.09
Spray chamber temp. (°C)	12	12	12	12
Hydrogen/ Helium Flow rate (mL/min)	5	3.5	None	8

5.4.5 Preliminary Enhancement

In total, 17 enhancement techniques were investigated for their abilities to enhance muddy footwear impressions on fabric. These techniques (previously presented in table 5.1) were first tested on impressions prepared from a mixture of soil (100g from each of the four selected soils) on white cotton and nylon/lycra. These two fabrics were chosen for practicality, costs and to observe differences between natural and synthetic fabrics. Techniques which offered vibrant enhancement were investigated further using the remaining fabrics within the study group.

5.4.6 Delivery of Enhancement Technique

Reagents were applied to the footwear impressions either by immersion, pouring or spraying. Preliminary experiments demonstrated that spraying with a very fine atomiser was necessary to avoid diffusion of the original muddy impression. Both an Ecospray® and Preval® spray unit were evaluated and best results were obtained by using the Ecospray® system supplied by Bluestar® as the use of a Preval® unit led to diffusion of the original muddy footwear impression on fabric.

5.4.7 Chemical Formulations

5.4.7.1 Ammonium Thiocyanate and Potassium Thiocyanate [3, 9, 13]

Ammonium and potassium thiocyanate behave very similarly under acidic conditions to give a dark red- brown colour when reacting with Fe^{3+} ions present in soil or dust.

Ammonium thiocyanate formulation: ammonium thiocyanate (3g) (Acros) was dissolved distilled water (15mL). Acetone (120mL) (Sigma) and concentrated nitric acid (8mL) (Riedel-de Haën) were added and the mixture stirred with a magnetic stirrer for a few minutes.

Potassium Thiocyanate formulation: potassium thiocyanate (15g) (Sigma) was dissolved in distilled water (15mL) and acetone (120mL) (Sigma) and stirred thoroughly with a magnetic stirrer. Dilute sulphuric acid (8.5mL) (Sigma) was added slowly to produce a milky mixture which eventually separated in two layers. The top layer was poured into a dark glass bottle.

Treatment of articles: The reagents were lightly sprayed over the impression using the Ecospray®. Controlled spraying was essential to avoid running of the original impression. The impressions were then examined for potential fluorescence using a Mason Vactron 40 Quaser and a green excitation filter (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 549nm filter (1% cut-on point).

5.4.7.2 Potassium Ferrocyanide [9]

Potassium Ferrocyanide formulation:
Solution A: hydrochloric acid (10mL) (Sigma) and ethanol (90mL) (Sigma).
Solution B: potassium ferrocyanide (5g) dissolved in distilled water (100mL).
The solutions had a shelf life of a few months if refrigerated.

Treatment of articles: The articles were sprayed lightly with Solution A using the Ecospray® and allowed to stand for 10-20 seconds. Then, while the sprayed area was still damp, the article was lightly sprayed with solution B ensuring not too spray heavily as this solution contains a large amount of water. Development of a blue colour denoted a positive reaction with Fe^{3+}.

5.4.7.3 Ammonium Pyrrolidinedithiocarbamate [9]

Ammonium pyrrolidinedithiocarbamate formulation:
Solution A: hydrochloric acid (1mL) (Sigma) and ethanol (9mL) (Sigma).
Solution B: ammonium pyrrolidinedithiocarbamate (1g) (Acros) and sodium citrate (3g) (Sigma) were dissolved in ethanol (50mL) (Sigma) and distilled water (50mL).

The solutions had a shelf life of about six months if refrigerated, although print clarity reduced with time.

Treatment of articles: The articles were sprayed lightly with solution A using the Ecospray® and allowed to stand until completely dry (about 30 seconds). The articles were then lightly sprayed with solution B. The second process was repeated until satisfactory results were achieved. Development of a black colour denotes a positive reaction with Fe^{3+}.

5.4.7.4 2,2'-Dipyridil [9]

2,2'-Dipyridil formulation: 2,2'-bipyridine (4g) (Acros) and ascorbic acid (1g) (Acros) were dissolved in methanol (100mL) (Sigma) followed by the addition of hydrochloric acid (3mL) (Sigma). This solution had a shelf life of about six months if refrigerated.

Treatment of articles: The articles were lightly sprayed with the prepared solution using the Ecospray®. Development of a red colour denotes a positive reaction with Fe^{3+}.

5.4.7.5 Phenanthroline Hydrosulfite [9]

Phenanthroline hydrosulfite formulation: phenanthroline hydrochloride (0.05g) (Acros) and sodium hydrosulfite (10g) (Sigma) were dissolved in distilled water (100mL).

Treatment of articles: The articles were lightly sprayed with the prepared solution using the Ecospray®. Development of a red-orange colour denotes a positive reaction with Fe^{3+}.

5.4.7.6 8-Hydroxyquinoline[2, 17]

8-hydroxyquinoline formulation: 8-hydroxyquinoline (0.5g) (Acros) was dissolved in acetone (90mL) (Sigma) and distilled water (10mL).

Treatment of articles: The articles were lightly sprayed with the prepared solution using the Ecospray®. The samples were observed visually and using fluorescence examination under UV light (254nm). A positive reaction results in fluorescence detectable in the ultraviolet (UV) light range.

5.4.7.7 Tetramethylbenzidine [10, 79]

Tetramethylbenzidine (TMB) formulation:
Solution A: TMB (1g) (Acros) dissolved in ethanol (200mL) (Sigma).
Solution B: ethanol (90mL) (Sigma), distilled water (90mL) and 30% hydrogen peroxide (20mL) (Merck).

Treatment of articles: The articles were sprayed lightly using the Ecospray® with solution A followed by solution B. Development of a blue-green colour denotes a positive reaction with Fe^{3+}.

5.4.7.8 Alizarin Red S [5]

Alizarin Red S formulation: Alizarin red S was added in excess to ethanol (100mL) (Sigma) until saturated.

Treatment of articles: The impressions were lightly sprayed using the Ecospray®. A positive reaction was recorded on an immediate colour change to red.

5.4.7.9 Bromophenol Blue (BB) and Bromocresol Green (BG) [5, 6, 8]

BB and BG formulations: BB or BG (1g) (Acros) was dissolved in methanol (95mL) (Sigma) and distilled water (5mL).

Treatment of articles: The articles were lightly sprayed using the Ecospray® to avoid diffusion and background staining as much as possible. If the enhancement was not satisfactory, the impressions were exposed to water vapour from a boiling kettle or steam iron [6]. Development of a blue (BB) or green (BG) colour denotes a positive reaction with Ca^{2+}.

5.4.7.10 DFO [28]

DFO formulation: Two methods using DFO were employed for the enhancement of muddy footwear impressions.

Method 1

DFO Formulation: DFO (0.25g) (BVDA) was dissolved in methanol (30mL) (Sigma) using a magnetic stirrer to produce a slurry. Acetic acid (20mL) (Sigma) was added and stirred until a clear, yellow solution was produced followed by the addition of HFE71DE (275mL) (3M Novec) and HFE7100 (725mL) (3M Novec).

Treatment of articles: The articles were immersed in working solution for a maximum of five seconds. The excess solution was allowed to drain back in the tray. The fabric was allowed to dry completely before being heated in an oven at 100^{o}C for 20 minutes without humidifying. Fluorescence examination was carried out using a green excitation source (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a band-pass 549nm filter (1% cut-on point).

Method 2

A black gelatin lifter was applied to the muddy footwear impression and removed immediately before treatment of the articles as for Method 1.

5.4.7.11 Physical Developer [28]

Physical Developer formulation:

Maleic Acid Solution: maleic acid (25g) (Sigma) was dissolved in distilled water (1L).

Silver Nitrate Solution: silver nitrate (10g) (Acros) was dissolved in distilled water (50mL).

Working Solution: iron (III) nitrate (30g) (Acros), ammonium iron (II) sulphate (80g) (Acros) and citric acid (20g) (BDH) were added and dissolved in the order given, in distilled water (900mL). The mixture was stirred until completely dissolved and for a further five minutes. Stock detergent solution (40mL) (supplied by CAST) was added and stirred for two minutes followed by the addition of the silver nitrate solution (50mL) and stirring for a further two minutes.

Treatment of articles: The article was immersed using unserrated forceps in the maleic acid solution for ten minutes. This was followed by immersion in the working solution with gentle rocking for 20 minutes. The article was then washed three times for five minutes in three different dishes containing distilled water ensuring that the distilled water was changed frequently. Development of a black/grey colour denotes a positive reaction.

5.4.7.12 Oil Red O (ORO) [49, 52]

ORO formulation:
Stain Solution: ORO (1.54g) (Acros) was dissolved in methanol (770mL) (Sigma). NaOH (9.2g) (Sigma) was dissolved in distilled water (230mL) and added to the ORO solution. The solution was then stirred and filtered to remove any undissolved solids.

Buffer Solution pH 7: Na_2CaO_3 (26.5g) (Sigma) was dissolved in distilled water (2L). 70% concentrate nitric acid (18.3mL) (Riedel-de Haën) was added slowly followed by more distilled water to make a total volume of 2.5L.

Treatment of articles: The articles to be examined were immersed in the stain solution and rocked gently for a period of 60 to 90 minutes. The articles were then immersed in the buffer solution for a few seconds to adjust the pH and rinsed in distilled water and dried. Development of a red colour denotes a positive reaction.

5.4.7.13 Safranin O [59]

Safranin O formulation: safranin (1g) (Acros) was dissolved in distilled water (1L) and stirred with a magnetic stirrer until all solid was dissolved to give a dark pink solution.

Treatment of articles: A black gelatin lifter was applied to the impression, lifted and photographed. Safranin solution was poured over the original impression and allowed to remain in contact for at least two minutes before rinsing thoroughly under running tap water. Water was allowed to drain from the fabric and left to dry overnight. A white gelatin lifter was then applied to the impression and left for several minutes before checking for potential fluorescence using a Mason Vactron 40 Quaser and a green excitation filter (band pass filter 473-548nm at 1% cut-on and cut-off points respectively) and viewed with a long pass 549nm filter (1% cut-on point). Development of a red fluorescent colour denotes a positive reaction.

5.4.7.14 Phosphorus Test (Ascorbic Acid Method) [62]

Phosphorus test formulation:
Sulfuric acid, H_2SO_4, 5N: concentrated H_2SO_4 (70mL) (Sigma) was added to distilled water (500mL).

Potassium antimonyl tartrate solution: $K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O$ (1.37g) (Acros) was dissolved in distilled water (400mL) in a 500-mL volumetric flask, diluted to a final volume of 500 mL and stored in a glass-stoppered bottle.

Ammonium molybdate solution: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (20g) (Acros) was dissolved in distilled water (500mL) and stored in a glass-stoppered bottle.

Ascorbic acid, 0.1*M:* ascorbic acid (1.76g) (Acros) was dissolved in distilled water (100mL). The solution was stable for about one week at 4^oC.

Working solution: the above reagents were mixed in the following proportions for 100mL of reagent: *5N* H_2SO_4 (50mL), potassium antimonyl tartrate solution (5mL), ammonium molybdate solution (15mL) and ascorbic acid solution (30mL). The mixing of solutions was ensured after the addition of each reagent. The reagent is stable for four hours.

Treatment of articles: The impressions were lightly sprayed using the Ecospray®. A positive reaction was recorded on the development of a vibrant blue colour.

5.4.7.15 Aluminium Test [80]

Aluminium test formulation:
Hexamine Buffer: 15% hexamine buffer was prepared by dissolving hexamine (75g) (Acros) in deionised water (400mL). 28% ammonia solution (16mL) (BDH) was added and the pH adjusted to the required value (pH 6.6 or 9.2) by the slow addition of 5M HC1 (drops) (Sigma).

Pyrocathechol Violet (PCV): The reagent was prepared by dissolving PCV (110mg) in deionised water (100 mL). The solution was stable for three months.

Treatment of articles: The impressions were lightly sprayed using the Ecospray®. A positive reaction was recorded on the development of a blue-purple colour.

5.4.8 Sequencing of Enhancement Techniques

Potassium thiocyanate (1), potassium ferrocyanide (2), ammonium pyrrolidinedithiocarbamate (3) and 2,2'-dipyridil (4) were the best performing enhancement techniques for muddy footwear impressions on fabric and were used in a sequential study using the road soil sample. Four representative fabrics (white cotton, white polyester, black polyester and leatherette) were selected as substrates. Since the techniques in general performed equally well, four random sequences of these techniques were selected as shown in table 5.5.

Table 5.5 - Sequential enhancement

Sequence Letter	Technique Sequence
A	1, 2, 3, 4
B	4, 3, 2, 1
C	3, 1, 4, 2
D	2, 4, 1, 3

5.5 Results and Discussion

5.5.1 Soil Sampling and Analysis

Figure 5.13 shows the main four locations of the soil sampling in Scotland that were used to prepare footwear impressions in mud.

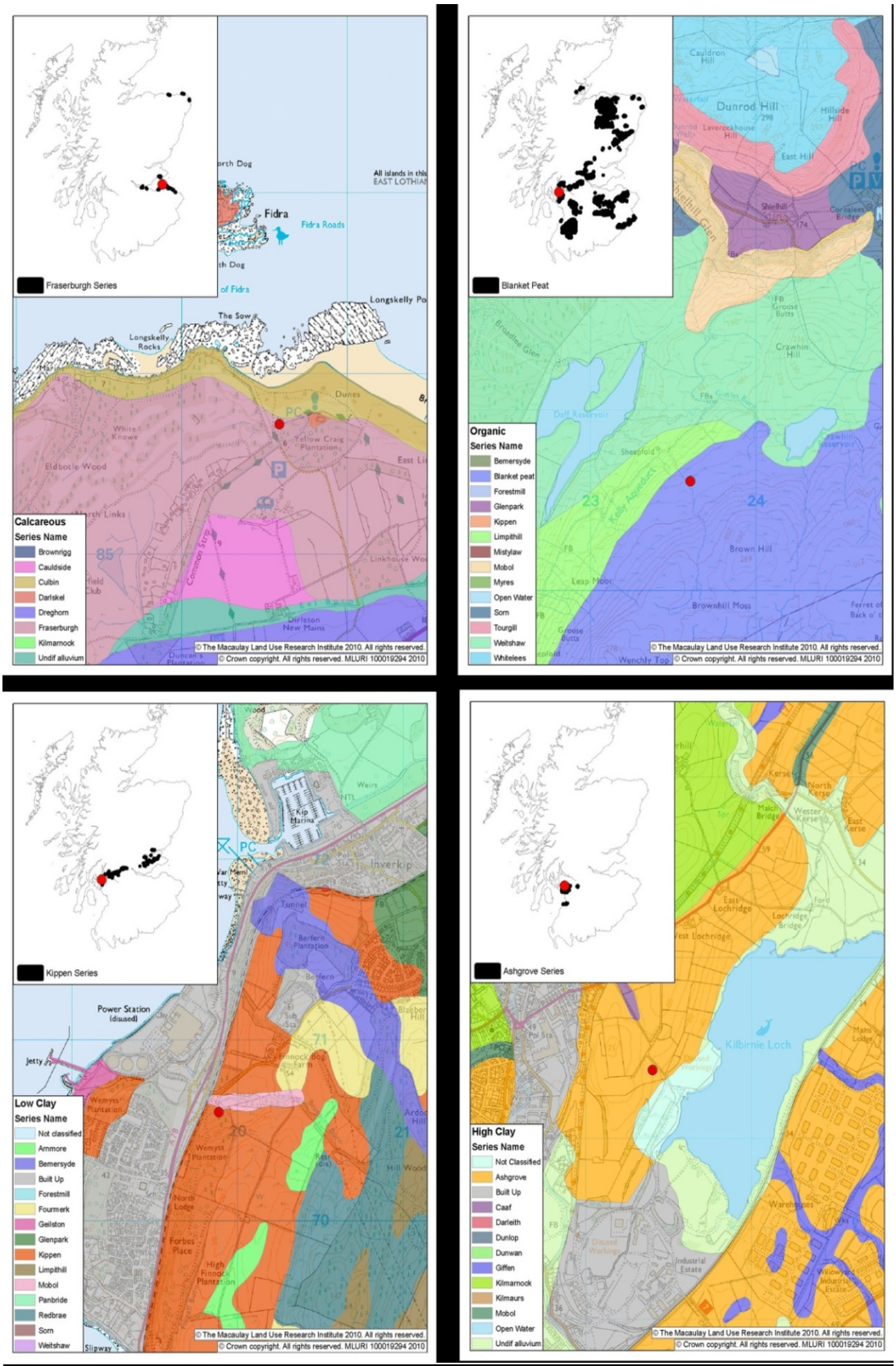


Figure 5.13 - Location of soils utilised in the study (clockwise from top left: North Berwick, Cornalees, Kilbirnie, Wemyss Bay).

The elemental profile of the four soils was undertaken by the James Hutton Institute in Aberdeen and is revealed in table 5.6 where the main element of interest, iron, is highlighted along with aluminium, calcium and phosphorus. The results demonstrated that the North Berwick soil has the lowest levels of iron in comparison to the other soils used in the study which may reveal some differences in the enhancement of that soil using iron targeting reagents.

Table 5.6 - Elemental analysis of soils

Element	Road sample NS58404 66408	Kilbirnie NS32495, 54282	Cornalees NS23473, 70754	North Berwick NT51591, 85718	Wemyss Bay NS19888, 70600
	Abundance (mg/kg of sample)				
Ag	0.23	0.26	0.11	0.03	0.05
As	6.00	7.13	4.09	5.30	2.58
Ba	225.92	210.55	127.48	21.68	32.98
Cd	0.94	0.85	0.09	0.12	0.04
Co	13.04	22.41	15.95	2.49	3.96
Cr	99.25	223.90	68.63	106.01	98.70
Cu	183.55	125.38	250.52	4.59	7.37
Hg	0.18	0.19	<0.15	<0.15	<0.15
Mo	8.96	5.98	0.31	3.39	2.95
Ni	38.44	64.11	43.31	10.25	9.47
Pb	164.15	151.97	14.74	16.67	19.55
Pt	0.05	<0.01	<0.01	<0.01	0.02
Se	0.75	1.16	0.30	0.23	0.52
Sr	60.36	93.14	122.32	83.31	3.69
Zn	730.05	367.29	102.33	37.00	31.34
Al	13030.00	27830.00	55750.00	2694.09	11930.00
B	59.65	67.27	137.32	15.62	29.02
Ca	13400.00	10660.00	30680.00	14450.00	222.90
Fe	35740.00	41300.00	31110.00	7470.17	21320.00
K	1372.94	2080.67	20290.00	510.43	1403.31
Mg	7149.38	4698.44	35280.00	1907.81	2608.52
Mn	597.54	1124.94	437.36	178.40	172.64
Na	1046.96	470.41	555.46	307.87	96.17
P	1262.60	2233.31	540.00	414.63	154.34
S	1071.15	1009.54	131.60	326.95	180.50
Ti	1779.03	1689.99	575.56	204.12	638.65

Table 5.7 presents the pH for each of the soil samples. All soils with the exception of the road soil sample, have a close to neutral pH (H_2O) with an average pH of 7.16. These values are relatively high in comparison to the mean pH recorded (pH=3.45) for Scottish mineral soils, suggesting that some management of the soil through the addition of lime may have occurred [81]. Liming of soils is the application of basic chemicals to neutralise acidity and increase bacterial activity. The North Berwick soil has the highest pH, due to its calcareous nature. The road soil is the most acidic (pH of 4.82), although it is still well within the frequency distribution for Scottish soils [81]. This is most likely due to the location where the sample was recovered where consistent vehicle traffic would be consistent with the release of hydrocarbons and carbon dioxide. The acidic pH of the roadside soil might have an impact on the effects of the enhancement reagents when compared to the other soils.

