Near Infrared Laser Dyes for the Detection of Latent Fingermarks

by

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Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

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NAME

DATE

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Abbreviations

^{13}C NMR	Carbon 13 spectroscopy
^{1}H NMR	Hydrogen-1 (proton) nuclear magnetic resonance spectroscopy
AA	Acetic acid
Ala	Alanine
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
CB	Cyanobloom
CCD	Charged-coupled device
$CdCl_3$	Deuterated chloroform
CdS	Cadmium sulfide
CdTe	Cadmium telluride
CdTe-MMT	Cadmium telluride montmorillonite
CTAB	Cetyl trimethylammonium bromide
Δ^9-THC	Δ^9-Tetrahydrocannabinol
DESI-MS	Desorption electrospray ionisation-mass spectrometry
DEUS	Digital enclosed ultra-violet imaging system
DFO	1,8-Diazafluoren-9-one
DMAB	p-Dimethylaminodbenzaldehyde
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethylsulfoxide
DNA	Deoxyribonucleic acid
Em	Emission
ESA	Europium-doped strontium aluminate
Ex	Excitation
FRET	Forster resonance energy transfer
FTIR	Fourier transform infrared spectroscopy

GD	Good development
Gly	Glycine
His	Histidine
IND	1,2-Indanedione
IND-Zn	1,2-Indanedione zinc
Leu	Leucine
LED	Light emitting diode
λmax	Wavelength of maximum absorbance or luminescence
LOD	Limit of detection
Lys	Lysine
MALDI-MSI	Matrix-assisted laser desorption ionization mass spectroscopy imaging
MeOH	Methanol
MAO	Monoamine oxidase
MHz	Megahertz
MMD	Multi metal deposition
mp	Melting point
ND	No development
NIN	Ninhydrin
NIR	Near-infrared
NMR	Nuclear magnetic resonance spectroscopy
OPSC	Optically pumped semi-conductor
Orn	Ornithine
PC UV	PolyCyano UV
PD	Physical developer
PD	Poor development
Phe	Phenylalanine
QD	Quantum dots
R6G	Rhodamine 6G

RAY	Rhodamine 6G, Ardrox™ and basic yellow 40 dye mixture
RH	Relative humidity
RP	Ruhemann's purple
RUVIS	Reflected ultra-violet imaging system
SDS	Sodium dodecyl sulfate
Ser	Serine
SMD	Single metal deposition
SPR	Small particle reagent
SPR UV	Small particle reagent ultra-violet
SSP	Sticky side powder
STaR 11	Styryl 11 and rhodamine 6G mixture
SWGFAST	Scientific working group on friction ridge analysis, study and technology
SWGIT	Scientific working group on imaging technology
TEC	Thenoyl europium chelate
TECTOPO	Thenoyl europium trioctylphosphine oxide
Thr	Threonine
THF	Tetrahydrofuran
TIFF	Tagged image file format
Tyr	Tyrosine
UTS	University of Technology Sydney
UV	Ultra violet
Val	Valine
VMD	Vacuum metal deposition
VSC	Video spectral comparator
Zn-RP	Zinc Ruhemann's purple complex

Abstract

The near infrared region (700 nm – 2000 nm) of the electromagnetic spectrum provides significant potential for fingermark detection. Many ubiquitous commercial surfaces give luminescent interferences that can present a challenge for latent fingermark enhancement. Background interference from these types of surfaces can be reduced when viewed in the near infrared region. The development of near infrared luminescent techniques for latent fingermarks would improve the possibility of imaging an exploitable fingermark. This research aimed to develop methods for near infrared detection of latent fingermarks across a number of different surface types and assess the effectiveness of the developed techniques by comparing them to conventional detection methods.

A mixture of two dyes, styryl 11 and rhodamine 6G (STaR 11), was coated onto a range of metal oxide powders to produce a luminescent fingerprint powder. This was applied as a dry powder for fingermarks on non-porous surfaces as well as a suspension for developing fingermarks on adhesive and wetted surfaces. The dry powder was successful in developing fingermarks and gave comparable results to a commercially available luminescent fingermark powder. The suspension for adhesive surfaces was able to develop fingermarks however when compared to the commercial method, the developed fingermarks were of significantly poor quality. The suspension for wetted surfaces, when used in conjunction with the EcoSpray® device (a pressurised sprayer which delivers the suspension in a fine mist to prevent fingermark damage), had shown significant promise when compared to conventional luminescent SPR. Ultimately, however, the suspension was unable to develop natural fingermarks, which affected its potential for routine use.

Styrylisatin was trialled as a potential near infrared luminescent amino acid sensitive reagent for the detection of latent fingermarks on porous surfaces. Styrylisatin was successfully synthesised, however there were several issues that made it unsuitable for use as a fingermark detection technique. Despite attempts to optimise the formulation, the sensitivity of styrylisatin to amino acids was not improved, thus it was not pursued any further.

The use of one-step luminescent cyanoacrylate (PolyCyano UV®) was also explored in this research and compared to conventional cyanoacrylate development subsequently stained with rhodamine 6G and STaR 11. PolyCyano UV® developed fingermarks were assessed for

development and visualisation under UV illumination as well as how they performed in a sequence. PolyCyano UV® developed fingermarks were applied successfully in sequence with rhodamine 6G and STaR 11. Sequencing allowed the developed marks to be visualised in the luminescence mode for two different visible wavelength regions as well as in the near infrared region, which was found to improve the possibility of imaging an exploitable fingermark.

A range of imaging systems are available to forensic laboratories, however, the suitability of these systems for near infrared imaging has not been explored in any published study. Four imaging systems (Condor, Fuji IS Pro, Poliview IV and VSC 6000) were compared based on their ability to image fingermarks developed with STaR 11 magnetic powder and cyanoacrylate developed fingermarks stained with STaR 11. Overall, the Poliview IV and VSC 6000 were found to give the best imaging capabilities of all the systems tested. Generally the VSC 6000 was better suited for well-developed fingermarks; however the Poliview IV produced better quality images for poorly developed fingermarks. The Fuji IS Pro was suitable as a lab based near infrared camera; however when used for field purposes it displayed a significant decrease in effectiveness.

The research has successfully developed a range of fingermark detection techniques that are luminescent in the near infrared region. These techniques can be used in conjunction with conventional techniques to improve and possibly increase the number of exploitable fingermarks.

Chapter 1:

Literature Overview

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1.1 Introduction

The detection of latent fingermarks is one of the most important aspects of forensic science. A wide range of techniques to detect and visualise latent fingermarks are available for different surfaces and different components of latent fingermarks. With the exception of some novel visualisation and development techniques, the majority of visualisation occurs within the visible region of the electromagnetic spectrum. However, within this spectral region, there is an increased likelihood that the surface will interfere with the treated fingermark (due to background luminescence or high contrast printing), which can lead to a decrease in contrast. Different imaging techniques have been developed for visualisation outside the visible region; e.g. Reflected Ultra-Violet Imaging System (RUVIS), hyperspectral imaging, Fourier Transform Infrared (FTIR) imaging and X-Ray Fluorescence. These techniques give superior contrast in treated fingermarks and significantly reduce interferences from the substrate, but they require expensive instrumentation and are currently not feasible for rapid visualisation.

The near infrared (NIR) region of the electromagnetic spectrum ranges from 700 to 2000 nm. Visualisation within this region has the advantage of fewer surface interferences, increased in contrast between a treated fingermark and the substrate, and the result can still be photographed using conventional forensic imaging systems [1]. When fingermarks are treated with dyes that absorb and fluoresce in the visible region, substrate interferences can decrease the effectiveness of the technique. It has been shown that most inks and dyes used on commercial surfaces do not exhibit luminescence emission within the NIR region [2]. There has been some research into near infrared emitting compounds as fingermark detection and developing reagents. This has included dye-coated nanopowders, cyanoacrylate stains and novel fingermark reagents for porous surfaces that emit in the NIR [1, 3, 4]. This chapter will examine the current fingermark detection techniques, investigate techniques that can visualise treated fingermarks outside of the visible region, and discuss the advantages of visualisation within these regions of the electromagnetic spectrum.

1.2 Fingerprints

1.2.1 Formation of Papillary Ridges

The fundamental principles of fingermark identification are that papillary ridges can be classified based on general pattern, and that the ridge characteristics, are immutable and unique to an individual. Papillary ridges begin forming in the ninth to tenth weeks of foetal development, with primary friction ridges developing in the dermal layer of skin. At about 14 weeks of gestation, sweat glands and sweat ducts begin to form from the ridges in the dermal layer. Secondary friction ridges form in the epidermis and develop at between 17 and 24 weeks gestation. The interface between the epidermal and dermal layers is the basis for the ridges seen on the surface of the skin (Figure 1-1) [5-7].

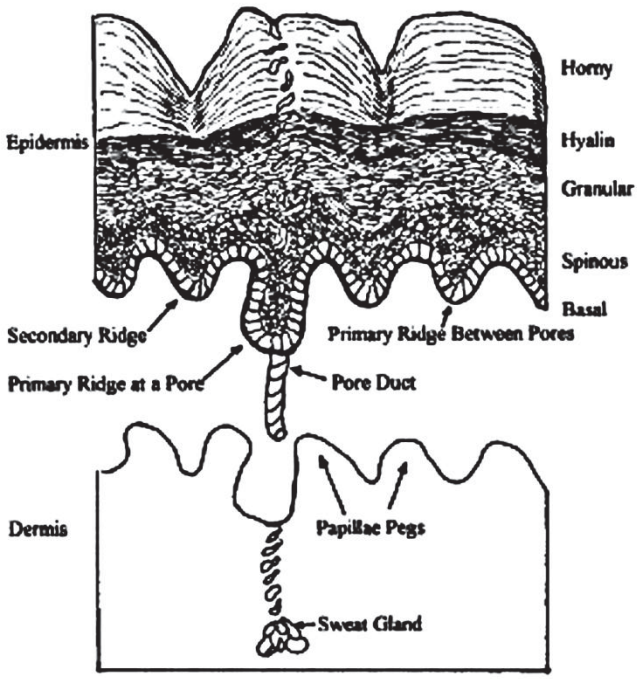


Figure 1-1: Cross Section of Fingerprint Ridge [8].

Fingermarks are also classified based on three general patterns: loops, arches and whorls. These are further subdivided into other distinct groups to help differentiate between the types of loops, arches and whorls (Figure 1-2). Genetics, environmental factors, drugs and disease affect patterning and arrangement of friction ridges [6]. The minutiae, however, are formed randomly from interactions within the womb. Types of minutiae used for identification include bifurcations, 'dots' (ridge unit) and ridge endings (Figure 1-3) [9]. The immutability of fingerprints can be attributed to a regenerative layer of cells acting as a template for the ridges. This layer exists the dermal layer, 1-2 mm beneath the surface of the skin. Even if an attempt to eliminate ridge characteristics was successful, the presence of permanent scars provides new characteristics for identification [10].

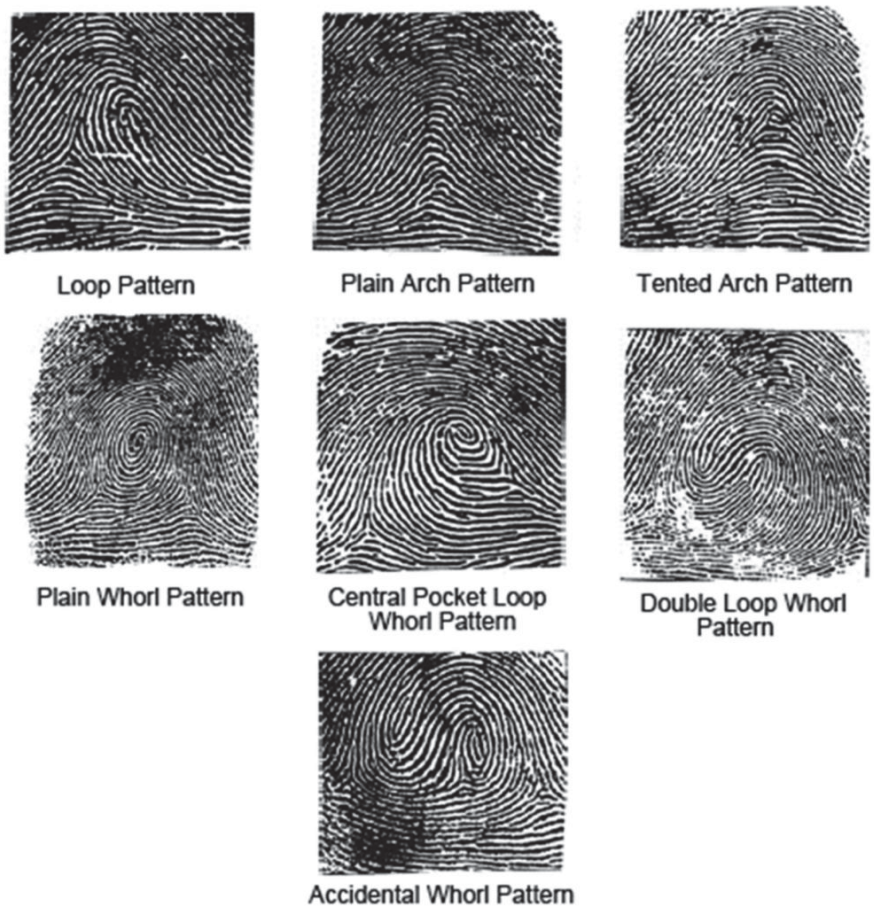


Figure 1-2: Types of general fingerprint patterns adapted from Hawthorne [11].

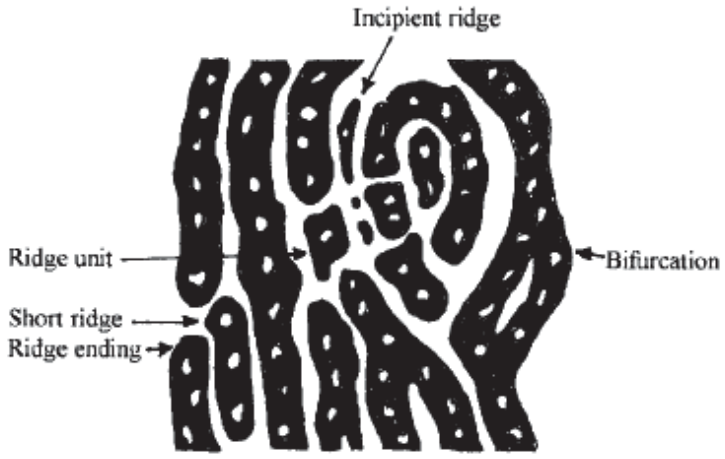


Figure 1-3: Examples of fingerprint minutiae [8].

1.2.2 Composition of Fingermarks

Fingerpads are especially susceptible to leaving marks as each ridge contains a single row of pores that connect to the sweat glands. Perspiration is excreted and deposited on the skin's surface through these pores. Sweat, along with oils from other parts of the body, contaminants such as dirt, bodily fluids and exogenous fatty compounds are transferred from the skin to the touched surface upon contact [10].

The three major glands responsible for the body secretions are eccrine, sebaceous and apocrine glands. Eccrine glands are found throughout the body, with 2-4 million distributed across the skin surface, but the highest densities are found in the palms and soles. These can collectively excrete 2-4 L of fluid per hour, 98% of which is water [12]. Eccrine perspiration contains numerous organic and inorganic constituents (Table 1-1).

Table 1-1: Major inorganic and organic components of latent fingermarks [12].

Source	Constituents	
	Inorganic	**Organic**
Eccrine Glands	Chlorides	Amino acids
	Metal Ions (Na^+, K^+, Ca^{2+})	Proteins
	Phosphates	Urea
	Ammonia	Uric Acid
	Water (>98%)	Lactic Acid
		Sugars
		Creatinine
		Choline
Sebaceous Glands		Glycerides (30-40%)
		Fatty acid (15-25%)
		Wax esters (20-25%)
		Squalene (10-12%)
		Sterol esters (2-3%)
		Sterols (1-3%)
Apocrine Glands	Water (>98%)	Proteins
	Iron	Carbohydrates
		Sterols

Sebaceous glands are typically located near regions containing hair follicles and are absent on the palms and soles of the feet. The sebum produced by the sebaceous glands travels into the follicular canal and then onto the skin's surface. Sebum is composed of a variety of organic compounds, with an individual's sebum composition influenced by factors such as

diet and genetics [12]. It is mainly comprised of glycerides (30-45%), wax esters (20-25%), fatty acids (15-25%), squalene (10-12%) and cholesterol and its esters (1-5%) [12]. Sebum composition is also dependent on the age of the donor. The rate of sebum excretion, the amount of fatty acids, and the ratio of wax ester to cholesterol have all been found to change with age; i.e. children tend to have higher concentrations of volatile fatty acids compared to adults [13].

The rate of eccrine sweating is dependent on the amount of water ingested but can also be due to diseases such as hyperhidrosis (increased sweating). Amino acids are commonly present, contributing 0.3 mg/mL - 2.59 mg/mL to the latent fingermark, making them a good target for chemical enhancement reagents. Proteins and peptides are also present in the deposit residues, and these are theorised to be one of the targets of cyanoacrylate fuming [6, 12]. Other constituents such as urea and lactate have also been detected in eccrine secretions but are not generally targeted in latent fingermark visualisation.

The apocrine glands are found mainly in the axillary regions. Few studies have been performed into the apocrine gland secretions because apocrine secretions are complicated by contamination from sebaceous and eccrine glands. In most instances only the eccrine and sebaceous glands contribute significantly to the latent fingermark deposit [12].

1.3 Current Detection of Latent Fingermarks

When a finger comes into contact with a surface it leaves a mark, which can be either visible or latent. Visible fingermarks can be further subdivided into:

- Positive fingermarks - formed by fingerprint ridges contaminated with a coloured substance such as blood;
- Negative fingermarks - where ridges remove surface material such as dust or flour;
- Indented fingermarks – formed by contact with malleable substance such as putty, which retains a three-dimensional image of the papillary ridges [14].

These marks can easily be visualised by optical examination techniques, although fingermarks in substances such as blood can be further visualised by chemical reagents such as diaminobenzidine (DAB) or amido black [15].

The most common fingermark evidence is latent fingermarks. Latent fingermarks are deposits of human secretions such as oils and perspiration that are invisible to the naked eye. A variety of techniques exist for the visualisation and enhancement of latent fingermarks and are commonly divided into: optical, physical and chemical.

These techniques are highly dependent on several factors that include the porosity, luminescent properties and colour of the surface the mark was deposited on, the type of secretion deposited on the surface and environmental conditions such as exposure to moisture and the age of the fingermark.

Because the formation of latent fingermarks is dependent on the surface upon which the fingerprint was deposited, detection and visualisation techniques are commonly classified by the type of surface. The three broad groups that all surfaces are divided into are porous, semi-porous and non-porous.

1.3.1 Porous Surfaces

Porous surfaces such as paper and cardboard will absorb some components of the eccrine and sebaceous secretions. Physical trapping of large constituents in the substrate pores and hydrogen bonding interactions fix the fingermark deposit to the surface for a long period of time [16]. The water soluble deposit is taken into the first few layers of the surface within seconds of deposition. Depth of penetration is dependent upon the type of paper the fingermark is deposited on; however, it has been shown that high quality fingermarks penetrate between 40-60 μm into a porous surface. The depth to which the aqueous layer penetrates is also dependent upon environmental conditions such as humidity and the porosity of the surface. During the early stages of absorption, water evaporates, leaving behind a mixture of residues including amino acids, urea and chloride [5]. While the diffused fraction is generally well preserved, it can be removed by exposure to water. The lipophilic components will remain on the surface for several days, depending on the ambient temperature, before diffusing into the surface. Under normal conditions a small amount of the lipophilic components will remain on the surface for years, and can still be detected [7].

Amino acid specific reagents are particularly useful for detection of fingermarks because of the amount of amino acid residues that remain on a porous surface. Common chemical

reagents for amino acid detection are 1,8-diazafluoren-9-one (DFO), 1,2-indanedione, ninhydrin and ninhydrin analogues. Ninhydrin is the oldest and most widely used chemical reagent for the detection of latent fingermarks on porous surfaces. The compound reacts with the amino acid component of a latent fingermark deposit to give a dark purple product known as Ruhemann's purple. This method, however, is very sensitive to external factors including acidity, temperature and humidity and must be controlled for optimal results to be obtained [7]. Ninhydrin-treated fingermarks can be further enhanced with a metal salt solution and immersed in liquid nitrogen for luminescence examination. 1,2-Indanedione can be combined with the metal salt in one solution [7]. When heat is applied, a visibly pink product is formed with the amino acids and this product is highly luminescent at room temperature. Recently 1,2-indanedione-zinc was determined to be the single best technique for the detection of latent fingermarks on porous surfaces [17].

Non-volatile water-insoluble sebaceous components are typically detected on porous surfaces by using physical developer (PD). Unlike amino acid reagents, PD can be used on surfaces that have been exposed to moisture. The fingermarks are visualised by the deposition of silver along the fingermark ridges, resulting in a visible dark grey to black colouration. PD is a very sensitive technique that uses unstable solutions with very short shelf lives. Since the deposited silver does not possess any luminescence properties, the technique is not suitable for dark surfaces. The other disadvantages of this technique are that it is destructive and the sensitive nature of the solution results in significantly variations in development quality. This is why the PD technique is often used only if the previous techniques did not produce any exploitable fingermarks [7]. There has also been research into other physical developer alternatives such as oil red O [18] and nile red [19]. While these methods are not universally accepted and require more research as an alternative to physical developer for old and wet samples, nile red is being recognised as a luminescent alternative to physical developer and oil red O [19].

1.3.2 Non-porous Surfaces

For non-porous surfaces such as plastics, glass and metallic surfaces, both water soluble and insoluble residues will remain on the surface. Consequently, fingermarks on these surfaces tend to be fragile, transient and can be 'cleaned up' relatively easily. The aqueous fraction

can be easily removed by water, while the lipophilic fraction will remain on the surface. Powders that adhere to the latent fingermark deposits are the most common technique for fingermark detection at crime scenes. Different types of powders can be applied depending on the substrate's colour, including black, white, silver or luminescent powders. The powdered fingermarks can then be lifted off the surface using adhesive film or gelatine lifters. Magnetic fingerprint powders have also been developed; these adhere to the latent fingermark, and are less destructive then conventional brushing techniques because the 'wand' does not come into contact with the fingermark. The major drawback of the powdering technique is that it is generally only effective on relatively fresh fingermarks because as the deposits dry out they become less receptive to powder adhesion [14]. This is the reason they are almost exclusively used at the scene and not in the laboratory.

Small particle reagent (SPR) is another common technique that is reserved for detecting fingermarks on wet, non-porous surfaces. SPR is a suspension of powdered molybdenum disulfide and detergent in water. The molybdenum disulfide adheres to the greasy, water insoluble components of the latent fingermark deposit. However, SPR is not particularly sensitive and is reserved for treatment of wet objects at the crime scene that cannot be readily transported to a laboratory [14] . Luminescent SPR has recently become commercially available which has addressed some of the issues associated with conventional SPR.

The most common and widely used non-porous latent fingermark detection technique is cyanoacrylate fuming. Cyanoacrylate esters are colourless monomeric liquids sold commercially as super glues. When heated, cyanoacrylate vapour is formed that reacts with certain eccrine and sebaceous components, selectively polymerising on the fingermark ridges to form a white polymer known as polycyanoacrylate (Figure 1-4).

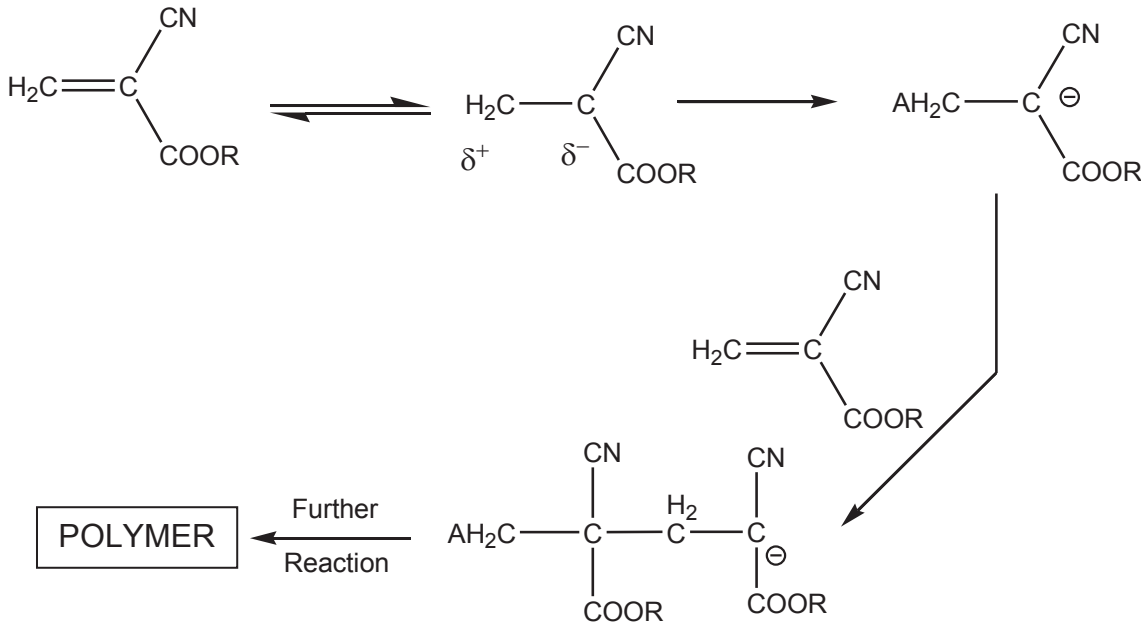


Figure 1-4: Cyanoacrylate ester structure and polymerisation mechanism [7].

Since polycyanoacrylate is a white solid, cyanoacrylate alone is not suitable for visualisation on all surfaces. While it is suitable on dark/black substrates, luminescent stains have been developed to help visualise cyanoacrylate developed fingermarks on surfaces that are coloured or white. Different optical techniques can help visualise unstained cyanoacrylate on difficult surfaces such as reflected ultra-violet imaging systems (RUVIS) [20] and coaxial illumination [21]. In the case of weak marks optical techniques may not be sufficient to enhance the cyanoacrylate developed fingermarks. As a result cyanoacrylate staining may need to be employed [22].

Cyanoacrylate staining of developed fingermarks aims to eliminate surface interferences by producing a luminescent fingermark that is visible using conventional imaging systems (e.g. Poliview and VSC). For a stain to be successful it must permeate the polycyanoacrylate deposited on the fingermark without altering it, and background staining must be kept to a minimum. The most popular luminescent stains are rhodamine 6G, Ardrox® 970-P10 and basic yellow 40 [6], though each of these exhibit shortcomings. Ardrox® 970-P10 is a skin irritant and Basic Yellow 40 has a very broad excitation spectrum, allowing a variety of visualisation parameters, but it has a very weak emission spectrum. All exhibit luminescence

emission within the visible region that can be obscured by any substrate'ls luminescence emission.

1.3.3 Semi-porous Surfaces

Semi-porous surfaces such as expanded polystyrene, latex gloves, glossy paper or polymer banknotes are the most difficult surfaces to on which to develop fingermarks as they do not suitably absorb the fingermark residues like porous surfaces, and because non-porous surface techniques such as cyanoacrylate stains will seep into the surface and create heavy background luminescence [23]. Vacuum metal deposition (VMD) and multi metal deposition (MMD) are commonly used on these surfaces [24]. There has been some research into the use of Fourier-transform infrared (FTIR) imaging as a suitable visualisation tool for cyanoacrylate developed fingermarks on semi-porous surfaces (such as polymer banknotes), including the use of specialised cyanoacrylates [25]. Other techniques that have shown some success on semi-porous surfaces include upconverter powders and near infrared powder suspensions [26].

1.3.4 Limitations of Current Techniques

The previously mentioned latent fingermark detection techniques are widely used in operational forensic laboratories. The main disadvantage of these techniques is that effectiveness in visualisation is largely dependent upon the substrate. For the majority of common surfaces (i.e. glass, plastic, paper), there are very few interferences and fingermarks can be visualised with little issue. However, a number of surfaces encountered in casework exhibit either high contrast printing (newspapers, magazines, labels) or strong luminescence emission (soft drink cans, packaging) that can interfere with the detection and visualisation of latent fingermarks. This can have an effect on the number of recoverable fingermarks and the quality of developed fingermarks. In an effort to minimise the surface dependency of fingermark detection techniques, numerous researchers have looked at developing new techniques for detecting and visualising latent fingermarks outside of the visible region. The remainder of this chapter will explore novel detection and visualisation techniques which

focus on areas outside the visible region as well as outlining the advantages that these techniques provide, when compared to conventional methods.

1.4 Ultra-violet Detection of Latent Fingermarks

The ultra-violet region has been used to image and recover forensic evidence since the early part of the 20th century [27]. It is routinely used in the forensic examination of documents, semen and bruises [28], however its application to fingermark detection and visualisation is very limited. Bramble *et al.* excited untreated latent fingermarks with short wave UV light, with emission in the long wave ultra-violet region, on white paper surfaces, as a replacement for visible luminescence of treated fingermarks. After UV luminescence of untreated fingermarks, the marks were then treated with ninhydrin or DFO. Significantly, after ninhydrin or DFO treatment there was no observed difference in enhancement between samples that were previously examined for UV luminescence and those that were not. Some drawbacks of this technique was that it did not provide any significant advantage over the current fingermark development techniques and DFO was more consistently successful in enhancement of fingermarks, and examinations times were longer than conventional methods [29]. Ben-Yosef *et al.* also found that the fingermark luminescence was dependent upon the type of secretion present, with eccrine-rich samples giving poorer results than sebaceous charged marks (i.e. the types of marks used by Bramble *et al.*). The results from this study showed that only a very small percentage of fingermarks (10% success rate) gave inherent luminescence. This was found to be due to the lower concentration of squalene (the luminescent constituent of the fingermark identified by Bramble *et al.*) in eccrine-rich samples [30]. These results indicate a limitation of this method as it relies on the fingermark to have a significant squalene concentration in order to be inherently luminescent. However the danger of exposing untreated latent fingermarks to ultra-violet light is that it can damage components of the latent fingermark and any possible DNA present in the fingermark [31]. There are also occupational health and safety issues arising from the use of UV light.

Akiba *et al*. further examined the luminescence of untreated latent fingermarks and determined that the strength of luminescence was dependent upon the irradiation time. The peak luminescence emission at 330 nm decreased with irradiation time while the 440 nm peak luminescence emission increased with radiation time [32]. Fingermarks developed on

white paper demonstrated strong luminescence when viewed in the ultra-violet region. In both cases only untreated fingermarks on white paper were visualised, this does not adequately assess the potential of the technique since conventional methods can develop fingermarks on this surface with little issue. However, Akiba *et al*. expanded on their original work by focusing on printed paper samples, it was determined that the UV luminescence for most ink toners was weak, therefore using a time resolved method and optical filters clear luminescent images of developed fingermarks were obtained [33].

A major drawback of using UV detection of latent fingermarks is the significant health and safety concern. Ultra violet light is harmful to both the eyes and skin; when combined with the high intensity of the laser, it increases the dangers considerably. The use of a reflected ultra-violet imaging system (RUVIS) does decrease the dangers associated with using ultra-violet lasers, due to the lower intensity light source used. RUVIS can be used to detect and image both treated and untreated fingermarks and can be used both at the scene and in the laboratory [20]. This system has also been used to effectively image fingermarks on post blast materials, which has proven difficult for conventional techniques. McCarthy found that the RUVIS device, while not offering the same image quality as a conventional DSLR, did possess greater portability and ease of use. It was recommended from this study that the RUVIS device should be considered as an initial development method prior to any physical or chemical detection method [34].

A study performed by Gibson *et al.* compared three UV searching and imaging systems for the recovery of latent fingermarks. Gibson *et al.* outlined that the effectiveness of UV imaging of latent fingermarks on nonporous surfaces was dependent on the angle between the surface and the light source, whereas for porous surfaces the effectiveness of the technique is dependent on the properties of the surface. Despite the issues identified, a digital enclosed ultra-violet imaging system (DEUS), custom built for this research, was found to be very effective for imaging fingermarks in the UV region. This work also determined that factors including illumination intensity, dynamic range of the imaging device, angle of the light and surface roughness, affected the quality of imaged fingermarks and should be considered when visualising in the UV region [35].

While there has been significant research into the development of UV imaging and imaging systems, the ultra-violet region is still underutilised for latent fingermark detection and visualisation. As previously mentioned issues such as; health and safety and potential

degradation of DNA prevent the wide spread use of UV detection of latent fingermarks. The other major factor is that a lot of substrates give strong luminescence interference in the visible region, as a result of UV excitation. This poses a significant drawback of UV imaging, compared to other regions of the electromagnetic spectrum, as the technique cannot be applied to all samples and in some cases requires specialised equipment for imaging. While there are advantages to visualising in the ultra-violet region, highlighted in the studies mentioned, currently the limitations of the technique prevent it from being a widely accepted examination technique.

1.5 Infrared Chemical Imaging

Chemical imaging in the mid-infrared region provides significant advantages to conventional techniques by inducing contrast by spectral absorption bands of different chemical components found in treated/untreated fingermarks. The use of Fourier transform infrared (FTIR) imaging has demonstrated significant potential as an alternative to traditional visualisation techniques, specifically with respect to cyanoacrylate-developed fingermarks. Tahtouh *et al.* [25, 36] explored the use of FTIR chemical imaging for a range of treated and untreated fingermarks. The most significant finding of this research was the ability to visualise a cyanoacrylate-developed fingermark on polymer banknotes, which is difficult using conventional imaging or development techniques. The significant reduction in background interferences was due to the selective imaging of the carbonyl group on the cyanoacrylate. The advantage of this technique was also demonstrated with the visualisation of fingermarks on aluminium cans that exhibit both strong luminescence and high contrast background [25, 36]. The radical increase in contrast demonstrates the advantage of visualising outside of the visible region due to the lack of background interferences in the infrared and the ability of focus on molecular bonds for fingermark targeting. A disadvantage of this technique is that since image acquisition times are long, often requiring the application of algorithms and image analysis to obtain suitable contrast, it would be unsuitable for rapid visualisation of fingermarks. Similarly the instrumentation used in this study is very expensive and would be unfeasible for operational forensic laboratories to implement.

The principle of FTIR chemical imaging was expanded in two separate studies, the first by Tahtouh *et al.* who examined novel cyanoacrylates for fingermark development in an effort to produce a strong isolated infrared spectral band to assist in infrared chemical imaging. While it was found that all cyanoacrylates were able to develop latent fingermarks, the bond stretching was not as intense as intended. One polymer, however, did show a shift in carbonyl absorption which allowed for greater contrast on polymer banknotes [37]. A study performed by De Grazia *et al.* examined the synthesis and application of diacetylene copolymers for latent fingermark development. The diactylenes copolymers are non-luminescent however, with the addition of FTIR chemical imaging, this allowed for greater contrast and background suppression for particularly difficult surfaces [38].

Infrared spectroscopic imaging of untreated latent fingermarks was examined by Crane *et al.*; fingerprints were deposited on a range of difficult surfaces and latent fingermarks were imaged by plotting individual infrared bands corresponding to different fingermark deposit components. Since most surfaces are composed of different chemical components than the latent fingermarks, a clear high contrast fingermark can be visualised. As stated in this article this technique works well when the location of the fingermark is known [39]. However the time required to generate the image is too long for it to be a viable imaging technique.

The other advantage of infrared chemical imaging is that, because it utilises spectroscopy to help image the fingermark, spectral data can also be obtained for the latent fingermark. This was demonstrated by Bartick *et al*. [40], who were able to isolate and target the components of the fingermark and generate an image of the fingermark. Significantly, this technique was able to visualise the fingermarks of children, which are notoriously difficult to image using conventional fingermark development and visualisation techniques.

The development of attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic imaging has also been used for chemical imaging of fingerprints. This technique is believed to be faster than traditional FTIR imaging and, like FTIR, can also provide the chemical properties of the latent deposits. Ricci *et al.* examined the use of ATR-FTIR on untreated latent fingermark deposits, focusing on the sebaceous material present. This technique was thus dependent upon the amount of material deposited, which means that it might not be effective for poor donors. This study only examined fingermarks that were placed directly on the ATR crystal and did not demonstrate the effectiveness of this technique on a range of different surfaces [41].

Infrared spectroscopic imaging of fingermarks can also be useful in separating superimposed fingermarks that have been developed by cyanoacrylate as demonstrated by Bhargava *et al*. [42]. From a casework perspective, this has significant applications as fingermarks can be imaged separately even if they overlap. Another significant application of this technique was the ability to detect trace evidence (such as explosive residues) [42] within individual fingermarks.

At this stage, infrared chemical imaging possesses significant potential in imaging fingermarks; however, the instrumentation is too expensive and the time required to image a whole piece of evidence is excessive. These are significant drawbacks that limit its potential for casework. Visualising in the NIR provides a compromise between visible and near-infrared imaging of fingermarks. NIR luminescent fingermarks can still be rapidly visualised by conventional forensic imaging systems and can reduce background interferences significantly.

1.6 Other Novel Imaging Techniques

X-ray fluorescence can be used for elemental analysis of latent fingermarks as well as for visualising fingermarks on non-porous surfaces. Worley *et al.* [43] examined the use of micro x-ray fluorescence to visualise latent and visible fingermarks by imaging the inorganic components of latent fingermark residues. Highly sebaceous fingermarks and those contaminated with hand lotion provided the best results. The analysis of the chlorine component of the fingermark gave a suitable image that could be extracted for the sebaceous fingermarks, while the lotion-contaminated fingermarks could be visualised by analysing potassium, chlorine, silicon, calcium and aluminium. However, the lotion fingermarks were visible when deposited on the surface, and elemental imaging did not provide any significant improvement over the visible image. Significantly, this study also examined a range of different cases in which childrens' fingermarks could be visualised (i.e. using saliva, touching bananas and sunscreen). While both the saliva and banana-enhanced fingermarks could be imaged, they did not give sufficient ridge detail for complete visualisation. The most significant results obtained were from fingermarks with sunscreen deposited on black paper and analysed for titanium; these gave good ridge detail and excellent contrast [43]. While the surfaces tested gave no background luminescence, there

was a significant amount of background 'noise' which prevented the recording of clear images. Another drawback of this technique is the long acquisition times required to image the fingermarks.

Raman spectroscopy is an analytical method used for the detection and characterisation of chemical compounds. Raman spectroscopy has been applied to forensic samples such as illicit drugs and explosives. Two published studies by Day *et al.* explored the use of Raman spectroscopy for the detection of drugs of abuse in both latent and cyanoacrylate fumed fingermarks. The technique was successful in the detection of exogenous compounds for both latent and fumed fingermarks; however, issues with locating the drugs within the fingermark made detection difficult. In both studies, it was recommended that this could be improved by using Raman mapping [44, 45]. Emmons *et al.* used Raman chemical imaging to detect and identify latent fingermarks that were doped with explosive residues. This study was successful in identifying the explosives in a non-destructive manner as well as providing addition information to the identification of a suspect. The resulting fingermark images were not as high in contrast as with other chemical imaging techniques, however, the ability to identify explosive contaminants in a fingermark is an important result [46]. A recent study by Connatser *et al.* applied surface-enhanced Raman imaging to untreated fingermarks. This technique involves improving the surface by applying metal nanoparticles (such as colloidal silver) to the surface and imaging the surface for chemical components. This study was the first to detect and image a latent fingermark using Raman based solely on the endogenous compounds present in a fingermark. While this work is preliminary, it does highlight a significant advance in available imaging and detection techniques for latent fingermarks [47].

Latent fingermarks may contain contaminants from illicit substances that a person has come into contact with. Several articles have already examined this [44, 45, 48], but few have explored the chemical imaging of latent fingermarks using mass spectroscopy. Ifa *et al.* [49] was able to image fingermarks using desorption electrospray ionization mass spectroscopy (DESI-MS) chemical imaging using sebum-rich, cocaine, and Δ^9-THC contaminated fingermarks. The advantage of this technique is that, on all the surfaces tested, there were no surface interferences so a high contrast fingermark was able to be visualised. This technique would be suitable for highly luminescent surfaces that cannot be visualised using conventional techniques, as the surface does not interfere with the fingermark. As with most chemical imaging techniques, this is in the early developmental stages and thus image acquisition times are too long for the method to be routinely used for casework [49].

Another emerging imaging technique is the use of matrix-assisted laser desorption ionization mass spectroscopy imaging (MALDI-MSI). Work conducted by Wolstenholme *et al.* involved the application of this technique to target endogenous lipids. High contrast images of groomed and ungroomed fingermarks could be obtained by targeting components such as cholesterol and oleic acid [50]. This work was expanded by Ferguson *et al.* who proposed a two-step method for the enhancement of latent fingermarks, first by powdering with a MALDI matrix that could be photographed under visible and UV light and then imaged with MALDI-MSI. Similar to the previous study, high contrast fingermarks were obtained from this work and the use of a powder means that it can be easily integrated into the current workflow of a crime scene investigator [51]. The main issue with this technique is that powders are commonly used on surfaces that cannot be removed from the crime scene and the MALDI-MSI is a lab-based instrument that is not widely available. Therefore the substrates on which this technique could be used are quite limited.

1.7 Near Infrared Detection of Latent Fingermarks

1.7.1 The Near Infrared Region

The infrared region ranges from 700 nm to approximately 10^6 nm and is divided into three sections near, medium and far infrared. The near infrared region (NIR) ranges from approximately 700 nm to 2000 nm (Figure 1-5). The advantage of visualising luminescent fingermarks in the NIR is that luminescence at these wavelengths from either the surface or the fingermark itself is very unlikely. Many ubiquitous commercial surfaces are difficult to image due to the presence of dyes or inks that are luminescent in the visible region. Conversely, in the NIR these dyes and inks are not luminescent, which results in a significant increase in contrast between the treated fingermark and the substrate. This indicates a significant advantage of visualisation in the NIR because, without interferences, the potential to obtain a high contrast fingermark is greatly increased.

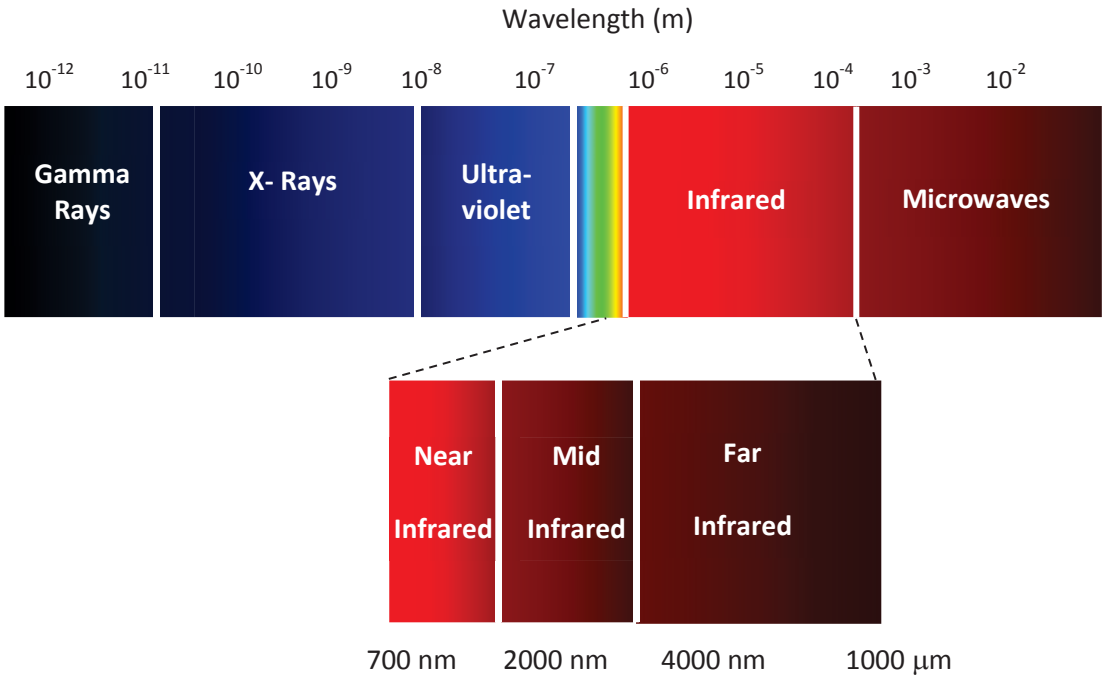


Figure 1-5: The electromagnetic spectrum.

Visualisation in the NIR has already been utilised in the biological imaging of cancer cells and immunoassays [52]. In a forensic context, most near infrared examinations are performed in the study of documents, and very few techniques have been applied for the detection of fingermarks.

1.7.2 Near Infrared Chemical Imaging

Chemical imaging is the combination of molecular spectroscopy and digital imaging which results in an image formed from spectral data. Image contrast is formed from the chemical differences between the surface and the fingermark [53]. The significance of this technique is that it can be used on both treated and untreated prints, it is a non-destructive and can detect fingermarks in absorption or luminescence modes [1]. Chemical imaging has been used for visualisation of treated latent fingermarks in the NIR region. NIR chemical imaging has significant advantages over visible chemical imaging because of the decrease in surface interferences [54]. Chemical imaging also provides the advantage of increased contrast of fingerprints developed on difficult backgrounds such as luminescent, dark and multi-coloured substrates [54]. Chemical imaging has the ability to reduce background interferences by

minimising luminescence and colour resulting in an increase in fingermark contrast. Chemical imaging has the advantage over digital manipulation because it can also provide quantitative, molecular and structural information for exogenous and endogenous compounds in the fingermark. Payne *et al.* examined the use of NIR chemical imaging on fingermarks developed using conventional detection techniques in both absorbance and luminescence modes. It was determined from this study that chemical imaging was specifically useful for weak fingermarks that could not be detected using conventional imaging techniques [55]. It was also superior in its ability to reduce the amount of background interferences from surfaces that had high contrast.

1.7.3 Development for Porous Surfaces

A study performed by Bleay et al. developed latent fingermarks with physical developer and visualised them with infrared filters [2]. IR filters were trialled in an attempt to reduce the amount of background interferences from multi-coloured backgrounds and found to be effective for PD developed fingermarks. While the use of IR filters was not successful on fingermarks developed using other techniques, it did illustrate an advantage of visualisation outside of the visible region. Since most printing inks are not visible in the near infrared, there are fewer interferences, resulting in a significant increase in contrast. This is important for porous surfaces, as inks on newspaper and printed documents will often obscure the treated fingermark when viewed in the luminescence mode. While this study did not develop any new techniques, it does clearly demonstrate the potential of visualising outside the visible region [2].

A similar approach was taken by Maynard *et al*. who examined the NIR luminescence of fingermarks developed using a range of common fingermark detection reagents, including 1,2-indanedione, physical developer, genipin, ninhydrin with zinc salt post-treatment and 1,8-diazafluoren-9-one (DFO) [1]. All developed fingermarks displayed some degree of NIR luminescence or emission with varied effectiveness. 1,2-Indanedione gave strong NIR luminescence emission for all surfaces tested, except for newsprint, which gave strong background luminescence. Ninhydrin and DFO had similar results, with poor luminescence in the NIR region. Physical developer was visible over the entire NIR region when viewed in absorption mode, which gave good contrast on white paper samples, while fingermarks

developed on newsprint could not be visualised as the newspaper ink obscured the treated fingermark. Genipin was the only development technique that could visualise the fingermarks in both absorption and luminescence modes when viewed in the NIR, resulting in superior contrast when compared to other development techniques [1].

Europium chelates for the development of latent fingermarks on porous surfaces were examined by Wilkinson, who synthesised a thenoyl europium trioctylphosphine oxide (TECTOPO) ligand for the detection of latent fingermarks on porous surfaces. The TECTOPO complex was lipid-sensitive and when compared to physical developer and DFO, the TECTOPO europium complex was found to be very selective for the latent deposits as well as providing luminescent images of the treated fingermarks, but performed poorly on older marks. A disadvantage of this technique was that it could not be used after DFO treatment and was not as sensitive as physical developer [56].

An alternative europium chelate was developed by Li *et al.*; europium, terbium, ortho-phthalic acid and ortho-phenanthroline is a multiple component chelate that can develop fingermarks on porous surfaces [57]. While this compound did not fluoresce in the NIR, the emission peak was significantly red-shifted (618 nm) with a large Stokes shift (265 nm) resulting in little to no background interference. This reagent could also be used to detect fingermarks on both porous and non-porous surfaces. However, exposure times were between 2 and 2.5 minutes, which meant that the compound itself does not exhibit luminescence emission as strong as other common fingermark detection techniques [57].

The development of novel amino acid specific reagents for the detection of latent fingermarks for visualisation in the NIR on porous surfaces has not been adequately explored. However, Jelly *et al.* [4] tested lawsone, which was shown to be strongly luminescent when viewed in the NIR and was also amino acid specific, resulting in good fingermark development.

1.7.4 Development for Non-porous Surfaces

While powdering is most commonly used at the crime scene to provide a high contrast fingermark that can be lifted, most powders only provide visible contrast between the

surface and the powder. There has, however, been significant development in the use of luminescent powders. This has several advantages in that it can increase contrast significantly compared to visible contrast and can still be imaged in the field using different camera filters.

Liu *et al.* examined the use of europium-doped strontium aluminate phosphors (ESAs) as a fingerprint powder for the development of latent fingermarks. An advantage of this method is the large Stokes shift between excitation and emission, but excitation with UV light has a number of health and safety concerns. Excitation with UV light and visualising in the red end of the visible spectrum results in a significant decrease in the amount of background luminescence, and thus gives a high contrast fingermark. While this technique gave high contrast fingermarks on all surfaces tested, the fingermarks had to be excited under UV light for two minutes and imaged within three minutes after excitation [58]. This technique is not good for field applications as the luminescence would have to be continually 'recharged' in order to photograph treated fingermarks.

The use of metal oxide nanopowders in combination with luminescent dyes has recently become a suitable alternative to conventional fingerprint powders. Choi *et al.* [3] examined the use of titanium dioxide powders coated with a perylene diimide dye. The dye-coated powder gave excellent luminescence in the NIR and a strong adherence to the oily residues of fingermark deposits [3]. The use of titanium dioxide nanopowder allows for tertiary detail development, and with the addition of perylene diimide dye the fingermark can be visualised using luminescence emission to improve contrast.

Maynard *et al.* [1] also developed styryl dye coated powders that were tested on a range of surfaces. As with the styryl dye cyanoacrylate stains, the coated nanopowders gave strong luminescence in the NIR. The styryl 9M coated titanium dioxide nanopowder gave excellent NIR luminescence emission, which allowed for a fingermark to be visualised on a soft drink label with a highly luminescent and patterned background that obscured the fingermark in the visible region. This research also determined that conventional fingerprint powders did not give any luminescence emission when visualised in the NIR [1].

Conventional luminescence relies on an emission wavelength longer than the excitation wavelength, with the difference between these energies being called the Stokes shift. Up-converters have a longer excitation wavelength than the emission wavelength (i.e. excitation

in the NIR, emission in the visible region). These materials have significant applications in the detection of latent fingermarks as the anti-stokes luminescent materials can develop fingermarks virtually free of background interferences. Ma *et al*. used sodium yttrium tetrafluoride doped with erbium and ytterbium ($NaYF_4$;Er,Yb) for use as cyanoacrylate stains as well as dry and wet powders. The use of these up-converters resulted in a significant decrease in background interferences with the most significant results obtained from polymer banknotes [26]. The disadvantages of this technique is that it requires complex sample preparation to prepare these up-converters, the use of NIR lasers for excitation has significant occupational health and safety concerns and there is a high cost associated with synthesising these materials

The use of infrared lighting for the imaging of pre-treated fingermarks has been explored by Kim *et al.* The use of a 940 nm emitting LED, and an adapted digital camera with conventional fingerprint powder was found to significantly reduce the background interferences from the surface. In the majority of cases, highly luminescent and high contrast backgrounds were found to have no effect on visualising the powdered fingermarks. The only limitation of this technique was that patterns including black and dark green pigments could not be effectively removed [59].

There has been extensive research into alternative cyanoacrylate stains that increase contrast of the treated fingermark by visualising outside the visible region. Wilkinson *et al*. examined the use of thenoyl europium chelate (TEC) complexes as cyanoacrylate stains. With a peak luminescence emission at 614 nm, the europium complex luminesces towards the red end of the visible spectrum. When compared to rhodamine 6G and Ardrox®, TEC provided greater ridge clarity, luminescence emission and ability to be absorbed into the cyanoacrylate, which resulted in superior development with less background staining. The combination of strong luminescence emission and a significantly red-shifted visualisation resulted in superior contrast when compared to conventional cyanoacrylate stains [60].

Mazzella and Lennard [61] combined styryl 7 with basic red 28 and basic yellow 40 in an attempt to extend the visualisation parameters of conventional cyanoacrylate stains. The combination was successful in that it resulted in the three dye mixture possessing a Stokes shift of 235 nm, which led to a decrease in background interferences. This is significant as it allows for visualisation across a much broader range of excitation and emission wavelengths, which decreases the potential for the background to interfere with the treated fingermark.

However, styryl 7 was unstable which meant that fresh solutions had to be prepared each time [61].

Maynard *et al.* [1] studied the use of styryl dyes (styryl 8 and styryl 9M) which are commercially used as laser dyes as cyanoacrylate stains. When applied to cyanoacrylate treated marks, there was a significant reduction in background luminescence compared to visualisation in the visible region. This study was also performed using NIR chemical imaging which proved to be excellent for visualisation of fingermarks [1]. Conventional cyanoacrylate stains were also tested for NIR luminescence and absorbance. It was determined that rhodamine 6G and basic yellow 40 gave weak NIR luminescence on fresh cyanoacrylate-developed fingermarks. When compared to the styryl 8 and 9M stained cyanoacrylate-developed marks, the luminescence emission was significantly less when viewed in the NIR.

Gentian violet is a technique already used for the detection of fingermarks on adhesive tapes and regular non-porous surfaces, though this technique has significant health risks due to the phenolic solution required for fingermark visualisation. This technique mainly stains the fingermark deposits, which can then be seen under normal lighting conditions. However, Bramble *et al*. determined that gentian violet gave strong luminescence emission in the NIR. Significantly, this means that gentian violet can be used to visualise fingermarks on both light and dark coloured surfaces by visible or NIR detection depending on the surface [62]. These fingermarks were excited by a laser. Visualisation in this region would also make gentian violet a suitable technique for fingermarks deposited on surfaces that have strong background luminescence in the visible region.

Fluorescin is a luminescent dye used in clinical diagnostics and has been suggested as an alternative to luminol for the detection of bloodstains. Fluorescin is oxidised to fluorescein in the presence of blood [63], and will give a luminescence emission in the NIR. Patonay *et al.* [63] examined the use of fluorescein to detect blood stains but were also able to develop fingermarks. The fingermarks developed were highly luminescent and gave excellent contrast. However, these marks were deposited on a black surface, which would still give good contrast when viewed under visible light.

The research outlined above covered either new NIR detection techniques or the application/modification of existing visualisation conditions for visualisation in the NIR. For the most part, these studies found that the NIR has significant potential as a region of

interest for fingermark visualisation. It does, however, highlight the general lack of research to find and optimise a NIR detection and visualisation techniques.

1.8 Conclusions

The majority of fingermark detection techniques currently used in casework involve imaging within the visible region of the electromagnetic spectrum. While these techniques can visualise fingermarks on most surfaces, the potential for background interference from the substrate is significantly higher within the visible region than outside this region. Recently with the development of new imaging systems, detection outside of the visible region has become more common and more widely available. Visualising outside of the visible region has significant potential as surface interferences are kept to a minimum. Near infrared luminescence has significant use as it can still be visualised using conventional forensic imaging systems without the need for expensive instrumentation, while still providing a significant reduction in background interferences present from the surface. While techniques such as FTIR imaging and other novel imaging systems can provide superior contrast, the associated costs are quite high, which is why techniques that use existing equipment are favoured.

1.9 General Research Objectives

The aim of this research project was to examine a range of laser dyes (focussing mainly on the styryl dyes) that are luminescent in the near infrared region and adapt them for fingermark detection or enhancement. This project will prepare and examine a range of near infrared luminescent fingermark detection techniques Comparisons with conventional fingermark development and enhancement techniques were used to determine the effectiveness of the new reagents. Donor, ageing and surface studies were also used to investigate the limitations of conventional techniques as well as assessing the parameters of the developed NIR techniques.

Based on the work of Mazzella and Lennard [61], the NIR dyes were combined with conventional reagents in an attempt to extend the visualisation parameters into the visible region. This project aimed to synthesise universal reagents that are strongly luminescent in the visible and near infrared regions and can be used on any surface without significant background interferences. This research explored the development of different detection techniques for latent fingermarks including a NIR luminescent powdering method (Chapter 2), NIR suspensions for adhesive and wetted surfaces (Chapter 3), NIR luminescent amino acid sensitive technique for porous surfaces (Chapter 4). Since the publication of Chadwick *et al.* [64]luminescent cyanoacrylates have increased in popularity. As a result the validation of PolyCyano UV and the sequencing of STaR 11 will also be explored in this thesis (Chapter 5).

Since this project examined techniques that could be used at both the crime scene and the laboratory, different imaging systems were assessed for their use in conjunction with the NIR development techniques. This is of specific importance with the NIR luminescent powders, as many conventional digital cameras have near infrared blocking filters. A specialised camera (Fuji IS Pro) that does not have the blocking filters was trialled and assessed based on the camera's ability to effectively visualise the NIR emission from treated fingermarks. Similarly, other laboratory imaging systems such as the video spectral comparator (VSC), Poliview and the Condor chemical imaging system was tested (Chapter 6).

Chapter 2:

Styryl Dye Coated Metal Oxide Powders for Latent Fingermark Detection

Chapter 2: Styryl Dye Coated Powders for Latent Fingermark Detection

2.1 Introduction

2.1.1 Nanotechnology and Nanostructured materials

Nano-structured materials such as nanopowders have been shown to be effective in developing latent fingermarks under a range of different conditions. These include commercially available nanopowders coated with a luminescent dye [3], colloidal gold for use in multi-metal deposition (MMD) [65], functionalized nanostructured materials such as quantum dots [66] and antibody conjugated gold nanoparticles [67]. The main advantage of using nano sized powders for latent fingermark detection is the ability to develop finer intrinsic features of the ridges as smaller particles tend to adhere more easily than larger particles [68] (due to the larger surface area to volume ratio). However, numerous studies have shown that the use of nanopowder and nano-structured materials introduces significant health and safety concerns [69-71] This section examines techniques that have utilized nanotechnology to develop latent fingermarks and outline the advantages that nanotechnology can bring to latent fingermark detection.

2.1.2 Development of Fingermarks on Non-porous Surfaces using Nanotechnology

Fingermark powdering is a 'physical' method of enhancement that relies on the mechanical adherence of fingermark powder particles to the moisture and oily components of friction ridge deposits [15]. Powdering is most commonly used at the crime scene as a quick and effective method of developing latent marks on fixed non-porous surfaces. Fingermark powders provide visible contrast through either absorption (e.g. black powder) or luminescence (e.g. Blitz Green®). Generally, the technique lacks sensitivity, particularly for

aged marks that have lost moisture. With the advent of nanotechnology, fingermark powders are more sensitive and are able to develop older fingermarks. The effectiveness of titanium dioxide nanopowder both as a dry powder and a wet powder suspension was evaluated by Wade [72]. Fingermarks that were not detected using conventional techniques could be developed with the nano-sized powder. An advantage of nanopowders is that luminescent dyes can easily be coated onto the surface of these powders, which can also significantly increase contrast. Choi et al. expanded on Wade's study and prepared a perylene diimide dye coated TiO_2 powder and compared it to commercially available magnetic fingerprint powders (Blitz Green® and Black Emerald®). While the luminescence was weaker for the TiO_2 powder there was significantly less background powdering which resulted in better overall ridge clarity when compared to the commercial powders [3].

Quantum dots (QDs) are highly luminescent nanoparticles that have a broad absorption range and a narrow emission band. These nanoparticles can be prepared to give a desired wavelength of emission [30]. Quantum dots also have the ability to luminesce up to 20 times brighter than available luminescent dyes [73]; these factors make latent fingermark detection a novel application of quantum dots. Menzel *et al.* were among the first to adapt this technology to latent fingermark detection by using a dioctyl sulfosuccinate capped CdS nanocrystals to develop untreated fingermarks, for use as a cyanoacrylate stain and as an alternative to sticky side powder (SSP) for developing latent fingermarks on the adhesive side of adhesive tape [74, 75]. All developed fingermarks gave strong luminescence and good selectivity for the fingermark deposits.

More recent studies include work performed by Dilag *et al.* who developed a CdS/chitosan nanocomposite powder to develop latent fingermarks. However when compared to conventional techniques, it did not provide any significant advantage [66]. Gao *et al.* synthesised cadmium tellurium montmorillonite (CdTe-MMT) nanocomposites and found a significant improvement in contrast and detection. A possible reason for this improvement was that the small particle size, (1.5 nm in diameter), gave better development, however the possibility of fresh and charged marks being used to give stronger results should not be discounted. The CdTe-MMT nanocomposite powder also showed higher chemical stability and a decreased tendency to oxidise in air, when compared to other quantum dots [76]. However, there are some problems with the quantum dots synthesised in this study. Firstly the small particle size causes substantial health and safety issues, the potential inhalation of these powders has been shown to diffuse in the nasopharyngeal, tracheobronchial and

alveolar regions of the respiratory tract [77]. This is combined with the cytotoxicity of cadmium, which has been shown to release Cd^{2+} into cells [78]. These health and safety concerns make these nanoparticles unsuitable for routine casework.

Quantum dots have also been shown to be an effective detection method for fingermarks in blood. Becue et al. synthesised cadmium telluride (CdTe) for the detection of weak fingermarks in blood and compared it to Acid Yellow 7. Results from this work, found that the quantum dots were as effective as Acid Yellow 7 for all surfaces tested. In addition to targeting blood fingermarks, the CdTe QDs were also able to target the latent components of the fingermarks [79]. This work was expanded on by Moret *et al.* who replaced the cadmium with zinc sulfide doped with copper (ZnS:Cu) in an effort to address the health and safety issues associated with cadmium. The ZnS:Cu QDs gave better results than Acid Yellow 7 and gave similar development quality to the CdTe QDs. The main advantage of the ZnS:Cu QDs was that it uses non-toxic zinc sulfide as its core, and since the QDs are delivered in an aqueous medium the hazards associated with the inhalation of nanopowders are therefore minimised [80].

Nanostructured materials can also be conjugated with antibodies to detect specific proteins or biological material found in fingermarks. Leggett et al. used gold nanoparticles tagged with an anti-cotinine antibody to detect cotinine (a nicotine metabolite) in groomed latent fingermarks donated by a smoker. The reagent not only developed clear fingermark ridges but also gave secondary information related to the donor's lifestyle [81]. Similar work has been conducted by Hazarika *et al.* and Wolfbeis. focussing on the development of fingermarks by targeting drug metabolites [82, 83] Spindler *et al.* conjugated anti-L-amino acid antibodies to gold-citrate nanoparticles to detect fingermarks on non-porous surfaces. Fingermarks were able to be developed up to 12 months; however, there were some cases of degradation from the solvent and background staining [67]. This work, however, highlights the significant potential that a nanotechnology (and antibody) based technique has in developing latent fingermarks.

2.1.3 Development of Latent Fingermarks on Porous Surfaces using Nanotechnology

Physical developer (PD) is the reagent of choice to visualise the water-insoluble components of latent fingermark residues on porous surfaces and is one of the first examples of using nanotechnology to develop fingermarks [84]. The development process requires several steps including an acid wash to remove $CaCO_3$ from the substrate and a silver redox solution, that results in the deposition of silver metal particles onto the ridges of the latent fingermark [84]. There are several issues with this technique including (but not limited to) solution instability, background development and low contrast on dark surfaces. Recently Jaber *et al.* developed a two-step process, application of functionalised gold nanoparticles and then silver physical developer. This results in 'negative' fingermarks (the gold and silver deposited on the paper as opposed to the ridges) which give high contrast between the fingermark and the substrate [85]. Despite physical developer's shortcomings it remains the technique of choice for porous surfaces that have been wet.

Multi metal deposition (MMD) was first introduced by Saunders [86] to detect latent fingermarks on both non-porous and porous surfaces. The treatment comprises two stages: the first is the immersion of the object to be treated in a solution containing colloidal gold as the active component; and the second is the enhancement of the detected fingermarks by the use of a modified physical developer redox solution [65]. The main advantage of this technique is that it can be used on a variety of difficult surfaces such as polyethylene and vinyl polymer-based surfaces. Recently Becue *et al.* prepared a modified MMD process that substituted the silver physical developer solution for a $Zn(NO_3)_2$ and dimethylene-borane solution [87]. The $Zn(NO_3)_2$ physical developer solution produced a UV luminescent ZnO film *in situ*, resulting in a dual reagent that gave high contrast fingermarks in the luminescence mode [87]. The main disadvantage of this technique is that it requires a significant amount of solution preparation and is very labour intensive compared to conventional techniques [88]. These issues lead to the development of MMD alternatives such as single metal deposition (SMD). Becue *et al.* functionalised gold nanoparticles with thiolated cyclodextrins to develop dark blue fingermarks after treatment [89]. Stauffer *et al.* expanded on this work by comparing the results to MMD developed marks and found that SMD not only gave similar development but was a more cost effective and less labour intensive process [90]. Gao *et al.* achieved similar success with the development of a single-metal nanoparticle deposition

(SND) method that used glucose stabilised gold nanoparticles. The development procedure was significantly less labour intensive than conventional methods and could be performed over a much broader pH range (2.5-5.0) [91]. Both MMD and SMD are presently being evaluated and optimised to make them more robust, more user friendly and less labour intensive [92].

2.1.4 Aims and Objectives

The aim of this study was to develop a NIR luminescent nanopowder by coating a range of commercially available metal oxide powders with NIR luminescent dyes. Based on the success of the dye combination of styryl 11 and rhodamine 6G (STaR 11) as a cyanoacrylate stain [64], this dye mixture was also coated onto nanopowders. Once an optimised nanopowder was developed it was compared to a conventional luminescent fingerprint powder currently used in routine case work (Blitz Green®). The effectiveness of both powders was examined by developing fingermarks on different surfaces, aged up to one month, and from male and female donors. Subject to the power being successful in this study, a pseudo-operational study was also performed to determine the ability of the NIR nanopowder to develop fingermarks with in-field equipment.

2.2 Materials and Methods

2.2.1 General Approach

A range of nano- and micro-powders were coated with styryl dyes to test their effectiveness as fingermark powders. Metal oxide powders were chosen because they are commercially available and previous studies have found that metal oxides can adsorb dyes readily to give a luminescent fingermark powder [3]. Optimisation of the powders was performed by comparing the luminescence of each powder by luminescence spectroscopy and examination

under a forensic light source (Polilight PL 500). Transmission microscopy of the treated fingermarks on glass was also used to determine the powder's effectiveness in terms of adherence to ridges. Once a suitable fingermark powder was developed, it was tested on a range of surfaces, over a period of up to one month with fingermarks from multiple donors, and the results compared to those from a popular luminescent magnetic powder Blitz Green®, that is currently used in the NSW Police Force [93]. In order for a fair comparison to occur only Blitz Green was compared to STaR 11 (not other non-luminescent powders). This was to ensure that the comparison was between two similar powders where the difference in quality would be due to the powder itself not outside influences (i.e. luminescent powder Vs non-luminescent powder, magnetic Vs non-magnetic powder). The optimised powder was also compared to Blitz Green® in a pseudo-operational scenario.

2.2.2 Materials

2.2.2.1 Reagents

Aluminium oxide nanopowder, <50 nm particle size (TEM) [CAS 1344-28-1], rhodamine 6G dye content 99% [CAS 989-38-8], titanium(IV) oxide, rutile powder <5 μm, ≥99.9% trace metal basis [CAS 1317-80-2], titanium (IV) oxide rutile nanopowder, <100 nm particle size, 99.5% trace metals basis [CAS 1317-80-2], zinc oxide, ReagentPlus® powder <5 μm particle size 99.9% [CAS 1314-13-2], zinc oxide nanopowder, <100 nm particle size [CAS 1314-13-2] were obtained from Sigma Aldrich and used as supplied.

Styryl 9M [CAS 82988-08-7], styryl 11 [CAS 92479-59-9] and styryl 13 [CAS 94507-05-8] were obtained through Exciton/Lastek and used as supplied.

Silver magnetic latent powder was obtained through Sirchie and used as supplied.

Blitz Green® magnetic fingerprint powder was obtained through Optimum Technology and used as supplied.

Reagent grade acetone [CAS 67-64-1], isopropanol [CAS 67-63-0], and methyl ethyl ketone [CAS 78-93-3] were obtained through Chem-Supply and used as supplied.

2.2.2.2 Instrumentation

A Branson 2210 sonicator was used to prepare the metal oxide nanopowders.

A Varian Cary Eclipse fluorescence spectrometer was used for measuring the luminescence of dye-coated nanopowders and dye solutions.

A Leica DMR microscope was used for transmission microscopy examination of powdered fingermark samples.

A Rofin Poliview IV fitted with a Polilight PL-500 was used for initial luminescence examination of powdered fingermarks and for comparison between the dye coated nanopowders and Blitz Green®. A Rofin Polilight PL-10 was used as a light source for the pseudo-operational study.

A Foster + Freeman Crime-lite (blue 430-470 nm and green 500-550 nm) were used for the pseudo-operational study.

A Fuji IS Pro digital camera with B+W 063 filter was used to image developed fingermarks in the NIR when performing the pseudo-operational study.

A Canon EOS 450D digital camera fitted with a HOYA 065 coloured filter was used to image developed marks in the visible region when performing the pseudo-operational study.

2.2.3 Methods

2.2.3.1 Preparation of dye solutions

Dye solutions were prepared according to Table 2-1 each solution was made fresh before being coated onto the nanopowders. These solutions were adapted from Chadwick *et al.* [64]. For luminescence examination of the dye solutions, 0.1 mL aliquots of each sample

were placed in a quartz fluorescence cuvette and diluted to 1 mL with the appropriate solvent.

Table 2-1: Dye solution formulations.

Dye Solution	Mass of Dye (g)	Volume of Acetone (mL)	Volume of Isopropanol (mL)	Volume of Methyl Ethyl Ketone (mL)
Styryl 9M	0.01	10.0	N/A	N/A
Styryl 11	0.01	10.0	N/A	N/A
STaR 11	0.01 of styryl 11 0.04 of rhodamine 6G	N/A	4.00	6.00
Styryl 13	0.01	10.0	N/A	N/A

2.2.3.2 Preparation of dye coated nanopowders

4 mL of the dye solution (Table 2-1) was added to 0.5 g of the metal oxide powder and the mixture was sonicated for five minutes. The suspension was then dried under reduced pressure by rotary evaporation. The powder was then placed on a watch glass and stored in desiccator in the dark until completely dry. Luminescence examinations of the powders were conducted by placing the powder in the Cary Eclipse solid sample holder and obtaining an emission spectra for different emission wavelength until the optimal conditions were found.

2.2.3.3 Comparison of the dye coated nanopowders

Once the powders were prepared they were evaluated on three factors: (i) luminescence, (ii) adherence to ridges and (iii) visualisation with forensic imaging systems. Firstly, luminescence spectral data were collected, then the powder was applied to a fresh fingermark on glass and viewed under a transmission microscope to determine its effectiveness in adhering to the fingermark ridges. Finally, the powdered fingermark was visualised under the Polilight to determine if the powder could be visualised using conventional forensic equipment. Once an

optimised powder was developed, it was coated onto silver magnetic powder with vigorous shaking.

2.2.3.4 Donor study

The surfaces used in this study were, glass microscope slides, Fanta® soft drink cans, laminate and polyethylene bags. These were sourced from the UTS forensic laboratory and were wiped down before use. Five fingermark donors (three male and two female, aged 18-25) were asked to deposit natural and charged marks on these surfaces. Charged marks were obtained by the donor rubbing their fingers across the nose and forehead prior to fingermark deposition. Natural fingermarks were deposited by the donor placing their fingers on the surface with no 'grooming' of the samples beforehand. Fingermark samples on glass, Fanta® soft drink cans and polyethylene were prepared according to Figure 2-1, as these surfaces were easily bisected after fingermark deposition. For fingermarks on laminate, a single fingermark was placed on the surface and re-deposited after ten minutes (to allow natural secretions to return to their typical concentrations). All samples were stored on a laboratory bench (20-22 °C and 30-40% relative humidity) and aged for periods ranging from fresh to one month. For comparison, each mark was split into two and each side was powdered with a different powder and imaged under the optimal imaging conditions for each technique. For Blitz Green® developed fingermarks, a 450 nm excitation and a 555 nm barrier bandpass filter were used, for STaR 11 developed marks a 530 nm excitation and a 700 nm barrier bandpass filter were used.

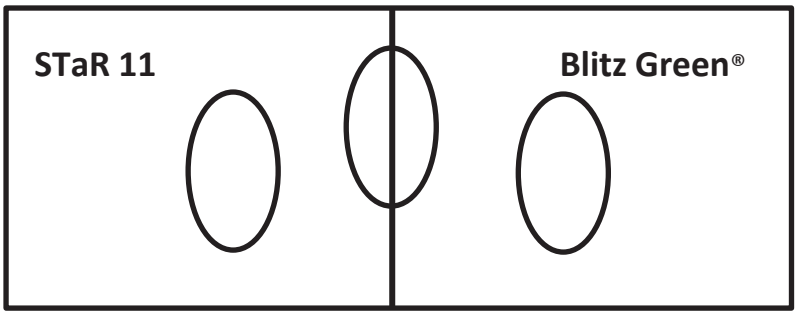


Figure 2-1: Preparation of fingermark samples for comparison.

2.2.3.5 Comparison of techniques

After imaging each fingermark, each 'half mark' was digitally stitched together to allow for a side-by-side comparison. For laminate samples since there was no 'half mark' used, each equivalent mark was digitally cut in half and placed together in a separate image. All digital stitching of fingermarks was performed using the GNU image manipulation program (GIMP). No other manipulations were made to the images after stitching. Each fingermark was then given two scores was based on two parameters: background powdering (contrast) and ridge detail (quality) (a total of 20 for all donors, per age, per substrate), using the McLaren scale [94]. Each powdered fingermark was compared to Blitz Green®, and given a score between 2 and -2 corresponding to the degree of enhancement the powder provides (Table 2-2).

Table 2-2: Comparison values.

