**University of Strathclyde**

**Department of Pure and Applied Chemistry**

# An Evaluation of the Mechanisms of Recovery of DNA and Fingerprints from Fire Scenes

**By**

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For Mum, Paul,

Gran, and Paul

# Abstract

Incidents involving the intentional or deliberate setting of a fire within a compartment are frequently difficult to investigate both because of the damage to the property in question and the apparent lack of forensic evidence which could be used to potentially identify a suspect. The recovery of such evidence in the form of DNA and fingerprints from a fire scene would therefore be advantageous.

During this project, replicate samples of DNA and fingerprints were deposited on both porous and non porous surfaces which were then exposed to laboratory controlled elevated temperatures for various time periods. In each case replicate DNA samples or replicate depleted series of fingerprint samples were used to produce robust data sets for subsequent statistical analysis. DNA and fingerprint samples were also exposed to a real fire environment using a fire training facility in order to simulate operational conditions.

The results obtained suggest that the optimum recovery method for low template DNA was to use a wet followed by a dry cotton swabbing action of the surface before combining the two swabs for extraction. When the DNA was exposed to elevated temperatures in a controlled environment, there was a greater possibility of recovering a full SGM Plus profile if the DNA had been absorbed into a porous rather than non porous surface and the surface exposed up to a maximum of 100˚C only. All of the samples which were exposed to the uncontrollable fire environment produced partial DNA profiles.

The survivability and chemical enhancement of fingerprints deposited on both porous and non porous surfaces was robustly investigated where 70 replicate fingerprints were examined in each case for each test condition. For porous surfaces the most efficient sequence of enhancement techniques was an initial visual examination, followed by a fluorescence examination prior to treatment with DFO, and finally PD. It was found that this sequence could be employed for both wet and dry articles. In the case of dry, non porous surfaces, visual examination followed by fluorescence examination should be utilised prior to undertaking superglue - BY40 treatment. Powder suspension should be substituted for superglue in the case of wet items.

# Acknowledgements

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# Publications and Presentations Related to this Research

## Publications

**Dominick, A.J.**, Welch, L.A., Nic Daéid, N., and Bleay, S.M., *Is There a Relationship Between Fingerprint Donation and DNA Shedding?* Journal of Forensic Identification, 2009. **59**(2): p. 133-143.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S.M., and Sears, V., *The Recoverability of Fingerprints on Paper Exposed to Elevated Temperatures – Part 1: Comparison of Enhancement Techniques.* Journal of Forensic Identification, 2009. **59**(3): p. 325-339.

**Dominick, A.J.**, Bleay, S.M., Nic Daéid, N., and Sears, V.G., *The Recoverability of Fingerprints on Paper Exposed to Elevated Temperatures – Part 2: Natural Fluorescence.* Journal of Forensic Identification, 2009. **59**(3): p. 340-355.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., Gibson, A.P., and Mehmet, S., *Identification of Heat Distorted Fingerprints using Sagem Metamorpho™ AFIS.* Journal of Forensic Identification, 2010. **Under Review**.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., and Edwards, P.R., *Compositional Analysis of Fingerprints on Metal exposed to Elevated Temperatures using Scanning Electron Microscopy.* Journal of Forensic Sciences, 2010. **Under Review**.

**Dominick, A.J.**, Nic Daéid, N., and Bleay, S.M., *The Recoverability of Fingerprints on Non Porous Surfaces Exposed to Elevated Temperatures.* Journal of Forensic Identification, 2010. **Under Review.**

## Oral Presentations

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., *The Recovery of Fingerprints from Fire Scenes.* Centre for Forensic Science Colloquia, University of Strathclyde, Glasgow, 27th March 2009.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., *Microscopic Analysis and Enhancement of Heated Fingerprints on Non Porous Surfaces.* International Fingerprint Research Group, Lausanne, Switzerland, 29th June 2009.

## Poster Presentations

**Dominick, A.J.**, Welch, L.A., Nic Daéid, N., and Bleay, S.M., *Is There a Relationship Between Fingerprint Donation and DNA Shedding?* Forensic Science Society Summer Conference, Crowne Plaza Hotel, Glasgow, 6-8th July 2007.

**Dominick, A.J.**, Welch, L.A., Nic Daéid, N., and Bleay, S.M., *Is There a Relationship Between Fingerprint Donation and DNA Shedding?* Analytical Research Forum, University of Strathclyde, Glasgow, 16-18th July 2007.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., *The Recoverability of Fingerprints on Paper Exposed to Elevated Temperatures.* WestCHEM Research Day, University of Glasgow, Glasgow, 30th May 2008.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., *The Recoverability of Fingerprints on Paper Exposed to Elevated Temperatures.* Forensic Science Society Autumn Conference, Robinson College Executive Centre, Wyboston, 31st October – 2nd November 2008.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., *The Recoverability and Examination of Fingerprints from Fire Scenes.* European Academy of Forensic Science, University of Strathclyde, Glasgow, 8-11th September 2009.

**Dominick, A.J.**, Nic Daéid, N., Bleay, S., Gibson, A.P., and Mehmet, S., *Identification of Heat Distorted Fingerprints using Sagem Metamorpho™ AFIS.* European Academy of Forensic Science, University of Strathclyde, Glasgow, 8-11th September 2009.

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# CHAPTER 1: INTRODUCTION

## 1.1 The Crime of Fireraising

Unlike many European countries, there is no criminal code in Scotland. Scots criminal law is based on rules pertaining to the "common law" (rules of such long standing that their origins cannot be traced). Professor David Hume made a study of High Court decisions where he was able to deduce and publish an account of common law in Scotland (Hume's *Commentaries*), which for many decades has been accorded authoritative status. Other highly regarded books are Archibald Alison's *Principles of the Criminal Law of Scotland* and J.H.A. Macdonald's *Practical Treatise on the Criminal Law in Scotland*. No other books on the subject have been regarded as sources of criminal common law and as such, the present Scottish common law consists of the deductions, conclusions, and opinions of Hume, Alison, and Macdonald together with alterations, additions and amendments that have been made to date by appropriate courts in the particular criminal proceedings before them [1].

In Scots criminal law, there are three crimes committed against property. These are malicious mischief, fireraising, and vandalism. Malicious mischief is the intentional or reckless damaging of a non heritable property belonging to another, without that owner's consent or permission. Fireraising is also the intentional or reckless damaging of a property belonging to another, in this case of heritable value, without that owner's consent or permission [1-2]. Traditionally, fireraising was a capital offence, however section 56 of the Criminal Procedure (Scotland) Act 1887 abolished this. Acts of fireraising cannot constitute vandalism [3].

To be guilty of most crimes, a defendant must have committed the criminal act (the *actus reus*) in a certain mental state (the *mens rea*). In common law, there are two types of *mens rea*, two core states of mind. These are *intention* and *recklessness* [1]. As a consequence there are two different crimes of fireraising based on the two types of *mens rea*. These are *wilful fireraising* and *culpable and reckless fireraising*.

Wilful fireraising is the crime of burning of property to any degree, no matter how small (*actus reus*). The fire must be raised *wilfully* with a purpose to destroy the property to which it is applied. Thus the *mens rea* of wilful fireraising is intention – actual intention to damage or destroy the property burned [1-3].

The *actus reus* of culpable and reckless fireraising does not differ from wilful fireraising; it is the *mens rea* which separates these two crimes. Wilful fireraising cannot be committed recklessly [1-3]. The case of *Byrne v HM Advocate* confirmed the existence of the crime of culpable and reckless fireraising as "mere negligence is not enough; the property must have been set on fire due to an act of the accused displaying a reckless disregard as to what the result of his act would be" [4].

Fireraising is equivalent to the crime of *arson* in English Law and the word originates from an Anglo-French word meaning the *act of burning* [5]. Arson is covered in English Law under legislation passed by Parliament and forms part of the Criminal Damage Act 1971 (section 1(3)) where an offence committed under this section, by destroying or damaging property by fire, would be charged as arson [6].

In the United States of America, most states by the 1950s had adopted either all or most of the Model Arson Law, which created some uniformity to the legislation across the country [5]. The crime of arson in the US has basically three elements:

1. There has been a burning of property. This must be shown to the court to be actual destruction, at least in part, not just scorching or sooting (although some US states include any physical or visible impairment of any surface). This also includes destruction by explosion.
2. The fire has been intentionally set. Proof of the existence of an effective incendiary device is adequate. Proof must be accomplished by showing specifically how all possible or accidental causes have been considered and ruled out.
3. The burning is shown to be started by malice, that is, with the intent of destroying property (i.e. starts a fire or causes an explosion with the purpose of destroying a building of another or one's own with fire) [5].

## 1.2 Tackling Fireraising

Fireraising is an expensive crime and is estimated to cost the economy £53.8 million per week (England and Wales only). Each week (based on data obtained from 2003), there are, on average, 2213 arson attacks, which result in 2 fatalities, 53 injuries, and damages or destroys 20 schools and colleges, 262 homes, 360 businesses and public buildings, and 1402 vehicles [7]. The conviction rate for incendiary fires remains less than 10% [8].

There are many agencies and organisations that come into contact with the fireraising problem or upon whom it has an impact. The main local and national contributors are the Home Office (which established the Arson Scoping Study [9]), the police, fire and rescue service, crime scene examiners, forensic scientists, insurers, and loss adjusters. The UK government has also set up various initiatives [7, 10-11] to try and tackle the issue. Each agency and organisation has its part to play within a specific investigation, though not all may be involved in each case. The fire and rescue service has an obligation to inform the police (if they are not already present) that the fire they have suppressed was *suspicious* to them. It is then the police's responsibility to call in the crime scene examiners and/or forensic scientists and other agencies as required [9]. In cases where deliberate ignition is suspected, the recovery of items of evidential value is obviously of importance and this may include the recovery of samples which may have an investigative relevance in that they can facilitate the identification of a suspect, and such evidence may include sources of DNA and fingerprints. The identification of the conditions within a fire scene where the recovery of fingerprints and DNA is a viable possibility is of obvious importance and is the focus of this work.

## 1.3 Fire and Heat

A fundamental and important property of fire is the production of heat. Most of the destructiveness of a fire is the direct result of the heat generated and transferred during the event. Heat transfer produces damage to structures and items within a compartment, intensifies the fire, is the means by which a fire spreads, and provides the greatest barrier to the extinguishment of the fire. When heat transfer is understood

in connection with fire development, the sequence and cause of the fire may become clear depending at which point suppression occurred. An understanding of the mechanisms and effects of heat on both exposed and protected areas within a scene is essential to effective sample choice when attempting to recover samples with potential DNA and fingerprint evidence. The way heat influences the initiation and spread of fire is explained below [5, 12] and visually in figure 1-1:

- Heat at the point of ignition.
  *The fundamental properties that influence the ignitability of an object are its density, thermal capacity and thermal conductivity. Taken together, these properties constitute the object's thermal inertia (ability of a given volume of a substance to store internal energy while undergoing a given temperature change). Ignition requires transfer of enough energy (or heat) quickly enough to overcome the thermal inertia and trigger sufficient combustion that it becomes self-sufficient.*

- Heat as it applies to the increase of the rate of chemical reactions.
  *Heat is energy in a kinetic form, or energy of molecular motion. Except at absolute zero (-273˚C) all matter contains heat because its molecules are in motion. Temperature is merely a means of expressing the relative amount of this energy that a body has. As heat is energy, an increase in heat is an increase in energy; therefore this provides an increase in the chemical reaction rates.*

- Heat transfer influencing the development and spread of fire when the additional conditions of available fuel and oxygen are met.
  *An integral part of every fire is the transfer of heat, both to the fuel and away from the areas of combustion. Heat can be transferred via three methods – conduction, convection, and radiation.*

  *Conduction is the transfer of heat energy through a solid material by contact between its moving molecules.*
  *Convection is the distribution of heat by means of a circulating medium or the transfer of heat to and from a moving medium. In most fires it is driven by*

*differences in density caused by temperature variations (buoyant flows). It occurs when the buoyant gases impinge upon a liquid or solid surface.*

*Radiation is the transfer of heat from one body to another without any contact or circulating medium. All objects above absolute zero radiate energy in the form of electromagnetic energy. When a fire is burning in a structure, all surfaces that face the fire will be heated by radiant heat, and when this surface temperature reaches the auto-ignition point of the material itself, it will ignite. This radiative ignition is a major factor in fire growth.*

The effects of heat on materials that survive the fire can bear indicators of the fire's intensity, duration, or direction of spread. By direct flame impingement and the transfer of heat, various 'patterns' can be left behind after the fire has been suppressed which may indicate the intensity, duration, and direction of spread of the fire. It can also reflect the effect of ventilation.

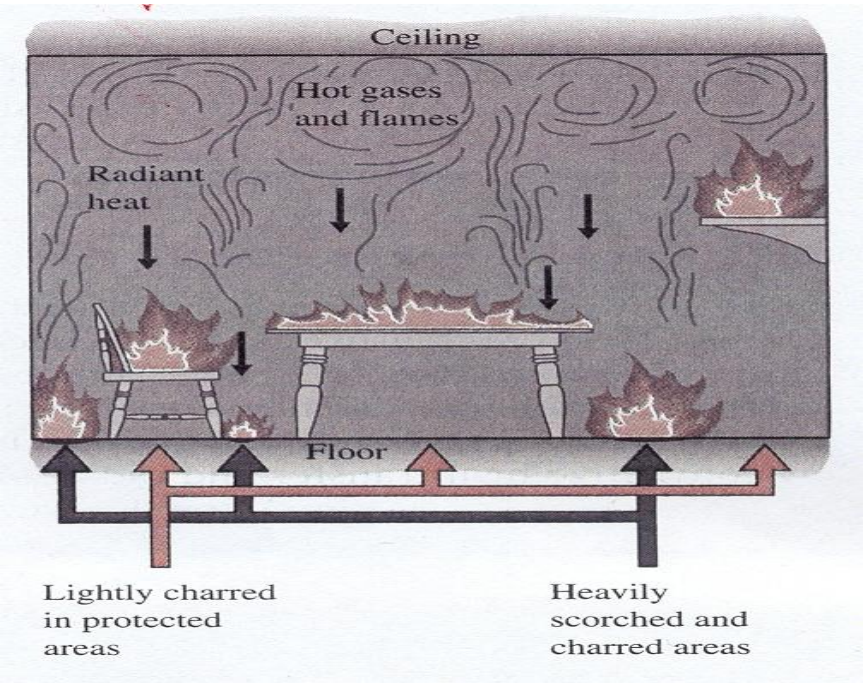



Figure 1-1 As fire extends across the ceiling, heat radiating downward from the ceiling layer chars and ignites exposed surfaces; areas under the chair and the table are protected until later in the fire [5]

## 1.4 Fire Behaviour

A fire which has occurred within a room with a normal fuel load will go through predictable stages of development. There are typically four stages – the initiation or incipient stage, growth and free-burning stage culminating in flashover, the post-flashover stage, and the decay stage and each of these stages will be described and discussed in turn.

### Initiation or Incipient Stage

In a confined fire, the initiation or incipient stage is regarded as the moment after ignition has occurred. The flames are only visible at the first item ignited. The air within the compartment has an ambient oxygen concentration of 21% [12] and the overall temperature within the compartment has not yet begun to rise. A plume of hot gases is evolved from the environment of the flame, the contents of which are dependent on the type of fuel which is burning, but will normally consist of soot (carbon and other solids), water vapour, carbon dioxide, carbon monoxide, sulphur dioxide, and traces of other toxic gases (as well as heated nitrogen from the surrounding air). This plume will then be carried to the upper part of the room by convection or buoyant flow to form a layer of hot gaseous materials at the ceiling level and will draw (or entrain) oxygen in at the bottom of the flame to sustain the combustion [5, 12-15]. This is shown in figure 1-2.

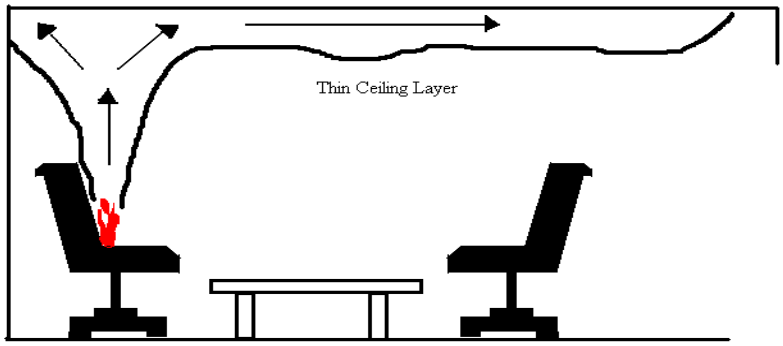



Figure 1-2 Early compartment fire developments [12-15]

**Growth and Free-Burning Stage**

The fire will grow in size as more fuel becomes involved. This occurs predominantly by convection and radiation (and occasionally by conduction), and promotes the development of the flame upwards and outwards from the initial burning item until nearby items begin to pyrolyse (undergo thermal decomposition) and become involved in the fire. The hot combustion products within the room begin to rise up and contribute to the already established ceiling layer of hot gases, and cool air flows into the room underneath this layer (figure 1-3). The oxygen content within the air in the room begins to become depleted as combustion increases and contains soot, smoke, and partially burned pyrolysis products (including toxic gases such as carbon monoxide, hydrogen cyanide, hydrogen chloride, acrolein, and also free radicals). These conditions are referred to as *preflashover* conditions [5, 12-15].