Table 5.7 - Soil analysis report

Location	Grid Position	pH (H2O)	pH (CaCl2)	% Weight Loss on Ignition	% Weight Loss on Ignition	% Weight Loss on Ignition	Soil Description
				@105°C	@450°C	@900°C	
Kilbirnie	NS 32495, 54282	7.05	6.80	1.17	8.21	10.14	Brown/black, lumpy
Cornalees	NS 23473, 70754	6.59	6.40	3.38	17.57	21.77	Grey, rocky
North Berwick	NT 51591 85718	7.75	7.57	3.35	0.82	10.10	Dark brown, sandy, sticky
Wemyss Bay	NS 19888, 70600	7.26	7.10	0.72	3.65	5.00	Red/light brown
Road soil sample	NS 58404 66408	4.82	4.16	1.23	4.21	5.36	Dark brown/black, thick mixture

5.5.2 Interaction of the Soils with Fabrics

The soil recovered from North Berwick was retained least by all fabrics, presumably due to the fine nature of the soil. All other soils had a similar retention on the various fabrics studied. Nylon/lycra appeared to retain the soils better than the other fabrics whereas leatherette appeared to be worst at retaining the soils. This reflects the permeability data of fabrics and suggests that the higher the permeability, the higher the retention of the soil. Smaller soil particles appeared to penetrate into the weave of the fabric for cotton and denim whereas larger soil particles were observed on the surface of the fabric. Similar phenomena were observed for the synthetic fabrics polyester and nylon/lycra, however for polyester most of the particles penetrated into the fabric.

5.5.3 Preliminary Enhancement

Alizarin Red S, Physical Developer and Oil Red O

Preliminary enhancement experiments were carried out on muddy impressions using a mixture of the test soils. Little or no enhancement was achieved with alizarin red S although results have been reported for enhancement of impressions on non-porous articles [4]. Fingerprints were successfully developed on paper with the physical developer formulation but no enhancement was observed for muddy footwear impressions on white cotton, or nylon/lycra. The muddy footwear impressions appeared to have been obliterated by the processing of the sample. Similarly the Oil Red O formulation used in this study did not develop any footwear impressions.

Safranin O

Safranin O, as sudan black and nile red, is believed to be absorbed by fatty components and is routinely used as a microbiological stain [25]. Safranin O enhancement was attempted on footwear impressions on fabric as suggested by Theeuwen [10] and Velders [59]. Very good results were reported for the enhancement of muddy footwear impressions on glass and some limited success on

glossy paper [10]. Safranin treatment of muddy footwear impressions on fabrics, however, did not produce any enhancement with only a slight visualisation observed after lifting with a gelatin lift as illustrated in figure 5.14.

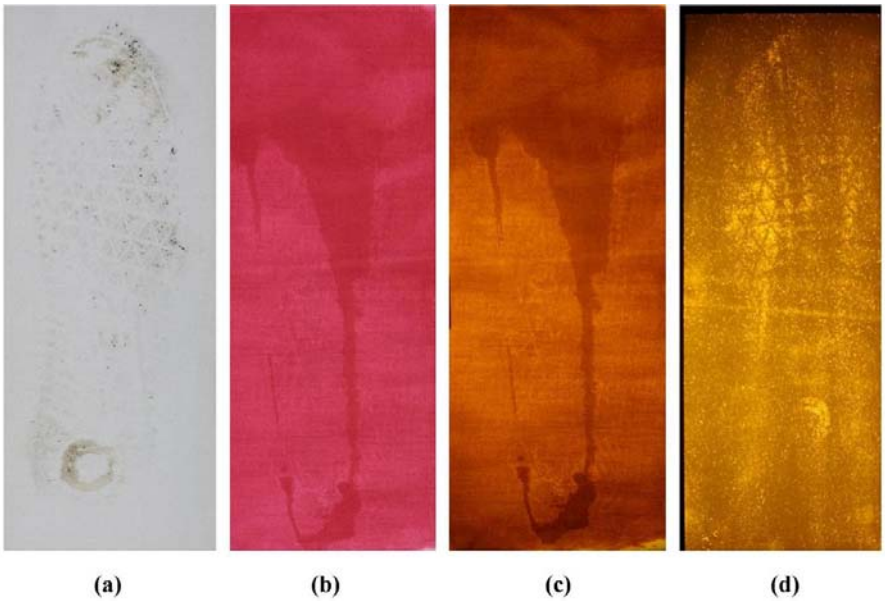


Figure 5.14 – Safranin treatment of a muddy footwear impression on white cotton: (a) 1 week old impression; (b) after safranin treatment; (c) fluorescence after safranin treatment (b); (d) fluorescence from white gelatin lift of (b)

Soils in Scotland are rich in phosphorus and aluminium and as such enhancement reagents which have an affinity for these two elements were also examined. However, no visual or fluorescent enhancement was achieved. The phosphorus test resulted in heavy blue background staining obliterating the original footwear impression. Background staining was minimised by lighter spraying, however, significant diffusion occurred as shown in figure 5.15. The aluminium test provided less background staining but only slight blue enhancement was achieved (figure 5.11) which did not improve what could already be observable visually.

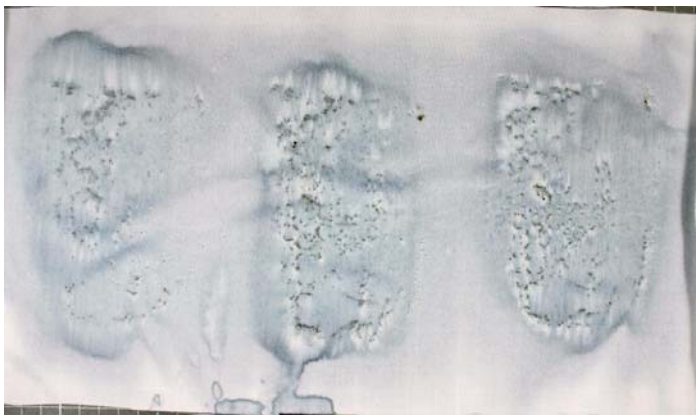


Figure 5.15 – Diffusion from the chemical enhancement for the detection of phosphorus on muddy footwear impressions on white nylon/ lycra (ascorbic acid method)

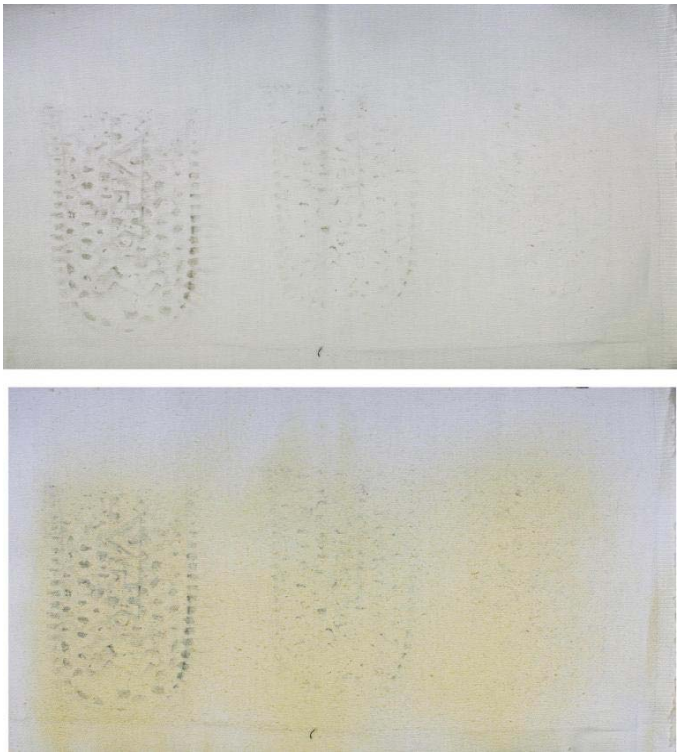


Figure 5.16 - Enhancement of muddy footwear impressions on white cotton for the detection of aluminium: (top) before enhancement; (bottom) after enhancement

Bromophenol Blue and Bromocresol Green

Researchers in Israel observed successful enhancement of dusty and muddy footwear impressions with bromophenol (BB) blue and bromocresol green (BG) [5, 6, 8]. However, poor enhancement was achieved on muddy footwear impressions on the fabrics in this study as illustrated in figure 5.17 and 5.18. Slight improvement was achieved by using steam from an iron [5, 6, 8]. These results agree with previous

research [27] which investigated these reagents using chalky soils from the south east of UK, with no successful enhancement on muddy footwear impressions.

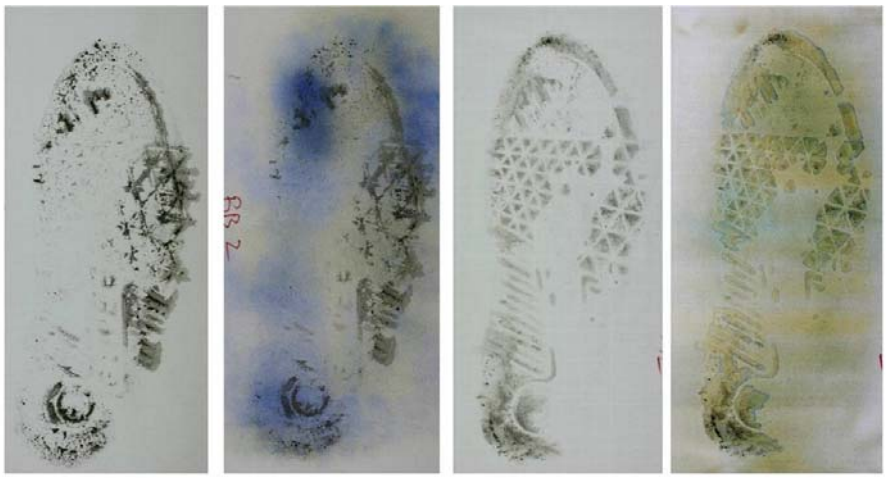


Figure 5.17 – Bromophenol blue enhancement of a muddy footwear impression on white cotton : (a) unenhanced; (b) enhanced; and white nylon/lycra: (c) unenhanced; (d) enhanced

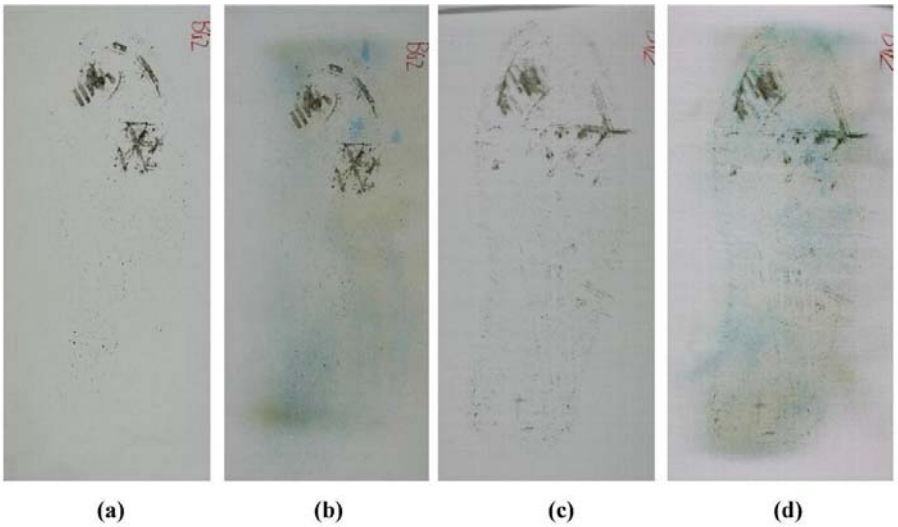


Figure 5.18 – Bromocresol green enhancement of a muddy footwear impression on white cotton: (a) unenhanced; (b) enhanced; and white nylon/lycra: (c) unenhanced; (d) enhanced

DFO

Gelatin lifting followed by DFO treatment was performed and figure 5.19 illustrates the gelatin lift under oblique lighting and the subsequent fluorescent enhancement with DFO under green light. The mirror-image obtained on the lift is not very clear due to fibres being lifted by the gelatin lifter. Slight improvement was observed with fluorescence, however, it was not very clear and some diffusion of the impression was also noted. Similar results were obtained with nylon/lycra.

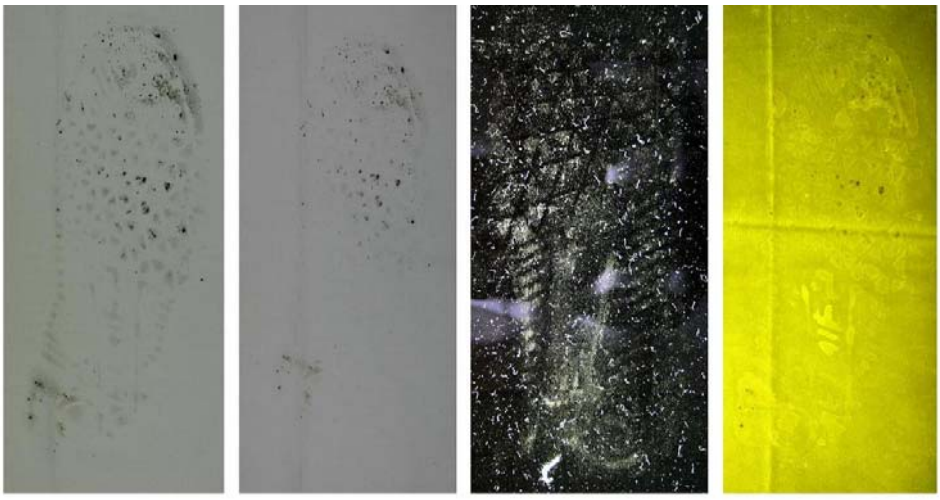
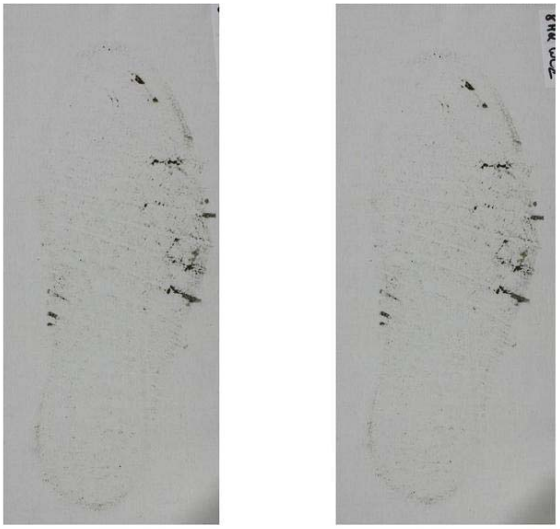


Figure 5.19 – DFO enhancement of a muddy footwear impression on white cotton: (a) fresh impression; (b) 1 week old impression; (c) gelatin lift of (b); (d) impression (b) under green light after application of gelatin lift and DFO treatment

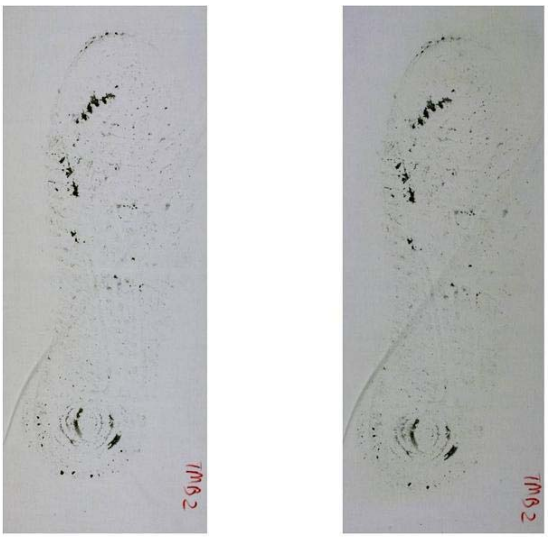
8-Hydroxyquinoline and Tetramethylbenzidine

Poor enhancement was observed with 8-hydroxyquinoline (figure 5.20 for white cotton) and UV fluorescence as suggested by Bodziak [12] and Brundage [2] failed to improve enhancement in this case. Similar results were observed with TMB (figure 5.21 for white cotton).



(a) **(b)**

Figure 5.20 - Enhancement of a muddy footwear impression on white cotton using 8-hydroxyquinoline: (a) unenhanced; (b) enhanced

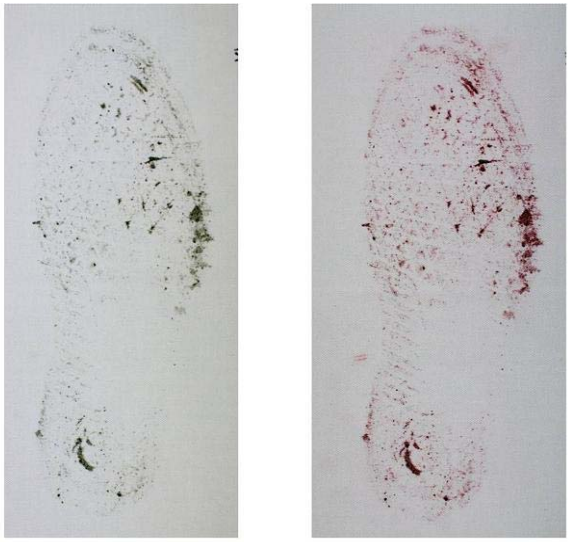


(a) **(b)**

Figure 5.21 - Enhancement of a muddy footwear impression on white cotton using TMB: (a) unenhanced; (b) enhanced

2,2'-Dipyridil

The enhancement achieved using 2,2'-dipyridl on muddy footwear impressions was weak on both cotton and nylon fabrics and the result for cotton presented in figure 5.22.



(a) (b)

Figure 5.22 - Enhancement of a muddy footwear impression on white cotton using 2,2'-dipyridil: (a) unenhanced; (b) enhanced

Potassium Ferrocyanide

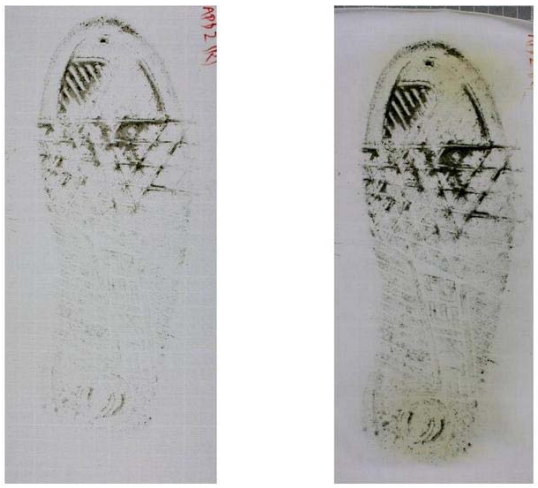
Enhancement with potassium ferrocyanide produced a weak blue colour change, presented in figure 5.23, however, this colour also stained the whole background over a period of time. Quick photography was therefore required. The blue colour observed was weak on both natural and synthetic fabrics.

(a) (b)

Figure 5.23 - Enhancement of a muddy footwear impression on white cotton using potassium ferrocyanide: (a) unenhanced; (b) enhanced

Ammonium Pyrrolidinedithiocarbamate

The enhancement results observed in this study with ammonium pyrrolidinedithiocarbamate (APD) were limited (figure 5.24). In contrast to the thiocyanate reagents, there were no toxic or odours emitted during application.



(a) (b)

Figure 5.24 - Enhancement of a muddy footwear impression on white nylon/lycra using APD: (a) unenhanced; (b enhanced

Phenanthroline Hydrosulfite (PHS)

The enhancement reaction provided a weak red colour on both cotton and nylon/lycra. Figure 5.25 presents these results for nylon/lycra.

(a) (b)

Figure 5.25 - Enhancement of a muddy footwear impression on white nylon/lycra using PHS : (a) unenhanced; (b) enhanced

Ammonium and Potassium Thiocyanate

Ammonium and potassium thiocyanate both gave similar results for the enhancement of muddy footwear impressions and are presented in figures 5.26 and 5.27 and reflected previous research by Froude [13]. Some diffusion and blurring occurred when Preval® sprayers were used, however, the best results were obtained using Ecosprayers®.