Numerical Value	Qualitative Equivalent
-2	Significant decrease in enhancement when compared to Blitz Green®
-1	Slight decrease in enhancement when compared to Blitz Green®
0	No enhancement when compared to Blitz Green®
+1	Slight increase in enhancement when compared to Blitz Green®
+2	Significant increase in enhancement when compared to Blitz Green®

A zero value can result if both techniques gave good development, if both techniques gave very poor ridge detail or if there was no development observed, so the zero values were further classified as indicated in Table 2-3 and Figure 2-2. This was developed in order to differentiate between scores given a zero because both techniques were able to develop the fingermark with good ridge detail (mainly present in fresh samples) and samples where neither technique was able to develop fingermarks (mainly present for the older samples).

These scores were collated according to the surface and at each point of the ageing study as it was determined that the age of the sample was the strongest factor which would affect fingermark development. Each fingermark was scored twice so the secondary scoring system may apply to the amount of background powdering or quality of ridge detail.

Table 2-3: Supplementary scoring system for zero values

Zero Score	Qualitative Equivalent
Good Development (GD)	Developed fingermarks give clear ridge detail and contrast
Poor Development (PD)	Developed fingermarks have very little ridge detail and poor contrast
No Development (ND)	Neither technique was able to develop fingermarks

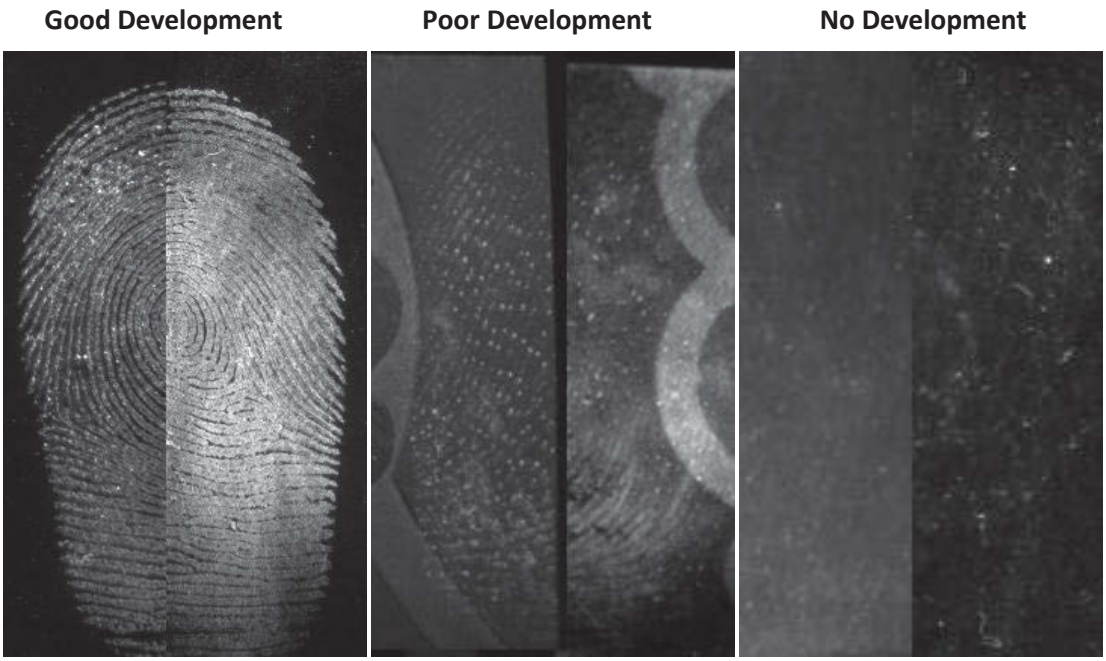


Figure 2-2: Examples of: (left) good development, (centre) poor development, (right) no development.

2.2.3.6 Pseudo-operational Study

To test the suitability of the STaR 11 nanopowder in a case-like scenario, a square room was divided into 4 quadrants, each containing a range of different surface types, i.e. painted wood, glass, metal, textured and untextured laminate. Prior to the experiment, all surfaces were wiped down and cleaned with commercial surface cleaner to ensure that any fingermarks found during the process were from a controlled source. A single fingermark donor then placed a number of charged and natural marks in different quadrants, ensuring that equivalent marks were placed in different quadrants (Figure 2-3). These marks were left for 16 hours before an examiner processed the scene. Two quadrants were powdered with Blitz Green®, while the other two quadrants were powdered with STaR 11. After this, each developed fingermark was photographed in the luminescence mode with a Canon EOS 450D fitted with a HOYA 065 coloured filter for Blitz Green® and STaR 11 luminescence emission in the visible region. NIR luminescence photography was then performed using a Fuji IS Pro fitted with a B+W 063 filter. The excitation light sources used were a 430-470 nm (for Blitz Green developed fingermarks) and 505-550 nm (for visible luminescence of STaR 11 developed fingermarks) Crime-lite and a Rofin Polilight PL-10 forensic light source was used for the NIR luminescence of STaR 11.

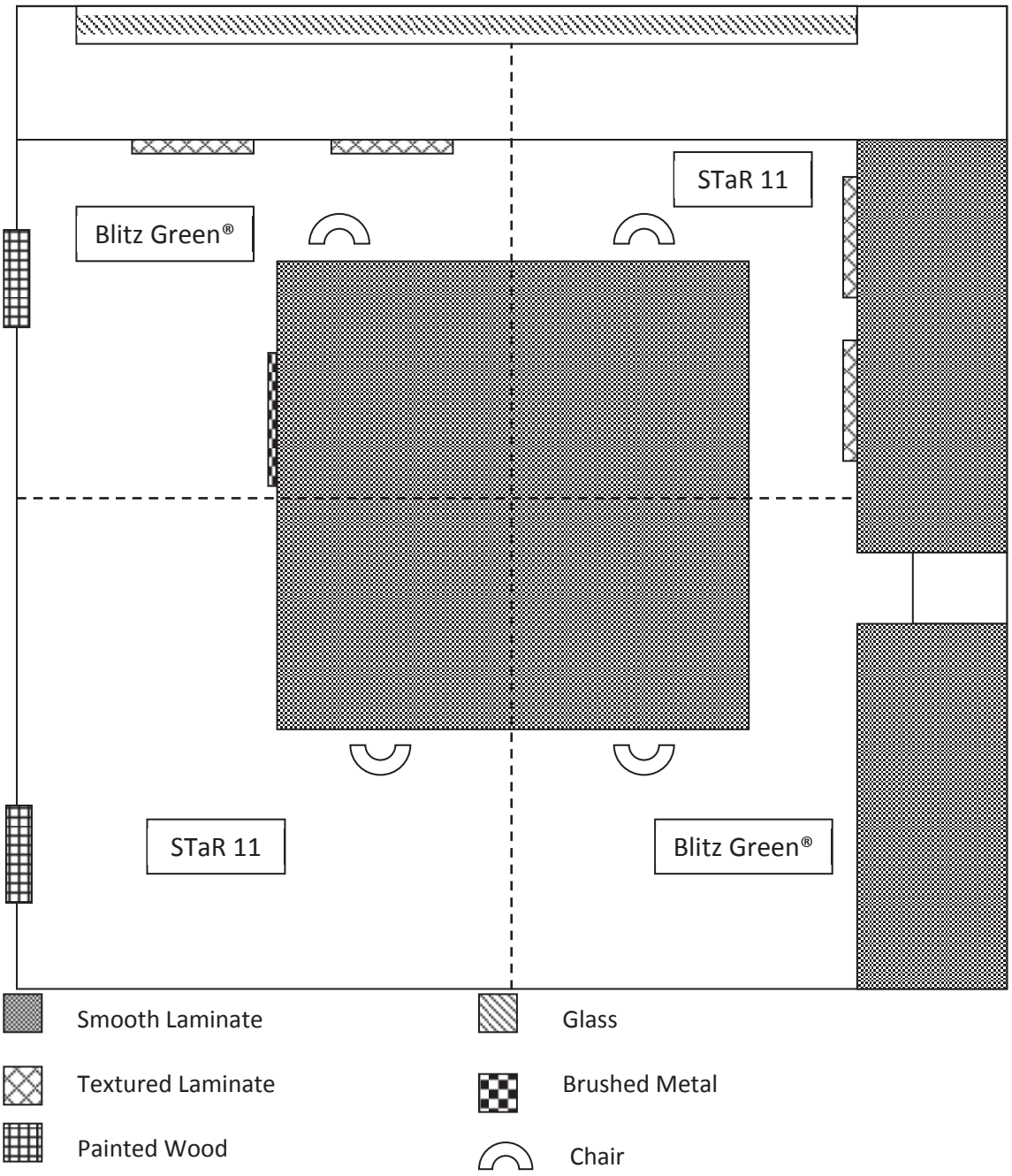


Figure 2-3: Schematic for pseudo-operational study.

2.3 Results

2.3.1 Powder Optimisation

Styryl 9M initially adhered to the powder well, exhibiting a pale purple colour; however, this faded to a light brown/grey colour in a matter of days. This was observed on all the metal oxide powders. Similarly, the styryl 13 coated powders; gave a pale purple colour, which changed to a dark blue/grey powder upon drying. When viewed under the Polilight, no luminescence was observed for either powder, which was confirmed by spectral data. It was hypothesised that the metal oxide acts as a catalyst for the degradation of the benzothioazolium group present in both the styryl 13 (Figure 2-4)and styryl 9M (Figure 2-5) dyes [95]. This degradation would explain the change in colour and the decrease in NIR luminescence as the highly conjugated structure would readily cleave into smaller molecules. A hypothesised degradation mechanism is illustrated in Figure 2-6. Styryl 11 does not have this functional group (Figure 2-7) and thus does not follow the same potential degradation mechanism as the other styryl dyes when in the presence of the metal oxide.

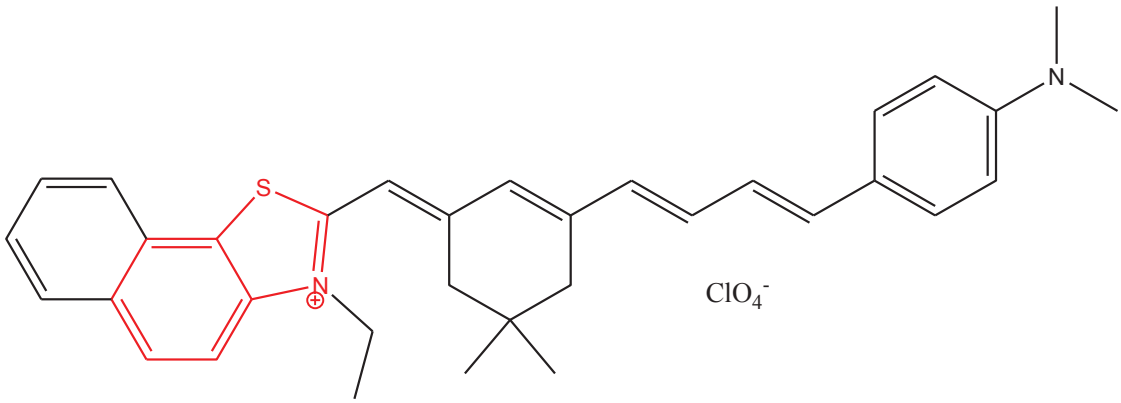


Figure 2-4: Styryl 13 chemical structure (benzothioazolium functional group in red).

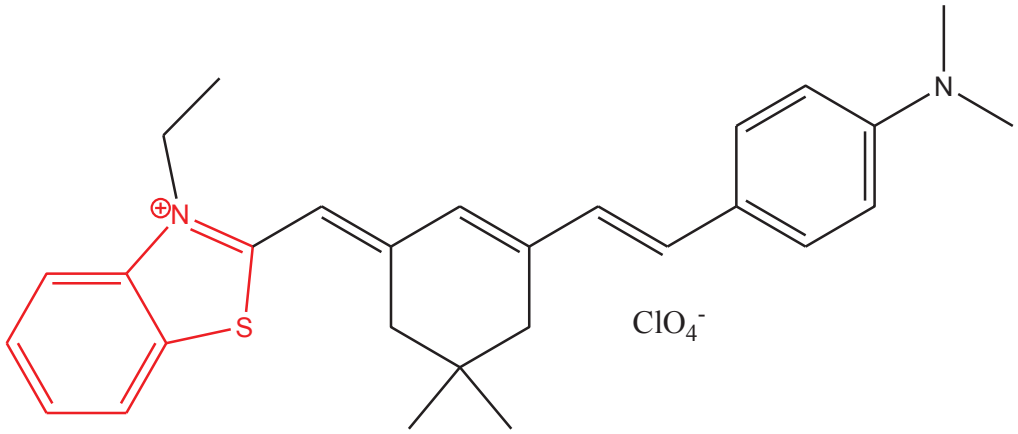


Figure 2-5: Styryl 9M chemical structure (benzothioazolium functional group in red).

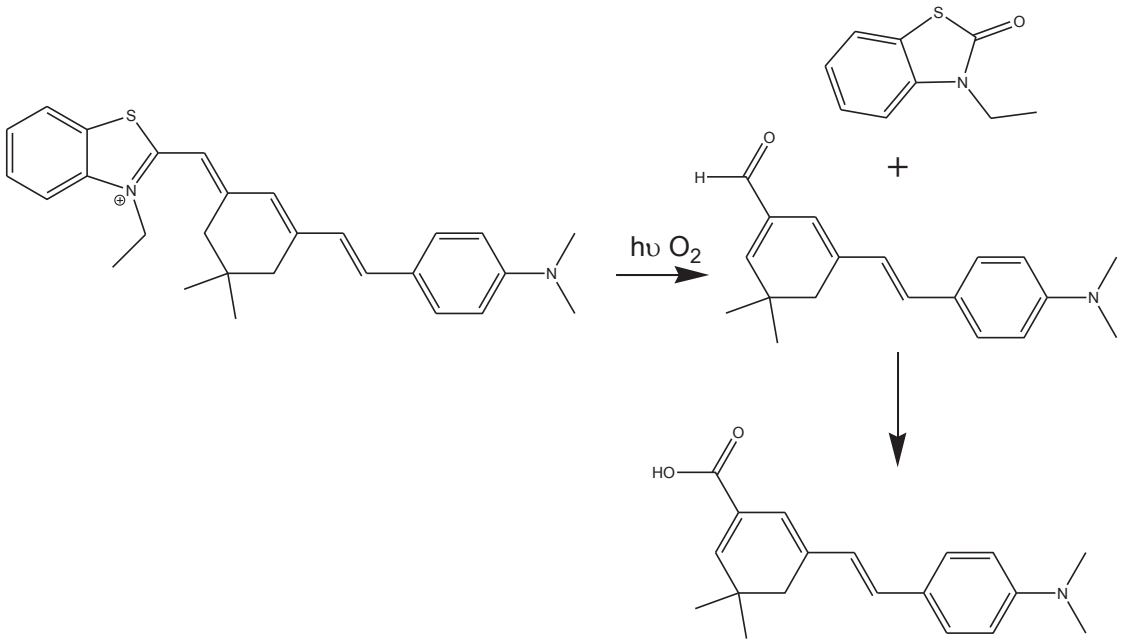


Figure 2-6: Hypothesised degradation of benzothiazolium based styryl dyes (adapted from [96]).

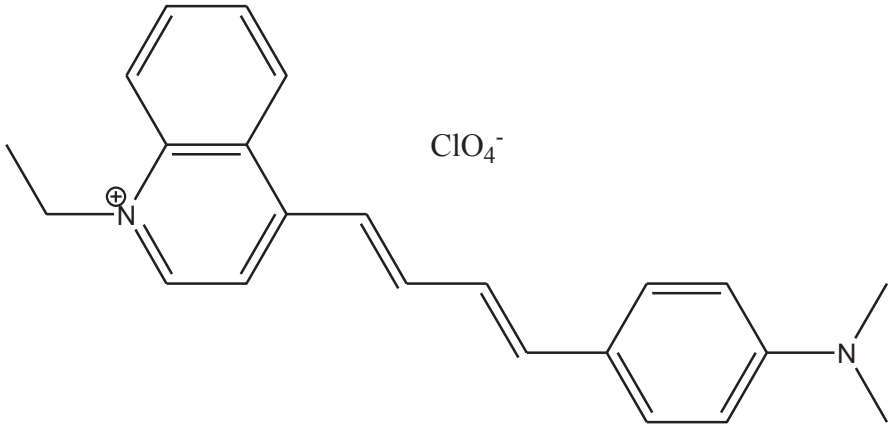


Figure 2-7: Styryl 11 chemical structure

The styryl 11 powders were a pale purple colour and were not strongly luminescent (Figure 2-8), with long exposures required to image the developed fingermark. The STaR 11 powders were a magenta colour and did not fade or degrade over time; the titanium dioxide powders were a darker purple colour than the aluminium or zinc oxide powders. Based on the spectral data of the nanopowders, the STaR 11 aluminium powder provided the strongest luminescence emission in both the visible and near infrared regions (Figure 2-9).

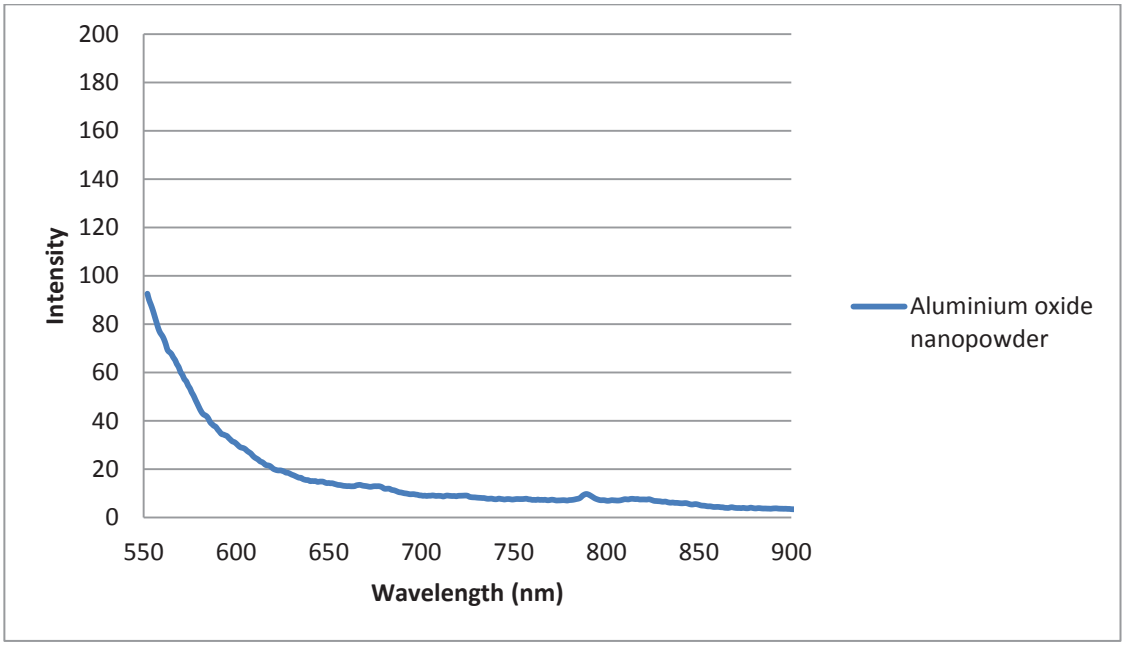


Figure 2-8: Luminescence spectra for styryl 11 coated aluminium oxide nanopowder (excitation 500 nm).

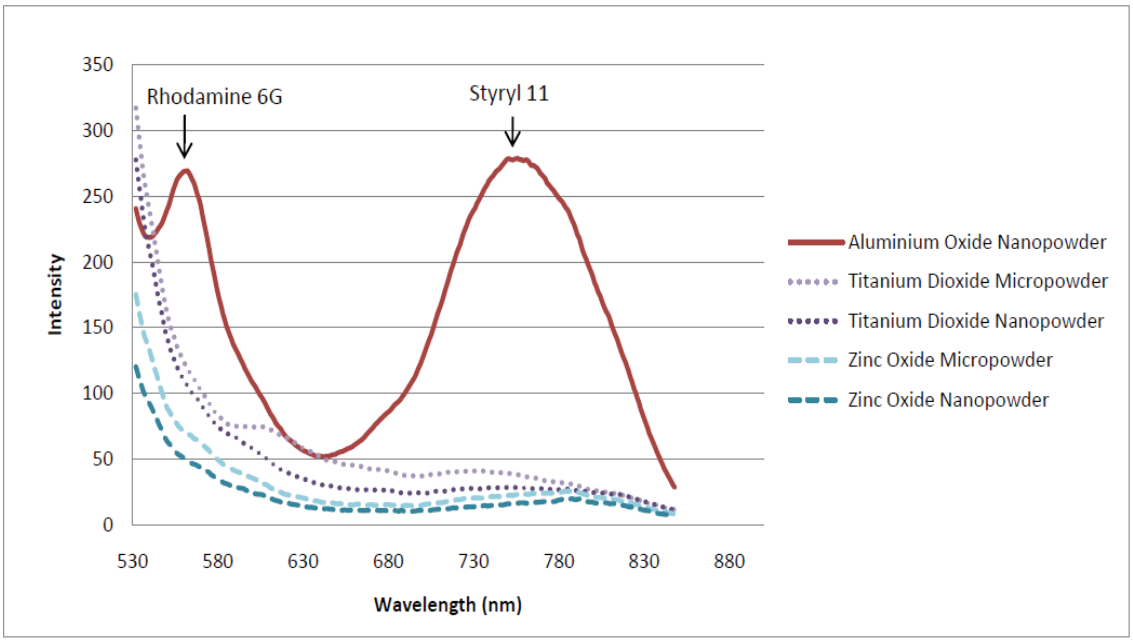


Figure 2-9: Luminescence spectra for STaR 11 coated nanopowders (excitation 500 nm).

A comparison between the luminescence spectra of styryl 11 and STaR 11 shows that there is a significant difference in NIR luminescence strength. This increase present in the STaR 11 sample was theorized to be due to Forster Resonance Energy Transfer (FRET) occurring between the two dyes. In this case, rhodamine 6G acts as a chromophore photon donor with absorbed photons transferred non-radiatively to the styryl 11 chromophore photon acceptor that then becomes excited [64]. Several experiments were conducted to determine the effect of concentration of rhodamine 6G on the luminescence intensity of styryl 11. From Figure 2-10 it can be seen that the relationship between concentration of rhodamine 6G and luminescence intensity of styryl 11 is not linear. Once the molar ratio of rhodamine 6G to styryl 11 is above 2:1, there is a significant decline in styryl 11 luminescence intensity. This can be explained by the formation of rhodamine 6G dimers and trimers at higher concentrations. Arbeola *et al.* found that in concentrated solutions (0.0205 M – 0.0490 M) of rhodamine 6G, there were higher concentrations of the rhodamine 6G dimers and trimers formed [97]. The increase in dimer and trimer formation would also have an effect on the efficiency of photon transfer as the orientation of the dipoles and the distance between rhodamine 6G and styryl 11 changes during this process. This change in efficiency of photon transfer would affect the luminescence of styryl 11 as the increased NIR luminescence intensity (compared to styryl 11 on its own) is dependent on transfer of photons from rhodamine 6G to styryl 11. With less photons transferred it would correlate to a decreased

luminescence intensity in the NIR, this is reflected in the results shown in Figure 2-10).While this result is not a confirmation of FRET, it provides significant evidence to indicate that there is a relationship between rhodamine 6G concentration and styryl 11 luminescence intensity.

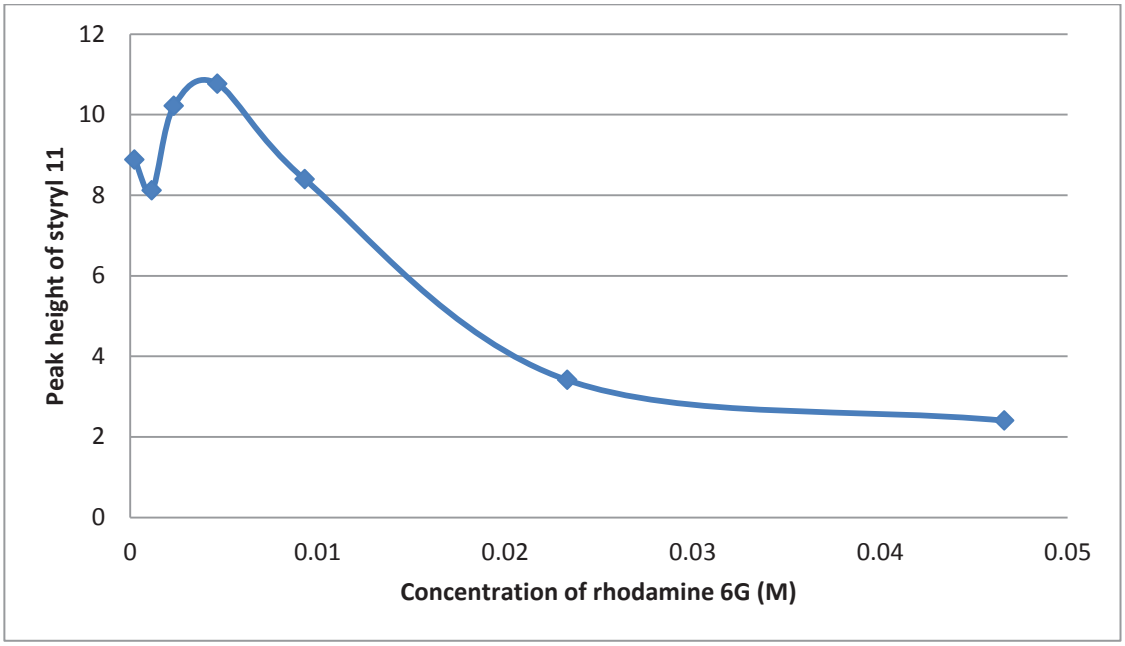


Figure 2-10: Styryl 11 luminescence intensity relative to concentration of rhodamine 6G.

Using the transmission comparison microscope, it could also be determined that the STaR 11 aluminium oxide powder adhered much better to fingermark ridges than the other metal oxide powders tested (Figure 2-11). A possible explanation for this is that the aluminium oxide powder used in this study was smaller (< 50 nm) than the other powders trialled (<100 nm), which potentially would give better development. This trend is also observed when comparing the micro and nanopowder (Figure 2-11); where regardless of the metal oxide, the size of the particle affected the quality of ridges developed. This had a direct effect on the luminescence of powdered fingermarks as well. Since more powder was placed on the ridges there was much greater luminescence and also greater contrast with the STaR 11 aluminium oxide powdered fingermarks (Figure 2-12). These comparisons also confirmed the spectroscopic data which showed that the aluminium oxide nanopowder had the strongest luminescence emission of all the metal oxides tested.

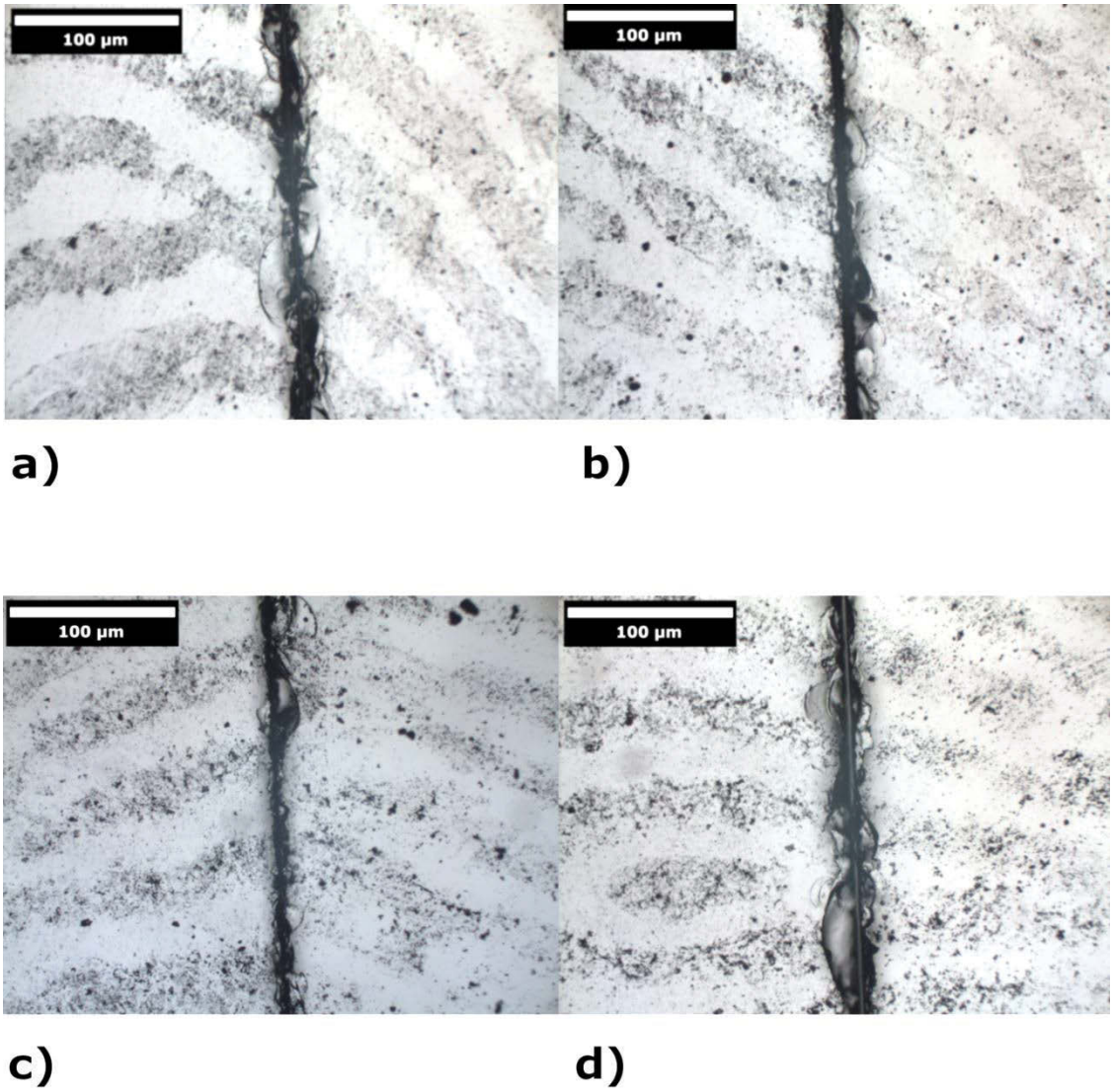


Figure 2-11: a) - (left) STaR 11 Al_2O_3 powder, (right) STaR 11 ZnO micropowder, b) - (left) STaR 11 Al_2O_3 powder, (right) STaR 11 ZnO nanopowder, c) - (left) STaR 11 Al_2O_3 powder (right) STaR 11 TiO_2 micropowder d) - (left) StaR 11 Al_2O_3 micropowder, (right) STaR 11 TiO_2 nanopowder.

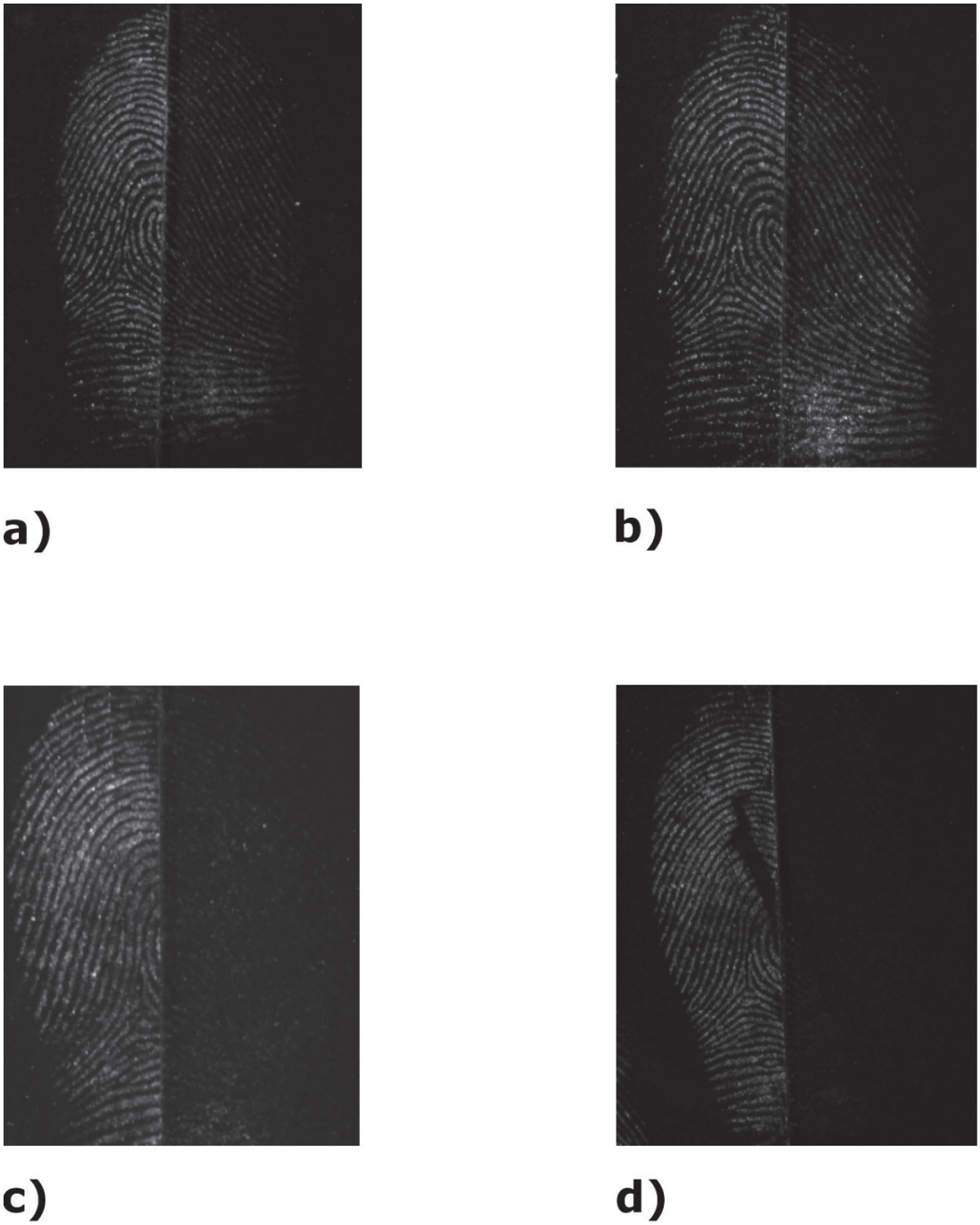


Figure 2-12: Luminescence photos of powdered fingermarks visualised with a 530 nm excitation and a 700 nm barrier band pass filter a) - (left) STaR 11 Al_2O_3 powder, (right) STaR 11 ZnO micropowder, b) – (left) STaR 11 Al_2O_3 powder, (right) STaR 11 ZnO nanopowder, c) - (left) STaR 11 Al_2O_3 powder (right) STaR 11 TiO_2 micropowder d) - (left) StaR 11 Al_2O_3 micropowder, (right) STaR 11TiO_2 nanopowder.

A rhodamine 6G aluminium oxide powder was also prepared to ensure that the NIR luminescence was from the presence of styryl 11 and not incidental luminescence from rhodamine 6G (Figure 2-13). From this it could be confirmed that the styryl 11 is responsible for the NIR luminescence.

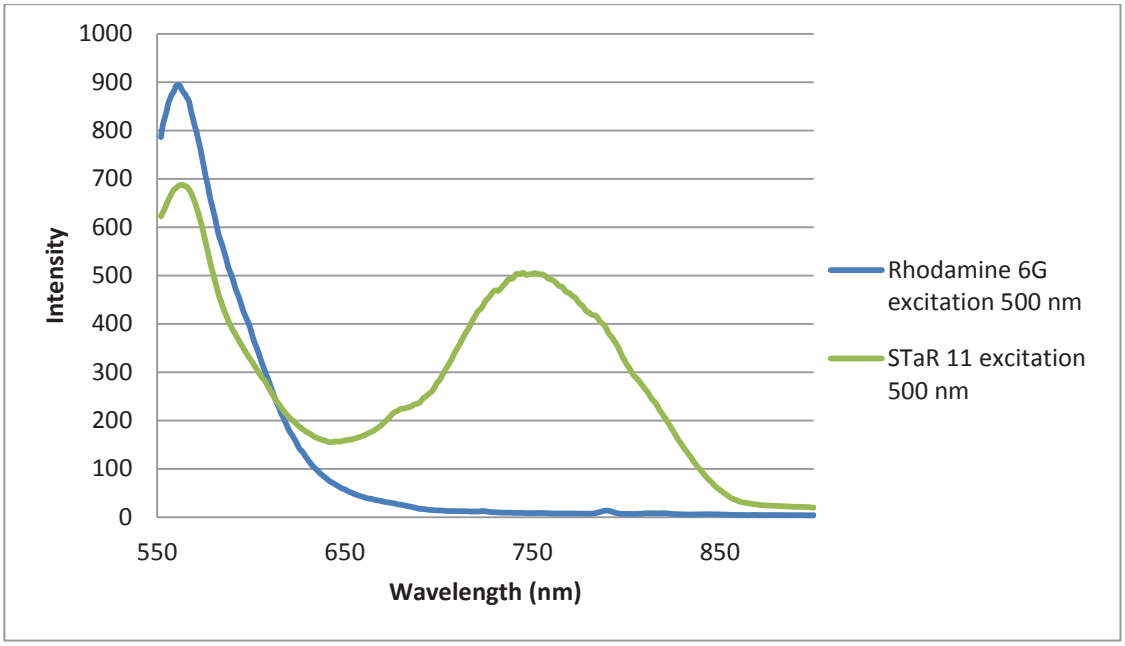


Figure 2-13: Comparison between STaR 11 and rhodamine 6G aluminium oxide nanopowders emission spectra.

STaR 11 was also coated onto neutral and acidic aluminium oxide. Emission spectra were recorded for all the STaR 11 aluminium oxide powder mixtures. A basic aluminium oxide micropowder was also coated with STaR 11 to see if particle size had a significant effect on luminescence (Figure 2-14). The absence and decrease of the emission peaks in the NIR for the neutral and acidic aluminium oxide nanopowders, show that the pH of the powder has a significant effect on the styryl 11 luminescence emission. The rhodamine 6G peak was also slightly blue-shifted and had a higher intensity compared to the conventional STaR 11 powder. The increased luminescence for basic aluminium oxide was due to the isoelectric point of aluminium oxide, which ranges from pH 8.2-9.1 [98]. When the pH of the metal oxide powder is greater than the isoelectric point, the negative charge density on the surface of aluminium oxide increases. This leads to increased adsorption of the dyes onto alkali aluminium oxide, compared to either the neutral or acidic aluminium oxide. Given that both rhodamine 6G and styryl 11 are cationic, they would absorb strongly to the negatively charged aluminium oxide surface. In this study, the alkali aluminium oxide powder used had a pH of 9.2. Similarly, the other metal oxides tested were neutral and therefore would exhibit weaker adsorption compared to alkali aluminium oxide, therefore resulting in weaker luminescence. The particle size did not have a major effect on the luminescence as both the nano- and micropowders give strong visible and NIR luminescence. The only slight difference is that that the micropowder has slightly stronger luminescence in the visible region and

slightly weaker luminescence in the NIR region. Since the aim of this work was to develop a NIR luminescent powder, the nanopowder was chosen for further testing based on the stronger NIR luminescence observed. It was also theorised that the nanopowder would allow for better adhesion of the dye coated nanopowder to the magnetic powder as the smaller particles have a larger surface area to volume ratio.

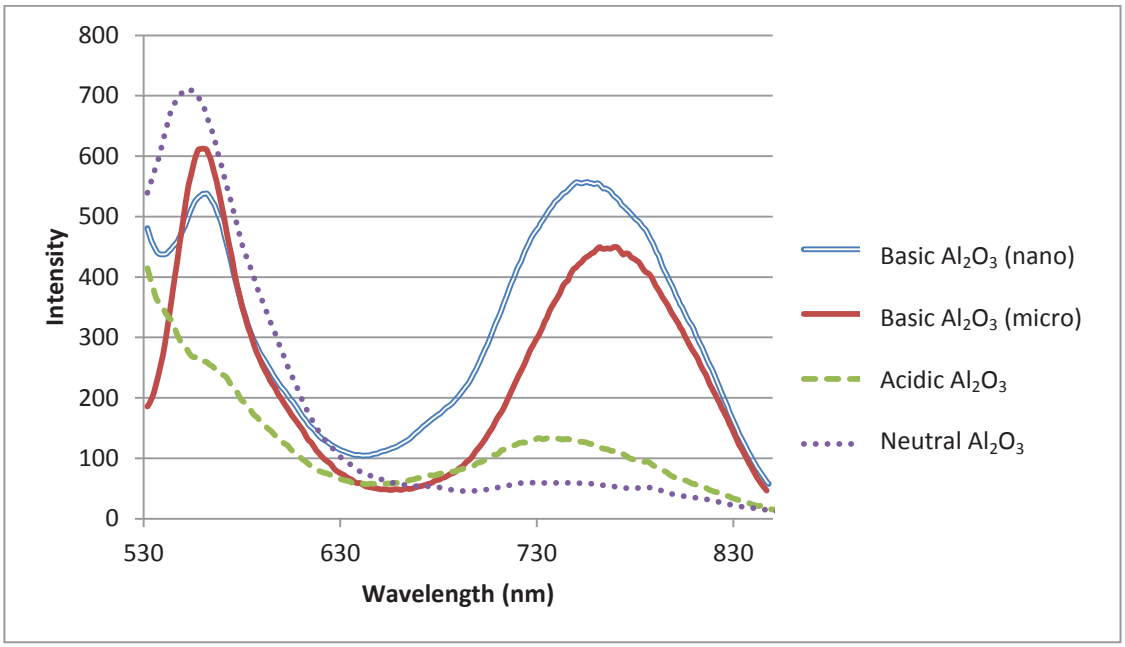


Figure 2-14: Emission Spectra for different aluminium oxide powders coated with STaR 11.

The STaR 11 aluminium oxide nanopowder was then coated onto silver magnetic powder, in an attempt to reduce damage to the ridges by brushing. Several different nanopowder to magnetic powder ratios were prepared and tested: 1:5, 1:10 and 1:25 mass ratios of STaR 11 Al_2O_3 to silver magnetic powder. Polilight images of developed fingermarks showed that the ratios tested did not have a significant effect on the luminescence emission intensity (Figure 2-15). However, when fingermarks were developed the 1:25 mass ratio powder deposited a significant amount of powder on the surface, which had to be removed before imaging the sample. Photomicrographs of the coated magnetic powders were taken to determine if the mass of nanopowder affected the adhesion to the magnetic powder. The mass ratio of 1: 10 gave the best coverage of the magnetic powder with the STaR 11 powder, without excess powder being deposited on the surface. Based on these photographs it was determined that the 1:10 mass ratio of nanopowder to magnetic powder provided the best adhesion and development of fingermarks and this ratio was used for further studies (Figure 2-16).

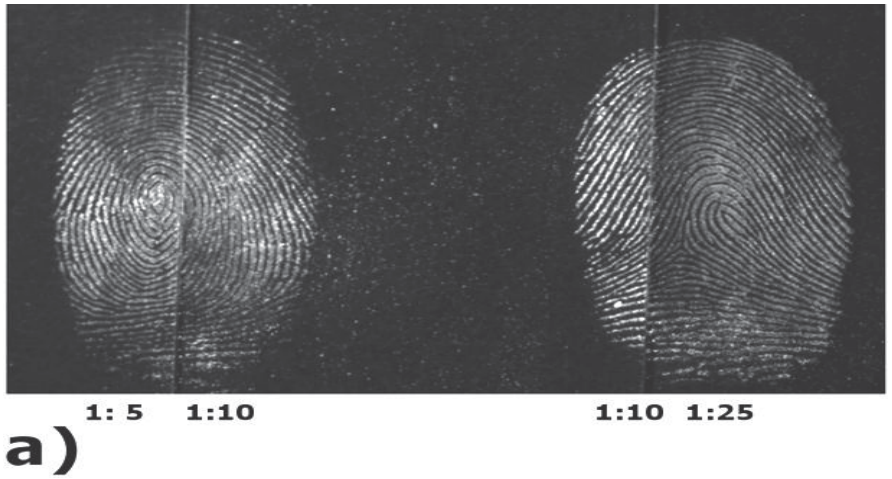


Figure 2-15: STaR 11 magnetic powder of different ratios - a) on glass, b) on laminate, c) on Fanta® soft drink cans.

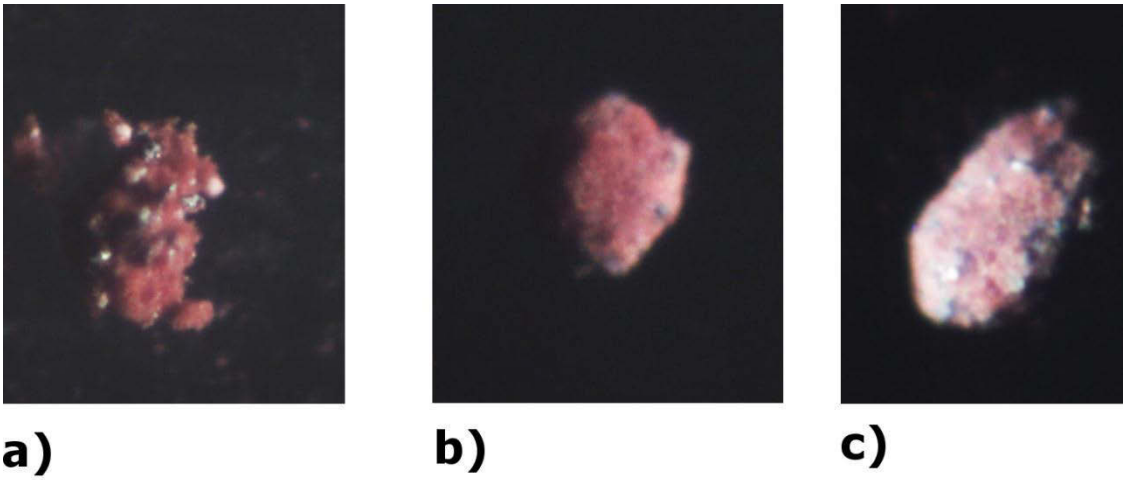


Figure 2-16: Microscopic images of STaR 11 Al_2O_3 magnetic powder (magnification 32x) - a) 1 : 5 STaR 11 : magnetic powder, b) 1 : 10 STaR 11 : magnetic Powder, c) 1 : 25 STaR 11 : magnetic powder.

2.3.2 Comparison Study

2.3.2.1 General results

The comparison study showed that the STaR 11 magnetic powder worked better on charged marks than natural marks and surfaces that are more textured (i.e. laminate). With surfaces that are smooth and glossy such as polyethylene bags, Blitz Green® performed better. Since Blitz Green® is so strongly luminescent; the background luminescence from Fanta® soft drink cans did not affect visualisation, which meant that this powder scored higher than STaR 11 on this surface. Generally, exposure times were longer for STaR 11 than with Blitz Green®; however, most were less than one second. This may have consequences for the use of STaR 11 magnetic powder at crime scenes; this will be explored further in section 2.3.3.

2.3.2.2 Glass

Overall, STaR 11 performed better than Blitz Green®, mainly due to the amount of background powdering from Blitz Green® which decreased contrast. This was more prevalent in the older samples (> 1 week). STaR 11 also performed better on older marks regardless of whether they were natural or charged. STaR 11 developed fingermarks did not exhibit high background development; however, they had lower luminescence strength (compared to Blitz Green®) which slightly decreased contrast (Figure 2-17, Figure 2-18). Blitz Green® did give better development for fresh charged and natural fingermarks regardless of gender. As Figure 2-19 shows there is a difference between female and male donors. Generally charged male marks gave better results for STaR 11, whereas the female charged marks gave variable results for samples up to 1 week old. After this time the trends were more consistent. Similarly with natural female marks, Blitz Green® gave much better results up until 1 week, after which STaR 11 was the preferred technique. This difference decreased over time with fingermarks older than one week giving positive values for STaR 11 developed marks deposited by females indicating that there was decreased donor dependency for older marks.

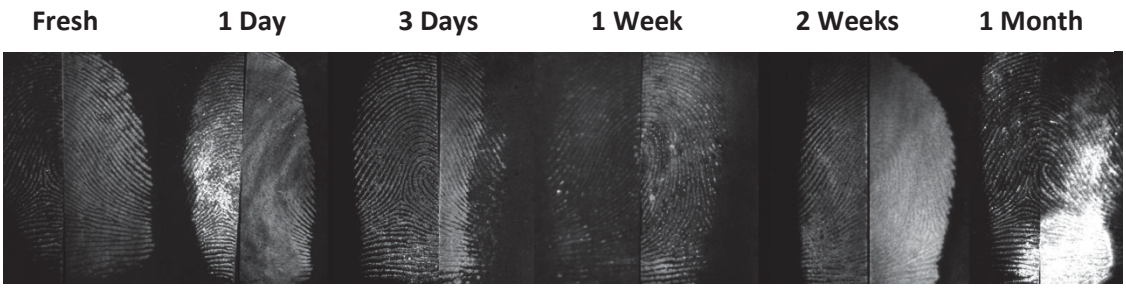


Figure 2-17: Representative charged fingermarks imaged in the luminescence mode (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on glass.

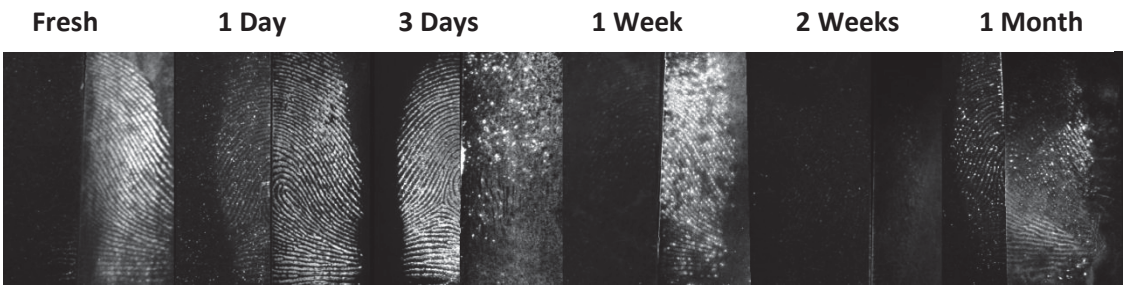


Figure 2-18: Representative natural fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on glass.

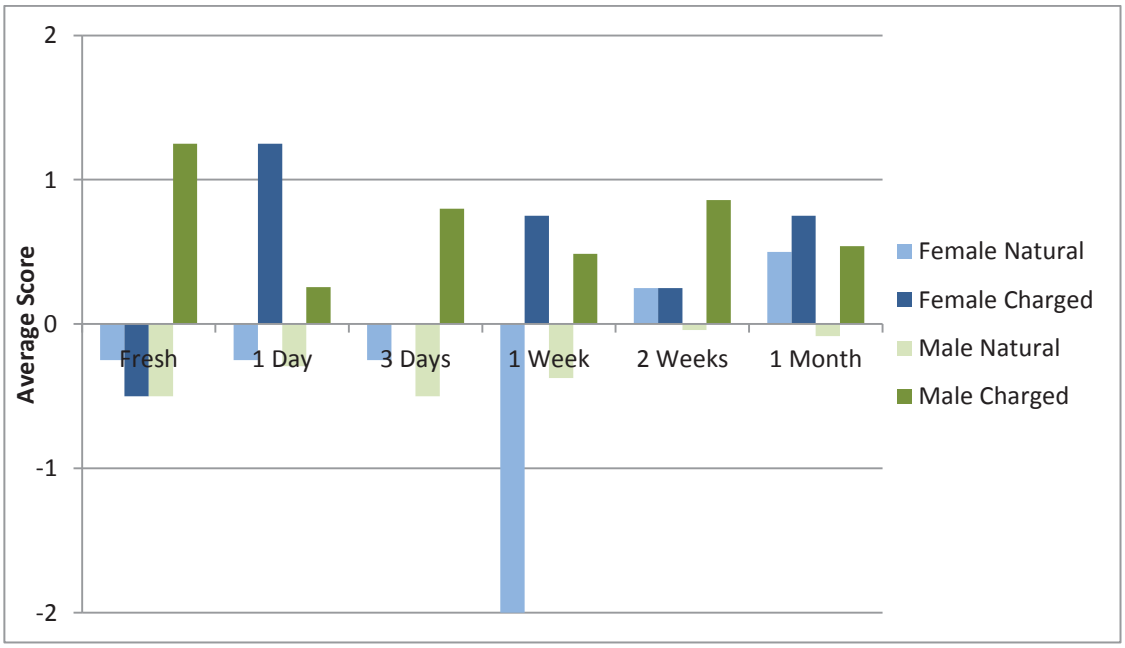


Figure 2-19: Comparison study between Blitz Green® and STaR 11 for all donors on glass (average McLaren scale values indicated).

An examination of the zero values (Figure 2-20) indicated that in the majority of cases, both powders gave good development regardless of fingermark age. Even with the older marks (>2 weeks) there were only two instances where there was no development. While the results in Figure 2-19 indicated that Blitz Green® performed slightly better for fresh and one day samples, the results from Figure 2-20 indicate that there were also a very high number of scores (50%) where both techniques gave the same development. This result would indicate that STaR 11 could still be used as a replacement for Blitz Green® for this surface.

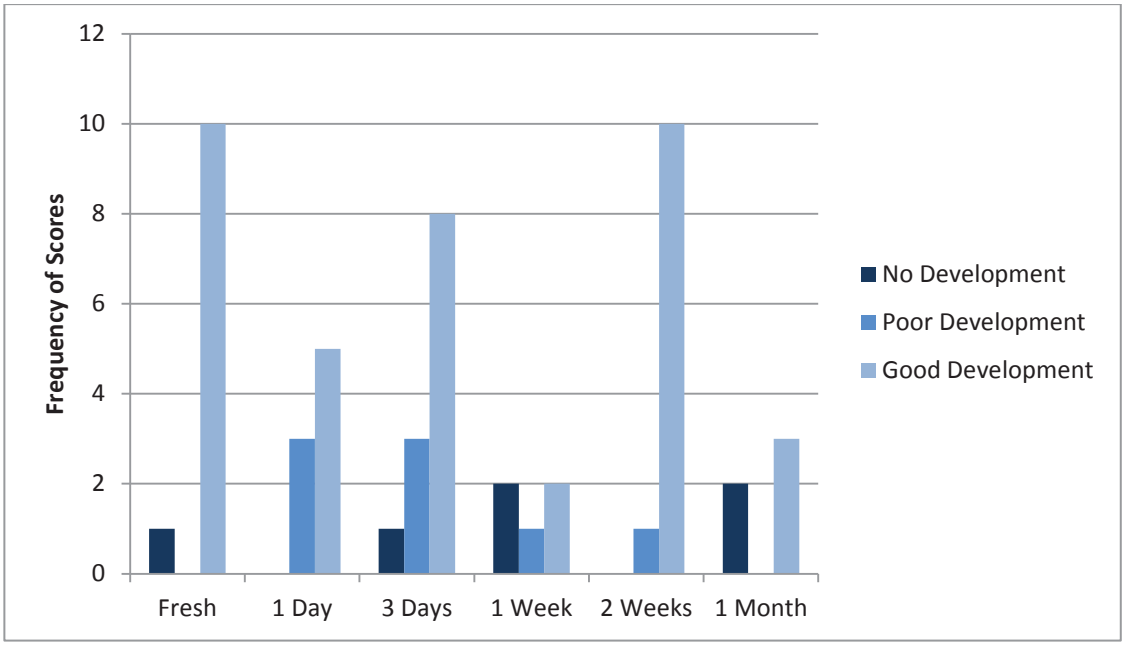


Figure 2-20: Classification of zero values between Blitz Green® and STaR 11 for all donors on glass.

2.3.2.3 Fanta® soft drink cans

Fanta® soft drink cans were chosen because they exhibit strong luminescence in the visible region and are multi-coloured, which affects fingermark contrast, whereas in the NIR there should be no interferences. Blitz Green®, however, was not luminescent in the same region as the substrate and, as a result, the background did not interfere with the visualisation of developed fingermarks (Figure 2-21 and Figure 2-22). Typically, similar development was observed for STaR 11 and Blitz Green®, with some samples giving poor and no development with both powders. Once again STaR 11 performed better with charged marks than with natural marks; although there were no significant trends observed between male and female donors (Figure 2-23).

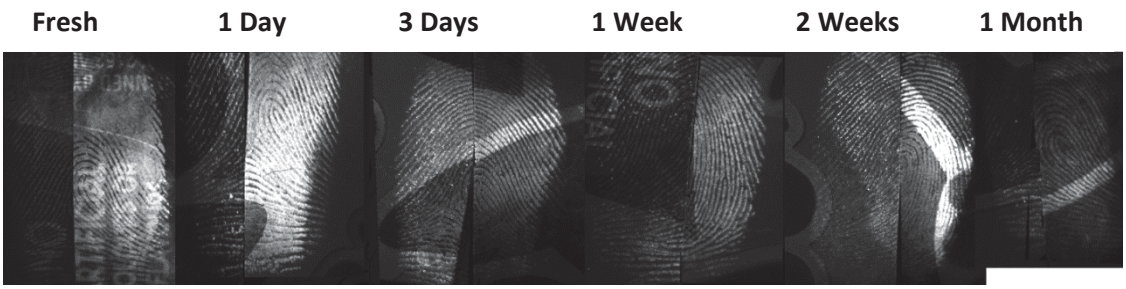


Figure 2-21: Representative charged fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on Fanta® soft drink cans.

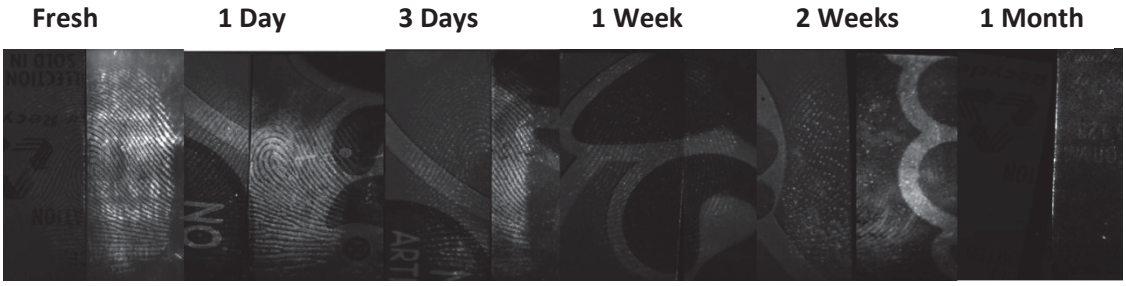


Figure 2-22: Representative natural fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on Fanta® soft drink cans.

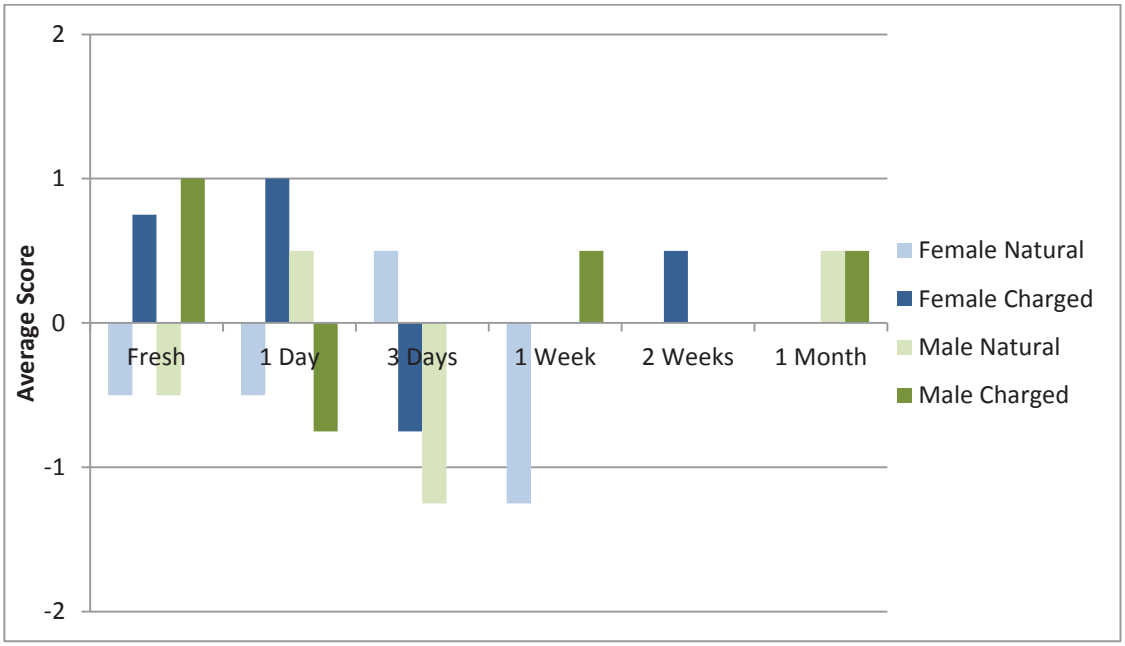


Figure 2-23: Comparison study between Blitz Green® and STaR 11 results for all donors on Fanta® soft drink cans (average McLaren scale values indicated).

An examination of the zero scores (Figure 2-24) indicates that Blitz Green® and STaR 11 gave the same quality of development up to two weeks, after this point there was a significant increase in the number of no development scores. This result suggests that any decrease in effectiveness of the powders was due to the ageing of the samples as opposed to any shortcomings of the powder itself. This result would be expected as Fanta® soft drink cans are a smooth metallic surface and the fingermark deposits could lose their moisture more rapidly on this surface. Combining this with the previous data it can be said that STaR 11 performs very similarly to Blitz Green®; however, development for both techniques is very dependent on the age of the fingermarks. Because of this, it should be noted that, for samples that are older than two weeks, it may be advantageous to develop fingermarks by an alternative method (e.g. cyanoacrylate fuming and staining with STaR 11 has shown to be successful in developing fingermarks on this surface [64]).

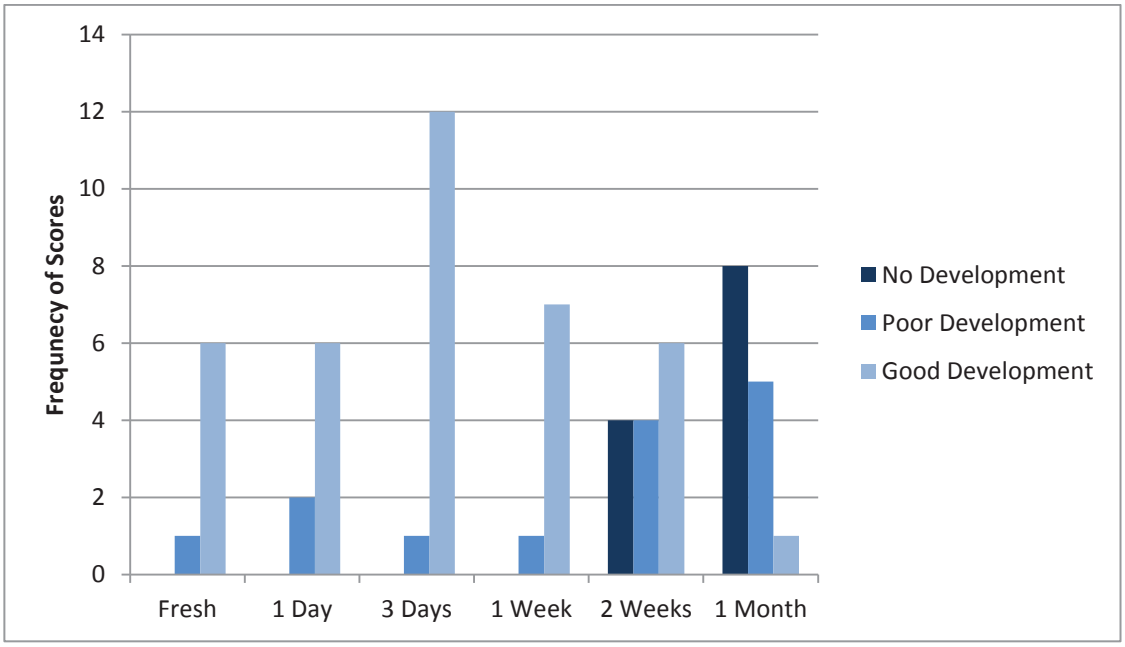


Figure 2-24: Classification of zero values between Blitz Green® and STaR 11 for all donors on Fanta® soft drink cans.

2.3.2.4 Laminate

For fingermarks that were developed on laminate, STaR 11 performed consistently better than Blitz Green®. This was because there was heavy background development observed in

the Blitz Green® samples – leading to reduced contrast – whereas STaR 11 gave minimal background powdering and good ridge detail. This is most likely due to the powder depositing in small grooves on the laminate surface. This became an issue for older samples for which there was an increase in the occurrence of negative development (where the background is luminescent and the fingermark is non-luminescent), significantly affecting the quality of fingermarks obtained (Figure 2-25 and Figure 2-26). Conversely, STaR 11 was strongly luminescent when a significant amount of powder was deposited on the fingermark as opposed to being deposited on the substrate. This is reflected in Figure 2-27 where STaR 11 performed better on the laminate in the majority of cases. There was no distinction between male and female marks, with both following similar trends for charged marks; however, the natural male marks tended to decrease in quality at a more rapid rate, with the one month marks unable to be developed, with both powders giving a similar development quality in the majority of samples.

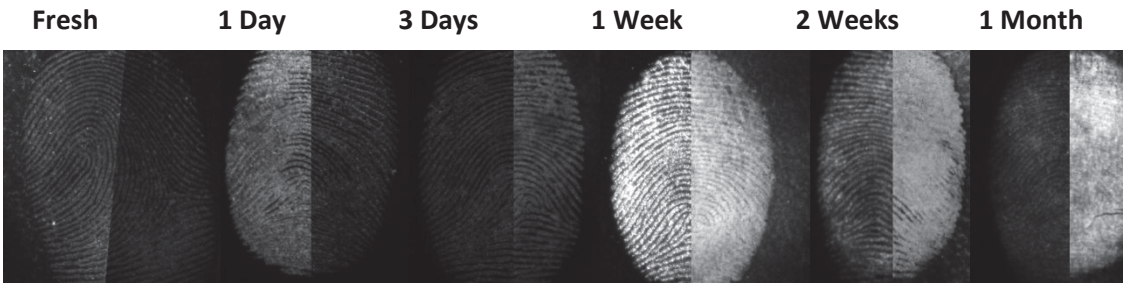


Figure 2-25: Representative charged fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on laminate.

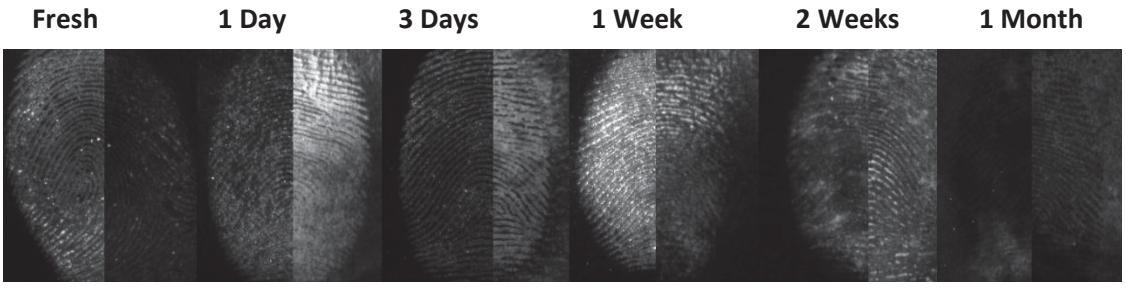


Figure 2-26: Representative natural fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from male donor on laminate.

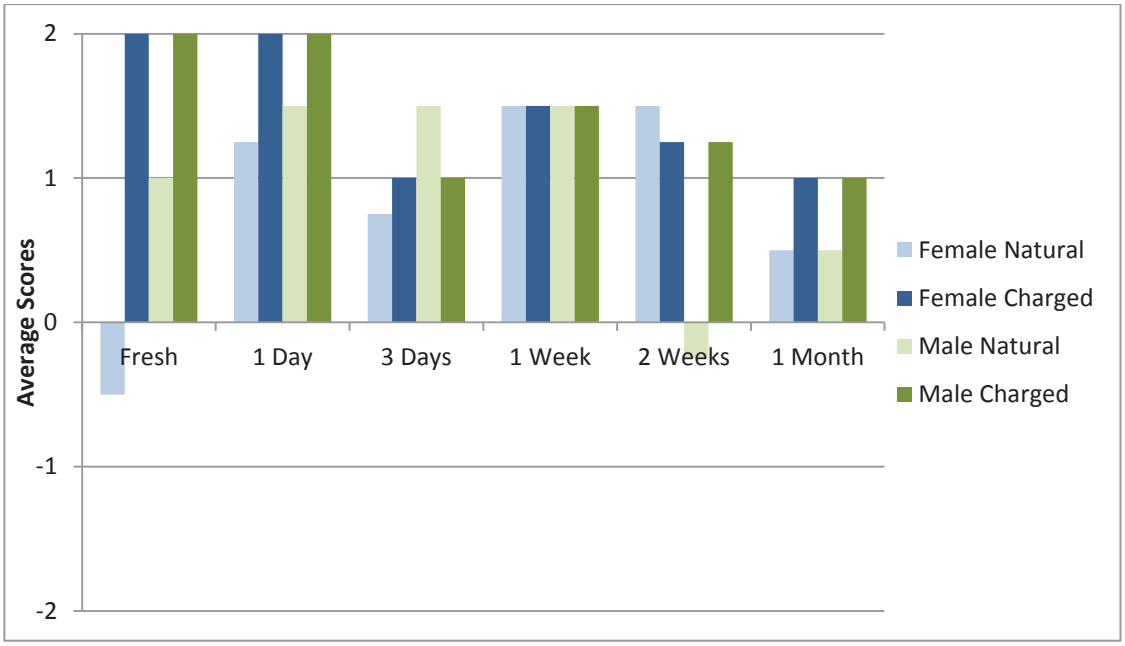


Figure 2-27: Comparison study between Blitz Green® and STaR 11 results for all donors on laminate (average McLaren scale values indicated).

The zero value data (Figure 2-28) for laminate would indicate that there were very few instances where STaR 11 and Blitz Green® gave the same quality of development (due to the low number of zero scores). The most significant result from this data is for the one month samples, which show a significant increase in the number of poor development samples. This would be most likely due to the loss of moisture in the fingermarks rather than the ability of the powders themselves. Based on all the data collected, it can be determined that STaR 11 would provide a significant advantage compared to Blitz Green® for this surface. The only instance where it might not be advantageous would be after 1 month, where it would be preferable to apply and alternate technique.

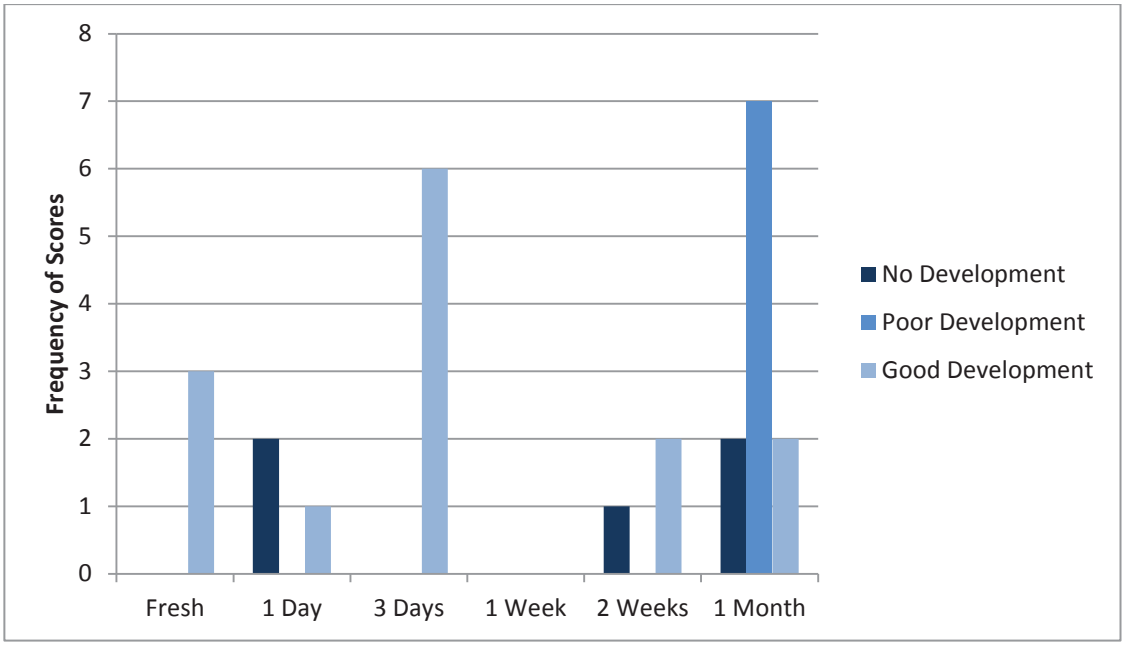


Figure 2-28: Classification of zero values between Blitz Green® and STaR 11 for all donors on laminate.

2.3.2.5 Polyethylene bags

Polyethylene bags are an increasingly common substrate recovered from crime scenes and are difficult to recover fingermarks from using fingerprint powders due electrostatic effects. There were major differences between male and female donors, with male donors giving better development with Blitz Green®, while female donors gave better results with STaR 11. For female donors, there was also a more significant difference between charged and natural fingermarks (Figure 2-29, Figure 2-30), whereas for male donors the difference was minimal. The results below indicate that overall Blitz Green® performed slightly better than STaR 11; however the difference is very minor as there was significant variability between donors of the same sex (Figure 2-31). No clear trend between the age of the sample and development quality could be derived from this data, suggesting that the age of the fingermark did not seem to appear to significantly contribute to the quality of development in these experiments.

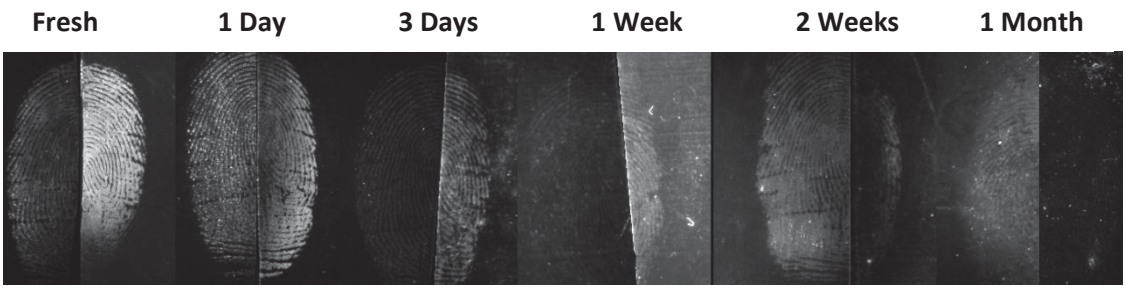


Figure 2-29: Representative charged fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from female donor on polyethylene bags.