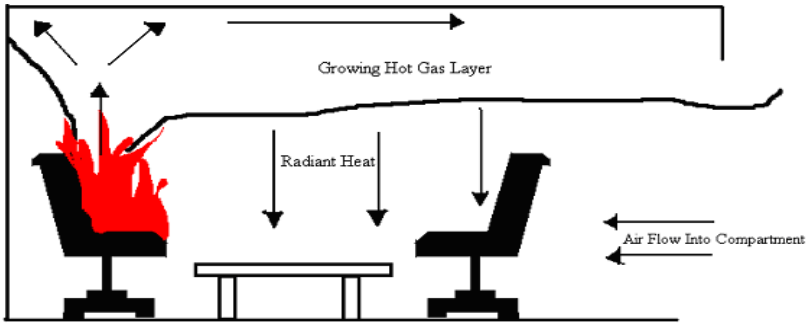



Figure 1-3 Preflashover conditions in compartment fire [12-15]

The ceiling layer's temperature will continue to rise and the layer will begin to descend as more fuel becomes involved in the fire. The radiant heat from this layer will also begin to affect other materials within the room and cause them to pyrolyse with their matrices to begin to thermally decompose releasing ignitable pyrolysis products. When the ceiling layer reaches approximately 600˚C [16-17], it is generating a sufficient amount of radiant heat to raise most other items, such as furniture and carpets, to their ignition temperatures and as such they become involved in flaming combustion and the compartment is considered to have reached *flashover* conditions (figure 1-4) [5, 12-15, 18].

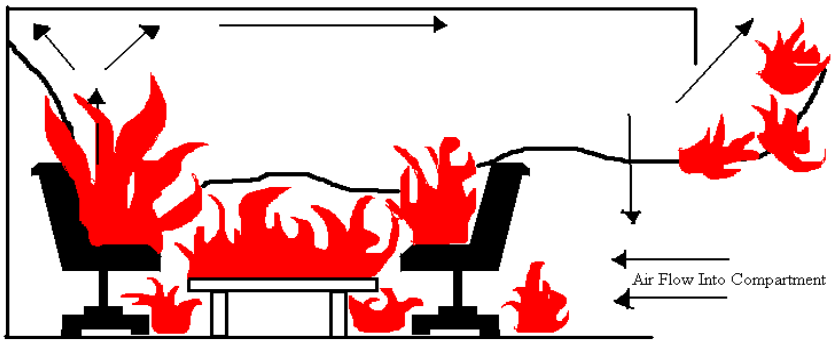



Figure 1-4 Flashover conditions in compartment fire [12-15]

When flashover is reached, the temperature in the room reaches its maximum. Flashover is often described as the moment when ‘a fire in a room becomes a room on fire’ [5, 12-15]. The fire becomes ventilation controlled at flashover as the requirement for oxygen to sustain the combustion process increases to its maximum. When this occurs, the production of carbon monoxide, smoke, and energy are at their highest values and the oxygen concentration in the air is depleted. Flashover marks a transition in which the fire development was previously dominated by the fuel materials available now becomes controlled by the ventilation openings (doors and windows) which influence the oxygen supply required to sustain combustion [12].

Figure 1-5 shows the heat release rate experienced in a typical small furnished room. In this figure, the critical heat release rate (and hot gas temperature) is reached and the room goes to flashover (approximately 600ºC). This is followed by steady state burning, and then a decay period. The fuel is exhausted and the fire dies down to smouldering embers before being extinguished.

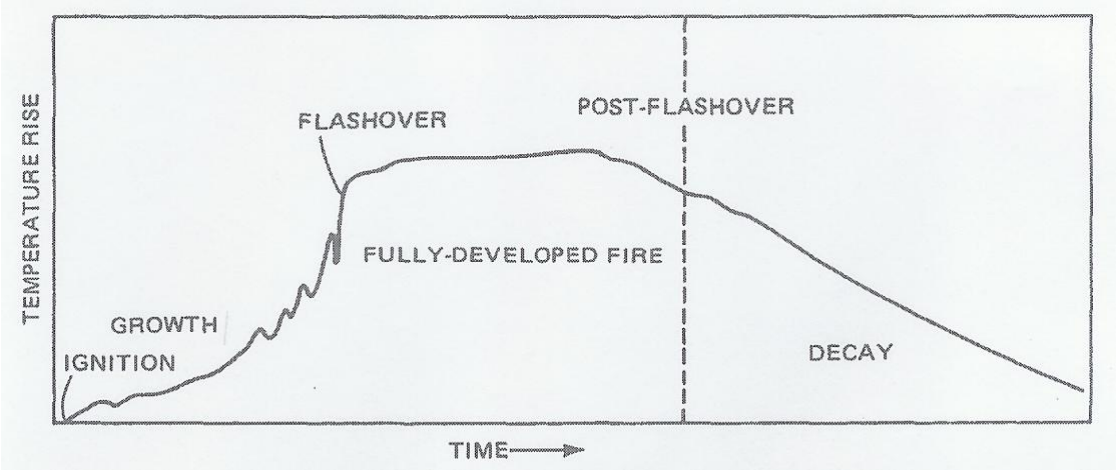



Figure 1-5 Total heat release rate versus time for a fire in a typical small furnished room [19]

**Postflashover Stage**

Once flashover has occurred (*postflashover*), all the items in the room are burning and full room involvement has occurred. While the burning of floor coverings is common, this may not extend under the target fuels or other shielding surfaces. Postflashover burning conditions in a room are both turbulent and dynamic. At this stage, the fire is ventilation controlled and all fuels in the fire will continue to burn as quickly as oxygen can be made available to them until they are consumed [5, 12-15]. This is shown in figure 1-6.

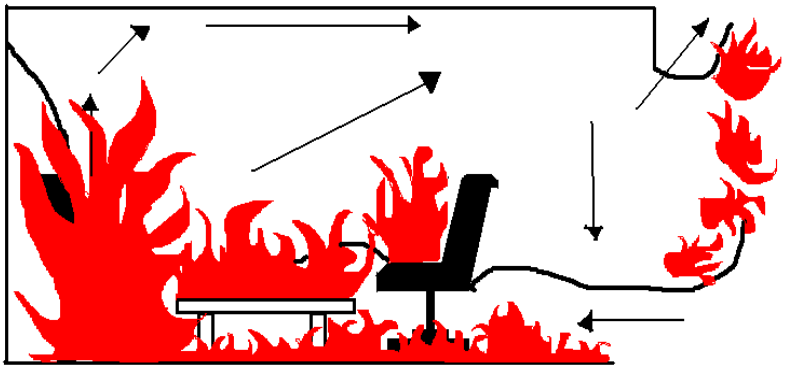


Figure 1-6 Postflashover or full room involvement in compartment fire [12-15]

Temperatures in postflashover fires can reach above 1000ºC [16]. The temperature at any time depends on the balance between the heat released within the room and the heat losses. These losses can be through either ventilation openings (such as windows and doors) via radiation and convection, and by conduction into the walls, ceilings, and floors [16]. Several studies have been conducted measuring the temperatures in postflashover fires [20-21] although the most widely referenced is the *Swedish curves* [22]. Each group of curves are for different ventilation factors ($F_v$) with different fuel loads (the amount of energy (in megajoules) produced per metre squared surface area of fuel – given in units of $MJ/m^2$). These curves show the change in temperature over time by varying the fuel load and ventilation and are given in figure 1-7.

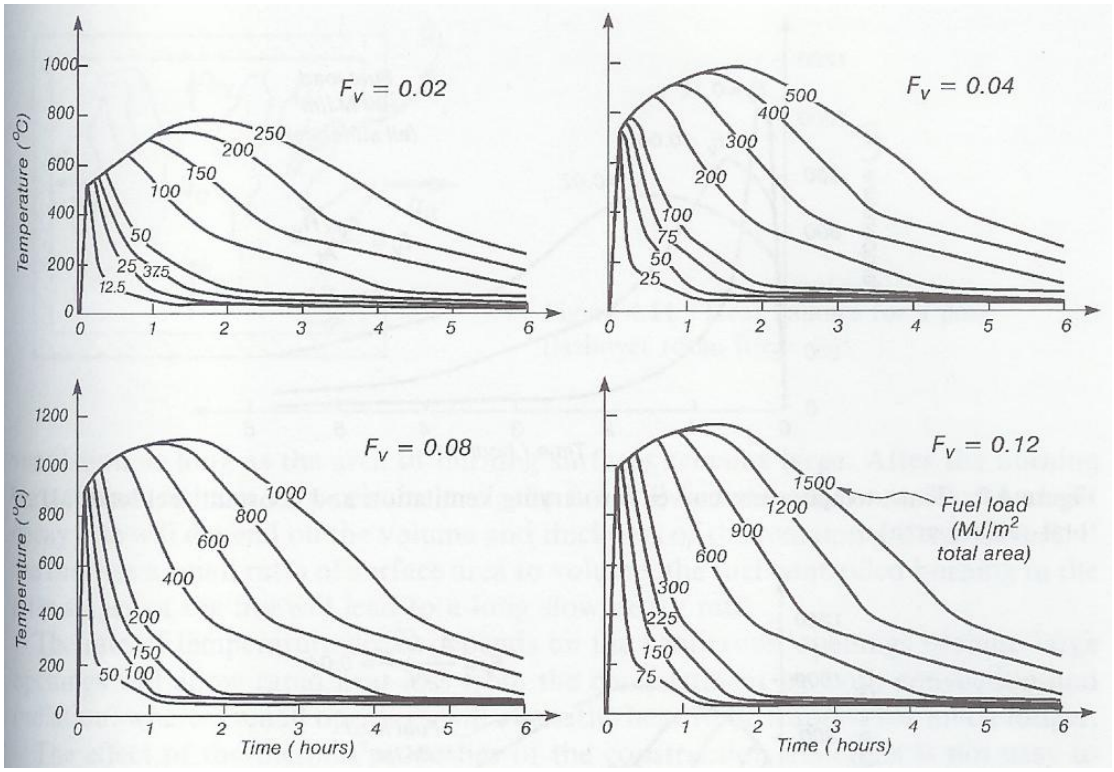



Figure 1-7 Swedish curves of temperature against time with varied ventilation and fuel load [16, 22]

**Decay Stage**

Eventually, the available fuel will become exhausted and open-flame burning begins to diminish. As a result, smouldering combustion becomes more prevalent in fuels which support such combustion and will continue as long as this fuel is present. Alternatively, if the oxygen concentration drops below approximately 16%, open-flame combustion will also decrease even in the presence of unburned fuel and may even stop completely if oxygen levels get below 5%. High temperatures may continue as long as there is suitable fuel, depending on the ventilation. If the room is inadequately ventilated, a fresh supply of oxygen can cause the fire to re-ignite very quickly. Such a scenario is sometimes called backdraft, flashback, or smoke explosion [5, 12-15].

## 1.5 Complications in Fire Scene Examinations

During a fire scene investigation, complications can arise which can affect the successful outcome of investigation and some of these are listed:

- A fire can be a complex event where its source is not obvious. Qualified fire scene investigators are essential. It may take several years and considerable cost to train such experts. As such, fires may be investigated by inexperienced examiners.
- The location and time of the fire may hinder the investigation. The fire may have destroyed foundations or load bearing walls causing access problems and an unstable scene. Also, if the fire occurs late at night, poor visibility may make it unsafe for an investigation to be undertaken straight away.
- The destructive nature of the fire complicates the recovery of evidence from the beginning. Also, fire suppression methods may additionally contribute to evidence destruction.
- Representatives of numerous agencies (e.g. fire service, police, etc.) are often present at the investigation for various different functions. With so many present, this could increase the risk of evidence loss.
- Responsibility for the fire investigation is bifurcated. While the fire service has the primary responsibility for establishing the fire's cause, if it is suspected that it was set deliberately, a crime has been committed and therefore the police must become involved.
- If the fire has been set deliberately, the conditions of the fire itself can make it less likely that evidence will survive the fire, thus making identification of the perpetrator more difficult [23].

Having an understanding of these factors as well as the mechanism of fire development including the potential temperatures achievable during compartment fires allows strategies to be developed to investigate the potential to recover evidence such as DNA and fingerprints.

## 1.6 Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid (DNA) is the material that carries genetic information. DNA exists within cells in the body in two forms – mitochondrial DNA and nuclear DNA. Mitochondrial DNA (mtDNA) exists in organelles called mitochondria and is passed down from parent to offspring through the maternal lineage of the family. For

example, a brother, sister, and mother will have the same mtDNA and as such it is not suitable for identifying an individual. MtDNA can be used for familial identity testing but this is carried out predominantly in the USA and other parts of Europe. MtDNA can also be used for human provenancing, which is the identification of a deceased unknown individual or the verification of the genetic history of an individual. Nuclear DNA exists within the nucleus of a cell and is more frequently used as a tool for the identification of individuals in criminal and other cases [24].

The nucleus of a human cell (figure 1-8) possesses 23 pairs of chromosomes which are inherited from our parents with each parent contributing half of each pair. Every somatic cell in the body has identical DNA, and every individual's DNA is considered to be unique (except for identical twins, triplets, etc. who originated from the same egg). Each one of the 46 chromosomes includes a single piece of double stranded DNA in which the two strands are wound around each other in a double helix formation. The double strands themselves are wound around a supporting structure of protein (figure 1-9) [24-27].

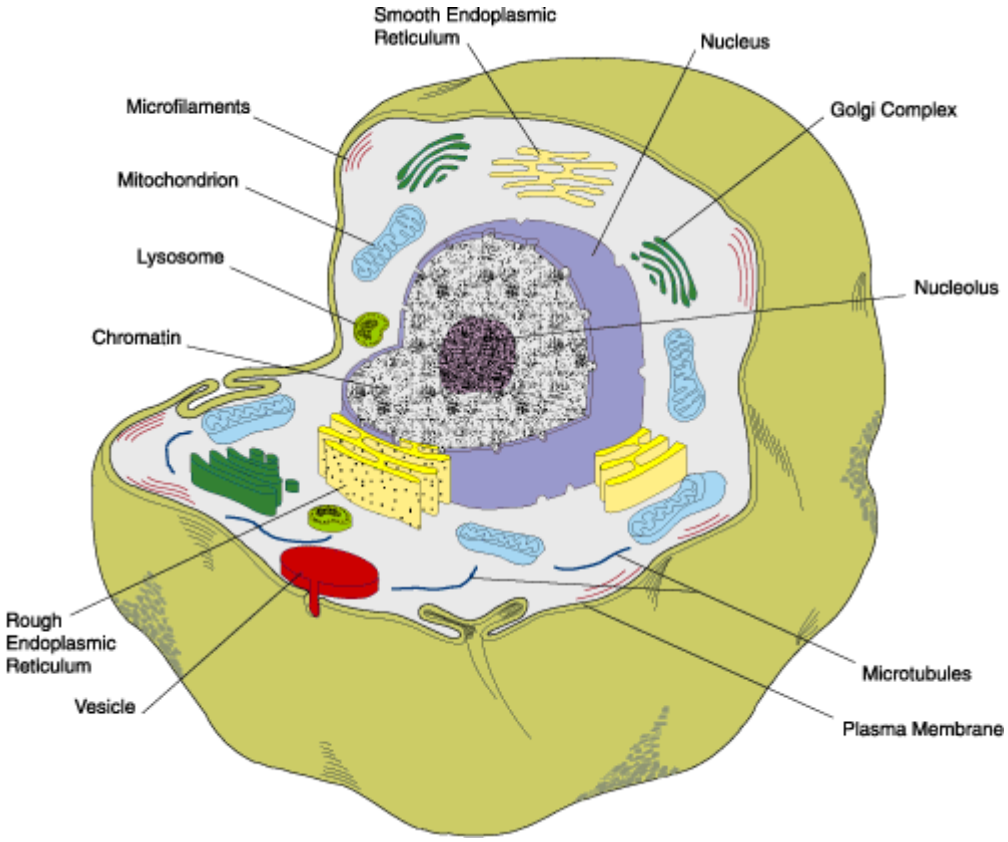



Figure 1-8 Illustration of a cell [28]

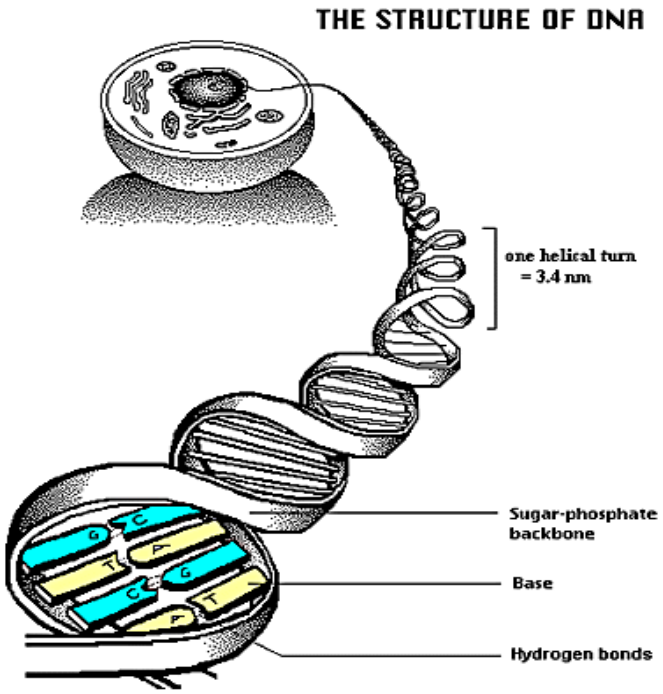



Figure 1-9 Illustration of DNA [29]

DNA is composed of a backbone of alternating sugar and phosphate molecules. The sugar is a 5' carbon ring deoxyribose which is linked via the phosphate group from the 5' carbon on one sugar to the 3' carbon on the following sugar. A nitrogen base is attached to every sugar in the chain. Two classes of base occur. One, a pyrimidine, has a 6-member ring of carbon and nitrogen. The other, a purine, is composed of fused 5- and 6-member rings. There are four types of base in DNA: adenine (A) and guanine (G), which are purines, and cytosine (C) and thymine (T), which are pyrimidines (figure 1-10).