Figure 5.26 - Enhancement of a muddy footwear impression on white cotton using ammonium thiocyanate: (a) unenhanced; (b) unenhanced

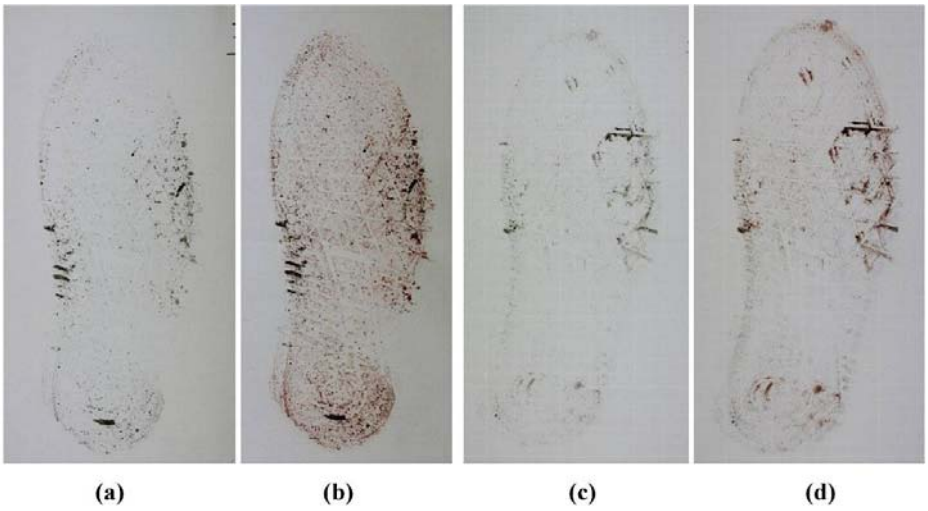


Figure 5.27 - Potassium thiocyanate enhancement of a muddy footwear impression on white cotton: (a) unenhanced; (b) enhanced; and white nylon/lycra: (c) unenhanced; (d) enhanced

Gelatin Lifting

Gel lifting may increase the background staining of subsequent chemical enhancement and thus decrease the contrast between the impression and the substrate [82]. As a consequence, gel lifting was not recommended prior to chemical treatment of fingerprints as it is less sensitive than the best chemical enhancement process on most surfaces. For footwear impressions, however, the effectiveness of chemical enhancement diminished greatly and gel lifting was suggested as one of the best enhancement processes. In this study, black (before chemical enhancement) and white (after chemical enhancement) gelatin lifting (BVDA) was attempted in preliminary experiments.

In general, although the impression was lifted before chemical enhancement, no further enhancement was achieved using a gelatin lifter. No dramatic increase in background staining was observed after attempted gelatin lifting. White gel lifting was unsuccessful after chemical enhancement on the fabrics used in this study. Although gelatin lifting was not successful, it did not appear to alter the original impression or effect subsequent chemical enhancement. The use of gelatin lifting before and/or after chemical enhancement may however assist in improving the contrast with the background. Research [10] has shown that black gelatin lifting followed by photography was one of the best enhancement techniques for both porous and non-porous substrates. The use of a BVDA gelatin lift scanner (GLS^{can}), if available, can help obtain distortion-free and perfectly illuminated images of the gelatin lift by scanning.

5.5.4 Chemical Formulation Modifications

Following preliminary experiments for the enhancement of muddy impressions on white cotton and nylon/lycra, the following techniques were selected for further investigation based on their performance in the preliminary investigation: potassium thiocyanate, ammonium thiocyanate, potassium ferrocyanide, ammonium pyrrolidinedithiocarbamate, 2,2'-dipyridil and phenanthroline hydrosulfite. Five out of these six enhancement techniques were recommended by Someha [9] for the enhancement of footwear impressions in mud and dust in Japan.

Several attempts were made to change the carrier solvent in formulations that utilised flammable and irritant solvents. Potassium and ammonium thiocyanate formulations were prepared using ethanol rather than acetone. Although ethanol (boiling point: 78^0C; flash point 17^0C) is also flammable, it is not an irritant and has a higher boiling point and flash point in comparison to acetone (boiling point: 56^0C; flash point -20^0C). Distilled water was replaced by ethanol in the formulation of potassium ferrocyanide and phenanthroline hydrosulfite since water can potentially lead to diffusion of the original impression in mud even with careful fine spraying. Neither of these modifications produced any improvement, either because the chemicals did not fully dissolve or because the enhancement achieved was poorer than with the recommended solvents.

However, a successful modification was achieved for 2,2'-dipyridil where ethanol was used instead of methanol. The enhancement obtained using ethanol appeared to be the same or better compared to the methanol formulation. As a result, the ethanol formulation for 2,2'-dipyridil was utilised.

5.5.5 In-depth studies of enhancement of footwear impressions in soil on fabric

The six techniques selected for further investigation were tested on impressions produced using all four soils on all test fabrics. Each technique targeted iron ions and the mean iron percentage concentration for each soil was calculated as 3.58, 4.13, 3.11, 0.75 and 2.13% for the roadside soil, Kilbirnie, Cornalees, North Berwick and Wemyss Bay soil respectively. The mean value for iron ions in Scottish mineral soils is reported as 2.5%, with most soils having values lower than 4% [81]. For organic soils the mean value is 0.97%, with most organic soils having values of less than 1% [81].

Wemyss bay soil was the most responsive soil to the chemical enhancement techniques. Weak colour enhancement with all the six techniques was observed on footwear impressions in mud prepared with the North Berwick soil. This can be explained by the fact that the soil was calcareous and the elemental analysis illustrated that the amount of iron present in this soil (0.75%) was considerably less in comparison to the other soils. Furthermore, once the muddy footwear impressions

prepared with this soil dried on the fabric, extra care was needed as the fine soil became loose on the fabric. This also required careful spraying to avoid damaging the impression.

In general, all of the six techniques provided suitable enhancement for all soils on white fabrics but enhancement was limited on dark and patterned fabrics due to poor contrast with the background. Little or no enhancement was achieved on denim and leatherette. Lighter spraying on the synthetic fabrics polyester and nylon/lycra was essential to avoid diffusion of the original muddy impression. 2,2'-dipyridil was the only technique, in addition to oblique lighting, to offer some colour enhancement on black fabrics by improving the contrast with the background, at least in the first impressions of the diminishing series. The enhancement of potassium and ammonium thiocyanate was a similar red colour and the enhancement obtained by using phenanthroline hydrosulfite was a very weak red-orange. As a result, ammonium thiocyanate and phenanthroline hydrosulfite were not included for the testing of the road soil sample.

The best enhancement on black polyester irrespective of soil type used was achieved using oblique lighting (Crime Lite® 82L) prior to any chemical enhancement (figure 5.28). Observations of the enhanced impressions with oblique lighting demonstrated inferior results to that observed before enhancement using oblique lighting. This was also reported during the enhancement of footwear impressions in blood using protein stains [83], however, the same effect was not observed on other black and dark fabrics.

The use of enhancement techniques for the detection of soil do not necessarily imply the presence of soil as the techniques target iron which might be present in other residues.

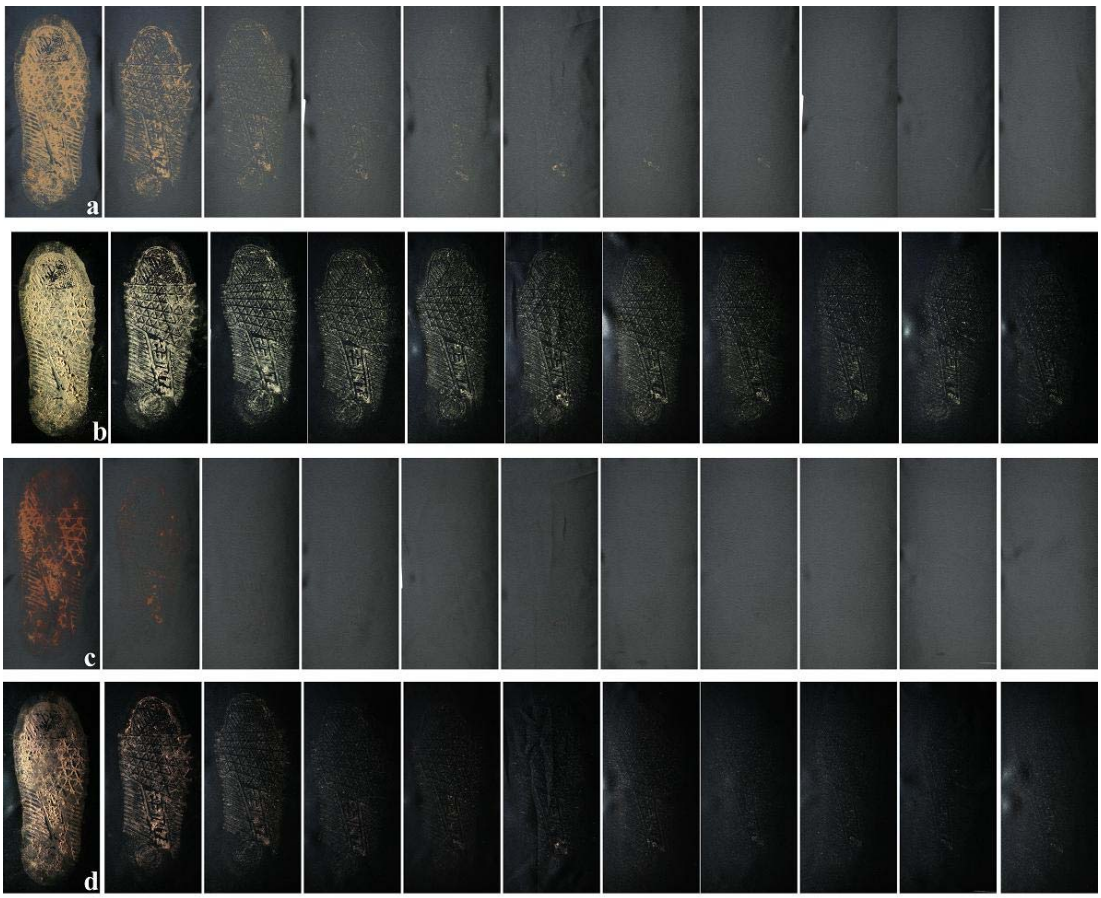


Figure 5.28 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on black polyester, with potassium thiocyanate: (a) 1 week old diminishing series; (b) oblique lighting observation of (a); (c) enhancement with potassium thiocyanate of (a); (d) oblique lighting observation of (c)

5.5.5.1 Phenanthroline Hydrosulfite (PHS)

Phenanthroline hydrosulfite was the least sensitive technique for the enhancement of footwear impressions in mud on fabrics producing a faint red-orange colour. Some weak enhancement was observed on the white fabrics with minimal or no enhancement on white nylon/lycra. Water was used as the main solvent which resulted in some diffusion across the synthetic fabrics even though it was a one-step spray process. No enhancement was observed on the dark and patterned fabrics as the contrast between the impression and the background was not improved (figure 5.29).

Little, if any, enhancement was achieved on the first, most loaded impression of the diminishing series for any soil on any fabric. Nonetheless, there was no deterioration of the original footwear impression after chemical treatment. Similar to other techniques, the best enhancement was achieved on Wemyss bay soil (figure 5.30), however, in general the enhancement did not provide much improvement over visual inspection of the original impression.

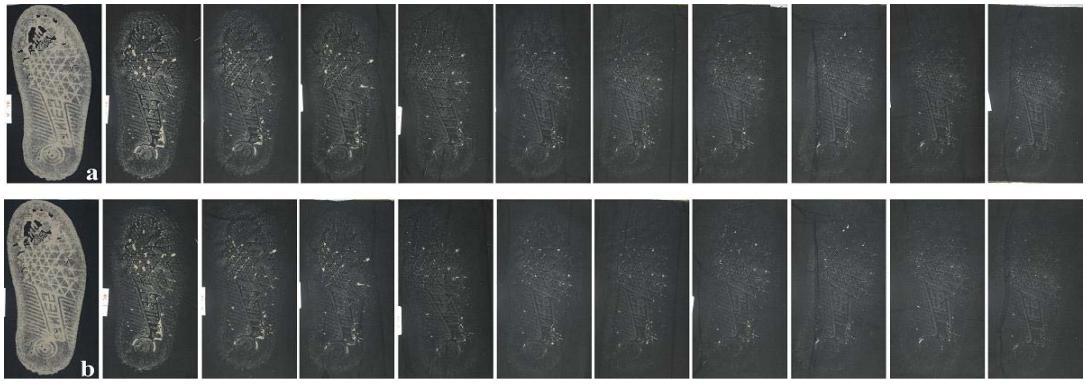


Figure 5.29 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Cornalees soil on black cotton, with PHS: (a) before; and (b) after enhancement

Figure 5.30 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on white cotton, with PHS: (a) before; and (b) after enhancement

5.5.5.2 Ammonium Thiocyanate

A vibrant red colour enhancement was observed for all footwear impressions in mud on white and patterned fabrics prepared from all the soils under test with the exception of the North Berwick soil (presumably due to low levels of iron). An example of the enhancement is provided in figure 5.31. The red colour change appeared after approximately 10 seconds.

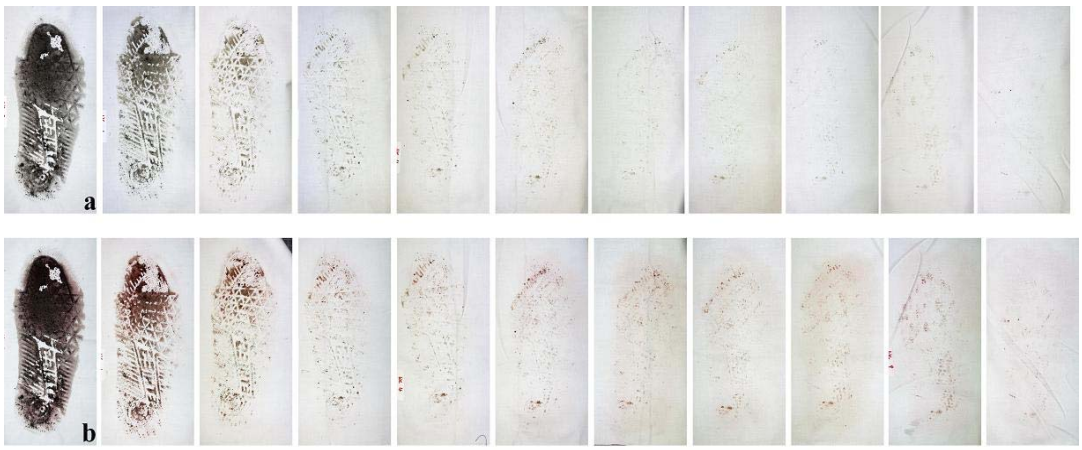


Figure 5.31 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Cornalees soil on white cotton, with ammonium thiocyanate: (a) before; and (b) after enhancement

The red coloured enhancement on dark fabrics provided poor contrast and in some instances the enhancement decreased the contrast of the impression on the dark background. This became more pronounced as the muddy footwear impression diminished. The red coloured enhancement faded considerably overnight on all fabrics for all soils except for impressions on white nylon/lycra. Re-application of the chemical permitted re-visualisation without detriment to the original footwear impression. Limited enhancement was observed on denim and leatherette as illustrated in figure 5.32.

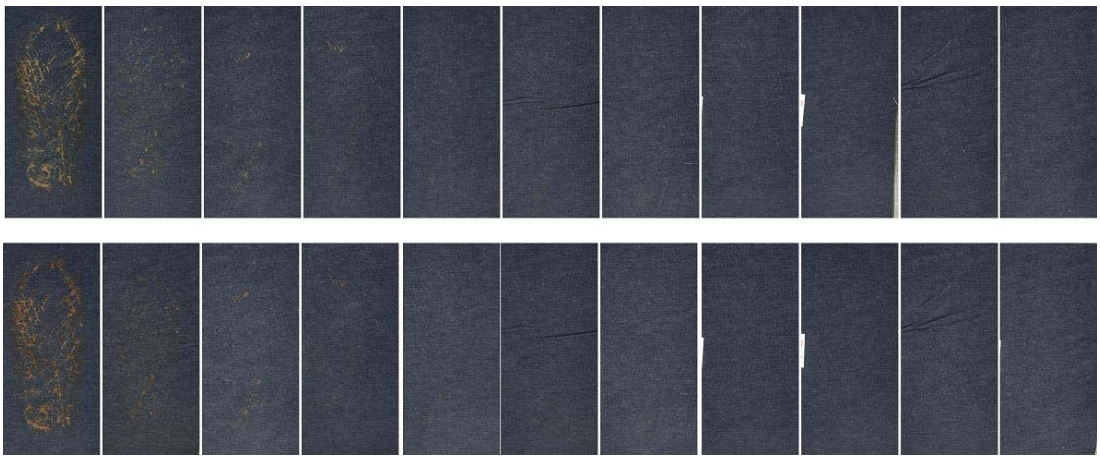


Figure 5.32 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with North Berwick soil on denim, with ammonium thiocyanate: (a) before; and (b) after enhancement

A yellow tinge developed around the muddy footwear impression on white polyester, whereas the same tinge was not observed when ethanol rather than acetone was utilised as the carrier solvent. However, the enhancement with the ethanol formulation produced a result which was not as vibrant and sharp as that obtained with the acetone formulation. Acetone also reacted with the plastic solvent container and the metal parts of the Ecospray® unit.

A recent study suggested that ammonium thiocyanate also provided the best results for the enhancement of muddy footwear impressions on vinyl flooring when compared to pyridyldiphenyl-triazine (PDT) and Ferrotrace® (a commercial version of PDT) [7].

5.5.5.3 Potassium Thiocyanate

Similar enhancement was achieved using both ammonium thiocyanate and potassium thiocyanate, however, the latter produced a brighter red colour which lasted longer before fading. This supports previous research [13] comparing ammonium and potassium thiocyanate which found both chemicals worked equally well for the enhancement of muddy footwear impressions. Additionally, the red colour developed faster (within 5 seconds) and there was no immediate yellow tinge observed on white polyester after enhancement. Furthermore, enhancement on black fabrics, denim and

leatherette appeared to deteriorate with poor contrast between the impression and the background in a similar way to results obtained using ammonium thiocyanate. Both thiocyanate techniques emitted unpleasant, toxic fumes and use a highly flammable, irritant solvent resulting in practical limitations in their use. Figure 5.33 and figure 5.34 illustrate the enhancement of Cornalees soil and Kilbirnie soil respectively with potassium thiocyanate on white cotton. When compared to ammonium thiocyanate enhancement in figure 5.31, it can be seen that potassium thiocyanate produces a stronger red colour.