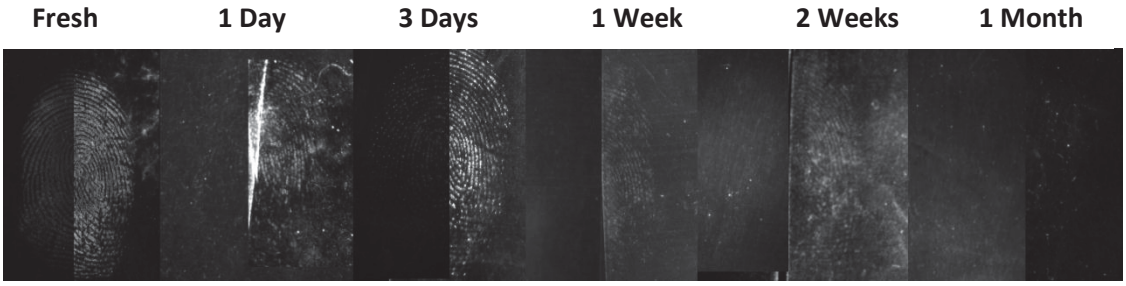


Figure 2-30: Representative natural fingermarks (developed with; LHS STaR 11 using excitation 530 nm and a 700 nm barrier bandpass filter, RHS BG using 450 nm excitation and a 555 nm barrier bandpass filter) from female donor on polyethylene bags.

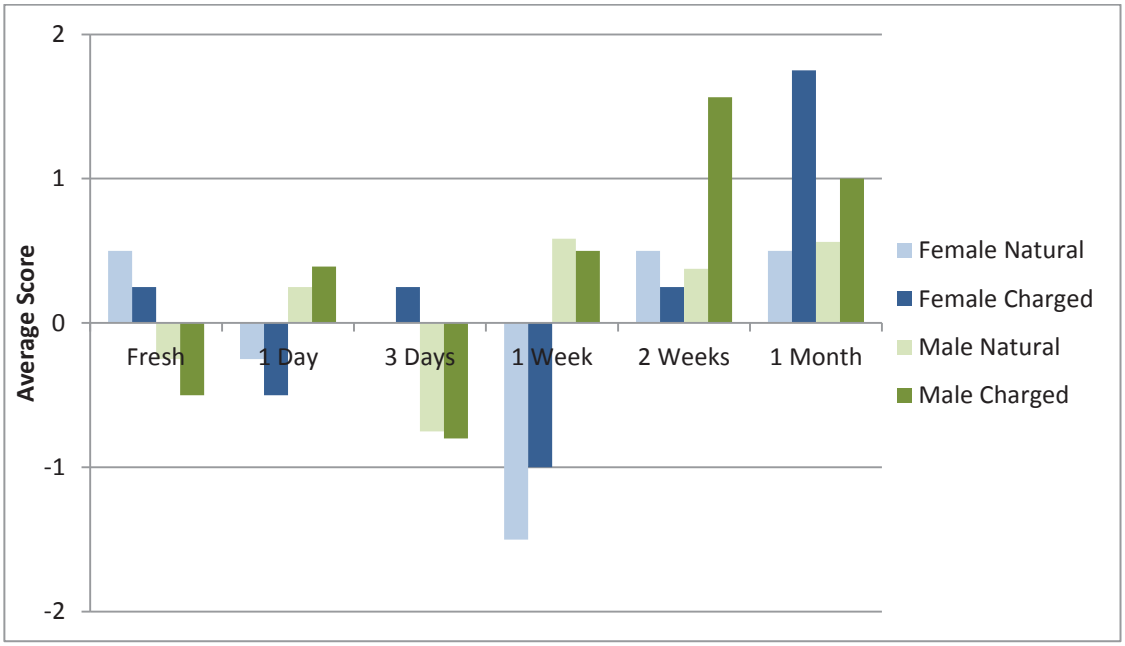


Figure 2-31: Comparison study between Blitz Green® and STaR 11 results for all donors on polyethylene bags (average McLaren scale values indicated).

The zero value data for polyethylene bags (Figure 2-32) show that of all the samples trialled in this study, this gave the highest number of poor and no development values overall. This result would indicate that using a fingerprint powder on this surface may not be the optimal development method. The results also indicate that the age of the fingermark had a significant effect on the quality of development, indicated by the large increase in poor and no development scores for samples aged greater than one week. This highlights a potential limitation of just using the McLaren scale in isolation when comparing the quality of fingermarks over time as the comparison scores on their own did not indicate any significant decrease in quality over time. It should also be noted that there was a large number of samples which gave the same quality of development up to three days. This would signify that if a powder is to be used on this surface it should only be used on relatively fresh samples.

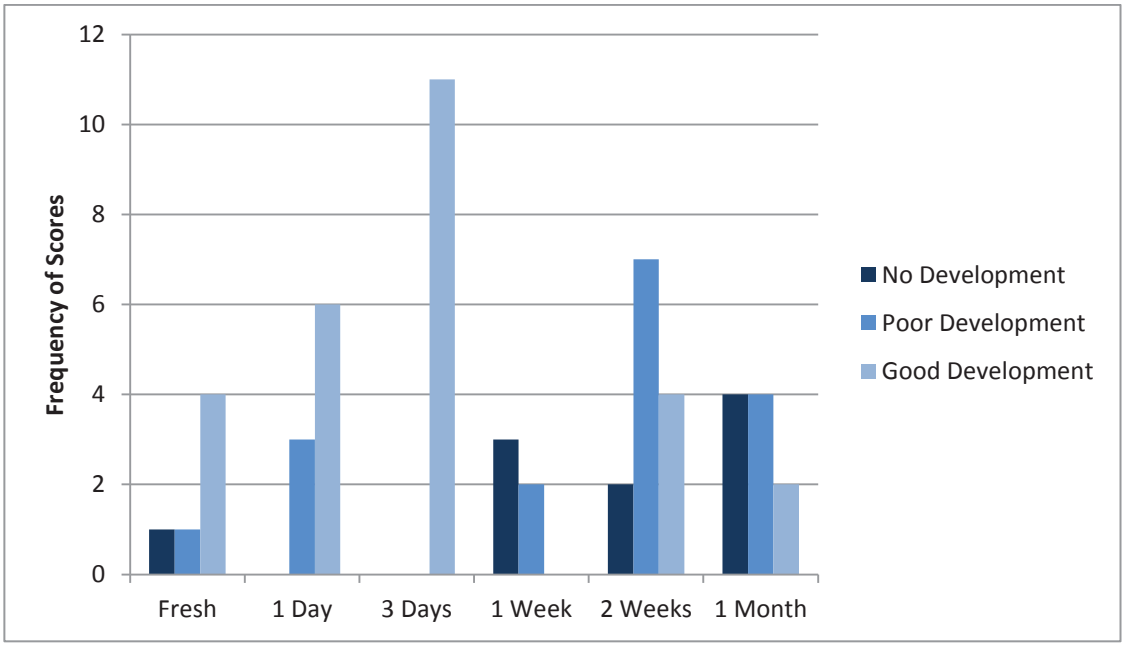
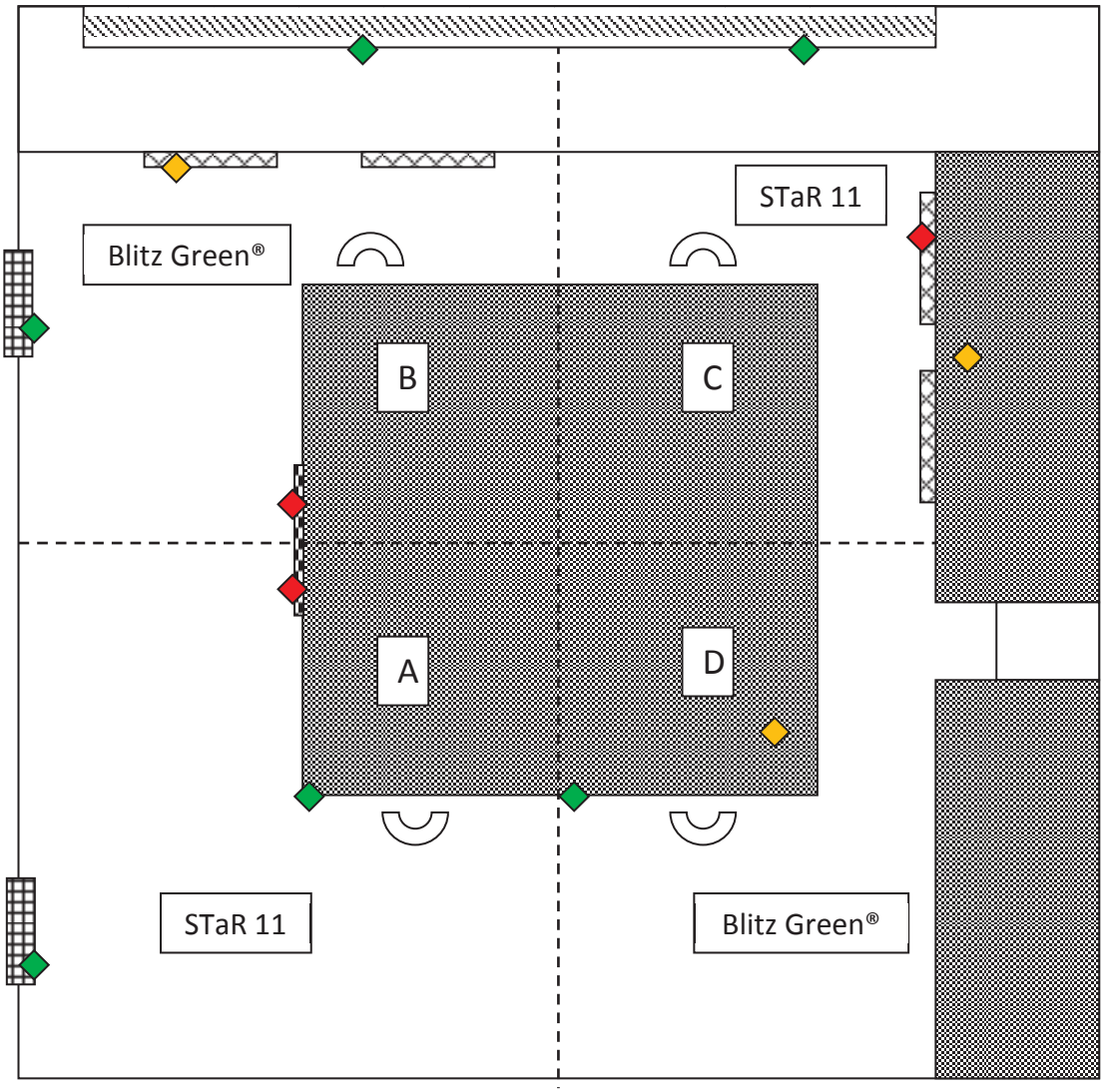


Figure 2-32: Classification of zero values between Blitz Green® and STaR 11 for all donors on polyethylene bags.

2.3.3 Pseudo-operational Study

In order to effectively assess the field capabilities of the STaR 11 powder a 'pseudo-operational' study was conducted. The results from this study are shown in Figure 2-33 and Table 2-4. Blitz Green® performed better overall as it was able to develop the most marks and developed marks could be photographed more easily. The only surface on which Blitz Green® could not develop marks was the metallic light switch. The primary reason for this was that the magnetic wand would come into contact with the surface and the powder would coat the surface, obliterating any ridge detail. STaR 11 could not develop marks on textured laminate or on the metallic light switch; these results were contradictory to the results obtained in the previous study, which found that STaR 11 performed between on textured surfaces. This could be attributed to an examiner bias for the comparison study, where the position of the fingermark was known, and therefore the powder could be applied directly to a fingermark. Whereas when the position of the fingermark is unknown a greater surface area is covered so the likelihood of 'over-powdering' increases. In terms of visual development, fingermarks powdered with Blitz Green® were easily seen under ambient lighting conditions, whereas STaR 11 developed marks were a pale pink colour and quite difficult to see with the naked eye. In both cases, contrast was significantly improved when viewed in the luminescence mode.



Fingermarks successfully powdered and photographed
Fingermarks successfully powdered but not photographed/poor ridge detail
Fingermarks could not be powdered or photographed

Figure 2-33: Results from the pseudo-operational study.

Table 2-4: Results from pseudo-operational study

Fingermark Location	Powder	Detected	Photographed - Vis/NIR
Door (Painted Wood)	Blitz Green®	Yes	Yes
Door (Painted Wood)	STaR 11	Yes	Yes/ Yes
Table Leg (Painted Metal)	Blitz Green®	Yes	Yes
Table Leg (Painted Metal)	STaR 11	Yes	Yes/Yes
Window (Glass)	Blitz Green®	Yes	Yes
Window (Glass)	STaR 11	Yes	Yes/No
Table (Untextured Laminate)	Blitz Green®	Yes	No
Table (Untextured Laminate)	STaR 11	Yes	No/No
Drawer (Textured Laminate)	Blitz Green®	Yes	No
Drawer (Textured Laminate)	STaR 11	No	No/No
Light Switch (Plastic and Metal)	Blitz Green®	No	No
Light Switch (Plastic and Metal)	STaR 11	No	No/No

When the developed marks were photographed in the luminescence mode neither powder gave good results for the laminate or metallic surfaces. STaR 11 developed fingermarks on glass were also unable to be photographed in the near infrared. A number of issues arose from photographing the STaR 11 developed fingermarks that affect its in-field capabilities. Firstly in order to visualise STaR 11 developed marks in the luminescence mode (visible or NIR) a very high-powdered light source is required. The Crime-lite and Polilight PL-10 are not powerful enough to excite strong luminescence from STaR 11. Additional work determined

that the PL-500 or a 530 nm laser gave significantly stronger luminescence and would be a stronger light source for excitation but, in the case of the laser, have issues with portability. The weak emission from STaR 11 also directly affected the exposure times for photography. Blitz Green® developed marks could be photographed without the use of a tripod and shutter speeds of under 1/10 second. Photographing STaR 11 developed marks in the NIR required a tripod and shutter speeds of up to 30 seconds to capture suitable had to be used. From an operational perspective this indicates a significant drawback of visualising in the NIR with Blitz Green® outperforming STaR 11 in terms of number of marks developed and number photographed. This is clearly illustrated in Figure 2-34 where the Blitz Green® developed fingermark has high contrast and clear ridge detail. The STaR 11 developed fingermark, however, has low contrast in both images, with the background interference eliminating ridge detail. A higher powered light source would allow the powders luminescence intensity to increase, this would decrease exposure times and potentially increase the quality of photographed fingermarks in the NIR.

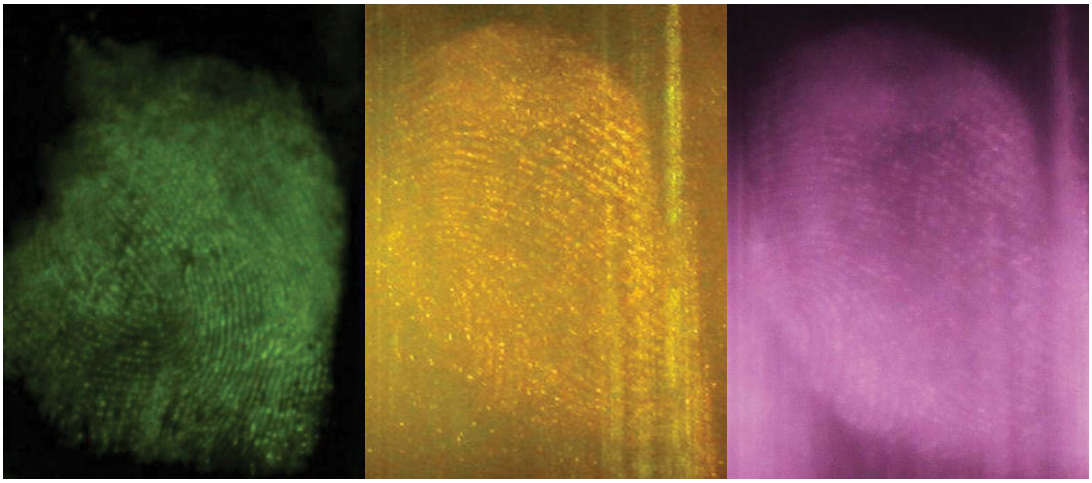


Figure 2-34: Fingermarks from pseudo-operational study on painted metal (left) Blitz Green®, (centre) STaR 11 visible region, (right) STaR 11 NIR.

The Fuji IS Pro used for photographing in the NIR also had several issues that affected the quality of photographed fingermarks. When photographing in the NIR the IS Pro uses a 'live-view' function that allows the NIR image to be seen for 30 seconds on the LCD screen. While this assists greatly in imaging it does not have the accessibility that a regular viewfinder has. Similarly the lens used did not have an automatic focus feature which meant that a lot of fine focusing was required in order to get clear ridge detail. From an operational perspective the advantages of imaging in the NIR do not outweigh the issues. The total time required to

image each fingermark is considerably longer (between five – ten minutes) than photographing Blitz Green® developed marks (one – two minutes). If a large number of fingermarks need to be imaged than it would not be feasible to image them all in the NIR.

Since this experiment was conducted, there have been several advances that could make imaging in the NIR faster and easier. Portable light sources are becoming increasingly more powerful with the advent of LED technology (i.e. Crime-lite 82S or Crime-lite XL) with intensities comparable to that of a portable laser [99]. This would assist in reducing exposure times for developed marks as the luminescence emission would be stronger The removal of the NIR blocking filter from conventional digital cameras has increased in popularity and can be performed through reputable companies. This allows for near infrared images to be seen through the viewfinder thus improving focusing and acquisition times.

2.4 Conclusions

The aim of this study was to develop a magnetic fingermark powder that has a luminescence emission in the NIR. Several different metal oxides of different particle sizes were coated with styryl dyes and an optimised powder was selected and mixed with a commercial magnetic powder. The results from this powder were compared with those obtained using a conventional luminescent fingermark powder (Blitz Green®).

STaR 11 - a mixture of the NIR laser dye styryl 11 and rhodamine 6G - coated on basic aluminium oxide nanopowder was found to be the most effective in terms of shelf life and NIR luminescence emission intensity. Other styryl dyes were either poorly luminescent or they rapidly degraded on the metal oxide powders, which made them unsuitable for routine fingermark development applications. From an operational health and safety perspective, the STaR 11 magnetic powder is a safer alternative to other nanopowders. Since the STaR 11 coated aluminium oxide nanopowder aggregates on the surface of the magnetic powder the particle size increases above dangerous levels, this reduces the potential inhalation risk.

The comparative study determined that STaR 11 magnetic powder was better suited for more textured surfaces as there was decreased background powdering and greater ridge detail when compared to Blitz Green®. For smooth glossy surfaces such as polyethylene bags,

Blitz Green® performed slightly better than STaR 11. For Fanta® soft drink cans and glass, both powders performed similarly. The effectiveness of STaR 11 did not significantly decrease when fingermarks were aged over time and, in some cases, this powder was better suited than Blitz Green for older marks. While these conclusions were made based on the results obtained it should be stressed that a limitation of this study was the small number of donors. Trends regarding male and female donors were made based on the data obtained in this research, however expanding the donor pool size to incorporate a larger number of males and females from different ages groups may alter the results and conclusions obtained in this research.

Based on the success of the comparison study, a pseudo-operational study was performed which compared the powders in a case-like scenario. This produced results contrary to those obtained in the comparison study, with STaR 11 unable to develop fingermarks on textured surfaces. This could be due to the controlled circumstances the comparison study was performed under, where fingermark positions were known. Whereas in the pseudo-operational trial, fingermarks were in uncontrolled, yet more realistic positions, as a result STaR 11 tended to over develop the background more than it did in the comparison study. Photographing developed marks in the NIR also posed a problem for STaR 11 developed marks as a very high powered light source was required to effectively image the fingermarks. The use of the IS Pro and portable light sources such as the Crime-lite severely limit the potential of STaR 11 as a fingermark powder as image acquisition times were significantly longer.

Despite this, STaR 11 has been shown to be an effective technique in developing fingermarks on non-porous surfaces that can provide visualisation of fingermarks under two different imaging conditions (visible luminescence emission and NIR luminescence emission). With the advent of more sophisticated imaging and illumination technologies the issues identified in the pseudo-operational study could be addressed and improved upon.

Chapter 3: Styryl Dye Coated Powder Suspensions

Chapter 3: Styryl Dye Coated Powder Suspensions

3.1 Introduction

3.1.1 Development of Latent Fingermarks on Adhesive Surfaces

Powder suspensions are a versatile method of detecting latent fingermarks on adhesive or wet surfaces. The first black powder suspension for adhesive surfaces was reportedly developed in Japan in the early 1990s. This simple mixture of water, detergent and powder would eventually be marketed as Sticky-side Powder™ by the Lightning Powder Company [100]. Sticky-side Powder™ (SSP) is a viscous suspension that is applied with a brush to develop fingermarks on adhesive surfaces. This technique has been assessed in a number of different studies; Wade found that TiO_2 mixed with Photo-Flo gave excellent results on both the adhesive and non-adhesive sides of the tape [72]. Williams and Elliott expanded on this work and developed a number of different SSP and small particle reagent (SPR) dual formulations and tested the methods of delivery (spraying, dripping, and immersion) using commercially available reagents. The optimal method of delivery was found to be spraying for both the adhesive and non-adhesive sides [101].

Schiemer *et al*. trialled a number of different techniques for the development of latent marks on dark adhesives surfaces. Ultimately, cyanoacrylate stained with a basic yellow/basic red 28 formulation was found to give the highest quality fingermarks. A white powder suspension was found to be a suitable alternative and could also be used in sequence with the cyanoacrylate fuming [102]. Wilson also found that staining the adhesive side of tape after cyanoacrylate fuming with a rhodamine 6G, Ardrox® and basic yellow (RAY) solution also gave superior results to gentian violet or black SSP [103]. A similar study was performed by Brzozowski who compared the development of WetWop™, Sticky-side Powder (both commercially sourced), and ethanol and phenolic gentian violet solutions. This study found that WetWop™ was the most effective technique while SSP was only effective for samples with rubber glue (results were poor for acrylic glue samples) [104].

Hollars *et al*. prepared a luminescent alternative to the commercial sticky side powders by mixing an industrial detergent (Liqui-Nox) and Ardrox®. This formulation allowed for luminescent examination of fingermarks on the adhesive side of black tapes. There were, however, some drawbacks with this formulations as some developed marks exhibited strong background luminescence and had to be photographed immediately after treatment because the luminescence would fade [105]. Wang *et al.* developed CdSe quantum dots to give luminescent fingermarks on the adhesive side of tapes. Clear ridge detail was observed with strong luminescence under 380 nm excitation [106]. The only issue with both of these studies is that neither compared the new luminescent SSP to a conventional technique.

3.1.2 Development of Latent Fingermarks on Wetted Surfaces

Small particle reagent (SPR) is a powder suspension that is applied to fingermarks on non-porous surfaces that are wet. SPR, first developed in the mid-1970s was patented by Morris and Wells in 1977 [100]. The technique is commonly applied by spraying the suspension on the surface. It is most commonly a suspension of molybdenum disulfide in a solution of the surfactant Tergitol™ [28]. Haque *et al*. examined iron oxide as a potential replacement for molybdenum disulfide and found that iron oxide has several advantages. The iron oxide suspensions were more selective than molybdenum disulfide SPR, had lower toxicity and irritability, and faster development [107]. Both techniques, however, are only suitable for pale coloured surfaces (since the developed marks are a grey-black colour). Frank *et al.* trialled a number of different white powders as an alternative to conventional small particle reagent. From this study, it was found that zinc carbonate in Tergitol™ gave the best development [108]. Particle size also played a significant role in the quality of development with smaller zinc carbonate particles (~2 μm) performing better than larger ones (~6 μm) [100]. Ishizawa *et al.* prepared a dual spray that developed latent fingermarks and fixed them to the surface; that could be used for both fingermarks on wet and dry surfaces [109]. A study by Kabklang *et al.* examined different formulations of both black and white SPR and found that the quality of developed fingermarks was determined by the type and proportion of particles and detergent, their ratio, and pH [110]. Daéid *et al.* compared a commercial white powder suspension (WetWop™) to vacuum metal deposition (VMD). This study found that VMD outperformed the powder suspensions on the majority of surfaces; however, since SPR is mainly used at the scene, it would still be the preferred technique for rapid visualisation in

the field [111]. Recently Sears and Downham reported that a thicker suspension, which is applied by brushing the suspension on the surface (as opposed to spraying) was highly successful. Suspensions of iron oxide and titanium dioxide (black and white SPR) gave very good results for fingermarks aged up to four months old [112].

Black and white small particle reagents are limited by the types of surfaces they can be applied on; as a result, luminescent alternatives have been trialled. Springer *et al.* prepared rhodamine 6G and basic yellow coated powder suspensions and found that basic yellow gave luminescent fingermarks of high quality [113]. Jasuja *et al.* coated zinc carbonate with various luminescent dyes and found that rhodamine 6G, rhodamine B and cyano blue were all able to develop latent fingermarks aged up to twelve days old. A significant result from this study was that luminescent fingermarks were able to be visualised on multi-coloured surfaces [114]. This was expanded upon by Sodhi and Kaur, who coated zinc carbonate with crystal violet dye which gave violet coloured fingermarks under white light and luminescence with 505 nm excitation. Results from this work indicated that the novel reagent gave increased contrast compared to conventional molybdenum disulfide SPR [115].

3.1.3 Aims and Objectives

Powder suspensions have been identified as an effective method for developing latent fingermarks on wetted and adhesive non-porous surfaces. However, most powder suspensions currently used in casework rely on contrast induced by absorption, which can be problematic for dark, multi-coloured and patterned backgrounds. The aim of this study is to develop a NIR luminescent powder suspension to develop latent fingermarks on both adhesive and wetted surfaces. Based on the previous study (Chapter 2), different dye coated metal oxide nanopowders were trialled in an attempt to get better development than with conventional methods. Once an optimised suspension was formulated, it was compared against conventional techniques (commercial Black Wet Powder™ and Fluorescent SPR).

3.2 Materials

3.2.1 Reagents

Aluminium oxide nanopowder, <50 nm particle size (TEM) [CAS 1344-28-1], rhodamine 6G dye content 99% [CAS 989-38-8], centrimonium bromide (CTAB) [CAS 57-09-0], titanium (IV) oxide, rutile powder <5 μm, ≥99.9% trace metal basis [CAS 1317-80-2], titanium (IV) oxide rutile nanopowder, <100 nm particle size, 99.5% trace metals basis [CAS 1317-80-2], zinc oxide, *ReagentPlus®* powder <5 μm particle size 99.9% [CAS 1314-13-2], zinc oxide nanopowder, <100 nm particle size [CAS 1314-13-2] were obtained from Sigma Aldrich and used as supplied.

Norton cloth tape – Yellow, Clingtape – black cloth tape, Norton trade painters masking tape, Clingtape silver duct tape and Bunnings brown packaging tape were all purchased from Bunnings Warehouse and used as supplied.

Styryl 9M [CAS 82988-08-7] and styryl 11 [CAS 92479-59-9], were obtained through Exciton/Lastek and used as supplied.

Sodium dodecyl sulphate (SDS) [CAS 151-21-3] was obtained through BDH Prolabo chemicals and used as supplied

Synperonic N [CAS 9016-45-9] was obtained through VWR international and used as supplied.

Triton X-100 [CAS 9002-93-1] was obtained through AJAX Finechem and used as supplied

Wet Powder™ and Small Particle Reagent UV were purchased through Optimum Technologies and used as supplied.

3.2.2 Instrumentation

A Varian Cary Eclipse fluorescence spectrometer was used for measuring the luminescence of the dye coated nanopowders and dye solutions.

A Leica DMR microscope was used for the transmission microscopy examination of powdered fingermark samples.

A Rofin Poliview IV fitted with a Polilight PL 500 was used for initial luminescence examination of developed fingermarks and for comparison between the dye coated nanopowder suspension and the commercial detection methods.

3.3 Methods – Sticky-side Powder

3.3.1 General Approach

The aim of this study was to prepare a nanopowder suspension that would be luminescent in the near infrared and that could be used for detecting latent fingermarks on adhesive or wet surfaces. Separate suspensions were prepared and optimised according to the type of surface they would be applied to. Once optimised suspensions were prepared a donor study was performed and a comparison against conventional techniques was performed.

3.3.2 Optimisation of Surfactants

Four different surfactants were trialled, cetyl trimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), synperonic N and triton x 100. These were chosen due to their different ionic properties and their availability in fingerprint laboratories (Table 3-1). The surfactants were trialled only on STaR 11 coated nanopowders as this dye gave the strongest luminescence of all the dyes tested in the previous study. The STaR 11 nanopowders were prepared according to section 2.2.3.2.

Table 3-1: Surfactants used in this study.

Surfactant	Ionic Nature
CTAB	Cationic
SDS	Anionic
Synperonic N	Non-ionic
Triton x 100	Non-ionic

For each surfactant, 0.1 M stock solutions were prepared; these were then diluted to working solutions (0.01 M, 0.005 M and 0.001 M), then mixed with the previously coated STaR 11 metal oxide powder. The STaR 11 powder was prepared as outlined in Section 2.2.3.2. The suspensions were then left overnight to dry and the resulting dry powder subjected to luminescence spectroscopy. Luminescence examinations of the powders were conducted by placing the powder in the Cary Eclipse solid sample holder and obtaining an emission spectra for different emission wavelength until the optimal conditions were found

Once a suitable surfactant was found, styryl 9M, styryl 11 and STaR 11 were all coated onto three metal oxides (aluminium, zinc, titanium). Luminescence spectra of each powder were collected and the most suitable powder surfactant combination was determined based on luminescence intensity.

3.3.3 Optimisation of NIR Sticky Side Powder

The optimised surfactant, dye and metal oxide combination was trialled on latent fingermarks. Fresh marks were deposited on cloth tape and the marks were treated with different suspensions by brushing; these were then viewed under the Poliview to determine the luminescence and ridge clarity. The development time was also optimised with fresh marks deposited on all tape surfaces (Figure 3-1) and with development times from five seconds to one minute were trialled. A single charged mark was placed on the tape surface and developed using the optimised sticky side powder and was then scored based on the quality of the development according to the Bandey scale (Table 3-2) [116].

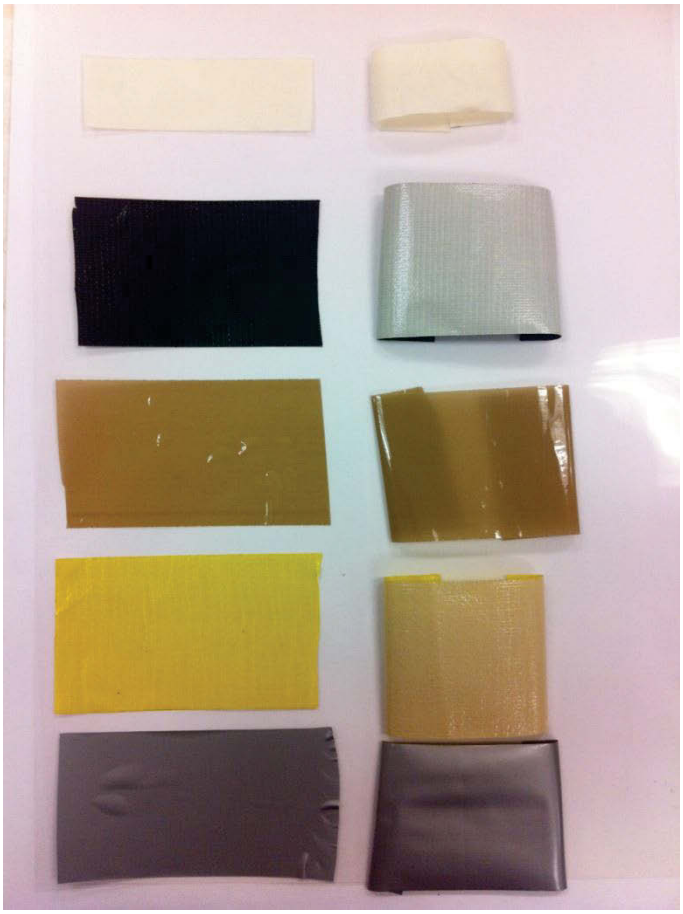


Figure 3-1: Tapes used in the comparison study with Wet Powder™.

Table 3-2: Bandey scale used for comparison [116].

Grade	Description
0	No development
1	No continuous ridges; all discontinuous or dotty
2	One third of the mark comprised of continuous ridges; remainder either show no development or dotty
3	Two thirds of the mark comprised of continuous ridges; remainder either show no development or dotty
4	Full development; whole mark comprised of continuous ridges

3.3.4 Donor Study NIR Sticky Side Powder

Once an optimised nanopowder suspension was developed, it underwent a comparison study with a commercial technique. For sticky side powder, this study covered a range of tapes (cloth, duct, gaffa, masking, and packing) (Figure 3-1) and fingermark samples from four donors (two male, two female) that were aged for up to one month. Tape samples were aged under two different conditions; after deposition some samples were stuck down to the back of the adhesive tape (referred to as stuck down samples), while other samples were left exposed to the environment (referred to as exposed samples). Each sample was cut in half, with each half being treated with STaR 11 SSP or Wet Powder™ (Figure 3-2). Each treated half was visualised under its respective optimal imaging conditions and digitally placed together for comparison. Wet powder™ developed marks were imaged using the Poliview system under white light. STaR 11 developed marks were imaged in the luminescence mode using a 530 nm excitation and 700 nm barrier band pass filter. All digital stitching of fingermarks was performed using GNU image manipulation program (GIMP). No other manipulations were applied to the images after stitching. Each fingermark was then given a score; the scoring system used for this study was the McLaren comparative scale [94] explained previously in section 2.2.3.5.

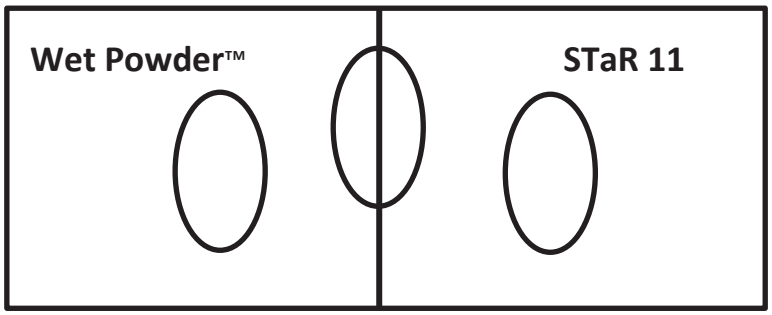


Figure 3-2: Preparation of samples for SSP comparison study.

3.4 Methods – Small Particle Reagent

3.4.1 Suspension Optimisation

A separate suspension had to be optimised for the small particle reagent. This involved optimising the surfactant concentration, mass of metal oxide nanopowder and concentration of luminescent dye. For the surfactant and luminescent dye concentrations, experiments were conducted using luminescence data obtained from the dried powders. Suspensions were prepared according to Table 3-3 and Table 3-4.In these trials the amount of aluminium oxide was kept constant (0.15 g per 10 mL of solution). For the surfactant trials, 0.1 M SDS solution was diluted to a new volume in order to give the final concentration given in Table 3-3. Once an ideal formulation was determined based on the luminescent data, the suspension was applied to charged latent fingermarks. Each suspension was sprayed onto a latent fingermark on a glass slide, excess suspension was removed by running the slide under tap water.

The results from these experiments resulted in a different approach having to be adopted as the luminescent data was not accurate at indicating the ability of the suspension to develop fingermarks. Ultimately the suspensions which had the strongest NIR luminescence emission intensity gave considerably poor development of latent fingermarks, whereas suspensions which had poorer NIR luminescence intensity gave better fingermark development. Therefore a different approach had to be taken. Charged fingermarks on glass microscope slides were treated with seven different suspensions in order to identify which factors had the greatest effect on development (Table 3-5).

Table 3-3: STaR 11 SPR-SDS concentration experiment.

	Control Suspension	**Suspension 1**	**Suspension 2**	**Suspension 3**	**Suspension 4**
Volume of 0.1 M SDS (mL)	10	10	10	10	10
Final Volume (mL)	70	200	100	50	20
Concentration of SDS (M)	0.014	0.005	0.010	0.020	0.050
Volume of STaR 11 (mL)	12.5	12.5	12.5	12.5	12.5

Table 3-4: STaR 11 SPR- STaR 11 concentration experiments.

10 mL 0.02 M SDS	**Solution 1**	**Solution 2**	**Solution 3**	**Solution 4**	**Solution 5**
Volume of STaR 11 (mL)	6	2	1	0.5	0.25
Final Volume (mL)	16	12	11	0.5	0.25
Styryl 11 Concentration (M)	8.73×10^{-4}	3.88×10^{-4}	2.09×10^{-4}	1.10×10^{-4}	5.68×10^{-5}
Rhodamine 6G Concentration (M)	313×10^{-3}	1.39×10^{-3}	7.59×10^{-4}	3.98×10^{-4}	2.04×10^{-4}

Table 3-5: STaR 11 SPR multivariate experiment parameters.

	Control	SPR 1	SPR 2	SPR 3	SPR 4	SPR 5	SPR 6
Volume of 0.1M SDS (mL)	10	10	10	10	10	10	10
Final Volume (mL)	70	**100**	**50**	70	70	70	70
Mass of Al_2O_3 (g)	1.5	1.5	1.5	**1.0**	**2.0**	1.5	1.5
Volume of STaR 11 (mL)	12.5	12.5	12.5	12.5	12.5	**5.0**	**20.0**

Once the key factor was identified this was further explored with additional experiments, Section 3.5.1 Table 3-6.

3.4.2 Comparison with Fluorescent SPR

Once an optimised suspension was developed, it was then compared with Small Particle Reagent UV (SPR UV), a commercially available fluorescent SPR. Before a comprehensive donor study was performed, initial comparison tests were undertaken. Charged and natural marks were deposited on glass microscope slides according to Figure 3-3. Each mark was then submerged in tap water for 24 hours before being dried and one half treated with SPR UV, the other half treated with STaR 11 SPR. After the suspensions were applied to the fingermarks, the surface was rinsed with water to remove excess powder. After treatment, each developed fingermark was photographed in the luminescence mode at each technique's respective optimal conditions. SPR UV was imaged using a 450 nm excitation and 555 nm barrier band pass filter, STaR 11 developed marks were imaged using a 530 nm excitation and a 700 nm barrier band pass filter. Each photographed mark was then digitally placed side by side for comparison.

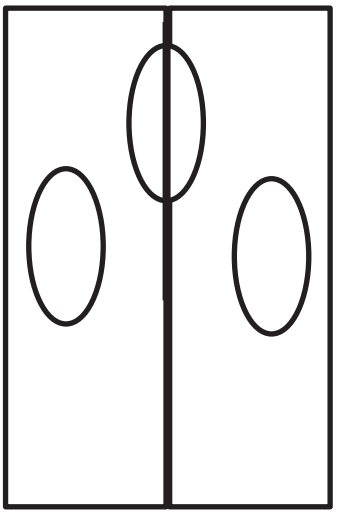


Figure 3-3: Preparation of fingermarks for comparison with Small Particle Reagent UV.

3.4.3 Optimisation of Delivery Method

Initial testing found that the traditional pump spray method resulted in a significant amount of background powdering; as a result, an alternate delivery method was trialled. The EcoSpray® is a commercially available gas-pressurised atomiser that can deliver liquids and suspensions in a fine mist. This was compared with the conventional spray pump bottle by depositing charged and natural marks on glass microscope slides and treating each mark with the optimised NIR suspension. Excess powder was removed by washing the surface with tape water before visualisation. Developed marks were compared by examination under the transmission microscope to determine the amount of powder being deposited on the fingermark ridges and in the luminescence mode to determine the quality of development that each technique provided.

3.4.4 Small Particle Reagent Dual Spray

A multi-step process was also trialled that involved first spraying the metal oxide suspension without any luminescent dye added, then applying a secondary spray that contained the luminescent dye. The advantage of this method was that developed fingermarks could be

imaged under both absorption and luminescence, which could significantly increase the number and quality of developed fingermarks. This was also attempted as there were several issues with aqueous suspensions and the luminescence of the NIR dye.

3.5 Results – Sticky-side Powder

3.5.1 Surfactant optimisation for STaR 11 Sticky Side Powder

STaR 11 coated onto aluminium oxide was used initially as this was the most effective nanopowder from the previous study. All STaR 11 powder suspensions gave luminescence in both the visible and NIR regions, indicating that the addition of a surfactant did not greatly alter the luminescence of STaR 11 when adsorbed onto the metal oxide powder surface. However for the CTAB, synperonic N and triton X-100 when the powders were being prepared there was a colour change observed. Traditionally, STaR 11 is a dark purple solution that becomes magenta upon adsorption to the metal oxide powder. The SDS suspension retained the purple colour on the powder and the aqueous component remained colourless. The other suspensions gave an orange coloured solution and powder (Figure 3-4, Figure 3-5). The darker purple colouration in Figure 3-5 is due to a decrease in the amount of water present, and reflects an increased amount of styryl 11 that remains unaggregated, compared to the more aqueous solution shown in Figure 3-4 which more magenta in colour. A possible reason for this is that the non-ionic and cationic surfactants were stripping the rhodamine 6G off the surface of the powder to give this orange colour. SDS was also the only surfactant that would readily suspend the STaR 11 coated nanopowder.

Figure 3-4: STaR 11 suspensions - (l-r) CTAB, synperonic N, triton X-100 and SDS).

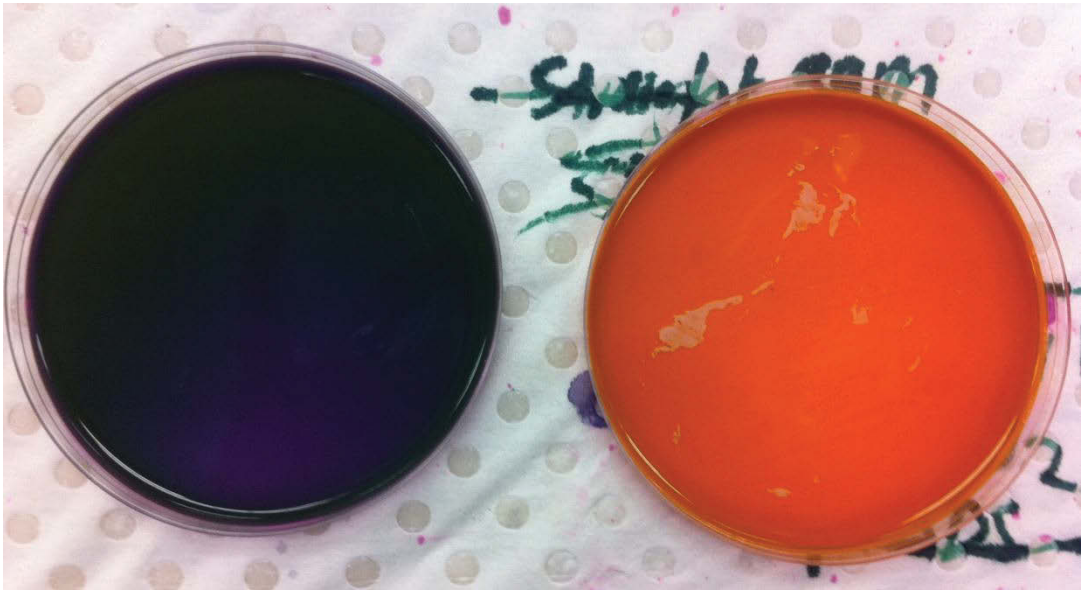


Figure 3-5: STaR 11 suspensions - (left) SDS, (right) synperonic N.

The choice of surfactant had a significant effect on the luminescence emission of STaR 11 (Figure 3-6). CTAB was found to give the strongest luminescence in the visible region but

exhibited comparatively poor NIR luminescence. The synperonic N, triton X-100 and SDS gave significant weaker luminescence in the visible region but slightly stronger NIR luminescence compared to CTAB. The major difference between the anionic and non-ionic surfactants was the significant shift in emission maxima. Synperonic N and triton X-100 had λ_{em} maxima shifted 20 and 8 nm, respectively from the λ_{em} maxima of SDS. However, SDS still gave the strongest luminescence in the NIR; a possible reason for this is that rhodamine 6G is a cationic dye and SDS is an anionic surfactant. Anionic surfactants such as SDS have been shown to decrease the formation of rhodamine 6G dimers [117] which would affect the luminescence of styryl 11 due to the potential FRET interaction between the dyes. Based on this result, SDS was chosen as the optimal surfactant for the remainder of the study.

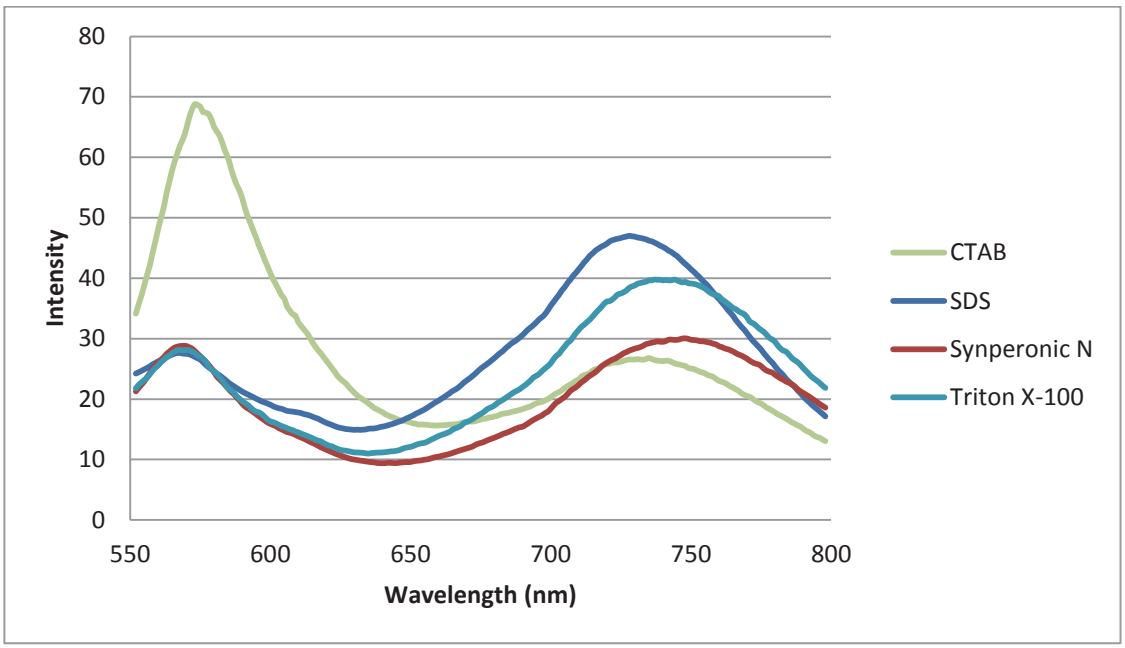


Figure 3-6: Emission spectra of STaR 11 coated Al_2O_3 nanopowder with different surfactants.

Based on this result; styryl 9M, styryl 11 and STaR 11 were all coated onto different metal oxides, then suspended in SDS and then the luminescence spectra was recorded (Figure 3-7, Figure 3-8, Figure 3-9). These results were found to be similar to those obtained from the powder study (Section 2.3.1); however, the styryl dyes appeared to be stabilised by the presence of SDS as the styryl 9M and styryl 11 powder suspensions did not fade as rapidly and gave some luminescence emission in the NIR. In all cases, aluminium oxide nanopowder gave the strongest luminescence compared to the other metal oxides. Comparing all the aluminium oxide nanopowder results, STaR 11 still gave the strongest NIR luminescence

emission and could also provide strong luminescence in the visible region. From these results, STaR 11 coated aluminium oxide nanopowder was used for the remainder of this study.

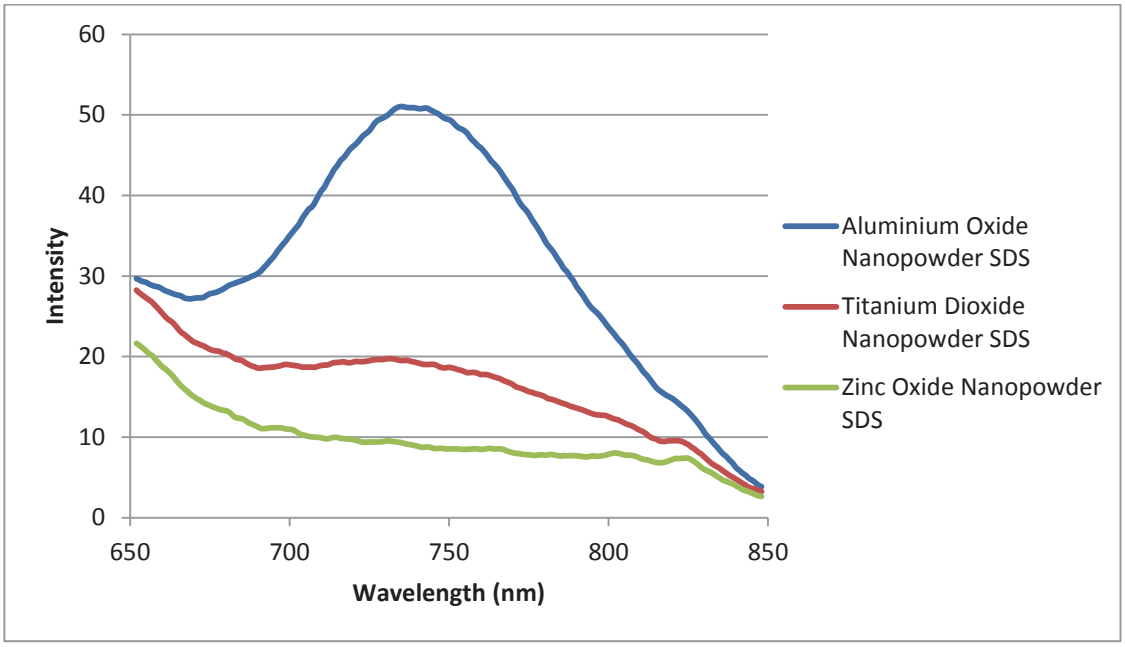


Figure 3-7: Emission spectra of styryl 9M coated nanopowders suspended in SDS (excitation 590 nm).

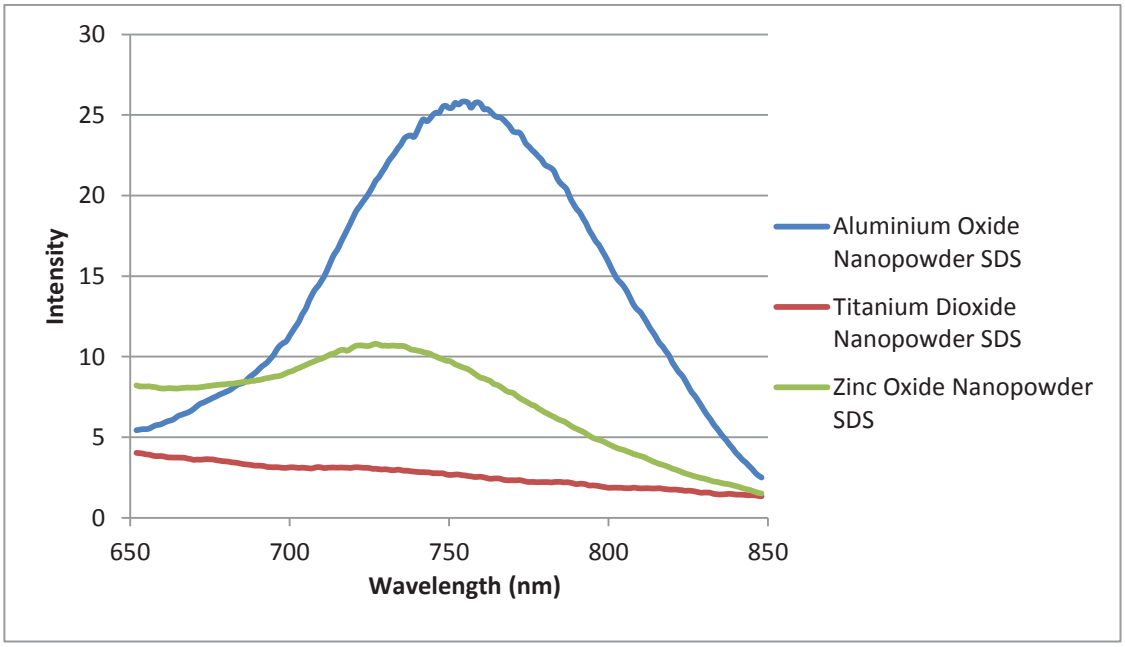


Figure 3-8: Emission spectra of styryl 11 coated nanopowders suspended in SDS (excitation 590 nm).

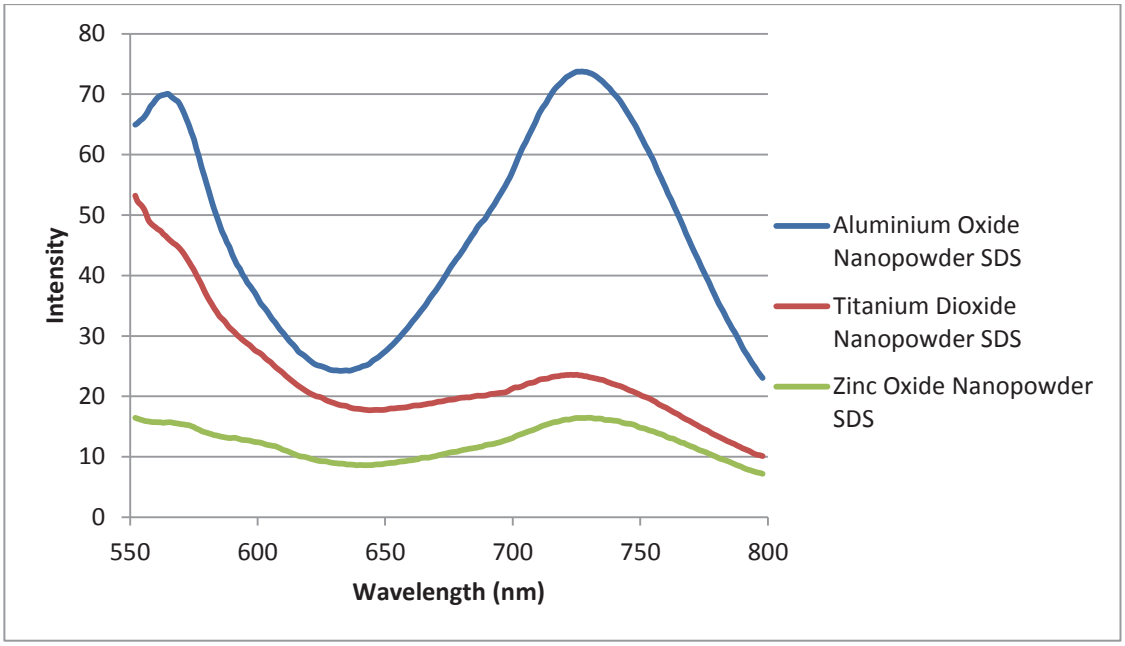


Figure 3-9: Emission spectra of STaR 11 coated nanopowders suspended in SDS (excitation 500 nm).

The STaR 11 dye solution was also added directly to commercial white Wet Powder™; however, the dye did not effectively coat the powder. Instead the organic dye solution and the powder suspension would separate into two layers. Developed marks were found to be poorly luminescent in both the visible and NIR regions; as a result, this was not explored further.

The next stage was to optimise the surfactant concentration. A range of different concentrations were trialled (0.1 M – 0.005 M) and the suspensions were applied to charged fingermarks to assess development. The higher concentration SDS samples tended to remove fingermarks from the surface, while the lower concentration samples failed to suspend the aluminium oxide and gave poorly developed fingermarks. From this, the optimal SDS concentration was determined to be 0.01 M. The final step in the surfactant optimisation was to determine the amount of aluminium oxide and STaR 11 (mixed with the 0.01 M SDS) required to develop luminescent fingermarks (Table 3-6).

Table 3-6: Al_2O_3 and STaR 11 optimisation experiments.

Suspension Number	Mass of Al_2O_3 (g)	Volume of 0.01 M SDS (mL)	Volume of STaR 11 (mL)
1	0.3	10	1
2	0.3	10	2
3	0.45	10	1
4	0.45	10	2
5	0.6	10	1
6	0.6	10	2
7	0.9	10	1
8	0.9	10	2

From this study, the main factor that determined the effectiveness of development was the mass of Al_2O_3. Lower mass suspensions gave very poor or no development for the majority of samples. The Al_2O_3 mass that gave the best development was 0.9 g; this gave high quality fingermarks for the majority of tapes tested. The volume of STaR 11 did have an effect on the luminescence strength, with the 2 mL samples exhibiting stronger NIR luminescence than the 1 mL samples. Based on these results suspension 8 was found to be the most effective in terms of development and NIR luminescence (Figure 3-10).

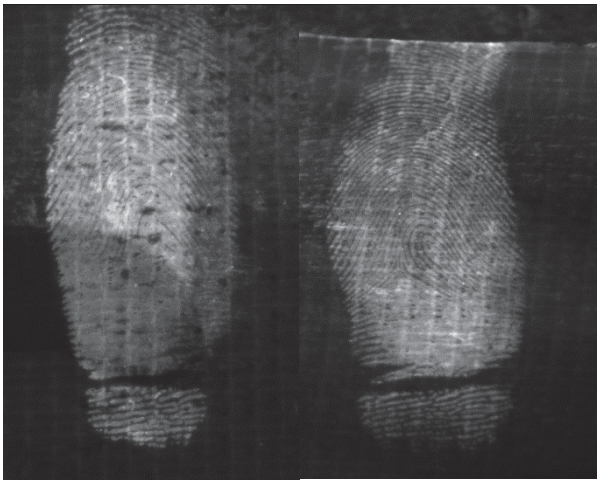


Figure 3-10: Fingermarks on gaffa tape developed with suspension 8 viewed in the luminescence mode (530 nm excitation, 700 nm barrier band pass filter) - (left) charged, (right) natural.

Once an optimised solution was determined, the final step was to look at development times for the adhesive tape samples. From Figure 3-11 and Figure 3-12 for the majority of samples a development time of 30 seconds gave the best overall development of samples regardless of the type of tape. This is shown in Figure 3-11 as a development time of 30 seconds gave the highest score for all tapes for a given development time. Shorter development times (5 – 10 seconds) resulted in poor ridge detail and poor luminescence. Development times of one minute, in some cases gave a high amount of background staining due to the difficulty in completely removing the suspension from the adhesive surface. Masking and packing tape gave very poor results regardless of development time; therefore, these were not considered. From Figure 3-12 it can be seen that cloth, duct and gaffa tape were the only tapes which show significant variability between the development times and at 30 seconds this gave the best development and luminescence of all the development times trialled.

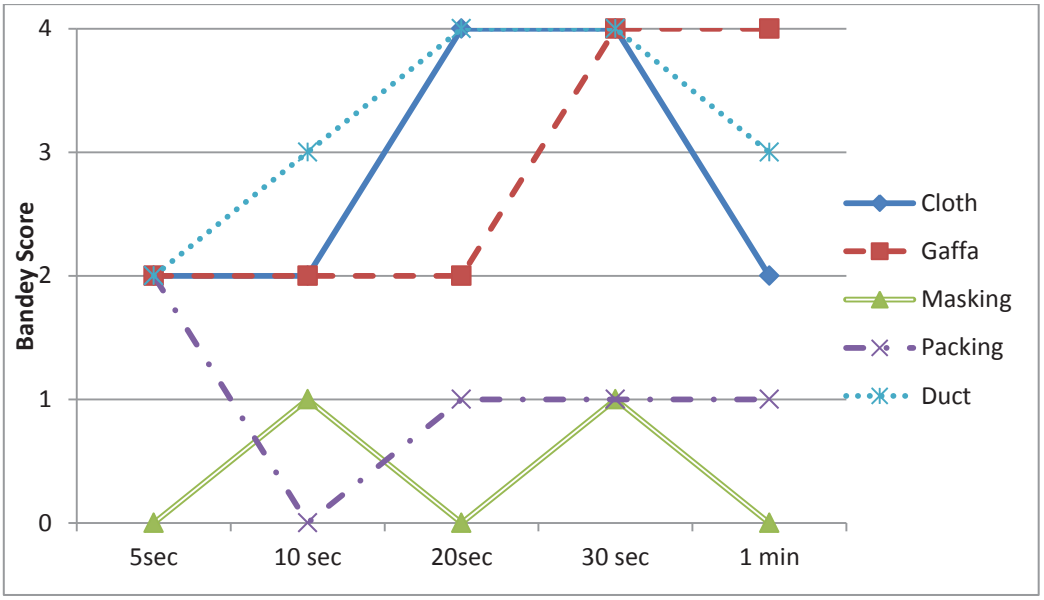


Figure 3-11: Development time results for STaR 11 SSP using the Bandey scale.

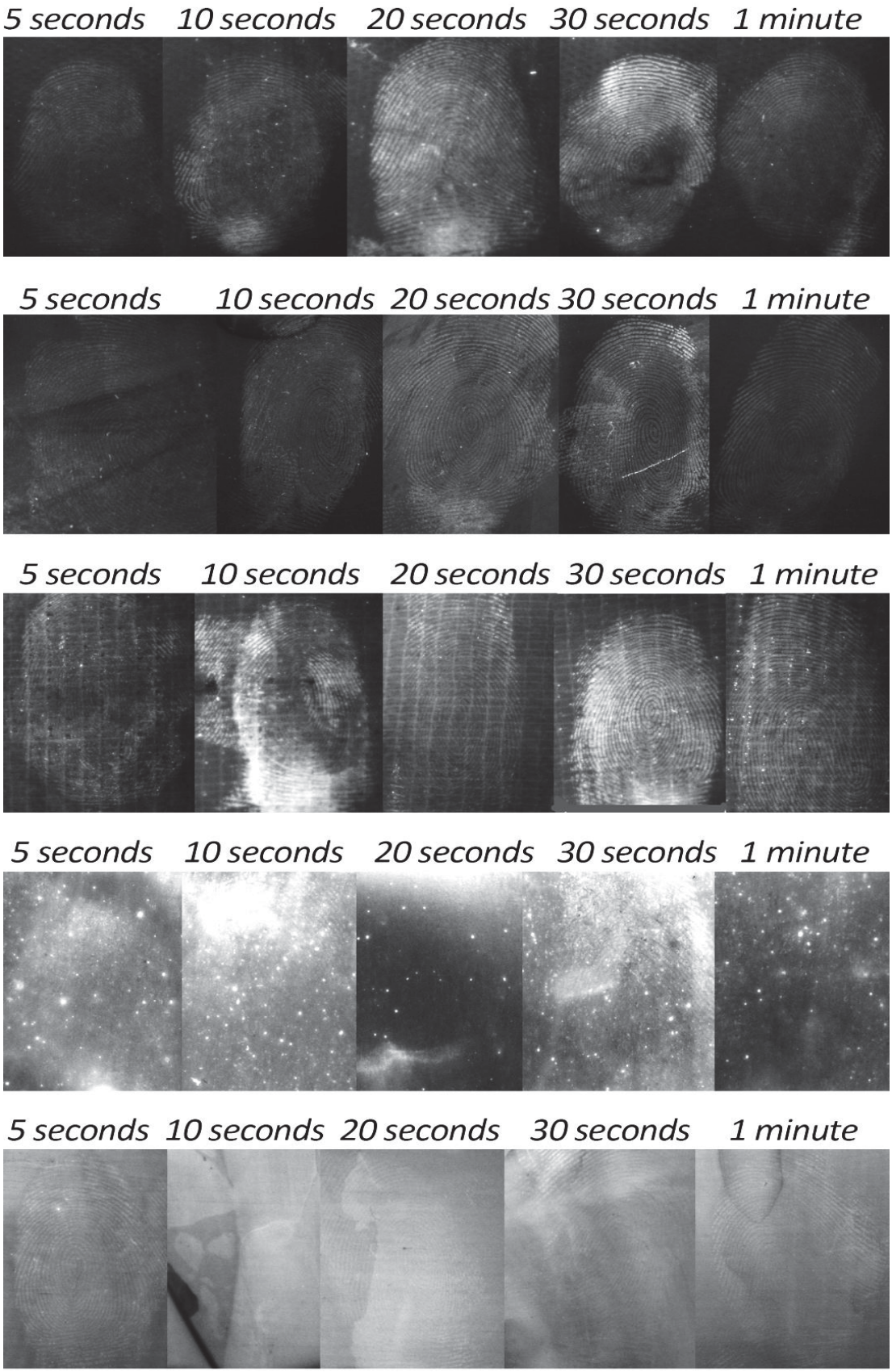


Figure 3-12: Optimisation of development time for STaR 11 SSP visualised in the luminescence mode 530 nm excitation, 700 nm barrier band pass filter (top – bottom) cloth, duct, gaffa, masking, packing.

3.5.2 Donor Study

3.5.2.1 Cloth tape

When compared to the commercial sticky side powder, STaR 11 SSP performed poorly on samples that were not fresh. This is reflected by the majority of negative values for samples aged from one day to one month (Figure 3-13). A comparison between stuck down, exposed samples, charged and natural marks indicated that there was no consistent trend between them. The only noticeable difference between the stuck down and exposed samples was stronger background development from the stuck down samples. This was observed for samples developed with Wet Powder™ aged for greater than three days (Figure 3-14, Figure 3-15); however, while there was a reduction in contrast, it did not significantly affect the scores. Similarly, there was no significant difference between male and female donors. This result would be expected as the fingermark components are less likely to degrade when stuck down, due to the fingermark deposits being protected from environmental damage, therefore giving similar target components to the fresh sample.

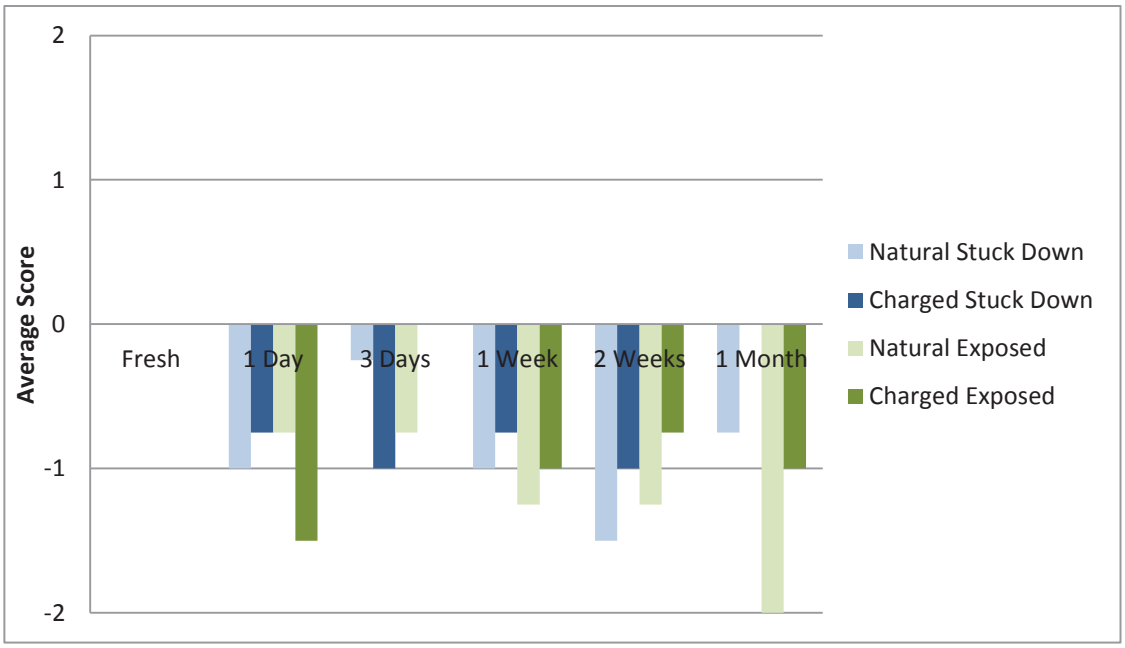


Figure 3-13: Comparison study results for all donors on cloth tape (average McLaren scale values indicated).

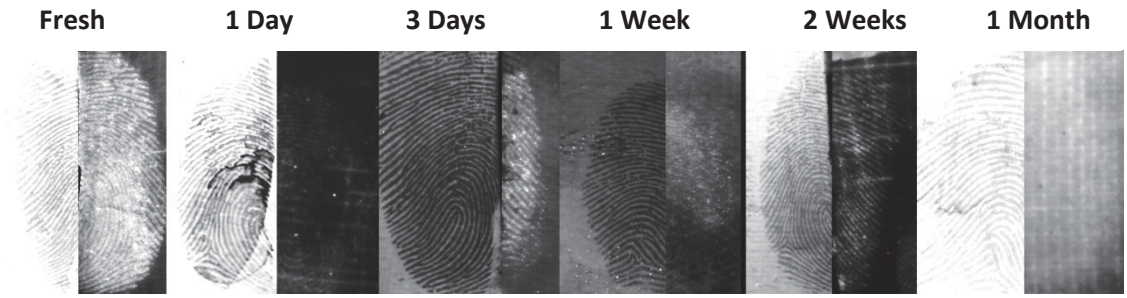


Figure 3-14: Representative charged fingermarks that were exposed to the environment (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a male donor on cloth tape.

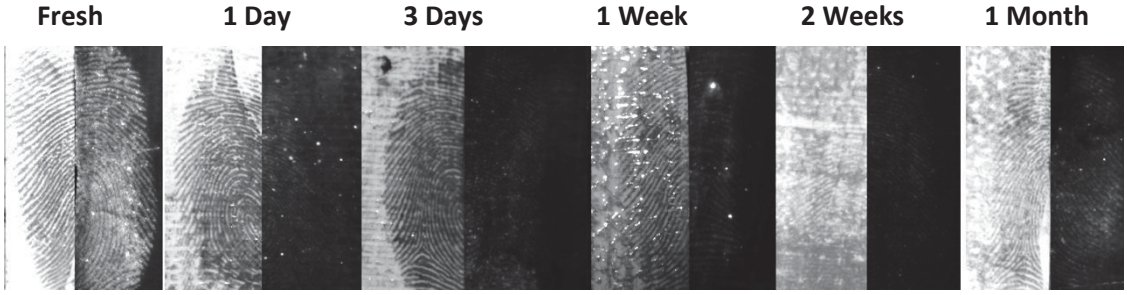


Figure 3-15: Representative charged fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a male donor on cloth tape.

An examination of the zero scores for this surface (Figure 3-16) indicate that there were no samples where neither technique could develop a fingermark and very few samples that gave poor development. This would indicate that the commercial technique is more than adequate for developing fingermarks on this surface. There were a large number of same development scores for this surface even up to one month. This would indicate that STaR 11 SSP performed slightly better than the results indicated in Figure 3-13, as marks could be developed of a similar quality to the commercial product. There was also no significant difference between samples that were exposed to the environment or those that were stuck down.

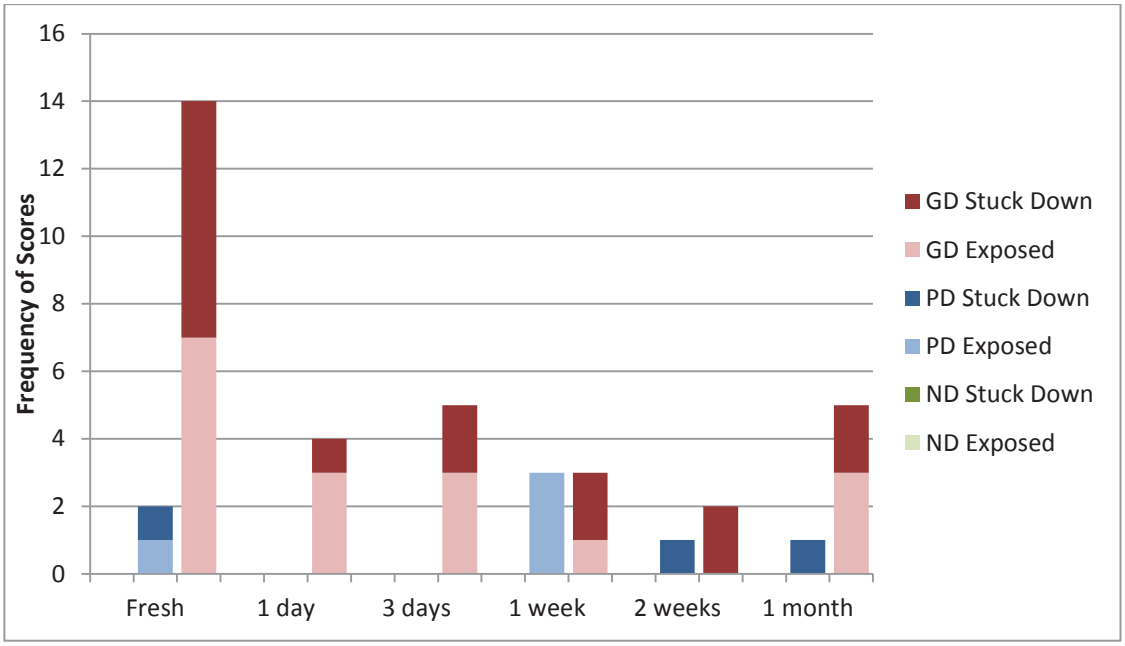


Figure 3-16: Classification of zero values for all donors on cloth tape (GD = good development, PD = poor development, ND = no development).

3.5.2.2 Duct tape

Of all the tapes trialled, duct tape gave the most promising results with the STaR 11 SSP, particularly for the samples that had been exposed to the environment. For these samples, STaR 11 performed consistently better than the commercial technique, for samples older than one day (Figure 3-17). When samples were exposed for longer than one day, there was a noticeable difference in the adhesive on the tape; it became glossier and more liquefied compared to the original matte appearance. This potentially is due to the different type of adhesive used - a rubber based adhesive that has poorer ageing characteristics may have been used on this tape, compared to conventional acrylic and block copolymer adhesives that have better ageing characteristics [118]. This change had an effect on the ability of Wet Powder™ to develop marks, which gave significantly more background development and lower contrast fingermarks. Conversely, STaR 11 SSP tended to only develop the fingermark with very little background staining (Figure 3-18). For samples that were stuck down the difference was not as obvious with a large number of STaR 11 samples (≈40 %) performing slightly worse than Wet Powder™. In very few cases did STaR 11 provide any increase in development quality (Figure 3-19).