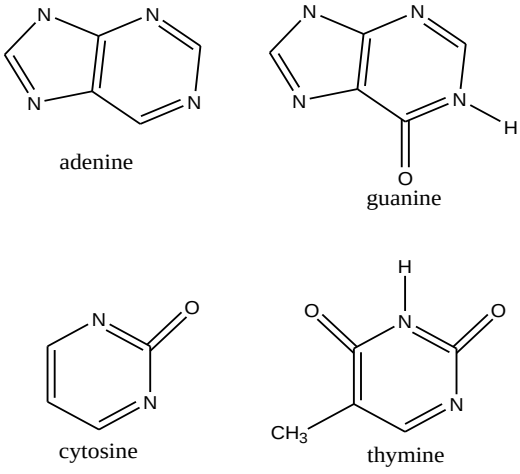



Figure 1-10 The four bases [24, 26]

The DNA strand is made up from individual units, called nucleotides, which comprise of a deoxyribose sugar, a phosphate and a base. Therefore there are four types of nucleotide corresponding to the four bases and these nucleotides can be linked to form a chain (polynucleotide). This is also referred to as the DNA sequence.

The strands of the double helix are held together by hydrogen bonding between the bases on adjacent strands. The cytosine and guanine bases form three hydrogen bonds and the thymine and adenine bases form two hydrogen bonds. Therefore, the two DNA strands of a pair do not carry the same base sequence but complementary sequences (figure 1-11). The pairing of the nucleotides is referred to as base pairs and this is the unit of measurement in determining the size of the DNA sequence.

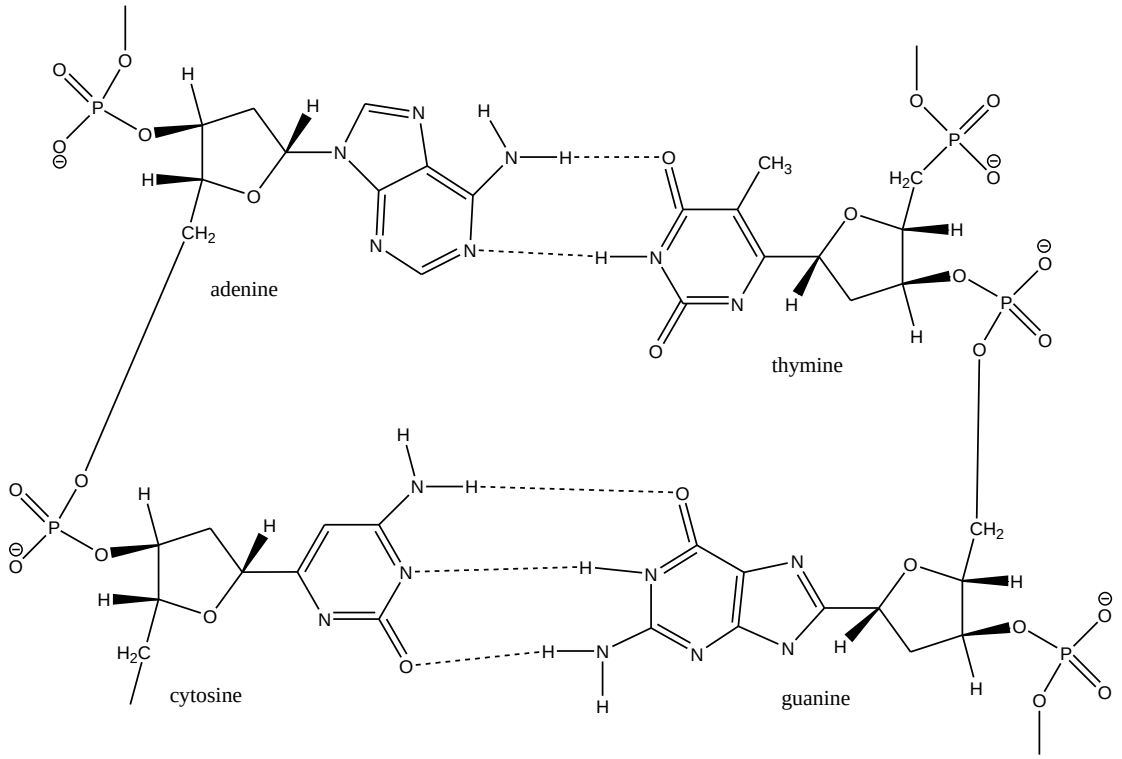



Figure 1-11 The polynucleotide sequence (dotted lines denote hydrogen bonding) [24, 26]

As previously mentioned, every human cell (except for erythrocytes (red blood cells) and spermatozoon (sperm cell)) has 23 pairs of chromosomes – 23 inherited from the mother and 23 inherited from the father. On each of these chromosomes there are coding regions and non-coding regions (which may also be referred to as non-protein-coding DNA). The coding regions are known as genes and these contain the

information necessary for a cell to make proteins. Each gene has two regions: the exons (protein-coding region) and the introns (intervening sequences). It is the exons in each gene which provide the information for the cell to make a specific protein and these areas also have the ability to change. The introns and the non-coding region of DNA are the focus of forensic testing. These areas are responsible for the small amount of differences between two individuals' DNA (polymorphism) and the DNA does not change during the lifetime of the person [24-25, 27].

Individual identity testing takes place at certain places along the chromosome (genetic locus). These genetic loci are in the same position on each of the chromosomes of the pair. The repetition of the DNA sequences at these loci is measured and the number of repeats is determined as the allele. This repeating sequence of DNA is known as a short tandem repeat (STR). For example, a chromosome inherited from the mother may have 13 base pair sequence repeats at locus TH01 and a chromosome inherited from the father may have 13 repeats at locus TH01. This person would be homozygote at this particular locus (same number of repeats (alleles) on each of the chromosome pair). If the chromosome inherited from the father had 15 repeats at locus TH01, the person would be heterozygous at this locus (different number of repeats (alleles) on each of the chromosome pair) [24-25, 27]. The alleles make up the DNA profile of an individual. This is described in the following flow chart (figure 1-12):

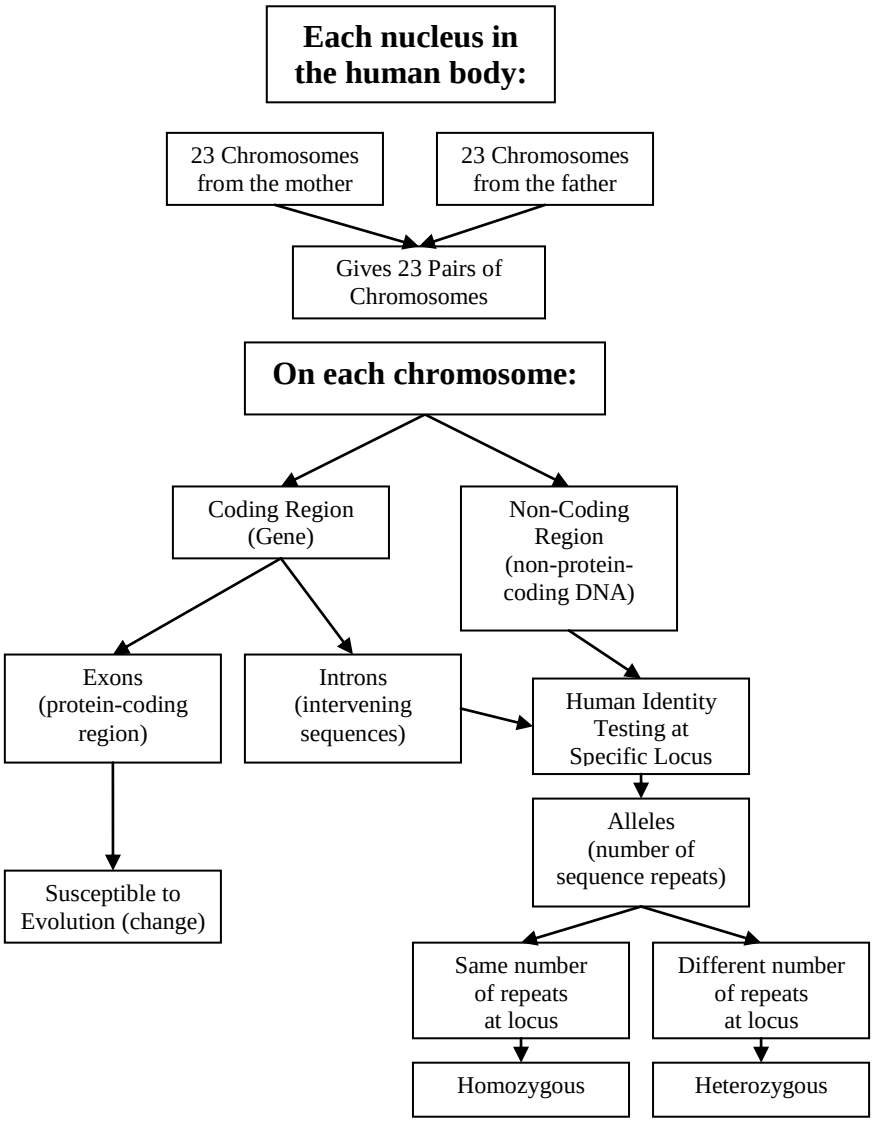



Figure 1-12 Flow chart showing where DNA is in the body and how it can be used

The following diagram (figure 1-13) shows the different regions of DNA on the chromosome:

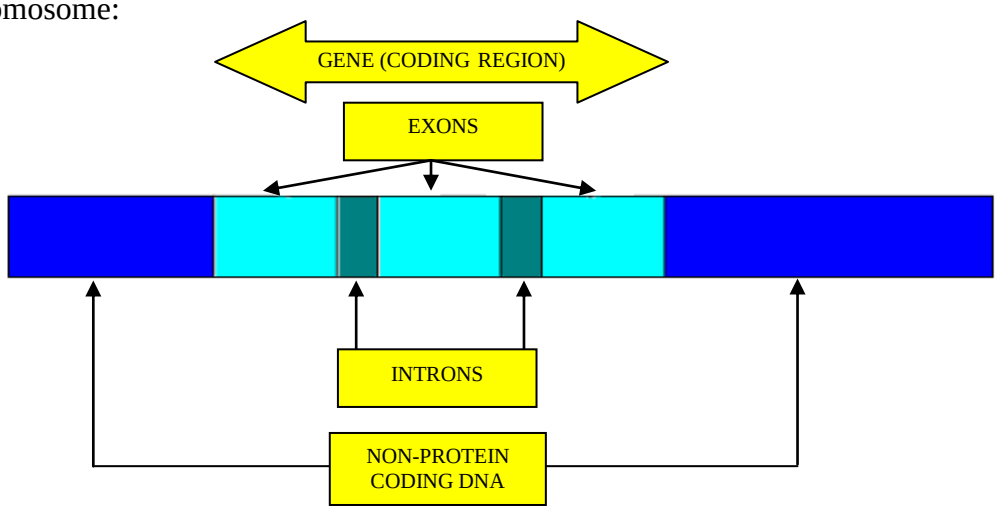



Figure 1-13 Various regions on the chromosome

### 1.6.1 Forensic DNA Applications

DNA fingerprinting or DNA profiling as it is now known was first described by Dr Alec Jeffreys in 1985 [30-32]. He discovered that certain regions of DNA contained sequences which were repeated and that these numbers of repeats varied from person to person. He developed a technique to examine these length variations and thus created the ability to perform human identity tests. There have been many advancements over 25 years since this technique was discovered culminating in the growth of the use of DNA profiling in both criminal investigations and civil cases such as paternity testing, disaster victim identification, and human trafficking.

The first use of DNA profiling in a forensic context was in 1986. Two young girls, Lynda Mann and Dawn Ashworth, were sexually assaulted and then murdered in 1983 and 1986. Both of these crimes were committed in the small village of Narborough in Leicestershire. The similarities between the crimes led police to believe it was that same perpetrator. A local man admitted to the murder of one of the girls but his blood sample was not a match to the semen found at either crime scene via DNA testing. Thus DNA testing proved this man's innocence when he may have otherwise been convicted. A mass screen to collect blood from all adult men in the three local villages to the crime scenes was undertaken. After over 4000 samples had been DNA profiled and compared to the semen samples, no match was found. Approximately a year later, a woman overheard a man in a bar bragging that he had given the police a blood sample for a friend named Colin Pitchfork. The police interviewed Colin Pitchfork and confronted him with this information. Upon this he admitted to the crimes. He was tried, convicted, and sentenced to life imprisonment for his crimes.

The UK National DNA Database (NDNAD), established in 1995, continues to provide the police service with a valuable intelligence tool. Currently 5.2% of the UK population are represented on the Database, compared with 0.5% in the USA, which makes it the largest in the world per head of population [33]. The NDNAD is a searchable storage facility for DNA profiles obtained from crime scenes and samples taken from individuals in police custody. The National Policing Improvement Agency (NPIA) has taken over Custodianship of the National DNA Database. The NDNAD Custodian Unit moved away from the Forensic Science Service (FSS) shortly before

the start of the 2006/07 year. This separation was essential, given the transformation of the FSS to a commercially oriented Government-Owned Company (GovCo) and the importance of retaining oversight and management of the NDNAD within a public sector environment was vital. The Custodian's role is ensuring execution of the quality systems for protecting the integrity of the Database and this is ever more important [34].

The core legislation in England and Wales that underpins the taking of samples and retention of DNA information is the Police and Criminal Evidence Act (PACE). This laid the ground for DNA taken from those charged to be retained. Before 2001 the police were required to destroy DNA samples and fingerprints from people against whom charges were dropped or not proceeded with. This category also included people found not guilty. PACE was amended in 2001 by the Criminal Justice and Police Act, which removed the obligation to destroy the DNA in the event of there being no prosecution or acquittal, so long as the samples had been lawfully taken. The Criminal Justice Act 2003 further amended PACE to allow the police to take DNA and fingerprints without consent from anyone arrested for a recordable offence and detained in a police station and is now part of the normal process when someone is taken into police custody [35-36]. There are no plans to introduce a universal compulsory or voluntary DNA database [33].

Scotland also has its own DNA Database, maintained by the Scottish Police Services Authority (SPSA) Dundee[1] [34, 37]. The Scottish DNA Database remains a distinct entity but exports 'copies' of its DNA profiles to the NDNAD.