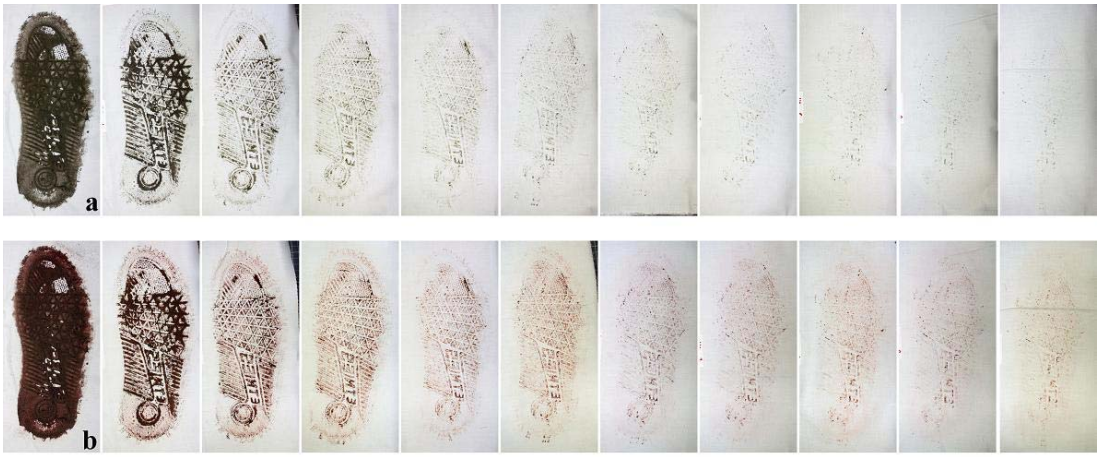


Figure 5.33 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Cornalees soil on white cotton, with potassium thiocyanate: before; and (b) after enhancement

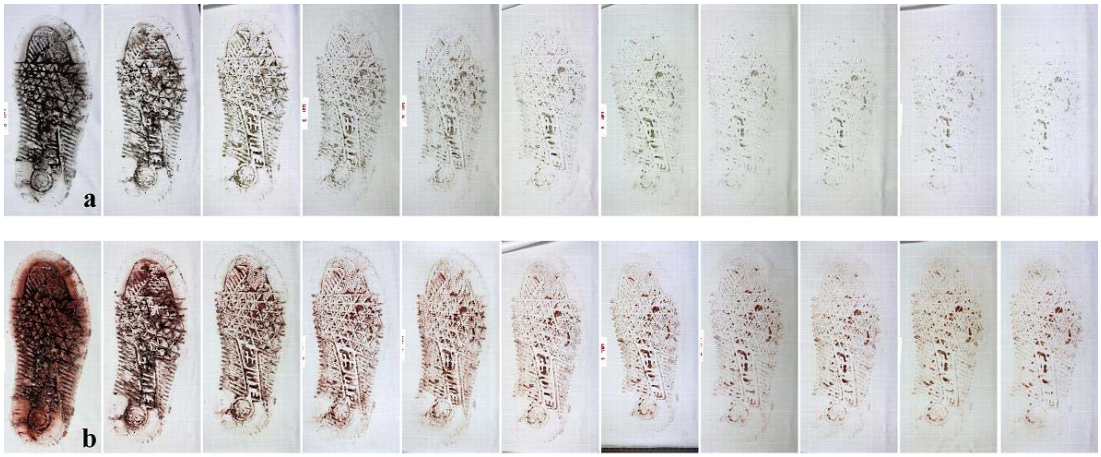


Figure 5.34 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Kilbirnie soil on white nylon/lycra, with potassium thiocyanate: (a) before; and (b) after enhancement

5.5.5.4 Potassium Ferrocyanide

The inclusion of water in the formulation of potassium ferrocyanide meant that enhancement may be prone to diffusion. The vibrancy and sharpness of this enhancement technique is less impressive compared to the thiocyanates, however, the reagent does not emit any toxic or offensive odours making it more operationally more useful. This technique, however, does require two sprayers making it slightly more cumbersome to use. The enhancement appeared to be more responsive to impressions deposited onto the synthetic fabrics (polyester and nylon/lycra) than the natural fabrics. The colour enhancement required a few seconds to appear after spraying, however, it did not diminish and in fact continued to develop over 24 hours post application of the reagent. On dark fabrics, illustrated in figure 5.35, the contrast between the blue colour and the background was difficult to visualise in comparison to light coloured fabrics (figure 5.36) and the enhancement did not greatly improve on what could already be seen visually. Very limited enhancement was obtained with the North Berwick soil across all fabrics, including light coloured fabrics.

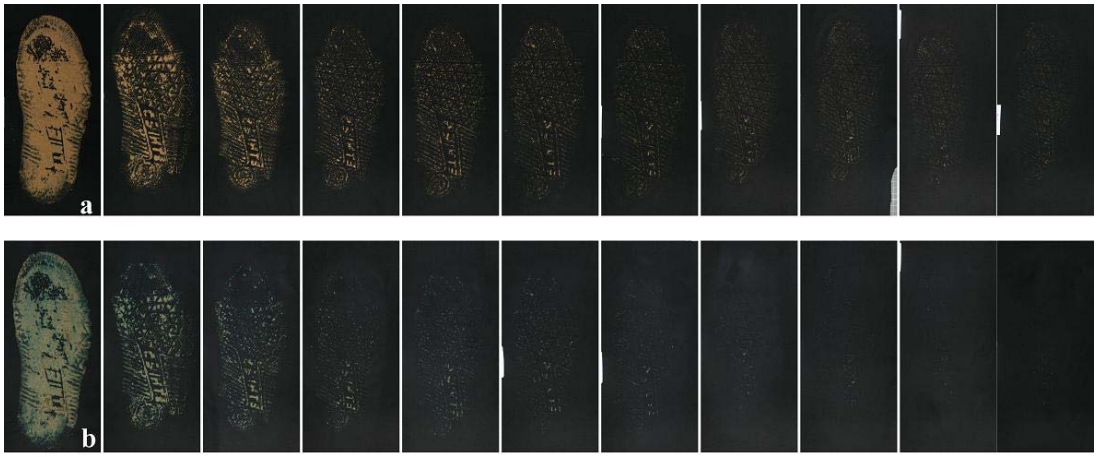


Figure 5.35 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on black nylon/lycra, with potassium ferrocyanide: (a) before; and (b) after enhancement

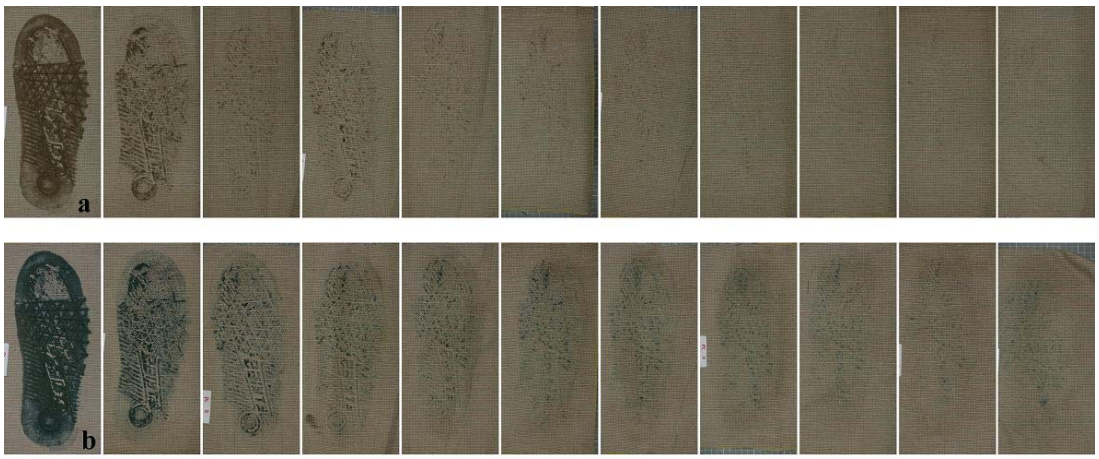


Figure 5.36 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Kilbirnie soil on pattered cotton, with potassium ferrocyanide: (a) before; and (b) after enhancement

5.5.5.5 Ammonium Pyrrolidinedithiocarbamate (APD)

The enhancement arising from this technique produced a black colour (illustrated in figure 5.37), and as a result, enhancement on dark fabrics was limited or in most cases non-existent due to poor contrast. The technique worked well on light coloured fabrics with slight diffusion on polyester, possibly due to the presence of distilled water in the formulation. The black enhancement appeared to be brighter on light coloured fabrics when observed the day after the reagents were applied. The technique requires two sprayers and does not emit any toxic fumes.

No enhancement was observed on dark fabrics and within one hour a white tinge developed on black nylon/lycra which obliterated the original footwear impression. The white tinge also developed on blank nylon/lycra controls indicating a reaction of the reagent with the fabric rather than with iron present in the soil. The brown-reddish colour of Wemyss Bay soil illustrated the good soil retention on the black nylon/lycra and a good contrast was obtained with the background prior to any chemical treatment (figure 5.38). Overall the technique worked well on all light coloured fabric for all soils except for the North Berwick soil.

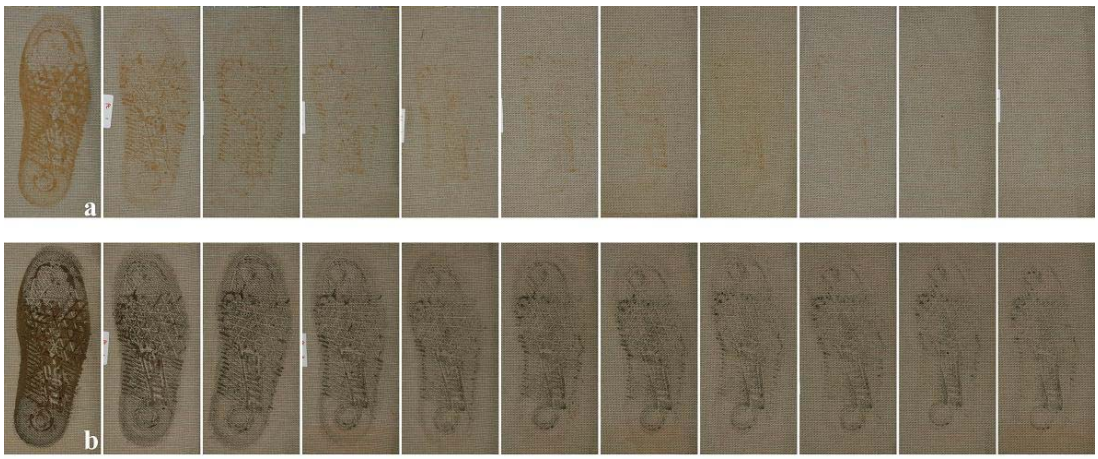


Figure 5.37 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on patterned cotton, with APD: (a) before; and (b) after enhancement

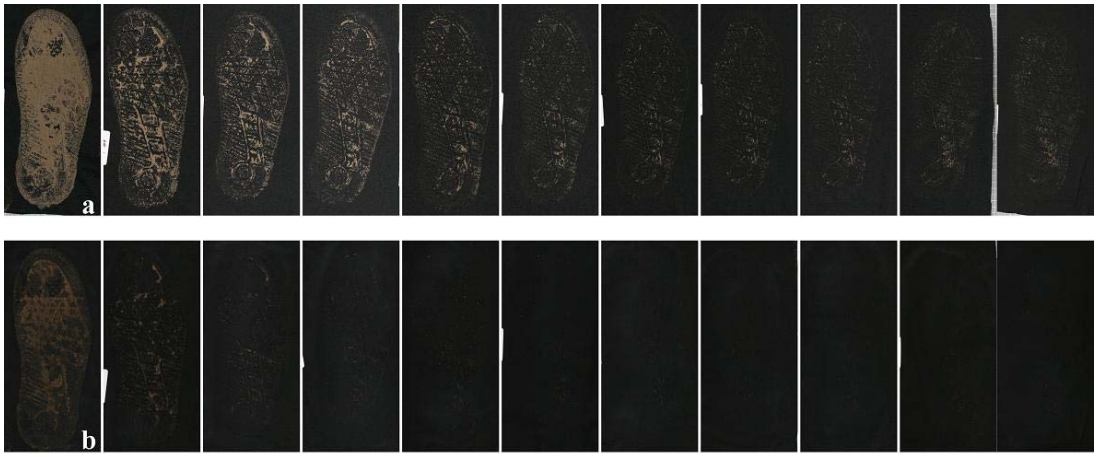


Figure 5.38 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Kilbirnie soil on black nylon/lycra, with APD: (a) before; and (b) after enhancement

5.5.5.6 2,2'-Dipyridil

All soils appeared to be responsive to this enhancement technique with vibrant red colours appearing on all fabric types. The diminishing series for dark coloured fabrics, however, only offered enhancement up to the third or fourth impression (figure 5.39).

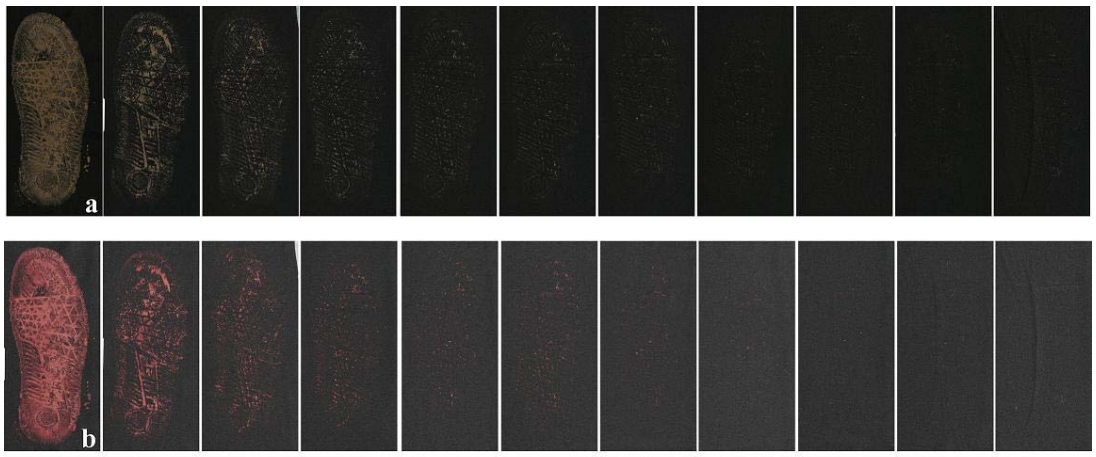


Figure 5.39 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Kilbirnie soil on black nylon/lycra, with 2,2'-dipyridil: (a) before; and (b) after enhancement

The colour change required 3 to 5 minutes to initially develop, reaching a maximum after about 24 hours. Strong colour development was observed on all light coloured fabrics up to the tenth impression in most instances as illustrated in figure 5.40 and figure 5.41.

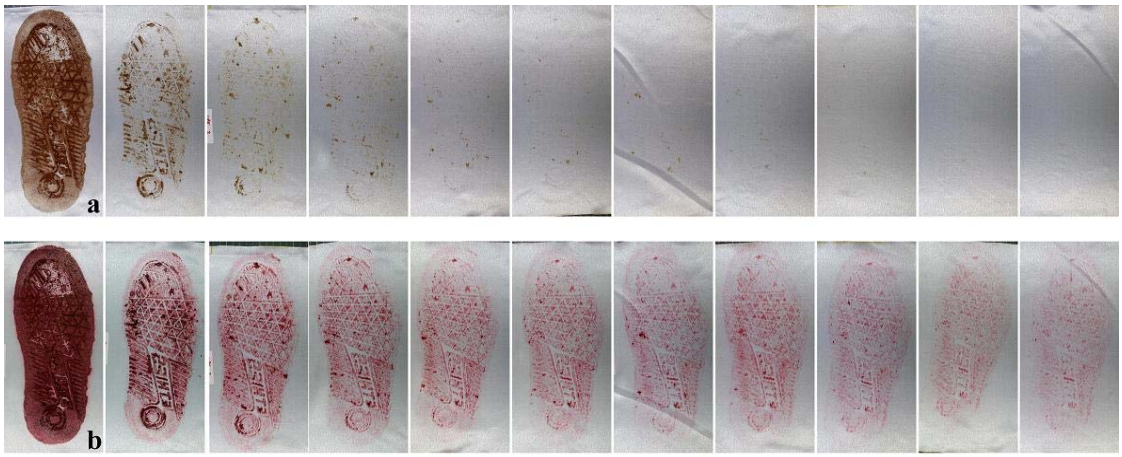


Figure 5.40 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on white polyester, with 2,2'-dipyridil: (a) before; and (b) after enhancement

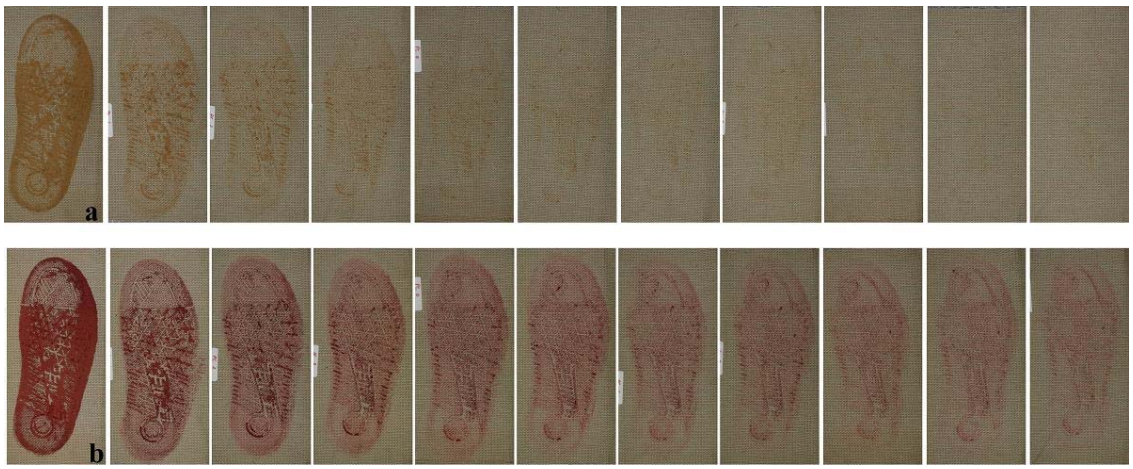


Figure 5.41 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with Wemyss Bay soil on patterned cotton, with 2,2'-dipyridil: (a) before; and (b) after enhancement

If further enhancement was necessary, re-application was carried out without any diffusion taking place. The carrier solvent was changed from methanol to ethanol making the technique less toxic and flammable and thus safer to use in confined spaces. In general, this technique provided the sharpest colour change in comparison to all other techniques tested in this study. This is in contrast to results by Someha [9] who reported potassium thiocyanate as the best enhancement technique, although, it is pointed out that 2,2'-dipyridil is the technique of choice in unventilated areas.

5.5.5.7 Road Soil Sample

Footwear impressions were prepared as previously described using the road soil sample. Ammonium thiocyanate and phenanthroline hydrosulfite techniques were omitted from the enhancement techniques for the road soil sample due to their poor enhancement capabilities across the other four soils. The other techniques, potassium thiocyanate, potassium ferrocyanide, ammonium pyrrolidinedithiocarbamate and 2,2'-dipyridil all revealed similar enhancement of footwear impressions prepared with the road soil sample as achieved with the other soil impressions Figures 5.42 – 5.45 illustrate some examples of the diminishing series with a road soil sample and the enhancement achieved with the various chemical reagents. In general, poor enhancement was observed on all dark fabrics and in most cases the contrast of the

impression was worse than for the original unenhanced impression, whereas good contrast was obtained on all light fabrics.