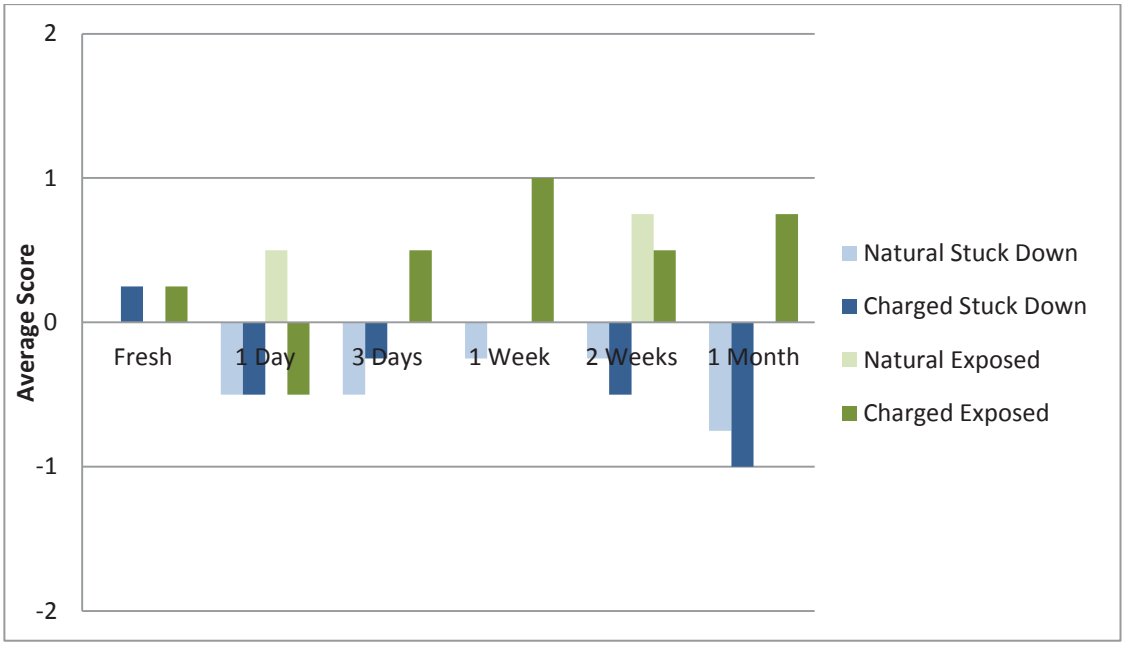


Figure 3-17: Comparison study results for all donors on duct tape(average McLaren scale values indicated).

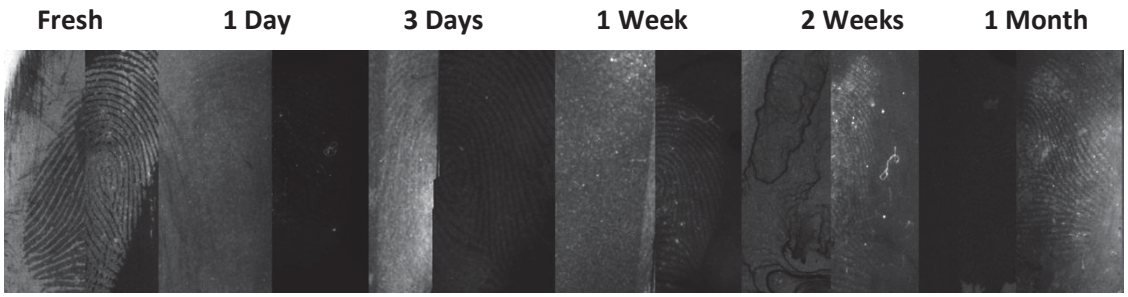


Figure 3-18: Representative charged fingermarks that were exposed to the environment (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a female donor on duct tape.

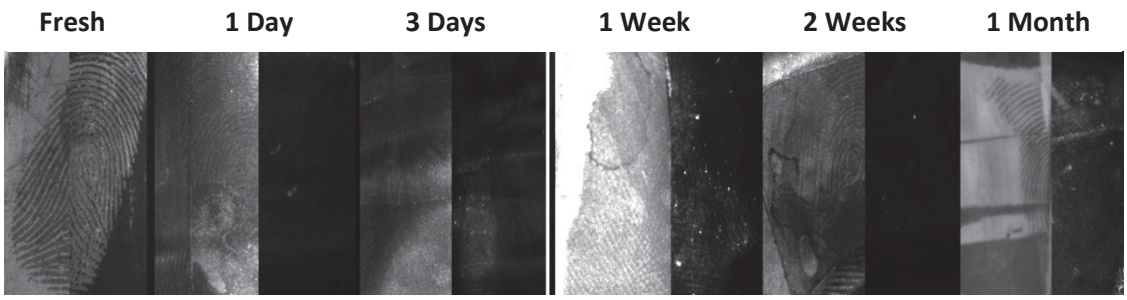


Figure 3-19: Representative charged fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a female donor on duct tape.

The zero score data from this surface (Figure 3-20) reinforces the significant difference in development between the samples that were exposed to the environment and those that were stuck down. This is shown by the higher number of poor and no development scores after one week for stuck down samples, in which both techniques performed similarly. Conversely, the exposed samples had very few zero scores, mainly due to the degradation of the adhesive for exposed samples causing an increase in background development for the Wet Powder™ developed fingermark samples. As with most tapes in this study fresh samples tended to give the highest number of good development scores.

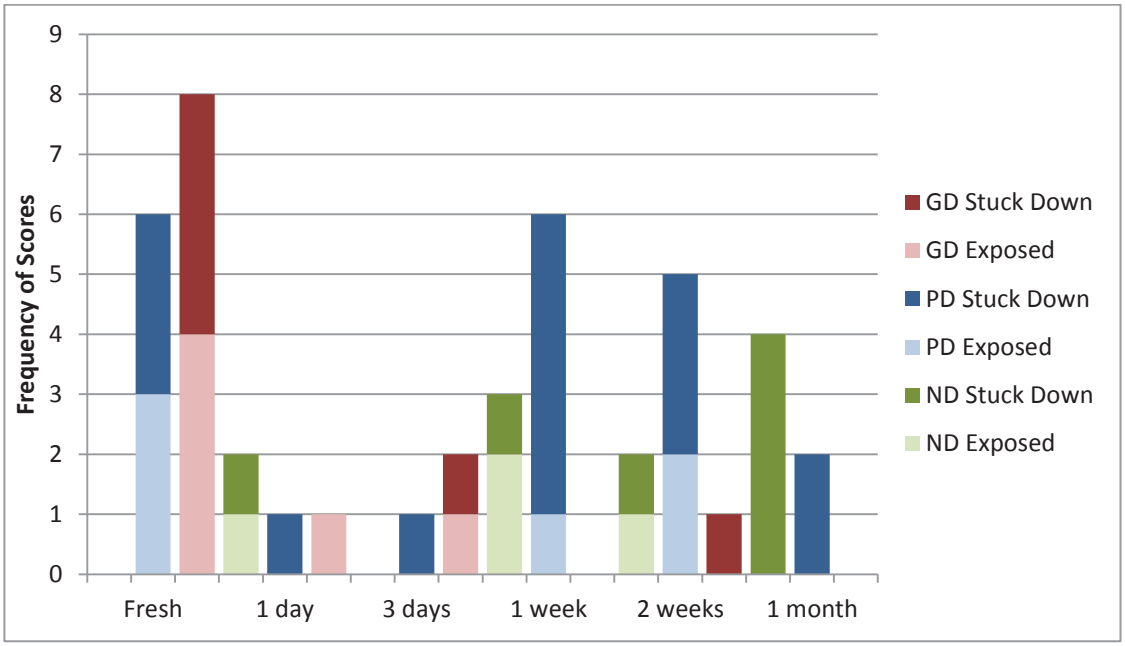


Figure 3-20: Zero values for all donors on duct tape (GD = good development, PD = poor development, ND = no development).

3.5.2.3 Gaffa tape

Gaffa tape is a common adhesive tape used for a variety of purposes. The texture of gaffa tape is very similar to cloth tape. This similarity in surface characteristics was reflected in the comparison study with results following a similar trend to cloth tape (Figure 3-21). Wet Powder™ performed better on the majority of samples for both exposed and stuck down samples. There was no significant difference between male and female donors or charged and natural marks; there was also no consistent increase or decrease in quality over the ageing period (Figure 3-22, Figure 3-23). Similarly to cloth tape, STaR 11 developed marks tended to perform better on the one month aged samples that were stuck down. However, the fact that the scores were not consistent throughout the ageing period would indicate that there is some loss or degradation of fingermark components even without being exposed to the environment.

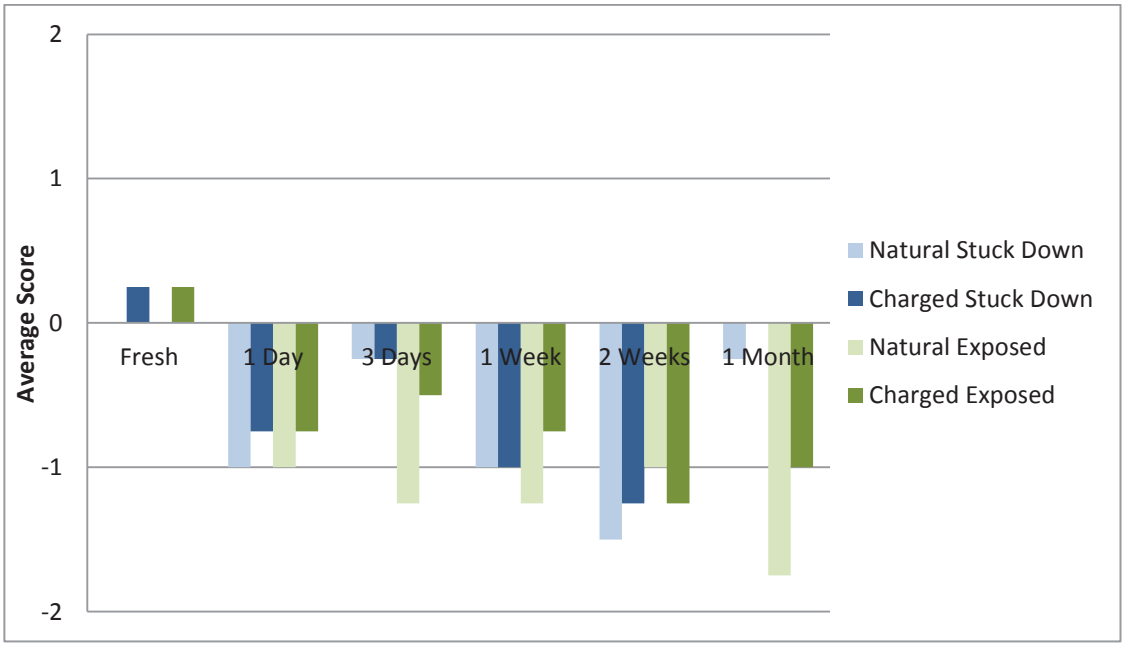


Figure 3-21: Comparison study results for all donors on gaffa tape (average McLaren scale values indicated).

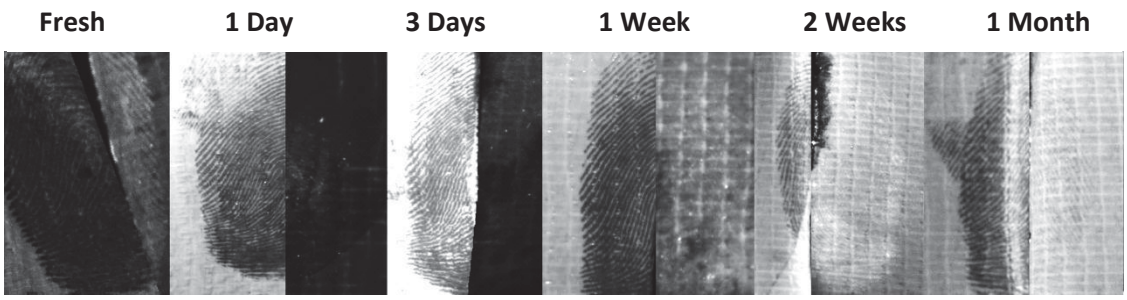


Figure 3-22: Representative natural fingermarks that were exposed to the environment (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a male donor on gaffa tape.

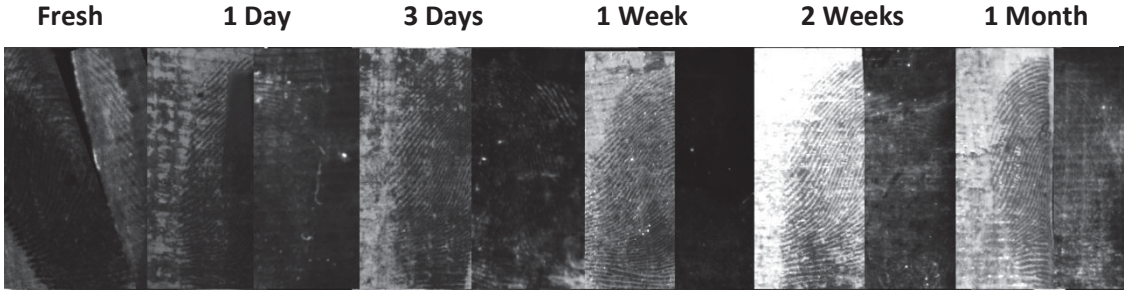


Figure 3-23: Representative natural fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a male donor on gaffa tape.

Similarly to cloth tape there were no instances where either technique did no develop a fingermark and very few samples that gave poor development (Figure 3-24). The majority of zero values came from both techniques providing the good development. There was no significant difference between samples that had been exposed to the environment or stuck down nor was there any discernible relationship between the age of the sample and the quality of development.

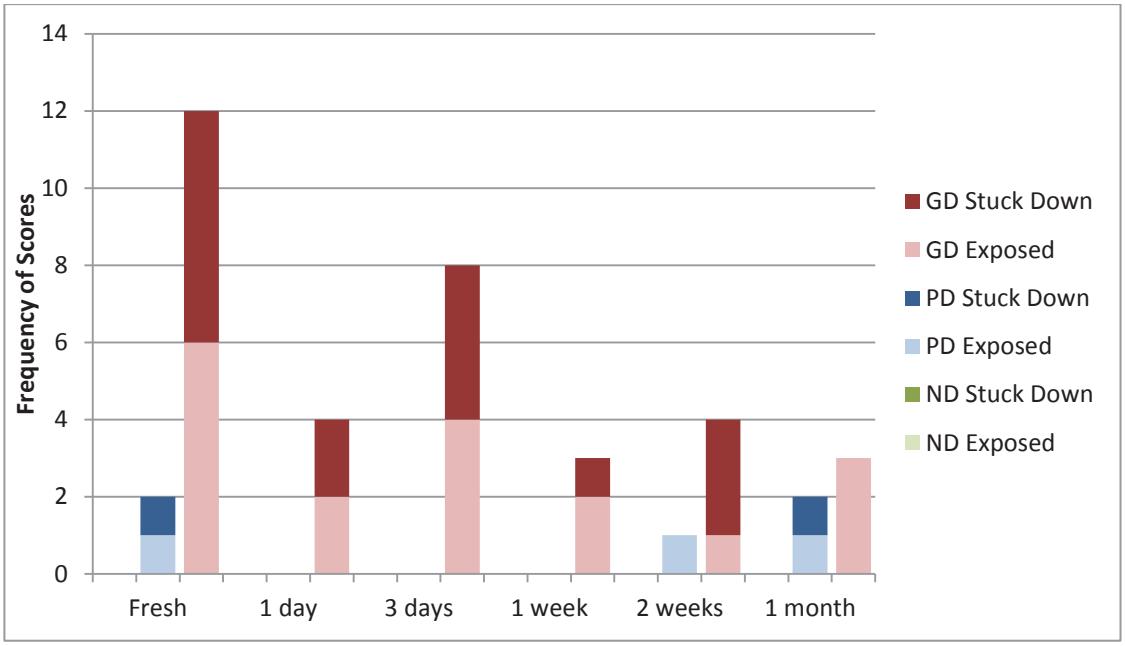


Figure 3-24: Classification of zero values for all donors on gaffa tape (GD = good development, PD = poor development, ND = no development).

3.5.2.4 Masking tape

Masking tape is a commonly used semiporous adhesive tape from which it can be difficult to recover fingermarks. The STaR 11 suspension performed very poorly on this surface when compared to Wet Powder™. Generally, the luminescent dye would seep into the surface and give high levels of background staining. Potentially the fingermark secretions had absorbed deeper into the surface than with other tapes and, as a result, the STaR 11 suspension was not sensitive enough to detect it; this was reflected in the scores for STaR 11 (Figure 3-25). Wet Powder™ was able to develop marks effectively throughout the entire aging period, particularly for the older samples (Figure 3-27, Figure 3-28).

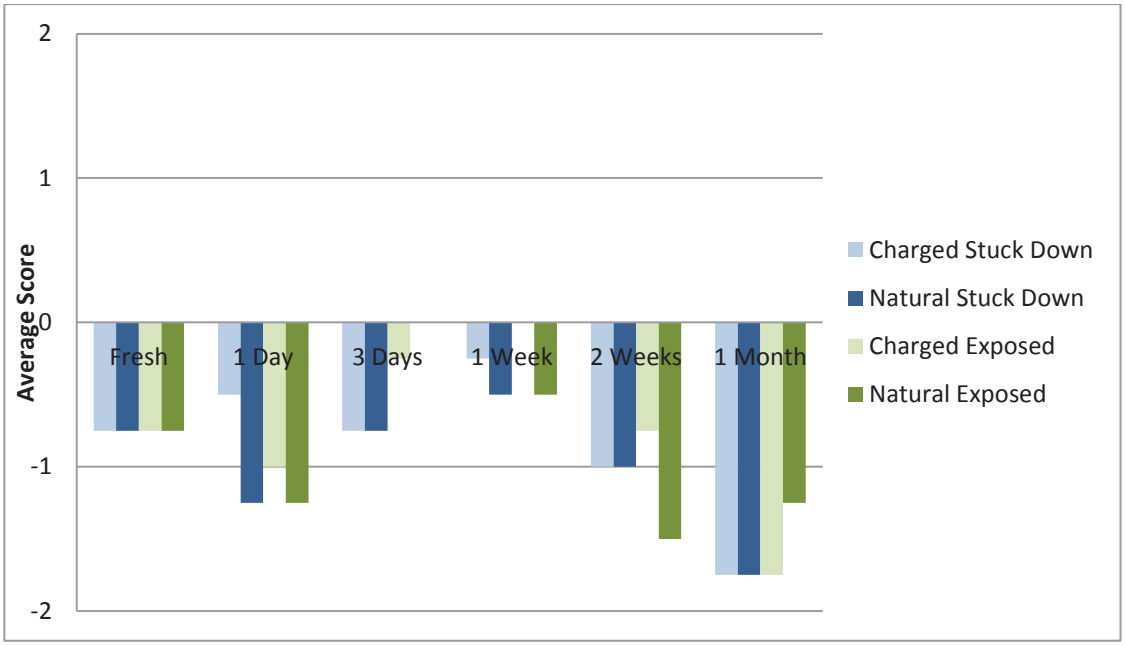


Figure 3-25: Comparison study results for all donors on masking tape (average McLaren scale values indicated).

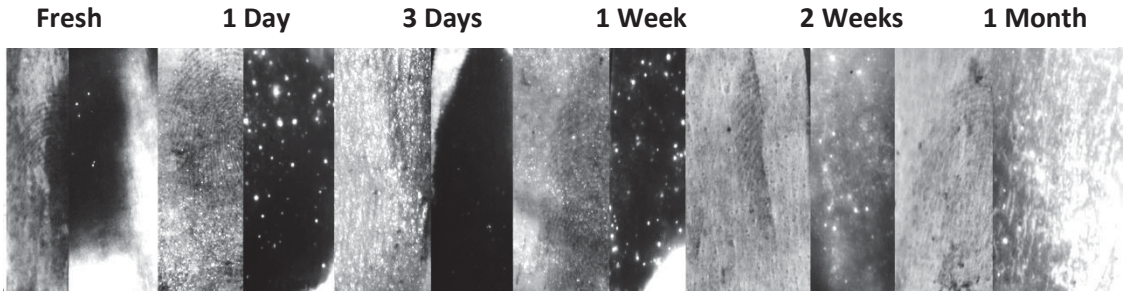


Figure 3-26: Representative natural fingermarks that were exposed to the environment (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a female donor on masking tape.

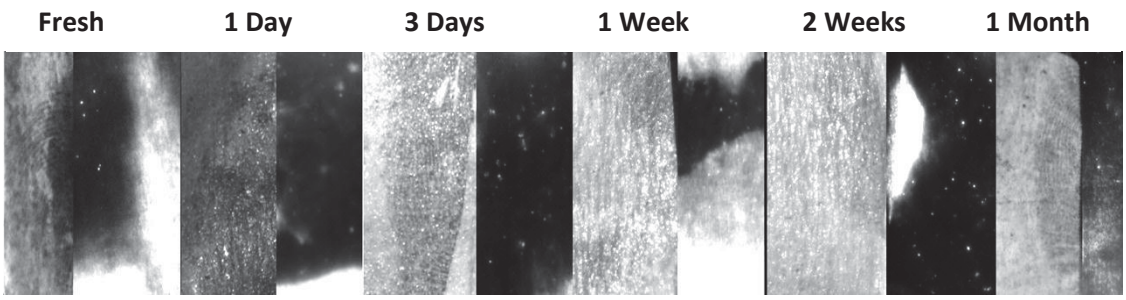


Figure 3-27: Representative natural fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from a female donor on masking tape.

The zero values from this surface indicate that there were very few instances where both techniques gave the same quality of development. Of all the tapes tested, this was also the tape that gave the highest number of no development scores with 25% of all samples tested giving no development (Figure 3-28). The number of no development scores tended to increase up to the one week samples, after which they dropped off dramatically. This coincided with the increase of negative values for the two week and one month samples, which indicates that STaR 11 is not a suitable technique for this surface. However, this result reinforces the issue with developing marks on this surface as the commercial technique failed to develop a quarter of all samples tested. As such, alternate methods of detection need to be explored for this substrate in order to maximise the number of recovered marks.

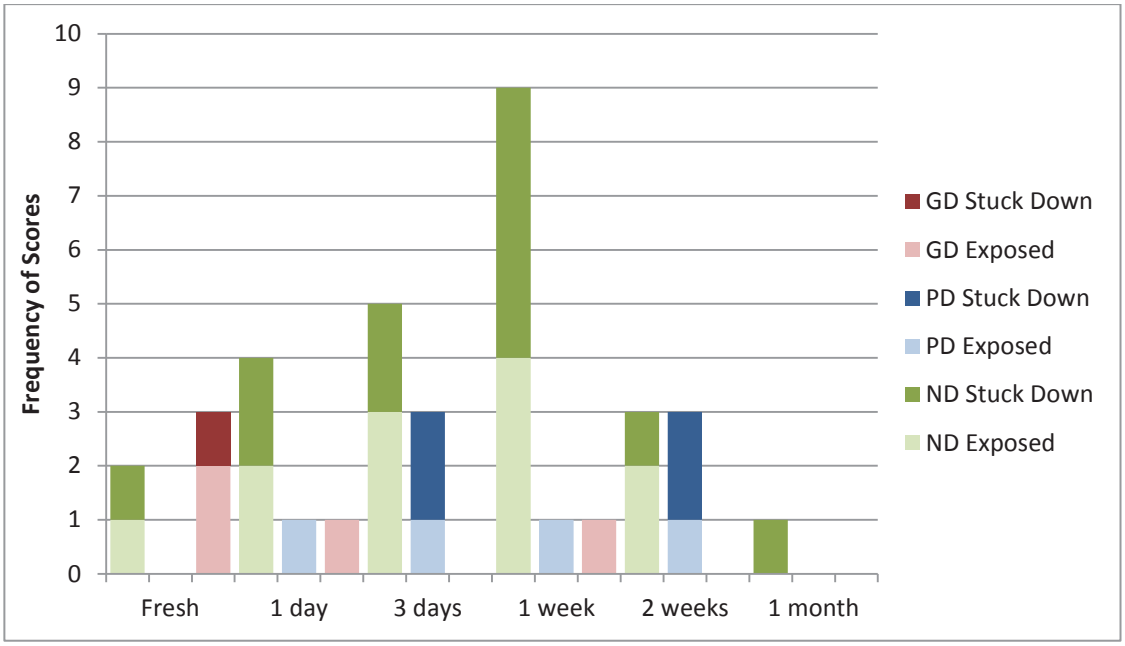


Figure 3-28: Classification of zero values for all donors on masking tape (GD = good development, PD = poor development, ND = no development).

3.5.2.5 Packing tape

Similarly to masking tape, this surface proved to be very difficult for STaR 11 SSP to develop fingermarks on, but for different reasons. In all cases, Wet Powder™ outperformed STaR 11 (Figure 3-29); this may be due to the surface of the tape, which is smooth and glossy. There were no cases where STaR 11 performed better than Wet Powder™ and in very few cases (<10 %) did STaR 11 develop marks of the same quality. Since STaR 11 is not as viscous as Wet Powder™ it would be more likely to wash marks off the surface; this is shown by the brush strokes present on some of the samples indicating that the fingermarks were most likely brushed off as a result of the STaR 11 treatment. There was a slight difference in the values for stuck down and exposed samples (Figure 3-30, Figure 3-31); samples that were stuck down gave the highest number of negative values and very few zero scores. Based on these results, STaR 11 SSP could not be used on this surface as no fingermarks with sufficient detail could be developed.

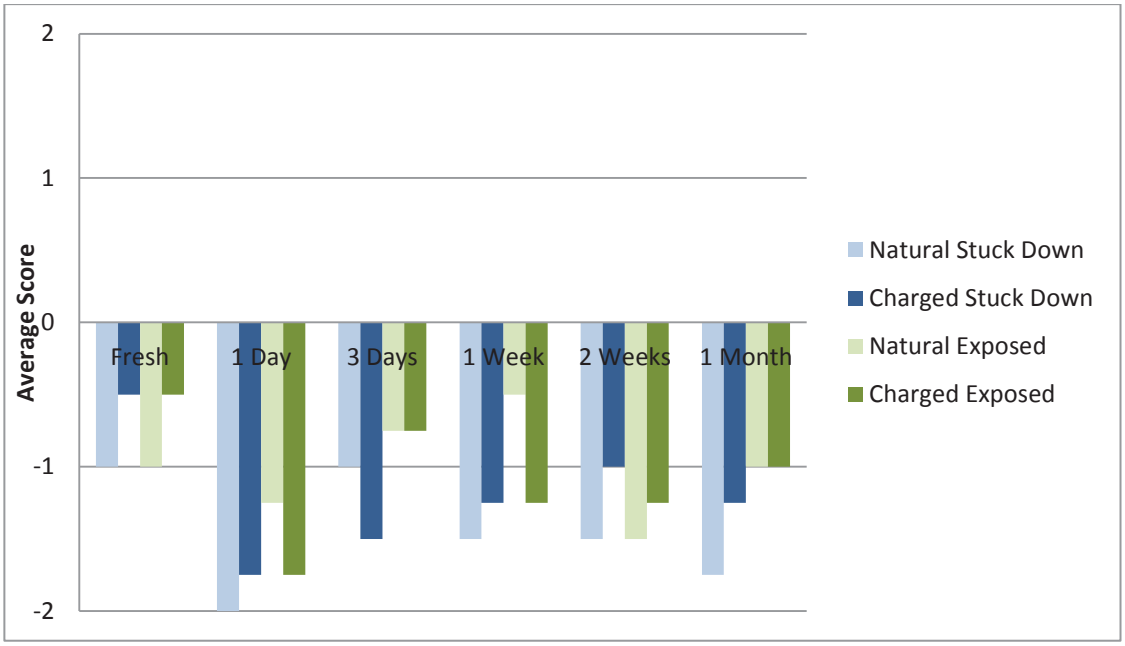


Figure 3-29: Comparison study results for all donors on packing tape (average McLaren scale values indicated).

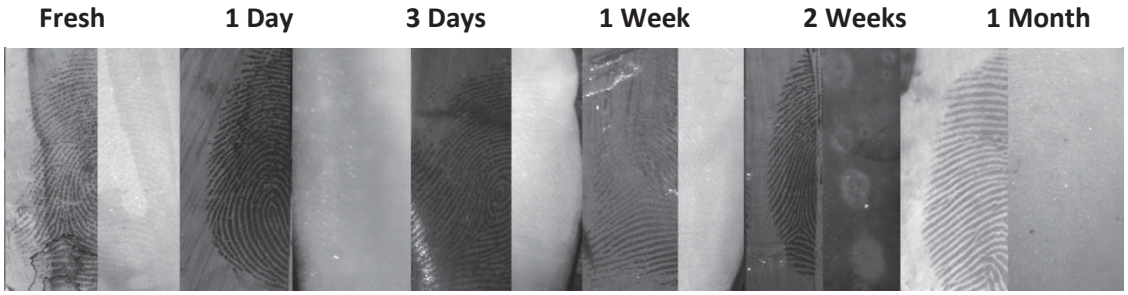


Figure 3-30: Representative natural fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from male donor on packing tape.

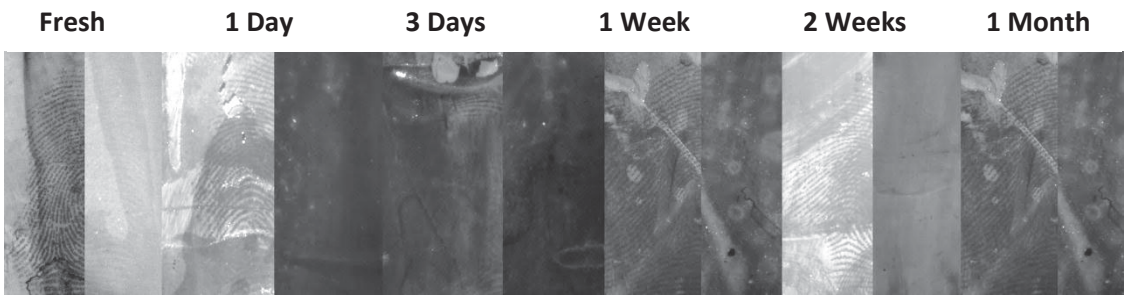


Figure 3-31: Representative natural fingermarks that were stuck down (developed with LHS Wet Powder™ viewed under white light, RHS STaR 11 viewed in luminescence mode excitation 530 nm, 700 nm barrier band pass filter) from male donor on packing tape.

An examination of the zero values firstly indicate that there were very few samples that gave a zero value (Figure 3-32). Combined with the previous data, this reaffirms that Wet Powder™ out-performed STaR 11 SSP for the majority of samples. There was no significant difference between samples that were exposed to the environment and the samples stuck down and no strong trend to indicate that age of the fingermark affected the quality of development.

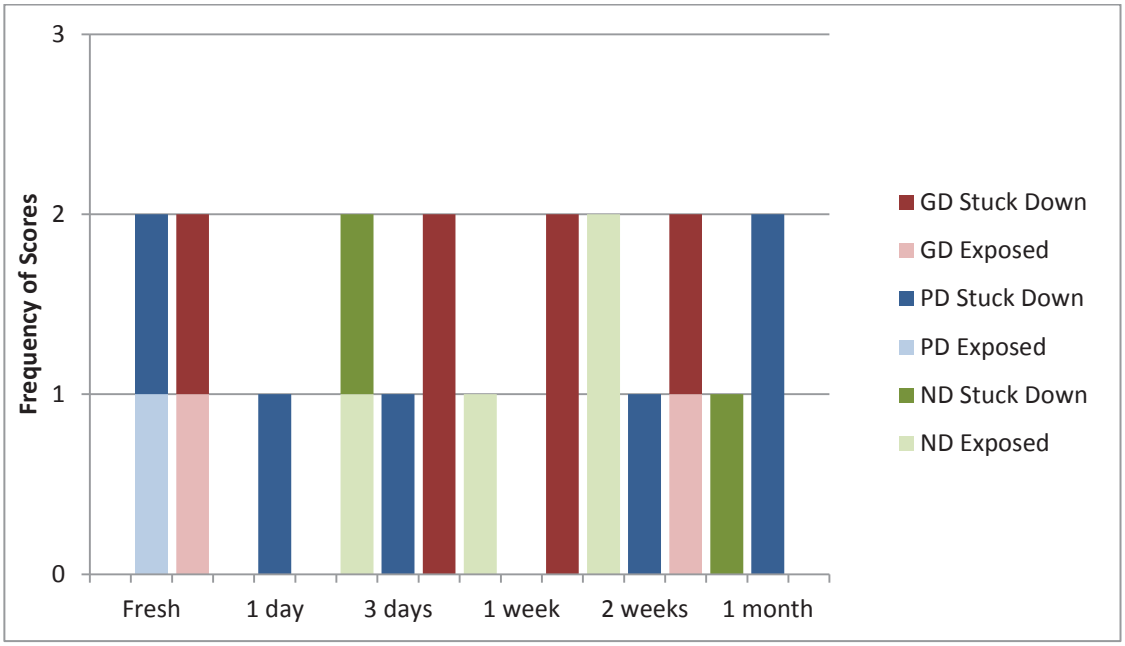


Figure 3-32: Classification of zero values for all donors on packing tape (GD = good development, PD = poor development, ND = no development).

3.6 Results - Small Particle Reagent

3.6.1 Surfactant Optimisation for STaR 11 Small Particle Reagent

Based on the previous work, STaR 11 suspensions (STaR 11 coated aluminium oxide with SDS as a surfactant) were optimised for use as a small particle reagent (SPR). When the STaR 11 sticky side powder formulation was used as an SPR, fingermarks were washed away; therefore surfactant, STaR 11 concentration and the amount of aluminium oxide had to be modified. Initial surfactant and dye tests were performed using the fluorescence spectrometer to see the effect of surfactant concentration on the luminescence of the dried powder. A control suspension was prepared from previous experiments and different SDS concentrations were compared against this suspension (Table 3-7). Surfactant solutions were diluted with deionised water, before the addition of STaR 11 to give a final volume.

Table 3-7: Surfactant concentration experiment.

	Control Suspension	**Suspension 1**	**Suspension 2**	**Suspension 3**	**Suspension 4**
Volume of 0.1 M SDS (mL)	10	10	10	10	10
Diluted Volume (mL)	70	200	100	50	20
Concentration of SDS (M)	0.0143	0.005	0.01	0.02	0.05
Volume of STaR 11 (mL)	12.5	12.5	12.5	12.5	12.5
Final Volume (mL)	82.5	212.5	112.5	62.5	32.5

Based on these results (Figure 3-33), it was found that an SDS concentration of 0.02 M gave the strongest luminescence emission in the visible region. None of the suspensions trialled gave any significant NIR luminescence emission; this indicated that either the higher concentration of water significantly decreased the quantum yield of styryl 11 or there was

not enough STaR 11 in the original solution in order to effectively coat the powder. This was the basis of the second experiment, which examined the amount of STaR 11 in the solution and the effect this had on luminescence.

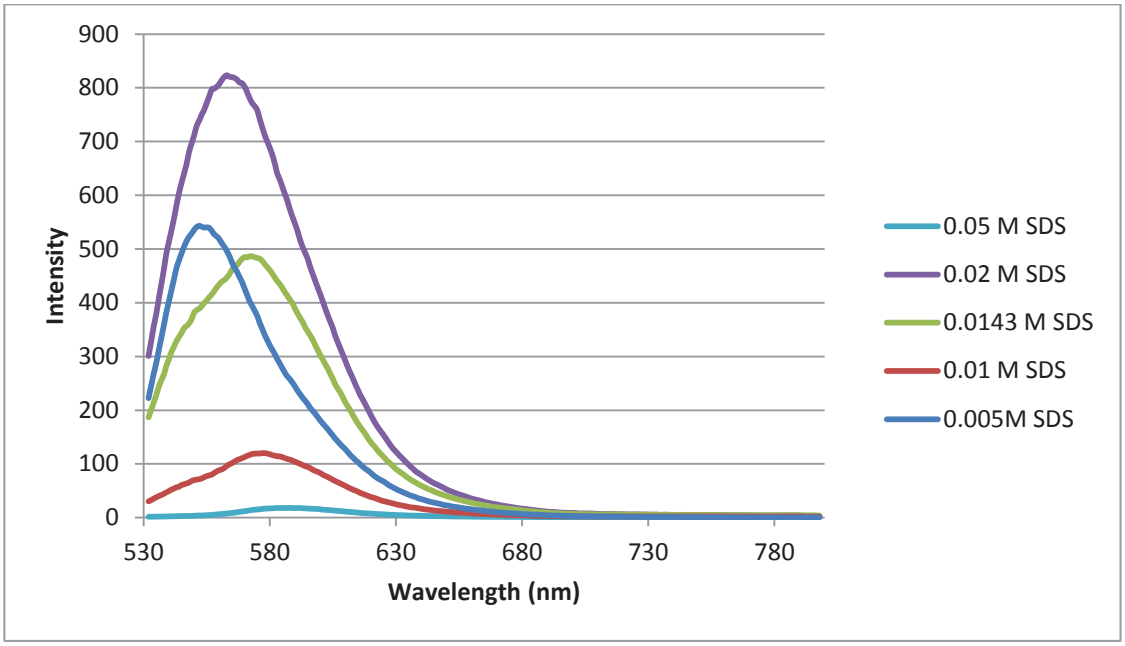


Figure 3-33: STaR 11 SPR - SDS concentration experiment.

In this experiment the surfactant concentration and aluminium oxide mass was kept constant (10 mL 0.02 M SDS, 2 g of Al_2O_3), with varying amount of STaR 11 added to the solution (Table 3-8). The suspensions were left to dry overnight and then luminescence data obtained from the dried powders.

Table 3-8: STaR 11 SPR- STaR 11 concentrations.

10 mL 0.02 M SDS	SPR 1	SPR 2	SPR 3	SPR 4	SPR 5
Volume of STaR 11 (mL)	6.00	2.00	1.00	0.50	0.25
Final Volume (mL)	16.00	12.00	11.00	10.50	10.25
Styryl 11 Concentration (M)	8.73×10^{-4}	3.88×10^{-4}	2.09×10^{-4}	1.10×10^{-4}	5.68×10^{-5}
Rhodamine 6G Concentration (M)	3.13×10^{-3}	1.39×10^{-3}	7.59×10^{-4}	3.98×10^{-4}	2.04×10^{-4}

The results from this experiment (Figure 3-34) indicated that the amount of STaR 11 had a significant effect on both the visible and NIR emission intensity, where the lower concentration samples resulted in strong visible and very weak NIR luminescence, whilst the contrary was found for the higher concentration samples. In order to compensate for this, the addition of 1 mL STaR 11 per 10 mL of 0.02 M SDS was chosen as the optimal STaR 11 concentration as this provided the best compromise between visible and NIR luminescence as well as minimising the amount of dye required.

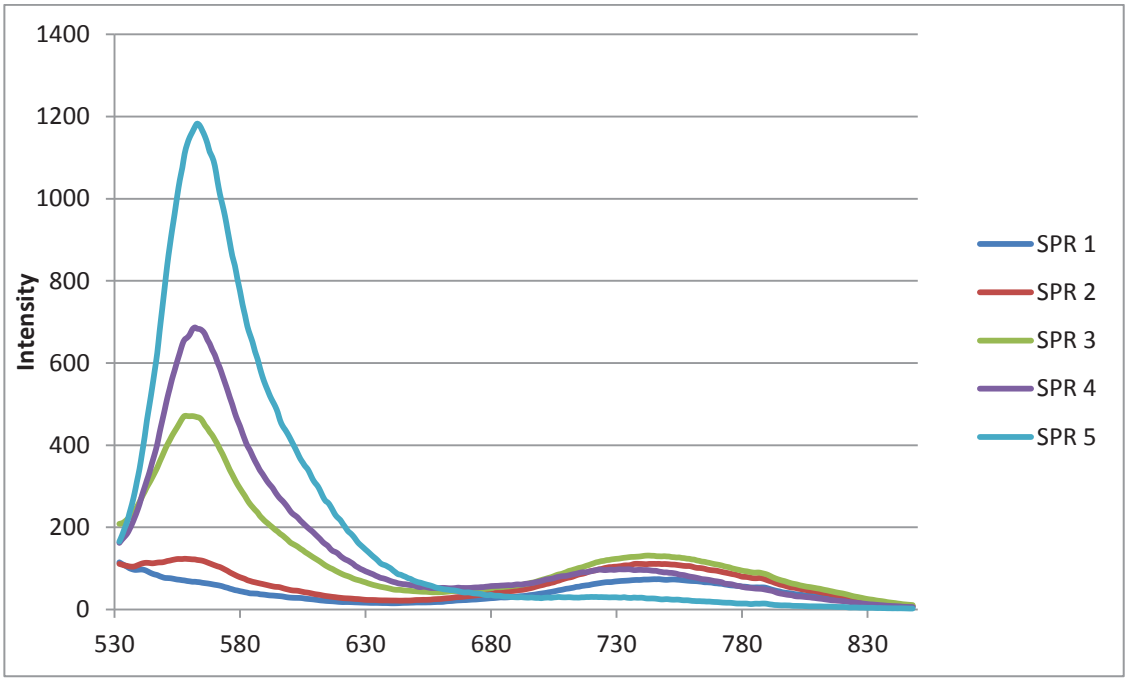


Figure 3-34: STaR 11 SPR - STaR 11 concentration luminescence emission spectra

3.6.2 Further Optimisation of STaR 11 SPR for Fingermark Development

The optimised suspension from the previous experiment was applied to charged marks on glass in an attempt to see if development was possible with these optimised conditions. However, using a 0.02 M solution resulted in fingermarks being washed away. Since luminescence data could only provide information on the luminescence strength and not on

the quality of development, it was decided that further experimentation would focus on the ability of the powder to develop fingermarks. As a result, another set of experiments had to be devised (Table 3-9). Using a control suspension several different parameters were altered and their effect on fingermark development was determined.

Table 3-9: STaR 11 multivariate experiment.

	Control	**SPR 1**	**SPR 2**	**SPR 3**	**SPR 4**	**SPR 5**	**SPR 6**
Volume of 0.02M SDS (mL)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Diluted Volume (mL)	80.0	**100**	**50**	80.0	80.0	80.0	80.0
Mass of Al_2O_3 (g)	1.50	1.50	1.50	**1.00**	**2.00**	1.50	1.50
Volume of STaR 11 (mL)	12.5	12.5	12.5	12.5	12.5	**5.00**	**20.0**
Final Volume (mL)	**92.5**	**112.5**	**62.5**	**92.5**	**92.5**	**85.00**	**100**

The transmission microscopy photos indicated that the factors that contributed significantly to the quality of development were the concentration of SDS and the amount of aluminium oxide present in the suspension. Lower SDS concentrations gave poor development compared to the control suspension (Figure 3-35a) with inconsistent ridge detail. The higher concentration SDS suspension did not exhibit any signifcant difference to the control suspension. The lower Al_2O_3 mass suspension (Figure 3-35c) was able to develop ridges effectively however the developed ridges had very poor contrast, whereas the higher Al_2O_3 mass suspensions was able to develop clear ridges with tertiary level detail and high contrast (Figure 3-35d). The amount of STaR 11 in the suspension did not appear to affect the development of fingermarks.

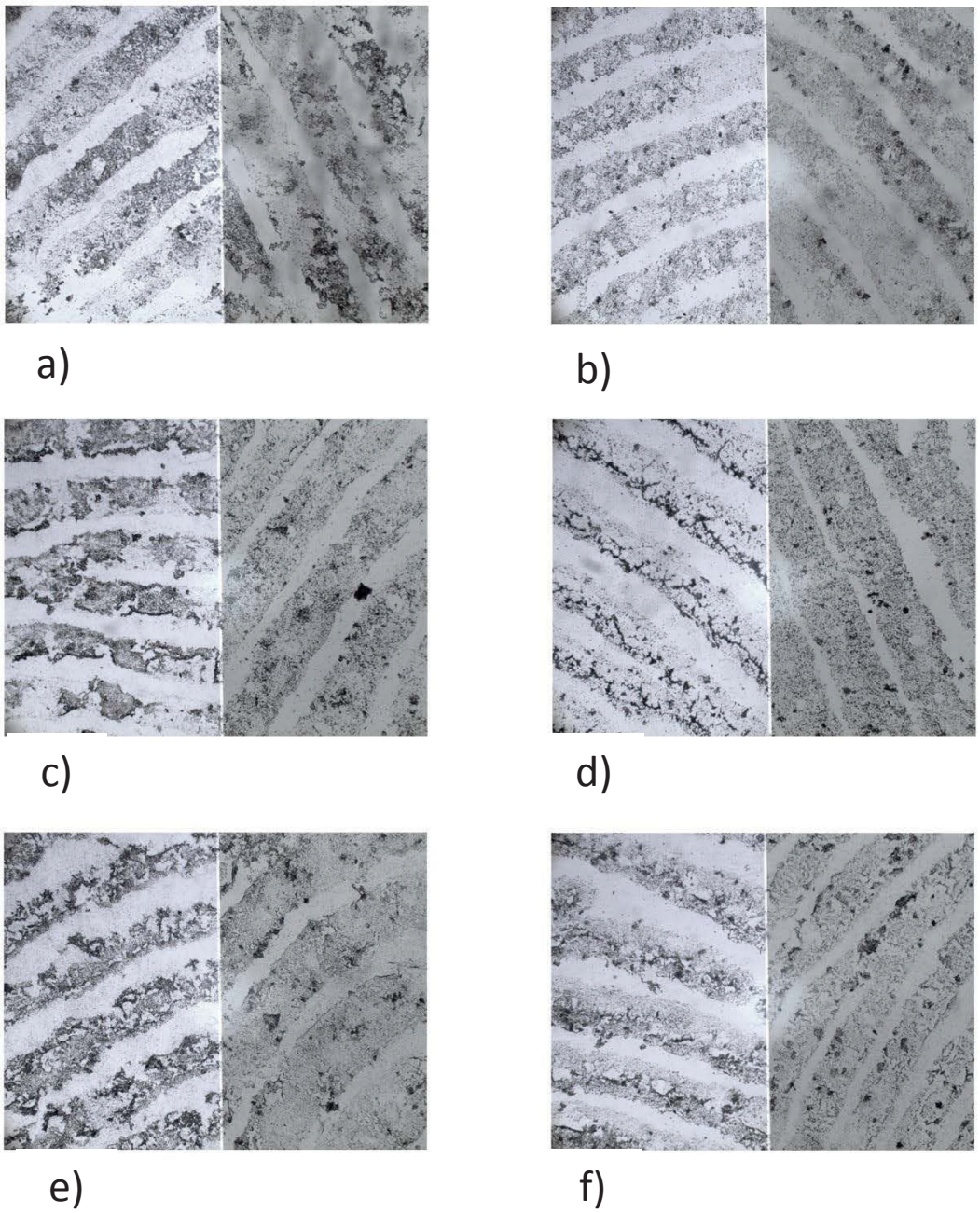


Figure 3-35: Transmission microscope photographs of STaR 11 developed samples, a) (left) control, (right) SPR 1, b) (left) control, (right) SPR 2, c) (left) control, (right) SPR 3, d) (left) control, (right) SPR 4, e) (left) control, (right) SPR 5, f) (left) control, (right) SPR 6.

The results from the white light examination of developed fingermarks indicated that, for all three parameters tested, less than the control concentrations gave poorer results (Figure 3-36), indicating that the control suspension trialled gave the best results. SPR 1 gave very weak development and poor contrast as well as some degree of background development. The developed marks were also a paler pink colour compared to the control (Figure 3-36a).

SPR 2 gave comparable results to the control solution, reaffirming that increasing the SDS concentration did not have a major effect on development. SPR 3 gave the poorest development of fingermarks under white light, with very low contrast and minimal ridge detail. It should also be noted that SPR 4 (higher Al_2O_3 mass) gave the same degree of background development, which partially obscured the fingermark (Figure 3-36d). SPR 5 gave the weakest contrast and this would be expected as this was the solution that had the lowest STaR 11 concentration (Figure 3-36e). There was no significant difference between the control SPR and SPR 6.

The final stage of comparison for this study was to look at the luminescence in the NIR. Interestingly, the concentration of SDS did not significantly affect the strength of the luminescence, which is contrary to the results previously obtained. This would indicate that the concentrations tested in this experiment were too low to exhibit any effect on the NIR luminescence. In the case of Al_2O_3 mass, while there was a significant difference in luminescence between SPR three and four this would mainly be due to poor development of the sample as previously mentioned. As expected, the concentration of STaR 11 did have an effect on the luminescence, with the lower concentration samples giving weaker luminescence compared to both the control and higher concentration samples (Figure 3-37e).

An examination of this data determined that the major factor that would affect the quality of development was the mass of Al_2O_3 in the suspension. As a result higher Al_2O_3 concentration suspensions were trialled (Table 3-10).

As the mass of aluminium oxide was increased, so too did the amount of background development; this is seen in Figure 3-38c and Figure 3-38d, which have clearly developed fingermarks but also a significant amount of background powdering is also present. Secondly, the amount of aluminium oxide did not have an effect on the luminescence, indicating that the concentration of STaR 11 was sufficient to coat the powder. The final conclusion was that the control suspension (2 g of aluminium oxide) gave very consistent development throughout the experiment and it produced minimal background powdering.

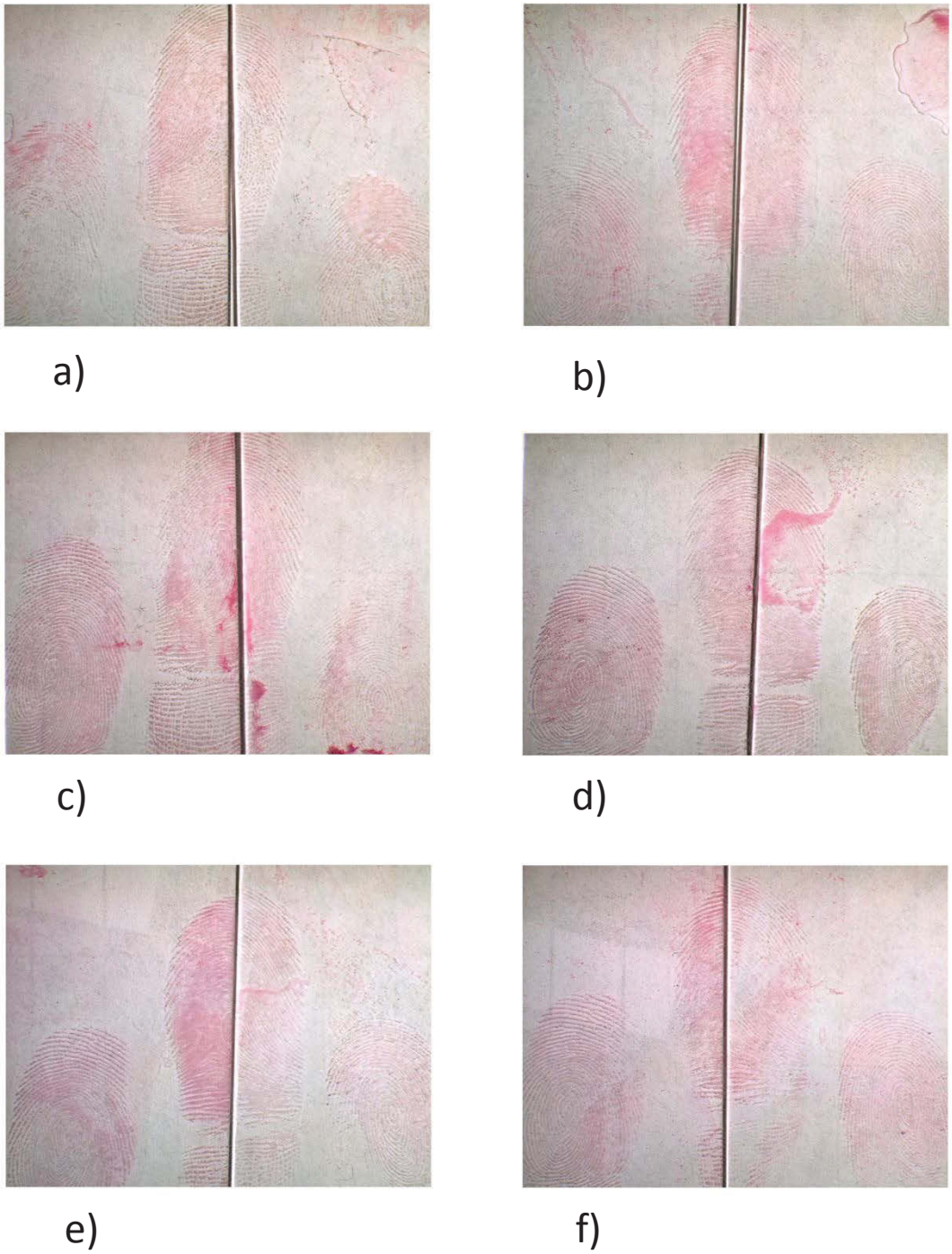


Figure 3-36: White light photographs of STaR 11 developed samples - a) (left) control, (right) SPR 1, b) (left) control, (right) SPR 2, c) (left) control, (right) SPR 3, d) (left) control, (right) SPR 4, e) (left) control, (right) SPR 5, f) (left) control, (right) SPR 6.

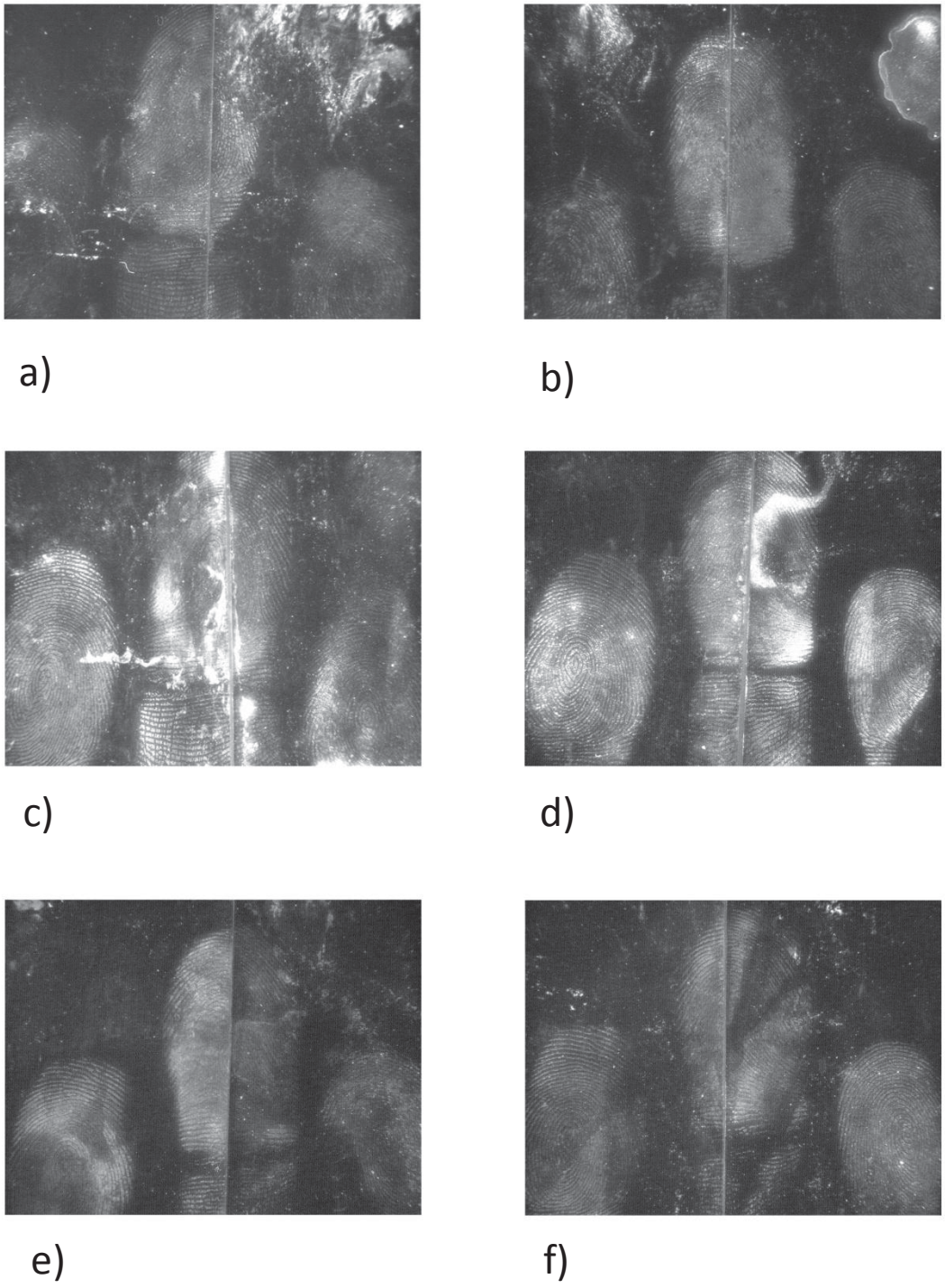


Figure 3-37: Luminescence photographs of STaR 11 developed samples, using excitation 530 nm and barrier band pass filter 700 nm - a) (left) control, (right) SPR 1, b) (left) control, (right) SPR 2, c) (left) control, (right) SPR 3, d) (left) control, (right) SPR 4, e) (left) control, (right) SPR 5, f) (left) control, (right) SPR 6.

Table 3-10: Further STaR 11 SPR Al_2O_3 mass optimisation.

	Control	**SPR 1**	**SPR 2**	**SPR 3**	**SPR 4**
Volume of 0.1 M SDS (mL)	10.0	10.0	10.0	10.0	10.0
Mass of Al_2O_3 (g)	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**
Diluted Volume (mL)	80.0	80.0	80.0	80.0	80.0
Volume of STaR 11 (mL)	12.5	12.5	12.5	12.5	12.5
Final Volume (mL)	92.5	92.5	92.5	92.5	92.5

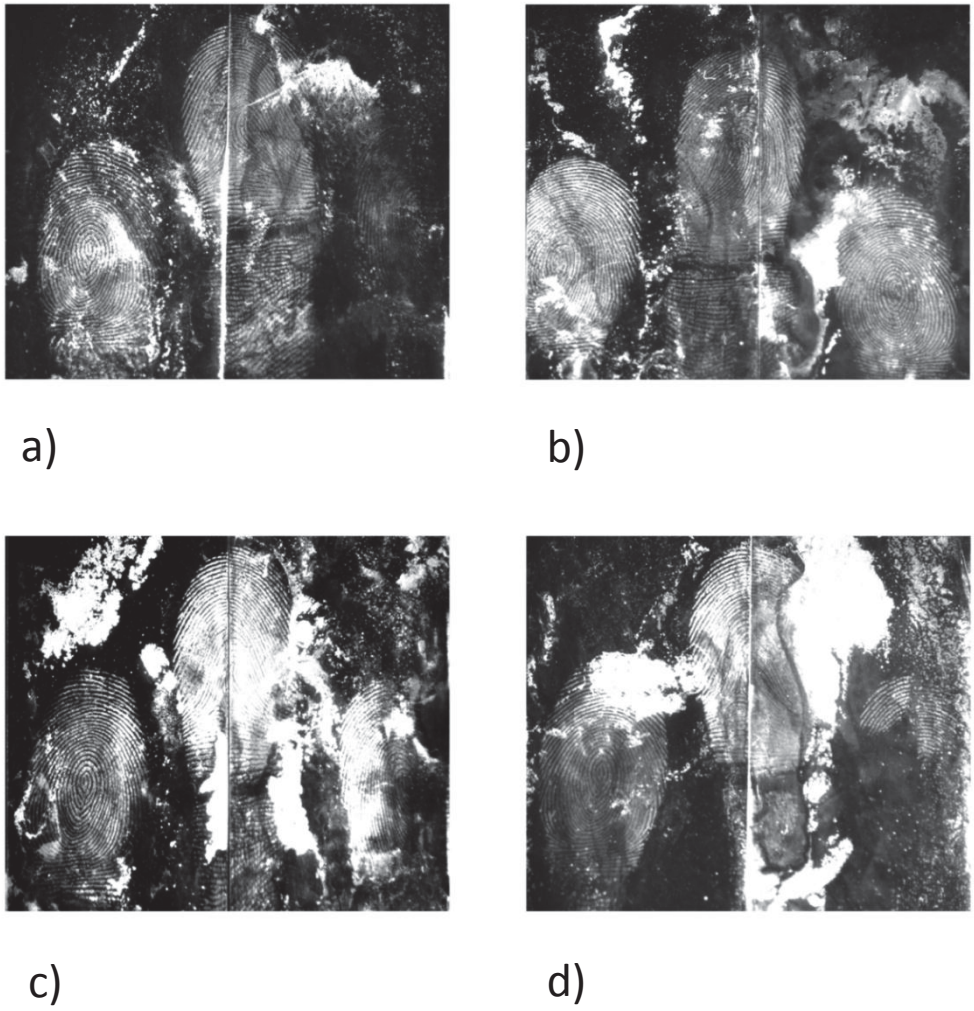


Figure 3-38: Luminescence photographs of STaR 11 developed samples, with excitation 530 nm and a barrier band pass filter 700 nm - a) (left) control, (right) SPR 1, b) (left) control, (right) SPR 2, c) (left) control, (right) SPR 3, d) (left) control, (right) SPR 4.

3.6.3 Comparison of STaR 11 SPR with Small Particle UV

Before a full donor study was undertaken, it was decided that several comparisons with Small Particle Reagent UV (SPR UV) were needed to ensure that a full donor study would be worthwhile. Fingermarks were deposited on glass slides. Charged and natural marks were submerged in water for 24 hours, then developed with each technique and compared using transmission microscopy, white light and luminescence examination.

Initial comparisons produced very promising results, with both techniques developing charged fingermarks with clear ridge detail. The transmission microscopy photographs also indicated that STaR 11 SPR was depositing more powder on the ridges than the conventional technique. However, natural marks exhibited a significant decrease in development quality for both techniques and this is shown in Figure 3-39 and Figure 3-40. These observations were reinforced when marks were viewed in the luminescence mode.

The main issue that arose with the STaR 11 formulation was that exposure times in the NIR were found to be in the range of five to ten seconds, compared to Small Particle Reagent UV, which required exposure times of less than 500 milliseconds. This highlighted a significant drawback of this technique as the majority of samples treated with SPR would be imaged at the scene. Based on the results obtained in the pseudo-operational trial, the long exposure times when imaging with lab based equipment would result in unrealistic exposure times using field equipment. The first issue believed to be causing this was the amount of powder being deposited on the fingermark ridges. It was hypothesised that, if more powder could be delivered to the fingermark ridges, this might improve both the exposure time and the ability to develop natural fingermarks. As a result, the wash step was removed from the sequence; however, this predictably resulted in a large amount of background development and very low contrast fingermarks.

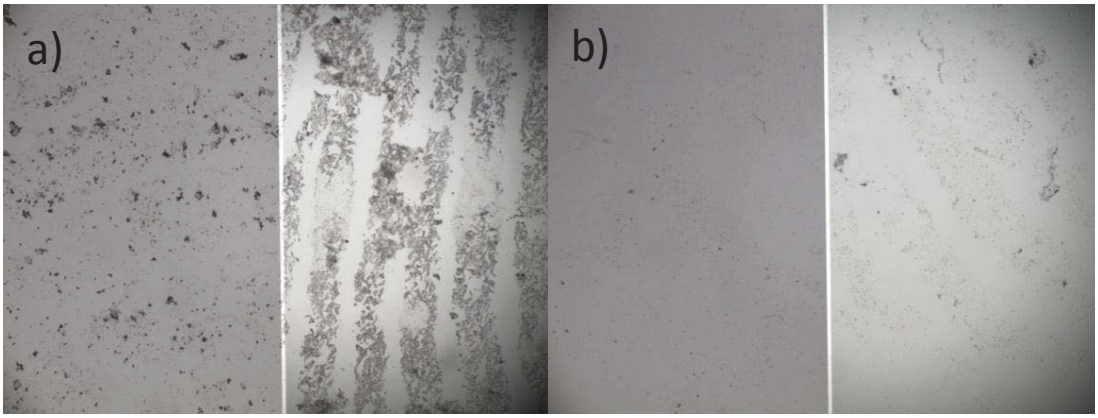


Figure 3-39: Transmission microscope photographs of a) charged fingermark developed with (left) SPR UV, (right) STaR 11 SPR and b) natural fingermark on glass developed with (left) SPR UV, (right) STaR 11 SPR.

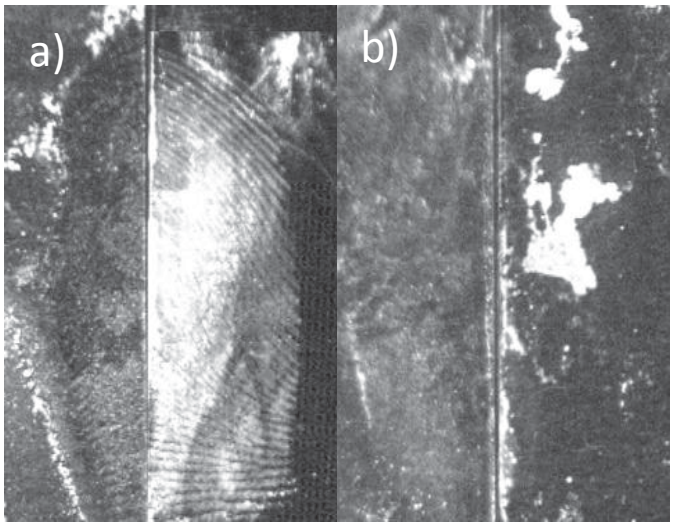


Figure 3-40: Luminescence photographs using a 450 nm excitation and 555 nm barrier band pass filter for SPR UV - 530 nm excitation and a 700 nm barrier band pass filter for STaR 11 - a) charged fingermark developed with (left) SPR UV, (right) STaR 11 SPR, b) natural fingermark on glass developed with (left) SPR UV, (right) STaR 11 SPR.

3.6.4 STaR 11 SPR Method of Delivery

SPR is typically applied using a pump spray bottle. While this can be effective, there are issues with consistency. The Ecospray® is a gas-pressurised reservoir that delivers the suspension in a fine mist. This is particularly beneficial for nanopowder suspensions as the nanopowders can be delivered without excess background development. In an effort to ensure that fingermarks were well coated, the Ecospray® was used to deliver the STaR 11 suspensions.

Comparisons of the pump spray bottle and Ecospray® showed that there was a definite improvement in the quality of developed fingermarks (Figure 3-41). Using the Ecospray® to deliver the suspensions resulted in a significant reduction in background development and gave high contrast fingermarks. Exposure times were also slightly improved as more powder was able to be deposited on the surface.

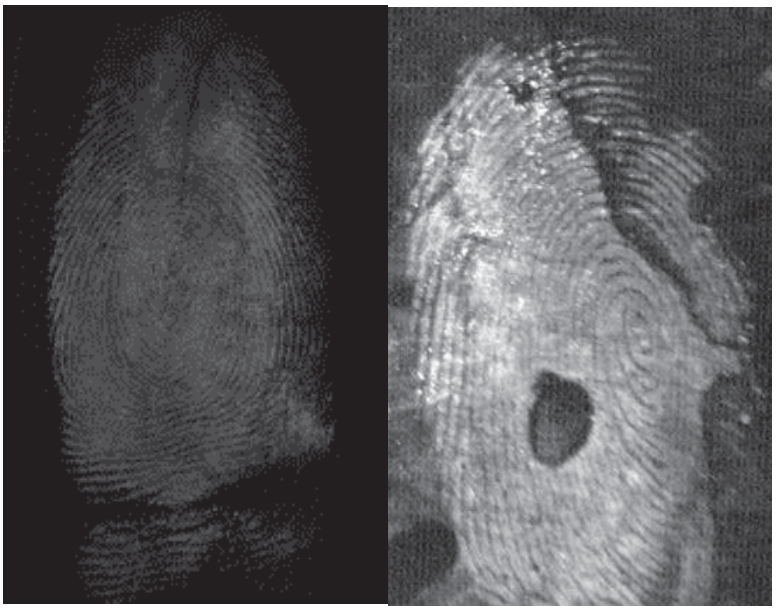


Figure 3-41: Developed charged fingermarks on glass viewed in the luminescence mode using excitation 530 nm and a 700 nm barrier band pass filter - (left) STaR 11 suspension sprayed using EcoSpray® Device (right) STaR 11 suspension sprayed using pump spray bottle.

Since background development was minimised with the Ecospray® and developed marks had greater ridge consistency, the mass of aluminium oxide was increased in an effort to increase the amount of powder being deposited on the ridges. Masses from two to five grams per 100 mL of surfactant solution were trialled and suspensions were applied directly to charged marks on glass. From the results shown in Figure 3-42, there was a marked improvement with increasing aluminium oxide mass. However, the higher mass suspensions (four and five grams) also had problems with blocking the aerosol spray head of the Ecospray® device. This became an issue as the nozzle had to be unblocked between each spraying application. Each fingermark was also imaged in the visible and NIR regions to determine whether there was a significant difference in luminescence strength and therefore exposure times of developed marks. While there was no major difference in development quality between the visible and

NIR imaged marks, the NIR imaged marks still had longer exposure times. Despite the slight improvement achieved with higher mass suspensions, the build-up of aluminium oxide around the nozzle posed a more severe problem and, as a result, the higher mass suspensions were not used in the comparison with the commercial SPR UV.

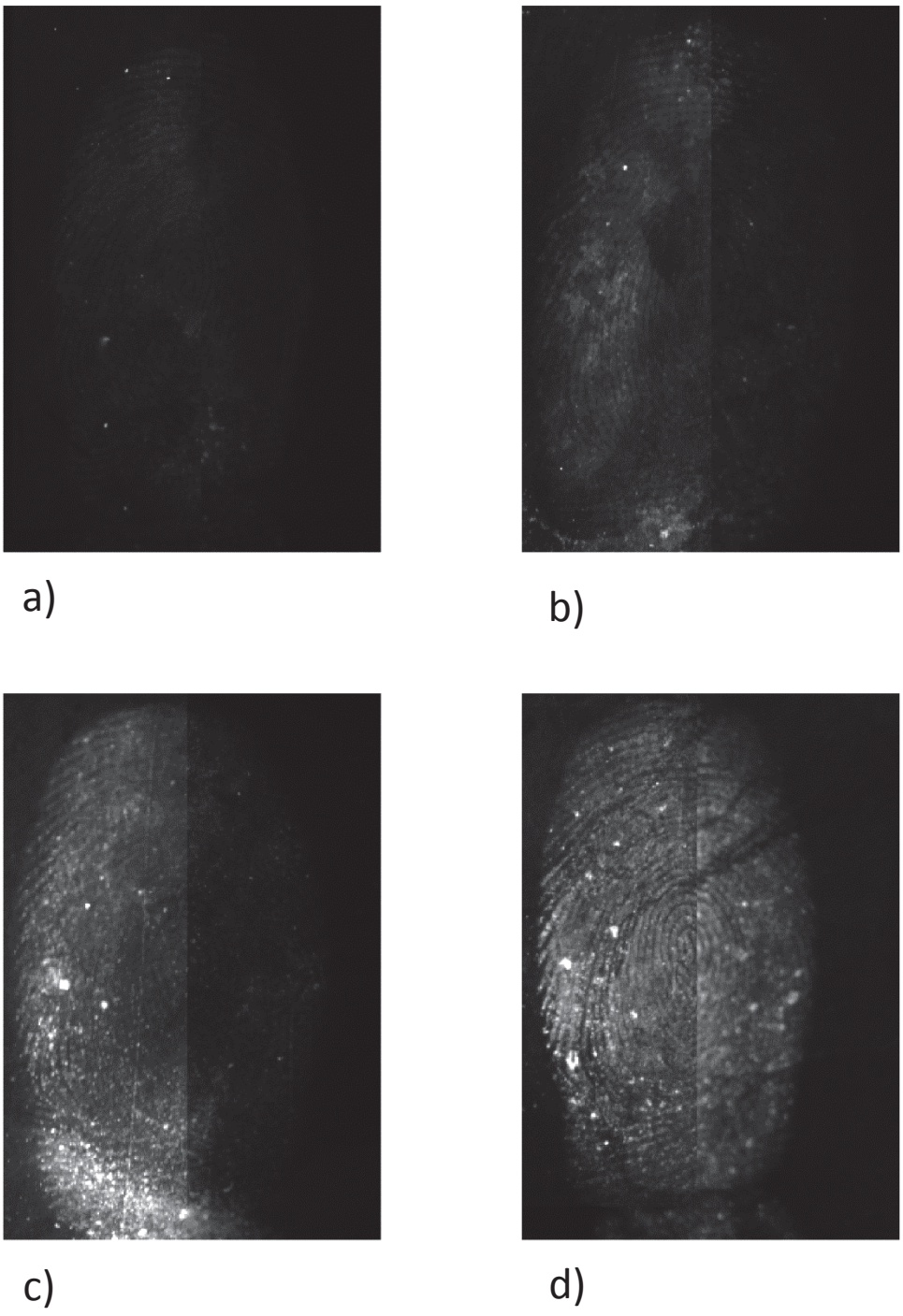


Figure 3-42: Comparison of different aluminium oxide mass using the Ecospray,®, viewed in the luminescence mode using - (left) STaR 11 505 nm excitation, 610 nm barrier band pass filter, (right) STaR 11 530 nm excitation 700 nm barrier band pass filter a) 2 g, b) 3 g, c) 4 g, d) 5 g.

When the samples were compared to those treated with SPR UV, there was a slightly better development for STaR 11 treated marks on aluminium (Figure 3-43); however, on both glass and polyethylene bags (Figure 3-44, Figure 3-45), SPR UV gave stronger luminescence and better ridge development. With the aluminium samples, it was observed that while neither technique gives tertiary level detail, the STaR 11 suspension gave some ridge detail (albeit low contrast) and very faint ridges could be seen for the natural marks. However, the SPR UV did not develop any fingermarks. The glass samples showed that, while neither technique could develop natural marks, the charged marks treated with SPR UV gave stronger luminescence and clearer ridge detail compared to those treated with STaR 11 SPR. The use of an Ecospray® device did not affect the ability to image natural fingermarks as there was no development for both the glass and polyethylene samples.