The main difference between the Scottish DNA Database and the NDNAD is the retention of DNA profiles [36]. Following a decision not to institute criminal proceedings against an individual or on the conclusion of proceedings against that person that did not result in a conviction, Section 18 of the Criminal Procedure (Scotland) Act 1995 states that all samples should be destroyed in these circumstances [38]. The NDNAD retains the DNA samples of innocent individuals. During 2006/07,

[1] Previously known as Police Forensic Science Laboratory Dundee (PFSLD) until 1st April 2007

23927 subject sample records were removed from the NDNAD. Of these, 23439 related to Scottish samples [34].

### 1.6.2 DNA Analysis

In order to obtain a DNA profile which can be placed onto or searched against a DNA database, the initial crime scene sample must be analysed. This is achieved through various steps which will be discussed in more detail as follows.

#### 1.6.2.1 DNA Retrieval

DNA is most commonly retrieved using a cotton swab [39] but there are various methods in which the swabs can be used and various different types of swabs on the market [40-41]. Some studies [39, 42-43] have previously been carried out into comparing these retrieval techniques and some have recovered more DNA (in ng) than other retrieval methods [39, 43]. An alternative method to using a swab has been developed and this involves using adhesive tape to retrieve any DNA [44] but there has been no study on its effectiveness compared to swabbing for touched surfaces.

#### 1.6.2.2 DNA Extraction

To undertake a DNA analysis, the first step is to extract the DNA from the sample. When the sample is recovered, the DNA will be present but still wrapped within its cellular proteins which are used to package and protect the DNA in the environment of the cell. These cellular proteins can inhibit the analysis of the DNA and as such, must be removed [24, 27]. There are many different types of extraction techniques [24, 26-27, 45-48], but only QiaAmp extraction will be used in this research [48].

#### 1.6.2.3 DNA Amplification

Human identity testing has benefited greatly from the discovery of a technique called the polymerase chain reaction (PCR). This was first described by Kary Mullis and the Human Genetics group at the Cetus Corporation (now Roche Molecular Systems) [49-

51]. Kary Mullis was awarded the Nobel Prize in Chemistry in 1993 for his invention, less than 10 years after it was first described [52].

**Denaturation**

The first part of the PCR process is the denaturing of the DNA. As previously described, DNA is double stranded and bound to each other through hydrogen bonding to form the double helix structure. Denaturing the DNA involves breaking these hydrogen bonds so that the DNA will be single stranded. This is achieved at 94˚C. The temperature is very important as it is just above the melting point of DNA; therefore it breaks the bonds but does not destroy the DNA. Each individual strand can then be used as a template to copy the DNA sequence [53].

**Annealing**

This part of the process occurs when the temperature is decreased which allows the oligonucleotide primers to anneal to their specific DNA target sequence. Annealing is achieved at 60˚C, when the temperature is low enough for the primers and single stranded DNA to bind but high enough that the primers do not bind to the wrong site. Each pair of primers flanks the required region of DNA, copying both strands [53].

**Extension**

The final step is achieved through an increase in temperature (to 72˚C) where the synthetic free bases (which are added before the thermal cycling process) are attached to the 3' end of the primer by means of using the original DNA single strand as a template. This will continue to occur until the next temperature cycle begins or until all cycles have been completed [53].

Without the ability to make copies of DNA, many DNA samples could never be analysed. This is due to the DNA being limited in quantity. PCR replicates specific regions of the DNA through an enzymatic process. This is achieved rapidly and sensitively, and is not limited by the quality of the DNA therefore it is ideally suited to forensic DNA analysis [24, 27, 53-54]. This copying process involves heating and

cooling the samples in a precise thermal cycling pattern of (typically) 28 cycles (in the presence of various components – commercial kits with pre-mixed components may also be used for PCR). During each cycle, a copy of the target DNA sequence is generated. These targets are defined by oligonucleotide primers that are complementary to the 3'-ends of the specific sequence required to be copied. The primers are the most important components of a PCR reaction. They are short DNA sequences that precede the loci to be copied. The loci are copied by attaching synthetic bases onto the primer to form a DNA sequence, therefore making a copy. DNA polymerase adds the bases in the proper order based of the target DNA sequence. Theoretically after 30 PCR cycles (assuming 100% efficiency), approximately one billion copies of the target DNA have been generated. These are sometimes referred to as 'amplicons'. The PCR is performed with the DNA that had been extracted from its original cellular state and is normally performed with a sample volume of 5-100μL, equivalent to 1-10ng target mass of DNA [24].

A typical example of a PCR thermal cycling temperature profile is shown below in figure 1-14.

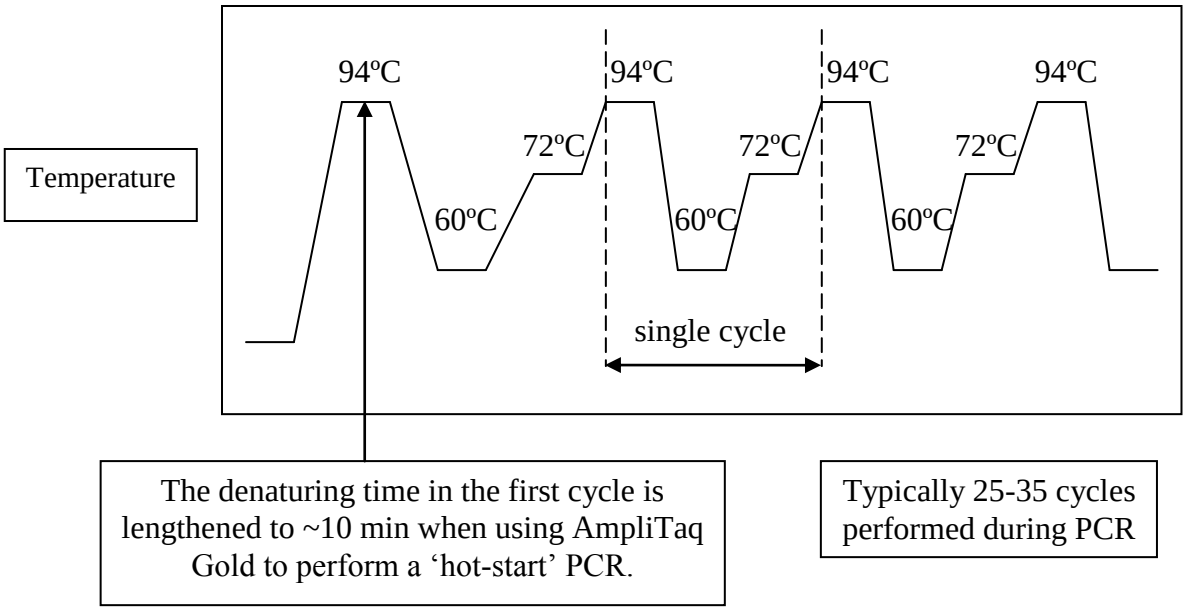



Figure 1-14 Thermal cycling temperature profile for PCR [24]

In human identity testing, STR PCR is monopolised. As previously stated, an STR is the short length of DNA comprising a number of tandemly repeated nucleotide sequences at a particular genetic locus. The number of repeats is called the allele and it is this polymorphism which can distinguish between two individuals [55]. In forensic casework, commercially produced multiplex (targets more than one region of DNA during PCR) PCR kits are utilised. These kits contain a number of oligonucleotide primers (each loci of interest will have pairs of primers specific to that locus in the kit) that bind to the specific loci on the DNA strand. STRs are more commonly analysed using electrophoreses and automated fluorescent detection systems [27]. Therefore, the primers are also fluorescently tagged in order to identify them when separation occurs during capillary electrophoresis. This is described in the following section.

#### 1.6.2.4 Capillary Electrophoresis and DNA Profiling

Capillary electrophoresis manipulates the negative charge of DNA. A fused silica (glass) capillary is filled with a viscous polymer solution and the PCR product is injected into it. A high voltage is applied across the capillary after injection. By electrical attraction, the DNA moves towards the positive pole. Small STR fragments move faster than larger fragments so the STRs sort themselves according to size. As the fragments have been tagged by a dye during PCR, a laser is used to detect the fragments by their fluorescence. The laser interfaces with the software package on a computer system and the STR fragments show up as peaks on the electropherogram where they can be interpreted later [24]. An example of an electropherogram is shown in figure 1-15.

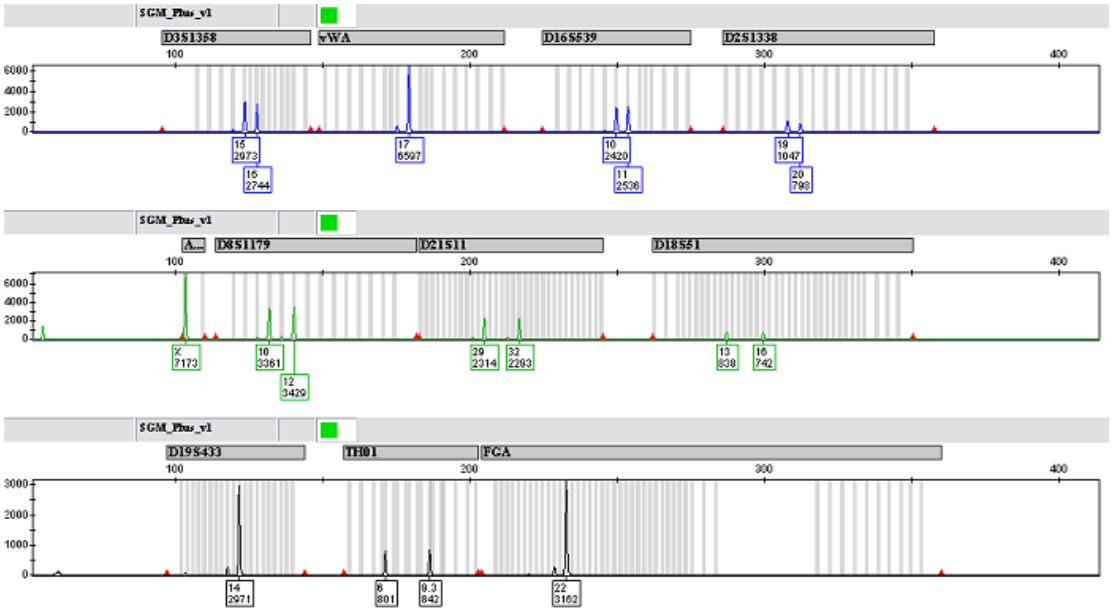



Figure 1-15 Example of an electropherogram

## 1.6.3 Real-Time (Quantitative) PCR

Instruments and assays are now available that can monitor the PCR process as it is occurring enabling 'real-time' data collection [24]. Real-time PCR, which was first described by Higuchi and co-workers also at the Cetus Corporation in the early 1990s, is sometime referred to as quantitative PCR (qPCR) as it analyses the cycle-to-cycle change in fluorescence signal resulting from the amplification of a target sequence during PCR [56-57]. A fluorescently tagged primer and a modified base are used in amplification. As the number of PCR cycles increases, the fluorescent signal is reduced due to the insertion of a fluorescence quencher. An example of this is shown in figure 1-16. A melt analysis (which is used as an internal standard) is also performed to confirm the amplification of target DNA and not contaminants[58].

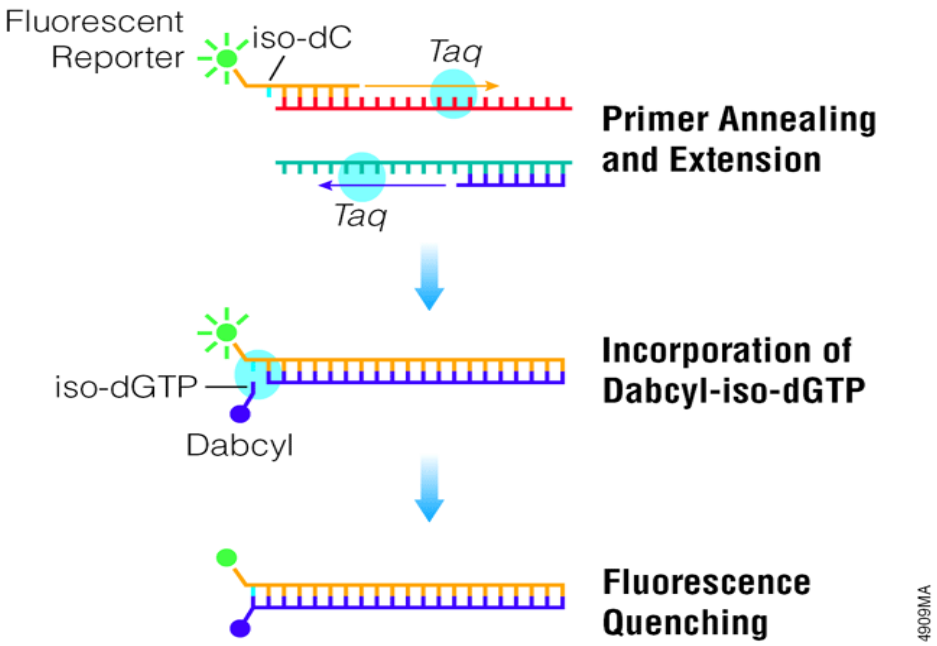



Figure 1-16 Schematic diagram illustrating the Plexor® real-time PCR process [58]

### 1.6.4 Low Template DNA (LT-DNA)

Low template DNA (LT-DNA) testing typically refers to DNA examination of less than 200pg of input [59], even as small as single cells [60]. Therefore, as the sample size is small, the collection of DNA must be controlled. This has been investigated and can be achieved by wearing protective clothing, wearing face masks, and wearing gloves [61-62], which should be changed regularly. Also, as the sample size is small, the sensitivity of the technique must be improved. This can be achieved by simply changing the number of cycles in the amplification stage of the DNA process [24, 26, 63-66]. The most common number of cycles used with samples greater than 200pg is 28 cycles. For LT-DNA, 28 cycles is not enough. A number of methods have been developed to deal with the LT-DNA problem. Protocols which involve increasing the number of PCR cycles are referred to as low copy number (LCN). Research has been carried out into this [63-67] and the optimum number of cycles suggested is 34 [65]. Other methods are used to increase the sensitivity and this can be achieved by concentrating the sample, increasing injection time, etc.

LT-DNA results have increased chance of allele dropout, allele drop-in, and there is also an increased risk of contamination (collection based and laboratory based) [24, 26]. Allele dropout is when an allele fails to amplify, allele drop-in is when additional

alleles are observed due to contamination, and due to the size of the sample, all LT-DNA work should be carried out in as sterile an environment as possible to avoid contamination and thus avoid allele drop-in [24].

Although a DNA profile may be obtained from LT-DNA evidence, it is not always possible to determine which type of cell it originated from [24, 63]. But this is also true for all DNA profiling. Remarkably, DNA profiles may be obtained from fingerprint residues due to cells which have been left on objects which have been touched [24, 68-72]. Therefore the ability to obtain DNA profiles from small amounts of biological material has expanded the types of samples available for analysis.

A recent independent study undertaken for the UK Home Office indicated that the science behind LT-DNA analysis was sound but that work into the interpretation of the results was ongoing. This will be overviewed and standardised by the Home Office's Forensic Science Regulator [73].

#### 1.6.4.1 DNA from Fingerprints

Aside from retrieving DNA from fingerprints in blood, the *Nature* letter by van Oorschot and Jones entitled 'DNA Fingerprints from Fingerprints' described primary DNA transfer onto objects touched by hands [69]. Since this breakthrough, other investigations into the transfer of trace DNA via skin contact have been conducted [60, 68, 70-72, 74]. As the sensitivity of DNA profiling has advanced, the examination of articles relating to a crime has increased to involve trace level transfer of DNA [24, 75].

The likely origin of DNA recovered from fingerprints is from skin cells that are also transferred at the moment of contact. Cells (which contain approximately 6pg of genomic DNA per cell [24]) called keratinocytes are found in the epidermis. The epidermis is comprised of five layers which are shown in figure 1-17. Cells are produced in the stratum basale before being pushed upwards through the other layers until they reach the stratum corneum, and slough off the skin surface. The process of cell generation through to sloughing is called differentiation [76].