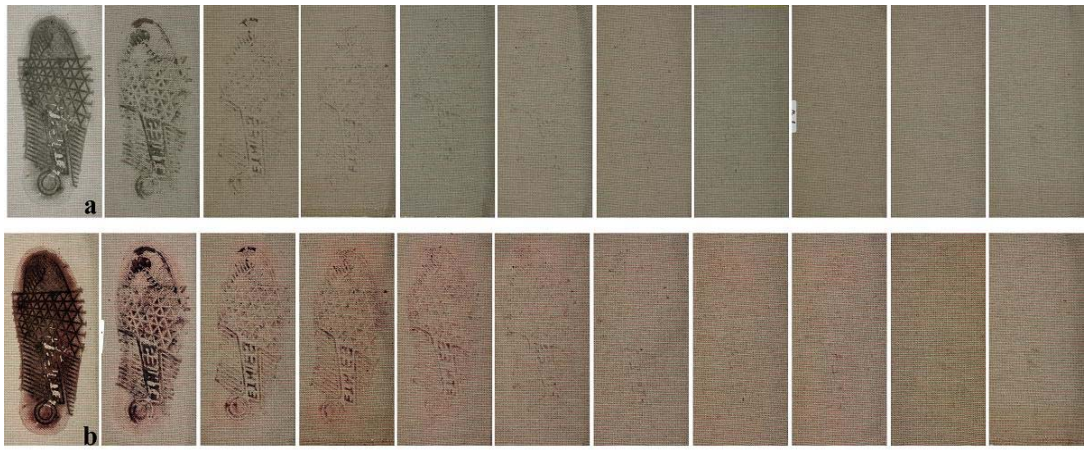


Figure 5.42 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with road soil sample on patterned cotton, with potassium thiocyanate: (a) before; and (b) after enhancement

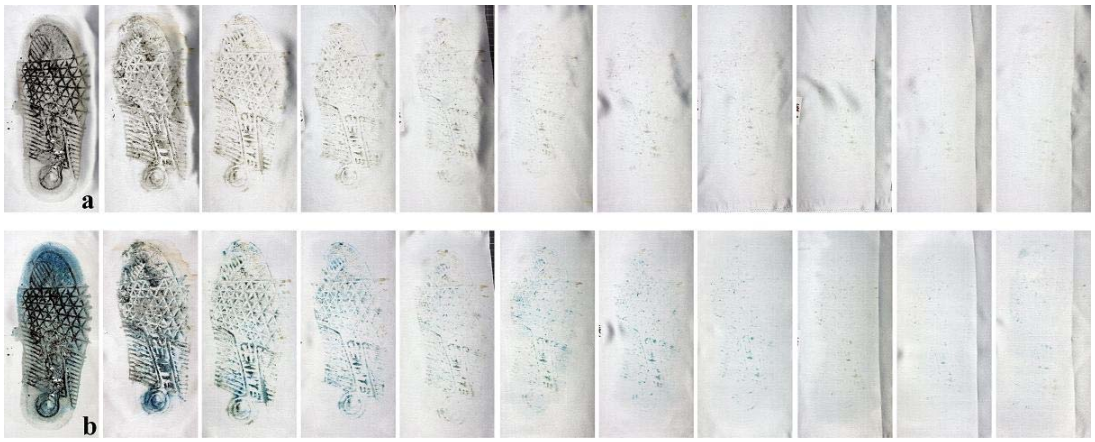


Figure 5.43 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with road soil sample on white polyester, with potassium ferrocyanide: (a) before; and (b) after enhancement

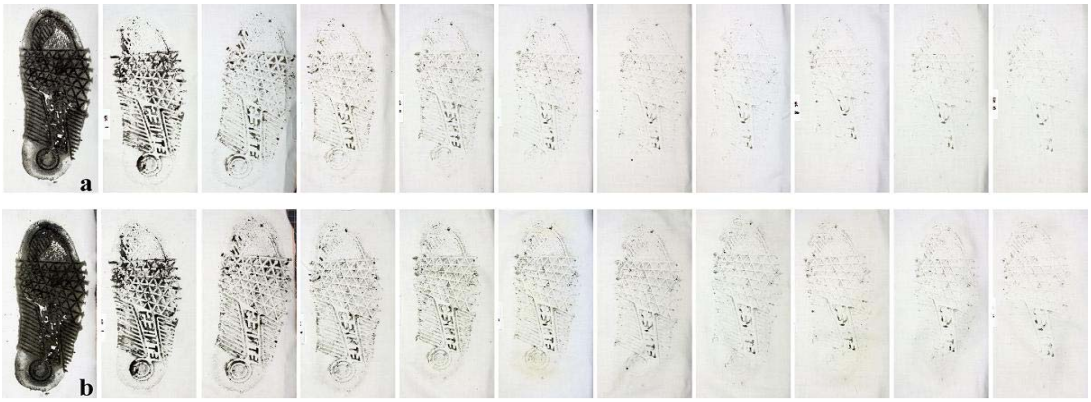


Figure 5.44 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with road soil sample on white cotton, with APD: (a) before; and (b) after enhancement

Figure 5.45 - Chemical enhancement of a diminishing series of footwear impressions in mud, prepared with road soil sample on black nylon/lycra, with 2,2'-dipyridil: (a) before; and (b) after enhancement

5.5.5.8 Enhancement of Fine Detail

It was not always possible to recover the fine detail of the footwear impression due to the nature of the fabric and the contaminant. The soil-based impressions appeared to diffuse into the fabric prior to enhancement and this interaction was confirmed through SEM analysis. Notwithstanding this the fine detail of the shoe pattern was visible in some cases with a variety of reagents as illustrated in figure 5.46.

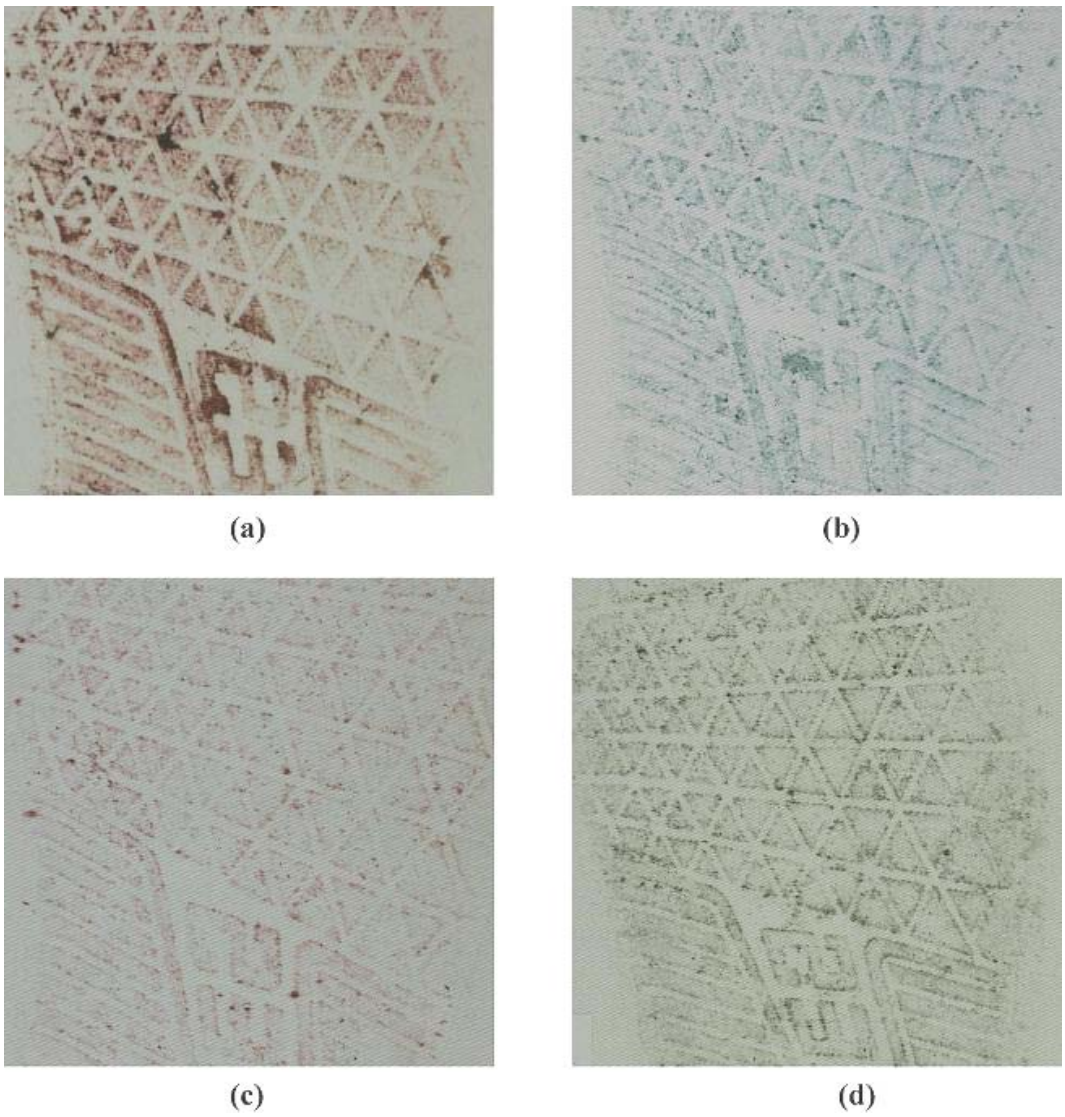


Figure 5.46 - Close-up detail of the enhancement of footwear impressions in mud on white cotton with: (a) potassium thiocyanate; (b) potassium ferrocyanide; (c) 2,2'-dipyridil and (d) APD

5.5.5.9 Sequencing of Enhancement Techniques

In order to investigate the effect of sequential application of the responsive enhancement reagents on impressions in soil, four random sequences of potassium thiocyanate, potassium ferrocyanide, ammonium pyrrolidinedithiocarbamate (APD) and 2,2'-dipyridil were selected for enhancement of footwear impressions prepared with the road dust sample as shown in table 5.8.

Table 5.8 - Sequence of soil enhancement reagents

Sequence Letter	Technique Sequence
A	potassium thiocyanate, potassium ferrocyanide, APD, 2,2'-dipyridil
B	2,2'-dipyridil, APD, potassium ferrocyanide, potassium thiocyanate
C	APD, potassium thiocyanate, 2,2'-dipyridil, potassium ferrocyanide
D	potassium ferrocyanide, 2,2'-dipyridil, potassium thiocyanate, APD

Sequence A

Potassium thiocyanate enhancement appeared to be stronger on white polyester than on white cotton producing a red enhancement of the impression. Some red enhancement was observed on black polyester and leatherette but the contrast between the impression and the background was poor. Different lighting conditions, such as oblique lighting, produced superior visualisation of the impression than potassium thiocyanate enhancement on black polyester and leatherette. The red enhancement developed into a blue colour when potassium ferrocyanide was applied but the overall enhancement did not improve. This effect occurred again with the application of APD changing the enhancement to a black colour. This might be important when the contrast with the background needs improving on a light surface, however, no improvement in the quantity of quality of the impression was observed. A slight improvement was observed when 2,2'-dipyridil was applied as the final technique to white cotton and polyester. A yellow tinge also developed on white polyester.

Sequence B

The red enhancement of 2,2'-dipyridil took about five minutes to develop and the enhancement appeared to be stronger on white polyester than on white cotton. The enhancement on black polyester and leatherette demonstrated overall poor contrast. The red colour reached a maximum after about 24 hours. 2,2'-dipyridil was followed by the application of APD and the red colour from 2,2'-dipyridil developed into a stronger red colour for both white cotton and polyester, however, the latter suffered

from some diffusion on application of the reagent. Potassium ferrocyanide and potassium thiocyanate were applied one after each other, however, no visual changes were observed. Figure 5.47 shows the sequential enhancement of white polyester using sequence B.

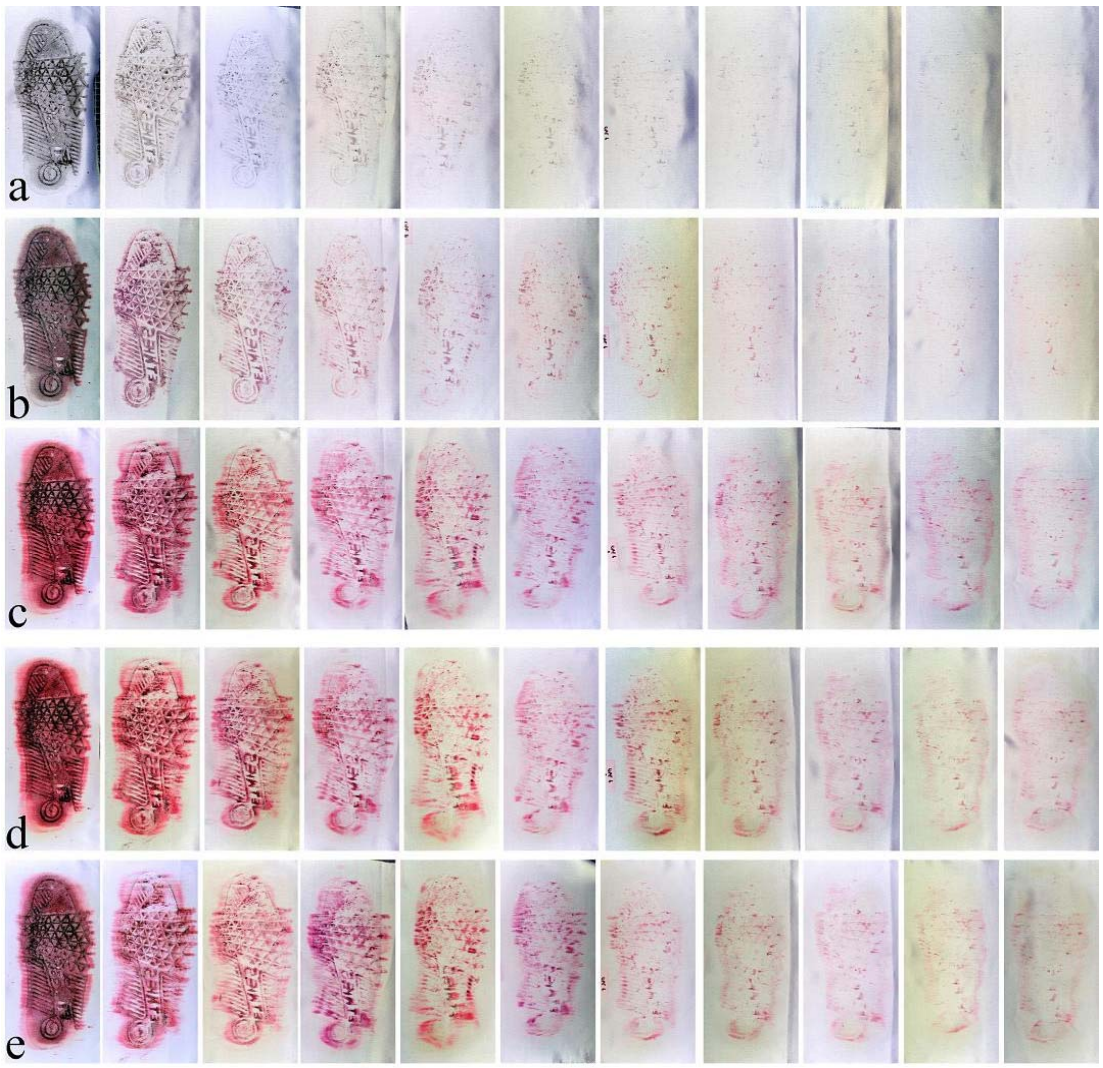


Figure 5.47 - Sequential enhancement (Sequence B) of a diminishing series of footwear impressions in mud, prepared with road soil sample on white polyester: (a) one week old muddy footwear impressions; (b) 2,2'-dipyridil; (c) APD; (d) potassium ferrocyanide; (e) potassium thiocyanate

Sequence C

APD provided reasonable enhancement on white cotton and polyester where a faster black colour developed on polyester. There was no visual enhancement on black polyester and leatherette due to poor contrast. There was no improvement when potassium thiocyanate followed APD except a slight red tinge on the first two impressions of the diminishing series on white cotton and white polyester. The development of the red colour on the application of 2,2'-dipyridil appeared to occur faster on the lighter coloured fabrics on application of the reagent. There was no improvement in the enhancement already achieved after the application of potassium ferrocyanide, however, a yellow tinge developed on the background of both the white cotton and polyester.

Sequence D

An initial blue colour developed on all fabrics after the application of potassium ferrocyanide. The colour was stronger on white polyester compared to white cotton. The enhancement on black polyester and leatherette appeared to slightly improve the visualisation of the footwear impressions. When 2,2'-dipyridil was applied, no red colour developed and the blue colour appeared stronger on all fabrics but less pronounced on black polyester and leatherette. A slight red colour developed on the lighter coloured fabrics overnight. Potassium thiocyanate enhancement improved the visualisation slightly with a blue/red colour developing on the white fabrics. Limited enhancement was observed on the dark fabrics and leatherette appeared to be damaged after the thiocyanate treatment, possibly due to a reaction with acetone in the chemical formulation. The application of APD did not improve the visualisation of the footwear impressions, however, a slight yellowing of the fabrics was observed.

Sequence Conclusions

Sequences A and D appeared to offer improved or continued enhancement throughout the whole sequence whereas the last technique/s of sequences B and C appeared to not improve the overall enhancement. It appears from this sequential study and from the previous enhancement studies using the four different soils that 2,2'-dipyridil alone provides the best enhancement on light coloured fabrics and in

some cases on darker fabrics. 2,2'-dipyridil can also be used in the enhancement of impressions on dark fabrics and can improve the contrast with the background. The use of more than one technique for enhancing the same footwear impression may result in diffusion leading to the obliteration of the original impression.

5.6 Conclusion

The six enhancement techniques discussed have demonstrated that it is possible to enhance footwear impressions in mud which are made on fabric. The best contrast was obtained on light coloured fabrics. Muddy impressions on black or dark fabrics were slightly enhanced, in some cases, at the beginning of the diminishing series but deteriorated as the series progressed. In general, superior colour enhancement was obtained on the synthetic fabrics however, these fabrics were more prone to diffusion of the original footwear impression in mud.

2,2'-dipyridil appeared to work well on all light coloured fabrics for impressions prepared with all soils used in this study and is a better alternative to the thiocyanates which emit toxic odours and use a highly flammable, irritant solvent. It was observed that 2,2'-dipyridil provided similar, if not better, results than the thiocyanates in most cases. This study has also demonstrated that, although sequential enhancement may improve the visualisation of footwear impressions in some cases, the use of 2,2'-dipyridil alone appears to be enough to offer good enhancement with limited diffusion. Observing the impressions under different lighting conditions, such as oblique lighting, prior to any chemical treatment also proved to be useful.

The soil from Wemyss bay produced the best enhancement results across all fabrics for the techniques investigated. The soil from North Berwick performed poorly, possibly due to its low iron levels and calcareous nature. The road side soil sample performed better than the soil from North Berwick.

The effect of pH did not seem to play a major role in the enhancement achieved. The higher retention of the soil-based impressions on synthetic fabrics was a result of the penetration of the soil particles into the fabric. Although the soil particles on natural fabrics appeared to rest on the surface, the enhancement achieved was comparable to

that obtained on the synthetic fabrics. Hence, the enhancement demonstrated dependence on the abundance of iron levels in soils, however, higher abundance did not necessarily imply better enhancement.

The soils used in this study appeared to have similar compositions although they were obtained from different parts of Scotland. The results cannot be generalised for the rest of the UK and further work is needed to address variables such as the consistency and colour of different soils.

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Chapter 6: Interpretation and Statistical Analysis of Chemical Enhancement Reagents for Footwear Impressions in Blood

6.1 Introduction

Footwear impressions were graded for quantity and quality of observable features; that is how much, post enhancement, of the footwear impression detail was visible (quantity) and how well the sharpness and contrast (quality) was achieved. Theeuwen *et al.* [1, 2] reported the grading results on the enhancement of impressions in blood and mud on porous and non-porous substrates. A study was conducted using three groups of volunteers. The first (postgraduate students) examined a wide series of impressions pre and post enhancement and was used to refine the methodology. Two further groups (practitioners and forensic science students) examined a smaller series using the refined methodology.

There are a number of limitations and complex issues that must be taken into account when interpreting the results of this chapter. Firstly, the limitations related to the enhancement of impressions in blood discussed in Chapter 3 are also valid here. Such an example is the use of a Program (P) setting for photography which might have affected the grading results obtained. Additionally, all practitioners were American but not all of them were certified footwear examiners. Individuals from different countries might perceive colour differently as they may have different sensitivities to colours. Although this grading work was performed in colour, it has now been established that future comparisons will be more robust if performed in black and white rather than colour images. Furthermore, the grading was done at the same time in the same room where peers might have discussed the gradings between them. Different results might have been obtained if the gradings were done independently.

6.2 Materials and Methods

A number of the enhanced footwear impressions in blood on fabric were graded by different groups of individuals. For each group, the footwear impressions (pre and post enhancement) were shown on a data projector screen in a dark room. The footwear impressions were graded after computer and monitor calibration using the following scale adopted from Theeuwen *et al.* [2].

Table 6.1 – Footwear impression grading system

Grading System	Description	Points
+++	Very much enhanced	3
++	Much enhanced	2
+	Somewhat enhanced	1
+/-	Unaltered	0
-	Deterioration	-1

The impressions enhanced with blood enhancement techniques were displayed (including repeats) and graded according to table 6.1. In each case, images of the footwear impression were observed and graded before and after enhancement for each fabric and technique.

Three groups of volunteers were asked to grade the images. The first group consisted of five postgraduate research students who graded a total of 2700 images (10 techniques x 9 fabrics x 6 repeats x 5 volunteers) and produced a combined score for quality and quantity. The second and third group consisted of 14 footwear examiners and 14 MSc forensic science students respectively. Both of these groups reviewed a reduced data set consisting of 5 fabrics and 14 blood enhancement techniques providing a total of 70 images. In comparison to the previous grading results, quality and quantity were graded separately for both of these groups.