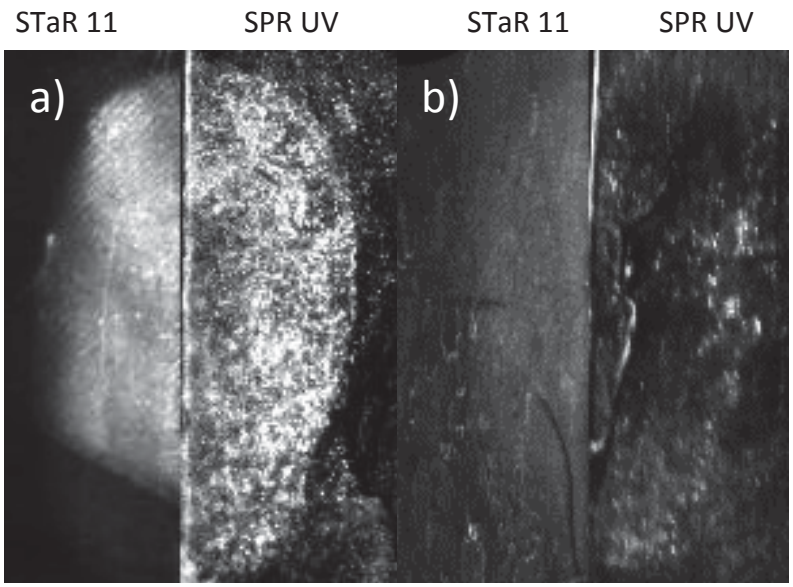


Figure 3-43: Developed fingermarks on aluminium sheeting viewed in luminescence mode using a 450 nm excitation and 555 nm barrier band pass filter for SPR UV - 530 nm excitation and a 700 nm barrier band pass filter for STaR 11 - (a) charged mark submerged in water for 24 hours, (b) natural mark submerged in water for 24 hours.

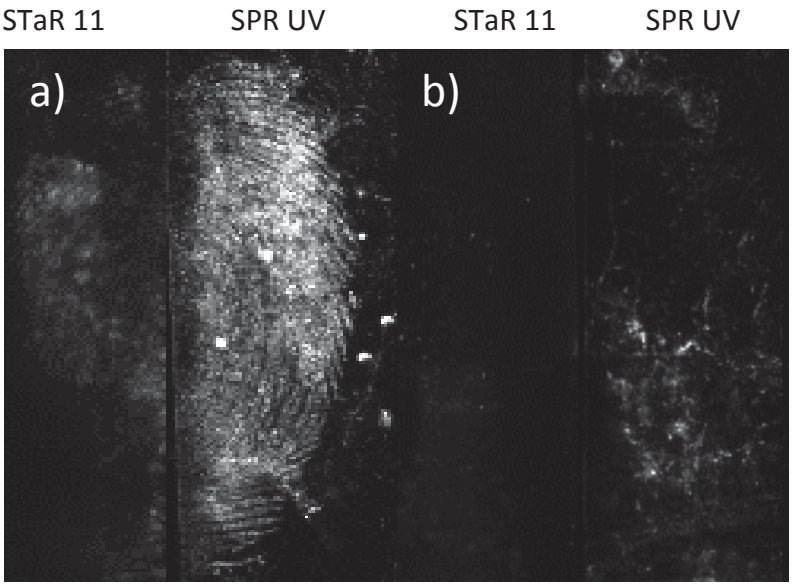


Figure 3-44: Developed fingermarks on glass microscope slides viewed in luminescence mode using a 450 nm excitation and 555 nm barrier band pass filter for SPR UV - 530 nm excitation and a 700 nm barrier band pass filter for STaR 11 - (a) charged mark submerged in water for 24 hours, (b) natural mark submerged in water for 24 hours.

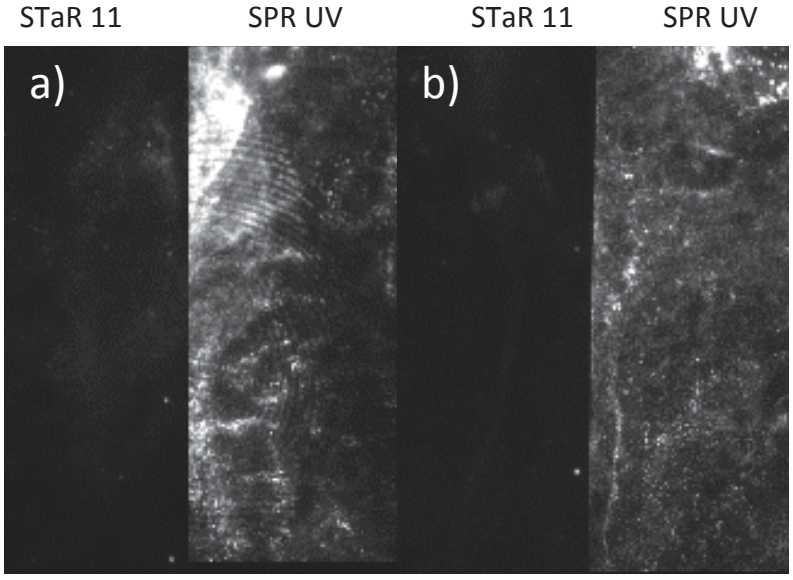


Figure 3-45: Developed fingermarks on polyethylene bags viewed in luminescence mode using a 450 nm excitation and 555 nm barrier band pass filter for SPR UV - 530 nm excitation and a 700 nm barrier band pass filter for STaR 11 - (a) charged mark submerged in water for 24 hours, (b) natural mark submerged in water for 24 hours.

The development time was another factor that needed to be optimised and could potentially be used to improve the quality of treated marks. Development times from five seconds to one minute were trialled on charged marks deposited on aluminium and glass. These results

(Figure 3-46) indicated that development quality was not dependent on the time the suspension was left on the fingermark. There was no specific time that gave superior development, nor was there a discernible relationship between longer development times and more powder being deposited on the surface.

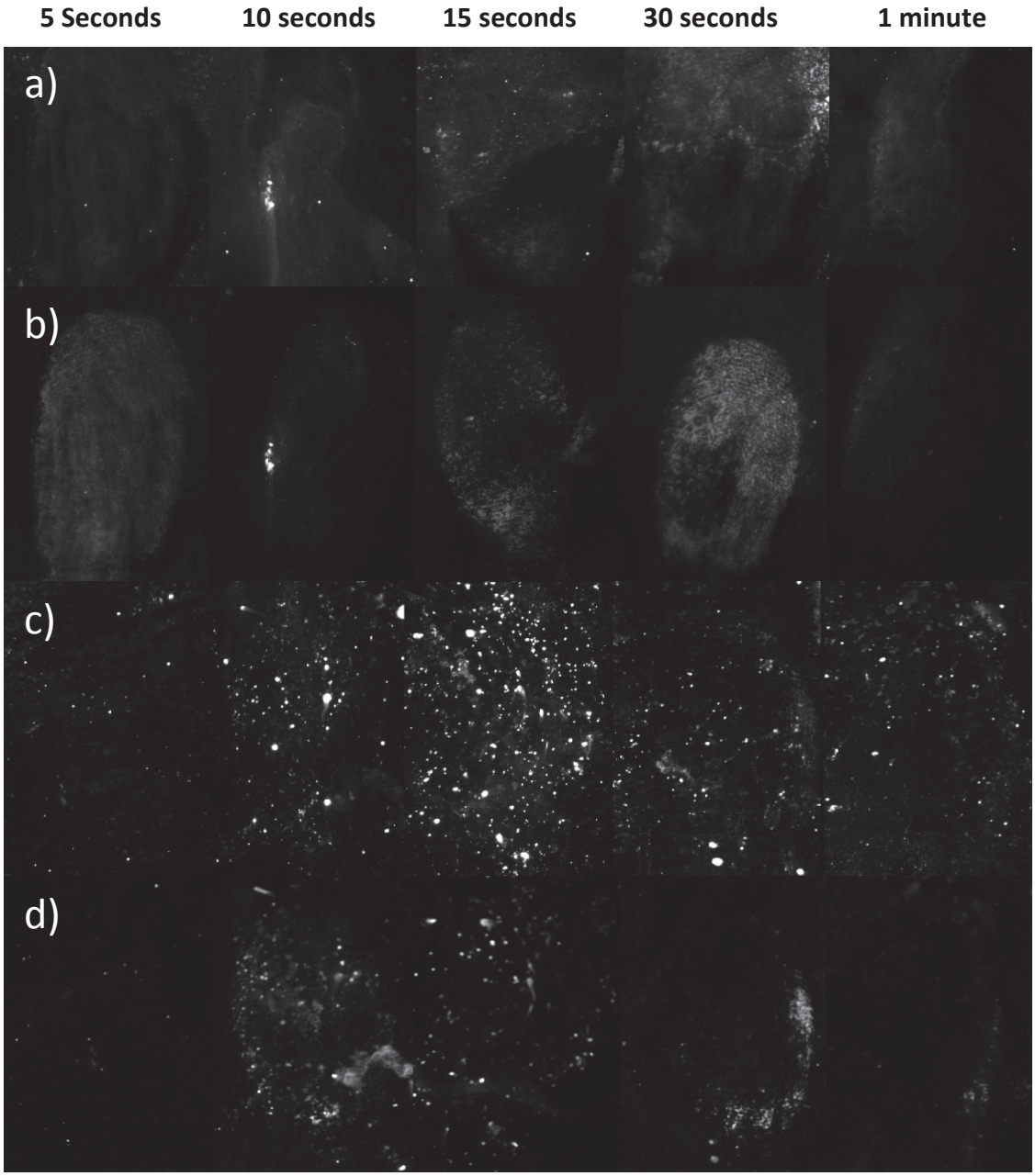


Figure 3-46: a) charged fingermarks on aluminium viewed in luminescence mode with 505 nm excitation and a 610 nm barrier band pass filter b) charged fingermarks on aluminium viewed in luminesce mode with 530 nm excitation and a 700 nm barrier band pass filter, c) charged fingermarks on glass viewed in luminescence mode with 505 nm excitation and a 610 nm barrier band pass filter d) charged fingermarks on glass viewed in luminesce mode with 530 nm excitation and a 700 nm barrier band pass filter.

Based on these results it was determined that the effectiveness of STaR 11 suspensions was not dependent on the application process, therefore the issues observed had to be chemical in nature. The quantum yield of styryl 11 is incredibly low in aqueous media and, due to the high water content in the suspension, it was determined that this would be the main contributing factor to the decreased NIR luminescence. As previously noted, more concentrated SDS solutions would remove the fingermarks off the surface. Once it was noted that water had an adverse effect on the NIR luminescence emission it was decided that a two-step spraying method should be pursued.

3.6.5 STaR 11 SPR Dual Spray

It was hypothesised that separating the dye from the powder suspension would eliminate the issue of decreased STaR 11 luminescence. First, an aluminium oxide suspension was sprayed onto the latent fingermark; the substrate was then washed with water to remove excess aluminium oxide. The developed fingermark was then air dried and sprayed with STaR 11 solution. The two-step method would potentially allow for fingermarks to be developed and imaged under three different conditions: first the white powder under white light, then in luminescence mode in both the visible and NIR regions. When fingermarks samples were treated with the dual spray technique fingermarks could be developed with the aluminium oxide suspension. However, after treatment with STaR 11, there was very little NIR luminescence present in the treated fingermark samples. When the dual spray was compared to SPR UV, both techniques gave very poor development. The luminescence from SPR UV was significantly stronger than with the dual spray method (Figure 3-47). Based on these result it was concluded that a NIR small particle reagent using STaR 11 was not achievable using the methods applied in this study.

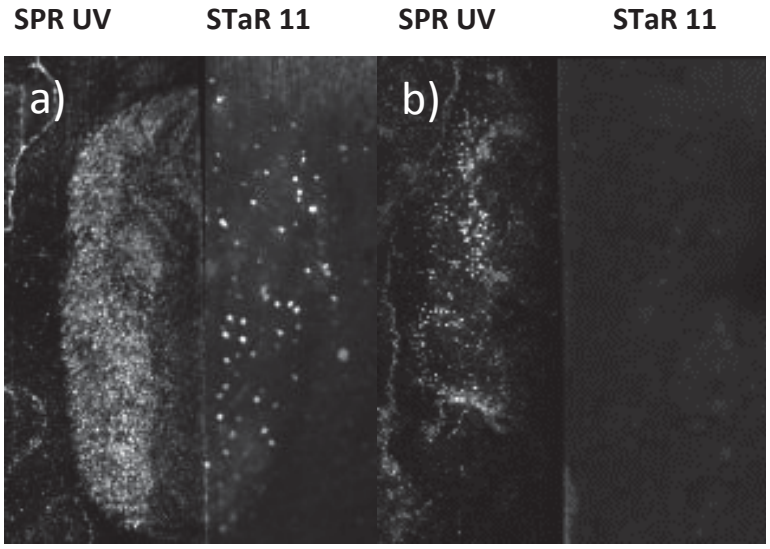


Figure 3-47: Developed fingermarks on glass viewed in luminescence mode using a 450 nm excitation and 555 nm barrier band pass filter for SPR UV - 530 nm excitation and a 700 nm barrier band pass filter for STaR 11 - (a) charged mark, (b) natural mark.

3.7 Conclusions

3.7.1 Conclusions for STaR 11 Sticky Side Powder

The aim of this study was to develop a NIR nanopowder suspension for the detection of latent fingermarks on adhesives surfaces that has the potential to be a useful alternative to conventional methods (such as commercially available suspensions). The ability to develop luminescent fingermarks in both the visible and near infrared regions on adhesive tape certainly has its advantages; however, the formulation used in this study was not ideal. The major issue with this method was that higher surfactant concentrations would allow for a thickened suspension (of a similar viscosity to that of Wet Powder™), however it would also remove the dye from the metal oxide. While lower surfactant concentrations would allow for easier application, it would also significantly decrease the quantum yield of styryl 11. The optimised suspension gave the best compromise between development quality and luminescence strength. This study had similar limitation to those stated in Section 2.4, as well as a few that were specific to this study. Firstly the optimisation of development times was only quantitatively performed on one occasion; repeat experiments were performed but not

quantified due to the similar result being obtained. The optimisation stage could be run again with a larger donor pool if a suitable alternate formulation was developed, however based on the formulation prepared in this study, these were the reported best conditions. Similarly due to the constraints of the project it would potentially more advantageous to pursue a non-NIR luminescent SSP as these may give better luminescent emission in surfactant solutions.

Compared to commercial Wet Powder™, the effectiveness of the STaR 11 suspension was also very dependent upon the type of tape the fingermarks had been deposited on. Semi-porous and glossy tape (i.e. masking and packing tapes, respectively) gave very poor development with the STaR 11 SSP and this treatment would not be suitable for these surfaces. Tapes such as cloth and gaffa tape gave comparable development quality; however, results were affected by the ages and environmental conditions the tape had been exposed to. Duct tape was the only tape where STaR 11 outperformed Wet Powder™, but only for samples that had been exposed to the environment. Due to these specific conditions, it is very unlikely that STaR 11 would replace or be used as an alternative to the commercial Wet Powder™ evaluated in this study.

3.7.2 Conclusions for STaR 11 Small Particle Reagent

The STaR 11 suspension for wet surfaces had similar drawbacks. It was only successful on charged marks and it would only develop marks less than 24 hours old. The main issue that arose from this method was that styryl 11 has a very low quantum yield in aqueous solutions, due to the formation of homoaggregates [119]. The formation of these homoaggragates in the solution would directly affect the luminescence of styryl 11 as well as the potential FRET interaction between styryl 11 and rhodamine 6G. Even after the powder was dried, these homoaggregates of styryl 11 would still be present on the surface of the powder and this would directly affect the NIR luminescence of any developed marks. When compared to the commercial SPR UV, there was a slight increase in development quality; however, very low luminescence resulted in alternate methods of delivery being explored.

While the use of an Ecospray® device did provide a significant advantage to the conventional pump spray bottle, a suitable NIR luminescent suspension for wet surfaces could not be adequately established and validated. Attempts to separate the luminescent dye from the

metal oxide suspension and develop a dual spray, did not increase the luminescence of developed marks. As a result, it was concluded that a NIR suspension using styryl dyes was not achievable due to the significant decrease in luminescence observed for the aqueous suspensions evaluated.

Since the effectiveness of a STaR 11 suspension was based on surfactant concentration, where higher concentration suspensions would remove fingermarks from the surface and lower concentration suspensions did not exhibit NIR luminescence, the study was concluded. Future work should look at using the Ecospray® device in combination with other luminescent suspensions that may or may not have an emission in the NIR.

Chapter 4:

Near Infrared Detection of Latent Fingermarks on Porous Surfaces

Chapter 4: Near Infrared Detection of Latent Fingermarks on Porous Surfaces

4.1 Introduction

4.1.1 Amino Acid Sensitive Reagents for Latent Fingermark Detection on Porous Surfaces

Amino acid sensitive reagents are the most common form of latent fingermark detection on porous surfaces and rely on the formation of coloured and/or luminescent products when in the presence of amino acids. The three most widely used reagents are ninhydrin, 1,8-diazafluoren-9-one (DFO) and 1,2-indanedione (IND).

The oldest and most established amino acid sensitive technique is ninhydrin (Figure 4-1), which forms a purple coloured complex when reacted with an α-amino acid. The reaction product is known as Ruhemann's purple (RP) [120]. Initially, ninhydrin was used as an amino acid visualisation tool for paper chromatography. It was not until 1954 that a method for the detection of latent fingermarks based on ninhydrin was proposed by Oden and von Hofsten [121]. This study proposed a 0.2% w/v solution of ninhydrin in acetone and acetic acid (0.4%) to spray on paper surfaces. Samples were allowed to air dry and then heated at 80°C for a few minutes. This procedure formed the basis for the standard operating procedures currently used in forensic laboratories [122]. Ruhemann's purple is a dark coloured product that can be visualised under ambient lighting conditions or with a combination of white light and a yellow-green observation filter [28]. In an attempt to form a luminescent product, a number of metal salt post treatments have been applied to ninhydrin. The most widely used metal salt post treatment is zinc chloride; this forms an orange complex that is luminescent when excited with a 490 nm light band, giving an emission peak at 550 nm. Luminescence can be enhanced by immersing the developed samples in liquid nitrogen, however, weak luminescence emission can be visualised at room temperature [28, 120, 123]. Lennard *et al.* trialled a range of metal salt complexes and found that the Zn-RP complex gave the strongest

luminescence when compared to other metal salt treatments. The only other potential metal salts that gave strong luminescence were cadmium and mercury which have significant health and safety issues, preventing them from replacing zinc chloride for routine use [123]. Since its development as a fingermark reagent, there have been numerous studies into the development of ninhydrin analogues that give coloured and luminescence development without the need for a metal salt post treatment (and without the need for cooling in liquid nitrogen). Despite the decades of research, there has been no widely accepted ninhydrin analog that has been implemented into standard operating procedures (the most promising is 5-methylthioninhydrin, however; more research is required [124, 125]).

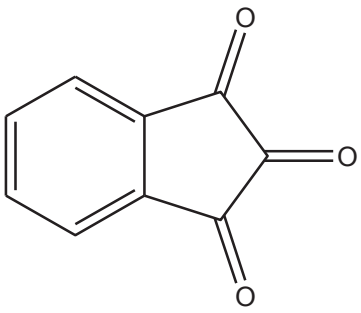


Figure 4-1: Ninhydrin structure (in its triketone form).

1,8-Diazafluoren-9-one (Figure 4-2) was developed in the 1950s by Druey and Schmidt; however, it was not until the late 1980s that its potential as a fingermark reagent was fully explored [126]. When reacted with α-amino acids, a luminescent product is formed (without the need for metal salt post treatment). When excited at 470 nm, the reaction product has an emission maximum at 570 nm. DFO developed fingermarks are a pale pink-purple colour when viewed under ambient lighting conditions. However, the greatest contrast is seen when viewed in the luminescence mode. DFO has been found to be effective when used in sequence with ninhydrin; however, DFO must be applied first as ninhydrin has a quenching effect on DFO's luminescent product [120]. A field trial performed by Wilkinson *et al.* determined that this sequence recovered more fingermarks using this sequence compared to ninhydrin then DFO [127]. Since its inception as a fingermark reagent, numerous formulations have been devised in attempts to replace CFC-based solvents, decrease background luminescence, and develop fingermarks on thermal paper [128-130]. While it was initially reported that humidity played a significant role in the effectiveness of DFO's luminescence emission, recent work has determined that pre-humidification provides no significant advantage [120].

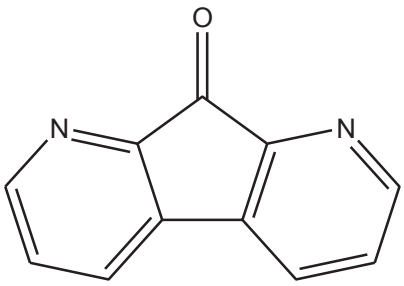


Figure 4-2: 1,8-Diazafluoren-9-one structure.

1,2-Indanedione (IND) (Figure 4-3) is the most recent widely accepted amino acid specific reagent for latent fingermark detection and when reacted with α–amino acids, provides a coloured product, similar to ninhydrin (under Australian conditions, different regions have varied success with the colouration of 1,2-indandione) and a luminescent product, similar to DFO. The use of IND as a fingermark reagent was first explored by Ramotowski *et al.* and the formulation was further optimised by Wallace-Kunkel *et al.* [131, 132]. After treatment, developed fingermarks are pink in colour and give strong luminescence emission when excited at 530 nm and using a 590 nm barrier band pass filter [28]. IND could also be combined with zinc salts (in one solution (IND-Zn)) to improve luminescence emission and decrease environmental sensitivity of the working solution. A recent study published by Berjedo *et al.* found that the difference between IND and IND-Zn to be minimal; however, work published by Spindler *et al.* determined that the addition of $ZnCl_2$ to the IND working solutions increased the formation of the desired reaction product due to the stabilisation of a highly reactive intermediate, particularly in low humidity environments [133, 134].

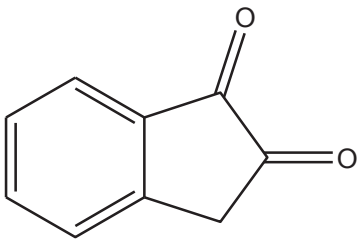


Figure 4-3: 1,2-Indanedione structure.

In the case of ninhydrin and indanedione, the generally accepted reaction mechanism between the reagents and amino acids is a Strecker degradation; the mechanism for Ruhemann's purple formation is shown in Figure 4-4 [135]. A similar mechanism has been proposed for the reaction between indanedione and amino acids [136]. It involves the

reduction on the triketo (or diketo for indanedione) group to form an azomethine ylide intermediate. The resonance stabilised intermediate then forms an amide that is further reacted with indanetrione to form the purple coloured Ruhemann's purple. The keto groups in the 2-position of both indanedione and ninhydrin are the reactive component of the molecule that undergoes Strecker degradation. As a result, many amino acid fingermark detection methods have these functional groups present, with different degrees of conjugation and other functional groups to promote Strecker degradation. Examples include lawsone and 5-methylthioninhydrin [4, 125].

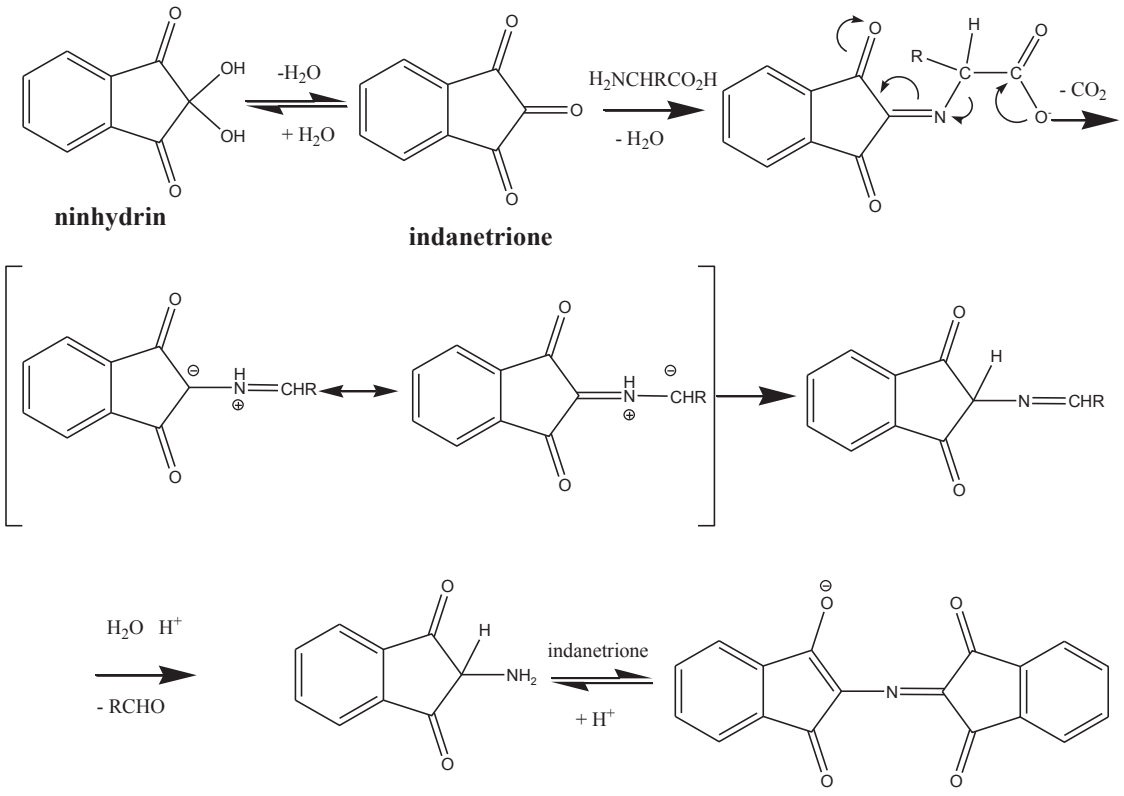


Figure 4-4: Reaction mechanism of ninhydrin with amino acids to form Ruhemann's purple [135]

4.1.2 Isatin and Isatin Analogues

Isatin (Figure 4-5) was used initially as an amino acid specific reagent for paper chromatography. Saifer and Oreskes published several articles outlining the effectiveness of isatin in differentiating proline from amino acid mixtures [137, 138]. The reaction products

between amino acids and isatin were outlined by Saifer and Oreskes. They found that, when compared to ninhydrin, isatin was not as sensitive, but it did give a broader range of colours that could be used to identify different classes of amino acids (i.e., primary and secondary amino acids) [137]. Isatin has also been used in sprays to develop TLC silica gel plates used to separate amino acids, with similar colour discrimination to that observed in paper chromatography [139]. A proposed reaction mechanism was outlined by Rehn *et al.* who hypothesised that isatin, when reacted with an α-amino acid, undergoes Strecker degradation to form the corresponding amide product (Figure 4-6) [140].

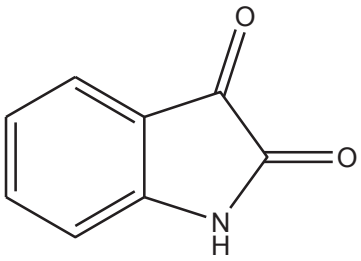


Figure 4-5: Isatin structure.

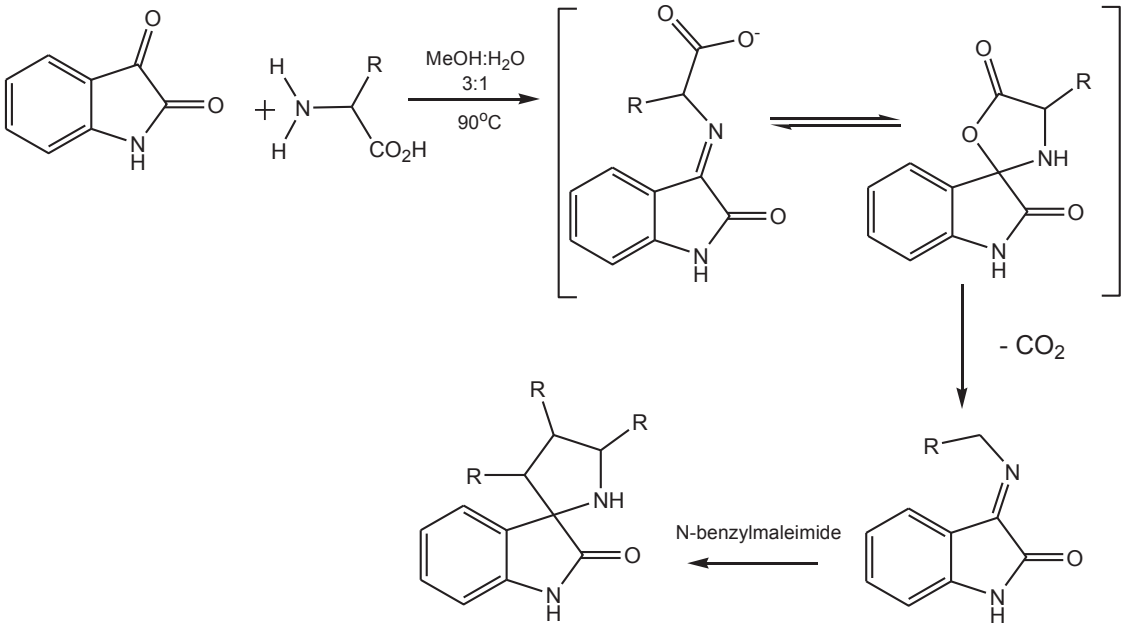


Figure 4-6: Proposed reaction scheme between isatin and a-amino acids adapted from Rehn et al.[140] .

The application of isatin for the development of latent fingermarks was first reported by Chan *et al.* This study found that isatin was able to develop luminescent fingermarks by reacting with the amino acids present in latent fingermarks. Luminescence emission intensity was increased with the addition of a metal salt post treatment (zinc chloride). When isatin was compared to DFO, IND-Zn and NIN, it was found that the conventional methods gave better development. While isatin was able to develop fingermarks that were luminescent at room temperature, unlike other techniques, the solvent used in the isatin formulation (dioxane) is significantly more hazardous than the solvents used in the formulations for the conventional fingermark detection techniques [141].

The preparation of styrylisatin (Figure 4-7) was published by Langenbeck *et al.* in 1957 [142]. Recently Van der Walt *et al.* and Manley-King *et al*. examined the use of styrylisatin as an inhibitor of monoamine oxidase (MAO) A and B [143, 144] Both synthesised a range of styrylisatin analogues and compared their inhibition abilities to isatin and other aniline analogues. This study found that styrylisatin had weaker binding affinity to MAO-A and MAO-B; however, it was still found to be a competitive inhibitor. The application of styrylisatin for the detection of latent fingermarks has not been explored in the available literature. Based on the work by Chan *et al,* it has been established that isatin can detect amino acids in fingermarks on porous surfaces [141]. The addition of a styryl group to the isatin molecule adds further conjugation, potentially shifting the luminescence peak emission of the reaction product into the NIR region. Based on this, its potential application for latent fingermark detection on porous surfaces was explored.

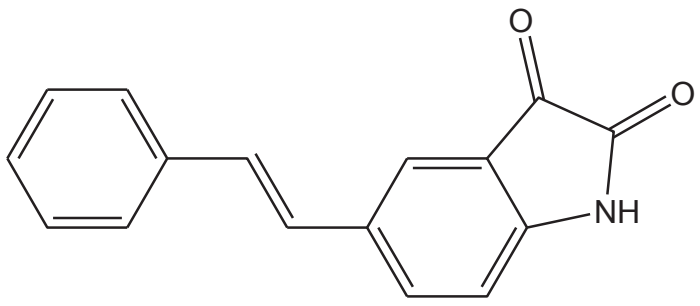


Figure 4-7: 5-Styrylisatin structure.

4.1.3 Aims and Objectives

Fingermarks on porous surfaces are very common in routine casework and, while the techniques outlined in the previous section are very selective for amino acids, each technique has its own limitations. A NIR detection method for latent fingermarks on porous surfaces could potentially increase the number of recoverable marks and make imaging easier (as substrate interferences such as printing would be largely negated in the NIR). Styrylisatin potentially has the ability to react with the amino acid components to give a coloured and NIR luminescent product. This study aimed to synthesise and characterise styrylisatin, and assess its ability to develop latent fingermarks on porous surfaces.

4.2 Materials

4.2.1 Reagents

Acetone-d_6 [CAS 666-52-4], L-alanine ≥98% [CAS 56-41-7], chloroform-d 99.8 atom %D containing 1% (v/v) TMS [CAS 865-49-6], diethyl ketomalonate 95% [CAS 609-09-6], dioxane ≥99.9% ACS [CAS 123-91-1], glycine *ReagentPlus®* ≥99% [CAS 3184-13-2], N-N-dimethyl formamide anhydrous ≥99% [CAS 68-12-2], dimethylsulfoxide-d_6 [CAS 2206-27-1], L-histidine *ReagentPlus®* ≥99% [CAS 72-19-5], L-leucine reagent grade ≥98% [CAS 61-90-5], L-lysine ≥98% [CAS 56-87-1], L-ornithine monohydrochloride ≥99% [CAS 3194-13-2], L-phenylalanine reagent grade ≥98% [CAS 63-91-2], L-serine *ReagentPlus®* ≥99% [CAS 56-45-1], tetrahydrofuran anhydrous inhibitor free ≥99.9%, [CAS 109-99-9], tin chloride reagent grade [CAS 7772-99-8], L-threonine reagent grade ≥98% [CAS 72-19-5], L-tyrosine reagent grade ≥98% [CAS 61-90-5] and L-valine reagent grade ≥98% [CAS 72-18-4] were obtained from Sigma Aldrich and used as supplied.

4-Nitrostilbene [CAS 4003-94-5] was obtained through Tokyo Chemical Industry Co. Ltd and used as supplied.

Glacial acetic acid reagent grade [CAS 64-19-7] and 32% hydrochloric acid analytical reagent grade [CAS 7647-01-0] were obtained from RCI Labscan and used as supplied.

Absolute ethanol 99.8% [CAS 64-17-5], methanol analytical grade [CAS 67-56-1], potassium hydroxide pellets [CAS 1310-58-3], sodium sulfate anhydrous [CAS 7757-82-6] and dichloromethane reagent grade [CAS 75-09-2] were obtained through BDH chemicals and used as supplied.

Dimethylsulfoxide reagent grade [CAS 67-68-5] and sodium acetate hydrate [CAS 41484-91-7] were obtained through Ajax Finechem and used as supplied.

Acetone analytical grade [CAS 67-64-1], acetonitrile analytical grade [CAS 75-05-8], chloroform reagent grade [CAS 67-66-3], diethyl ether reagent grade [CAS 60-29-7] and zinc chloride laboratory reagent [CAS 7646-85-7] were obtained through Chem Supply and used as supplied.

Sodium hydroxide pellets laboratory grade for analysis [CAS 1310-73-2] were obtained through Merck and used as supplied.

4.2.2 Instrumentation

A John Morris Electro Thermal Melting Point Apparatus was used to determine the melting point of synthesised products.

An Agilent Technology 500MHz (^{1}H Frequency) DDR2 Premium Shielded NMR spectrometer operating with a 1 second pulse delay and 90 degree spin angle was used for the characterisation of synthesised products.

A Nicolet Magna IR-760 spectrometer was used to characterise synthesised products.

A Craic 20/20 UV-visible-NIR microspectrophotometer was used to obtain the absorbance spectrum for styrylisatin.

A Foster + Freeman VSC 6000 was used to visualise developed fingermarks and obtain luminescence emission spectra of styrylisatin and styrylisatin reacted with amino acids.

4.3 Methods

4.3.1 General Approach

Styrylisatin was synthesised using an adapted method from Langenbeck *et al.* and characterised using Fourier transform infrared spectroscopy (FTIR), and proton (H^1) and carbon (C^{13}) nuclear magnetic resonance spectroscopy (NMR). Once the product was successfully synthesised, a physical and chemical examination of the synthesised styrylisatin was also undertaken, i.e. solubility, pH effects and melting point. The reactivity of styrylisatin with 11 amino acids commonly found in fingermarks was examined. Luminescence emission measurements on paper were used to give an indication of the effectiveness of the reaction.

4.3.2 Synthesis of Styrylisatin

The synthesis of styrylisatin started with 4-nitrostilbene (Figure 4-8a), which underwent a reduction to form 4-aminostilbene (Figure 4-8b). This intermediate was then reacted with diethyl ketomalonate to give the desired 5-styrylisatin (Figure 4-8c).

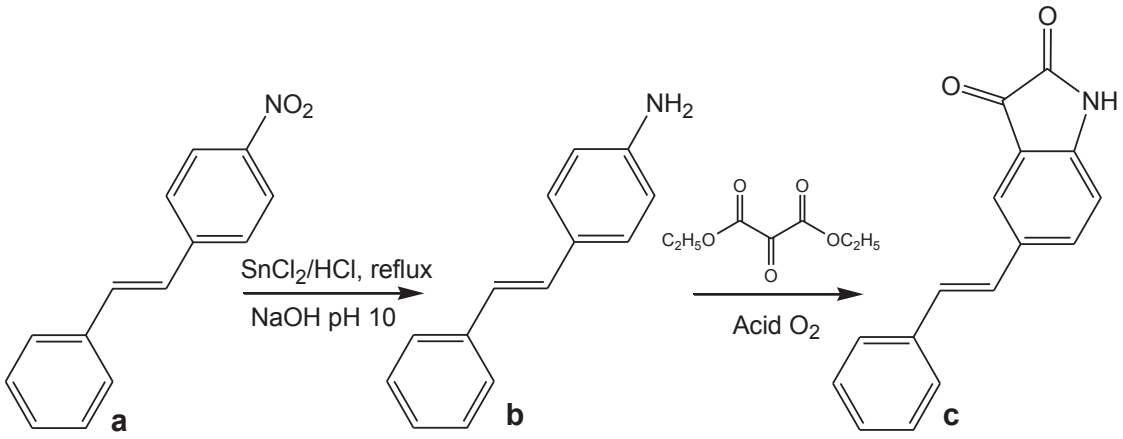


Figure 4-8: 5-Styrylisatin synthesis route.

4.3.2.1 Reduction of 4-nitrostilbene to 4-aminostilbene

4-Aminostilbene was synthesised using an adapted method from Hanna *et al.* [145]. A solution of hydrochloric acid (HCl) (9 mL) and tin chloride ($SnCl_2$) (7.1 g) was added dropwise to a solution of glacial acetic acid (AA) (28 mL) and 4-nitrostilbene (1 g). This mixture was sealed and stirred overnight at room temperature. The cloudy yellow suspension was filtered and washed with 10 mL of glacial acetic acid. The precipitate was collected and suspended in 300 mL of H_2O. This suspension was then basified to pH 10 using 40% aqueous sodium hydroxide. The crude product was extracted using 2 x 25 mL of chloroform ($CHCl_3$), dried with anhydrous sodium sulphate (to remove any water), before being decanted into a round bottom flask and evaporated under reduced pressure to give a yellow powder. The solid was recrystallised using hot ethanol to give a yellow crystalline product.

4.3.2.2 Formation of 5-styrylisatin from 4-aminostilbene

A solution of 4-aminostilbene (1 g), diethyl ketomalonate (1 g) and glacial acetic acid (8 mL) was heated (120 °C) and stirred under reflux for four hours. The solution turned dark red and excess acetic acid was evaporated under reduced pressure. The viscous solution was then basified with 20% potassium hydroxide (KOH) solution, which formed a yellow suspension. This mixture was then refluxed for four hours under a stream of oxygen to promote oxidation. After cooling, the mixture was filtered and HCl was added to the solution until a red precipitate was formed (pH 2). The precipitate was washed with distilled water and dried to give crude styrylisatin. Recrystallisation was performed on the crude product with ethanol to give a dark red crystalline product. Once the product was purified FTIR, ^{1}H NMR and ^{13}C NMR were performed to confirm the final product. The spectra from all stages of the reaction can be found in Appendix iii. The key identifying factors for characterisation were the presence of a keto and amide group in the final product spectrum. The loss of a primary amide stretch in the FTIR and peaks in the NMR of the final product indicated the purity of the final product.

4.3.3 Physical and Chemical Properties of Styrylisatin

After characterisation, a suitable solvent system had to be developed that could be used to safely apply the reagent paper to potentially develop fingermarks. Factors that had to be taken into account included: solubility of styrylisatin in the solvent, occupational health and safety, volatility of the solvent, and effect of the solvent on amino acids (i.e., no dissolution of available amino acids).

4.3.4 Reactivity with Amino Acids – Porous Surfaces

Since the reaction between any fingermark reagent and amino acids present in fingermarks will occur on a porous substrate, it was decided that all studies should be performed on filter paper first (as this is essentially a pure cellulose substrate, without additives). Eleven amino acids (Table 4-1) were used to assess the reactivity of styrylisatin with amino acids. The relative abundances in deposited marks were obtained from Ramotowski and Yamashita *et al.* [12, 146]. A 0.1 M stock solution was prepared for each amino acid by dissolving the amino acid in deionised water; lower concentration solutions (0.01 – 1 $x10^{-4}$ M) were prepared using serial dilution. A 5 μL aliquot of each amino acid was spotted onto ashless #42 Whatman filter paper and left to dry before treatment. After the solubility of styrylisatin was determined, different solvents systems were trialled, mimicking those already used in current fingermark detection techniques (Table 4-2). When these solvent systems was deemed unsuccessful, dissolution of styrylisatin in DMSO was used to apply to the amino acid spots on filter paper.

Table 4-1: Amino acids used for this study [12, 146].

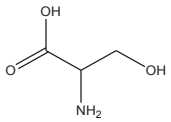
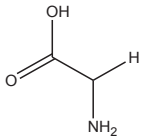
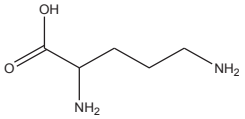
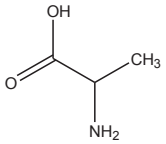
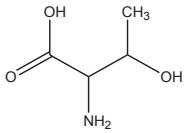
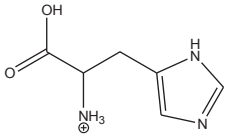
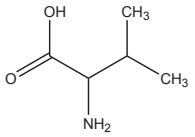
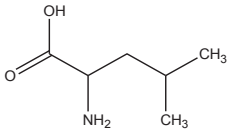
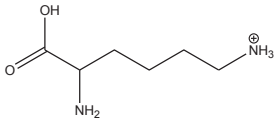
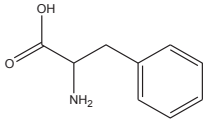
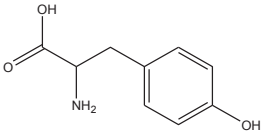
Amino Acid	Abundance in Fingermarks (μmol)	Structure
Serine (Ser)	0.106	
Glycine (Gly)	0.071	
Ornithine (Orn)	0.029	
Alanine (Ala)	0.018	
Threonine (Thr)	0.018	
Histidine (Hi)	0.013	
Valine (Val)	0.013	
Leucine (Leu)	0.011	
Lysine (Lys)	0.011	
Phenylalanine (Phe)	0.006	
Tyrosine (Tyr)	0.005	

Table 4-2: Solvents used and concentrations of fingermark reagents.

Fingermark Reagent	Solvents	Concentration of Fingermark Reagents
DFO	Dichloromethane Methanol Acetic acid HFC-4130 carrier solvent	Stock solution 0.044 M Working solution 0.004 M
Indanedione	Ethyl acetate Acetic Acid HFE-7100 carrier solvent	Stock solution 0.044 M Working solution 0.004 M
Ninhydrin	Ethanol Ethyl acetate Acetic acid HFE-7100 carrier solvent	Stock solution 0.402 M Working solution 0.026 M
Isatin	Dioxane Acetone	Working solution 0.034 M

4.4 Results and Discussion

4.4.1 Synthesis of Styrylisatin

The synthesis of 4-aminostilbene outlined by Hanna *et al.* [145] had to be modified to reduce the volume of solvents used and increase the yield. This synthesis recommended the use of THF in the reaction mixture while stirring overnight. It was determined that the use of THF did not affect the overall reaction and was removed from the procedure. This method also suggested the use of NaOH pellets to increase the pH of the reaction mixture. It was determined that this method was too imprecise and as a result a 40% aqueous NaOH solution was used instead; this was the same method that Van Der Walt reported [143]. Four extractions of 25 mL of chloroform were used to remove the crude product from the suspension; the recommended two extractions resulted in lower yields.

4-Aminostilbene was obtained in a yield of 53%: mp 149-151 °C; literature mp 151 °C [147]. ^{1}H-NMR (Agilent 500 MHz) ($CDCl_3$) δ 6.668 (d, 2H, J = 8 Hz), 6.914 (d, 2H, J = 16.5), 7.020 (d, 2H, J = 16.5), 7.187-7.217 (t, 1H, J = 15 Hz), 7.308-7.339 (t, 2H, 15.5 Hz), 7.464 (d, 2H, J = 8

Hz). ^{13}C-NMR (Agilent, 500 MHz) ($CDCl_3$) δ 115.187, 125.119, 126.086, 126.872, 127.737, 128.044, 128.575, 137.935, 146.132.

The Langenbeck method was altered to increase the yield. For the oxidation step, oxygen was passed through the reaction mixture instead of the recommended compressed air. Ten percent KOH resulted in a large volume of solution for the final step and this resulted in very low yields. As a result, 20% KOH was used which increased the yield of the final product.

Styrylisatin was obtained in a yield of 60%: mp 262-265 °C; literature mp 264-266 °C [142]. ^{1}H-NMR (Agilent 500 MHz) (DMSO d-6) δ 6.937 (d, 2H, J = 7.5 Hz), 7.247-7.278 (t, 3H, J = 7.75 Hz), 7.360-7.390 (t, 2H, J = 7.75 Hz), 7.581 (d, 1 H, J = 7 Hz), 7.784 (s, 1H), 7.819 (d, 1H, J = 8 Hz), 11.129 (s, 1 H). ^{13}C-NMR (Agilent, 500 MHz) (DMSO-d-6) δ 118.453, 122.220, 126.534, 127.167, 127.937, 128.821, 132.304, 126.348, 137.114, 159.690, 184.551.

4.4.2 Physical and Chemical Properties of Styrylisatin

A range of solvents were used to dissolve styrylisatin (0.01 g) in 5 mL of solvent; as can be seen from Table 4-3, styrylisatin was not very soluble in most of the solvents tested. The only solvent that completely dissolved styrylisatin at this concentration was dimethyl sulfoxide (DMSO); this was expected as this was the reported solvent used for NMR characterisation [143]. Non-polar and polar protic solvents (with the exception of methanol) were unsuitable solvents as they did not dissolve styrylisatin. Compared to isatin, the additional conjugation present in the styrylisatin molecule significantly affected the solubility, as it was not soluble in the common solvent systems reported in the literature (acetone and dioxane) [137, 141].

Table 4-3: Solubility of styrylisatin (soluble = all styrylisatin was dissolved, sparingly soluble = styrylisatin not completely dissolved, insoluble = styrylisatin remains undissolved).

Solvent	Solubility of Styrylisatin	Polarity [Polarity Index][71]
Acetone	Sparingly Soluble	Polar (aprotic) [5.1]
Acetonitrile	Sparingly Soluble	Polar (aprotic) [5.8]
Chloroform	Insoluble	Non-polar [4.1]
Dichloromethane	Sparingly Soluble	Polar (aprotic) [3.1]
Diethyl ether	Insoluble	Non-polar [2.8]
Dimethyl formamide	Sparingly soluble	Polar (aprotic) [6.4]
Dimethyl sulfoxide	Soluble	Polar (aprotic) [7.2]
Dioxane	Insoluble	Non-polar [4.8]
Ethanol	Insoluble	Polar (protic) [5.2]
Ethyl Acetate	Insoluble	Polar (aprotic) [4.4]
Methanol	Sparingly Soluble	Polar (protic) [5.1]
Tetrahydrofuran	Insoluble	Polar (aprotic) [4.0]

The pH also played a significant role in the structure of styrylisatin; in acidic conditions, styrylisatin is a deep red colour. As pH increases, this fades to a yellow colour. It is hypothesised that, as the solution becomes more alkaline, the nitrogen deprotonates and results in an isatin salt being formed. To ensure that styrylisatin does not convert to the salt form when reacting with amino acids, it was determined that only acidic conditions would be used for testing the reactivity of styrylisatin and amino acids.

The absorbance of styrylisatin was measured using microspectrophotometry; the spectrum shown in Figure 4-9 shows a very broad absorption band from 425-550 nm. Luminescence emission data of styrylisatin solid was also collected on the VSC 6000 at various broad-band wavelengths across the visible region (Figure 4-10). The Cary Eclipse Spectrometer gave very poor results and could not be used for this study. As can be seen from this data, styrylisatin on its own has luminescence emission at the red-end of the visible spectrum (λ_{max} 662 nm for 485-610 nm excitation) and into the NIR (λ_{max} 717 nm for 515-640 nm excitation). This information strengthens the possibility that a reaction product with an amino acid would shift the luminescence emission further into the NIR.

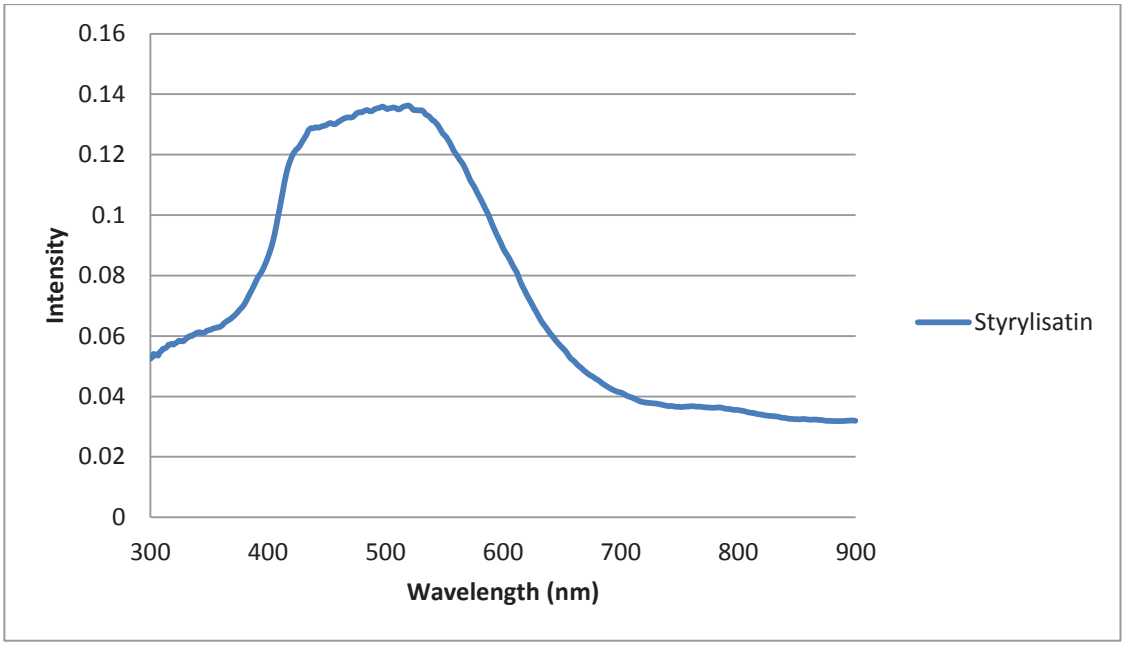


Figure 4-9: Absorbance spectrum of styrylisatin.

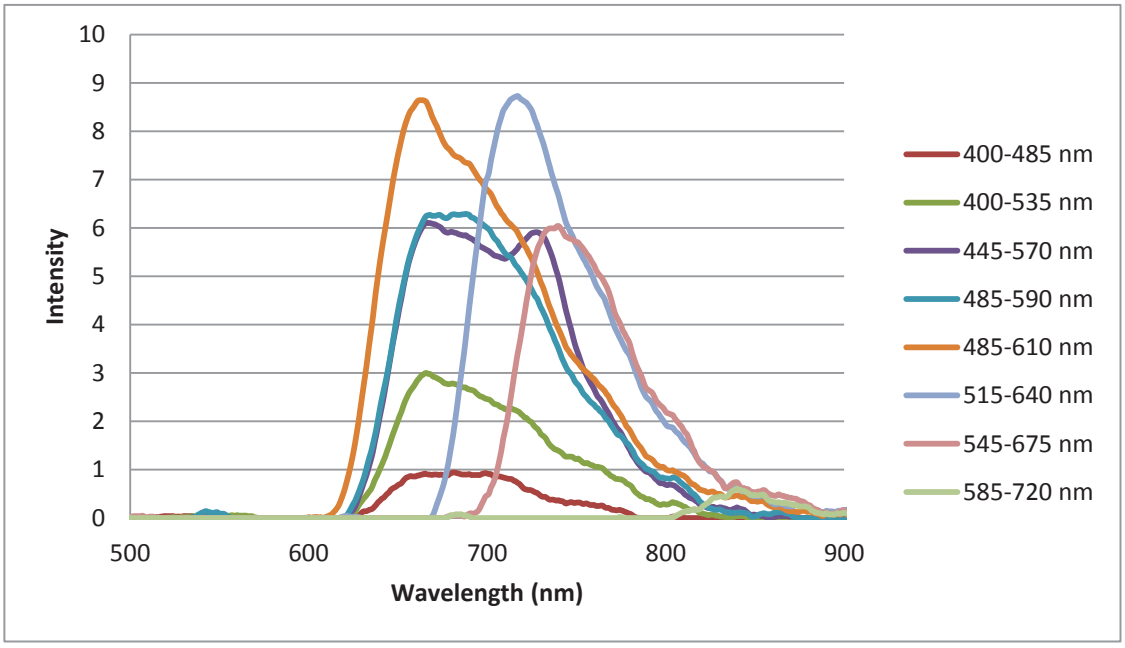


Figure 4-10: Luminescence emission data from solid styrylisatin for different excitation wavelengths.

4.4.3 Reactivity with Amino Acids – Porous Surfaces

Based on the physical and chemical data presented in section 4.4.2, it was determined that the best solvent to use would be DMSO as this was able to easily dissolve styrylisatin. Initial tests with this solvent worked well; a colour change was visible for 0.01 M amino acid spots (Figure 4-11) and luminescence emission was detected for concentrations from 0.01 M for some amino acids; however, when applied to fingermarks there was no development. This would be expected as the concentrations of amino acids in fingermarks are significantly less than the concentrations tested in this sample. Different amino acids also gave a difference in colour and luminescence emission limits of detection (LOD) (Table 4-4). The limit of detection was determined subjectively based on the images obtained and luminescence spectra were obtained for the highest LOD for all amino acids. From this data, it could be inferred that a reaction was occurring between the amino acids and styrylisatin. However, there was no consistent trend between the colour of the amino acid spot and the luminescence emission limit of detection. Further optimisation was performed in order to increase the sensitivity of styrylisatin. Another issue with the 0.05 M solution was the high amounts of background staining present (Figure 4-11); this would indicate that the solution used is too concentrated and lower concentration solutions may decrease the amount of background staining and potentially increase the contrast between the developed amino acid spots and the substrate.

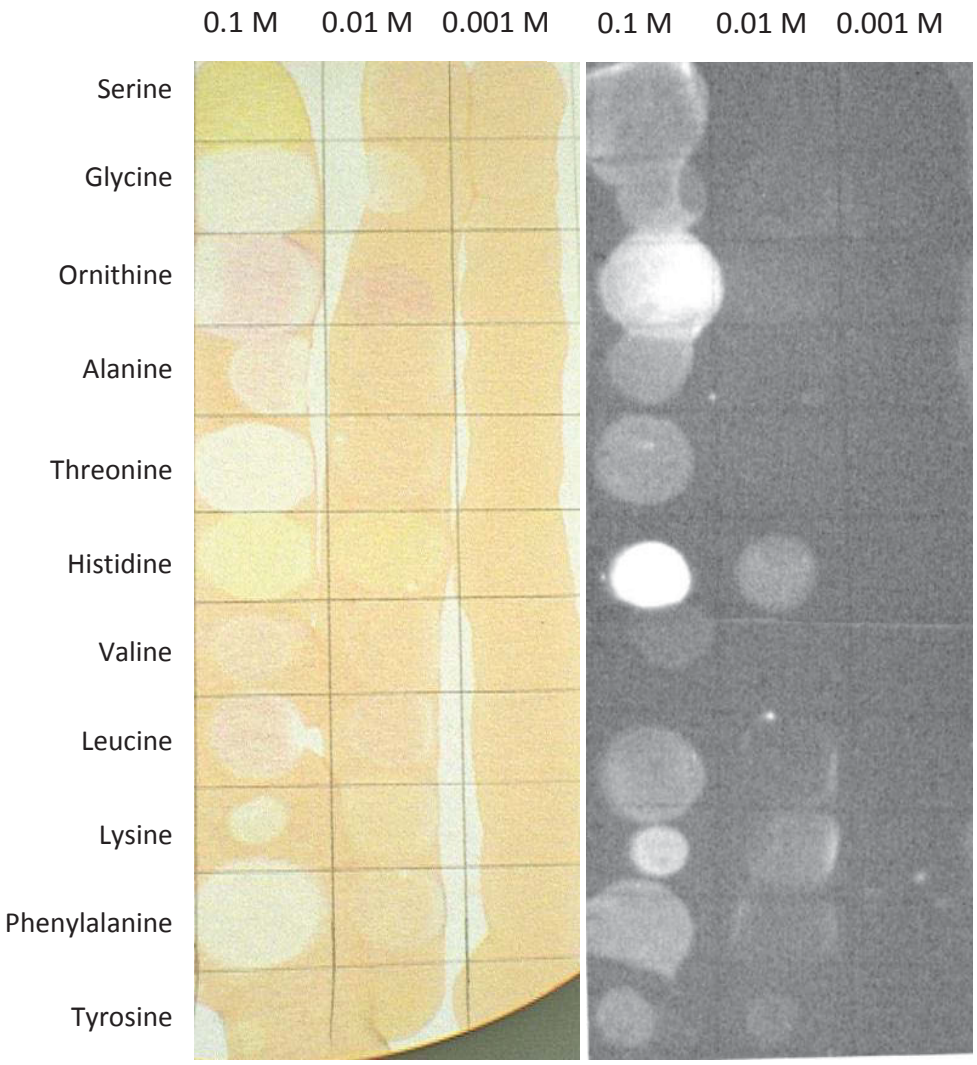


Figure 4-11: Amino acid spot test results for 0.05 M styrylisatin in DMSO after heating at 180°C for 10 seconds.

Table 4-4: Colour changes and luminescence emission limits of detection for styrylisatin in DMSO reacted with the amino acids selected.

Amino Acid	Colour	Luminescence LOD (M)
Serine	Yellow	0.10
Glycine	Yellow	0.01
Ornithine	Pink	0.10
Alanine	Pink	0.10
Threonine	Pink	0.10
Histidine	Yellow	0.01
Valine	Pink	0.10
Leucine	Pink	0.01
Lysine	Yellow	0.01
Phenalanine	Pink	0.01
Tyrosine	Yellow	0.01

In an effort to reduce background staining and increase sensitivity for amino acid detection, different concentrations of DMSO were trialled (Table 4-5). For each of the concentrations, luminescence emission spectra were taken for each of the amino acids at 0.1 M. The peak luminescence intensity of each amino acid was recorded and graphed in Figure 4-12. The higher concentration samples gave the strongest luminescence for the majority of the amino acids tested; however, the relationship between styrylisatin concentration and luminescence emission intensity was not linear for all amino acids. In some cases, the 0.016 M solution gave strong luminescence and, in the case of serine and tyrosine, the luminescence intensity was stronger than for the higher concentration samples. This would indicate that styrylisatin has an affinity for different amino acids and concentration was not the only factor that determined the optimum formation of the reaction product. From this data, it could be determined that styrylisatin was most reactive to histidine as this gave consistently higher luminescence emission intensities than for the other amino acids tested. Potentially, this could be due to the presence of an electrically charged side chain (at physiological pH, the amino acid will be protonated) that could favour a reaction with styrylisatin. Similarly, lysine

has an electrically charged side chain and also gave quite strong luminescence. While the luminescence emission intensity varied between concentrations of styrylisatin solution, there was no change in the limit of detection of styrylisatin to the amino acids tested.

Table 4-5: Concentrations of styrylisatin in DMSO that were tested in this study (*concentration is similar to that of indanedione and DFO working solutions, †concentration is similar to that of ninhydrin working solution).

Mass of Styrylisatin (g)	0.050	0.040	0.030	0.020	0.010
Volume of DMSO (ml)	5.00	5.00	5.00	5.00	5.00
Concentration (M)	0.040*	0.032	0.024†	0.016	0.008

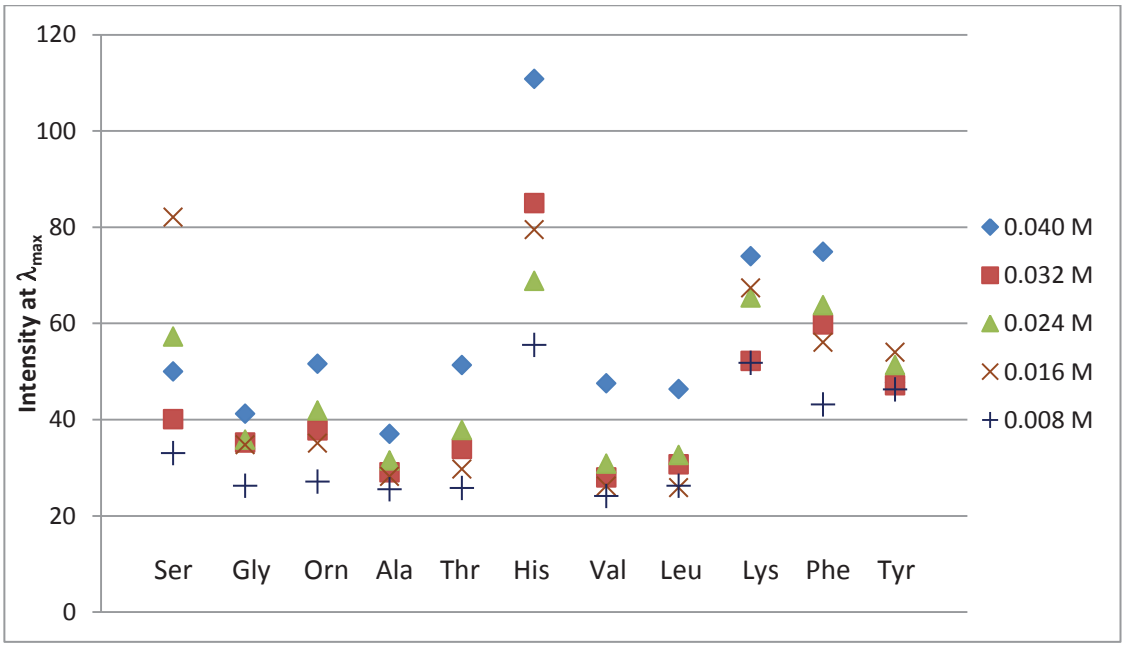


Figure 4-12: Peak luminescence emission intensities for styrylisatin at different concentrations reacted with the selected amino acids (see Table 4-1 for abbreviations).

Another factor that was examined was the pH of the solution. As previously mentioned, alkali solutions resulted in the formation of an isatin salt that cannot react with amino acids. Therefore, a small pH range of 2.5-4 was tested to determine the optimal pH. Above a pH of 4, the solution tended to change to an orange colour, which would indicate the formation of the isatin salt, which should be avoided as it would potentially decrease reactivity.

The result from this experiment determined that pH was not a significant factor for development conditions for the DMSO solution as changes to pH did not consistently improve detection sensitivity across all of the amino acids tested (Figure 4-13). Luminescence was not greatly affected for all of the amino acids and it did not change the overall trend observed in the previous experiment. Amino acids such as histidine, lysine and ornithine exhibited the greatest change with pH; however, these were not consistent between the amino acids (as seen with histidine and lysine, which gave similar results in the previous experiment; these now gave contrary results). Based on these experiments, it was decided that changes to both concentration and pH could not increase the sensitivity of styrylisatin. Therefore, other avenues needed to be explored in order to produce a reaction between styrylisatin and amino acids at a concentration more representative of the concentration observed in latent fingermarks.

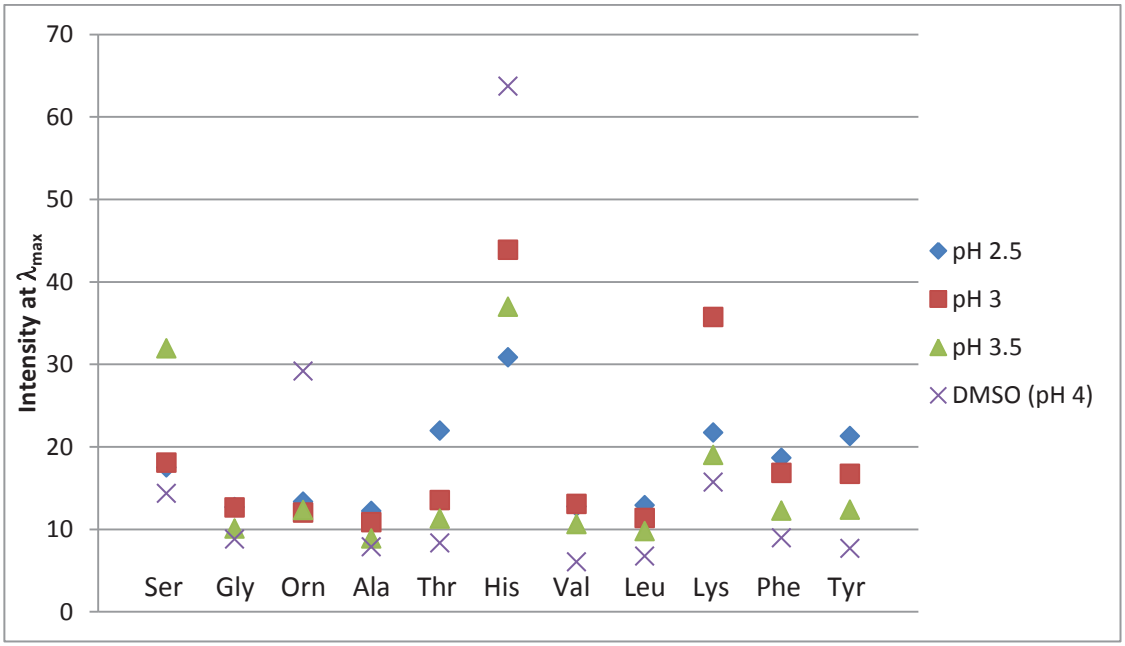


Figure 4-13: Peak luminescence emission intensities for styrylisatin at different pH reacted with the chosen amino acids (see Table 4-1 for abbreviations).

4.4.4 Alternative Solvents and Solvent Systems for Styrylisatin

An issue was the risks associated with the use of DMSO as a carrier solvent. DMSO is hazardous when inhaled or when in contact with the skin. Attempts to use alternate solvents

yielded poor results as very few solvents were able to dissolve styrylisatin completely. Long heating times were required to completely evaporate all the solvent off the paper, which in some cases caused the styrylisatin applied areas to turn a dark brown colour. Less toxic alternatives such as diethyl ether, DMF and THF were trialled but solubility was still quite poor. Since an alternate solvent could not be used in this instance, the next stage was to minimise the amount of DMSO used in the working solution. This was performed by dilution with methanol, as styrylisatin was still slightly soluble in methanol; methanol is volatile and is less hazardous than other solvents trialled. Methanol is also used in the DFO formulation and has no significant effect on amino acids; it was therefore deemed a suitable solvent in this case. Six solutions were prepared (Table 4-6) with the same concentration of styrylisatin (0.008 M). This concentration was chosen as it was the least effective when using DMSO only and therefore any improvement could be attributed to the new solvent system. Luminescence emission spectra were collected and luminescence images taken of each sample for comparison.

Table 4-6: Styrylisatin dilution study solutions.

Solution	Volume of DMSO (ml)	Volume of MeOH (mL)
1	5	0
2	4	1
3	3	2
4	2	3
5	4	1
6	0	5

Dilution with methanol did not precipitate styrylisatin out of the solution. When applied to the amino acid spots, luminescence emission was seen for all the amino acids tested, indicating that methanol did not change the reactivity of styrylisatin. The results shown in Figure 4-14 and Figure 4-15 indicate that methanol was effective in diluting the DMSO solution and also increased the sensitivity. These results also indicated that the methanol solution on its own was able to develop strong luminescence emission (when compared to the DMSO solution) for the majority of amino acids tested. Increasing the amount of methanol in the solution also increased the clarity of the amino acid spots, as can be seen in Figure 4-15a and Figure 4-15f. This would indicate that the DMSO caused the amino acids to

diffuse on the paper which, in the case of fingermark detection, could potentially eliminate ridge detail. It should be noted that the methanol solution (solution 6) gave the higher amount of background staining and the visible colour changes reported in Table 4-4 were not seen for the methanol-only solution. All luminescent amino acid spots were a similar yellow colour to those exhibited by only some of the amino acids when reacted with the DMSO solution. There were no pink spots developed when methanol was used.

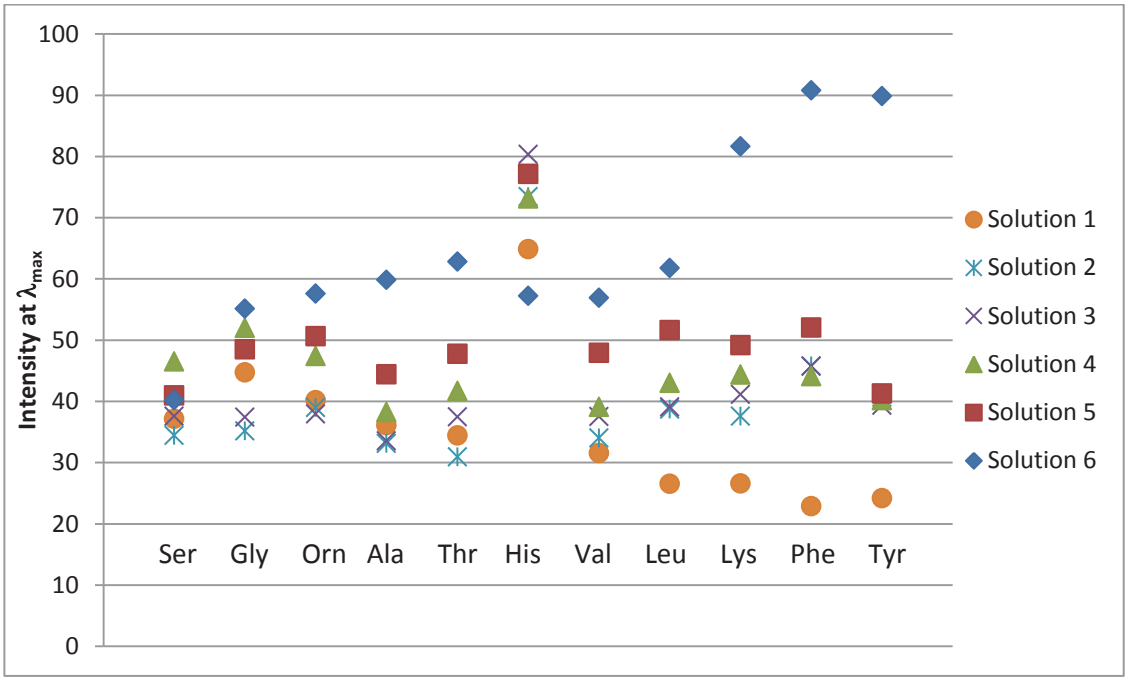


Figure 4-14: Luminescence emission peak intensity for amino acids treated with styrylisatin with different DMSO:MeOH ratios (See Table 4-1 for abbreviations).

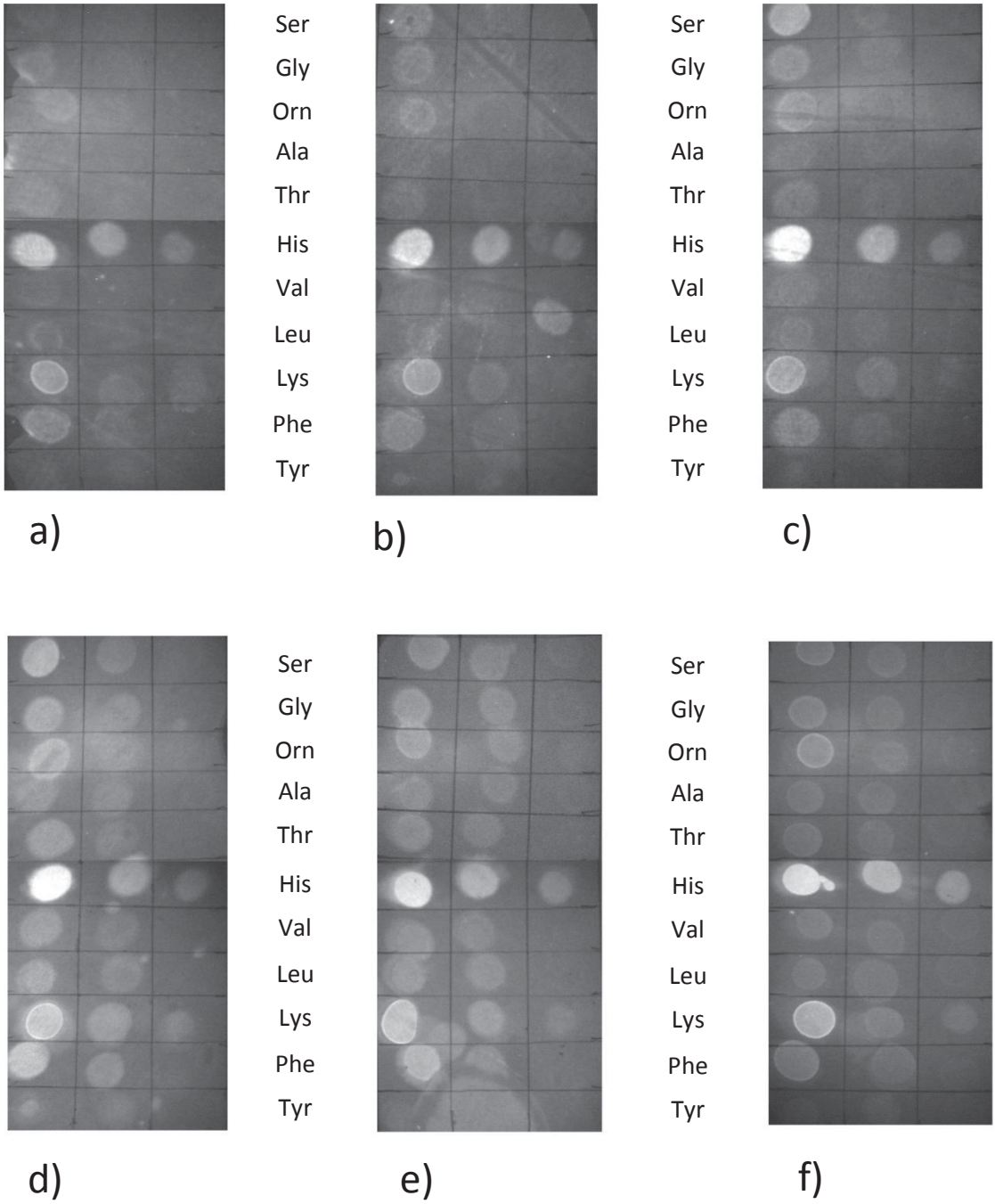


Figure 4-15: Styrylisatin developed amino acid spots viewed in the luminescence mode (excitation 485-590 nm, barrier longpass filter 715 nm) - a) Solution 1, b) Solution 2, c) Solution 3, d) Solution 4, e) Solution 5, f) Solution 6. (see Table 4-1 for abbreviations).