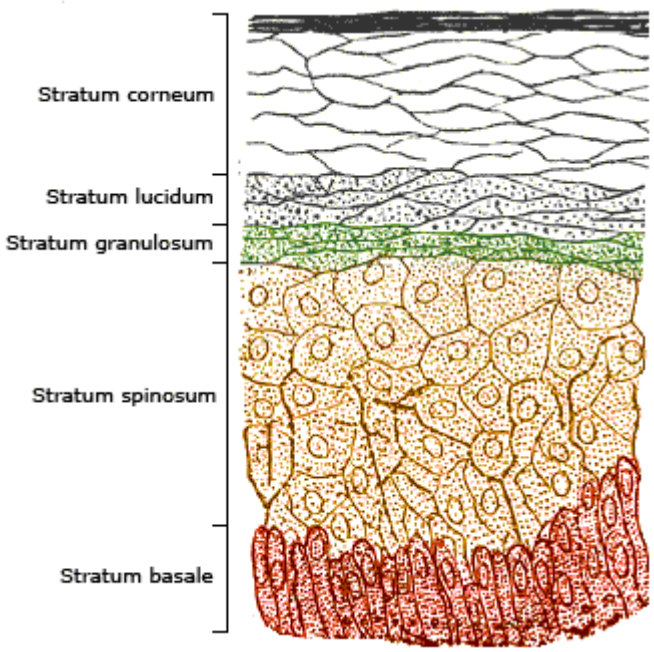



Figure 1-17 Illustration of the epidermal layers [77]

During the process of differentiation, the keratinocyte cells undergo a programmed cell death, with the nuclei of the cells also undergoing alterations and eventually disappearing from the cells. Studies have shown that the distribution of DNA will gradually change and decrease in the nuclei throughout the differentiation process and it is also thought that the DNA is degraded into smaller molecules [78]. A more recent study has shown that DNA is present in these dead skin cells in single stranded and fragmented form and as such, it is conceivable that DNA profiles can be generated from these DNA fragments transferred during the deposition of a fingerprint [79].

### 1.6.5 DNA at Fire Scenes

The heat that a fire will exert on its surroundings will also impact the DNA recoverability from items within the scene. Very little literature exists on the effects of heat on DNA recovery and the research that has been done was undertaken on DNA rich blood samples, and not the trace levels of DNA found in fingerprints.

Kell *et al.*, concluded that amplifiable DNA could be recovered from blood samples that had been recovered from a fire scene [80]. Tontarski *et al.*, have recently published research into the recovery of DNA from fire scenes, but again their DNA

source was blood [81]. They stated that there was a 'lack of DNA typing results for samples affected by high temperature and/or close proximity to the ignition source. In particular, six samples from two of the hottest points in the fire did not yield DNA profiles'. Notwithstanding this, they report obtaining full DNA profiles on samples exposed to temperatures up to 300˚C.

Two other papers have been published describing the recovery of DNA from exploded pipe bombs [82-83]. Esslinger *et al*., reported a 45% identification success rate from the bomb fragments using STR analysis. Foran *et al*., reported a 50% success rate using mtDNA. These low explosive bombs can reach temperatures of 2500-3000˚C, however the duration of these high temperatures is very short.

There are many different fire suppression methods with the most common being water. Water does not affect DNA, and cases of skeletal remains submerged in water for three years has still yielded a DNA profile [84]. It is not known if the pressure that the water exerts on the sample when it leaves the hose during fire suppression has an effect on DNA recovery.

Surface thermal effects observed on the physical evidence surviving within a fire scene can also provide information of the temperatures DNA deposited at the scene may have experienced. This may prove to be a useful tool to the forensic scientist, as it could be a waste of resources to process an item for DNA when such samples may not be viable after exposure to the temperatures indicated by the post fire indicators. Having knowledge relating to the viable recovery of DNA profiles exposed to different temperatures on a range of common substrates (porous and non porous) encountered in fire scenes is therefore advantageous.

## 1.7 Fingerprints

Fingerprints are produced from the contact between the ridges present on our fingers and a substrate. The pattern transfer is achieved by the deposition of the secretions from our glands or contaminants present on the finger surface. These ridges are more commonly referred to as friction ridges as it is suggested that their primary purpose is

to enhance grip. The skin which is found on the fingers, palms, and soles is specifically referred to as volar skin. Like all skin, volar skin comprises of two layers – the epidermis (previously discussed in section 1.6.4.1) and the dermis, and it is the these layers which provide the friction ridges with their various patterns and shapes [75].

The development of friction ridge is initiated at a very early stage in the development of the foetus within the womb. The development of the foetus is charted in terms of its estimated gestational age (EGA). At around 5-6 weeks EGA, the foetus's hand will start to develop, with fingers developing at approx 6-7 weeks EGA. At that time, volar pads appear on the palm, which are swellings of mesenchymal tissue, followed by pads developing on the fingers (figure 1-18) [75-76, 85-88].

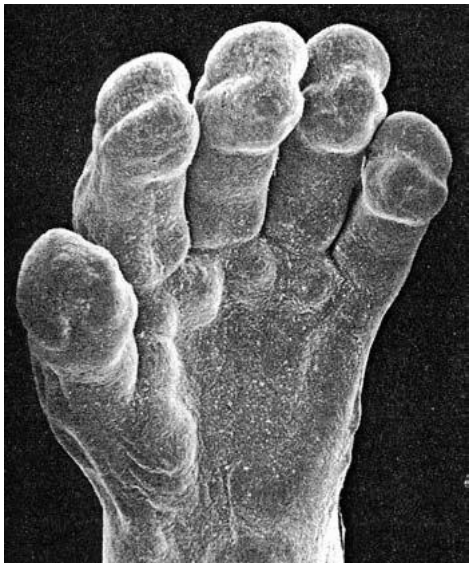


Figure 1-18 SEM view of the hand of a foetus, displaying prominent volar pads [76, 89]

By 16 weeks EGA, the mesenchymal tissue swelling is invisible, as the growth of the hands has enveloped the pads (regression stage of the volar pads). It is in weeks 11-20 EGA that the friction ridge development occurs (the volar pads provide the bedding for that development). Primary ridges on the dermis with fully formed ridge endings are the first to develop. These can be thought of as ridge units, as each are associated with a sweat pore (figure 1-19) [75-76, 85-88].

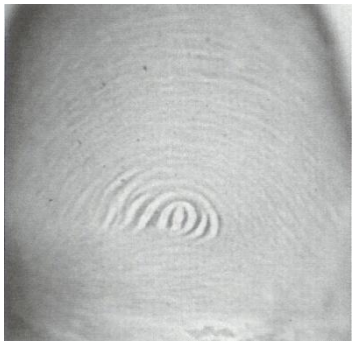


Figure 1-19 The dermal surface of the apex of the index finger of a foetus (11 weeks EGA) [75]

During the development, ridges will start to become visible from the apex of the finger, the tip of the finger, and the distal interphalangeal flexion crease (the first joint underneath the apex of the finger). The developments of the ridges from the three different regions occur at different speeds until they converge and cover the dermal surface (figure 1-20) [75-76, 85-88].

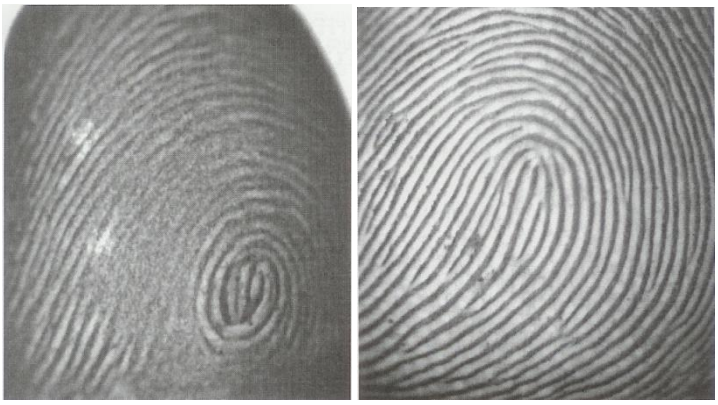


Figure 1-20 Dermal surface of the index finger of a foetus, showing various development fronts (left) and showing the final fingerprint pattern (right) [75]

Between weeks 15-17 EGA, secondary ridges start to develop between the primary ridges, and continue until week 24 EGA. Further development leads to the construction of bridges between the primary ridges and the secondary ridges, which divides the dermal ridges. These divisions are referred to as papillae pegs or dermal papillae. At 24 weeks EGA, the development of the dermis is complete. A diagrammatic view of this development is shown in figure 1-21.

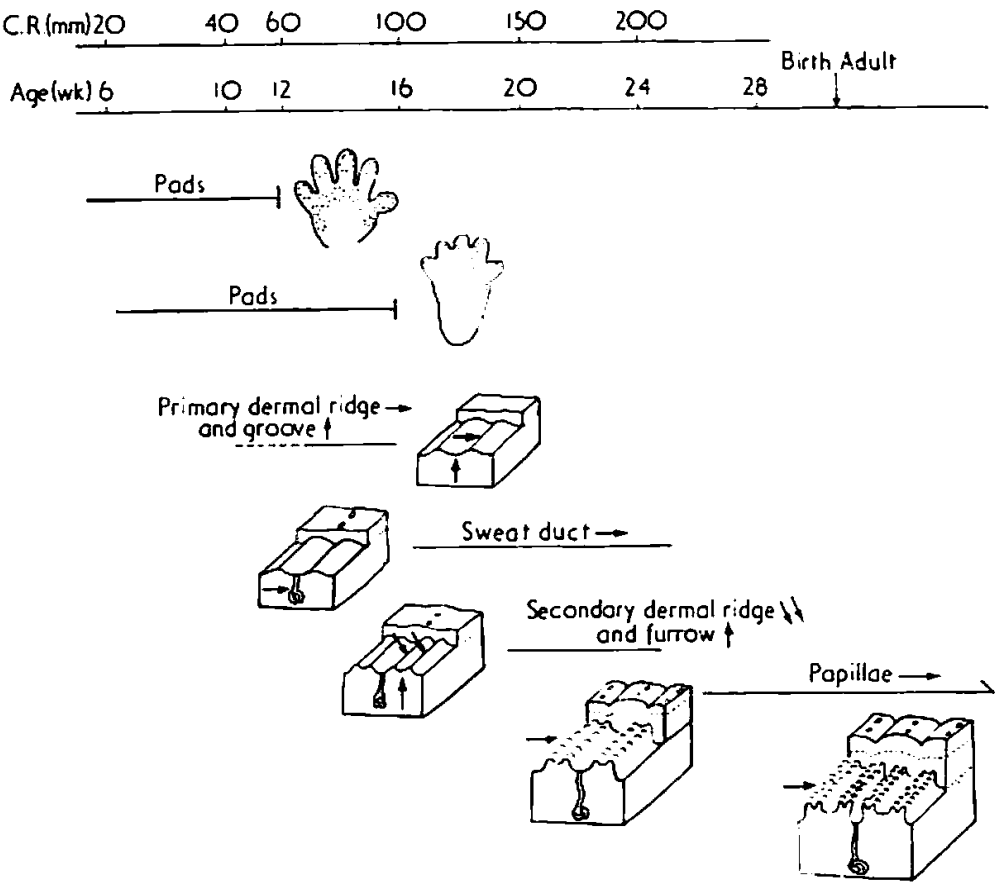



Figure 1-21 Diagrammatic representation of dermal ridge morphogenesis
(CR is crown rump which is the length from the crown of the head to its rump) [75, 87]

These friction skin ridges can be classified into three levels of detail. First level detail refers to the overall pattern, second level detail refers to the major path deviations (or minutiae), and third level detail refers to the intrinsic or innate ridge formations [75]. These levels of detail are used in identifying individuals from their fingerprints.

### 1.7.1 Fingerprint Identification

There have been many uses for fingerprints throughout history. Fingerprints were found on ancient pottery and cave paintings in Asia, Europe, and North America, where they may have denoted authorship or identity [90]. Archaeological evidence from seventh-century China shows fingerprints embossed in clay seals which were used to sign documents, and this practice may have been as old as the Former Han dynasty (202 BCE – 220CE). From China the practice spread to Japan, Tibet, and India, where fingerprints were used as seals [90]. The use of fingerprints in these ways suggests that fingerprint patterns were believed to be associated with specific

individuals [90-92]. The idea that fingerprints could be used for identification started in the 1850s [90]. The person attributed with this idea is unclear and Sir Francis Galton, Sir William James Herschel, Sir Edward Richard Henry, and Dr. Henry Faulds have all made contributions to the field [90-94]. Of them, Dr. Henry Faulds was the first to suggest the use of fingerprints for the detection of criminals by publishing a letter on 28th October 1880 in Nature stating 'When bloody finger marks or impressions on clay, glass, etc. exist, they may lead to the scientific identification of criminals' [95]. Approximately one hundred years later in 1983, the UK Home Office accepted that no two individuals have the same fingerprints [90].

**First Level Detail**

Individuals generally have a mixture of pattern types on their fingertips, with some correlation between the left and right hands. There is also evidence that the general fingerprint pattern may be genetically determined [96]. While the loop pattern is the most common pattern, classification of individuals by assigning a pattern type to each of the ten fingers in an ordered fashion, serves as a first line of differentiation [26, 85, 96]. The main types of pattern are shown in figure 1-22.


Figure 1-22 Types of fingerprint pattern [96]

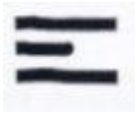
**Second Level Detail**

Identification of an individual using fingerprints relies on pattern matching followed by the detection of certain ridge characteristics (also known as Galton details, points of identity, or minutiae) and the comparison of the relative positions of these minutiae points with a reference fingerprint [26, 85, 97].

A single rolled fingerprint may have as many as 100 or more minutiae (points) that can be used for identification purposes. The number of minutiae found on a

fingerprint impression depends on the area of the finger that the fingerprint was deposited from. For example the area containing the fingerprint pattern will contain more minutiae per square millimetre than the area near the tip of the finger [97]. Various examples of the minutiae are shown in table 1-1.

Table 1-1 Minutiae characteristics [97]

| Basic and composite ridge characteristics (minutiae) | | | |
|---|---|---|---|
| Minutiae | Example | Minutiae | Example |
| ridge ending |  | bridge |  |
| bifurcation |  | double bifurcation |  |
| dot |  | trifurcation |  |
| island (short ridge) |  | opposed bifurcations |  |
| lake (enclosure) |  | ridge crossing |  |
| hook (spur) |  | opposed bifurcation/ridge ending |  |

Using this second level detail, a numerical approach to fingerprint identification was established. This development was attributed to Edmond Locard who proposed that the minimum number of minutiae used for identification was as follows [75]:

1. If more than 12 concurring points are present and the fingerprint is sharp, then the certainty of identity is beyond debate.

2. If 8 to 12 concurring points are involved, then the case is borderline, and the certainty of the mark will depend on:
   - The sharpness of the fingerprint
   - The rarity of its type

- The presence of the centre of the finger (core) and the triangle (delta) in the exploitable part of the fingerprint
- The presence of the pores
- The perfect and obvious identity regarding the width of the papillary ridges and valleys, the direction of the lines, and the angular value of bifurcations

  In these instances, certainty can only be established following discussion of the case by at least two competent and experienced specialists.

3. If a limited number of minutiae are present, the fingerprint cannot provide certainty for identification, but only a presumption proportional to the number of minutiae available and their clarity.

In the UK, the numerical identification system was originally Locard's 12 point standard but this changed to a 16 point standard in 1924 following the publication of photographs which purported to show two fingerprints from different people with 16 common characteristics. New Scotland Yard disagreed with six of these characteristics but in the belief that 10 were identical, recommended that the standard should be raised to 16 points [98]. The fingerprints, which prompted this change, were later found to have been altered and in fact did not contain 10 points of agreement. Nonetheless the 16 point standard remained until 2001 in England and Wales and until 2006 in Scotland. Nowadays, the UK will still use second level detail as a method of comparison but will now also take into account first and third level detail (which is discussed in the following section) [98]. This is referred to as the non-numeric fingerprint standard, where there is now no concurring point standard. The practice in other countries varies from non-numeric (like the UK) to a fixed number which is usually lower than 16 (The Netherlands, Slovakia and some other European countries still use the 12 point system) [98].

**Third Level Detail**

The current fingerprint identification system operating in the UK at the moment utilises poroscopy and edgeoscopy (often referred to as non-numeric fingerprint identification system). It can also be referred to as the 'holistic approach' where all visible features are used to assist in identification. This approach involves the

examination of the pores and shapes of the edges of the ridges. The concept of poroscopy was developed by Dr. Edmond Locard in 1912. He stated that the sweat pores within the ridges were variable in size, have various forms (i.e. elliptical, oval, square, etc.), can take up various positions along the ridge, and can have variable frequency along the ridge (figure 1-23). This concept, coupled with that of edgeoscopy (which was suggested by Salil K. Chatterjee in 1962), and refers to the individuality of the ridge edges, is what makes up the third level detail for identification [88].

Figure 1-23 An example of third level detail, clearly showing the pores and edges of the ridges [88]

Fingerprints are an important category of physical evidence for positive identification or individualisation [23] and have been used in identification for over 100 years. A fingerprint pattern does not change and it is relatively easy to capture [85, 92]. Fingertips may be scarred or cut, but the resultant fingerprint can still contain enough information to link it with its owner [99].

### 1.7.2 Visible Fingerprints

The detection of fingerprints by close visual examination should always be undertaken before any subsequent analysis. These fingerprints are normally photographed before any other work is undertaken as they may be lost or not detected after further fingerprint enhancement is carried out. An example is shown in figure 1-24 [100].