The resultant data was analysed using SPSS (version 18) where the model chosen assumed that the responses (grading scores) were continuous and normally

distributed. The Kolmogorov-Smirnov (KS) test was performed for each fabric-technique combination to determine whether the grading scores are normally distributed or not. The individual fabric-technique combination revealed a normal distribution. This facilitated the use of an analysis of variance as the appropriate statistical test. A two-way ANOVA statistical test (95% confidence level, 0.05) was performed to investigate the effect of the independent variables (fabric type and enhancement technique) on the dependent variable (grading score) to identify any significant interaction effects.

6.3 Results and Discussion

6.3.1 Grading of Footwear Impressions by Research Students

Initial grading experiments were carried out using a small group of postgraduate research students in order to provide a baseline of assessment of the grading process. This process was later refined for the two further study groups (practitioners and forensic science students).

The ANOVA results associated with the grading scores obtained are revealed in table 6.2. The independent variables and all the interaction effects were revealed to contribute to the variation in the grading scores obtained. This is evidenced by the significant p values obtained in each case and presented in table 6.2.

The profile plots for the fabric and volunteer are provided in figure 6.1a and b and illustrate the overall variation in the grading results produced across the volunteer group. Volunteer 4 (V4) overall was the most stringent in grading the footwear impressions and as a consequence produced the lower scores in almost all cases and volunteer 1 (V1) produced consistently higher scores than others. Notwithstanding this, all volunteers returned the same general trends in relation to the grades produced for each fabric and all agreed that leather produced the least favourable enhancement of impressions. Furthermore, the figure 6.1b indicates that, footwear impressions in blood are most likely to be enhanced best on black polyester and patterned cotton and that overall, luminol provided an enhancement that obtained the highest average grading score as illustrated in figure 6.2.

Table 6.2 – ANOVA table in terms of grading by research students

Tests of Between-Subjects Effects

Dependent Variable: Grading

Source	Type III Sum of Squares	Df	Mean Square	F	Sig. P Value
Corrected Model	2799.205[a]	161	17.386	60.055	.000
Intercept	1155.459	1	1155.459	3991.153	.000
Technique	392.043	9	43.560	150.465	.000
Fabric	645.496	8	80.687	278.707	.000
Volunteer	43.229	4	10.807	37.330	.000
Technique * Fabric	1613.211	72	22.406	77.393	.000
Fabric * Volunteer	34.851	32	1.089	3.762	.000
Technique * Volunteer	46.069	36	1.280	4.420	.000
Fabric * Volunteer * Technique	142.947	288	.496	1.880	.000
Error	609.187	2310	.264		
Total	4617.000	2760			
Corrected Total	3551.339	2759			

a. R Squared = .829 (Adjusted R Squared = .795)

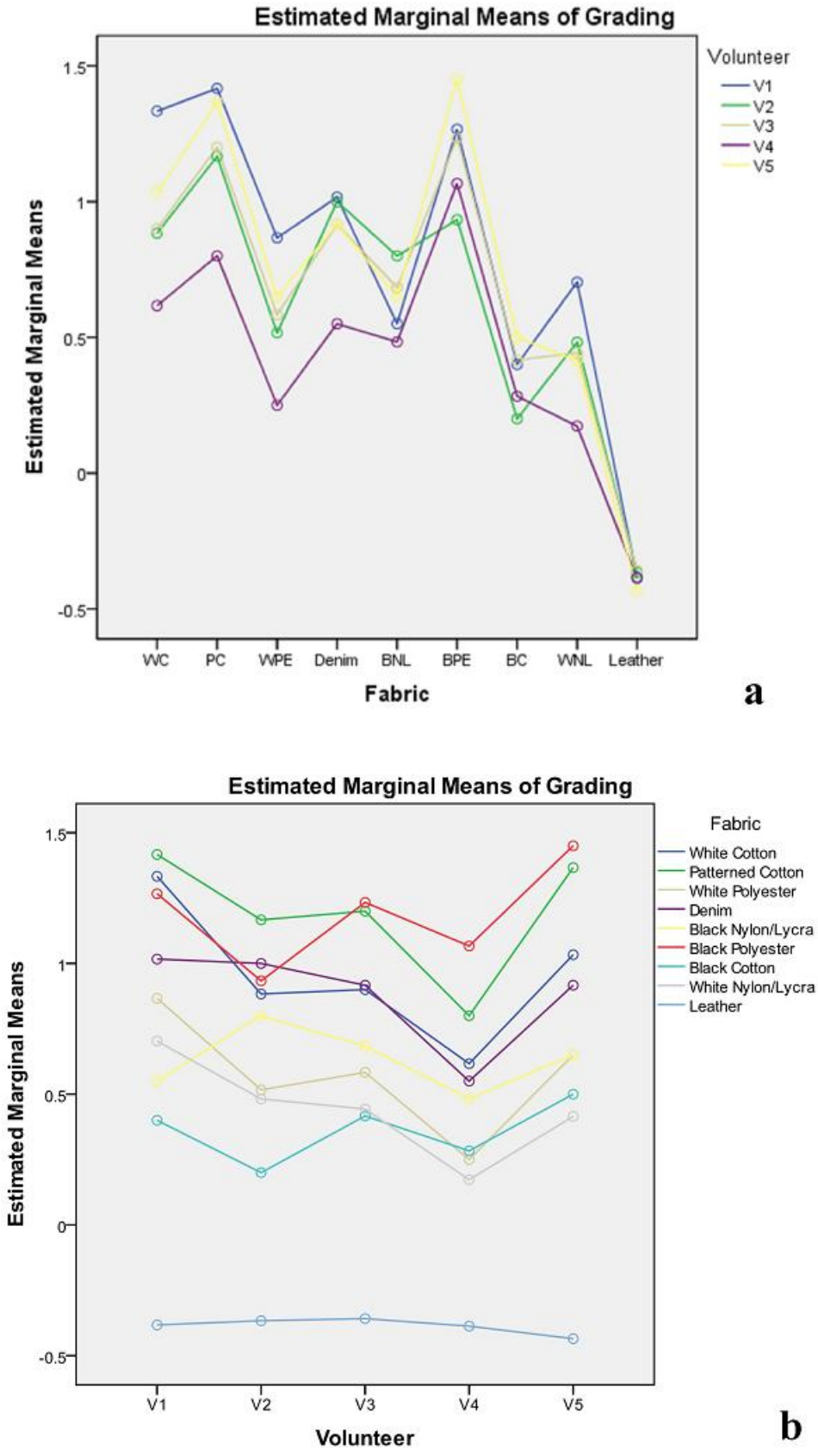

Figure 6.1 – Profile plots: (a) fabric by volunteers and (b) volunteers by fabric

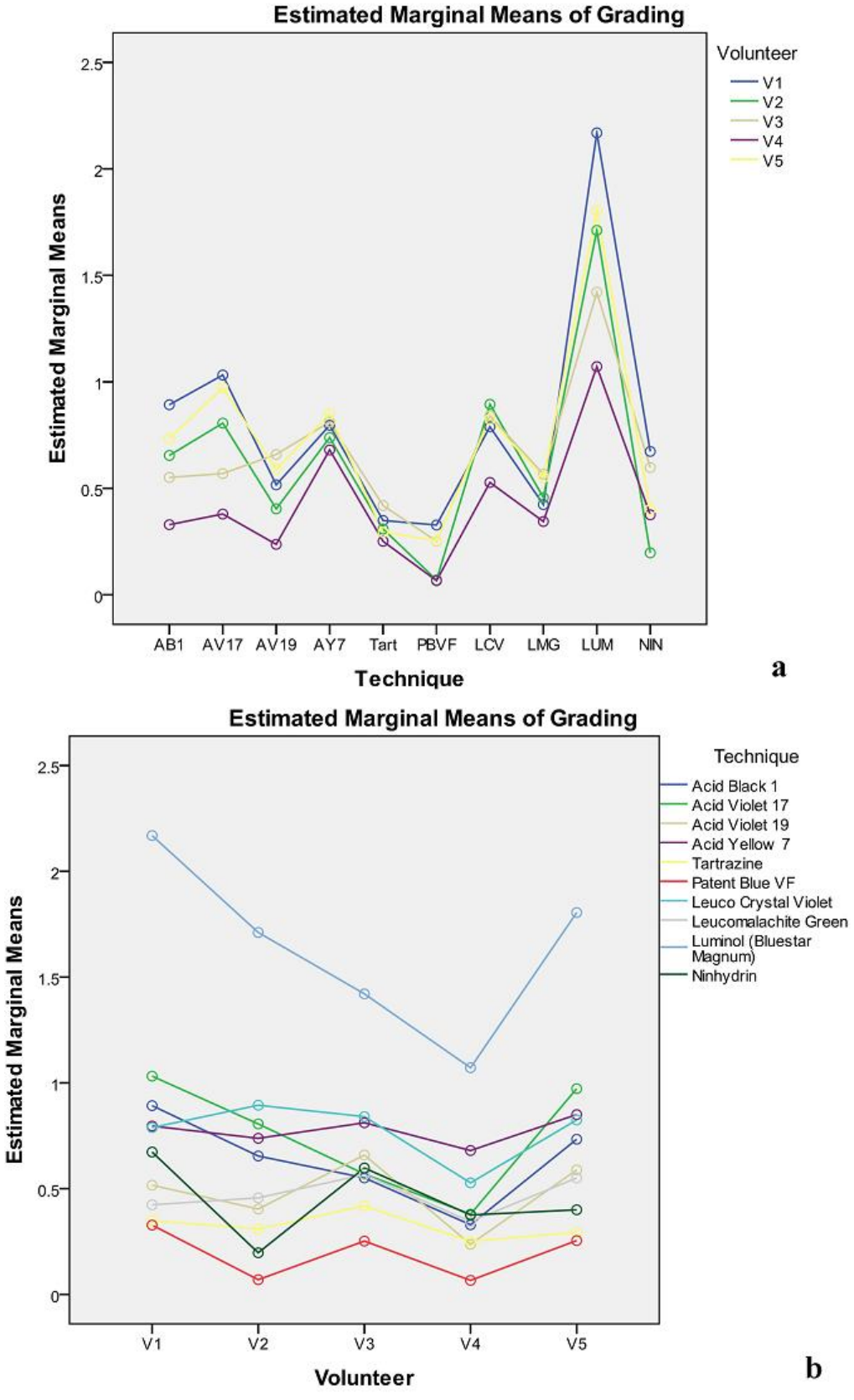

Figure 6.2 - Profile plots: (a) technique by volunteers and (b) volunteers by technique

Figure 6.3 shows that other enhancement techniques might demonstrate superior enhancement on a given fabric. For example, the fluorescence from acid yellow 7 provided better enhancement results on black fabrics when compared to luminol. For clarity purposes, some of the techniques were removed from the profile plot in figure 6.3 which illustrates the interaction effects of technique, fabric and volunteers on the grading scores obtained [3].

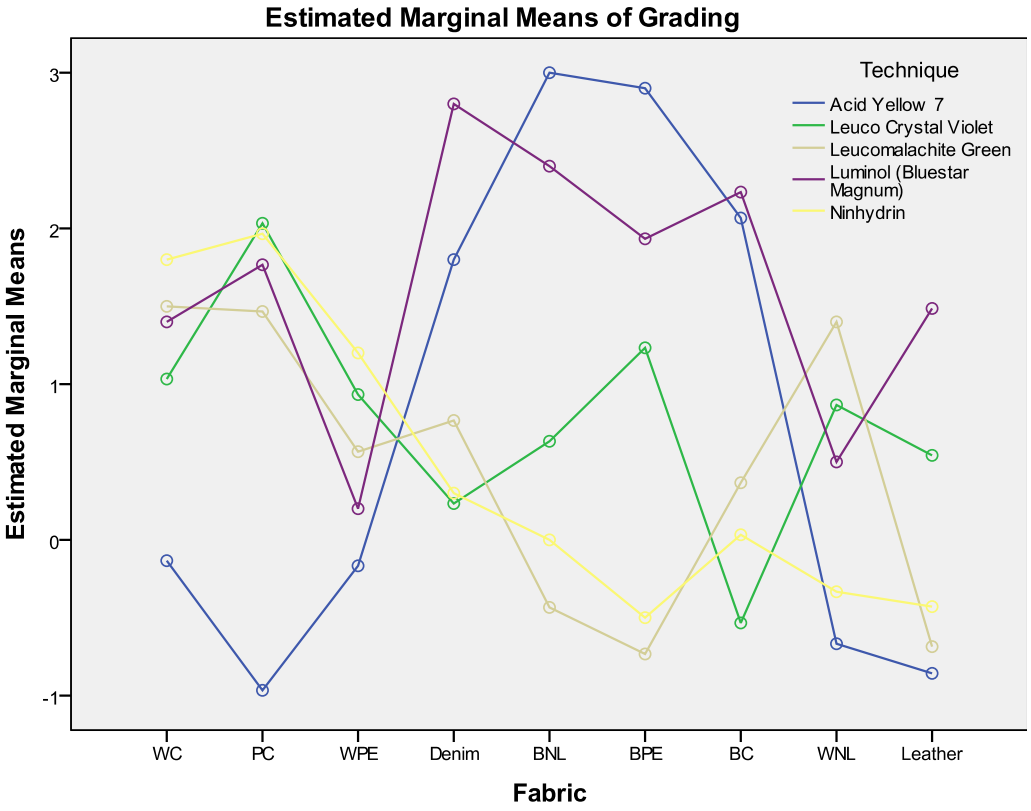

Figure 6.3 - Profile plots (fabric by technique)

Further exploration of the results was undertaken as follows:

Grading by fabric: The grading results for each fabric and technique combination per volunteer were totalled and then the total number obtained divided by 54 (9 fabrics x 6 repeats) as presented in table 6.3. The results confirm the observations in figure 6.3 and demonstrate that impressions in blood are least likely to be enhanced on leather whereas the opposite is true for impressions on black polyester and patterned cotton. This high value for black polyester is due to the successful results obtained with protein stains and oblique lighting.

Table 6.3 – Grading results by volunteer and fabric (research students, n=5)

	White Cotton	Patterned Cotton	Black Cotton	White Polyester	Black Polyester	White Nylon	Black Nylon	Denim	Leather
V1	1.5	1.6	0.4	1.0	1.4	0.8	0.6	1.1	-0.4
V2	1.0	1.3	0.2	0.6	1.0	0.5	0.9	1.1	-0.4
V3	1.0	1.3	0.5	0.6	1.4	0.5	0.8	1.0	-0.4
V4	0.7	0.9	0.3	0.3	1.2	0.2	0.5	0.6	-0.5
V5	1.1	1.5	0.6	0.7	1.6	0.5	0.7	1.0	-0.4
Total	**5.3**	**6.6**	**2.0**	**3.2**	**6.6**	**2.5**	**3.5**	**4.9**	**-2.1**
Average (Out of 3)	**1.1**	**1.3**	**0.4**	**0.6**	**1.3**	**0.5**	**0.7**	**1.0**	**-0.4**

Grading by enhancement reagent: The grading results obtained were also explored by enhancement technique as illustrated in table 6.4. In this case, the grading results for each volunteer and fabric per technique were summed and divided by 30 (6 repeats x 5 volunteers). Patent blue VF (acid blue 1) proved to be the worst technique overall for the enhancement of footwear impressions in blood on fabric. Luminol was revealed to work best with good enhancement of impressions on denim and leather in comparison to poor enhancement of all other techniques. This provides a further refinement of the results presented in figure 6.2 which indicated that luminol was the best performing technique overall. The values highlighted in blue illustrate the best technique for a particular fabric according to the grading scores obtained. For example acid yellow 7 provided the best enhancement of footwear impressions in blood on black cotton, polyester and nylon/lycra. This is due to the fluorescence advantage which provided a better enhancement than luminol in each of these cases. In contrast, acid yellow 7 performed poorly on light coloured fabrics due to background fluorescence. Ninhydrin appeared to give suitable enhancement results on light coloured fabrics according to the grading results.

Table 6.4 – Grading results by fabric and technique (research students, n=5)

	AB1	AV17	AV19	AY7	Tart	PB VF	LCV	LMG	Lum	NIN
WC	1.6	1.4	0.7	-0.1	0.0	0.2	1.0	1.5	1.4	1.8
PC	1.7	1.8	1.8	-1.0	-0.1	0.4	2.0	1.5	1.8	2.0
BC	0.0	0.0	-0.3	2.1	0.5	-0.7	-0.5	0.7	1.9	0.0
WPE	0.9	0.7	1.1	-0.2	0.1	0.1	0.9	0.6	0.2	1.2
BPE	0.7	1.9	0.8	2.9	2.1	1.6	1.2	-0.7	1.9	-0.5
WNL	0.4	0.8	0.7	-0.7	0.0	0.7	0.9	1.4	0.5	-0.3
BNL	0.0	0.3	-0.6	3.0	1.1	0.0	0.6	-0.4	2.4	0.0
Denim	1.3	0.8	0.8	1.8	0.0	0.1	0.2	0.8	2.8	0.3
Leather	-1.0	-1.0	-0.8	-1.0	-0.7	-0.7	0.8	-0.8	1.7	-0.4
Total	5.6	6.7	4.3	6.9	2.9	1.7	7.2	4.5	14.6	4.0
Average (out of 3)	0.6	0.7	0.5	0.8	0.3	0.2	0.8	0.5	1.6	0.4

Even though individual combinations of enhancement technique and fabric type demonstrated a normal distribution, the overall data set was not parametric (confirmed by the Kolmogorov-Smirnov test), Spearman's correlation test, the non-parametric equivalent of the Pearson correlation, was used to check for correlations between fabric, volunteer and technique. The test provides a measure of the magnitude and direction of the association between the variables where the correlation coefficients vary between -1 and +1. The closer the value is to ±1, the stronger is the correlation. The Spearman correlation uses the same calculations as the Pearson correlation but converts the scores into ranks before performing the test allowing the correlation to be tested even when there is a non-linear relationship [3]. Significant correlations are marked with an asterisk * at the 0.05 level and with a double asterisk ** at the 0.01 level. Table 6.5 illustrates the correlation results for the volunteers grading scores arranged by fabric. The results indicate a strong, positive and significant correlation for the grading scores when arranged by fabric. The correlation is significant at the 0.01 level (**) except in one case where it is significant at the 0.05 level [(*) V1 and V4 for white polyester]. Table 6.6 shows the correlation results arranged by enhancement technique where again a strong, positive and significant correlation was obtained for the grading scores. All correlations are significant at the 0.01 level (**).