4.4.5 Optimisation of Styrylisatin Development Solution and Conditions

The methanolic styrylisatin solution was able to increase sensitivity for the amino acids with 0.001 M amino acids giving some degree of luminescence emission; however, there was an increase in the amount of background luminescence emission observed for samples treated with this formulation. Lower concentration solutions were trialled (Table 4-7) Table 4-1 and applied to amino acid spots on filter paper.

Table 4-7: Styrylisatin concentrations in methanolic solutions.

Solution No.	Concentration (M)
1	6.4×10^{-3}
2	5.6×10^{-3}
3	4.8×10^{-3}
4	4.0×10^{-3}
5	3.2×10^{-3}
6	2.4×10^{-3}

While decreasing the concentration did increase the contrast between the amino acid spot and the background, the sensitivity also decreased at concentrations lower than 4.0×10^{-3} M. These solutions also showed a decrease in preferential development, which was observed with the DMSO based samples, with each amino acid giving similar luminescence emission intensities. The solution that gave the best compromise between sensitivity and contrast was the 4.0×10^{-3} M solution. Each solution was pipetted onto a latent fingermark on filter paper to see if it could be detected; however, no luminescence emission was observed under these conditions.

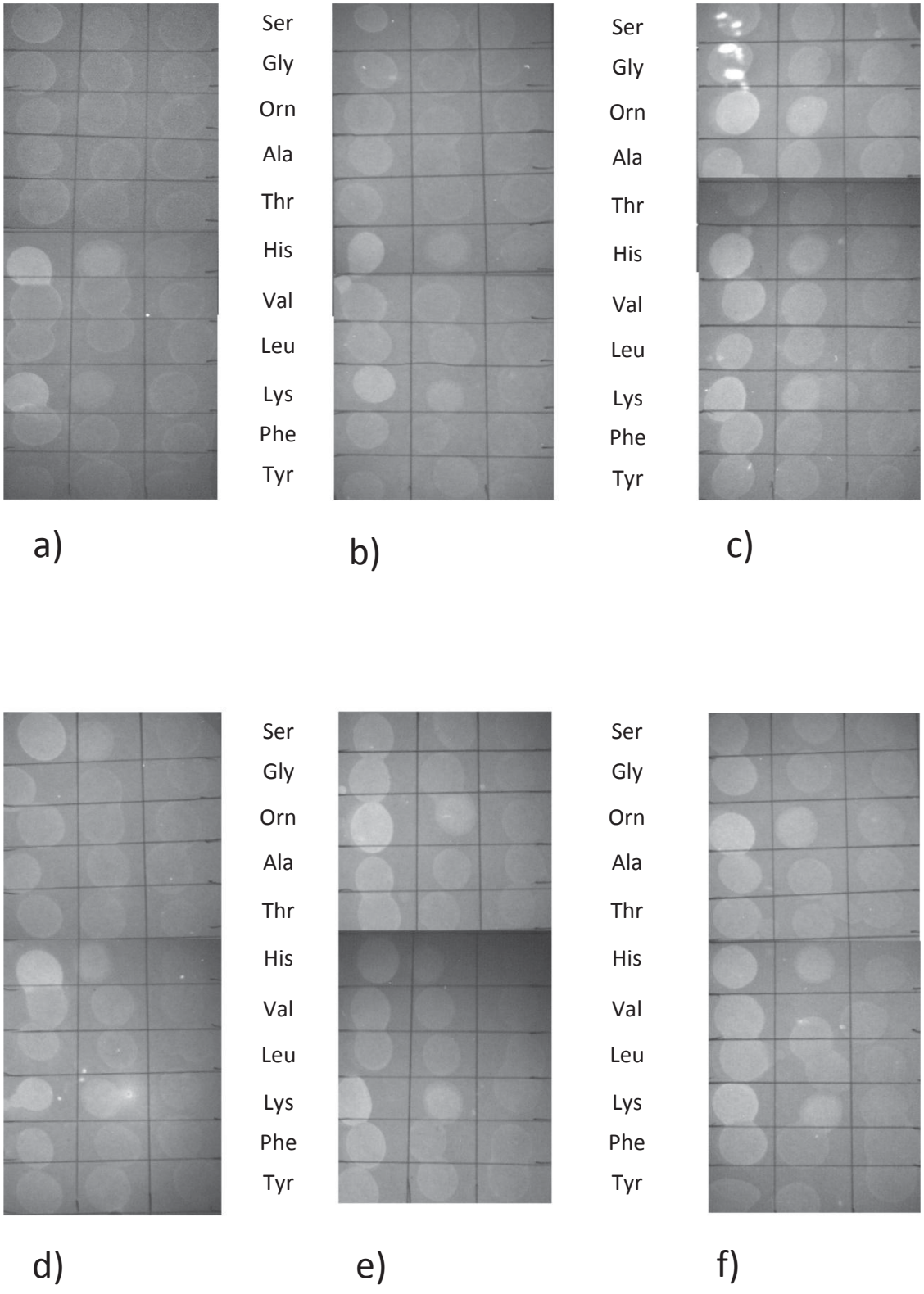


Figure 4-16: Amino acid spots developed with methanol-based styrylisatin solutions viewed in the luminescence mode (excitation 485-590 nm, barrier longpass filter 715 nm) - a) 6.4 x 10^{-3} M , b) 5.6 x 10^{-3} M, c) 4.8 x 10^{-3} M, d) 4.0 x 10^{-3} M e) 3.2 x 10^{-3} M, f) 2.4 x 10^{-3} M. (see Table 4-1 for abbreviations).

The temperature at which the amino acid spots were developed was also optimised. Heating is required for indanedione and DFO development to accelerate the reaction. Previous samples had been heated at 180 °C using a heat press. This is a quick and efficient way of heating the sample. However, using this method tended to increase the amount of background staining and the unreacted styrylisatin darkened the paper, reducing contrast between the amino acid spots and the background. To assess the effectiveness of temperature, treated samples were developed in an oven for 30 minutes at different temperatures (Table 4-8) and viewed in luminescence mode for comparison.

Table 4-8: Styrylisatin development temperature experimental conditions.

Sample Number	Development Temperature (°C)
1	100
2	120
3	150
4	180

Temperature was found to be a significant factor in the development of the amino acids spots, with higher development temperatures giving more intense luminescence emission. This is shown in Figure 4-17, where contrast improved with increasing temperature. It should also be noted that sample 4 (Figure 4-17d) was imaged using a 600 ms exposure time, which, compared to the 1.4 second exposure time for the other samples tested, indicates a significant increase in luminescence emission intensity. Oven development generally decreased the amount of background interference observed when compared to samples developed with the heat press. This could potentially be due to direct contact with the heat source over-developing the styrylisatin. When samples were left in the oven for over one hour, over-development was beginning to be observed, which reduced contrast. Samples left in the oven for longer than one hour darkened to a brown colour and there was no contrast observed between the amino acid spot and the background.

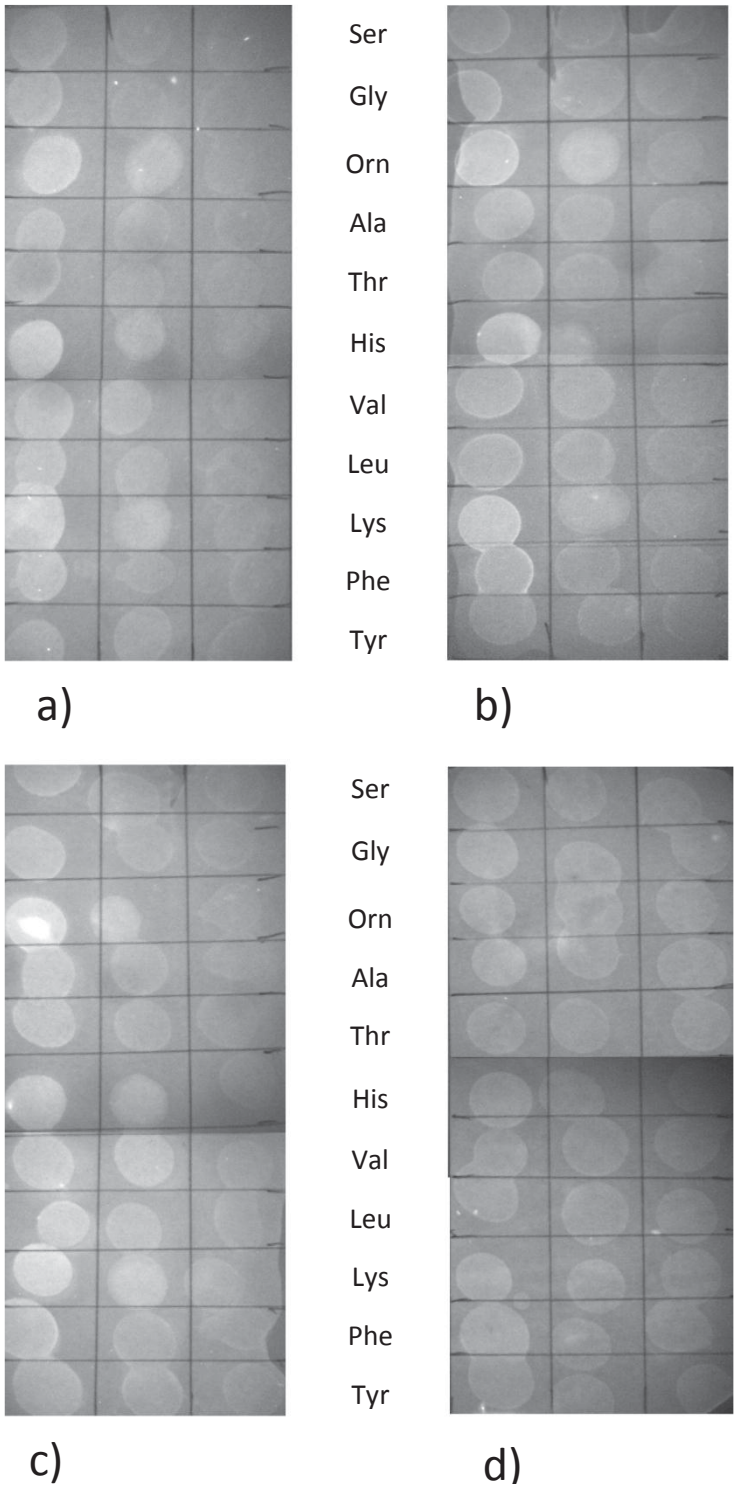


Figure 4-17: 4.0 x 10^{-3} M styrylisatin in MeOH spot tests viewed in the luminescence mode (excitation 485-590 nm, barrier longpass filter 715 nm). Development temperature - a) 100 °C, b)120 °C, c) 150 °C, d)180 °C (with a 30-minute development time). NB Samples a,b,c had exposure times of 1.4 seconds, sample d had an exposure time of 600 ms.

An eccrine charged fingermark (hand was placed in a latex glove for 15 minutes before deposition) was deposited on each sample being developed at a different temperature. For samples three and four, weak development was observed. While 150 °C produced no ridge detail, the outline of a fingermark could be seen. The 180 °C sample, however, showed partial ridge detail and acceptable contrast between the substrate and the fingermark (Figure 4-18). These results were very preliminary, but showed that development was possible with heavily eccrine charged fingermarks.

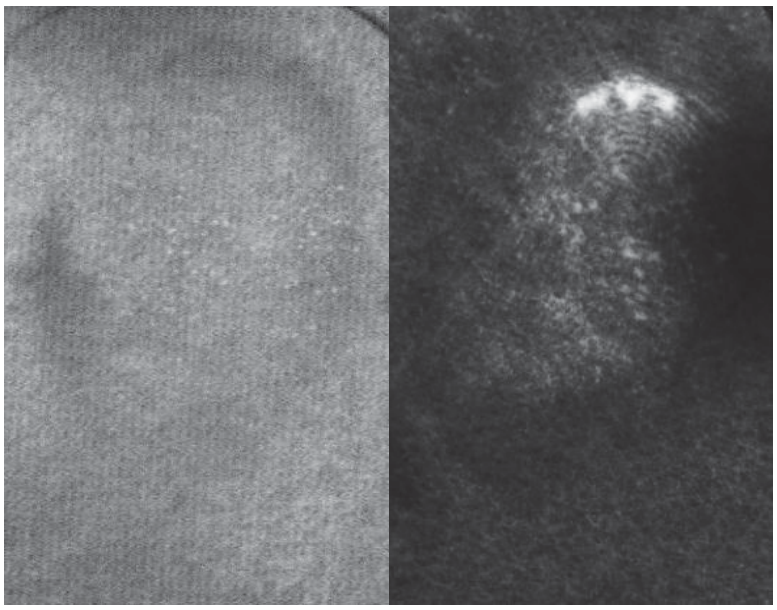


Figure 4-18: Styrylisatin developed fingermarks viewed in the luminescence mode (excitation 485-590 nm with a 715 nm barrier longpass filter), (left) developed at 150 °C, (right) developed at 180 °C.

Since styrylisatin was able to partially develop a fingermark, it was thought that post-treatments such as metal salt addition may assist the reaction or form coordination complexes. For luminescence emission examination, most fingermark detection techniques rely on the addition of a metal salt post treatment (though it is only essential for ninhydrin) to enhance developed fingermarks. Zinc chloride is the most common metal salt post treatment (in Australia), in the case of ninhydrin development, it is applied after the ninhydrin solution has dried to create a luminescent complex. In the case of indanedione-zinc, the zinc acts as a Lewis acid catalyst which promotes the reaction to completion. Both -treatments were attempted using the zinc chloride formulations provided by Stoilovic and Lennard [28]. The only difference observed with the metal salt post treatment was a darkening of the 0.1 M amino acid spots; luminescence was not affected at the lower concentrations. Cooling the samples (as per ninhydrin development) did not affect the luminescence intensity.

The final factor that might have increased the potential of styrylisatin for fingermark development was the addition of a buffer to control the pH of the development solution. As previously discovered, alkali solutions of styrylisatin converted it to its salt form, which would not be ideal for development. Three solutions of styrylisatin were prepared using a sodium acetate/acetic acid buffer for a pH range of 4-6. Altering the pH did not greatly alter the effectiveness of the styrylisatin solution in reacting with amino acids as each solution gave similar sensitivity for the amino acid spots (Figure 4-19). Increasing the pH, however, did lead to an increase in background luminescence, which reduced contrast (Figure 4-19c). This is potentially due to the formation of the styrylisatin salt that would not react with amino acids and would just stain the filter paper. The solutions were also more prone to 'charring' than for the other samples tested. The addition of a buffer caused the paper to brown faster in the oven, which essentially destroyed the samples tested.

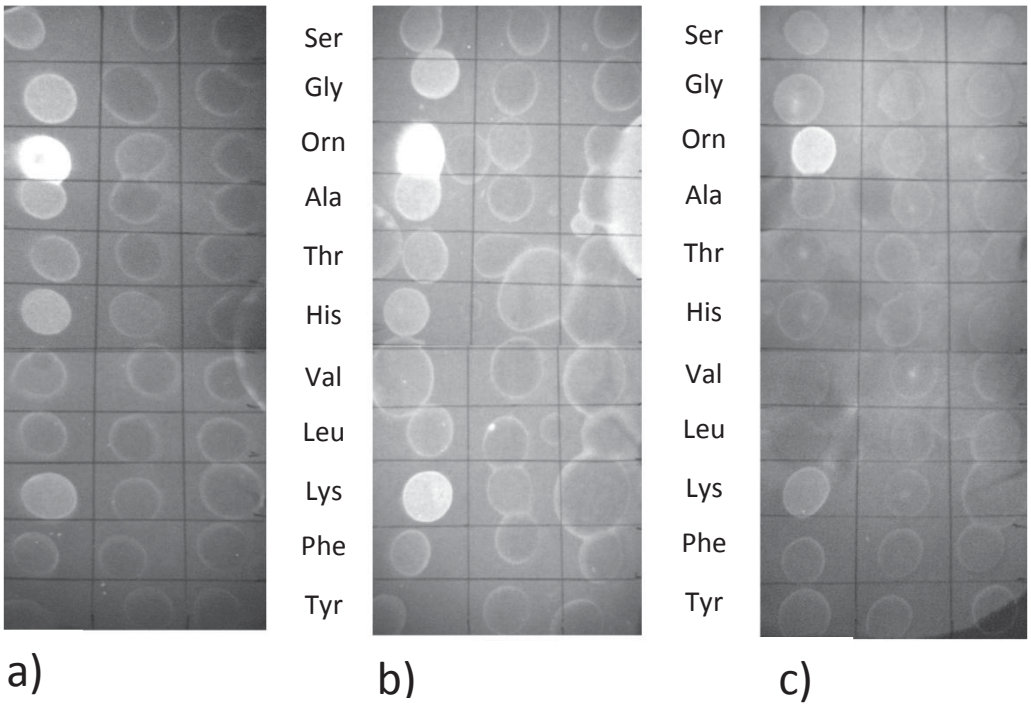


Figure 4-19: 4.0 x 10^{-3} M styrylisatin in MeOH spot tests viewed in the luminescence mode (excitation 485-590 nm, barrier longpass filter 715 nm). pH of solution - a) 4, b) 5, c) 6.

A comparison between eccrine and sebaceous charged fingermarks was also performed to determine if styrylisatin was able to target other components of the fingermark. The sebaceous mark was prepared by rubbing three fingers on the nose and forehead before depositing a fingermark on the paper. Eccrine charged marks were prepared by keeping the hand in a glove for fifteen minutes before depositing a mark on the paper. The sebaceous fingermark was unable to be developed, whereas the eccrine mark gave good ridge detail. Natural marks (no charging of the fingers with secretions) were unable to be developed (Figure 4-20). This result is not unexpected as the sebaceous components of fingermarks contains very little amino acids and is predominately composed of fatty acids and oils (Table 1-1). However, for the technique to be considered for the routine detection of latent fingermarks, it needs to be able to detect low concentrations of amino acids. On this requirement styrylisatin lacks the necessary sensitivity to be considered a feasible chemical enhancement reagent for latent fingermarks on porous surfaces.

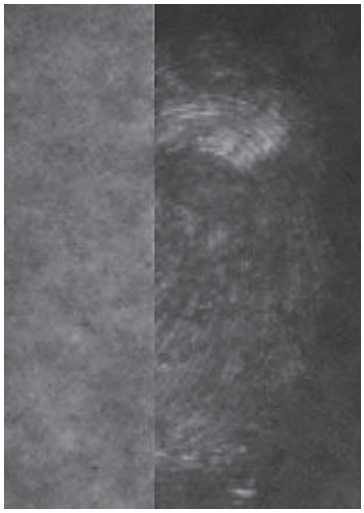


Figure 4-20: Fingermarks developed with styrylisatin solution viewed in the luminescence mode(excitation 485-590 nm, barrier longpass filter 715 nm). (left) Sebaceous charged fingermark, (right) eccrine charged fingermark.

This result confirmed that styrylisatin was unsuitable for the detection of amino acids in latent fingermarks. The major factor that attributed to this was the fact that only highly eccrine charged marks could be detected with this technique, while natural marks (i.e. marks that would be generally encountered in casework) gave no results. It was hypothesised that the concentration of amino acids had to be very high in order for a luminescent product to be

formed. This would explain the luminescence emission of amino acid spots and strongly charged eccrine marks, but not natural and sebaceous charged fingermarks. The final step was to determine if the luminescence occurred due to a reaction between styrylisatin and an amino acid or if it was precipitation of unreacted styrylisatin. An experiment was conducted to test this hypothesis, the results of which are shown in Figure 4-21. These results would indicate that styrylisatin does react with amino acids, not water. However, the unreacted styrylisatin did give weak luminescence emission. Based on this observation, a comparison between serine spots at different concentrations and blank styrylisatin was performed. The results of this experiment are shown in Figure 4-22, amino acids with a concentration lower than 0.1 M exhibited significantly lower luminescence emission. Similarly both the blank and H_2O spots gave very similar luminescence to the lower concentration amino acid spot tests. This would indicate that styrylisatin is not an effective amino acid sensitive technique as it is not readily able to react with amino acids at more representative concentrations to those found naturally in fingermarks. This result explains the development of eccrine charged fingermarks, as they would have a higher concentration of amino acids than natural or sebaceous charged fingermarks.

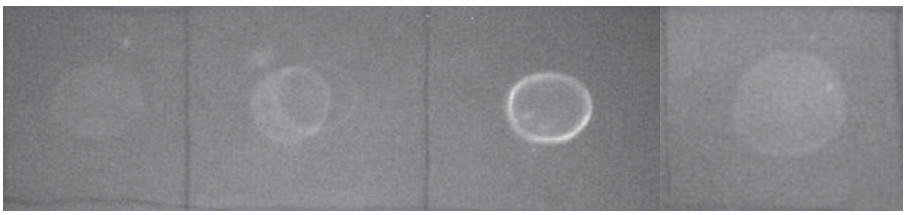


Figure 4-21: Images of (l-r) styrylisatin in DMSO, styrylisatin in MeOH with water spot, styrylisatin in MeOH with 0.01 M serine spot, Styrylisatin in MeOH spot on filter paper viewed in the luminescence mode with an excitation of 485-590 nm and barrier longpass filter 715 nm.

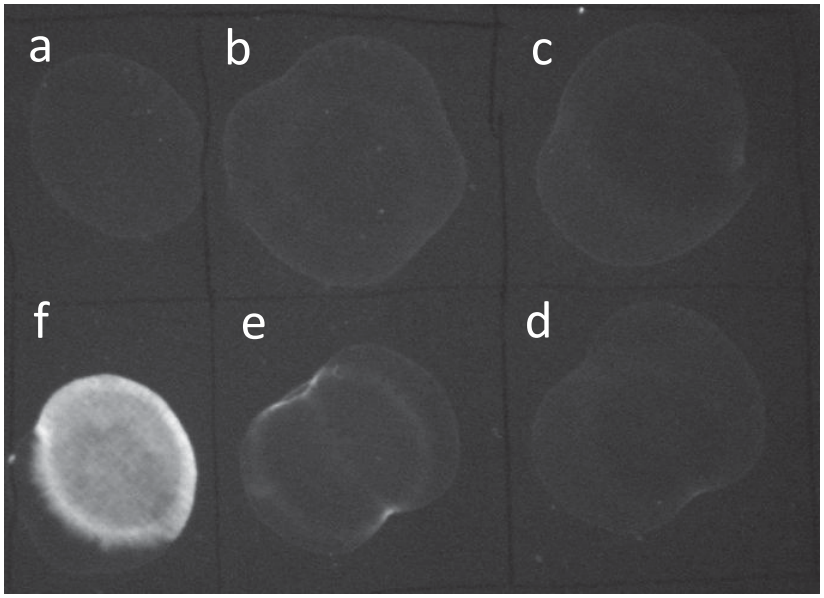


Figure 4-22: Styrylisatin reactivity comparison viewed in the luminescence mode with an excitation of 485-590 nm and barrier longpass filter 715 nm (a) deionised water , (b) blank, (c) 0.0001 M serine, (d) 0.001 M serine, (e) 0.01 M serine, (f) 0.1 M serine.

Attempts were made to characterise the reaction product between styrylisatin and serine. This was performed by mixing styrylisatin and serine in a 1:1 mole ratio and removing aliquots of the reaction solution. Aliquots were taken after: initial mixing, 30 minutes of stirring at room temperature, 15, 30 minutes and 1 hour after heating at 180 ^{o}C. These samples were dried under a stream of nitrogen and analysed by NMR. However, a characteristic spectrum could not be obtained for proton or carbon NMR. Generally there was no change in appearance of the solution or in the NMR peak positions.

4.4.6 Styrylisatin and NIR Luminescent Amino Acid Sensitive Techniques.

Styrylisatin was used in this study as it was believed that the additional conjugation to an already established amino acid sensitive reagent (isatin) would give a reaction product that was luminescent in the NIR region. Based on the results of this study, a potential reaction product was obtained; however, it was only formed with very high amino acid concentration spots. This section will explore potential reasons for the low sensitivity of styrylisatin to amino acids and outline potential challenges with finding a NIR luminescent amino acid

sensitive technique. Firstly, the size of the molecule plays a significant role in its reactivity. Conventional amino acid sensitive techniques such as ninhydrin and indanedione are small indane molecules substituted with three and two keto- groups respectively, on the 5-membered rings. In both cases, the molecules themselves are essentially colourless and non-luminescent; however, when reacted with amino acids they form a highly conjugated molecule that is a coloured product (for ninhydrin) or a coloured-luminescent product (for indanedione).

These factors make these reagents very effective for fingermark detection. Conversely, styrylisatin (and potentially any NIR luminescent amino acid sensitive technique) requires higher degrees of conjugation in order for the final reaction product to be luminescent in the NIR region. This directly affected two factors relating to fingermark detection; firstly, the molecule needs to be small enough so that it can orientate itself to react with the amino acids present. Styrylisatin has a stilbene and benzene chain attached to the reactive isatin group which makes the molecule very bulky (compared to other reagents of this type). A hypothesised reaction product between styrylisatin and amino acids would occur via a Strecker degradation pathway, which is very dependent on molecular geometry to proceed to completion. The extended conjugation in styrylisatin almost doubles the size of the molecule (compared to isatin), which significantly decreases the chance of a successful collision between the amino acid and styrylisatin and the chance for the reaction to proceed to completion. Therefore low concentration amino acid solutions/spots would have a lower probability of a successful reaction product forming due to the size of styrylisatin. Increasing the concentration of styrylisatin would increase the chances of a successful collision; however, as was observed in this study, increasing the styrylisatin concentration leads to an increase in background staining and overall reduced contrast between the reacted amino acid and the background.

It was observed that increasing the heat of the reaction tended to increase the formation of the reaction product, which is consistent which conventional reaction mechanics. However, samples were not heated above 180 ^{o}C due to the potential thermal development of fingermarks/amino acids [148] which would have given misleading results and the potential destruction of the paper substrate. This observation highlights a significant drawback of any potential fingermark detection technique with high levels of conjugation that require large molecular sizes. A compromise has to be made between molecule size/degree of conjugation before it can be deemed a suitable reagent for fingermark detection. This becomes a problem

for amino acid sensitive reagents for the NIR detection of latent fingermarks since the degree of conjugation is a major factor in the ability of the reaction product to be visualised in the NIR.

The majority of NIR luminescent materials are used as dyes for lasers or biological applications. These materials are also coloured, which limit their use for fingermark detection. As previously mentioned, the ideal fingermark detection method has a non-luminescent/non-coloured starting material and a luminescent/coloured final reaction product. For a NIR luminescent reaction product to be formed, the starting material needs to have some degree of conjugation in order to ensure that the reaction product will be NIR luminescent. This is problematic as the colour/luminescence of the starting material and the final reaction product needs to be sufficiently different so that high contrast fingermarks can be obtained under both luminescence and non-luminescence conditions. Similarly, the additional conjugation makes the starting material more likely to stain the surface; therefore, in order to obtain suitable contrast between the unreacted amino acid sensitive reagent and the reaction product, there has to be a significant change in the overall molecular structure. The addition of conjugation alone will be unlikely to provide enough difference between the reacted and unreacted material to give suitable contrast. If a computational chemistry approach was to be taken, increasing the reactive sites on a highly conjugated molecule would decrease the effect of molecular geometry. However, the synthesis of molecules with multiple reactive sites increases the difficulty in sourcing and synthesising these materials as well as increasing the probability of unwanted side reactions.

Styrylisatin is a highly conjugated analogue of isatin, which has previously been shown to develop fingermarks [141]; however, the additional conjugation of styrylisatin severely affected the solubility and reactivity towards amino acids. Isatin was shown in the previous study to be soluble in a number of solvents suitable for fingermark detection. However, styrylisatin was insoluble in many organic solvents, which limited its ability to be applied to latent fingermarks. DMSO was the only solvent that readily dissolved styrylisatin, and methanol would only dissolve it in very low concentrations. As explained previously, this was not ideal for promoting a reaction between styrylisatin and amino acids. Similarly, the addition of carrier solvents such as HFE and HFC could not be performed as they would precipitate styrylisatin out of solution. Based on this study, any future work examining the application/testing of a NIR luminescent fingermark detection reagent, should firstly examine its chemical properties before assessing its suitability for latent fingermark detection.

Another potential reason for the poor solubility and reactivity was the heterocyclic N ring present in both isatin and styrylisatin.

Potentially the best use of a NIR luminescent method for the detection of latent fingermarks may be by targeting other endogenous components of the latent fingermarks such as proteins or fatty acids. Ideally, a NIR alternative to physical developer would be a more advantageous application for NIR detection. The lipid dye nile red, has been shown to be an effective alternative to physical developer [149] and nile blue (an analogue of nile red, which has also been used for histological applications) has been used for magnetic powdering to give NIR luminescence [150]. These approaches are worthy of further investigation as an alternative means of inducing NIR luminescence in latent fingermarks.

4.5 Conclusions

Styrylisatin is a highly conjugated analogue of isatin, which has previously been used for biological applications. This study attempted to use styrylisatin for the detection of amino acids in latent fingermarks deposited on porous surfaces. While a luminescent product was formed, it was only formed with very high amino acid concentrations (0.1 M) after heating to about 160 ^{o}C. A range of different solvents, pH's and reaction conditions were trialled and, while this increased the limit of detection, only highly eccrine charged fingermarks could be visualised. Styrylisatin was only readily soluble in DMSO and methanol in very low concentrations, and could not be diluted in carrier solvents such as HFC and HFE, which also limits its use on latent fingermarks. Attempts to characterise the reaction product between styrylisatin and serine were made; however, the product could not be suitably identified. Based on the results obtained, it was determined that styrylisatin was unsuitable for latent fingermark detection at this time.

The NIR detection of latent fingermarks on porous surfaces is potentially highly advantageous as substrate interferences such as printing and background luminescence would be essentially negated. This study has shown that, in order for an effective amino acid sensitive technique to be produced, there needs to be a careful balance between the amount of conjugation to shift the reaction products luminescence emission into the NIR region and the size of the molecule to ensure that the molecule can remain soluble in common solvents and

can readily undergo a reaction with amino acids with a high level of sensitivity. Alternatively it may be better to target other components of the fingermark, such as proteins or sebaceous components, to induce NIR luminescence.

Chapter 5: Assessment of PolyCyano UV & sequencing of STaR 11 for cyanoacrylate staining

Chapter 5: Assessment of PolyCyano UV & Sequencing of STaR 11 for Cyanoacrylate Staining

5.1 Introduction

5.1.1 Development of Latent Fingermarks by Cyanoacrylate

The cyanoacrylate fuming technique, effective on most non-porous substrates such as glass and plastic was first developed by the Criminal Identification Division of the Japanese National Police agency in 1978. Since the initial reporting of the cyanoacrylate (Super Glue) fuming method in the United States in 1982, the method has become a prominent means of detecting latent fingermarks on non-porous surfaces [7, 151]. When a fingermark is exposed to cyanoacrylate in the vapour form, a white polymer forms along the fingermark ridges with very little material deposited on the substrate [152]. The polymerisation mechanism is divided into three steps: initiation, propagation and termination. The reaction is initiated by a medium-hard nucleophile (such as fatty acids and proteins) [153], in which the nucleophile attacks the first carbon containing the double bond, with an electron transferring to the double-bonded oxygen. The negative charge is then held at the second carbon and distributed between the cyano and ester groups. Water acts as a catalyst for the polymerization process; as the cyanoacrylate polymer grows it, in turn, serves as the nucleophile and continues to propagate the polymerization by attacking the cyanoacrylate monomer. These steps are illustrated in Figure 5-1. The polymerization process is terminated either when the monomer supply is exhausted or the propagating anions react with a terminating agent such as a positively charged hydrogen ion [151, 153].

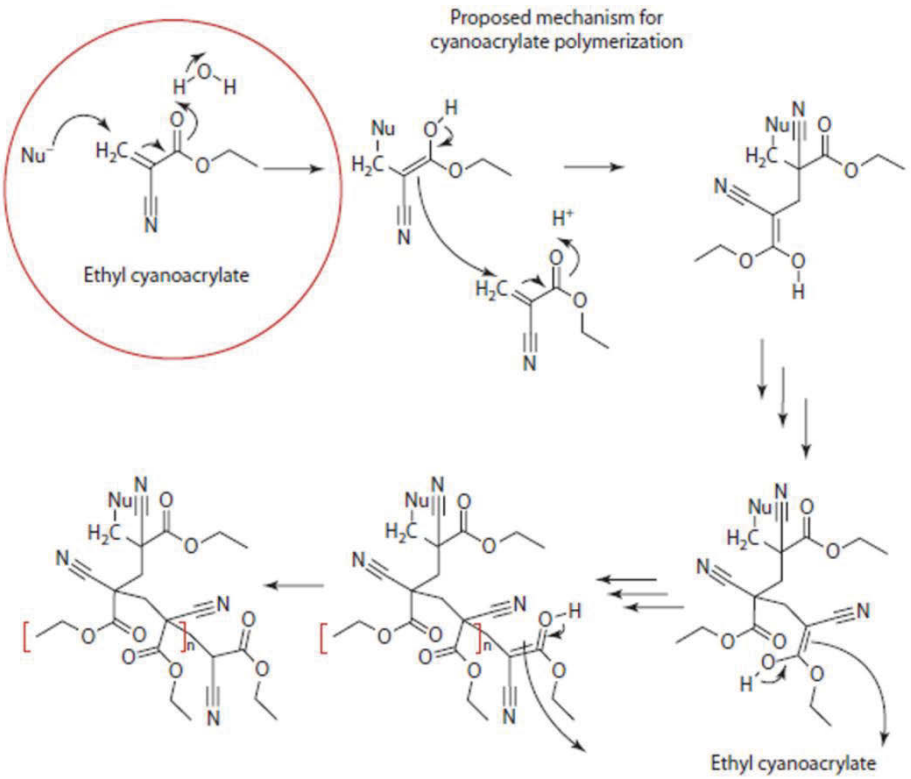


Figure 5-1: Proposed mechanism of cyanoacrylate polymerisation [151, 153].

This method is very successful at developing latent fingermarks and is the most widely used process for the laboratory development of fingermarks on non-porous surfaces [7]. However, there are several factors that have been found to greatly affect the success of these methods including the environmental conditions the exhibit was stored under and the initial composition of the fingermark. There have been several studies into negating these factors including: altering the vaporising temperature, re-humidifying the sample and applying a chemical pre-treatment to the fingermark [152, 154, 155].

A recent study by McLaren *et al.* examined a process to reintroduce moisture to a dried fingermark to improve cyanoacrylate development [94]. The results from this study found that a pre-treatment with the vapour from 10% w/v aqueous methylamine solution significantly improved subsequent cyanoacrylate development. It was hypothesised that the observed improvement in cyanoacrylate development after methylamine pre-treatment was

due to two main effects; the action of methylamine as a Lewis base initiator; and an increase in water content [94]. This work was confirmed in another study performed by Montgomery *et al.* who found that methylamine pretreatment improved the polymer morphology of the cyanoacrylate developed fingermarks. This improvement was found to give a similar polymer structure to cyanoacrylate developed fresh fingermarks [156]. However, the health and safety issues associated with methylamine (highly flammable and corrosive) are an issue that would need to be addressed before implementing such a treatment into an operational setting. While there has been a significant amount of research into the basic chemical properties of cyanoacrylate, Lewis highlights that there is a need to optimise the fuming method in a manner than supports high detection efficiency and developed print quality [151].

5.1.2 Cyanoacrylate Enhancement Techniques

Although cyanoacrylate is a widely used and reliable fingermark detection and enhancement technique the developed marks often lack contrast and can be difficult to visualise, particularly on light coloured surfaces. Techniques to increase contrast include FTIR imaging [36], fingerprint powder [15] and - most commonly - applying a luminescent stain. The advantage of using a luminescent stain is that the dyes will be trapped in the cyanoacrylate polymer, significantly enhancing contrast. Different stains can also be used depending on the surface [22]. Commonly used stains include rhodamine 6G, Ardrox® and basic yellow 40, each with different excitation and emission wavelengths. There has been a wide range of studies examining the effectiveness of these dyes as well as proposing alternative methods and dye mixtures [60, 61, 157].

The concept of a luminescent cyanoacrylate polymer has been investigated since the 1980s; however there was very little success. The first reported successful one-step cyanoacrylate fuming process was by Weaver and Clary who combined methyl cyanoacrylate with dye from the styryl family. This work was published and patented, the resulting product is marketed as CN-Yellow™ [158]. Another commercial product is Lumicyano™, which is available through Global Forensics Ltd. Both products are a luminescent cyanoacrylate that can be excited under UV light to give a luminescent fingermark. Due to availability these products were not

tested, however, it would advantageous in future studies to perform a comparison between each luminescent cyanoacrylate treatment.

PolyCyano UV is a one-stage glue/stain process for developing luminescent fingermarks without the need for further chemical treatment; after fuming, developed marks can be imaged using long-wave UV light. This product is developed and sold through Foster + Freeman, this product was chosen due to its availability and the emergence of its use in operational forensic laboratories. This technique has several advantages: the one-step fuming and staining process is more efficient, minimises the use of hazardous chemicals and could be used on surfaces that cannot be stained (i.e. semi porous surfaces). A similar method was proposed by Takatsu, who used p-dimethylaminobenzaldehyde (DMAB) crystals as a vapour staining method of visualising cyanoacrylate developed fingermarks. However they proposed it as a two-step method, DMAB exhibited strong luminescence under UV radiation [159]. The material safety and data sheet for PolyCyano UV confirms that DMAB was used in this product [160]. This product was previously evaluated by Hahn and Ramotowski and compared to different luminescent cyanoacrylate stains. This study found that the PolyCyano UV produced luminescent fingermarks of comparable quality to the stained fingermarks on a range of different surfaces. Similar to conventional cyanoacrylate the effectiveness of PolyCyano UV was found to be very dependent on the surface itself [161]. A limitation of this study was that it used a modified fuming cabinet to deliver the PolyCyano UV powder into the glue dish and did not use the commercially available equipment.

5.1.3 Aims and Objectives

PolyCyano UV is a commercially available luminescent cyanoacrylate and requires validation before it can be accepted into operational forensic laboratories. Since this is a newly developed technique, the performance of PolyCyano UV in a sequence should also be determined as the luminescent properties of PolyCyano UV may interfere with the luminescence of any cyanoacrylate stains subsequently employed. The aim of this study was to determine how effective PolyCyano UV was in developing marks when compared to conventional superglue fuming. This was performed by optimising the fuming conditions then comparing the technique to conventional cyanoacrylate (Cyanobloom). Fingermarks developed using both cyanoacrylates were examined under a range of different conditions

including white light examination, UV excitation (for PolyCyano UV), post rhodamine and STaR 11 staining, and by developing fingermarks aged up to two months. A secondary aim of this study was to determine how well STaR 11 performed in a sequence with other staining techniques and whether the luminescent strength of STaR 11-developed marks was affected by other pre-treatments.

5.2 Materials

5.2.1 Reagents

PolyCyano UV and Cyanobloom were purchased through Foster + Freeman and used as supplied.

Rhodamine 6G dye content 99% [CAS 989-38-8] was obtained from Sigma Aldrich and used as supplied

Styryl 11 [CAS 92479-59-9] was obtained through Exciton/Lastek and used as supplied.

Reagent grade isopropanol [CAS 67-63-0] and methyl ethyl ketone [CAS 78-93-3] were obtained through Chem-Supply and used as supplied.

5.2.2 Instrumentation

A Varian Cary Eclipse fluorescence spectrometer was used for measuring the luminescence of the pre- and post-fumed PolyCyano UV.

A Foster + Freeman MVC1000/D cyanoacrylate fuming cabinet was used to fume all samples throughout the study.

A Foster + Freeman VSC 6000 was used to image all the developed samples.

5.3 Methods

5.3.1 General Approach

This study was divided into two sections; (i) optimisation of development conditions and (ii) a donor and sequencing study. This first section aimed to compare different development conditions to the reported optimised conditions to determine whether this had an effect on the development quality and luminescence of the developed fingermarks. Once the conditions were optimised, PolyCyano UV was compared to Cyanobloom (a non-luminescent Foster + Freeman cyanoacrylate product) and stained with different luminescent stains in a sequence. The cyanoacrylates were compared at different stages of the staining sequence based on the quality of development of fingermark ridges and contrast between the fingermark and substrate.

5.3.2 Optimisation of Development Conditions

Table 5-1 outlines the manufacturer's recommended development conditions for PolyCyano UV; for the optimisation study, only the mass, humidity and fuming times were altered. Temperature was not changed as there were only two temperatures available on the MVC 1000/D cabinet (180 ^{o}C and 230 ^{o}C) and the lower temperature would not completely vaporise the PolyCyano UV.

The recommended settings were used as a baseline comparison (Table 5-1), each parameter was optimised individually; for initial tests (Table 5-2). Fresh marks (charged and natural) from a single donor were deposited on glass, aluminium foil, Fanta® soft drink cans and polyethylene bags in duplicate. Luminescence spectroscopy and thermogravimetric analysis was performed on PolyCyano UV powder to determine the most effective visualisation and heating conditions.

Table 5-1: Parameters Recommended by Foster + Freeman for PolyCyano UV.

Parameter	Recommended Value
Temperature (°C)	230
Mass of PolyCyano UV (g)	0.6 (for MVC1000/D cabinet)
Humidity (%)	80
Fuming Time (minutes)	25
Visualisation Conditions (nm)	Ex = 365, Em = 415-510

Table 5-2: Parameters used for optimisation.

Mass of PolyCyano UV (g)	Relative Humidity (%)	Fuming Time (min)
0.5	70	15
0.6	75	25
0.7	80	35

5.3.3 Donor and Sequencing Study

For the sequencing study, four donors (two male and two female) deposited three sebaceous charged and natural fingermarks on three surfaces: aluminium, glass and polyethylene bags. Samples were aged from fresh to two months. Each set of three marks was split into two with one half developed with PolyCyano UV and the other half developed with Cyanobloom (fumed under the same conditions as PolyCyano UV) (Figure 5-2). After fuming, each developed sample was imaged under a range of imaging conditions and compared according to Figure 5-3.

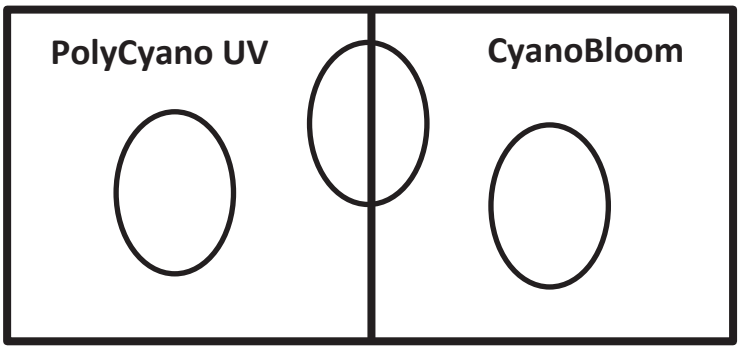


Figure 5-2: Preparation of samples for donor and sequencing study.

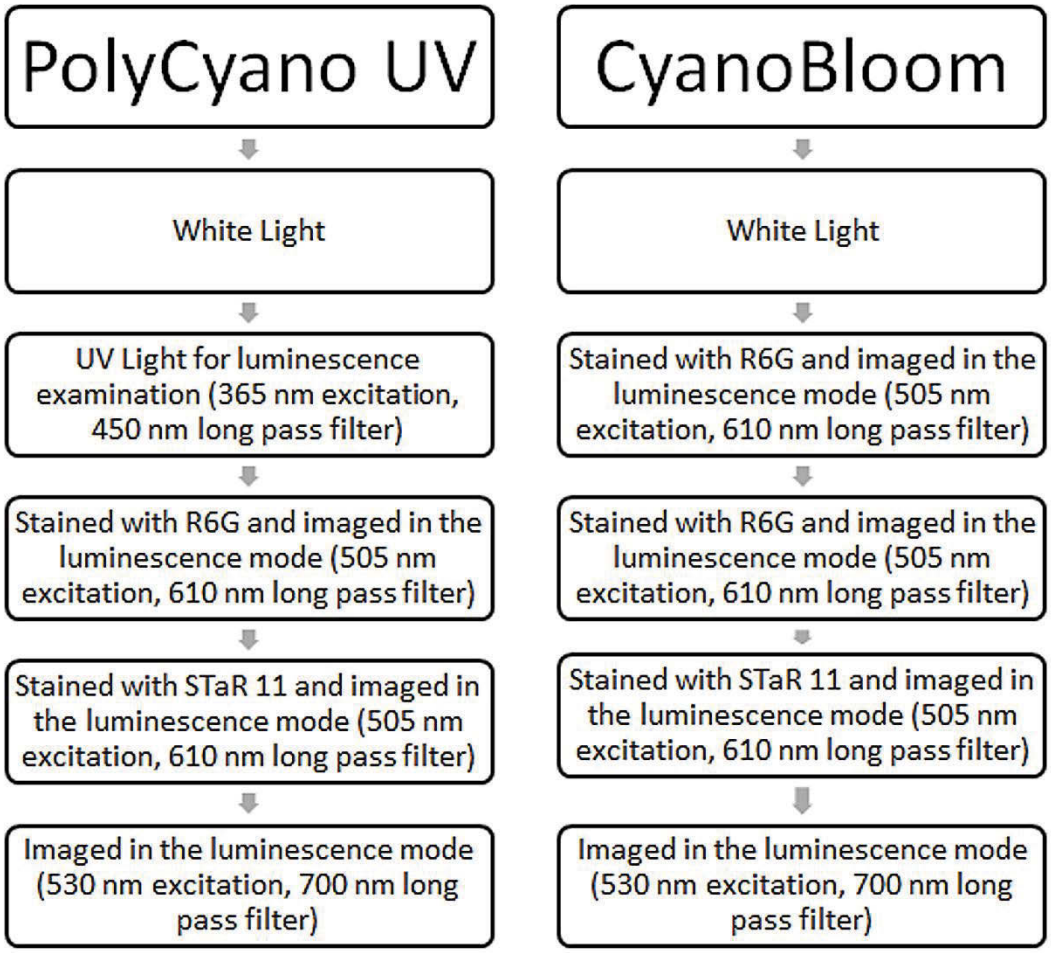


Figure 5-3: Comparison stages for PolyCyano UV comparison and sequence study.

Samples were stained with rhodamine 6G first and after luminescence examination of the rhodamine 6G developed marks, were subsequently stained with STaR 11. The formulations for both staining techniques are outlined in Table 5-3. This was done in an attempt to understand how well STaR 11 performed in a sequence of conventional staining methods as well as whether the presence of other staining techniques would lead to a decrease in quality.

Table 5-3: Preparation of 200 mL solutions for cyanoacrylate stains used in this study.

Cyanoacrylate Stain	Mass of Rhodamine 6G (g)	Mass of STaR 11 (g)	Volume of Methyl Ethyl Ketone (mL)	Volume of Isopropanol (mL)	Volume of water (mL)
Rhodamine 6G	0.02	N/A	30	20	150
STaR 11	0.2	0.05	30	20	150

5.3.4 Comparison Technique

After developing and imaging, the split samples were digitally place side by side for comparison. All digital stitching of fingermarks was performed using GNU image manipulation program (GIMP). No other manipulations were made to the images after stitching. Each three fingermark impression was then given a score, using the McLaren scale [94] Table 5-4.

Table 5-4: Comparison scoring system.

Numerical Value	Qualitative Equivalent
-2	Significant decrease in enhancement when compared to Cyanobloom
-1	Slight decrease in enhancement when compared to Cyanobloom
0	No enhancement when compared to Cyanobloom
1	Slight increase in enhancement when compared to Cyanobloom
2	Significant increase in enhancement when compared to Cyanobloom

In an effort to remove ambiguity of a zero score, a supplementary scoring system had to be implemented. Table 5-5 outlines the differentiation between the samples when a zero score is given.

Table 5-5: Supplementary scoring system.

Zero Score	Qualitative Equivalent
Good Development (GD)	Developed fingermarks give clear ridge detail and contrast
Poor Development (PD)	Developed fingermarks have very little ridge detail and poor contrast
No Development (ND)	Neither technique was able to develop fingermarks

5.4 Results and Discussion

5.4.1 Mass of PolyCyano UV Optimisation

The amount of PolyCyano UV was a significant factor in quality of development and luminescence (Figure 5-4). The higher mass samples had observable luminescence quenching

and over development, with this effect particularly noticeable on glass. The only surface that produced better fingermark development with 0.7 g of PolyCyano UV was Fanta® soft drink cans, as the other masses trialled gave reverse development on this substrate. However, this surface is very uncommon in casework, and as a result the improvement that 0.7 g provided was not enough to warrant it being the optimal mass for development of all samples. Post fuming there was also a significant amount of unevaporated residue remaining in the foil dish, from a cost point of view this would want to be minimised. The 0.5 g samples performed the best on the majority of surfaces and, as a result, it was decided that this was the optimal mass from this study. It should be noted that the mass of PolyCyano UV is also dependant on other influences such as the cabinet size and number of samples fumed, this optimisation only covers cabinets of this size.

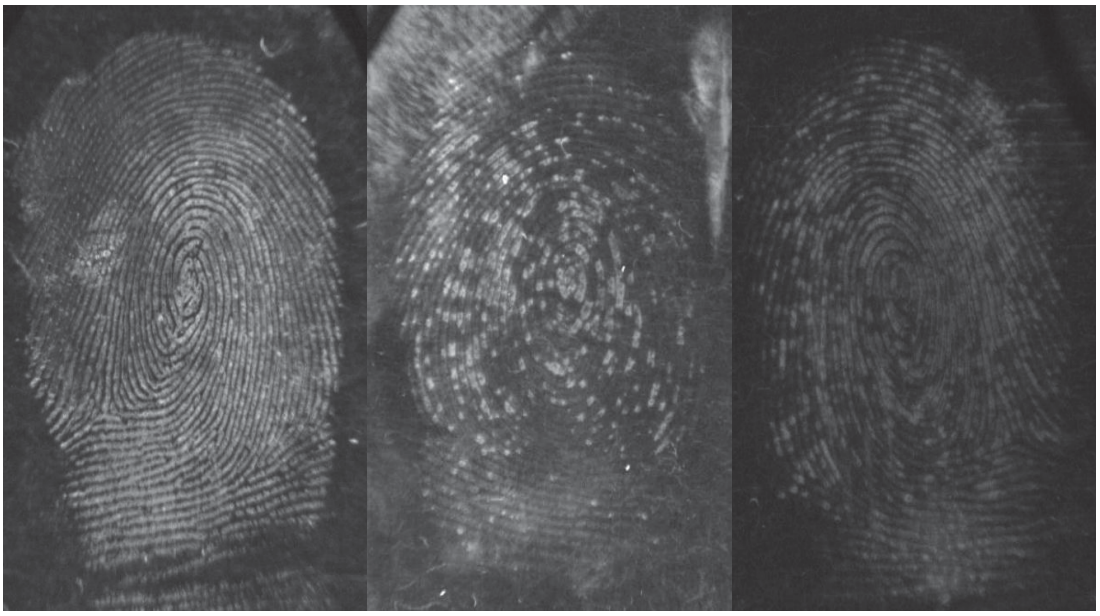


Figure 5-4: Natural fingermark on polyethylene bags developed with; (left) 0.5 g, (centre) 0.6 g, (right) 0.7 g PolyCyano UV.

5.4.2 Relative Humidity Optimisation

Humidity is an important factor when developing fingermarks by cyanoacrylate: the amount of moisture in the deposit can significantly affect the quality of development. The main issue

that arose with this optimisation was ensuring that the cabinet could reach the ideal humidity. It was recommended by Foster + Freeman that a relative humidity (RH) of 90% would increase the luminescence of developed marks, however the cabinet used in this study could not reach over 85% relative humidity. While 75% and 80% RH gave very similar development (Figure 5-5), some samples developed at 80% RH gave strong over-development (particularly for glass samples). Of the parameters trialled, 75% RH was determined to give the best overall development and was the most practical as the cabinet could reach this humidity without issue.

Figure 5-5: PolyCyano UV developed natural fingermark on polyethylene bags; (left) 70% RH, (centre) 75% RH, (right) 80% RH.

5.4.3 Fuming Time Optimisation

Of all the parameters tested, the fuming time played the most significant role in the quality of development (Figure 5-6). Samples that were fumed for 15 minutes tended to be underdeveloped and exhibit poor luminescence, while 35 minute samples gave good development but exhibited poor luminescence (possibly due to luminescence quenching). A fuming time of 25 minutes was determined to give the best compromise between

development and luminescence quality while also allowing for a high throughput of samples in a day, with the total analysis time including humidification, fuming and purging was 45 minutes.

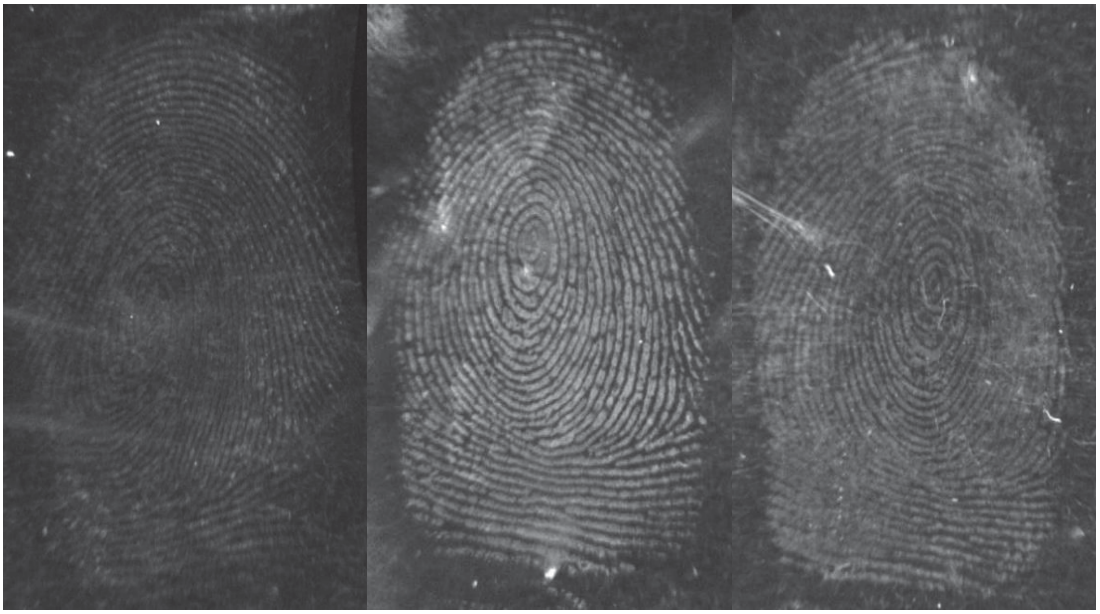


Figure 5-6: PolyCyano UV developed natural fingermark on polyethylene bag; (left) 15 minutes, (centre) 25 minutes, (right) 35 minutes.

Based on these results the optimised settings that were used for the donor study are outlined in Table 5-6. (Note that the visualisation conditions are as specified by the manufacturer and were not further evaluated).

Table 5-6: Optimised Parameter for PolyCyano UV.

Parameter	Recommended Value
Temperature (°C)	230
Mass of PolyCyano UV (g)	0.5 (for MVC1000/D cabinet)
Humidity (%)	75
Fuming Time (minutes)	25
Visualisation Conditions (nm)	Ex = 365, Em = 415-510

5.4.4 Physical and Chemical Properties

The most noticeable difference between PolyCyano UV and Cyanobloom is that PolyCyano is solid cyanoacrylate polymer whereas Cyanobloom is a liquid cyanoacrylate monomer. The thermogravimetric analysis results (Figure 5-7) show that PolyCyano UV must be heated above 208 °C to be completely evaporated. The temperature settings on the MVC 1000/D are for 180 °C and 230 °C; therefore, to ensure that enough PolyCyano UV is evaporated, the 230 °C setting must be used. This could be explained by the fact that the polymer would require a higher temperature to depolymerise and vaporise, compared to the monomer that has a higher volatility. The luminescence data of pre- and post-fumed PolyCyano UV showed that when excited by UV light the luminescent emission is very strong and broad (Figure 5-8) (emission range 390 – 430 nm, λ_{max} = 400 nm). This indicates that a range of visualisation conditions can be used if there are interferences from the surface. While there is a slight decrease in intensity for the post fumed samples it is not significant enough to prevent visualisation of developed marks.

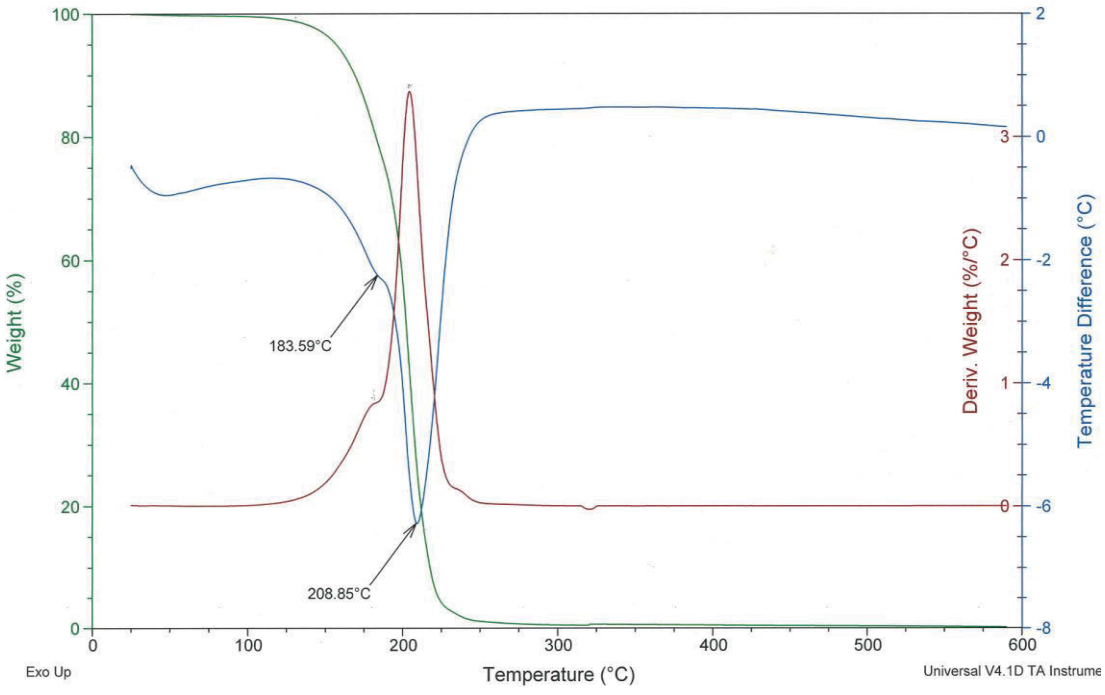


Figure 5-7: Thermogravimetric analysis of PolyCyano UV.

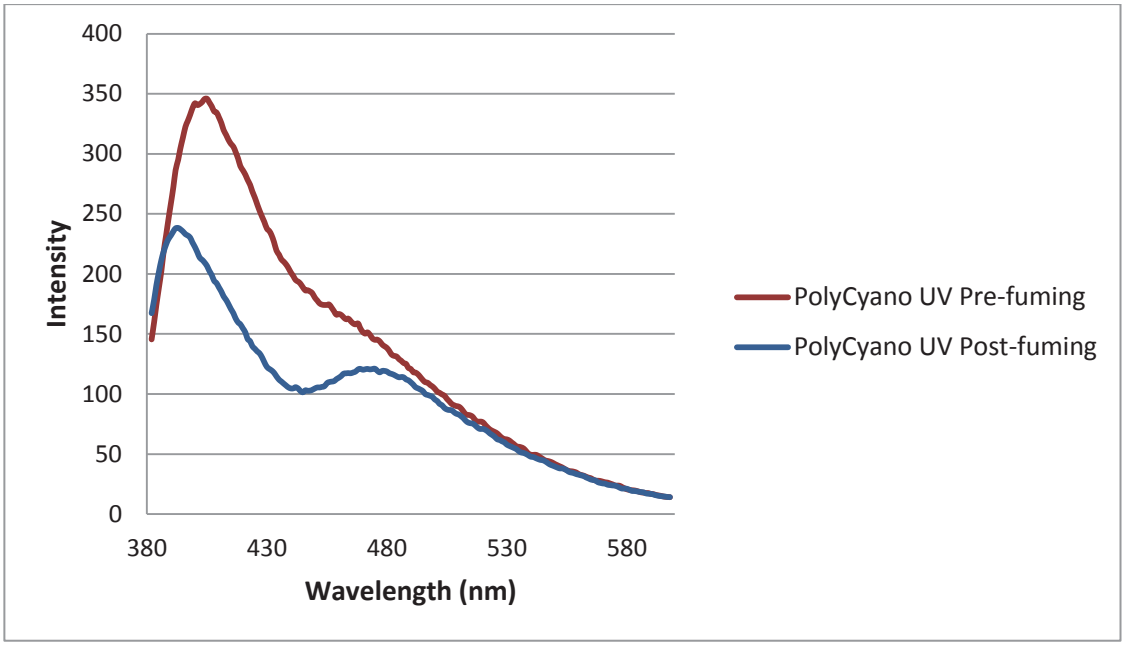


Figure 5-8: Luminescence emission spectra of PolyCyano UV (excitation 365 nm).

5.4.5 Donor and Sequencing Study – White Light Examination

Each sample was examined under white light after development to see how effective each cyanoacrylate was at developing latent fingermarks. Based on the results shown in Figure 5-9 there were only minor differences between both cyanoacrylates, there was a slight difference in colour of developed fingermarks as PolyCyano UV developed marks gave a pale yellow while Cyanobloom gave white fingermarks. There was a degree of degradation with age of the samples (particularly for the two month samples) however the trend was not consistent for most donors. With regards to the effectiveness of each cyanoacrylate, PolyCyano UV only outperformed Cyanobloom on glass samples, Cyanobloom developed samples tended to be over-developed (excess cyanoacrylate deposited on the ridges) (Figure 5-10) this resulted in a significant reduction of ridge detail, however this may be due to the donors overloading the fingermarks with secretions. This was observed for older samples with only fresh marks giving better development for Cyanobloom.

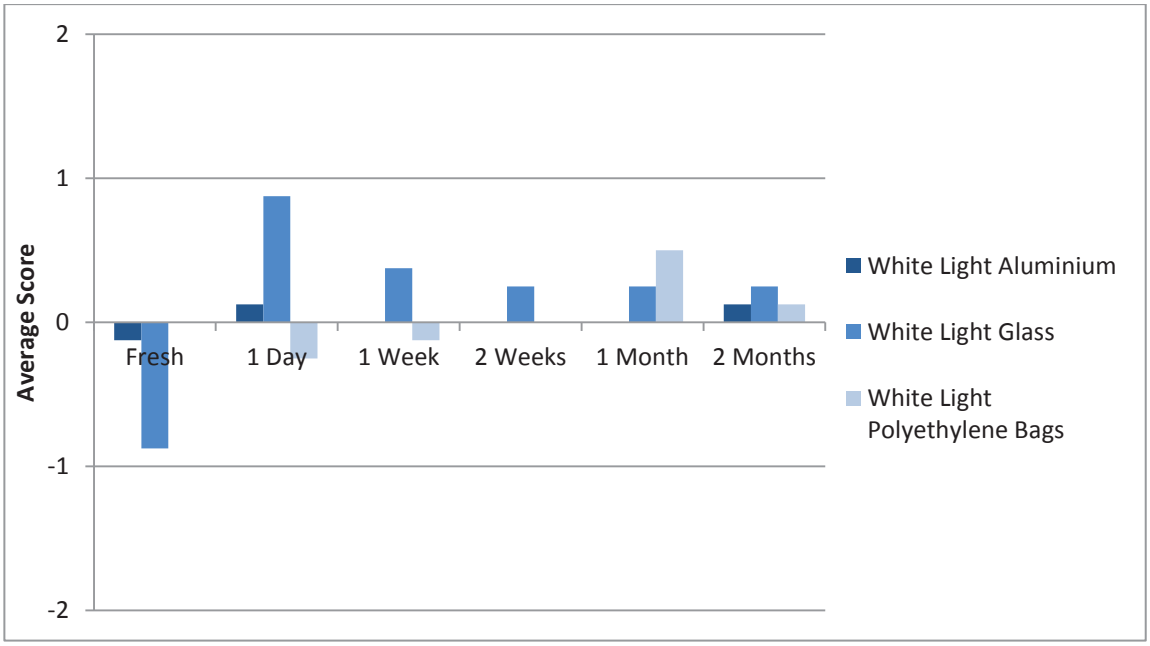


Figure 5-9: Comparison results for all surfaces under white light examination (average McLaren scale values indicated).

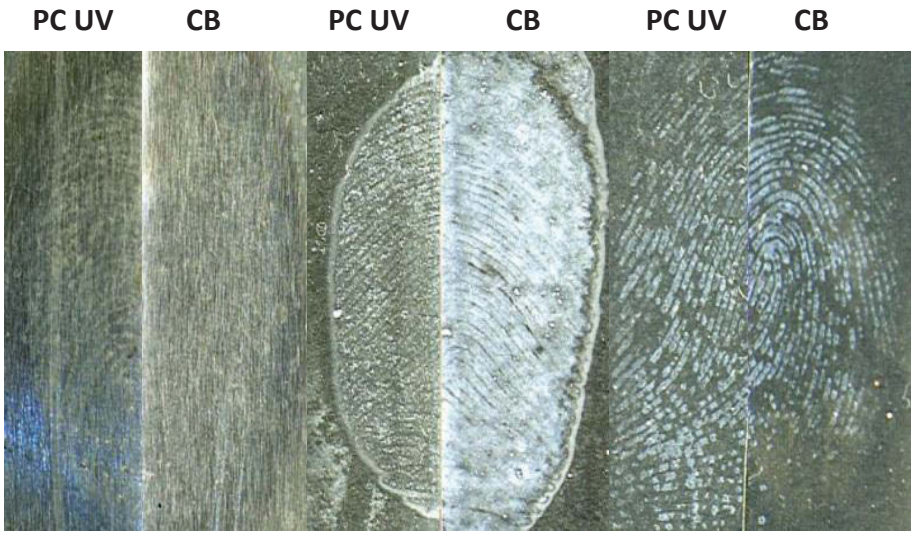


Figure 5-10: Representative fingermarks viewed under white light, developed with PolyCyano UV (PC UV), and cyanobloom (CB) on, (left) aluminium, (centre) glass, (right) polyethylene bags.

All the zero scores for this comparison were graphed (Figure 5-11); the graph illustrates the distribution of the zero scores over time and by surface type. Each column in the graph is divided by the number of scores allocated to good development (GD), poor development (PD) and no development (ND). This allows the development quality to not only be monitored over time but also monitored amongst samples of the same age. Fingermarks that were

developed on aluminium tended to have very poor contrast due to the colour and reflective nature of the surface. This however could be overcome by using an optical enhancement technique such as RUVIS or coaxial illumination. This resulted in a large number (two-thirds of all aluminium samples) of poor and no development scores (Figure 5-11). Polyethylene bags gave good development for most samples but a noticeable decrease in quality (particularly for the cyanobloom samples) was seen over the course of the study. Generally development gave incomplete ridges but sufficient detail could still be extracted from the fingermark (Figure 5-10) with both cyanoacrylates giving similar development. Based on the white light examination there was no clear difference between the cyanoacrylates in their ability to develop fingermarks on the surfaces tested. This would be expected as the addition of a molecular dye such as DMAB is unlikely to alter the mechanics of the development process.

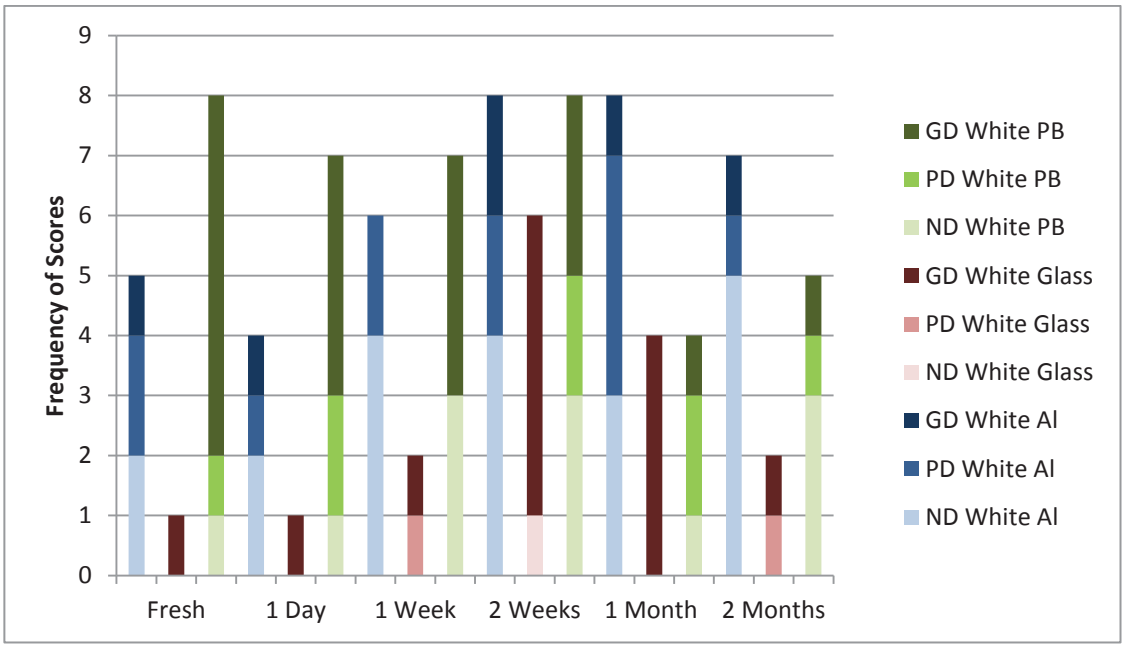


Figure 5-11: Zero values for all surfaces under white light examination (GD = good development, PD = poor development, ND = no development).

5.4.6 Donor and Sequencing Study – UV Examination and Rhodamine Post-Treatment

The main advantage of PolyCyano UV over the traditional cyanoacrylate method is the ability to visualise developed fingermarks in the luminescence mode under UV light without the need for a staining post treatment. However, as the results in Figure 5-12 indicate, the UV luminescence of PolyCyano UV was not as intense as for rhodamine 6G stained Cyanobloom. For both aluminium and polyethylene bags there was a significant decrease in quality compared to the white light examination; this is reflected by the increase in negative values across all ages. For both surfaces, the PolyCyano UV developed marks exhibited very low contrast and, when compared to rhodamine 6G, there was a significant decrease in quality Figure 5-12. Staining samples with rhodamine 6G did significantly decrease the number of no development scores for the aluminium samples. This would be due to the increase in contrast between the fingermark ridges and the surface when viewing samples in the luminescence mode. PolyCyano UV developed fingermarks on glass also exhibited a slight decrease in quality when compared to the rhodamine stained marks, however, because there were some samples that had been overdeveloped this decrease was not as significant. There were also some cases where the luminescence of PolyCyano UV was quenched, possibly due to slight overdevelopment (Figure 5-13). The only trend that could be ascertained from the age of the samples was the increase of no development score over time (Figure 5-14). Another potential limitation of the visualisation of PolyCyano UV is that UV excitation of substrates potentially can generate background luminescence. Compared to excitation at longer wavelengths (i.e. staining with rhodamine 6G) this poses a potential disadvantage of PolyCyano UV. These results indicate that, based on luminescence, PolyCyano UV does not provide any significant advantage over conventional cyanoacrylate and rhodamine 6G staining.

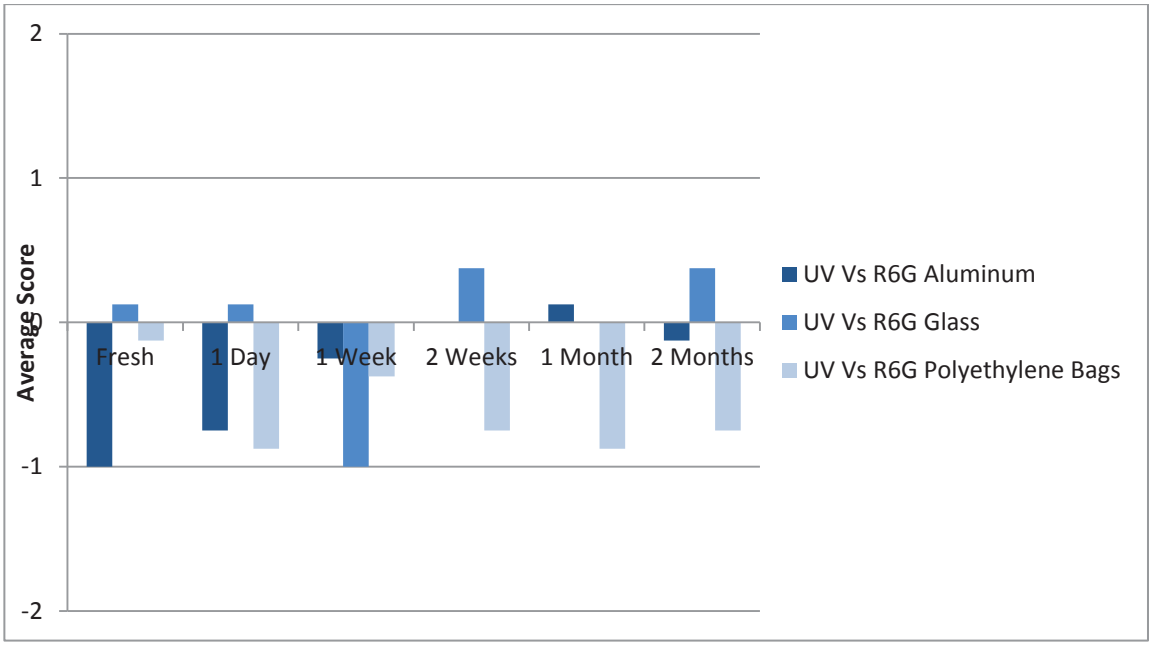


Figure 5-12: Comparison values for all surfaces between PolyCyano UV and Cyanobloom post rhodamine 6G staining (average McLaren scale values indicated).