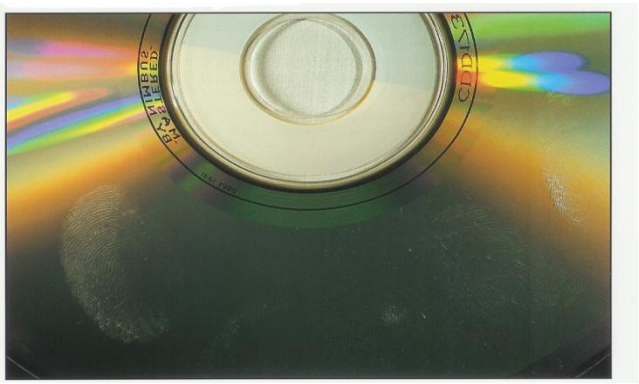


Figure 1-24 A visible fingerprint on a compact disc [101]

### 1.7.3 Latent Fingerprints

The term latent literally means "present and capable of becoming (though not now) visible, obvious, or active" [75]. The latent fingerprint is the most common source of fingerprint evidence and also the one that is most problematic: it is present but invisible [75]. Each primary ridge is associated with a sweat pore. This is the source of perspiration on the hands. The composition of human perspiration has been studied and reported extensively in literature [90]. However, knowing the precise contents of the perspiration does not accurately represent the nature of what is actually deposited onto substrates from the fingers and palms. In operational scenarios, numerous contaminants are present in the latent fingerprint deposit, including material from other glands, cosmetics, perfumes, and food residues [26, 90, 100].

The three major glands responsible for the fingerprint deposits are the eccrine, apocrine, and sebaceous glands (table 1-2). The eccrine glands are usually found throughout the body, but the highest densities are found in the palms and soles. The sebaceous glands are typically localised to regions containing hair follicles, as well as the face and scalp. The apocrine glands are primarily found in the axillary regions (e.g. armpits). In most instances, only the eccrine and sebaceous glands contribute significantly to the latent fingerprint deposit [90].

Table 1-2 Components of fingerprint deposit [75, 90, 100, 102]

| | **Source** | **Inorganic Constituents** | **Organic Constituents** |
|---|---|---|---|
| **water-soluble deposits** | Eccrine Glands | chlorides, metal ions, ammonia, sulphate, phosphate | amino acids, proteins, urea, lactic acids, sugars, creatinine, choline, uric acid |
| | Apocrine Glands | iron | proteins, carbohydrates, cholesterol |
| **non-water-soluble deposits** | Sebaceous Glands | | glycerides, fatty acids, hydrocarbons, alcohols |

### 1.7.4 Enhancement of Latent Fingerprints

Fingerprint enhancement techniques have been developed through many years of research. In the UK, the main research into development techniques is performed by the Home Office Scientific Development Branch (HOSDB). This organisation produced the Manual of Fingerprint Development Techniques (MoFDT) which is distributed to all fingerprint enhancement laboratories in the UK, and distributed to international laboratories on request.

Two main factors must be considered before a latent fingerprint can be enhanced. These are primarily the surface that is being processed, and the glandular nature of the secretion which is left behind on that surface. The following table (table 1-3) indicates what processes are used for different secretions [75, 101].

Table 1-3 Processes and the glandular secretions they detect (abbreviations in brackets) [101]

| Sebaceous (Fatty) Material | Eccrine (Aqueous) Material |
|---|---|
| Visual Examination | Visual Examination |
| Powders | Powders |
| Physical Developer (PD) | Ninhydrin (NIN) |
| Vacuum Metal Deposition (VMD) | 1,8-diazafluoren-9-one (DFO) |
| Small Particle Reagent (SPR) | Vacuum Metal Deposition (VMD) |
| Gentian Violet | Fluorescence Examination |
| Solvent Black 3 | Radioactive Sulphur Dioxide |
| Iodine | Superglue |
| Radioactive Sulphur Dioxide | |

In almost all circumstances it is the surface upon which the fingerprints are deposited which determines the sequence of processes to be used, as the glandular deposit is a variable that cannot easily be determined. By using a sequence of processes, or using a process which will enhance both sebaceous and eccrine deposits, attempts can be made to enhance any traces of fingerprint deposits present on the surface. For example, when processing dry paper, the sequence of DFO and ninhydrin which detect eccrine deposits can then be followed by physical developer which detects sebaceous deposits. Or in the case of polythene bags, vacuum metal deposition could be used to enhance for both sebaceous and eccrine deposits [101].

There are three main types of surface classified in fingerprint development: porous, semiporous and non porous. Examples of these types of surface and their interactions with the latent fingerprint deposit are shown in table 1-4.

Table 1-4 Types of surfaces and their interactions with the latent fingerprint deposit [75]

| Porous | Semiporous | Non porous |
|---|---|---|
| Porous surfaces absorb the water-soluble deposit very quickly (within seconds) of deposition | Semiporous surfaces absorb the water-soluble deposit slowly after deposition (mins to hrs) | Non porous surfaces do not absorb any part of the latent fingerprint deposit |
| The non-water-soluble deposit stays on top of the surface for a longer period (a half day to a day) | The non-water-soluble deposit stays on top of the surface much longer (a day to several days) | The water-soluble deposit and non-water-soluble deposit, as an emulsion mixture, stays on top of the surface for a very long time (until degraded) |
| A small amount of the non-water-soluble deposit stays on the surface for a significant period | A small amount of the non-water-soluble deposit stays on the surface for a significant period | Latent deposits remain on the surface and are very fragile (fingerprints easily smudged) |
| **Typical examples:**<br>Paper<br>Cardboard<br>Some fabrics (e.g. cotton)<br>Untreated wood, etc. | **Typical examples:**<br>Certain types of plastic<br>Waxed surfaces<br>Certain types of wall paints and wallpapers<br>Varnished wood, etc. | **Typical examples:**<br>Certain types of plastic<br>Glass<br>Metal surfaces<br>Glazed ceramics<br>Glossy paints, etc. |

The research work described in this thesis involved the examination of fingerprints deposited on porous and non porous surfaces only. A selection of the more commonly available enhancement techniques were chosen for examination according to the HOSDB guidelines in MoFDT. The detection techniques used in the project are described in the next section.

### 1.7.4.1 Detection Techniques for Porous Surfaces

#### Ninhydrin

Ninhydrin (2,2-dihydroxy-1,3-indanedione) (figure 1-25) reacts with primary and secondary amines which are present in the eccrine component of latent fingerprint deposits which is typically composed of a range of amino acids as well as other components (table 1-5 and figure 1-26) [26, 75, 90, 101, 103].

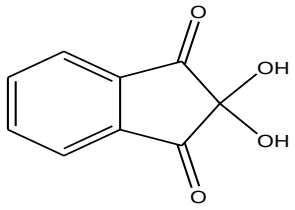
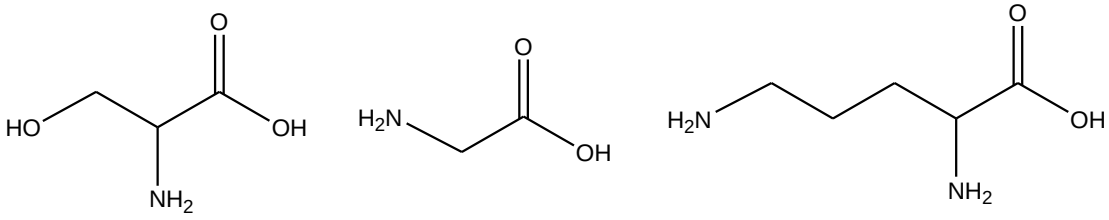


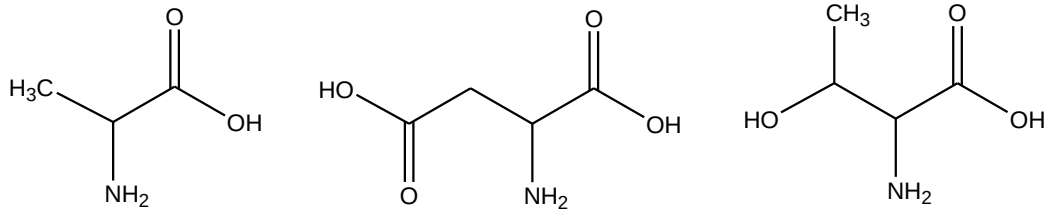

Figure 1-25 Structure of ninhydrin [75]

Table 1-5 Quantities of major amino acids found in a single wet thumb fingerprint [75]

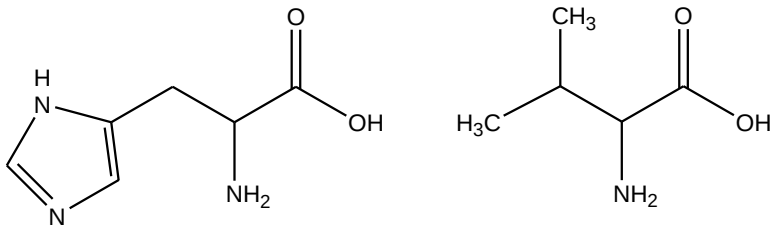
| Amino Acid | Amount (µmol) | Serine Ratio |
|---|---|---|
| Serine | 0.106 | 100 |
| Glycine | 0.071 | 67 |
| Ornithine | 0.034 | 32 |
| Alanine | 0.029 | 27 |
| Aspartic Acid | 0.023 | 22 |
| Threonine | 0.018 | 17 |
| Histidine | 0.018 | 17 |
| Valine | 0.013 | 12 |




Serine (Ser) Glycine (Gly) Ornithine (Orn)




Alanine (Ala) Aspartic Acid (Asp) Threonine (Thr)




Histidine (His) Valine (Val)

Figure 1-26 Structures of the major amino acids found in a typical latent fingerprint [75]

The use of ninhydrin as a fingerprint developer was first suggested by Odén and von Hofsten [104] and is the most popular technique for developing fingerprints on paper and cardboard. The chemical reaction is shown in figure 1-27 [75, 90, 102, 105].

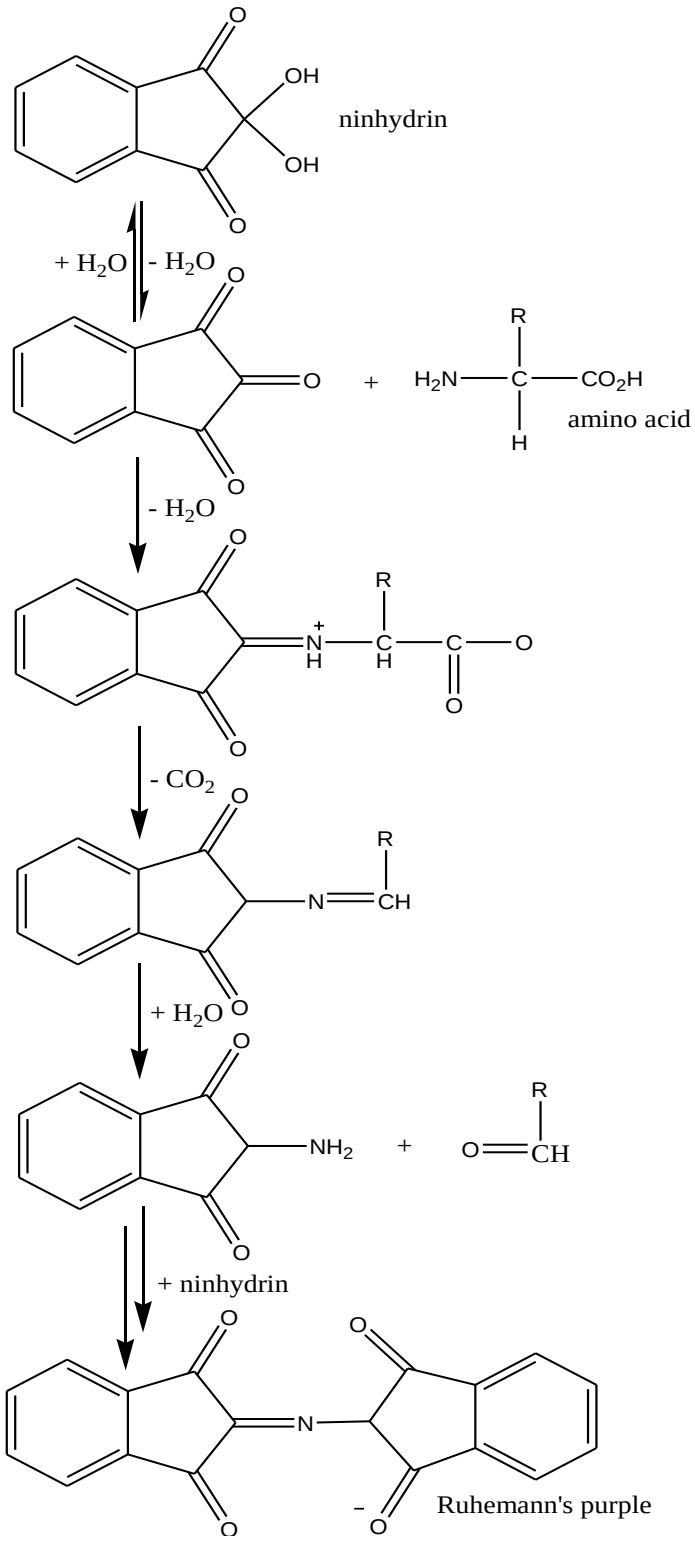



Figure 1-27 Mechanism for the reaction between ninhydrin and an amino acid, resulting in the formation of a product known as Ruhemann's purple [75, 90, 105]

Use of heat and humidification accelerates the reaction so that the majority of fingerprints develop within a few minutes. A small percentage of fingerprints will continue to develop after treatment and may take several weeks to develop fully [26, 75, 90, 101, 103]. An example of ninhydrin enhanced fingerprints is shown in figure 1-28.

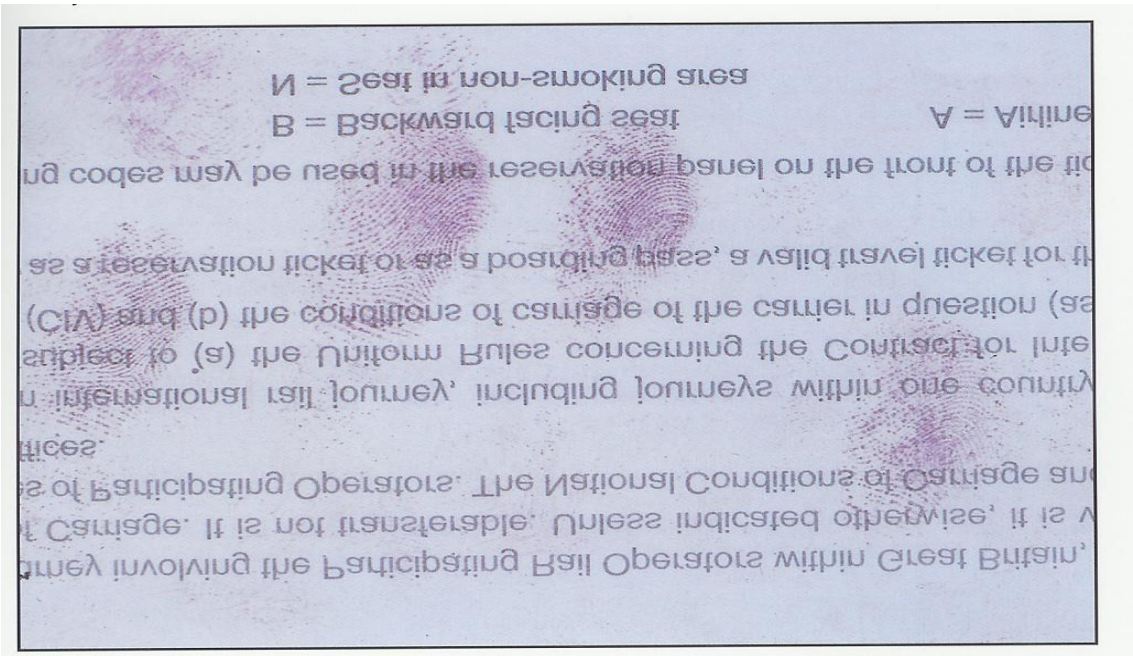


Figure 1-28 Ninhydrin enhanced fingerprints on a receipt [101]

**1,8-diazafluoren-9-one (DFO)**

The compound 1,8-diazafluoren-9-one (DFO) is an amino acid sensitive reagent that gives a pale pink/purple coloured reaction product (lighter than the colour obtained with ninhydrin) [75, 101]. This reaction is shown in figure 1-29.