Table 6.5 – Correlations of grading scores by fabric (V represents volunteer)

Fabric		V1	V2	V3	V4	V5
White Cotton	V1	1.000	.825**	.749**	.823**	.825**
	V2	.825**	1.000	.750**	.798**	.764**
	V3	.749**	.750**	1.000	.761**	.747**
	V4	.823**	.798**	.761**	1.000	.814**
	V5	.825**	.764**	.747**	.814**	1.000
Patterned Cotton	V1	1.000	.852**	.904**	.855**	.912**
	V2	.852**	1.000	.846**	.862**	.841**
	V3	.904**	.846**	1.000	.841**	.832**
	V4	.855**	.862**	.841**	1.000	.841**
	V5	.912**	.841**	.832**	.841**	1.000
Black Cotton	V1	1.000	.824**	.813**	.795**	.674**
	V2	.824**	1.000	.757**	.819**	.721**
	V3	.813**	.757**	1.000	.785**	.689**
	V4	.795**	.819**	.785**	1.000	.668**
	V5	.674**	.721**	.689**	.668**	1.000
White PE	V1	1.000	.635**	.524**	.295*	.584**
	V2	.635**	1.000	.563**	.426**	.611**
	V3	.524**	.563**	1.000	.555**	.646**
	V4	.295*	.426**	.555**	1.000	.487**
	V5	.584**	.611**	.646**	.487**	1.000
Black PE	V1	1.000	.879**	.867**	.878**	.868**
	V2	.879**	1.000	.876**	.887**	.883**
	V3	.867**	.876**	1.000	.900**	.891**
	V4	.878**	.887**	.900**	1.000	.894**
	V5	.868**	.883**	.891**	.894**	1.000
White NL	V1	1.000	.722**	.576**	.438**	.745**
	V2	.722**	1.000	.671**	.482**	.689**
	V3	.576**	.671**	1.000	.561**	.594**
	V4	.438**	.482**	.561**	1.000	.475**
	V5	.745**	.689**	.594**	.475**	1.000
Black NL	V1	1.000	.917**	.905**	.851**	.878**
	V2	.917**	1.000	.941**	.855**	.895**
	V3	.905**	.941**	1.000	.863**	.841**
	V4	.851**	.855**	.863**	1.000	.801**
	V5	.878**	.895**	.841**	.801**	1.000
Denim	V1	1.000	.811**	.816**	.740**	.820**
	V2	.811**	1.000	.838**	.867**	.937**
	V3	.816**	.838**	1.000	.830**	.849**
	V4	.740**	.867**	.830**	1.000	.881**
	V5	.820**	.937**	.849**	.881**	1.000
Leather	V1	1.000	.794**	.852**	.862**	.942**
	V2	.794**	1.000	.748**	.750**	.806**
	V3	.852**	.748**	1.000	.805**	.829**
	V4	.862**	.750**	.805**	1.000	.797**
	V5	.942**	.806**	.829**	.797**	1.000

Table 6.6 – Correlations of grading scores by enhancement technique (V represents volunteer)

Technique		V1	V2	V3	V4	V5
AB1	V1	1.000	.842**	.730**	.696**	.789**
	V2	.842**	1.000	.825**	.817**	.838**
	V3	.730**	.825**	1.000	.804**	.807**
	V4	.696**	.817**	.804**	1.000	.773**
	V5	.789**	.838**	.807**	.773**	1.000
AV17	V1	1.000	.771**	.681**	.738**	.738**
	V2	.771**	1.000	.733**	.809**	.802**
	V3	.681**	.733**	1.000	.807**	.806**
	V4	.738**	.809**	.807**	1.000	.824**
	V5	.738**	.802**	.806**	.824**	1.000
AV19	V1	1.000	.799**	.853**	.783**	.887**
	V2	.799**	1.000	.708**	.718**	.757**
	V3	.853**	.708**	1.000	.770**	.883**
	V4	.783**	.718**	.770**	1.000	.793**
	V5	.887**	.757**	.883**	.793**	1.000
AY7	V1	1.000	.917**	.967**	.962**	.940**
	V2	.917**	1.000	.913**	.902**	.863**
	V3	.967**	.913**	1.000	.959**	.912**
	V4	.962**	.902**	.959**	1.000	.936**
	V5	.940**	.863**	.912**	.936**	1.000
Tart	V1	1.000	.849**	.892**	.808**	.821**
	V2	.849**	1.000	.824**	.858**	.848**
	V3	.892**	.824**	1.000	.808**	.796**
	V4	.808**	.858**	.808**	1.000	.781**
	V5	.821**	.848**	.796**	.781**	1.000
PB VF	V1	1.000	.741**	.765**	.633**	.873**
	V2	.741**	1.000	.770**	.545**	.805**
	V3	.765**	.770**	1.000	.642**	.761**
	V4	.633**	.545**	.642**	1.000	.609**
	V5	.873**	.805**	.761**	.609**	1.000
LCV	V1	1.000	.887**	.867**	.722**	.864**
	V2	.887**	1.000	.913**	.753**	.837**
	V3	.867**	.913**	1.000	.680**	.828**
	V4	.722**	.753**	.680**	1.000	.775**
	V5	.864**	.837**	.828**	.775**	1.000
LMG	V1	1.000	.801**	.851**	.810**	.841**
	V2	.801**	1.000	.881**	.789**	.805**
	V3	.851**	.881**	1.000	.853**	.791**
	V4	.810**	.789**	.853**	1.000	.837**
	V5	.841**	.805**	.791**	.837**	1.000
LUM	V1	1.000	.711**	.699**	.740**	.662**
	V2	.711**	1.000	.827**	.758**	.758**
	V3	.699**	.827**	1.000	.786**	.816**
	V4	.740**	.758**	.786**	1.000	.693**
	V5	.662**	.758**	.816**	.693**	1.000

NIN	V1	1.000	.889**	.853**	.842**	.731**
	V2	.889**	1.000	.869**	.848**	.801**
	V3	.853**	.869**	1.000	.803**	.753**
	V4	.842**	.848**	.803**	1.000	.812**
	V5	.731**	.801**	.753**	.812**	1.000

In tables 6.5 and 6.6, the positive correlations indicate that the grading scores may be a good indicator of the enhancement techniques performance.

This first survey did not differentiate between the grading of quantity and quality of the footwear enhancement and differences were noted between the grading results of volunteers. During discussion with the volunteers, it was found that some considered improved sharpness as an enhancement whereas others only took into consideration the colour of the visual enhancement as an improvement. As a consequence the grading of each of these qualities was carried out independently with group 2 (practitioners) and group 3 (forensic science students).

6.3.2 Grading of Footwear Impressions by Practitioners

In total, 14 practitioners took part in the study. Similar to the previous grading results, a two-way ANOVA statistical test (95% confidence level, 0.05) was performed to investigate the effect of the independent variables (fabric and enhancement technique) on the dependent variable (grade scoring) to identify any significant interaction effects. The results are presented in table 6.7 and table 6.8 for impression quality and impression quantity grading scores respectively. The independent variables and most of the interaction effects contributed to the variation as illustrated by the grading scores obtained.

The data set as a whole was found to be non-parametric and a Friedman statistical test (equivalent of a parametric one-factor repeated measures ANOVA) was performed to assess whether a significant difference between the two sets of grading results, quality and quantity, existed. The results indicated there is no significant difference at the 0.05 level in the grading scores of quality and quantity by the practitioners. The profile plots for the quality and grading scores are presented in figure 6.4 highlighting the similar means for the two data sets with no or little difference.

Table 6.7 - ANOVA table in terms of quality grading for practitioners

Tests of Between-Subjects Effects

Dependent Variable: Impression Quality

Source	Type III Sum of Squares	df	Mean Square	F	Sig. p-value
Corrected Model	1635.202[a]	303	5.397	20.905	.000
Intercept	1474.290	1	1474.290	5711.022	.000
Fabric	113.251	4	28.313	109.676	.000
Technique	352.596	13	27.123	105.067	.000
Volunteer	13.510	13	1.039	4.026	.000
Fabric * Technique	1072.292	52	20.621	79.880	.000
Fabric * Volunteer	19.949	52	.384	1.486	.017
Technique * Volunteer	63.604	169	.376	1.458	.001
Error	174.508	676	.258		
Total	3284.000	980			
Corrected Total	1809.710	979			

a. R Squared = .904 (Adjusted R Squared = .860)

Table 6.8 - ANOVA table in terms of quantity grading for practitioners

Tests of Between-Subjects Effects

Dependent Variable: Impression Quantity

Source	Type III Sum of Squares	df	Mean Square	F	Sig. p-value
Corrected Model	1650.246[a]	303	5.446	19.430	.000
Intercept	1521.266	1	1521.266	5427.137	.000
Fabric	110.402	4	27.601	98.465	.000
Technique	350.691	13	26.976	96.238	.000
Volunteer	15.405	13	1.185	4.228	.000
Fabric * Technique	1081.427	52	20.797	74.192	.000
Fabric * Volunteer	18.284	52	.352	1.254	.114
Technique * Volunteer	74.038	169	.438	1.563	.000
Error	189.488	676	.280		
Total	3361.000	980			
Corrected Total	1839.734	979			

a. R Squared = .897 (Adjusted R Squared = .851)

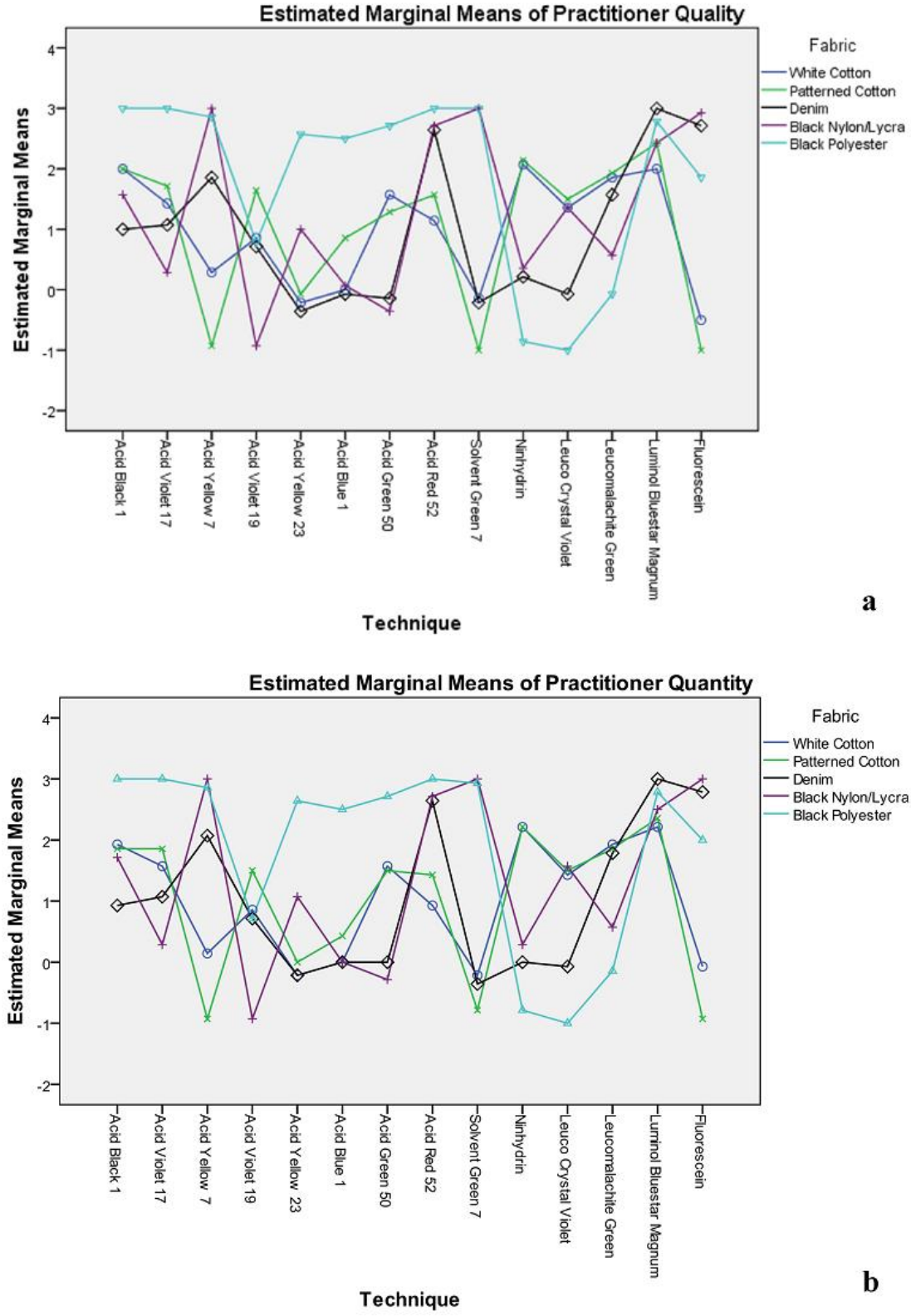

Figure 6.4 - Profile plots for practitioners: (a) quality and (b) quantity

6.3.3 Grading of Footwear Impressions by Forensic Science Students

14 MSc forensic science students at the University of Strathclyde undertook the same grading test as the practitioners. This cohort of students had received some basic information in relation to the enhancement and examination of footwear impressions. The same statistical tests (as previously described) were utilised for the analysis of the quality and quantity of grading scores obtained from the MSc forensic science students. For quality grading all main effects and only one pairwise interaction effect (fabric*technique) were significant at the 0.05 level of significance (table 6.9). In contrast, for quantity grading, all main effects and pairwise interaction effects (table 6.10) were significant at the 0.05 level of significance (p-value less than 0.05). In other words, the independent variables and most of the interaction effects contribute to the variation in the grading scores (quality and quantity) obtained.

Table 6.9 - ANOVA table in terms of forensic science students quality grading

Tests of Between-Subjects Effects

Dependent Variable: Impression Quality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1112.112[a]	303	3.670	19.432	.000
Intercept	790.204	1	790.204	4183.604	.000
Fabric	82.827	4	20.707	109.628	.000
Technique	178.710	13	13.747	72.781	.000
Volunteer	14.110	13	1.085	5.746	.000
Fabric * Technique	785.545	52	15.107	79.980	.000
Fabric * Volunteer	13.145	52	.253	1.338	.061
Technique * Volunteer	37.776	169	.224	1.183	.076
Error	127.684	676	.189		
Total	2030.000	980			
Corrected Total	1239.796	979			

a. R Squared = .897 (Adjusted R Squared = .851)

Table 6.10 - ANOVA table in terms of forensic science students quantity grading

Tests of Between-Subjects Effects

Dependent Variable: Impression Quantity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1193.526[a]	303	3.939	20.161	.000
Intercept	907.397	1	907.397	4644.244	.000
Fabric	113.220	4	28.305	144.871	.000
Technique	205.646	13	15.819	80.964	.000
Volunteer	18.903	13	1.454	7.442	.000
Fabric * Technique	793.237	52	15.255	78.076	.000
Fabric * Volunteer	16.265	52	.313	1.601	.006
Technique * Volunteer	46.254	169	.274	1.401	.002
Error	132.078	676	.195		
Total	2233.000	980			
Corrected Total	1325.603	979			

a. R Squared = .900 (Adjusted R Squared = .856)

Correlation tests again indicated that there is a strong, positive and significant correlation for the grading scores when arranged by fabric or technique. In comparison to the profile plots for the practitioner quality and quantity grading scores (figure 6.4), there is a higher degree of variation in the forensic science students profile plots for quality and quantity (figure 6.5).

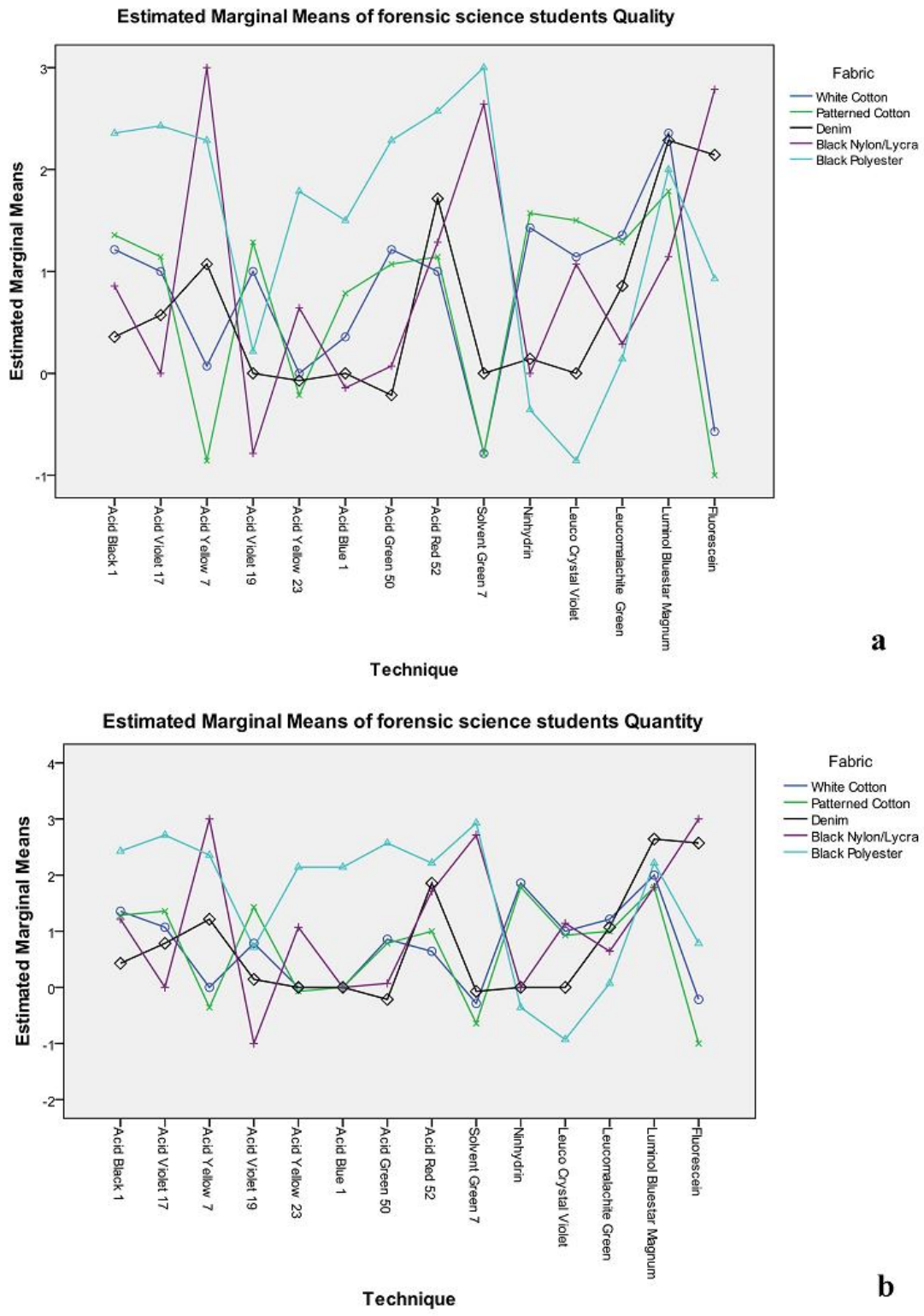

Figure 6.5 - Profile plots for forensic science students: (a) quality and (b) quantity

6.4 Conclusion

The profile plots for practitioners indicated a slight variation between the grading of quality and quantity enhancement in contrast to the high degree of variation in the grading from the forensic science students. There also appears to be a high variation amongst the students in comparison to the agreement between the practitioners.

Taking into account the limitations of this work, the results appear to suggest that individuals who have obtained training or education related to footwear examination are better suited for the job rather than individuals without any training or certification.

Nonetheless, there are similarities within the two groups and with the research students grading. Black polyester obtained high grading due to its excellent enhancement with protein stains and oblique lighting. Luminol appeared to be the best overall technique, however, acid yellow 7 provided better enhancement on the black fabrics of cotton, polyester and nylon/lycra.

6.5 References

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Chapter 7: Conclusions and Future Work

7.1 Conclusions

This is the first systematic study to examine both a wide range of enhancement techniques and the effect of the interaction between the fabric surface and the contaminant on the subsequent enhancement. In addition, impressions were prepared using a whole footwear sole rather than a small section of the sole.

Studies [1-11] relating to footwear impression enhancement have focused on the chemical mechanisms underlying specific techniques or the enhancement efficiency with little or no obvious regard to potential variables in the preparation of test footwear impressions. The results obtained from enhancement comparisons of footwear impressions without taking into consideration the variable of force can be misleading. Consequently, a mechanical stamping rig was developed and used to control the delivery pressure of the mark. Other variables, such as the amount of contaminant on the footwear sole, were also carefully controlled. This facilitated the direct comparison between techniques across the different fabrics and contaminants.

The enhancement achieved appeared to be partly dependent on the surface topography of the fabric although this was limited for impressions in urine. Footwear impressions in blood on synthetic fabrics and enhanced with protein stains appeared to have less background staining than natural fabrics, however, the opposite was found to be true when peroxidase reagents were utilised. The success of enhancement on footwear impressions in mud depended on both the soil type and on the fabric surface topography.

For the enhancement of footwear impressions in blood, luminol was the best performing enhancement technique overall, however, acid yellow 7 and fluorescein appear to offer better results and less diffusion than luminol for the enhancement of footwear impressions in blood on black cotton, polyester and nylon/lycra. The use of alginates is an available technique for the *in situ* recovery of blood impressions which might be suitable for use on fabric surfaces, such as leather and denim, where other techniques struggled to achieve enhancement.