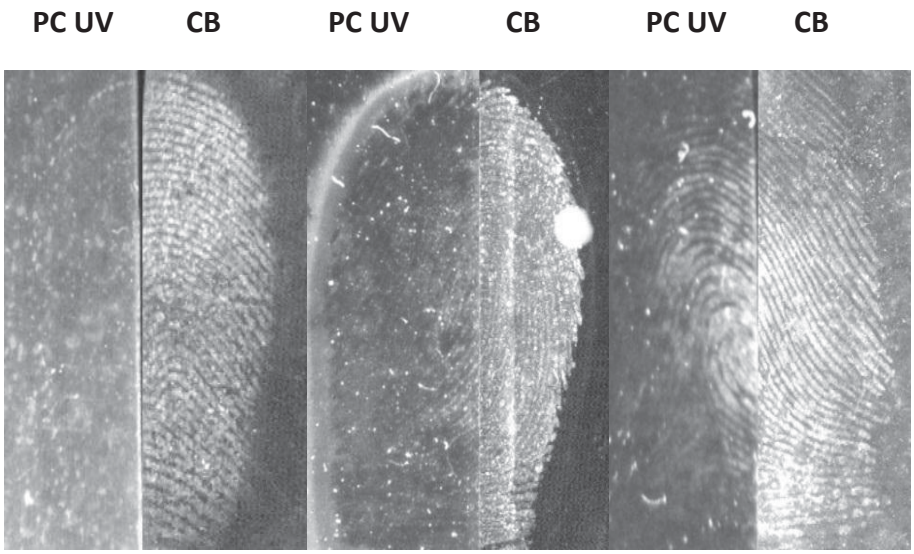


Figure 5-13: Representative fingermarks viewed in the luminescence mode (PolyCyano UV 365 nm excitation 400 nm longpass filter, Cyanobloom 505 nm excitation, 610 nm barrier bandpass filter) developed on (left) aluminium, (centre) glass, (right) polyethylene bags.

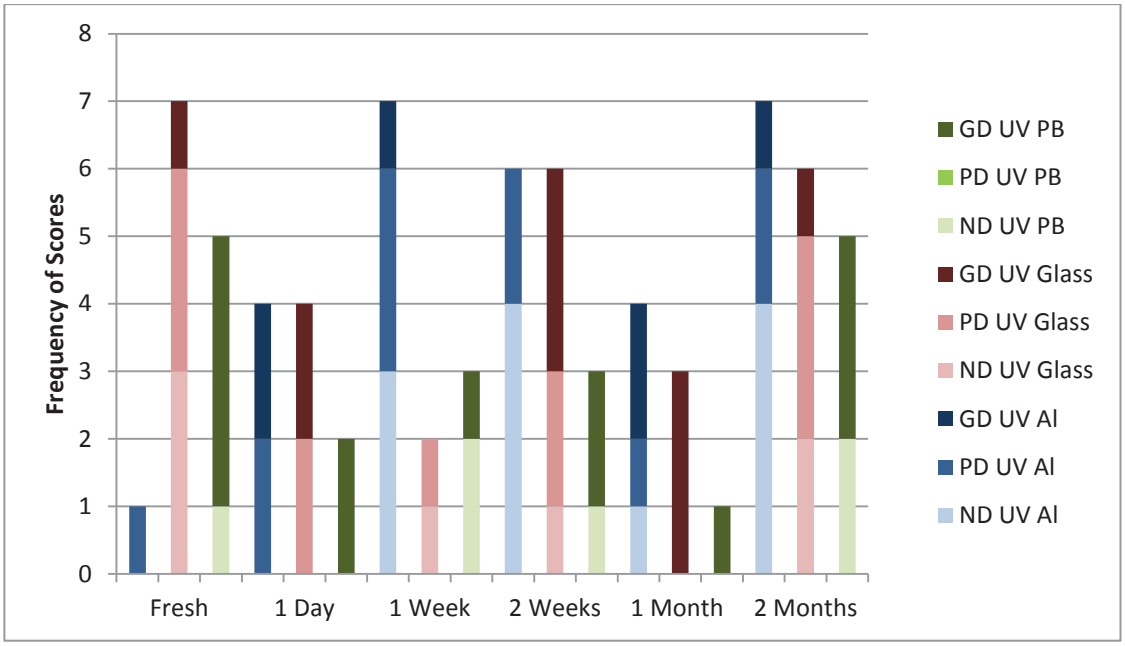


Figure 5-14: Zero values for all surfaces between PolyCyano UV under UV examination and CyanoBloom after rhodamine 6G staining. (GD = good development, PD = poor development, ND = no development).

When PolyCyano UV was used in sequence and stained with rhodamine 6G, there was a significant improvement in the luminescence of most samples (compared to the unstained UV luminescence). When these marks were compared to Cyanobloom and rhodamine 6G stained samples, the PolyCyano UV marks gave better development, in the majority of cases. This can be attributed to two factors; firstly, when examined under white light PolyCyano UV tended to give better development, therefore when these samples were stained with rhodamine 6G it would be expected to outperform Cyanobloom. Secondly, the only issue that affected the quality of development when examined under UV light was the weaker luminescence of PolyCyano UV when compared to rhodamine 6G. Therefore, upon staining with rhodamine 6G the luminescence of PolyCyano UV should also increase. This is shown in the average values shown in Figure 5-15. This trend was seen on all surfaces, with aluminium exhibiting the greatest change in scores (35% negative values for UV examination to 4% post staining). A comparison between PolyCyano UV pre- and post-staining demonstrated the significant increase in quality observed when staining with rhodamine 6G (Figure 5-15). These results indicate that PolyCyano UV is very effective when used in a sequence; the luminescent dye in the cyanoacrylate polymer does not decrease the luminescence strength of rhodamine 6G (Figure 5-16). This comparison also had a significantly high amount of zero scores, which is to be expected as the same enhancement technique was applied to both sides so any variability would be quite minimal (Figure 5-17). However, the main advertised advantage of

this technique is that it should develop luminescent marks without the need the further staining. While it was successful to a certain extent, the luminescent dye in PolyCyano UV was not as effective as rhodamine 6G.

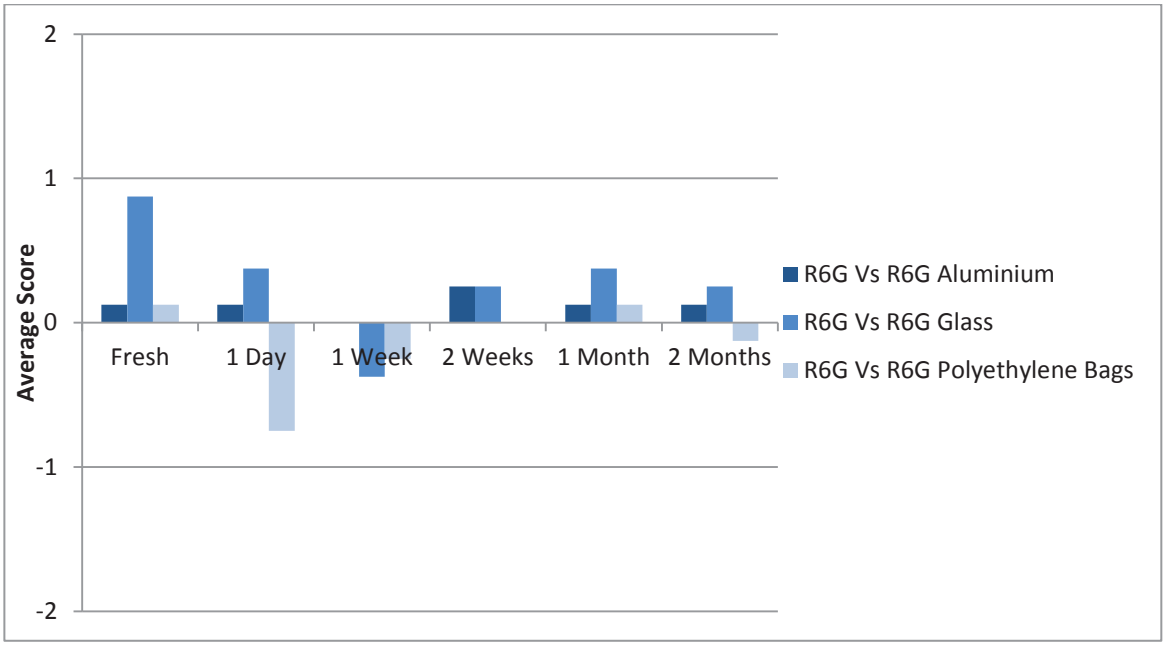


Figure 5-15: Comparison results for all surfaces between PolyCyano UV after rhodamine 6G staining and Cyanobloom after rhodamine 6G staining (average McLaren scale values indicated).

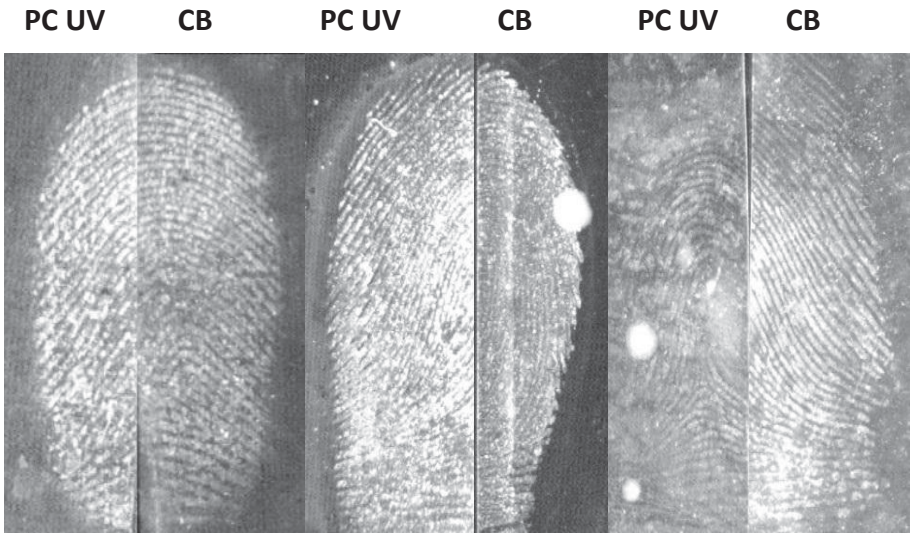


Figure 5-16: Representative fingermarks stained with rhodamine 6G viewed in the luminescence mode (505 nm excitation, 610 nm barrier bandpass filter) developed on (left) aluminium, (centre) glass, (right) polyethylene bags.

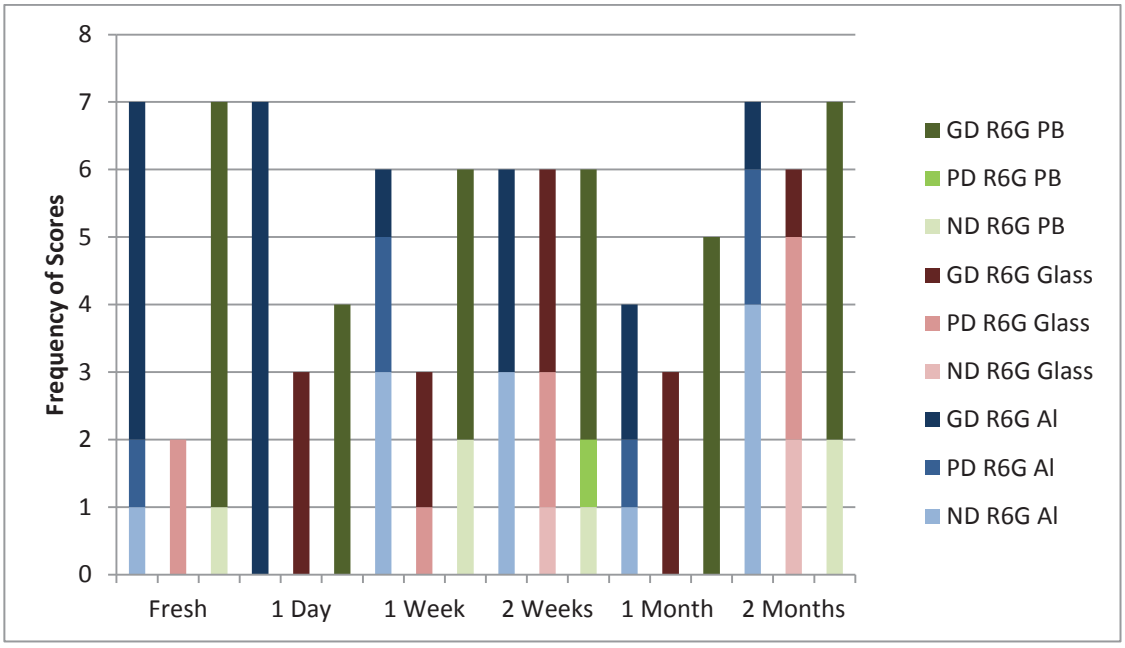


Figure 5-17: Zero values for all surfaces for comparison between PolyCyano UV and Cyanobloom stained with rhodamine 6G (GD = good development, PD = poor development, ND = no development).

5.4.7 Donor and Sequencing Study – Sequencing with STaR 11

For this study two separate comparisons were performed:

1. Both cyanoacrylates were stained with STaR 11 to see if the luminescent polymer decreased the effectiveness of luminescence from STaR 11;

2. The performance of STaR 11 stained PolyCyano UV versus Cyanobloom stained with rhodamine 6G.

The results from the first comparison are shown in Figure 5-18 and Figure 5-19. From these results there are very minor differences between the cyanoacrylates. This comparison also had a large number of zero values, 81%, 69% and 83% for aluminium, glass and polyethylene bags respectively (Figure 5-20). The only surface that showed a consistent trend was the polyethylene bags, which indicated that Cyanobloom performed better than PolyCyano UV

for the majority of samples. These results indicate that STaR 11 can perform very well in a sequence with either cyanoacrylate product and the luminescence of STaR 11 is not affected by other previous staining. This highlights the potential of STaR 11 as a replacement for rhodamine 6G as a cyanoacrylate stain.

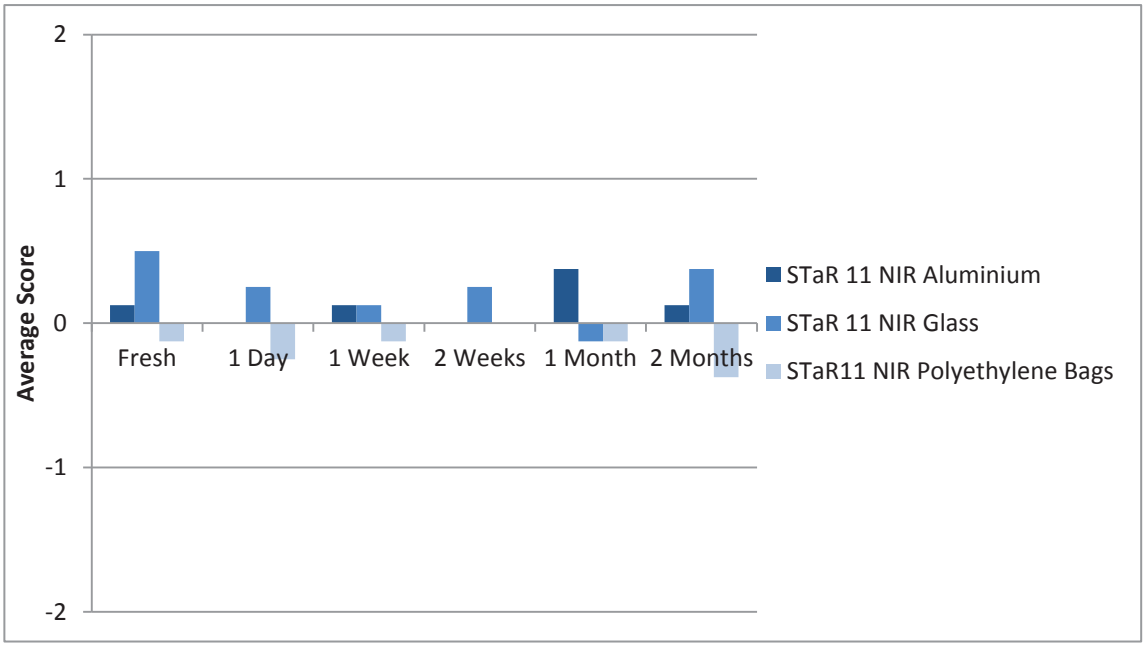


Figure 5-18: Comparison values for all surfaces between PolyCyano UV and Cyanobloom post staining with STaR 11 viewed in the NIR (average McLaren scale values indicated).

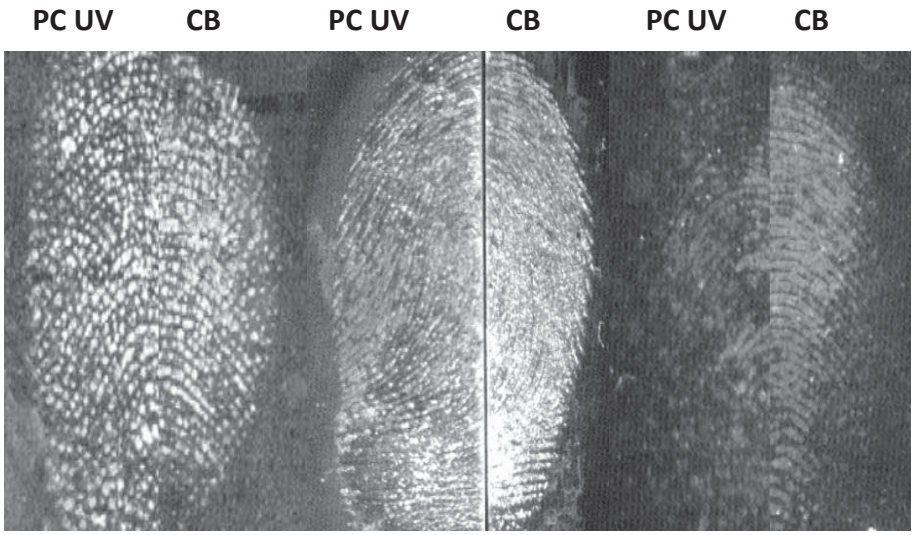


Figure 5-19: Representative fingermarks stained with STaR 11 viewed in the luminescence mode (530 nm excitation, 700 nm barrier band pass filter) developed on (left) aluminium, (centre) glass, (right) polyethylene bags.

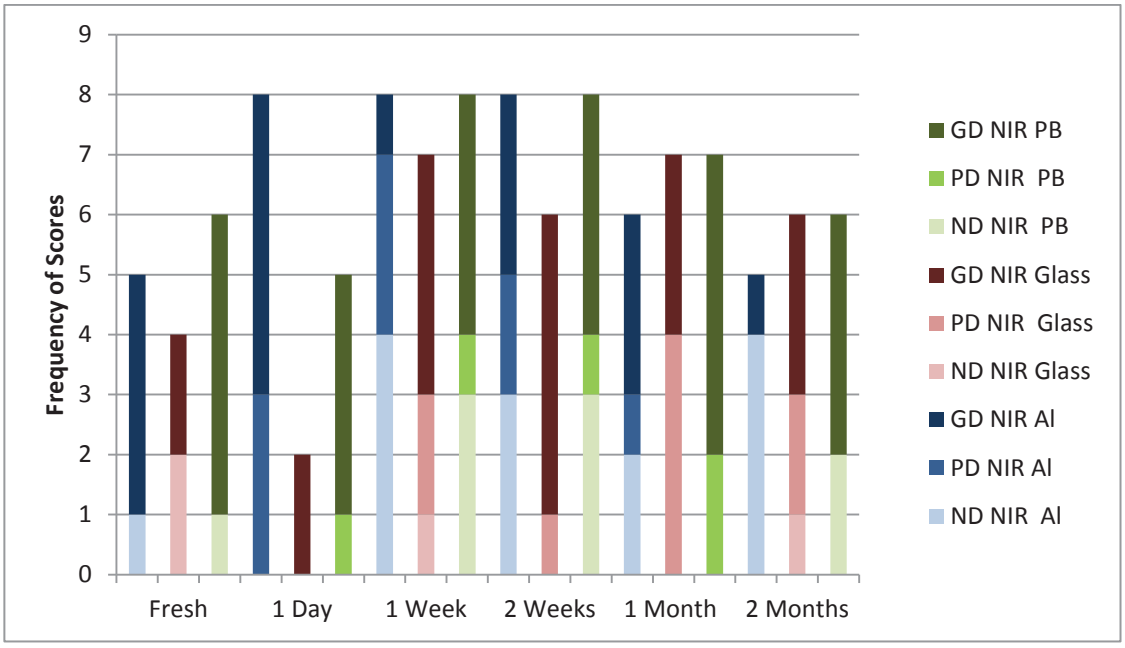


Figure 5-20: Zero values for all surfaces after Polycyano UV and Cyanobloom have been stained with STaR 11 and viewed in the NIR (GD = good development, PD = poor development, ND = no development).

When STaR 11 stained marks were compared to rhodamine 6G stained marks, there was only very minor improvement when visualising in the NIR (Figure 5-21, Figure 5-22). Polyethylene bags were once again the only surface which indicated that rhodamine 6G was better than STaR 11 at visualising developed marks; however the scores were very close to zero indicating that the enhancement was only minimal. Rhodamine 6G stained fingermarks on glass and aluminium tended to exhibit a degree of background staining that resulted in reduced contrast, whereas this was not the case with STaR 11 stained marks. Similarly to the previous comparison there were a large number of zero scores, 69%, 56% and 75% for aluminium, glass and polyethylene bags respectively (Figure 5-23). These results indicate that in the majority of cases - regardless of age, donor or surface - STaR 11 will give a similar enhancement quality compared to rhodamine 6G.

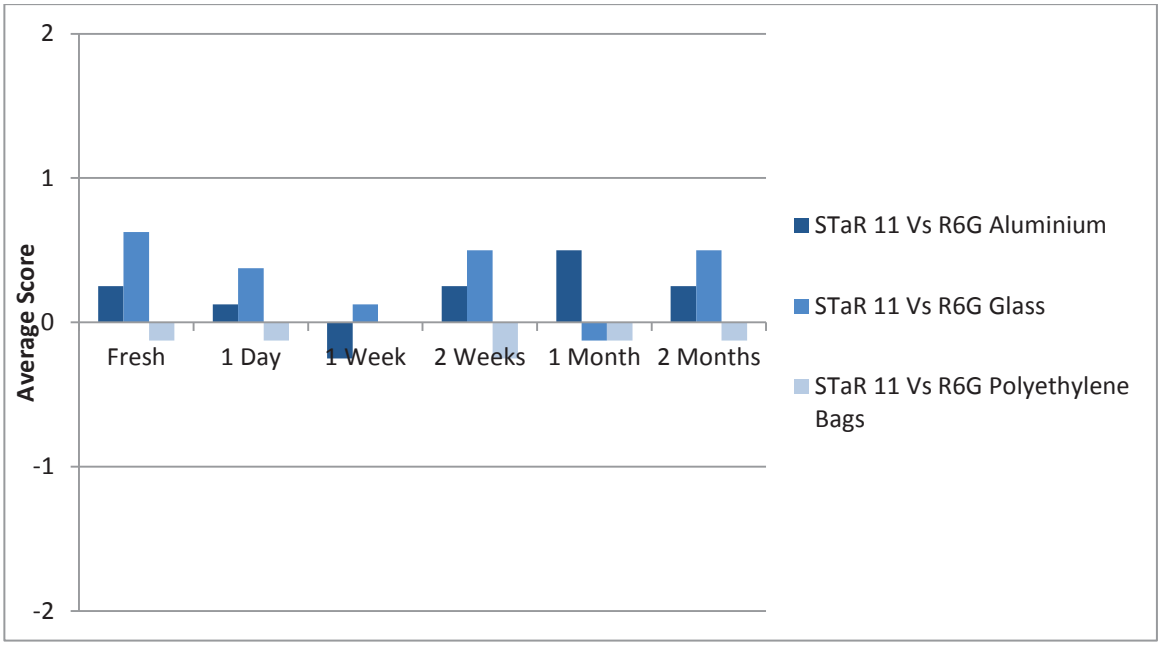


Figure 5-21: Comparison values for all surfaces between PolyCyano UV post staining with STaR 11 viewed in the NIR and Cyanobloom post staining with rhodamine 6G (average McLaren scale values indicated).

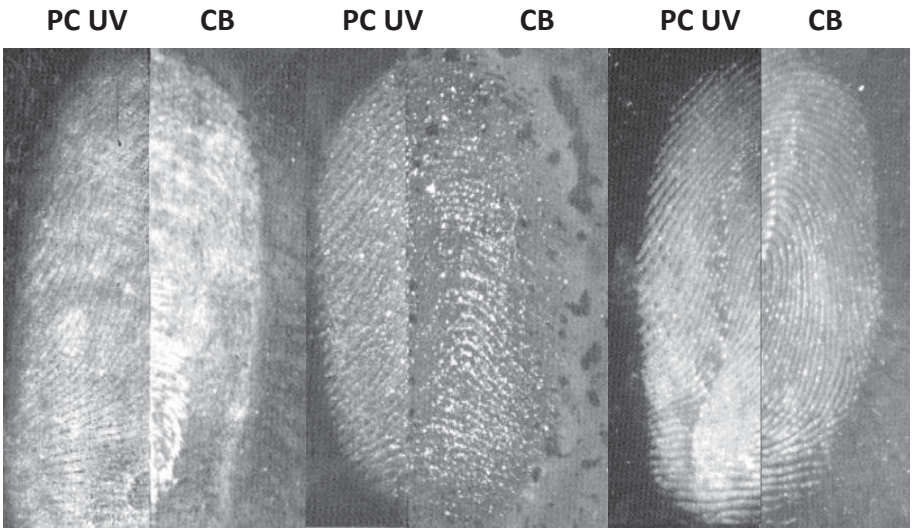


Figure 5-22: Representative fingermarks PC UV stained with STaR 11, CB stained with rhodamine 6G viewed in luminescence mode (505 nm excitation, 610 nm barrier band pass filter) developed on (left) aluminium, (centre) glass, (right) polyethylene bags.

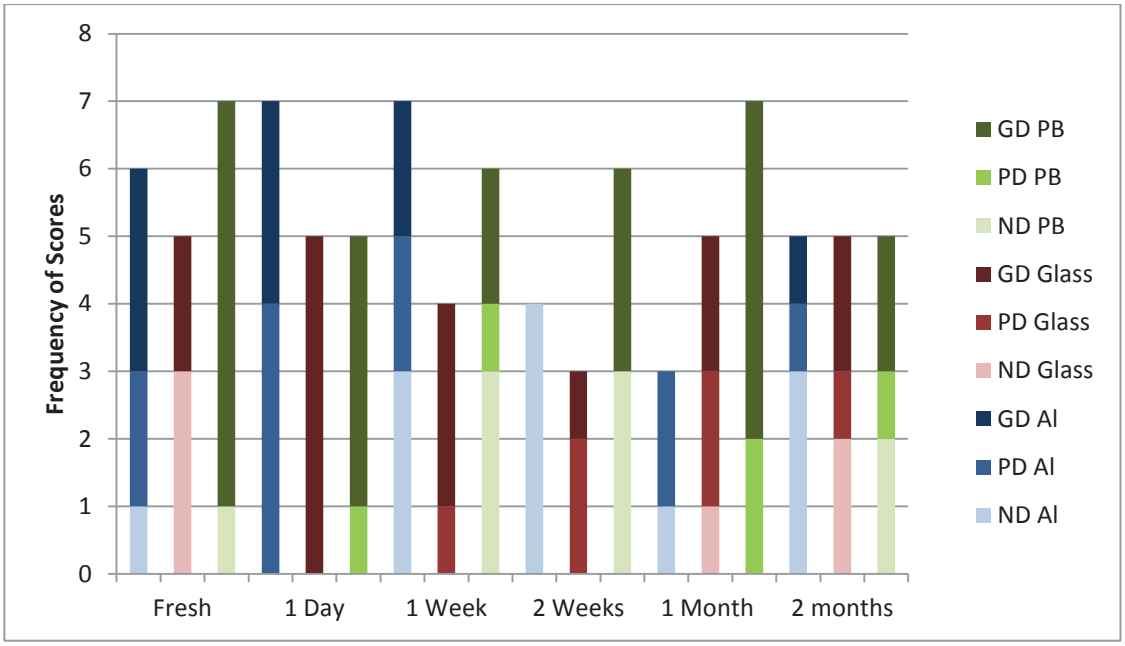


Figure 5-23: Zero values for all surfaces after comparison with STaR 11 stained Polycyano UV and rhodamine 6G stained Cyanobloom (GD = good development, PD = poor development, ND = no development).

5.4.8 Discussion Regarding Overall Performance of PolyCyano UV

This study has demonstrated that while PolyCyano UV is able to develop fingermarks of a similar quality to conventional cyanoacrylate developed fingermarks. However, the advantage of being able to visualise developed fingermarks in the luminescence mode without the addition of luminescent stains is not as effective as previously reported. The UV luminescence emission observed for developed fingermarks in this study was poorer than the rhodamine 6G stained fingermarks, in the majority of cases. While the luminescence was significantly improved upon, with the addition of rhodamine 6G post treatments the overall performance of PolyCyano UV was underwhelming. Considering the cost associated with PolyCyano UV, compared to Cyanobloom or other commercially available cyanoacrylates, it would not be advantageous for it to replace conventional methods. The health and safety issues associated with luminescent stains are quite minimal and can be easily controlled in an operational forensic laboratory.

The only potential use of PolyCyano UV would be for a cyanoacrylate fuming device that is used primarily at crime scene as this would remove the use of storing and transporting dangerous and hazardous chemicals. Once again the cost is a major factor which would need to be considered, based on this study it was found that for a MVC 1000D cabinet, 0.5 g was the optimal mass, it recommended by Foster + Freeman that 0.5-1.0 g be used per cycle (depending on the number of exhibits). PolyCyano UV costs approximately $150 AUD per 10 g, while Cyanobloom cost $6-7 AUD for a 20 g bottle. For a large volume of samples it would be incredibly costly for PolyCyano to be used as a replacement to conventional cyanoacrylate. Factoring into account the cost of luminescent dyes and solvents, the PolyCyano UV is a costly alternative that does not give justifiable results based on price alone.

5.5 Conclusions

PolyCyano UV is marketed as a one-step luminescent cyanoacrylate; this study showed that while UV luminescence could be an advantage, however the luminescence was noticeably poorer than conventional cyanoacrylate stained with rhodamine 6G. It was found that when used in sequence PolyCyano UV gave better development compared to conventional cyanoacrylate. When stained with rhodamine 6G PolyCyano UV in some cases provided better enhancement than the conventional cyanoacrylate stained with rhodamine 6G. This could be due to variability between fuming cycles or slight variations in the amount of cyanoacrylate being vapourised. It is unlikely that PolyCyano UV would replace conventional cyanoacrylate for fuming, due to the cost associated with the product. PolyCyano UV could be used for cases which require DNA examination after fuming (as PolyCyano UV does not damage DNA like rhodamine 6G staining can [162]) or in cases where staining would damage the surface (i.e. semiporous surfaces).

The effectiveness of STaR 11 when used in sequence was also examined and it was found to give comparable results to rhodamine 6G stained fingermarks. STaR 11 could develop fingermarks in both the visible and NIR regions for both cyanoacrylate trialled. Fingermarks of ages up to two months could be imaged with STaR 11 and in some cases the STaR 11 developed marks gave better development than rhodamine 6G on its own. Using an examination sequence of white light, UV luminescence, luminescence from rhodamine 6G staining and luminescence from STaR in both the visible and NIR regions allows for imaging of

developed fingermarks at five different spectral regions. This could potentially increase the likelihood of recovering exploitable fingermarks.

Chapter 6: Assessment of Imaging Systems for Visualisation in the Near Infrared

Chapter 6: Assessment of Imaging Systems for Visualisation in the Near Infrared

6.1 Introduction

6.1.1 Forensic Imaging Systems

The first camera designed specifically for fingermark examination was developed in the early 20th century and since its development there have been numerous forensic imaging systems to aid in the visualisation of latent fingermarks [163]. Developed fingermarks that rely on contrast induced by absorption (e.g. black powder or ninhydrin developed fingermarks) can be imaged under white light using commercial cameras and equipment. Luminescent fingermarks, however, require specialised light sources and scientific grade cameras in order to obtain a suitable image for identification. As ridge detail can be lost or distorted with low resolution images, it has been recommended by the Scientific Working Group on Friction Ridge Analysis, Study and Technology (SWGFAST) that images be captured at a resolution of no less than 1000 ppi [164], which can be beyond the capabilities of some conventional digital imaging equipment.

In Australia one of the most widely used forensic imaging systems for visualising developed fingermarks is the Poliview, which incorporates a Polilight forensic light source. The 'Unilite' was developed at the Australian National University in the 1980s. This was further developed and commercialised by Rofin Pty Ltd as the Polilight [28]. The Polilight is a high-intensity filtered light source specially developed for forensic science, it provides a range of monochromatic bands (350 – 650 nm), as well as white light. While the Polilight was initially developed as an alternative to lasers for the detection of developed fingermarks, it has been effective in visualising and discriminating biological fluids such as semen, blood and saliva, as well as chemical based lubricants [165, 166]. The Poliview combines the Polilight with a Peltier cooled charged-coupled device (CCD) camera and macro lens to allow for long exposure times without interference due to thermal electronic camera noise [167].

The Video Spectral Comparator (VSC) is a digital imaging system developed by Foster + Freeman principally for the examination of questioned documents. The VSC has the ability to illuminate samples with white, UV, IR and banded (400-700 nm) light. Similar to the Poliview IV, the VSC contains a high resolution CCD camera that allows for rapid imaging of latent fingermarks. Since the VSC was produced mainly for document examination, it also contains a grated spectrometer, which allows for absorbance, reflectance, transmission and luminescence emission spectra to be obtained from any sample. The most recent incarnation of the VSC (the VSC 6000 HS) also includes a hyperspectral imaging function for increased detection and imaging capabilities [168, 169]. The range of illumination sources present in the VSC, means that it has been used for many different forensic applications, including imaging carbon paper documents, classification of pen inks and passport verification [169-171].

The Condor is a visible-NIR hyperspectral imaging (chemical imaging) system that combines both molecular spectroscopy and digital imaging [172]. The images are captured in a data cube, where each pixel of an image contains a full spectrum; this process can be performed for a range of wavelengths. This has an advantage over conventional imaging systems as contrast can be induced by visible and spectral means. This is particularly useful for fingermarks on patterned substrates or weakly luminescent fingermarks [172]. Spectral contrast, however, requires the application of a data processing technique called chemometrics, which is the application of mathematics and statistics to extract information from chemical data [173]. While in many cases this technique has been shown to be useful for improving contrast in developed fingermarks [55, 174], chemometrics was not applied in the study presented here. Chemical imaging is not limited to imaging in the visible region, but has been utilised to detect fingermarks with other analytical techniques such as FTIR, Raman and DESI-MS [25, 40, 46].

The demand for portable imaging systems is very high in response to the increase in scene-based techniques and the development of luminescent powders. Conventional digital cameras contain a NIR blocking filter in the camera to reduce IR interference from natural light. This filter can be removed by camera manufacturers, but results can vary between vendors, resulting in inconsistent performance. As a result, Fuji released a camera in 2007 called the IS Pro. This camera was an advancement on the previously released Finepix S3 Pro UVIR and IS-1 camera and had a wider wavelength range than conventional digital cameras (400-900 nm) [175]. The IS Pro is fitted with a CCD sensor that allowed a live image preview

for images taken in the UV and NIR (a first for this type of camera). NIR cameras have many forensic applications including the photographing of tattoos on mummified remains, bite marks and footwear impressions [176, 177].

There has been very little literature published on the comparison of imaging systems, with no literature on comparison of systems focussing on NIR imaging. Comparisons of imaging systems are difficult to undertake due to the cost associated with the systems themselves. The choice of imaging system is also largely dependent on the needs of the laboratory, there have been some studies into the comparison of different forensic equipment which were used as a guide for the study presented in this chapter. Wilkinson and Watkin performed a comparison between forensic light sources, the Polilight, the Luma Light and the Spectrum 9000 in terms of light intensity, ease of use and quality of filters. However, this study only compared the technical capabilities of the instruments, not their effectiveness in visualising forensic samples [178]. A similar study by Dalrymple compared the capabilities of a Coherent TracER optically pumped semi-conductor (OPSC) laser and the Rofin Polilight –Flare- Plus. This comparison was performed using a range of biological samples including: saliva, semen and blood as well as treated and untreated fingermarks. This study found that both techniques had significant advantages; however, no light source was deemed to be better than the other in all cases [179].

This review highlights a gap in the current literature. While the imaging systems mentioned (Poliview, VSC 6000, Condor and Fuji IS Pro) have been shown to be effective at imaging developed fingermarks it would be advantageous for operational forensic laboratories to have a reference guide for different imaging systems and whether certain fingermark development techniques are better suited to a specific imaging system. This study will only examine the effectiveness in visualising STaR 11 developed fingermarks in the NIR only.

6.1.2 Aims and Objectives

The advancement in imaging technologies has made imaging in the NIR easier and more available to operational forensic laboratories. At this time, there has been no objective comparison between these imaging systems that specifically focuses on their ability to image samples in the NIR. The aim of the study was to develop optimal viewing conditions for

fingermarks developed with cyanoacrylate and stained with STaR 11, and STaR 11 magnetic powder developed fingermarks. Once these conditions were obtained, developed fingermark samples on a range of surfaces were imaged using all four imaging systems tested:

1. Condor;
2. Fuji IS Pro;
3. Poliview IV; and
4. VSC 6000 HS.

All the images were than combined and compared based on several factors including: ease of use, quality of image, exposure/run time for images, and limitations of each system. This study also aims to be a guide to the available imaging systems and assist with selecting the best imaging system for different sample types.

6.2 Materials

6.2.1 Reagents

CyanoBloom was purchased through Foster and Freeman and used as supplied.

Aluminium oxide nanopowder, <50 nm particle size (TEM) [CAS 1344-28-1] and rhodamine 6G dye content 99% [CAS 989-38-8] were obtained from Sigma Aldrich and used as supplied.

Styryl 11 [CAS 92479-59-9] was obtained through Exciton/Lastek and used as supplied.

Reagent grade isopropanol [CAS 67-63-0] and methyl ethyl ketone [CAS 78-93-3] were obtained through Chem-Supply and used as supplied.

6.2.2 Instrumentation

A Foster + Freeman MVC1000/D cyanoacrylate fuming cabinet was used to produce the cyanoacrylate developed fingermark samples in this study.

A ChemImage Condor coupled with a Mini CrimeScope MSC 400 light source was used to image developed fingermarks in the NIR (Figure 6-1). The Condor was used at the Australian Federal Police Forensic & Data Centres facility in Canberra.

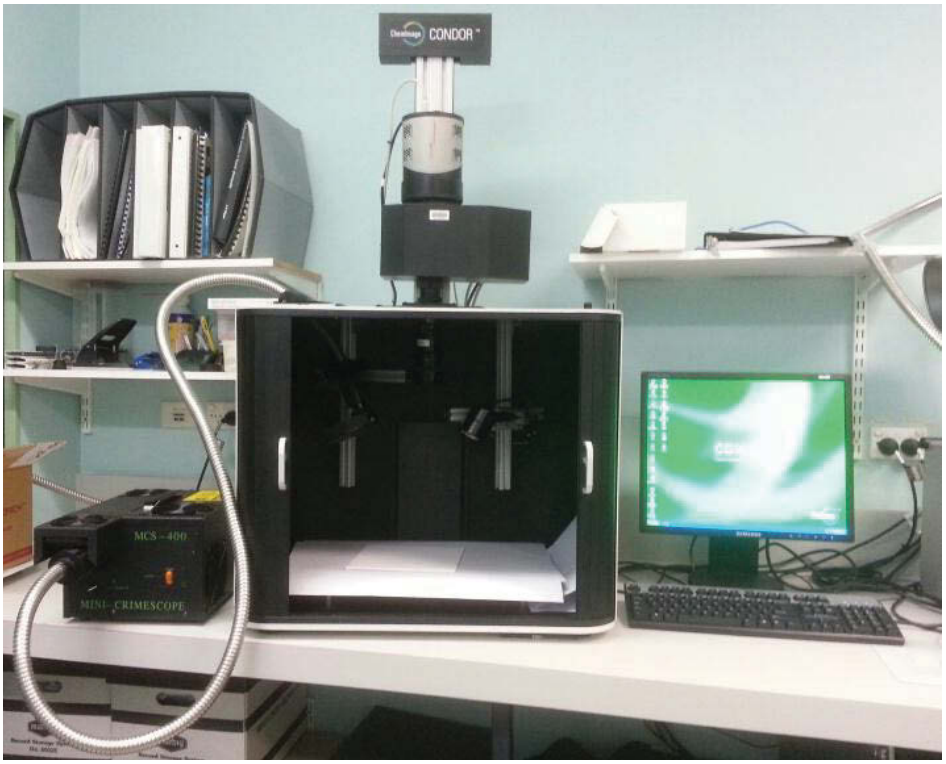


Figure 6-1: The Condor chemical imaging system used in this study.

A Foster and Freeman VSC 6000/HS (Figure 6-2) and VSC Suite version 6.6 software was used to image developed fingermarks in the NIR.

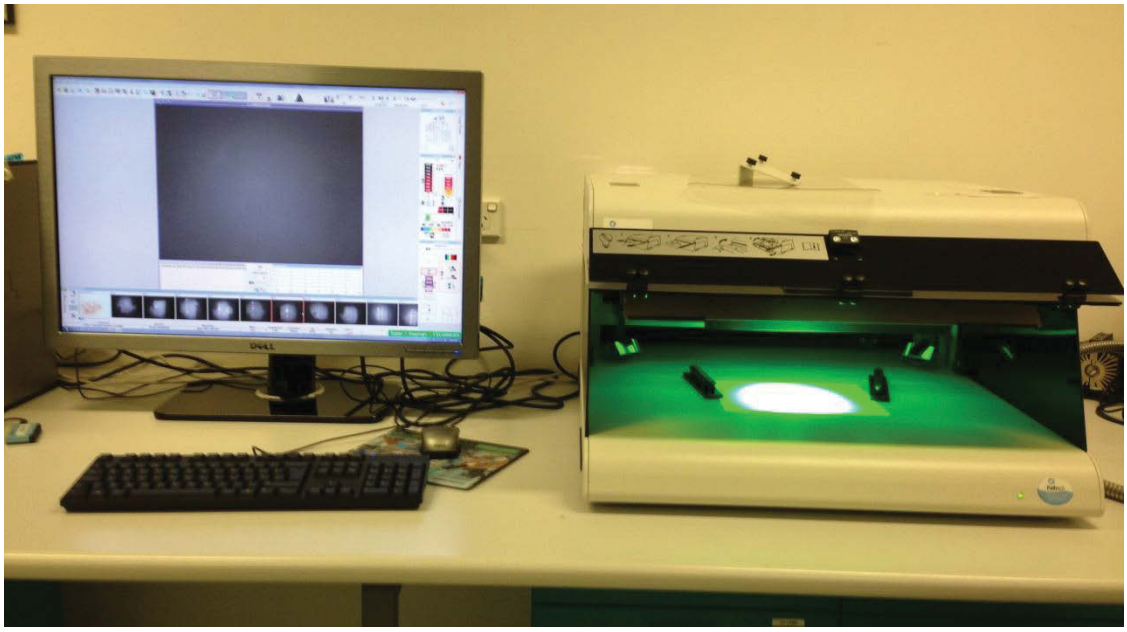


Figure 6-2: VSC imaging system used in this study.

A Fuji IS Pro with B+W 063 filter (Figure 6-3) was used to image developed fingermarks in the NIR. The light source used with this imaging system was the Polilight PL 500.

Figure 6-3: Fuji IS Pro with B+W 063 filter used in this study.

A Rofin, Poliview system (AF Micro Nikkor 60mm lens, Q imaging Peltier cooling CCD camera) using V++ imaging software and incorporating a Polilight PL 500 (Figure 6-4) was used to image developed fingermarks in the NIR.

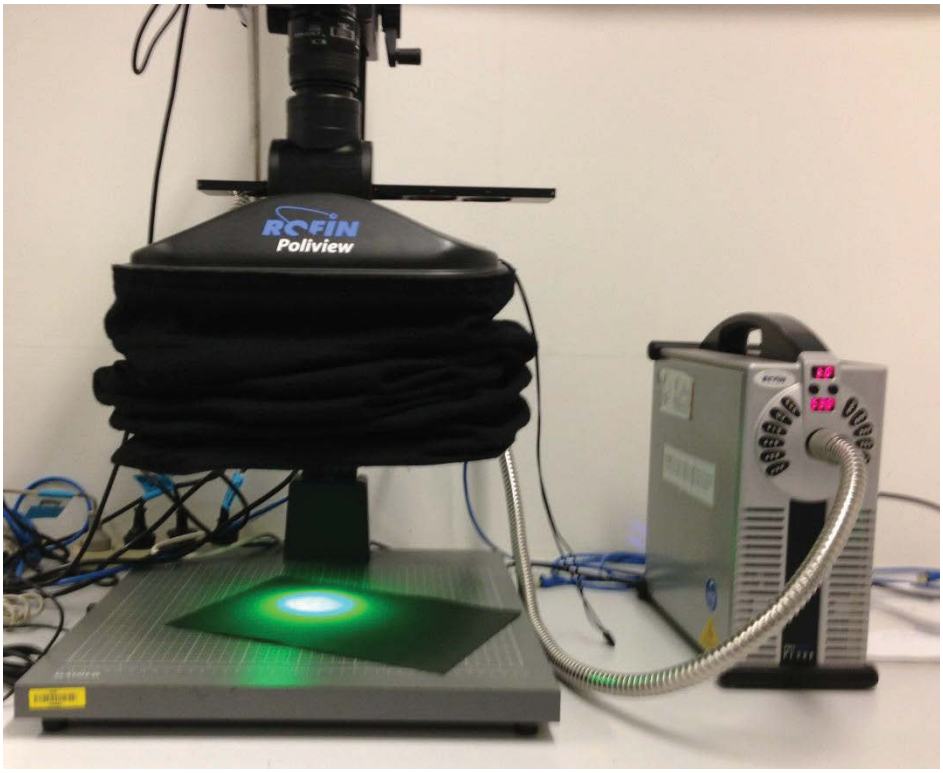


Figure 6-4: Rofin Poliview IV system used in this study.

6.3 Methods

6.3.1 General Approach

This study aimed to compare the currently available forensic imaging systems and assess their ability to visualise STaR 11 developed fingermarks. The basis of the comparison is the hardware available image capture, not additional software manipulations that could be made to the images after they have been recorded. As a result, no 'darkroom' adjustments (such as brightness and contrast) were made to any image, and chemical imaging post-processing was not performed on any image. The reason for this, and the limitations that this put on the study itself, will be explored in section 6.4.5.

6.3.2 Preparation of Fingermark Samples

Surfaces (Table 6-1) were cut into 76 mm by 26 mm rectangles (the size of a glass microscope slide), so they could be transported without damaging the developed ridges. Surfaces that were pliable were wrapped and secured around a glass microscope slide. These surfaces were chosen based on the frequency that they are encountered in casework, luminescence emission from the substrate, and substrate pattern. A single donor deposited charged and natural fingermarks in a depletion series of three for each surface tested (Figure 6-5). Two sets of these samples were prepared. One set was fumed with cyanoacrylate and stained with STaR 11, and the other set was powdered with STaR 11 magnetic powder. In both cases, samples were stored under laboratory conditions (20-22 °C and 30-40% relative humidity) for 24 hours before any treatments were applied. The STaR 11 powder and cyanoacrylate stain were prepared in the same manner as outlined in 2.2.3.2 and 5.3.3 respectively.

Table 6-1: Surfaces used in the imaging system comparison study.

Samples used in this study
Glass microscope slides
Aluminium sheeting
Polyethylene bags
Fanta® soft drink can
Schweppes® lemonade soft drink label
Sunkist® soft drink label
Orange glossy cardboard

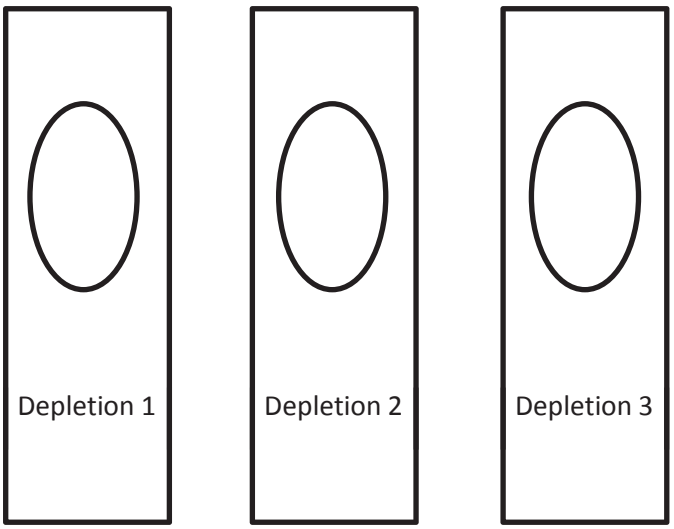


Figure 6-5: Preparation of samples using a depletion series.

6.3.3 Imaging of Samples

The developed fingermarks were imaged at their respective optimal conditions for each imaging system (Table 6-2). The best possible image was captured for each sample and used for comparison purposes. The best possible image was obtained using the settings that gave the greatest contrast and ridge detail.

Table 6-2: Imaging conditions for each surface and imaging system.

Imaging System	Surface	Excitation Wavelength (nm)	Emission Wavelength (nm)
Condor (10 averages)	Aluminium Glass slide Polyethylene bag	535	700-701 bandpass filter
	Fanta soft drink can Orange cardboard Schweppes soft drink label Sunkist soft drink label	535	750-751 bandpass filter
Fuji IS Pro	Aluminium Fanta soft drink cans Glass slide Orange cardboard Polyethylene bag Schweppes soft drink label Sunkist soft drink label	530	RG 830
Poliview	Aluminium Glass slide Polyethylene bag	530	700 barrier bandpass filter
	Fanta soft drink can Orange cardboard Schweppes soft drink label Sunkist soft drink label	530	750 barrier bandpass filter
VSC 6000	Aluminium Glass slide Polyethylene bag	515-590	725 long pass filter
	Fanta soft drink can Orange cardboard Schweppes soft drink label Sunkist soft drink label	533	780 long pass filter

Images from the VSC and Fuji IS Pro were in the JPEG file format and the Poliview images were in TIFF format. Images captured on the Condor were saved in TIFF spectral format and converted to a TIFF image file using Chemimage Xpert software.

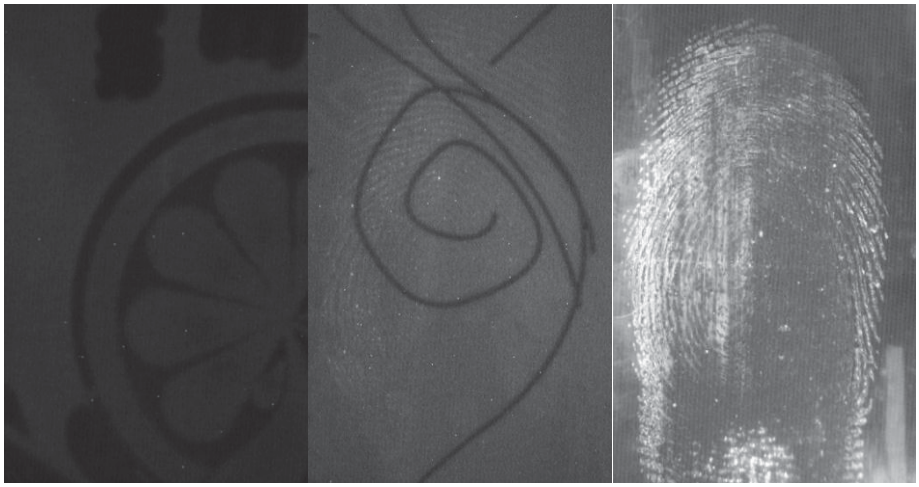
6.3.4 Comparison of Imaging Systems

Two scoring systems were used for comparison, the first was an adaptation of the Bandey scale [116], this scale aimed to take into consideration both the development of the fingermark and the contrast obtained from the image (Table 6-3, Figure 6-6).

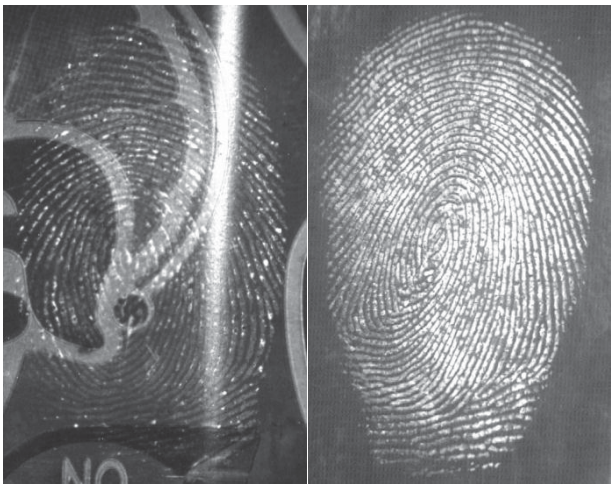
Table 6-3: Adapted Bandey scale used in the comparison study.

Grade	Description
0	No development
1	General fingermark pattern visible, minutiae not visible
2	General fingermark pattern visible, minutiae is visible, but less than half the fingermark is developed
3	General fingermark pattern visible, minutiae is visible, greater than half the fingermark is developed, but still incomplete.
4	General fingermark pattern visible, minutiae is visible and fingermark is fully developed.

After the images were scored using the above system, all four images from the one sample were combined to give a composite fingermark (Figure 6-7). This composite fingermark was used for a ranking score. Since each imaging system had different file types and resolutions, each quadrant was copied into a 300 ppi TIF file using the GIMP program. No other alterations to the images were made. Each imaging system was given a rank between 4 and 1, where 4 gave the best development and contrast and 1 gave the poorest development and contrast. For samples that did not give any development, a score of zero was given. All scoring was performed by one examiner.



Score = 0 Score = 1 Score = 2



Score = 3 Score = 4

Figure 6-6: Representative images for each score.

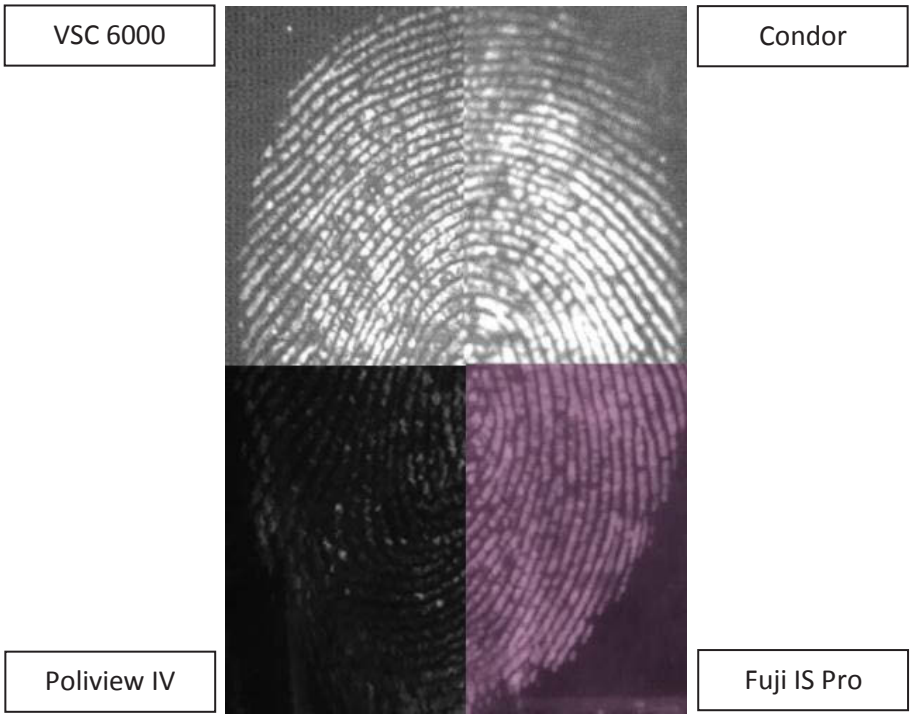


Figure 6-7: Composite image used for ranking system.

Each rank was then graphed and tallied in order to determine which imaging system gave the best overall development and contrast. The reasoning behind using the two comparison systems is that the first scoring system assesses the quality of a developed fingermark, while the ranking system assesses the relative performance of an imaging system, compared to the other imaging systems. Both systems provide different information about the overall performance of each imaging system.

6.4 Results and Discussion

6.4.1 Image Acquisition and Set Up

The three lab-based imaging systems (Condor, Poliview and VSC 6000) all had easy set up as the light source and the camera were already fixed. The IS pro had to be mounted onto a desk with an adjustable stand. The Polilight PL 500 also had to be put into a fixed position to ensure that the intensity of the light remained the same for all samples. Both the Poliview IV and VSC 6000 have live-view capabilities built into the systems, allowing for direct feedback

from the system during adjustment of the focus and exposure times. This resulted in much faster image acquisition times than the other two systems. The samples imaged with the Poliview IV were excited using a Polilight PL 500, a high intensity light source with a bandwidth of approximately 40 nm for each coloured light band [28]. The intensity of the light can be increased or decreased depending on the luminescence emission intensity of the sample. In this study, the most intense light setting was used in an attempt to minimise exposure times. The VSC 6000 uses a spot lamp with adjustable filters for varied bandwidth, ranging from 20 nm to 75 nm. A disadvantage of the VSC 6000 is that the spotlight cannot be adjusted for size or intensity. However, the ability to vary the bandwidth of the excitation light source was suitable compensation for the absence of an intensity dial.

When visualising in the NIR, the viewfinder in the Fuji IS Pro cannot be used and while it does possess a live-view function that is only operational for a 30 second time period before resetting. While this is certainly an advantage over other NIR cameras that do not possess live view capabilities, the 30-second time restriction can be troublesome especially when fine focussing. While these issues were minimised by placing the camera and the light source on a fixed mount, if this camera were to be used at the scene the image acquisition times would be significantly longer.

As part of the chemical imaging software each pixel of an image also contains spectroscopic data. As a result, image acquisition times are expected to be longer than with conventional imaging systems. This was observed in this study as run times for image collection were, in some cases over 50 times longer than for the other imaging systems. It should also be noted that the images on the Condor were only collected over a one nanometre wavelength range. The Condor can image over a range of 400-1100 nm; therefore, the run times for image acquisition with the Condor are largely dependent upon the wavelength range chosen for examination.

Based on the image acquisition and set up times both the Poliview IV and VSC 6000 provide the best conditions for rapid visualisation of NIR luminescent fingermarks. However, the Condor allows for a greater range of wavelengths and also provides spectroscopic data that could be useful for the identification of exogenous compounds.

6.4.2 Comparison Study

The results from the scoring system (Figure 6-8) indicated that the VSC 6000 gave the highest number of high quality fingermarks. The images obtained from the VSC 6000 had very high contrast and the quality of ridges was very clear. The VSC 6000 also had the lowest number of no development scores (as did the Poliview IV), indicating that it was also very good at imaging weakly developed or poor contrast fingermarks, in particular with the samples that exhibited strong background luminescence emission. A possible reason for this was that the excitation bandwidths could be adjusted to either increase the intensity of the STaR 11 treated fingermark or decrease the luminescence interference from the substrate.

The Poliview IV gave the second highest overall score and the margin between the VSC 6000 and the Poliview IV was very small (214 and 211 respectively). This result would be expected as both of these systems are lab-based imaging systems. The IS Pro gave the third highest overall score indicating that, as a laboratory based imaging system, the IS Pro is very effective. This, however, is not the intended use of the IS Pro. If the IS Pro was used as a portable imaging device the result would have been significantly worse.

Finally the Condor had the lowest score, though the difference between the IS Pro and the Condor was very small (190 and 188 respectively). This result is not unexpected as the comparison was made on the image captured alone, not the image obtained after post processing. Images were taken over a very short wavelength range in an effort to reduce run times. If the wavelength range was increased, chemometrics could be applied to the spectral data to increase contrast. The application of chemometrics to Condor images has previously provided a significant advantage compared to other imaging methods. In some cases, it was possible to detect and enhance fingermarks that could not be imaged by conventional imaging methods/systems [55, 174].

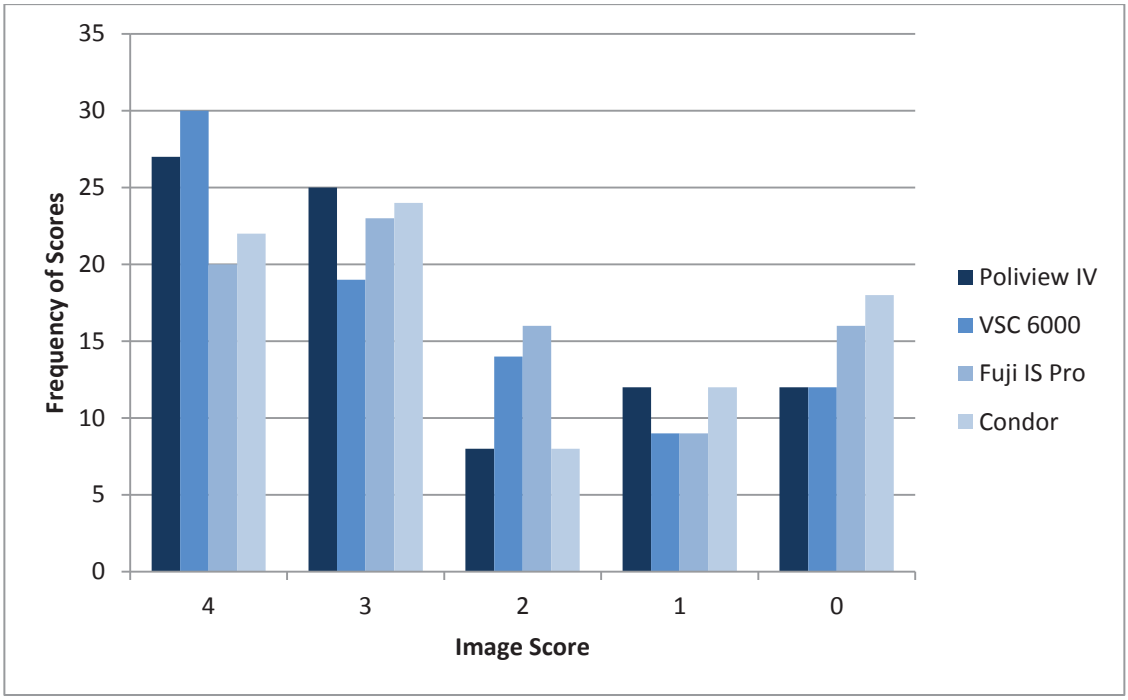


Figure 6-8: Imaging system comparison study results.

An examination of the individual scores indicated that, while the VSC 6000 had the highest overall score, the Poliview IV was able to give more marks of higher quality (i.e. scores of 3 or 4). Whereas the VSC 6000 was able to image weaker marks (scores of 1 or 2), interestingly both imaging systems had the same number of zero scores (no development); however, they were not the same set of 12 samples. This result would indicate that the imaging systems are only a tool for examinations and are limited by the development techniques. This will be explained further in Section 6.4.5.

As previously mentioned, a possible reason behind the low score for the Condor images was the fact that the comparison was made solely on the image without post processing. For weak samples, the Condor gave very 'grainy' images that reduced both ridge detail and contrast; this resulted in these samples getting a zero score (where other imaging systems gave scores of 1 or 2). This would indicate that the Condor, without post processing would not be suitable for imaging weakly developed or low contrast fingermarks. This result would probably change with the application of chemometrics or by using a broader range of wavelengths for examination.

Overall, these results indicate that the Poliview IV and the VSC 6000 give similar image contrast and quality. To further differentiate the imaging systems, the image capture/exposure times were compared.

6.4.3 Comparison of Exposure Times

Forensic imaging systems in operational use will be used to process a large volume of samples. As a result, rapid imaging is a desirable feature. As expected, exposure time was found to be very dependent on the luminescence emission intensity of the sample; weakly luminescent samples had longer exposure times than strongly luminescent samples. Since the same samples were used across all imaging systems and were not altered in any way, the variation between exposure times should only be a result of the system itself, not on the developed fingermark. Average exposure times for each system were tabulated and are shown in Table 6-4. Generally, the samples that exhibited strong luminescence emission from the substrate (Fanta soft drink cans, orange cardboard) had longer exposure times than the samples with no background interferences. A possible reason for this is that, for all imaging systems except the Fuji IS Pro, longer wavelength filters had to be used in order to sufficiently supress the background; therefore longer exposure times had to be used in order to effectively image the developed fingermark. Using a longer wavelength filter for the substrates with strong luminescence interference meant that images were collected past the peak luminescence emission wavelength of STaR 11. This meant that luminescence was going to be weaker and therefore longer exposure times were required.

Table 6-4: Average of exposure times for the different imaging systems.

Sample	Poliview IV Average (s)	VSC 6000 Average (s)	Fuji IS Pro Average (s)	Condor Average (s)
Aluminium	1.94	0.18	0.48	223
Fanta Can	4.77	2.27	0.26	167
Glass	1.72	0.04	0.34	115
Orange Cardboard	12.8	3.82	0.55	152
Polyethylene Bags	3.26	0.04	1.20	150
Soft Drink Label Black	4.99	3.00	0.58	130
Soft Drink Label Orange	10.6	3.13	1.19	218

The Fuji IS Pro, overall, gave the lowest average exposure time for the samples tested. A possible reason for this observation is that the Polilight used to excite the samples was closer to the sample than the excitation source for other imaging systems. This increased intensity of the light source could have increase the emission intensity of STaR 11 developed fingermarks and therefore reduced the exposure times will discussed further in Section 6.4.5. However, as previously mentioned, the Fuji IS Pro does not use the viewfinder for NIR imaging; as a result, focussing times and 'framing' of the samples takes significantly longer than with the other imaging systems. While focussing and framing times were not included in the averages above (for any system), it should be noted that the IS Pro was significantly longer to set up than either the Poliview IV or the VSC 6000.

The VSC 6000 had shortest average exposure times for the lab based imaging systems with very short exposure times for the glass and polyethylene bag samples. The most likely reason for this is that the VSC 6000 uses longpass filters instead of the bandpass filters used in the other imaging systems [169]. This would, ultimately, have an effect on exposure times since the longpass filter allows for more luminescence emission from STaR 11 to be visualised due to the broader wavelength that emission intensities can be observed in. The issue with longpass filters is that for substrates with strong broadband luminescence emission, it can decrease the contrast when viewing in the NIR. In an effort to minimise this, longer wavelength filters (780 nm as opposed to 725 nm) had to be used; this resulted in a decrease in luminescence for STaR 11 as the peak emission is at a shorter wavelength (730 nm). The

Poliview IV followed a similar trend; however, exposure times were longer for all samples. Since bandpass filters were used with the Poliview IV, it would be expected that longer exposure times would be required, when compared to longpass filter. Longpass filters are available for the Poliview IV; however, they were not used in this comparison as the filters available were not optimal for STaR 11 visualisation and images using these filters had higher amounts of background interferences.

The Condor exposure times were significantly longer than the other imaging systems used in the comparison. This result was expected as the image collection process of the Condor requires images to be collected over a wavelength range (in this case 1 nm) and a number of averages be taken of each image. Increasing the wavelength range may have assisted with contrast but it would have also increased the exposure times. Unlike the other imaging systems, the background luminescence emission did not greatly affect the exposure times for each sample as they were all very similar. Similar to the Fuji IS Pro, there was no 'live-view' function on the Condor. Instead a test image was used at a shorter exposure time to focus, and the exposure time was then adjusted to allow for suitable contrast. This was not included in the exposure time comparison but should be noted as it would be required for all samples being imaged.

Based on the exposure times alone, it would be fair to conclude that the Fuji IS Pro was the most rapid imaging system available. However, as previously mentioned the absence of a true 'live-view' function increases the focussing and framing times. Similarly, in this study the Fuji IS Pro was placed in a fixed position with a fixed Polilight light guide to decrease these focussing and framing times. If the Fuji IS Pro was used as a portable imaging system, the focussing and framing times would have been significantly longer.

6.4.4 Ranking Comparison

After the images were stitched together in quadrants, each imaging system was given a rank based on the quality of development and the results were graphed (Figure 6-9). The general trend was very similar to the scoring system used previously; however, the difference between the imaging systems was more pronounced when using the ranking system. This data indicated that, in the majority of cases, the VSC 6000 gave the highest quality images,

with the Poliview IV being the next best imaging system; however, the difference in scores was significant. The VSC 6000 also never received a rank of 1. This would indicate that, of all the imaging systems used in this study, the VSC 6000 gave higher quality images than at least one other imaging system.

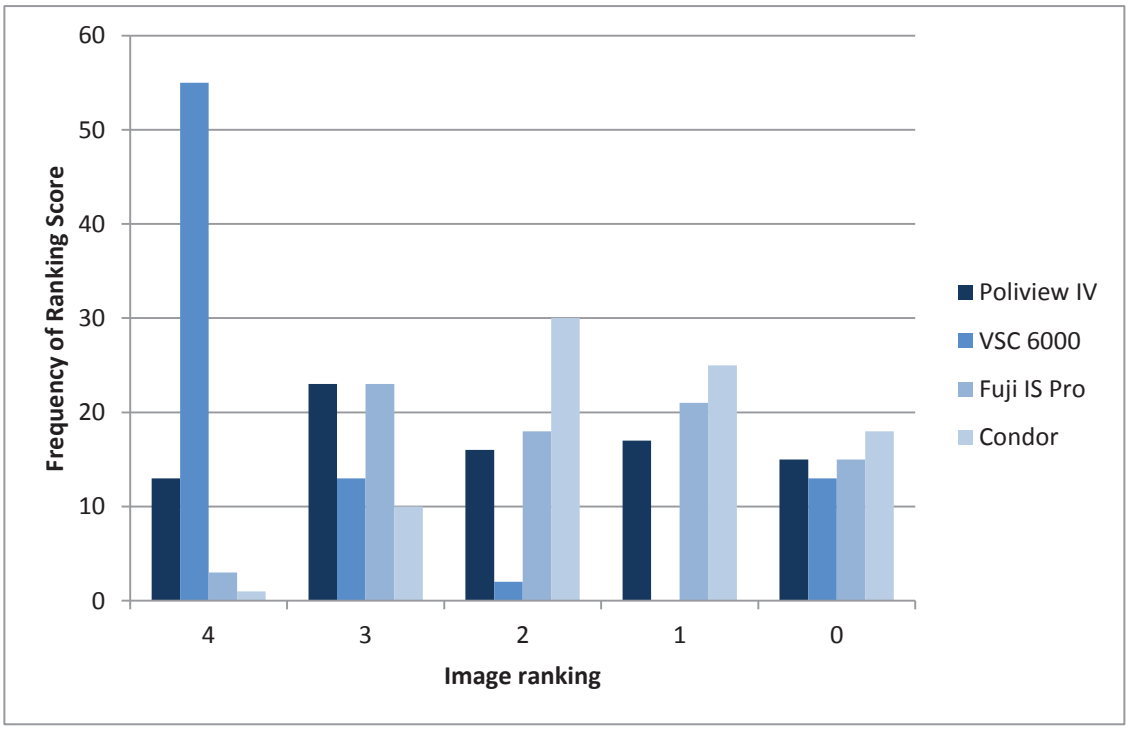


Figure 6-9: Ranking comparison results (4 – 1 best image to worst image, 0 – no development).

The Poliview IV had a significant difference in scores compared to the VSC 6000. A major reason for this is that, when placed side by side with the VSC images, the Poliview IV images had significantly lower contrast, despite the images showing good ridge detail. This trend could also be seen when comparing the Poliview IV images with strongly luminescent samples, as the other techniques gave higher contrast. This explains the wider spread of ranks for the Poliview than with any other system. Interestingly, the Poliview IV had the highest ranks than the other imaging systems for samples that were weakly developed or had poor contrast (Figure 6-10). This result highlights a significant advantage of the Poliview IV, since well-developed marks will easily be imaged regardless of the imaging system. However, there is a greater use for an imaging system which will enhance weakly developed fingermarks. In this instance, the Poliview IV may not be the strongest performer overall, but is better suited for imaging fingermarks that cannot be visualised by the other imaging systems.

Figure 6-10: Representative images of: (left) poor contrast from the Poliview IV images and (right) good development from the Poliview IV image viewed in the luminescence mode for different imaging systems (clockwise from top left; VSC 6000, Condor, Fuji IS Pro and Poliview IV).

The Fuji IS Pro and Condor were the two lowest ranked imaging systems respectively. This was found to be for the same reasons stated in the previous scoring comparison method. The Fuji IS Pro had very poor contrast for weakly developed samples and the Condor images were very grainy, which decreased contrast significantly.

6.4.5 Limitations of the Comparison Method

The comparison of different imaging systems is not without limitations and, as a result, several assumptions had to be made in order for the experiment to be performed consistently. The aim of this experiment was to determine the effectiveness of each imaging system based solely on the imaging capabilities of the hardware itself. This meant that software improvement of the images by post processing was not employed. This limitation meant that several functionalities that are a key feature of the imaging system (such as the chemical imaging component of the Condor) were not tested. The implication was that the Condor was at a disadvantage since it was not being compared on all its capabilities. It is highly unlikely that the Condor would be purchased without using the chemical imaging

capabilities. It has been shown in previous studies that the post processing of chemical images can significantly increase contrast from the captured images [174]. According to the Scientific Working Group on Imaging Technology (SWGIT), images adjustments are permitted, so long as the original image is kept and the processing technique does not introduce artefacts that add misleading information [180]. In theory, these adjustments could be made to all the images to improve the imaged fingermark and this may have decreased the difference in scores. This, however, introduces another issue in that the image enhancements are limited by the skill of the image processing software and the user. This would introduce another level of subjectivity as the adjustments would be made to the satisfaction of one examiner, not by an objective measure.

The subjectivity of the study itself is also a limitation that affected the results obtained, specifically with the image capturing. Since there is no objective way to measure the 'best possible image' for each sample before capturing an image, it had to be based on a single examiner's judgement. While attempts to minimise bias were used, any examination that required a subjective judgement will have some level of bias. The Poliview IV images are a specific instance where bias was minimised. While overall the results showed that the Poliview IV images were generally of a high quality, when compared to the other systems, contrast was quite poor. It would have been advantageous to re-run the Poliview IV samples (altering the distance from the excitation source, adjusting the gain etc.) in an attempt to have similar contrast to the other images, however, this would have been manipulating the data to get a desired outcome. As a result, this may have led to an underestimation of the Poliview IV system.