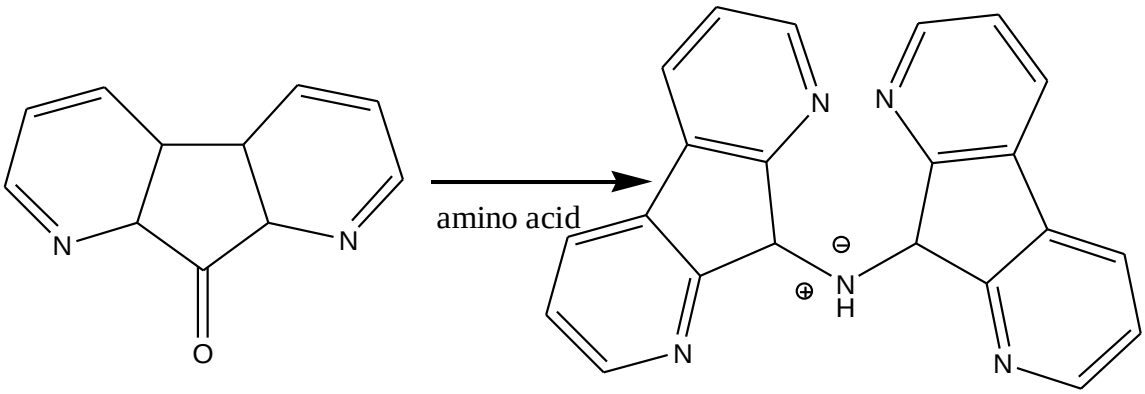



Figure 1-29 Suggested reaction between DFO and an amino acid to produce a luminescent product [106-107]

The advantage of the reagent is that developed fingerprints show a strong room temperature luminescence [75, 108]. Ideally after enhancement, the DFO stained fingerprints are viewed under a light of an appropriate colour [109] as the resultant product is highly fluorescent. Fluorescence is the property that some chemicals possess of being able to absorb light of a specific wavelength, and then convert and emit some of this absorbed energy into visible light of a different, longer wavelength. If a sequential treatment process is to be carried out, DFO should be used before ninhydrin [90]. DFO is more sensitive than ninhydrin and as a consequence, reacts with far smaller traces of amino acids than ninhydrin [108]. However, the use of ninhydrin is necessary after DFO enhancement as ninhydrin, apart from reacting with amino acids, is also capable of reacting with other substances such as milk, urine, and blood [90]. Therefore it has the capacity to develop some additional fingerprints if used after DFO. Figure 1-30 shows an enhanced fingerprint by DFO and appropriate lighting [101].


Figure 1-30 DFO and light enhanced fingerprints on a train ticket [101]

**Physical Developer (PD)**

Physical developer (PD) is an aqueous reagent containing silver ions, a ferrous/ferric redox (reduction/oxidation) system, a buffer (citric acid), and a cationic surfactant (generally *n*-dodecylamine acetate) which interacts with the sebaceous, insoluble deposits of the fingerprint [110]. As a consequence, PD can be effective even if the surface has been wet (unlike ninhydrin and DFO). A grey silver product is obtained when the ferrous ions reduce the silver ions to silver metal. Ferric ions are present to hold back the reaction and citric acid complexes with the ferric ions to maintain a low pH. The redox reaction is shown in figure 1-31 [75, 101, 103].

$$Fe^{2+}_{(aq)} + Ag^{+}_{(aq)} \rightleftharpoons Fe^{3+}_{(aq)} + Ag^{0}_{(s)}$$

Figure 1-31 The redox reaction that is the chemical basis for the PD process [75]

The action of the surfactant is to inhibit the premature deposition of silver metal by trapping randomly generated silver particles, as they are formed within positively charged micelles, as shown in figure 1-32 [75, 90].

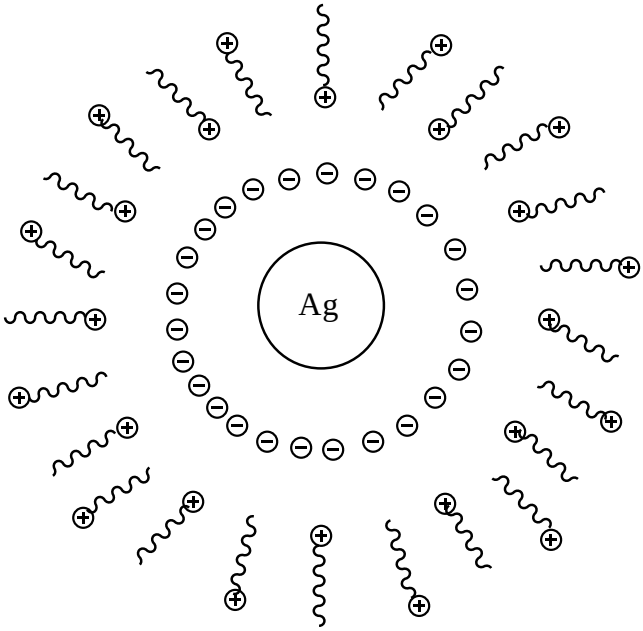



Figure 1-32 In the PD reagent, cationic surfactant molecules form positively charged micelles, each encapsulating a negatively charged silver colloid (silver colloid surrounded by negatively charged citrate ions). These positively charged micelles stabilise the reagent by repelling nearby silver ions and other micelles [75, 90]

The surface can be submerged in the PD solution and removed when sufficient development has occurred. This may take up to 1 hour and by comparison is more time consuming than ninhydrin or DFO. PD treatment can be used after ninhydrin and can detect latent fingerprints that ninhydrin may not have enhanced. PD developed fingerprints are shown in figure 1-33 [101].


Figure 1-33 PD enhanced fingerprints on an account book [101]

## 1.7.4.2 Detection Techniques for Non Porous Surfaces

### Fingerprint Powders

The simplest and most commonly used method for enhancing latent fingerprints on non porous surfaces is powder dusting. This is a *physical* method of enhancement that relies on the mechanical adherence of the fingerprint powder particles to the humid, sticky, or greasy substances in the fingerprint deposit – with fresh fingerprints it is the aqueous (eccrine) component and with older fingerprints it is the sebaceous component in the deposit which is targeted. Fingerprints which have been enhanced can also be easily lifted using tape or gel lifters. The general concept is to use a light

coloured powder (such as aluminium) on a dark surface and vice versa. Metallic oxides, sulphides, and carbonates have commonly been used as colorants, offering a wide range of possible colours for different backgrounds [75, 90]. Many types of powder have been compared to each other with the most effective powder on smooth surfaces being aluminium powder [111]. Figure 1-34 shows fingerprints enhanced using two different powders, selected to contrast with the background.

Figure 1-34 An aluminium powder lift (top) and black magnetic powder on a magazine (bottom) [101]

**Superglue**

Superglue vapour, ethyl cyanoacrylate, reacts with certain eccrine and sebaceous deposits of the latent fingerprint. The vapour selectively polymerises on the fingerprint to form polycyanoacrylate, which appears as a white deposit [75]. This reaction is shown in figure 1-35.

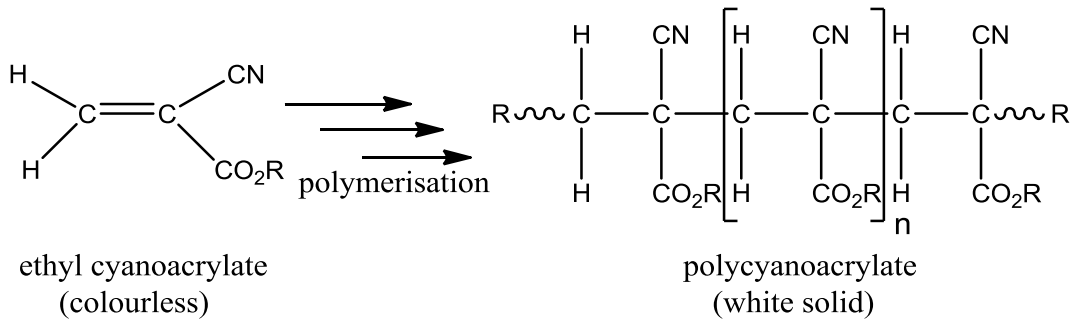



Figure 1-35 Ethyl cyanoacrylate polymerisation reaction that results in the formation of a hard white polymer known as polycyanoacrylate [75]

The item is placed in a cabinet where the optimum supergluing conditions of atmospheric pressure, normal room temperature, and 80% relative humidity can be maintained, and is then exposed to cyanoacrylate vapour. This technique develops fingerprints on a range of non porous surfaces and it is common to use a fluorescent dye (which is applied either by spraying or by full submersion within a dye tank after removal from the superglue cabinet) and subsequent fluorescence examination to visualise the maximum number of fingerprints. This means that the superglue can be viewed under an alternative light source, such as a Quaser (which can provide light of different wavelengths). This is helpful when looking at different types of dye, such as Basic Yellow 40 (BY40) and Basic Red 14 (BR14) which require different light wavelengths to fluoresce (figure 1-36) [26, 101, 103].

Figure 1-36 Basic red 14 dyed superglue on a tiled wall [101]

**Vacuum Metal Deposition (VMD)**

Vacuum metal deposition (VMD) can be used on smooth non porous surfaces such as polythene. The process starts with the evaporation of a small quantity of gold which deposits a very thin, discontinuous layer across the surface. The next stage is the deposition of a thicker layer of zinc. Where gold has diffused into the fingerprint deposits, they are not available for subsequent zinc deposition to occur. As a consequence the fingerprint remains undeveloped and can be clearly resolved against a continuous zinc background (figure 1-37) [26, 75, 90, 101, 112].

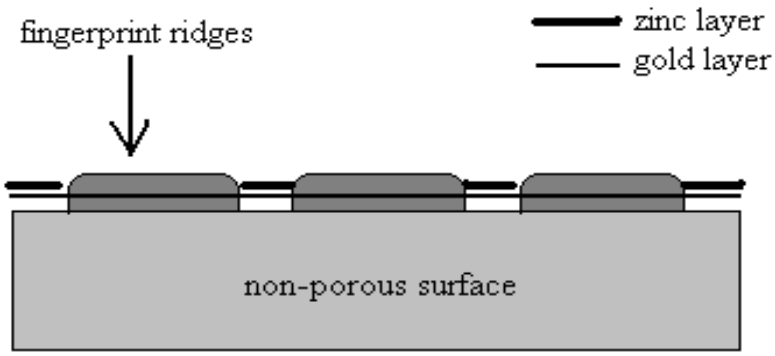



Figure 1-37 The principle of fingerprint development by VMD [75, 112]

An enhanced fingerprint by VMD is shown in figure 1-38 below.

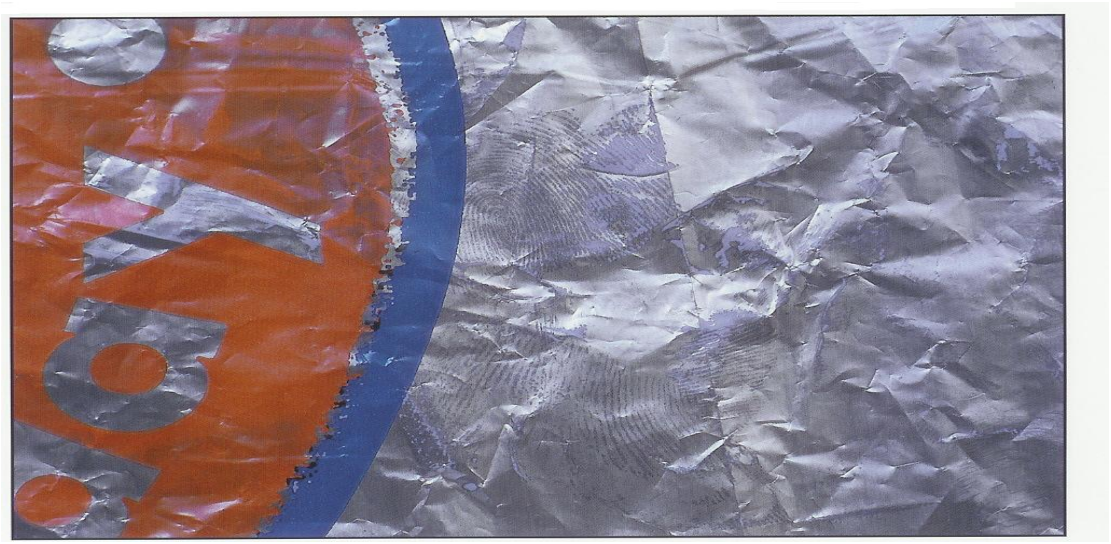


Figure 1-38 VMD on a high density polythene bag [101]

**Powder Suspension**

Powder suspension involves the suspension of a fingerprint powder in a detergent solution which can be used on non porous surfaces [113]. It is an extremely effective and easy to use fingerprint enhancement technique. It can also be used as an enhancement technique for adhesive tapes [114]. The powder and detergent are mixed together until a thin paint consistency is achieved. This is then brushed onto the item, left for 10-15 seconds then washed off. If using a black powder on a light surface then the enhanced fingerprints will be dark grey/black in appearance or a white powder on a dark surface will give light grey/ white fingerprints [75].

Powder suspension has many forms – there are the commercial products Wetwop™ (Armor Forensics, Jacksonville, Florida), Wet Powder™ (Evident, Union Hall, Virginia), which are both carbon based products, and Adhesive-Side Powder™ (Sirchie, Youngsville, North Carolina), which is iron based. HOSDB have also formulated their own powder suspension which is iron based [114] and they recommend iron based formulations for non porous surfaces [115]. A powder suspension enhanced fingerprint is given in figure 1-39.

Figure 1-39 Powder suspension enhanced fingerprint on uPVC

### 1.7.4.3 Detection Techniques for both Porous and Non Porous Surfaces

#### Fluorescence Examination

As previously mentioned, when an item has undergone superglue treatment, it is normally followed by a fluorescent dyeing process but fluorescence can also be used as a stand-alone technique to examine items for fingerprints prior to any chemical enhancement. Fluorescence is a form of luminescence which is mostly observed as an optical phenomenon in cold bodies. This occurs through the absorption of light of one wavelength (a photon) that results in the emission of light of a different wavelength (a different photon). This involves a change in energy and this energy difference is normally converted to heat or molecular vibrations. The energy in a single photon determines its wavelength. When a molecule absorbs a photon its energy is transferred to electrons that become excited. Electrons in this excited state are very unstable and rapidly lose this excess energy, emitting a photon with less energy, and a longer wavelength [116]. Enhancement of fingerprints using fluorescence can be achieved by either the latent fingerprint fluorescing, or the background surface fluorescing with the fingerprint absorbing light, leaving it black against the now fluorescing background. Examples of this are shown in figure 1-40 [90, 101, 103, 109].

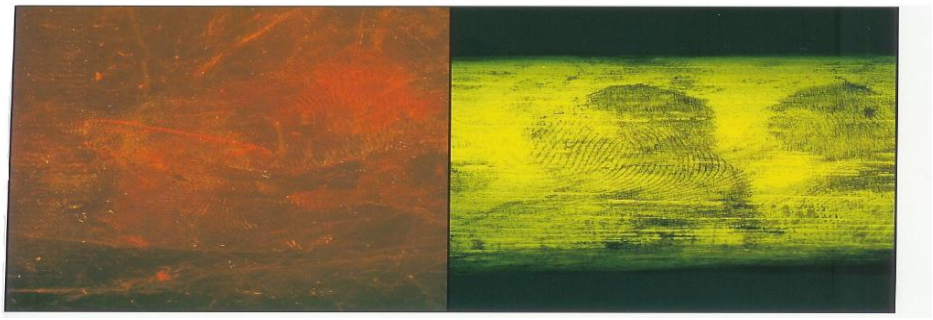


Figure 1-40 Fluorescent examination on a low density polythene bag (left) and blood on a wooden axe handle (right) [101]

### 1.7.5 Fingerprints at Fire Scenes

There is a general assumption that fires will result in the destruction of fragile evidence such as DNA and fingerprints [117]. Research [5, 118-119] has demonstrated that this is not always the case and that:

- Fingerprints are more likely to survive if the item has been exposed to temperatures less than 300˚C.
- If the surface of the item has been protected in some way from the direct effects of heat and smoke, survival rates for fingerprints are increased.
- The fingerprints are easier to develop on items which are relatively clean or lightly soot covered. Techniques are available for the removal of heavily sooted items but this lessens the chances of recovering a fingerprint.
- More fingerprints will be recovered on items, or regions of the item, which had remained dry during the fire suppression efforts [117-123].