Footwear impressions in urine on light coloured fabrics where enhanced with 1,2-indanedione, ninhydrin, DMAC and DFO where DFO provided the best enhancement overall with no tangible advantage observed when the reagents are used in sequence. Furthermore, it has been demonstrated that non-destructive lighting techniques can provide suitable a means of enhancing impressions in urine and that the use of alternative or oblique lighting should be performed first before any chemical treatment.

In the case of soil-based footwear impressions on fabrics, 2,2'-dipyridil appeared to work well on all light coloured fabrics for impressions prepared with all soils used in this study and might be a suitable alternative to the thiocyanates which emit toxic odours and use a highly flammable, irritant solvent. Other techniques demonstrated good enhancement on all light coloured fabrics, however, all techniques struggled to achieve any sort of enhancement on dark coloured fabrics. The enhancement process did not appear to be dependent on the penetration of the soil particles into the fabric or the soil pH.

The grading system appeared to work well in this study, however, there is room for improvement or development of a better scale which can be devised with support from operational practitioners.

7.2 Recommendations for the Enhancement of Footwear Impressions on Fabric

Chemical treatment of impressions on fabrics may result in background staining and furthermore repeated application, as in sequential processes, increases the occurrence of diffusion and the obliteration of the original impression in some cases. Although sequential enhancement was investigated in this study, it appears that choosing the best single chemical technique for that particular fabric provides better results. This is particularly the case for impressions in blood. For impressions in urine and mud, sequential enhancement techniques can be used however the initial enhancement technique generally provided good results. A suggested sequential process is provided in figures 7.1 to 7.3 based on results and observations using the fabrics mentioned in this study.

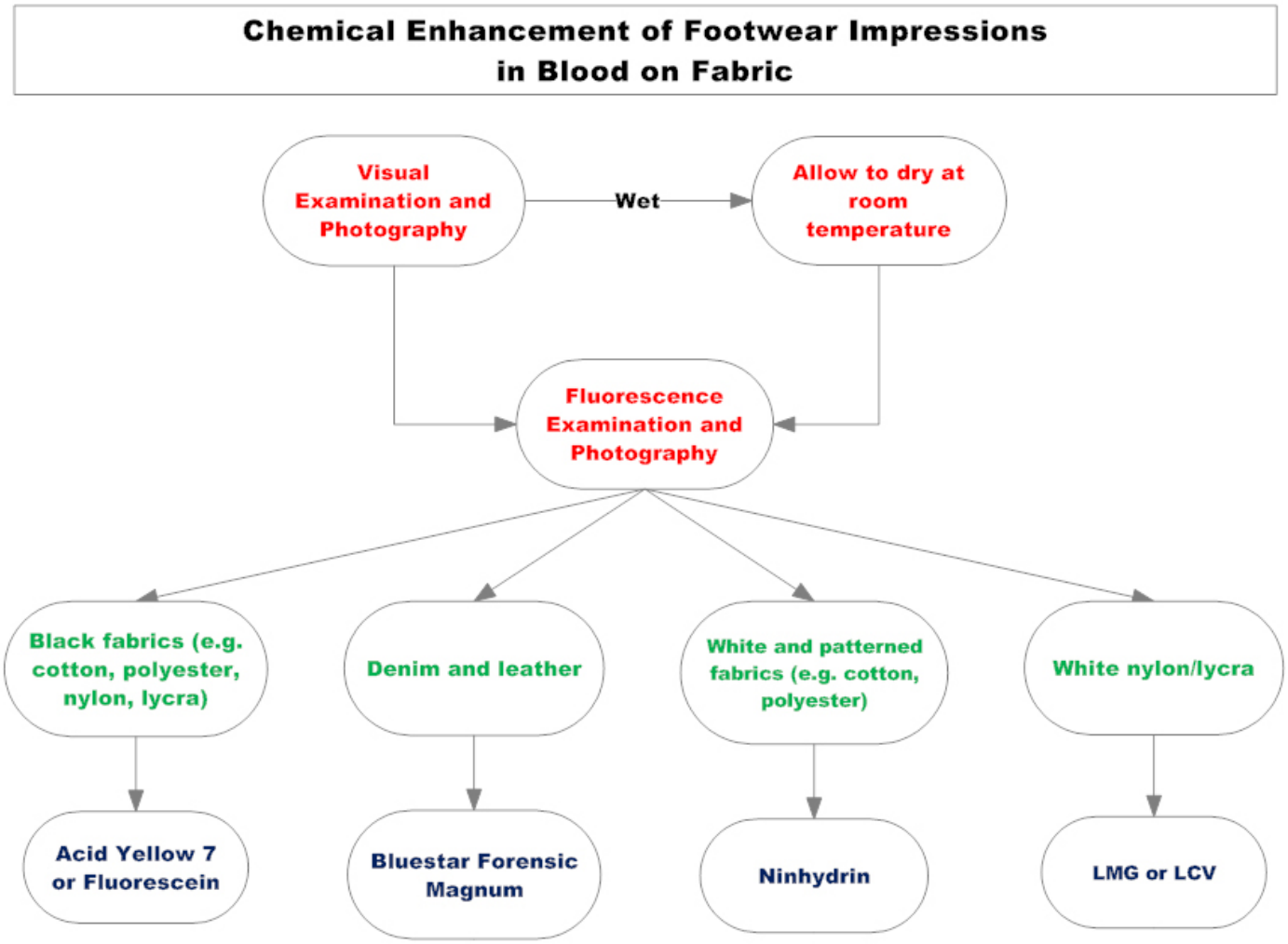

Figure 7.1 – Sequential chemical process for the enhancement of footwear impressions in blood on fabric

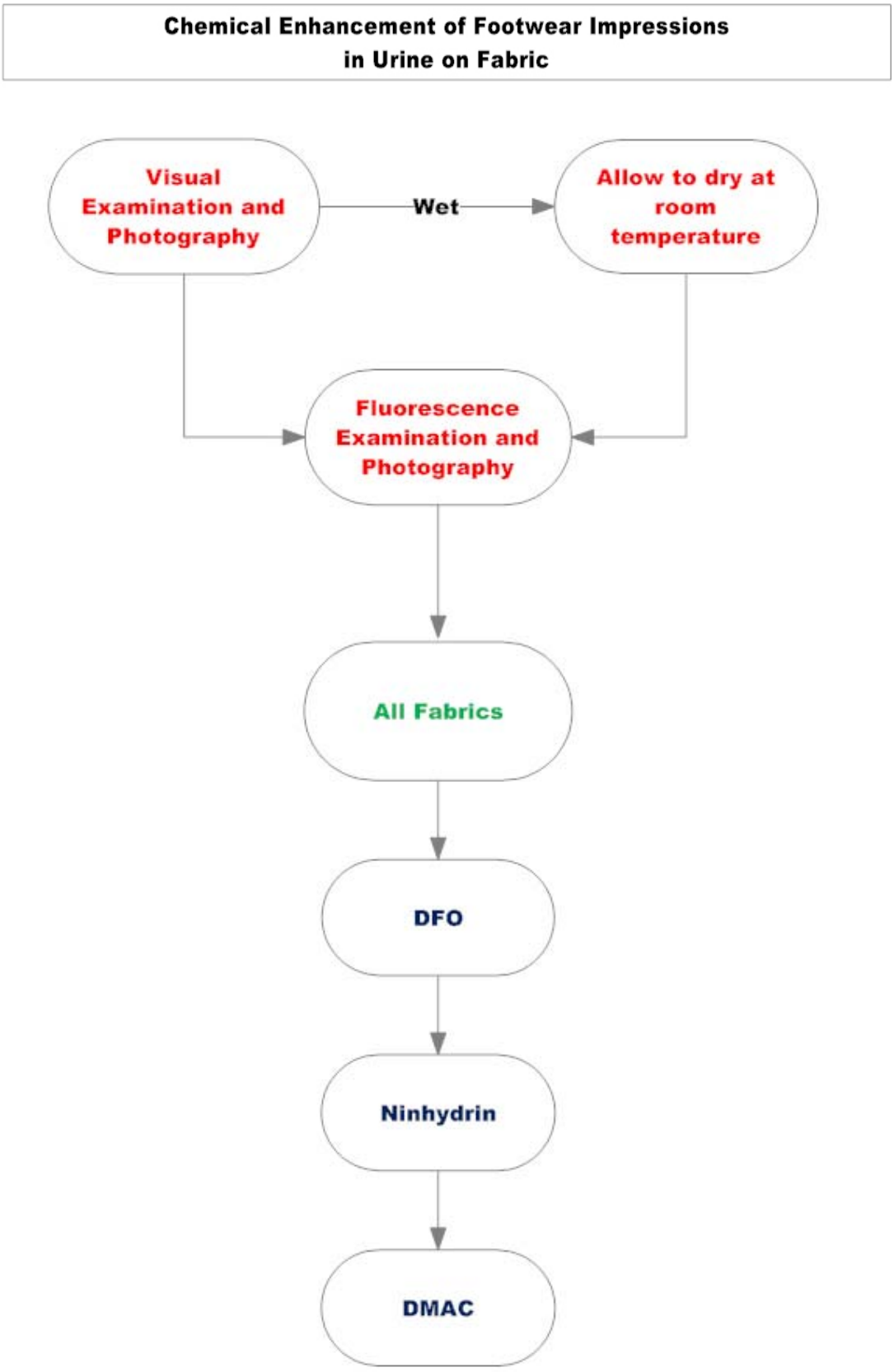

Figure 7.2 - Sequential chemical process for the enhancement of footwear impressions in urine on fabric

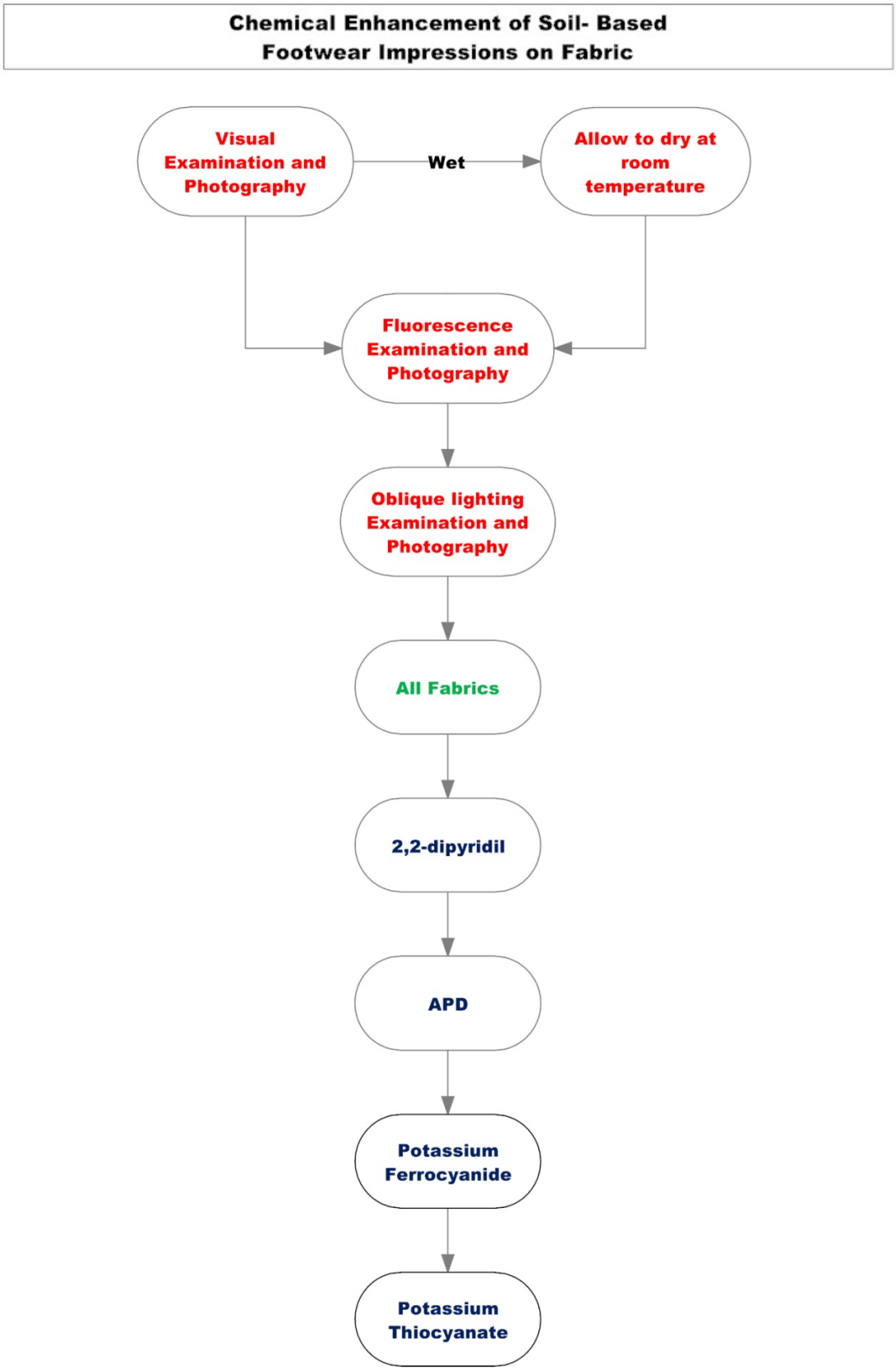

Figure 7.3 - Sequential chemical process for the enhancement of soil-based footwear impressions on fabric

7.3 Recommendations for Future Work

7.3.1 Protein Stain and Peroxidase Reagent Formulations

The use of various protein stains for the enhancement of footwear impressions in blood has been investigated. These methods are cheap, readily available and easily applied. Future work involving the mixture of two or more protein stains in the same formulation warrant further investigation as increased enhancement and fluorescent properties might be obtained. Further research should also investigate improving the formulations of peroxidase reagents and have effective means for employing at the crime scene as some formulations are cumbersome to prepared and apply.

In addition, utilising a methanol-based protein formulation and increasing the concentration of protein stains in solution might provide superior fluorescence on fabric surfaces such as denim and leather as well as limit background staining on other fabrics.

7.3.2 Ninhydrin Analogues and Natural Products

There are a number of ninhydrin analogues, some of which (DFO and 1,2-indanedione) have been investigated in this study. Their versatile use has demonstrated that they are suitable for the enhancement of impressions in both blood and urine. Limited research has investigated the possibility of implementing other ninhydrin analogues such as 5-methylthioninhydrin and 5-methoxyninhydrin [12-14] or the use of natural products for the enhancement of latent impressions [15-18]. While Kent [19] suggests that it is important ‘not to be be seduced into giving up well-tried and documented methods (such as ninhydrin) by the superficial attraction of a “new” technique until we have reliable data’, this should not prevent the systematic investigation of potential new reagents based upon the ninhydrin structure since further research in this area would be advantageous.

7.3.3 Contaminants and Substrates

The use of footwear impressions in contaminants such as milk and creams warrant further investigation. CAST has carried out preliminary work and reported that powder suspensions worked on over 50% of contaminants on surfaces such as laminate flooring [20]. Blind trials, where the contaminant is unknown would further enhance and develop the sequential approach.

The recommendations presented in this work are based on the fabric types and colours used in the study. Further research is necessary to investigate differences, if any, in the chemical enhancement techniques' efficiency when using fabrics supplied from the same (inter) and different (intra) manufacturers and in particular where variations of the warp and weft of the fabric occur. This work should also be expanded to incorporate other complex surfaces such as skin or surfaces with variable porosity.

The soils used in this study were obtained from different parts of Scotland and as such cannot be generalised for all of the UK. Further work is needed to address different soils from all over the UK. For example, Scottish soils have high iron content when compared to English soils and the results obtained in this study may vary when used to treat soil-based footwear impressions in England.

7.3.4 Alginates

The use of alginates warrants further research, mainly related to the effect of alginate on the footwear impression in blood and the substrate surface interaction. The use of alginates might also prove a useful technique for lifting soil-based footwear impressions or impressions in other contaminants. Moreover, an understanding of the mechanism of lifting would be beneficial.

7.3.5 DNA

Although previous research has established that chemical techniques do not affect subsequent DNA profiling, further work needs to expand upon this as this may vary depending on variables such as the substrate and the time elapsed before profiling.

7.3.6 Footwear Grading System

Although there exists a harmonised ENFSI conclusion scale, there is no such harmonised scale for grading impressions before and after enhancement. The scale is necessary to provide a harmonised approach to footwear impression enhancement research. The work undertaken in this study provides some support to the efficacy of a grading system and has modified the system already reported in the literature. Further investigation with practitioners will develop the robustness of the proposed scale and its value in operational trials for comparison of footwear enhancement techniques. Contaminants other than blood should also be considered.

7.3.7 Operational Trials

A next logical step in this work would be the implementation of the suggested enhancement techniques with an operational context. Pseudo operational trials (where staged samples are produced within an operational context) and operational trials would be recommended. This would allow for insight as to where the particular techniques will fit in the overall forensic strategy which may be different from the results obtained in this study.

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Appendix 1

PARTICIPANT INFROMATION SHEET

This project is part of a PhD study which will focus on the recovery, enhancement and lifting of footwear impressions from difficult surfaces such as clothing and human skin. Most techniques utilised for the visualisation and enhancement of fingerprints can also be utilised for footwear impressions. If for example, somebody is assaulted and has been kicked and stamped upon, footwear impressions might develop on the outside and inside of the fabric.

To have a better understanding of the forces involved in stamping, pressure plates can be utilised where volunteers are asked to stamp onto the plates. Data is collected via a computer. Once this data is collected and analysed, a suitable average force involved in stamping can be obtained. This allows for the design of a rig to reproducibly deliver footwear impressions of the desired force on to the test substrates.

You will be asked to stamp onto 2 substrates with different densities (soft and hard). The forces will be measured automatically. For each substrate, a minimum of 10 stamps is required for statistical data to be robust. If needed a five minute rest period between stamping on each substrate. The tests will take place at the Bioengineering Unit, Wolfson Centre at the University of Strathclyde in September depending on the availability of the Bioengineering Unit. The whole procedure should not take more than 15 minutes per participant.

The data from the investigation will be stored and retained until the study is finished. The data will allow for a better understanding of the forces involved in stamping. Your data will be made available to you if you want to see it after the experiments are concluded.

For any queries during or after the investigation please contact:

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Or

Mrs Gwen McArthur, Secretary to the University Ethics Committee
g.mcarthur@strath.ac.uk

CONSENT FORM – FOOTWEAR STAMPING PROJECT

Please read the following points and sign at the end if you agree.

- I am in good physical health and know of no illness which will prevent me from participating in this study
- I understand that my participation in the study is voluntary
- I am aware of what participation involves
- All questions regarding the investigation has been satisfactorily answered
- I understand I can withdraw from participating at any time without giving a reason and can ask that data collected relating to my participation be destroyed
- I understand that all data given will be treated with the utmost confidentiality
- Where relevant, I give permission for the investigators involved to maintain records of the investigation should a follow-up to the investigation be conducted in the future, or a
  further investigation be undertaken.

_____ _____

Name Date

_____

Signature

Age: _____

Height: _____

Weight: _____

Sex: _____

Appendix 2

CALCULATIONS FOR THE FOOTWEAR RIG

Work done = $\int_0^{0.1} F\ dx$

where F_p = 3500 sin (2πft) [f = 5Hz if the sine curve cycle has a time of 0.2s)

$\int_0^{0.1} F_p\ dt$ = $\int_0^{0.1}$ 3500 sin (2πft) = [- $\frac{3500}{2\pi f}$ cos (2πft)] = 222.8Ns

$F_{av} = \frac{222.8}{0.1}$ = 2228J

Work done = F_{av} d = 2228 x 0.03 = 66.84J

Given that PE = KE, PE = mgh and m = 10.2 kgs

$h = \frac{PE}{mg} = \frac{66.84}{10.2 \times 9.81}$

h = 0.67m