The fundamental aim of this project was to look at the effectiveness of each imaging system in its ability to visualise NIR luminescent fingermark. It should be noted that these results only apply to NIR visualisation. Samples were not viewed or compared in the visible region. As a result, the effectiveness of each system in visualising conventional luminescent fingermark detection techniques cannot be determined from this information. In order to gain a full understanding of the advantages and limitation of each imaging system, a study should be performed on conventional fingermark detection techniques in the visible region in order to gain a greater comparison between the systems.

Another issue that may have skewed the results was that different imaging systems used different excitation light sources. The Poliview IV and Fuji IS Pro both used the Polilight PL

500; however, the distance between the light and the sample could not be kept consistent between the different systems. This would have had an effect on the luminescence emission intensity of the samples and thus the exposure times of the images captured. Similarly, the VSC 6000 and the Condor used alternate light sources that have different intensities to the bands provided by PL 500. In the case of the VSC 6000, it would be unrealistic to use an external light source in order to image samples as this set-up is unlikely to be employed by operational forensic laboratories. While the effect may not have been significant, the different light source may have slightly altered the results and this is therefore highlighted as a limitation of this study.

6.5 Conclusion

Imaging systems are important tools in the forensic examination of latent fingermark. Therefore the effectiveness of these imaging systems needs to be determined, particularly when new techniques (such as NIR detection of latent fingermarks) become available. This study looked at four different forensic imaging systems (Condor, Fuji IS Pro, Poliview IV and VSC 6000) and assessed their ability to image STaR 11 developed fingermark samples in the NIR. Overall, it was determined that all four systems were able to image STaR 11 developed fingermarks. The two main factors that influenced the effectiveness of the imaging system were the quality of the development and the substrate the fingermark was placed on. If the fingermark was weak or STaR 11 poorly developed the fingermark, the Poliview IV or the VSC 6000 would be the most effective imaging systems. For samples that exhibit high luminescence interference from the surface, the VSC 6000 provides greater control over the excitation and emission wavelengths than any other system. The Fuji IS Pro one of only a few commercially available NIR SLR cameras. This camera was very effective in imaging STaR 11 developed samples; however, its poor live-view functionality results in long set-up and focus times that potentially limit its field use. The Condor is a chemical imaging system and while the chemical imaging capabilities were not explored in this study, chemical imaging must be used if the best possible results are to be obtained from weak fingermarks. The long run times for samples limits its use for routine examinations.

This study however has several limitations, the most significant being that only the NIR imaging capabilities were compared. This meant that in some cases (particularly with the

Condor), that the full extent of the imaging capabilities was not considered. However, the reasoning behind this is that the study looked at the NIR imaging capabilities; in theory, imaging in the NIR should remove the need for image post treatment as substrate interferences should be minimised. Similarly, in order to keep the conditions as consistent as possible, images were not altered with any digital darkroom adjustments (e.g. brightness and contrast). There were however, some factors that could not be controlled and would have affected the results. The forensic light sources were not kept consistent throughout the experiment, this was due to availability and practicality (i.e. the VSC 6000 has an inbuilt light source; therefore it is unlikely that examiners would use a Polilight PL 500 instead). Similarly, different filters were used between the systems; a long pass filter was used for the VSC 6000 examination, while all other imaging systems used a band pass filter. This would have affected the exposure times for the VSC 6000 imaged samples. Again this was an unavoidable inconsistency.

Overall, this study showed that all of the forensic imaging systems tested have NIR imaging capabilities; however, the effectiveness of each system is quite variable. The VSC 6000 and Poliview IV both provide excellent image quality, the VSC 6000 gave the best results overall, however the Poliview IV gave better results for the weaker developed marks. Based on this they are the preferred lab-based imaging systems. The Fuij IS Pro is an excellent NIR digital camera; however, it is better suited for lab-based imaging than field use. While the full capabilities of the Condor were not explored in this study, previous research indicates that the use of chemical imaging would be better suited for weak samples that could not be imaged with other forensic imaging systems.

Chapter 7: Future Work, Recommendations and Conclusions

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7.1 Future Work and Recommendations

The near infrared region provides a significant opportunity for fingermark detection techniques, of which, only a small area was explored in this research. With regards to NIR luminescent dyes, there are a variety of different dyes that are commercially available. These dyes could be applied as cyanoacrylate stains or coated onto nanopowders to give strong luminescence further into the NIR region than the dyes tested in this study. For instance, there are a number of carbocyano based laser dyes, which are available at a lower cost compared to the styryl dyes and still provide peak luminescence emissions in the NIR region. These potentially could be combined with luminescent dyes in the visible region (similar to the STaR 11 formulation) for broader luminescence emission, which would assist in the detection and enhancement of latent fingermarks.

A major area for further research regarding this project is the expansion of donors, surfaces and ages for all successful reagents developed (STaR 11 powder and cyanoacrylate stains). The conclusions reached for these techniques were based on the data obtained, which was very limited and therefore should be treated with caution. This is a significant limitation of this work, however given the small amount of current research in this area it was a conscious decision to have smaller studies that cover as many fingermark detection techniques as possible instead of focussing on one or two types of techniques. At this stage it would be unrealistic to begin using these reagents for casework as more rigorous testing needs to be applied. To strengthen these results these studies should be escalated to a validation/pseudo operational study phase. According to the draft IFRG guidelines [181], this would include the use of a larger donor pool, more realistic samples and substrates. The use of a depletion series and reproduction of the results obtained in this work would be of significant advantage for any further work.

With regards to the STaR 11 powder formulation, a physical mixture of STaR 11 and Blitz Green was prepared and gave strong luminescence emission over a very broad range. This powder was able to give strong luminescence when using a 360 nm excitation with a 700 nm barrier bandpass filter (Stokes shift of 340 nm). This would reduce any background interferences but could also provide luminescence emission in the blue and yellow regions of the visible spectrum (in addition to the NIR luminescence emission). This was not explored in this research as there was no significant improvement in the NIR luminescence emission when compared to STaR 11 on its own. The potential for this powder however needs to be further explored. Similarly, there has been an increase in the number of NIR cameras and portable light sources available which may improve the field capabilities of STaR 11 aluminium oxide nanopowder.

As previously mentioned there are a wide range of commercially available NIR dyes that are not based on the styryl structure. These should be explored for use in the development of a NIR suspension. These other NIR dyes potentially may perform better in aqueous suspensions than styryl 11. This would assist in the both the SSP and SPR based formulation as the dye may not be as sensitive to water as styryl 11 was and as a result give stronger luminescence. Similarly it could be coated of different nanopowders such as titanium dioxide, which has previously been shown to be very effective in suspensions [101]. The sensitivity to surfactants and water made the STaR 11 SSP give poorer results compared to Wet Powder™. The consistency of the STaR 11 SSP could be improved with the addition of more nanopowder, which was not possible was not possible with the suspension developed in this study. The use of Ecospray® was explored in this research, however its potential as a delivery method for suspensions was not fully explored due to the poor results from the STaR 11 SPR suspension. The Ecospray® device could easily be used to replace the conventional pump-spray bottles to deliver a more consistent suspension mist which could give better developed marks. This device could potentially give stronger results and if paired with an effective luminescent suspension (NIR or otherwise) and would be a suitable alternative to the current method of delivery.

Styrylisatin was synthesised successfully, however, it was deemed to be an ineffective amino acid sensitive reagent for fingermark detection. It is unlikely that this conclusion could be altered with additional studies. Based on this it would be more advantageous to explore other biological stains or biologically reactive molecules. There are a number of NIR luminescent dyes used for biological applications, a review by Peng and Draney outlines that

new NIR luminescent dyes have been developed in an effort to address the low quantum yields, poor water solubility and poor photostability. This review also outlined a number of dyes which could be used for the visualisation of proteins and DNA, this potentially could be applied to latent fingermark detection on both porous and non-porous surfaces [182]. Given the water solubility of these dyes, combined with their affinity for biological material, a NIR luminescent blood reagent may be an avenue worth exploring. A similar area to explore would be the use of a NIR luminescent protein stain for use as an alternative to physical developer.

The comparison between PolyCyano UV and Cyanobloom determined that PolyCyano UV generally gave poorer results when compared to Cyanobloom stained with rhodamine 6G. This study used a very limited number of surface types, in an effort to broaden the comparison and strengthen the conclusion from this study more surfaces should be trialled with a larger number of donors. Similarly, there are a number of other commercially available luminescent cyanoacrylates which could give differing results to those obtained in this study and may perform better than PolyCyano UV. PolyCyano UV did not affect the luminescence of rhodamine 6G which still gave good results. An area where these luminescent cyanoacrylates would provide a significant advantage is in the development of latent fingermarks on semi-porous surfaces such as glossy magazines and polymer banknotes. A small study was attempted, however, it required additional optimisation that did not fit with the scope of the project. This is an important area for future work that should be conducted in order to effectively determine whether luminescent cyanoacrylate can develop fingermarks on surfaces that would be damaged if stained with conventional cyanoacrylate stains. With regards to the sequencing of STaR 11 this work reaffirmed the conclusions of a previously study [64], that STaR 11 could be used as a replacement for rhodamine 6G. However, if needed, STaR 11 could still be used in sequence with rhodamine 6G without affecting either stains luminescence emission intensity.

The comparison between forensic imaging systems confirmed that all systems were suitable for visualisation in the NIR. Both the VSC 6000 and Poliview IV gave the strongest performance overall, however, the VSC 6000 generally gave better results all round, the Poliview IV was better at visualising weakly developed fingermarks. The Fuji IS Pro was better suited as a lab based camera due to the issues with focussing and poor live-view functionality when used as a field-based camera. However, when compared to the lab-based imaging systems it generally gave poorer results. The Condor gave the poorest results in this study,

the main reason for this is that the full capabilities of the Condor were not used. To expand on this work, the use of chemometrics would need to be applied to samples in order to truly assess the abilities of the Condor. Similarly, these results are only representative of how these systems perform in NIR visualisation, imaging in the visible region may give different results to those obtained in this study. As previously mentioned, commercial cameras can be adapted for imaging in the NIR, a small trial was performed using an adapted Nikon D90 and was found to give better field functionality than the IS Pro.

7.2 Conclusions

As outlined in Section 1.9, the aim of this research was to investigate the use of near infrared dyes for various areas of latent fingermark detection. This study has shown that there are numerous advantages of using a NIR luminescent detection or enhancement method, most notably in reducing the interferences from the substrate and improving the quality of developed marks deposited on difficult surfaces. When compared to other techniques that visualise fingermarks outside the visible region, the NIR detection methods do not require the use of specific instrumentation or post-processing of images.

The development of a luminescent dye mixture of styryl 11 and rhodamine 6G (STaR 11) highlighted the potential for universal imaging of developed fingermarks (i.e. luminescent in both the visible and NIR regions). This research has shown that NIR detection techniques can be used in conjunction with or as a replacement for current fingerprint powders or cyanoacrylate stains. The donor and ageing studies determined that the techniques were able to develop and enhance fingermarks up to 1 month for powder development and 2 months of cyanoacrylate staining. As these were the maximum ageing times considered, the reagents may be able to develop older marks than those examined in this study. When compared to the conventional methods there were very few instances where the conventional technique performed significantly better than the NIR method. However, the ability to visualise in two different regions of the electromagnetic spectrum is a significant advantage that no other commercial technique can provide.

While NIR luminescent suspensions for adhesive and wetted surfaces were developed, when compared to the commercial techniques they failed to provide any significant advantage.

Similarly for the development of a novel NIR luminescent detection technique for fingermarks on porous surface also gave very poor results. The sensitivity of styrylisatin was very low for amino acids which resulted in only high concentrations of amino acids giving a luminescent product. In these cases, while the results obtained did not show any significant improvement, it should not exclude the possibility for NIR detection for these techniques As previously mentioned this study focussed on the styryl dyes and the use of other NIR luminescent dyes may give more promising results as well as targeting different fingermark components for porous surfaces.

Ultimately, this research has laid the groundwork for future work with regards to the detection of latent fingermarks in the NIR region. There are still many different areas to explore which could expand and improve upon the work discussed throughout this thesis.

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Appendices

Appendix i - **Full Donor Study Results from STaR 11 Nanopowders**

Table i-1: Comparison study results for all donors on glass

	Female		**Male**	
	Natural Glass Mean ± SD	**Charged Glass Mean ± SD**	**Natural Glass Mean ± SD**	**Charged Glass Mean ± SD**
Fresh	-0.25 ± 0.50	-0.50 ± 2.1	0.33 ± 1.03	-0.17 ± 0.75
1 Day	-0.25 ± 0.50	1.25 ± 0.5	-0.25 ± 1.4	1.00 ± 0.00
3 Days	-0.25 ± 0.50	0.00 ± 0.82	0.00 ± 0.52	0.50 ± 0.50
1 Week	-2.00 ± 0.00	0.75 ± 0.96	0.50 ± 1.4	1.00 ± 0.89
2 Weeks	0.25 ± 0.96	0.25± 0.50	0.67 ± 0.52	0.17 ± 0.41
1 Month	0.50 ± 0.58	0.75 ± 0.96	1.00 ± 0.52	1.00 ± 0.63

Table i-2: Comparison study results for all donors on Fanta® soft drink cans

	Female		**Male**	
	Natural Fanta® Soft Drink Can Mean ± SD	***Charged Fanta® Soft Drink Can Mean ± SD***	***Natural Fanta® Soft Drink Can Mean ± SD***	***Charged Fanta® Soft Drink Can Mean ± SD***
Fresh	-0.50 ± 1.29	0.75 ± 0.96	-0.17 ± 0.75	1.00 ± 0.63
1 Day	-0.50 ± 0.58	1.00 ± 0.00	-0.17 ± 0.75	0.67 ± 0.82
3 Days	0.50 ± 0.58	-0.75 ± 1.50	0.17 ± 0.41	0.17 ± 0.41
1 Week	-1.25 ± 0.96	0.00 ± 0.82	-0.83 ± 1.17	0.00 ± 0.63
2 Weeks	0.00 ± 0.00	0.50 ± 1.00	0.17 ± 0.75	0.00 ± 0.63
1 Month	0.00 ± 0.00	0.00 ± 0.82	-0.17 ± 0.75	0.17 ± 0.41

Table i-3: Comparison study results for all donors on laminate

	Female		Male	
	Natural Laminate Mean ± SD	**Charged Laminate Mean ± SD**	**Natural Laminate Mean ± SD**	**Charged Laminate Mean ± SD**
Fresh	-0.50 ± 1.41	2.00 ± 0.00	0.50 ± 0.55	1.33 ± 0.52
1 Day	1.25 ± 1.15	2.00 ± 0.00	0.83 ± 1.17	1.50 ± 0.55
3 Days	0.58 ± 1.00	1.00 ± 1.41	0.67 ± 1.63	0.83 ±0.98
1 Week	0.58 ± 1.50	1.50 ± 0.58	1.50 ± 0.55	1.50 ± 0.55
2 Weeks	0.58 ± 1.25	1.25 ± 0.50	0.33 ± 1.51	1.17 ± 0.41
1 Month	0.50 ± 1.00	1.00 ± 1.15	0.00 ± 0.00	1.50 ± 0.55

Table i-4: Comparison study results for all donors on polyethylene bags

	Female		Male	
	Natural Polyethylene Bag Mean ± SD	**Charged Polyethylene Bag Mean ± SD**	**Natural Polyethylene Bag Mean ± SD**	**Charged Polyethylene Bag Mean ± SD**
Fresh	0.5 ±0.58	0.25 ± 0.96	-0.33 ± 1.0	0.17 ± 0.75
1 Day	-0.25 ±0.5	-0.5 ± 1.3	0.00 ±0.89	-0.33 ± 1.0
3 Days	0.00 ± 1.4	0.25 ± 0.5	-0.50 ± 0.55	-0.33 ± 0.52
1 Week	-1.5 ± 1.0	-1 ± 1.2	-0.83 ± 0.41	-0.17 ± 0.98
2 Weeks	0.50 ± 0.58	0.25 ± 0.5	-0.17 ± 0.41	-0.50 ± 0.55
1 Month	0.50 ± 1.0	1.75 ±0.5	-0.50 ± 0.84	-0.33 ± 1.0

Appendix ii - **Full Donor Study Results for STaR Sticky-Side Powder Study**

Table ii-1: Comparison study results for all donors on exposed cloth tape

	Female		Male	
	Natural Cloth Tape Mean ± SD	**Charged Cloth Tape Mean ± SD**	**Natural Cloth Tape Mean ± SD**	**Charged Cloth Tape Mean ± SD**
Fresh	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
1 Day	-1 ± 0.00	-1 ± 0.00	-0.5 ± 0.71	-2 ± 0.00
3 Days	0 ± 2.83	0.5 ± 0.71	-1.5 ± 0.71	-0.5 ± 0.71
1 Week	1.5 ± 0.71	-1 ± 1.41	-1 ± 1.41	-1 ± 0.00
2 Weeks	-2 ± 0.00	-0.5 ± 0.71	-0.5 ± 0.71	-1 ± 1.41
1 Month	-2 ± 0.00	-0.5 ± 0.71	-2 ± 0.00	-1.5 ± 0.71

Table ii-2: Comparison study results for all donors on cloth tape which was stuck down

	Female		Male	
	Natural Cloth Tape Mean ± SD	**Charged Cloth Tape Mean ± SD**	**Natural Cloth Tape Mean ± SD**	**Charged Cloth Tape Mean ± SD**
Fresh	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
1 Day	-1 ± 1.41	-1.5 ± 0.71	-1 ± 0.00	0 ± 0.00
3 Days	0 ± 1.41	-2 ± 0.00	-0.5 ± 0.71	0 ± 0.00
1 Week	0 ± 0.00	-1 ± 1.41	-2 ± 0.00	-0.5 ± 0.71
2 Weeks	-1.5 ± 0.71	-1.5 ± 0.71	-1.5 ± 0.71	-0.5 ± 2.12
1 Month	-1 ± 0.00	0 ± 0.00	-0.5 ± 0.71	0 ± 0.00

Table ii-3: Comparison study results for all donors on exposed duct tape

	Female		Male	
	Natural Duct Tape Mean ± SD	**Charged Duct Tape Mean ± SD**	**Natural Duct Tape Mean ± SD**	**Charged Duct Tape Mean ± SD**
Fresh	0 ± 0.00	0 ± 0.71	0 ± 0.00	0.5 ± 0.71
1 Day	-0.5 ± 0.71	-1 ± 1.41	1.5 ± 0.71	0 ± 1.41
3 Days	0.5 ± 0.71	1 ± 0.00	-0.5 ± 0.71	0 ± 2.83
1 Week	0 ± 0.00	1 ± 1.41	0 ± 0.00	1 ± 1.41
2 Weeks	1.5 ± 0.71	1 ± 1.41	0 ± 0.00	0 ± 0.00
1 Month	0 ± 0.00	1 ± 1.41	0 ± 0.00	0.5 ± 0.71

Table ii- 4: Comparison study results for all donors on duct tape which was stuck down

	Female		Male	
	Natural Duct Tape Mean ± SD	**Charged Duct Tape Mean ± SD**	**Natural Duct Tape Mean ± SD**	**Charged Duct Tape Mean ± SD**
Fresh	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.71
1 Day	0 ± 1.41	-0.5 ± 0.71	-1 ± 0.00	-0.5 ± 0.71
3 Days	0 ± 1.41	-0.5 ± 0.71	-1 ± 0.00	0 ± 1.41
1 Week	0 ± 2.83	0.5 ± 0.71	-0.5 ± 0.71	-0.5 ± 0.71
2 Weeks	-0.5 ± 2.12	-0.5 ± 0.71	0 ± 0.00	-0.5 ± 2.12
1 Month	-1 ± 0.00	-1 ± 1.41	-0.5 ± 2.12	-1 ± 0.00

Table ii- 5: Comparison study results for all donors on exposed gaffa tape

	Female		Male	
	Natural Gaffa Tape Mean ± SD	**Charged Gaffa Tape Mean ± SD**	**Natural Gaffa Tape Mean ± SD**	**Charged Gaffa Tape Mean ± SD**
Fresh	0 ± 0.00	0.5 ± 0.71	0 ± 0.00	0 ± 0.00
1 Day	-1 ± 0.00	-0.5 ± 0.71	-1 ± 0.00	-1 ± 1.41
3 Days	0.5 ± 0.71	-1 ± 1.41	-2 ± 0.00	0 ± 0.00
1 Week	-1 ± 0.00	-1 ± 0.00	-1.5 ± 0.71	-0.5 ± 0.71
2 Weeks	-2 ± 0.00	-1.5 ± 0.71	0 ± 0.00	-1 ± 1.41
1 Month	-2 ± 0.00	-0.5 ± 0.71	-1.5 ± 0.71	-1.5 ± 0.71

Table ii- 6: Comparison study results for all donors on gaffa tape which was stuck down

	Female		Male	
	Natural Gaffa Tape Mean ± SD	**Charged Gaffa Tape Mean ± SD**	**Natural Gaffa Tape Mean ± SD**	**Charged Gaffa Tape Mean ± SD**
Fresh	0 ± 0.00	0.5 ± 0.71	0 ± 0.00	0 ± 0.00
1 Day	-1 ± 1.41	-1 ± 0.00	-1 ± 0.00	-0.5 ± 0.71
3 Days	-0.5 ± 0.71	-1 ± 0.00	0 ± 0.00	0.5 ± 0.71
1 Week	-0.5 ± 0.71	-1.5 ± 0.71	-1.5 ± 0.71	-0.5 ± 0.71
2 Weeks	-2 ± 0.00	-1 ± 1.41	-1 ± 1.41	-1.5 ± 0.71
1 Month	-1 ± 1.41	0.5 ± 0.71	0.5 ± 0.71	-0.5 ± 0.71

Table ii- 7: Comparison study results for all donors on exposed masking tape

	Female		Male	
	Natural Masking Tape Mean ± SD	**Charged Masking Tape Mean ± SD**	**Natural Masking Tape Mean ± SD**	**Charged Masking Tape Mean ± SD**
Fresh	-1 ± 0.00	-0.5 ± 0.71	-0.5 ± 0.71	0 ± 0.00
1 Day	-0.5 ± 0.71	-0.5 ± 0.71	-2 ± 0.00	-1 ± 0.71
3 Days	-0.5 ± 0.71	-0.5 ± 0.71	0.5 ± 0.71	-2 ± 0.00
1 Week	-0.5 ± 0.71	0 ± 0.00	-0.5 ± 0.71	-1.5 ± 0.00
2 Weeks	-1 ± 1.41	-1 ± 1.41	-2 ± 0.00	0 ± 0.71
1 Month	-1 ± 1.41	-2 ± 0.00	-1.5 ± 0.71	-1.5 ± 0.71

Table ii- 8: Comparison study results for all donors on masking tape which was stuck down

	Female		Male	
	Natural Masking Tape Mean ± SD	**Charged Masking Tape Mean ± SD**	**Natural Masking Tape Mean ± SD**	**Charged Masking Tape Mean ± SD**
Fresh	-1 ± 0.00	-0.5 ± 0.71	-0.5 ± 0.71	-1 ± 0.00
1 Day	-0.5 ± 0.71	0 ± 0.00	-2 ± 0.00	-1 ± 1.41
3 Days	-1 ± 0.00	-1 ± 1.41	-0.5 ± 0.71	-0.5 ± 0.71
1 Week	0 ± 0.00	0 ± 0.00	-1 ± 1.41	-0.5 ± 0.71
2 Weeks	-1 ± 1.41	-0.5 ± 0.71	-1 ± 1.41	-1.5 ± 0.71
1 Month	-2 ± 0.00	-1.5 ± 0.71	-1.5 ± 0.71	-2 ± 0.00

Table ii- 9: Comparison study results for all donors on exposed packing tape

	Female		Male	
	Natural Packing Tape Mean ± SD	**Charged Packing Tape Mean ± SD**	**Natural Packing Tape Mean ± SD**	**Charged Packing Tape Mean ± SD**
Fresh	-1 ± 0.00	-0.5 ± 0.71	-1 ± 0.00	-0.5 ± 0.71
1 Day	-1 ± 1.41	-2 ± 0.00	-1.5 ± 0.71	-1.5 ± 0.71
3 Days	-1.5 ± 0.71	-0.5 ± 0.71	0 ± 0.00	-1 ± 1.41
1 Week	-1 ± 0.00	-1 ± 0.00	0 ± 0.00	-1.5 ± 0.71
2 Weeks	-2 ± 0.00	-1 ± 1.41	-1 ± 1.41	-1.5 ± 0.71
1 Month	-1 ± 1.41	-1.5 ± 0.71	-1 ± 0.00	-0.5 ± 0.71

Table ii- 10: Comparison study results for all donors on packing tape which was stuck down

	Female		Male	
	Natural Packing Tape Mean ± SD	**Charged Packing Tape Mean ± SD**	**Natural Packing Tape Mean ± SD**	**Charged Packing Tape Mean ± SD**
Fresh	-1 ± 0.00	-0.5 ± 0.71	-0.5 ± 0.00	-0.5 ± 0.71
1 Day	-2 ± 0.00	-1.5 ± 0.71	-2 ± 0.00	-2 ± 0.00
3 Days	-1.5 ± 0.71	-2 ± 0.00	-0.5 ± 0.71	-1 ± 0.00
1 Week	-1.5 ± 0.71	-1 ± 1.41	-1.5 ± 0.71	-1.5 ± 0.71
2 Weeks	-1 ± 1.41	0 ± 0.00	-2 ± 0.00	-2 ± 0.00
1 Month	-2 ± 0.00	-1 ± 1.41	-1.5 ± 0.71	-1.5 ± 0.71

Appendix iii - **Spectra Used in the Characterisation of Styrylisatin.**

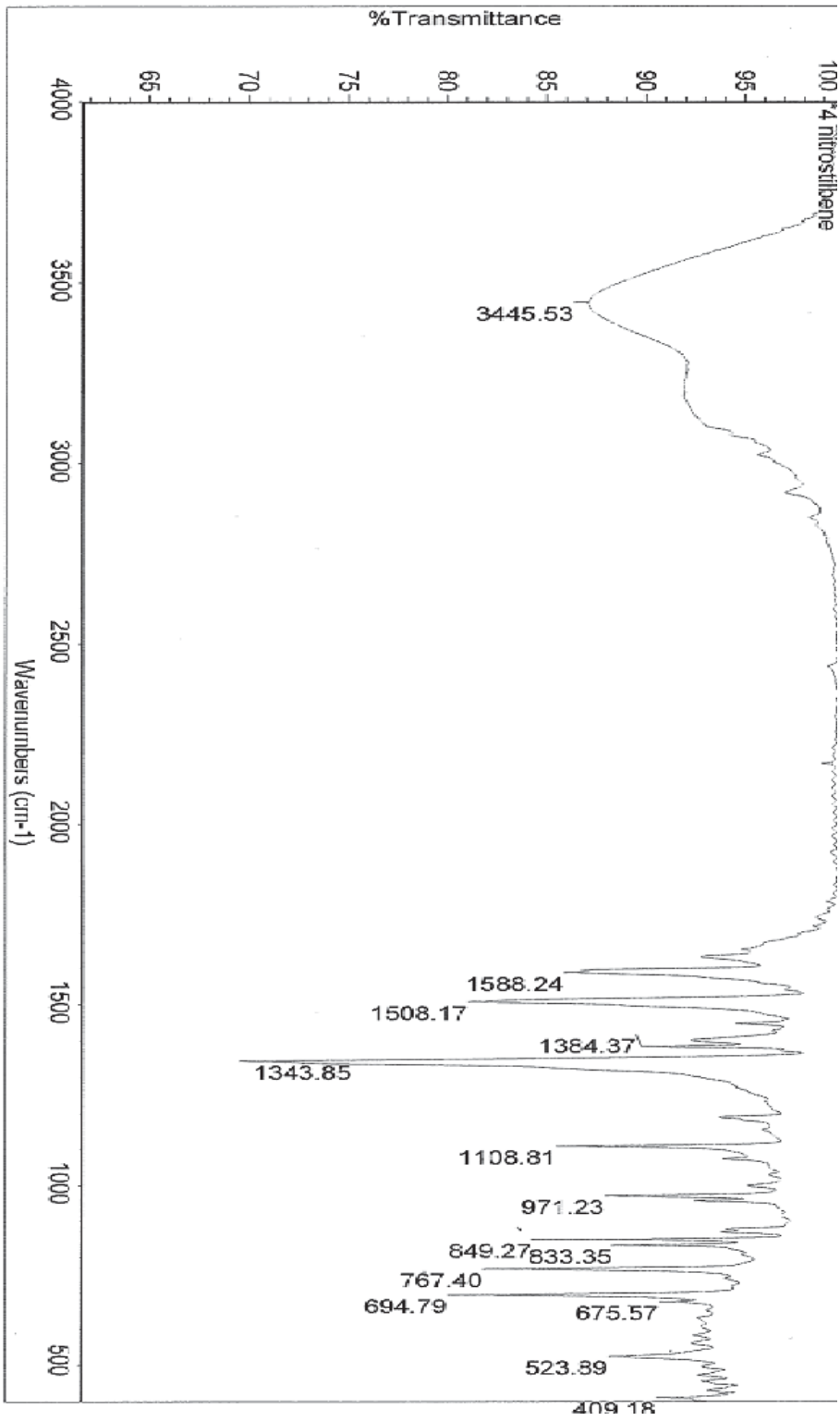

Figure iii-1: FTIR spectrum of 4-nitrostilbene

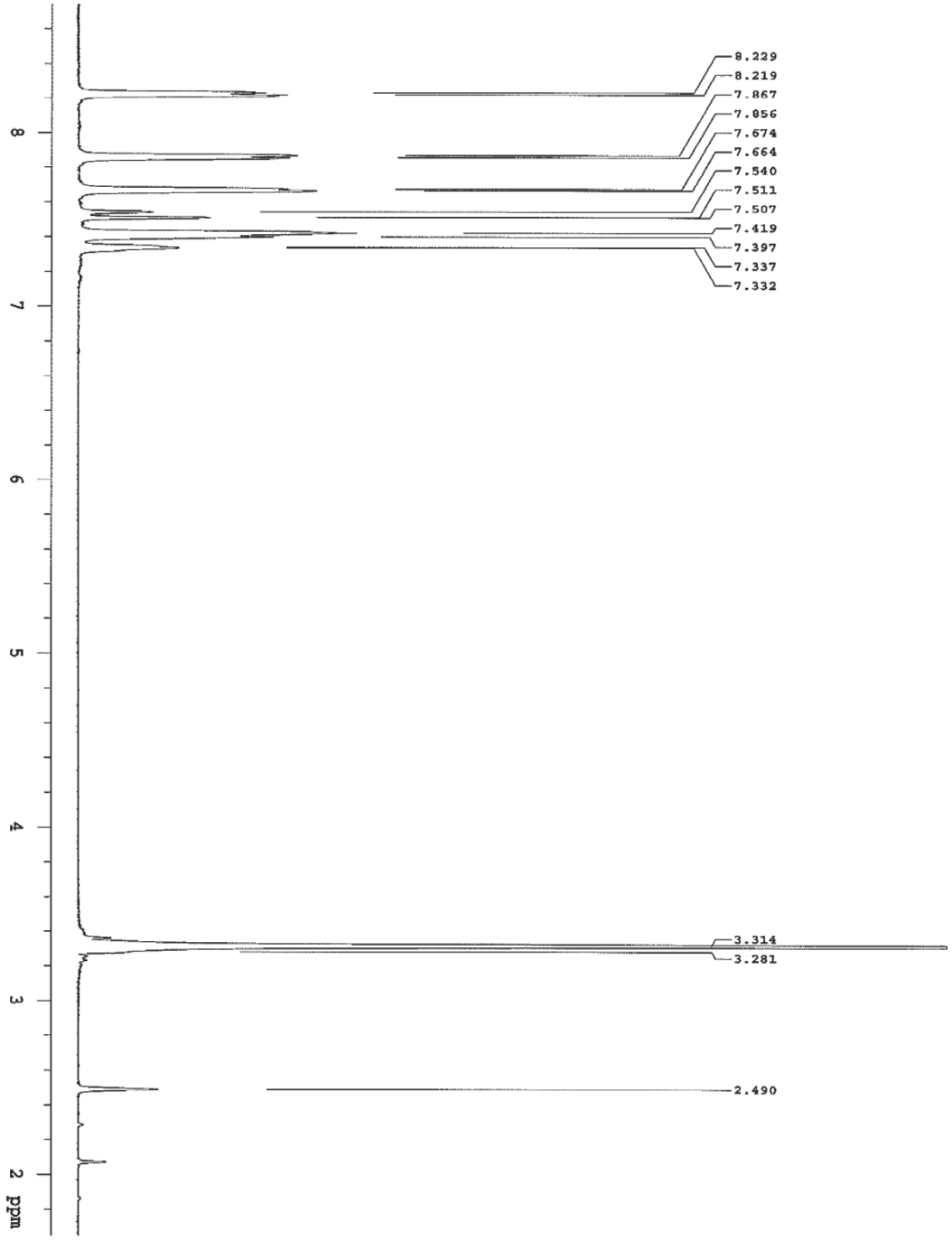

Figure iii-1: ^{1}H NMR spectrum of 4-nitrostilbene

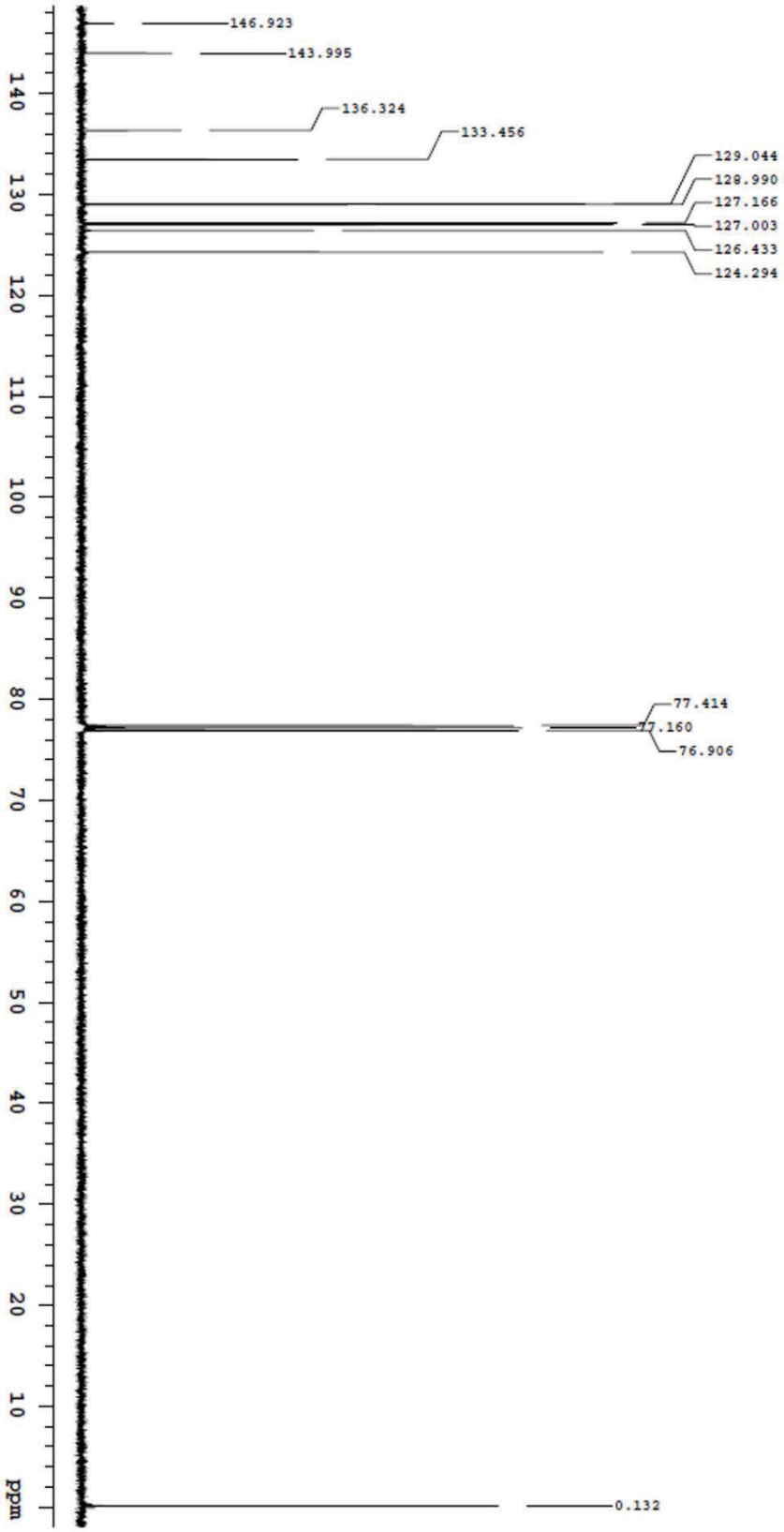

Figure iii- 2: ^{13}C NMR spectrum of 4-nitrostilbene

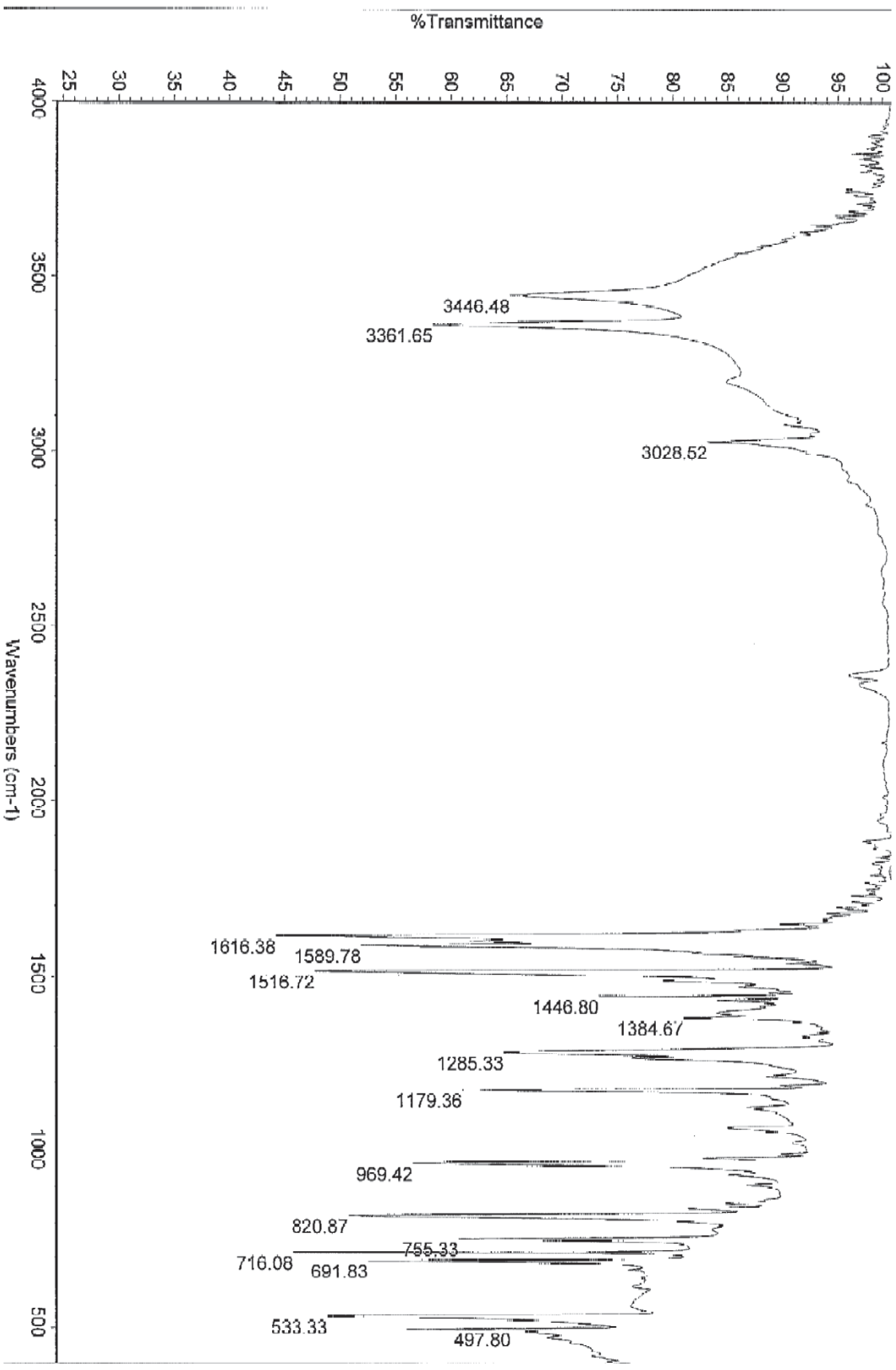

Figure iii-3: FTIR spectrum of 4-aminostilbene

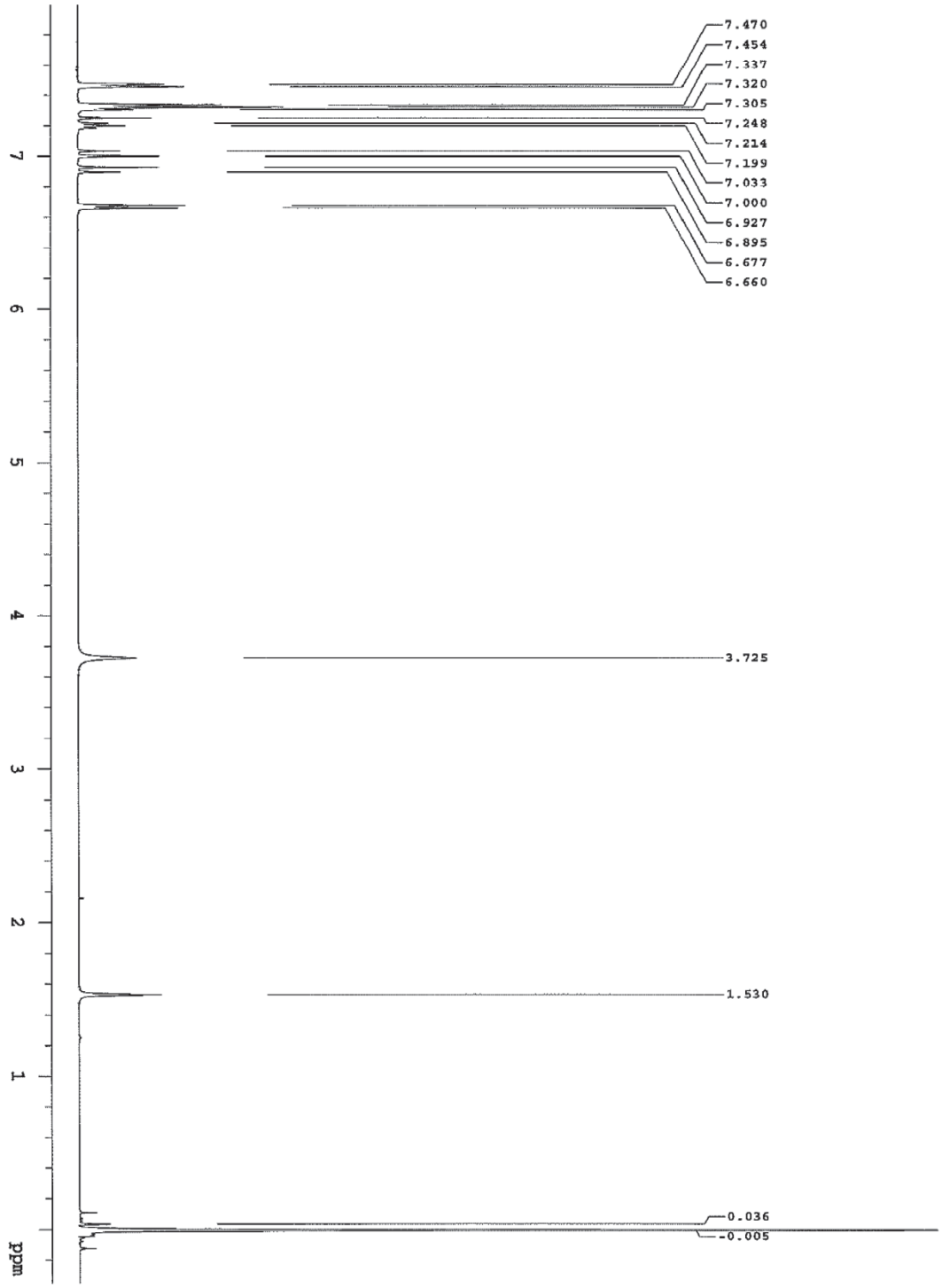

Figure iii- 4: ^{1}H NMR spectrum of 4-aminostilbene

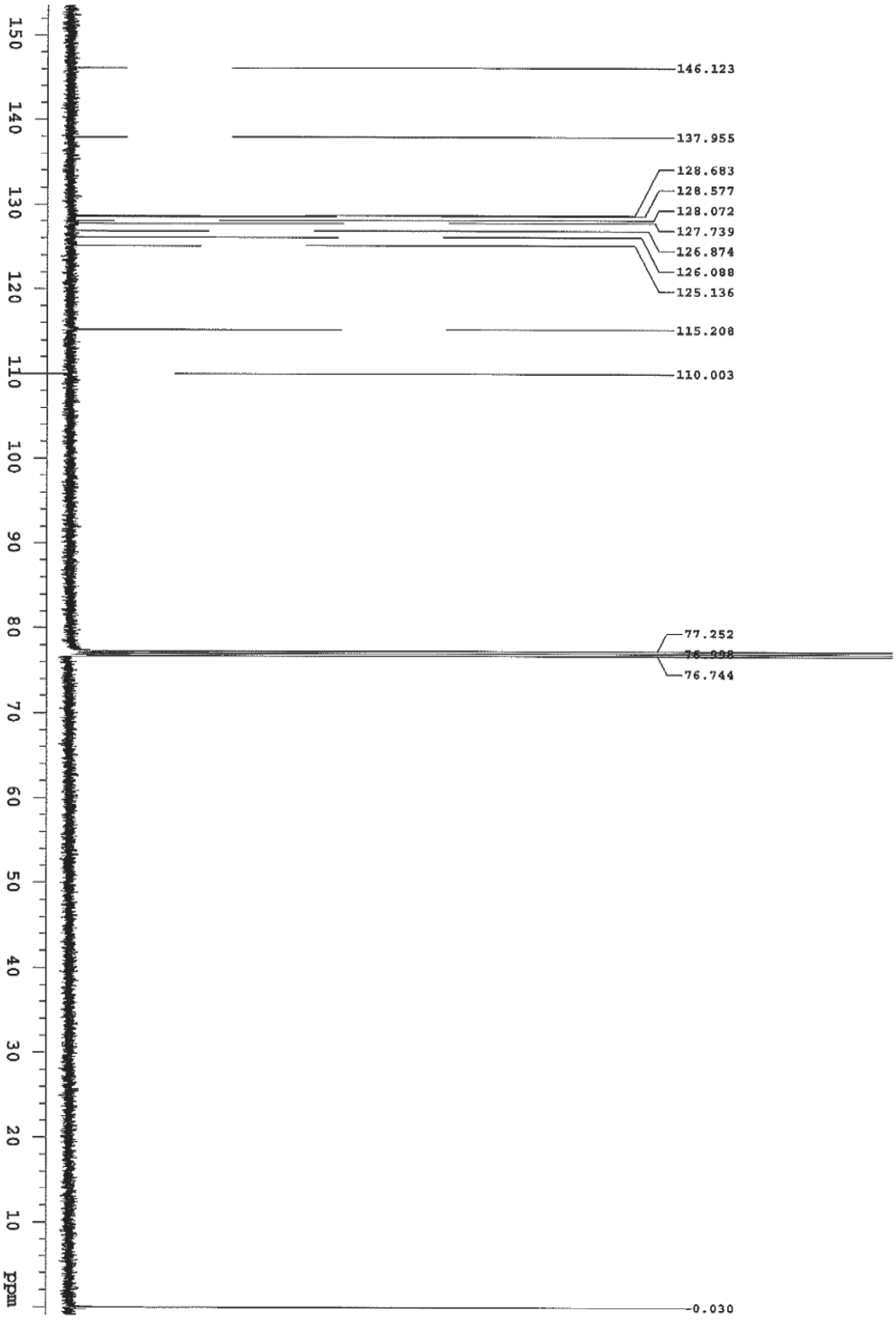

Figure iii- 5: ^{13}C NMR spectrum of 4-aminostilbene

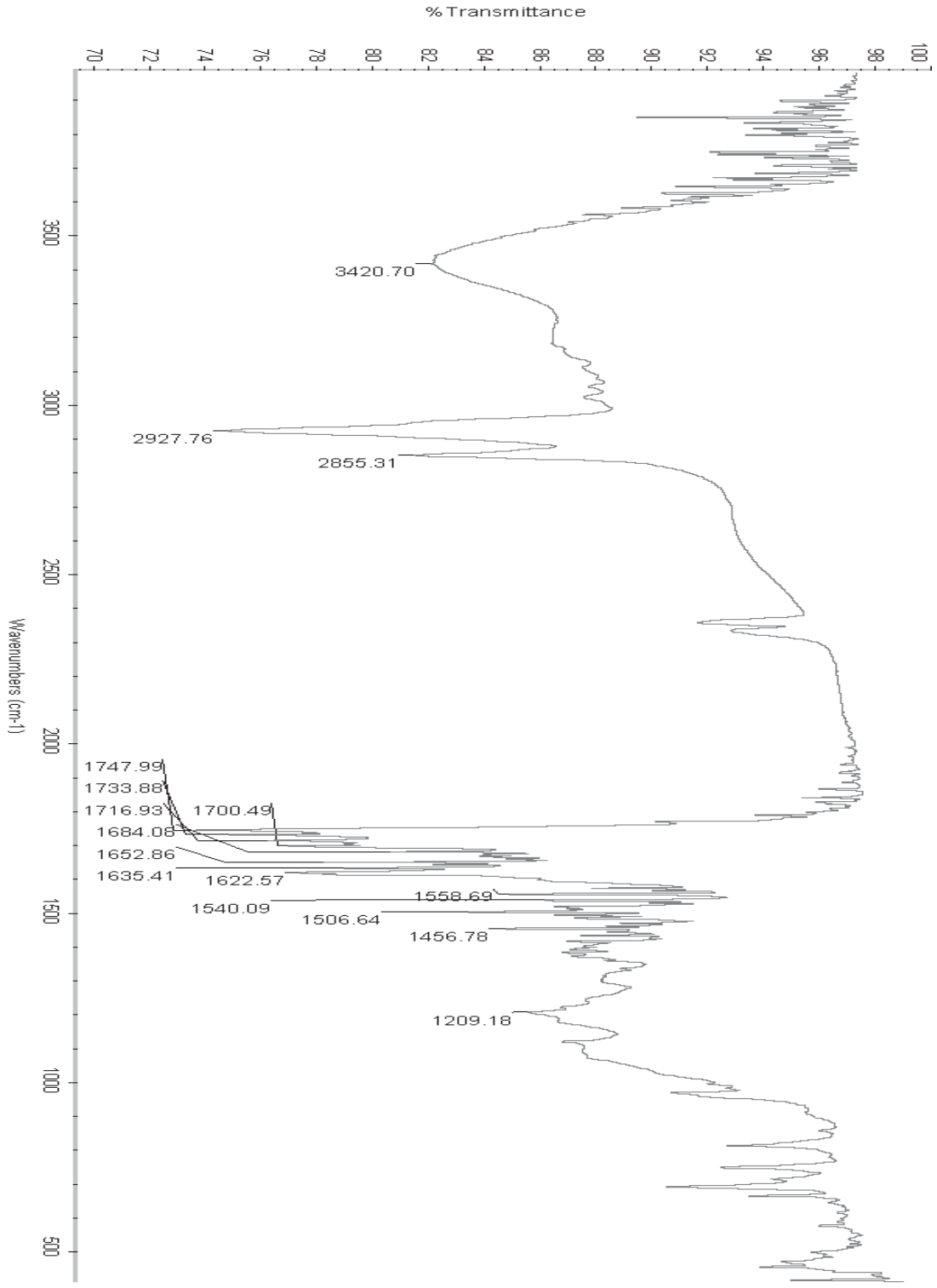

Figure iii- 6: FTIR spectrum of styrylisatin

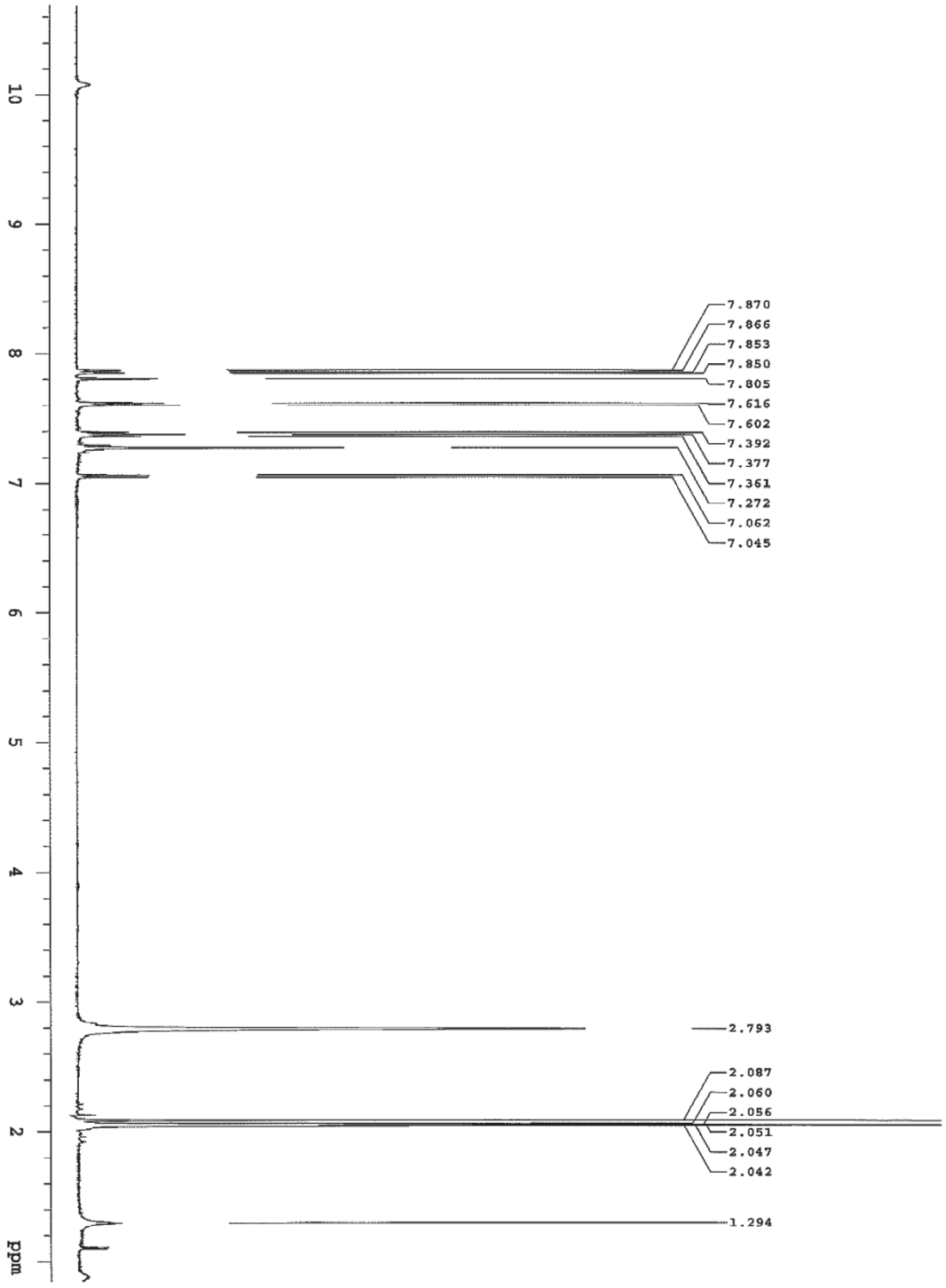

Figure iii- 7: ^{1}H NMR spectrum of styrylisatin

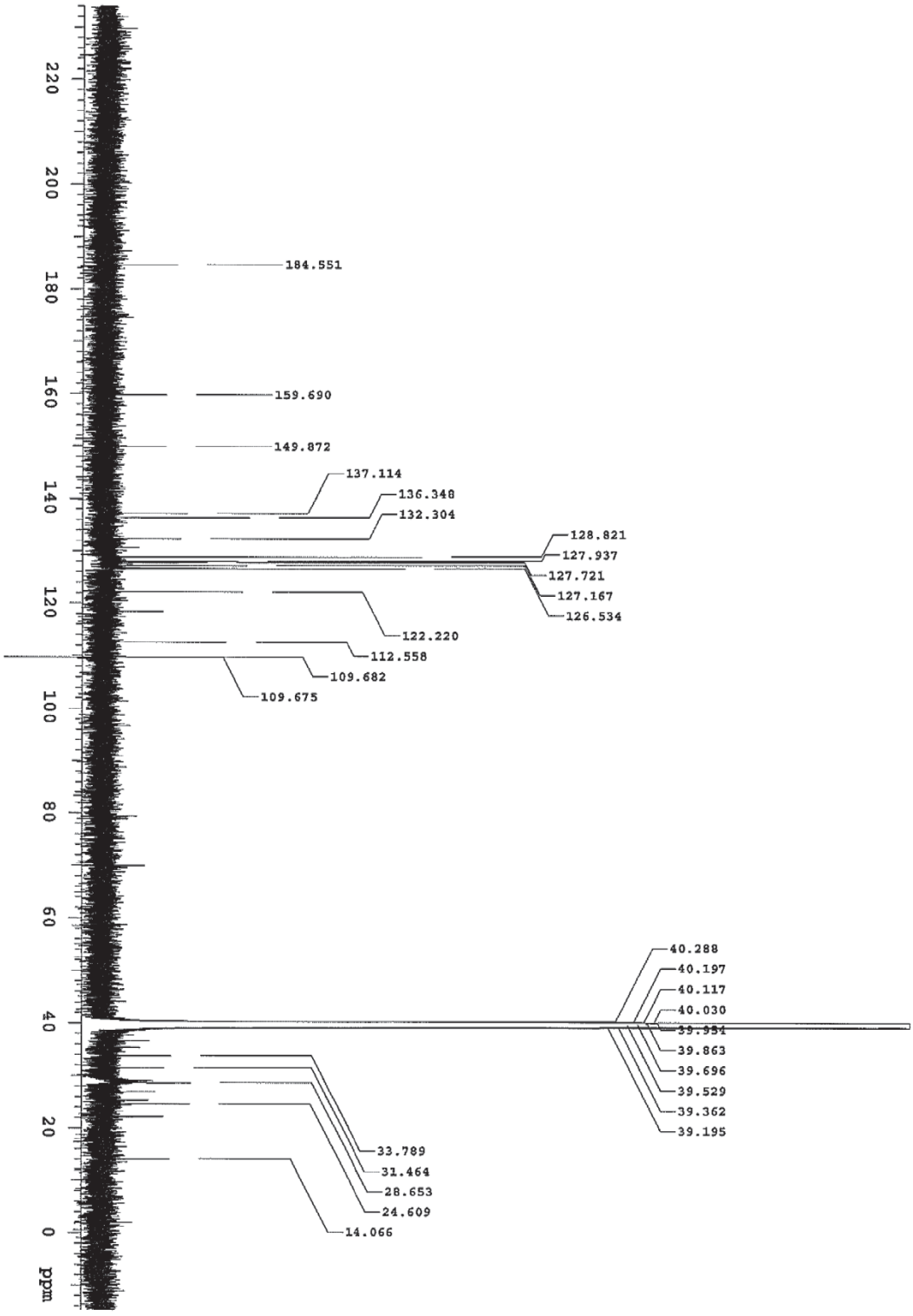

Figure iii- 8: ^{13}C NMR spectrum of styrylisatin

Appendix iv - **Comparison Study Results for PolyCyano UV Cyanobloom Sequencing Study**

Table iv- 1: Average comparison values for white light comparison between PolyCyano UV and Cyanobloom

	Aluminium		**Glass**		**Polyethylene bags**	
	Male Mean ± SD	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**
Fresh	-0.25 ± 0.96	0.00 ± 0.00	-1.00 ± 0.82	-0.75 ± 0.96	0.00 ± 0.00	0.00 ± 0.00
1 Day	0.50 ± 0.57	-0.25 ± 1.26	0.75 ± 0.50	1.00 ± 0.00	0.00 ± 0.00	-0.50 ± 1.00
3 Days	-0.25 ± 0.50	0.25 ± 0.50	0.75 ± 0.50	0.00 ± 1.41	-0.25 ± 0.50	0.00 ± 0.00
1 Week	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
2 Weeks	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.82	0.50 ± 0.58	0.75 ± 0.50	0.25 ± 0.50
1 Month	0.25 ± 0.50	0.00 ± 0.00	0.25 ± 0.96	0.25 ± 0.96	0.50 ± 0.58	0.50 ± 0.50

Table iv- 2: Average comparison values for UV excitation for PolyCyano UV and rhodamine 6G stained Cyanobloom

	Aluminium		**Glass**		**Polyethylene bags**	
	Male Mean ± SD	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**
Fresh	-1.25 ± 0.50	-0.75 ± 1.50	0.25 ± 0.50	0.00 ± 0.50	-0.25 ± 0.50	0.00 ± 0.82
1 Day	-1.50 ± 0.58	0.00 ± 0.82	0.75 ± 0.50	-0.50 ± 0.58	-0.50 ± 0.58	-1.25 ± 0.50
3 Days	-0.50 ± 1.00	0.00 ± 0.00	-0.50 ± 1.29	-1.50 ± 1.00	-0.75 ± 0.50	0.00 ± 0.82
1 Week	0.25 ± 0.50	-0.25 ± 0.50	-0.25 ± 1.50	1.00 ± 0.82	-0.25 ± 0.50	-1.25 ± 0.50
2 Weeks	0.25 ± 0.50	0.00± 1.63	-0.75 ± 0.50	0.75 ± 0.50	-0.75 ± 0.50	1.00 ± 0.00
1 Month	0.00 ± 0.82	-0.25 ± 0.50	0.00 ± 0.82	0.75 ± 0.50	0.00 ± 0.00	0.00 ± 1.00

Table iv- 3: Average comparison values for rhodamine 6G for PolyCyano UV and rhodamine 6G stained Cyanobloom

	Aluminium		Glass		Polyethylene bags	
	Male Mean ± SD	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**
Fresh	0.00 ± 0.00	0.25 ± 0.50	0.75 ± 0.50	1.00 ± 0.50	0.00 ± 0.00	0.25 ± 0.50
1 Day	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.82	0.75 ± 0.50	-0.75 ± 0.96	-0.75 ± 0.96
3 Days	0.00 ± 0.82	0.00 ± 0.00	0.00 ± 0.82	-0.75 ± 1.50	-0.25 ± 0.50	-0.25 ± 0.50
1 Week	0.25 ± 0.50	0.25 ± 0.50	0.00 ± 0.00	0.50 ± 0.58	0.00 ± 0.00	0.00 ± 0.82
2 Weeks	0.50 ± 0.58	-0.25± 1.25	0.25 ± 0.96	0.50 ± 0.58	0.25 ± 0.50	0.00 ± 0.50
1 Month	0.25 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 1.50

Table iv- 4: Average comparison values for STaR 11 stained PolyCyano UV and rhodamine 6G stained Cyanobloom

	Aluminium		Glass		Polyethylene bags	
	Male Mean ± SD	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**
Fresh	0.25 ± 0.50	0.25 ± 0.50	0.75 ± 0.96	0.50 ± 1.00	-0.25 ± 0.50	0.00 ± 0.00
1 Day	0.00 ± 0.00	0.25 ± 0.50	0.25 ± 0.50	0.50 ± 0.58	-0.25 ± 0.50	0.00 ± 0.82
3 Days	-0.50 ± 1.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 1.41	0.00 ± 0.82	0.00 ± 0.00
1 Week	0.75 ± 0.50	-0.25 ± 0.50	0.00 ± 0.82	1.00 ± 0.82	0.00 ± 0.00	-0.50 ± 0.57
2 Weeks	1.00 ± 0.82	0.00± 0.82	-0.50 ± 0.58	0.25 ± 0.50	0.00 ± 0.00	-0.25 ± 0.00
1 Month	0.75 ± 0.96	-0.25 ± 0.50	0.75 ± 0.96	0.25 ± 0.50	0.25 ± 0.50	0.25 ± 0.58

Table iv- 5: Average comparison values for STaR 11 stained PolyCyano UV and STaR 11 stained Cyanobloom

	Aluminium		**Glass**		**Polyethylene bags**	
	Male Mean ± SD	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**	**Male Mean ± SD**	**Female Mean ± SD**
Fresh	0.50 ± -0.25	-0.25 ± 0.50	0.50 ± 0.96	0.50 ± 0.96	-0.25 ± 0.50	0.00 ± 0.00
1 Day	0.00 ± 0.00	0.00 ± 0.00	-0.25 ± 0.50	0.75 ± 0.50	-0.25 ± 0.50	-0.25 ± 1.26
3 Days	0.25 ± 0.00	0.00 ± 0.00	0.25 ± 0.00	0.00 ± 0.00	-0.25 ± 0.50	0.00 ± 0.00
1 Week	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
2 Weeks	0.25 ± 0.50	0.50± 1.00	-0.25 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-0.25 ± 0.00
1 Month	0.50 ± -0.25	-0.25 ± 0.50	0.50 ± 0.50	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.96

Appendix v - **Imaging System Bandey Scale Comparison Results.**

Table vi- 1: Adapted Bandey scale used in this comparison study

Grade	Description
0	No development
1	General fingermark pattern visible, minutiae not visible
2	General fingermark pattern visible, minutiae is visible, but less than half the fingermark is developed
3	General fingermark pattern visible, minutiae is visible, greater than half the fingermark is developed, but still incomplete.
4	General fingermark pattern visible, minutiae is visible and fingermark is fully developed.

Table v- 1: Adapted Bandey scale scores for fingermarks developed on aluminium

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	3	2	1	2
CC2	3	2	1	3
CC3	3	2	1	3
CN1	1	1	0	1
CN2	1	1	0	2
CN3	3	3	2	3
PC1	4	4	4	4
PC2	4	4	4	4
PC3	4	4	4	4
PN1	4	4	2	4
PN2	4	4	3	4
PN3	4	4	3	2

Table v- 2: Adapted Bandey scale scores for fingermarks developed on Fanta® cans

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	4	4	4	4
CC2	4	4	4	4
CC3	4	4	4	4
CN1	3	4	2	3
CN2	2	3	3	3
CN3	3	3	3	3
PC1	4	4	4	4
PC2	4	4	4	4
PC3	4	4	4	4
PN1	0	1	0	0
PN2	0	0	0	0
PN3	0	0	0	0

Table v- 3: Adapted Bandey scale scores for fingermarks developed on glass

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	4	3	4	4
CC2	3	3	3	3
CC3	3	3	3	3
CN1	3	2	2	3
CN2	3	3	2	3
CN3	3	2	2	3
PC1	4	4	1	3
PC2	4	4	3	4
PC3	4	4	3	3
PN1	0	1	1	0
PN2	0	0	0	0
PN3	0	0	0	0

Table v- 4: Adapted Bandey scale scores for fingermarks developed on orange cardboard

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	3	3	3	3
CC2	1	1	2	1
CC3	2	2	2	1
CN1	1	0	0	0
CN2	0	0	0	0
CN3	0	0	0	0
PC1	3	3	3	3
PC2	4	4	3	4
PC3	3	4	3	3
PN1	2	3	3	2
PN2	1	3	3	1
PN3	1	2	3	1

Table v- 5: Adapted Bandey scale scores for fingermarks developed on polyethylene bags

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	2	2	2	1
CC2	3	3	3	2
CC3	3	3	3	3
CN1	4	4	4	4
CN2	4	4	4	4
CN3	4	4	4	4
PC1	1	0	0	0
PC2	2	0	0	0
PC3	1	0	0	0
PN1	1	1	0	0
PN2	0	1	0	0
PN3	0	0	0	0

Table v- 6: Adapted Bandey scale scores for fingermarks developed on black soft drink labels

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	4	4	4	3
CC2	3	3	2	3
CC3	4	3	3	3
CN1	3	2	2	2
CN2	3	3	1	3
CN3	2	2	2	2
PC1	4	4	4	4
PC2	4	4	3	4
PC3	3	3	2	3
PN1	0	0	1	0
PN2	1	1	2	0
PN3	1	2	3	1

Table v- 7: Adapted Bandey scale scores for fingermarks developed on orange soft drink labels

Sample	Poliview IV Score	VSC 6000 Score	Fuji IS Pro Score	Condor Score
CC1	4	4	4	4
CC2	3	4	3	3
CC3	3	3	3	2
CN1	3	2	1	1
CN2	3	2	1	1
CN3	2	2	2	1
PC1	4	4	4	4
PC2	4	4	4	4
PC3	3	4	4	3
PN1	0	4	4	1
PN2	1	3	3	1
PN3	2	1	2	0

Appendix vi – **Imaging System Comparison Ranking Results**

Table vi- 2: Ranks for fingermarks developed on aluminium (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	1	2
CC2	2	3	1	4
CC3	4	3	1	2
CN1	4	3	1	2
CN2	4	2	1	3
CN3	4	3	1	2
PC1	4	3	1	2
PC2	1	4	2	3
PC3	1	4	2	3
PN1	1	4	2	3
PN2	2	4	1	3
PN3	3	4	1	2

Table vi- 3: Ranks for fingermarks developed on Fanta® cans (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	1	2
CC2	2	4	3	1
CC3	2	4	1	3
CN1	1	4	3	2
CN2	1	4	3	2
CN3	1	4	3	2
PC1	2	4	1	3
PC2	2	4	1	3
PC3	3	4	1	2
PN1	0	4	0	0
PN2	0	0	0	0
PN3	0	0	0	0

Table vi- 4: Ranks for fingermarks developed on glass (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	4	2	1	3
CC2	3	4	2	1
CC3	1	4	2	3
CN1	3	4	2	1
CN2	3	4	2	1
CN3	3	4	2	1
PC1	4	3	1	2
PC2	4	3	1	2
PC3	3	4	1	2
PN1	0	4	3	0
PN2	0	0	0	0
PN3	0	0	0	0

Table vi- 5: Ranks for fingermarks developed on orange cardboard (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	2	1
CC2	3	4	2	1
CC3	2	4	3	1
CN1	0	0	0	0
CN2	0	0	0	0
CN3	0	0	0	0
PC1	3	4	2	1
PC2	1	4	3	2
PC3	3	4	2	1
PN1	2	4	3	1
PN2	1	4	3	2
PN3	1	4	3	2

Table vi- 6: Ranks for fingermarks developed on polyethylene bags (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	2	1
CC2	2	4	3	1
CC3	2	4	3	1
CN1	1	4	3	2
CN2	1	4	3	2
CN3	1	4	3	2
PC1	0	0	0	0
PC2	0	0	0	0
PC3	0	0	0	0
PN1	4	3	0	0
PN2	0	0	0	0
PN3	0	0	0	0

Table vi- 7: Ranks for fingermarks developed on black soft drink label (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	2	1
CC2	3	4	1	2
CC3	4	3	1	2
CN1	4	3	1	2
CN2	3	4	1	2
CN3	2	4	3	1
PC1	3	4	1	2
PC2	3	4	1	2
PC3	3	4	2	1
PN1	3	2	4	0
PN2	4	0	3	0
PN3	2	3	4	1

Table vi- 8: Ranks for fingermarks developed on orange soft drink label (4-1, best to worst image, 0 = no development)

Sample	Poliview IV Rank	VSC 6000 Rank	Fuji IS Pro Rank	Condor Rank
CC1	3	4	2	1
CC2	1	3	4	2
CC3	3	4	2	1
CN1	1	4	3	2
CN2	2	4	3	1
CN3	2	4	3	1
PC1	1	4	3	2
PC2	1	4	3	2
PC3	2	4	3	1
PN1	2	4	3	1
PN2	4	3	2	1
PN3	0	4	0	0

Appendix vii - List of Related Publications and Presentations

Articles

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *"Styryl dye coated metal oxide powders for the detection of latent fingermarks on non-porous surfaces."* Forensic Science International, (2012). **219**(1–3): p. 208-214

Oral presentations (presenters underlined)

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Near infrared laser dyes for detection of latent fingermarks on non-porous surfaces* – presented at International Fingerprint Research Group (IFRG) Meeting, 13th – 17th June 2011 – Linköping, Sweden

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Near infrared laser dyes for latent fingermark detection* – presented at the University of Technology Sydney, University of Canberra, Australian Federal Police Research and Development Workshop, 28th - 29th July 2011 – Canberra, Australia

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Styryl dye coated metal oxide nanopowders for the detection of latent fingermarks on non-porous surfaces* – presented at the 19th World Meeting of the International Association of Forensic Sciences (IAFS) 12th - 17th September 2011 – Funchal, Portugal

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Styryl dye coated metal oxide nanopowders for the detection of latent fingermarks on non-porous surfaces* – presented at the 6th European Academy of Forensic Science (EAFS) Conference, 20th – 24th August 2012 – The Hague, The Netherlands

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, X. Spindler and C. Roux. *STaR 11 coated aluminium oxide nanopowders for the detection of latent fingermarks on adhesives and wet surfaces* **-** presented at the 6th European Academy of Forensic Science (EAFS) Conference ,20th – 24th August 2012 – The Hague, The Netherlands

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Styryl dye coated metal oxide nanopowders for latent fingermarks on non-porous surfaces* **–** presented at the 21st International Symposium on the Forensic Sciences, 23rd - 27th September 2012 – Hobart, Australia

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, X. Spindler and C. Roux. *STaR 11 coated aluminium oxide nanopowder suspensions for the detection of latent fingermarks on adhesives and wet surfaces* - presented at the 21st International Symposium on the Forensic Sciences , 23rd - 27th September 2012 – Hobart, Australia

S. Chadwick, P. Maynard, L. Xiao and C. Roux. *Validation of PolyCyano UV: A one step fluorescent cyanoacrylate polymer* - presented at the 21st International Symposium on the Forensic Sciences , 23rd - 27th September 2012 – Hobart, Australia

Poster Presentations

S. Chadwick, P. Maynard, P. Kirkbride, C. Lennard, A. McDonagh, X. Spindler and C. Roux. *Styryl dye coated metal oxide powders for the detection of latent fingermarks on non-porous surfaces* **–** presented at the 1st Doctoral Summer School in Forensic Science and Criminology, 27th – 29th August 2012 – Arolla Switzerland