Depending on the surface the latent fingerprint has been deposited on, the fingerprint may become visible during the fire. Fingerprints may still be recovered after fire suppression methods such as water, foam and dry powder [118, 124]. Using an effective soot removal technique, followed by an effective fingerprint enhancement technique, the probability of finding latent fingerprints are increased if they survive after the fire and the fire suppression methods [5, 117, 119, 125]. Various soot removal techniques have been reported in the literature [63-76].

### 1.7.5.1 Soot Removal Techniques

#### 1.7.5.1.1 Soot Removal Techniques for Both Porous and Non Porous Surfaces

**Brushing**

Where the recipient surfaces have experienced temperatures of approximately 100-200˚C, a simple form of soot removal is to simply brush off the excess soot from the item. Studies have shown that the soot particles can adhere to the fingerprint deposit and by brushing off the excess particles (using a very soft brush to minimise damage), the fingerprint can be enhanced. This can be effective for porous and non porous surfaces [117, 119-120, 126].

**Rubber**

A soft pencil eraser can be used to remove excess soot but it is reported that it should only be used on fingerprints which appear to be 'baked on' to the surface, otherwise the fingerprint may be rubbed away with the soot deposit [117, 119-121, 123].

**Liquid Latex**

A recent publication by the Metropolitan Police [127] discussed the use of liquid latex as a soot remover. The latex is applied to a heavily sooted surface and allowed to dry before peeling away from the surface. This method of soot removal is inexpensive, fast, and effective and is reported to not affect further forensic examinations.

#### 1.7.5.1.2 Soot Removal Techniques for Porous Surfaces

**Absorene**

Absorene is a paper and book cleaner. It is a pink dough which is worked in the hands until soft and pliable. It is then rubbed over the surface of the item which removes the soot. Studies have shown that Absorene is effective on paper and painted wood but

care must be taken when using it as the rubbing action may remove the fingerprints [117, 119-121, 123].

**Dry Cleaning Sponge**

A dry cleaning sponge works in a similar fashion to a normal pencil eraser. Again care must be taken not to 'rub off' the fingerprint during the removal process although it is a very quick technique to employ [117, 120].

**Clean Film**

Clean Film is a stonework and masonry cleaner which is applied as a paste and left to 'modify' for 24 hours. The tape is peeled off when it has a brown discolouration. Although it is an effective soot remover, it is time consuming to use and leaves a residue on the surface which could interfere with subsequent enhancement techniques [117, 120].

**1.7.5.1.3 Soot Removal Techniques for Non Porous Surfaces**

**Rinsing**

Rinsing the item by simply running it under water has also been previously studied. This method has found to remove much of the excess soot from the item but further brushing was required afterwards. The rinsing method removes the debris from the fingerprint without damaging the fingerprint itself [117, 119-120, 123, 126, 128-130].

**Lifting Tape**

On some non porous surfaces the fingerprint may be enhanced by a fine layer of soot, before excess soot becomes layered on top. Normal fingerprint lifting tape has been used to remove excess soot from such items. This technique can be used on its own or can be used after a water rinse which allows for a better contrast between the fingerprint and the background which simplifies the recording of the fingerprint. This

technique has been recommended for ceramic tiles, glass and metals in particular [117, 120-121, 129-130].

**Gel Lifters**

As an extension of normal fingerprint lifting tape, gel lifters were also investigated. This technique was found to be uneconomical as many gel lifters were required to lift a sufficient amount of soot. Although this method could be used, other methods for non porous surfaces were more effective [121, 123].

**Isomark**

Isomark is a commercial name for a polymeric casting material which can be used to cast fingerprints, toolmarks, etc. to produce three-dimensional casts. It has also shown to be effective at removing soot on surfaces such as steel, and melamine [119, 121, 123].

**0.4M Sodium Hydroxide**

Washing the non porous surface with a 0.4M solution of sodium hydroxide followed by lifting tape was demonstrated to be effective in removing some soot. It was most effective on heavily sooted aluminium and other techniques were better on different surfaces and different levels of sooting [117, 119-121, 123]. 5-Sulphosalicylic acid has been used for fingerprints in blood prior to using a sodium hydroxide wash to fix the fingerprint, however research showed that good quality fingerprints were recovered both with and without the fixative application [131].

**Organic Solvents (Xylene, Chloroform, and Phenol Chloroform)**

Using organic solvents such as xylene, chloroform, or phenol chloroform were not shown to be very successful. Only xylene removed the top layer of soot, requiring further treatment with another method, such as lifting tape [117, 120].

## 1.8 Aims and Objectives of this Research

There is at present little published information on the optimised methods for recovery of fingerprints and DNA from fire scenes. No recorded attempt has been made to carry out systematic research of the temperatures and timescales that both latent fingerprints and DNA can survive and the best technique(s) for the recovering of DNA from fingerprints is not reported in the literature. Similarly, the best technique(s) for the development of latent fingerprints post fire have neither been robustly or systematically established. With this in mind, there are four main aims of this PhD research project.

**1. Investigation of the survivability of DNA at elevated temperatures**

From a review of the literature no systematic research was identified which reported the recoverability and subsequent profiling of DNA exposed to elevated temperatures. This research aims to systematically investigate whether DNA deposited on paper (as the porous substrate) and glass (as the non porous substrate) could survive when exposed to elevated temperatures at various exposure times. Samples containing DNA were also placed into a simulated fire scene to investigate the survivability of the DNA under real fire conditions.

**2. Investigation of the survivability of latent fingerprints at elevated temperatures**

Although some studies have been carried out into fingerprint recovery from fire-affected items, few of these have used large numbers of fingerprints or subjected the fingerprints to repeated testing. The work of Deans [118] demonstrated that fingerprints could be repeatedly obtained from a wide variety of articles retrieved from fire scenes. Bradshaw *et al.*, [117] investigated the number of latent fingerprints that could survive high temperature exposure with a minimum of 1 hour exposure. However many fires are actually extinguished before this length of time. None of these studies have used sufficient numbers of fingerprints in repeated tests to enable a detailed statistical analysis of the results to be undertaken.

This research will aim to robustly establish whether various ages of fingerprints deposited on paper, uPVC, glass, and ceramic and exposed to various temperatures for various exposure times could survive and, if so, to determine the most effective technique for their enhancement. Experiments were devised to include a high number of repeat samples (70 fingerprints in each case) for each set of tests to generate the data sets. In total over 100,000 individual fingerprints were examined during the study. The results from these laboratory controlled experiments were applied to samples that were placed within a simulated fire scene in order to assess their effectiveness under actual fire conditions.

### 3. Investigation of the recoverability of DNA from fingerprints

Different recovery methods for retrieving DNA had been examined previously [41, 132] but a new recovery technique 'minitaping' [44] has not been compared to these existing methods. This work investigated minitaping as a DNA recovery technique for LT- DNA.

No research had been published addressing whether a relationship existed between the amount of DNA shed from the fingertips and the quality of the fingerprint that the same person would deposit. A study was undertaken to identify whether such a relationship existed.

### 4. Production of a best practice guide

The culmination of this research will be the production of a 'best practice' guide in collaboration with the Home Office Scientific Development Branch. This guide will be distributed to all police scene examination branches and fingerprint enhancement laboratories within the UK and will also be available on their website for download for the international community. This aim of this guide is to provide information to practitioners who regularly investigate fires regarding the conditions and techniques under which the recovery of fingerprints and DNA is viable and dispel common beliefs that fingerprints and DNA will not survive in such circumstances.

## 1.9 Thesis Outline

Chapter 1 of the thesis has outlined the theory behind fire development and the fireraising problem. It also discussed the theory behind fingerprints and DNA and included the relevant literature currently available related to the various aspects of this research establishing the need and purpose of the work.

Chapter 2 describes the experimental procedures undertaken with respect to the DNA analysis carried out during the study. This chapter also includes the results and discussion of the DNA analysis undertaken including the optimal LT-DNA recovery method, the quantity of DNA recovered from fingerprints, the relationship between fingerprint donation and DNA shedding, and the effects of heat exposure on LT-DNA.

Chapter 3 provides the experimental procedures, results, and discussion for the studies undertaken examining the effect of heat exposure on fingerprints deposited on paper. Three chemical enhancement techniques were tested for eccrine, sebaceous and natural fingerprint deposits. The results were explored statistically and the optimum enhancement techniques for eccrine, sebaceous and natural fingerprints were defined for paper at each temperature and exposure time. Natural fluorescence of fingerprints on paper exposed to certain temperature was observed and a study into this phenomenon is also discussed in this chapter.

Chapter 4 examines the effect of heat exposure on fingerprints deposited on three non porous surfaces – uPVC, glass, and ceramic. The surfaces were chosen so that high exposure temperatures could be achieved and because of their common occurrence in domestic and commercial properties. The experimental, results, and discussion are all presented in this chapter. Scanning electron microscopy was undertaken to examine the degradation of components of fingerprint deposits after heating. This is discussed within this chapter also.

Chapter 5 describes the experimental procedure for the DNA and fingerprint samples that were exposed to a real fire environment. This chapter also discusses in detail the

results obtained for the analysis of the recovered samples and compares these with the results of the laboratory controlled experiments in chapters 2, 3, and 4.

Chapter 6 provides a conclusion to the thesis which amalgamates and condenses the results of chapters 2, 3, 4, and 5. It provides detailed conclusions to the research. It also has a section which discusses further work that could be undertaken. The conclusion chapter is followed by the bibliography and appendix.

# CHAPTER 2: DNA

## 2.1 Experimental

### 2.1.1 Determination of the Most Efficient DNA Retrieval Method

It was necessary to investigate the DNA recovery technique which would yield the best DNA profiles. This was achieved by employing the technique of Lowe *et al.*, [66]. This involved a donor holding a glass boiling tube for 30 seconds, 15 minutes after they have washed their hands. The glass boiling tubes had been subjected to 254nm UV radiation of energy 999,900μJ/cm$^2$ within a CL-1000 Ultraviolet Crosslinker (UVP, San Gabriel, California) for 10 minutes to eradicate any DNA present before being placed into similarly radiated ziplock bags and given to the donor. A single donor was used for this study. The donor was free to carry on with everyday duties except from wearing laboratory gloves during this 15 minute period.

Three different types of swabs were used to retrieve the 'touch' DNA. These were Cotton Tip Woodstick swabs (Technical Service Consultants Ltd, Heywood, Lancashire, product code TS6-H), Sterile Omniswabs (Whatman, Brentford, Middlesex, cat no. WB100035), and Sterile Foam Tipped Applicators (Whatman, Brentford, Middlesex, cat no. WB100032).

The following swabbing techniques were investigated:

1. Dry swab only
2. Wet swab only
3. Wet and dry swab combination (both wet and dry swab extracted together)
4. Dry swab after wet swab (dry swab only extracted)

Swabs of the entire tube surface were taken and this was repeated three times on separate boiling tubes for each technique. Wet swabs were moistened using room temperature molecular grade distilled water. The whole swab head was then placed into 1.5mL tubes for DNA extraction to be undertaken.

The second retrieval technique to be compared with the swabbing techniques was minitaping. This method is in use within the Scottish Police Services Authority (SPSA) Glasgow[2] [44]. Minitapes are made from Sellotape® Double Sided Tape 15mm by 10m (Henkel Customer Adhesives, Winsford, Cheshire, code 484344) and A4 OHP Photocopier Film (Lloyd Paton Limited, Chorlton, Manchester). The film was firstly subjected to UV radiation as previously described before one side of the tape was stuck to the film. The film was cut using a previously UV irradiated guillotine (Stationary Box, Warrington, Cheshire) to 20mm by 70mm.

When taping the boiling tubes, the backing was removed from the other side of the tape and the adhesive side patted onto the boiling tubes until the whole surface had been taped. The backing was disposed of and the whole tape cut up into strips using UV irradiated scissors before being placed into 1.5mL tubes for the DNA extraction procedure.

#### 2.1.1.1 DNA Extraction

Following retrieval, the DNA was extracted using a QIAGEN QIAamp® DNA Micro Kit (QIAGEN, Crawley, West Sussex, cat no. 56304). QiaAmp is a silica-column based method, developed by Qiagen, which removes the cellular materials after the cells have been broken open (lysed). The DNA extract is then passed through a small column containing miniscule beads which the DNA will adhere to under certain conditions. All the other material is washed off the column. The conditions are then changed so that the DNA can now be released from the column (eluted). The DNA when it is isolated this way is of a high quality and in double-stranded form [27, 48, 133-134]. A negative control of either an unused swab or minitape was taken through the extraction protocol, and the extract processed through the amplification and capillary electrophoresis stages of the DNA analysis.

All solutions were provided with the kit apart from ethanol (Bamford Laboratories, Norden, Rochdale) and DNA storage buffer. The buffer was made up (into a 50x concentrated solution) by adding Tris Base (242g) (Promega Corporation, Madison,

[2] Previously known as Strathclyde Police Forensic Science Laboratory (SPFSL) until 1st April 2007

Wisconsin, product no. H5133), acetic acid (57.1mL) (BDH, Poole, Dorset), and made up to 1L with distilled water. This was diluted down to a 1x concentrated working solution. The following protocol [48] was undertaken inside a Captair Bio workstation with UV lamp (erlab, Salisbury, Wiltshire):

1. Remove whole swab tip (or cut full minitape into squares). Place into 1.5mL tube.
2. Add 20µL Proteinase K to the tube.
3. Add 400µL Buffer ATL. Vortex immediately for 10sec.
4. Incubate 56˚C for 1 hour, vortex for 10sec every 10 minutes.
5. Briefly centrifuge after incubation.
6. Add 400µL Buffer AL, spiked with 1µL of 1µg/µL carrier RNA in Buffer AE.
7. Vortex for 15sec.
8. Incubate 70˚C for 10 minutes, vortex for 10sec every 3 minutes.
9. Briefly centrifuge after incubation.
10. Add 120µL of ethanol. Vortex for 15sec and briefly centrifuge.
11. Transfer entire lysate to Qiagen spin column.
12. Spin for 1 minute at 8000rpm.
13. Add 500µL AW1 to the spin column.
14. Spin for 1 minute at 8000rpm.
15. Remove the spin column to a fresh tube.
16. Add 500µL AW2 to the spin column.
17. Spin for 1 minute at 8000rpm.
18. Remove the spin column to a fresh tube.
19. Spin for 3 minutes at 14000rpm, until membrane is dry.
20. Place spin column in fresh 1.5mL tube.
21. Add 30µL[3] DNA storage buffer to the spin column.
22. Incubate at room temperature for 1 minute.
23. Spin for 1 minute at 14000rpm.
24. Store extract collected in 1.5mL tube at 4˚C.[4]

[3] The 30µL extract volume was selected to allow three repeats of each extract to be amplified (using a volume of 4.5µL in the PCR) whilst also leaving extract left over for any additional reruns required
[4] AL, ATL, AW1, and AW2 are solutions provided with the QIAGEN QIAamp® DNA Micro Kit

### 2.1.1.2 DNA Amplification

Following extraction, the samples were amplified using an AMP*Fl*STR® SGM Plus® kit (Applied Biosystems[5], Warrington, Cheshire, cat no. 4307133). The kit amplifies at 10 different loci and also the Amelogenin locus to determine sex (XX is female, XY is male). These 10 loci are D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, and FGA. As mentioned previously in section 1.6.2.3, the oligonucleotide primers are bound to specific regions of DNA. These primers are fluorescently tagged in order to detect the STR alleles which are separated during capillary electrophoresis. In the SGM Plus® kit, the four dyes used are 5-FAM (blue), JOE (green), NED (yellow), and ROX (red) [135].

The samples were run on an ABI 2720 Thermal Cycler (Applied Biosystems, Warrington, Cheshire). The thermal cycling temperature programme is given in table 2-1.

Table 2-1 Thermal cycler programme for Qiagen extracted samples

| **Thermal Cycler Programme** | **AMP*Fl*STR® SGM Plus®** | **Cycles** |
|---|---|---|
| Hot-Start | 95ºC for 11min | 1 |
| Denaturing | 94ºC for 1min | }34 |
| Annealing | 59ºC for 1min | |
| Extension | 72ºC for 1min | |
| Adenylation | 60ºC for 60min | 1 |
| Cool-Down | 4ºC for 4min | 1 |

For PCR, 4.5µL of each sample was added to a 0.25mL tube which included 8µL of SGM Plus® master mix. This master mix was made up by adding Reaction Mix (5.16µL), Primer (2.58µL), and AmpliTaq Gold® (0.26µL) per sample. All DNA extracts were run in triplicate. A positive control of 0.10ng/µL human male genomic DNA in 0.05% $NaN_3$ and buffer (labeled as Control DNA 007 and supplied with the kit) was also run with each batch of samples. All the samples were centrifuged before being placed in the thermal cycler.

[5] Merged with Invitrogen on 21st November 2008 to form Life Technologies

The SGM Plus manual [135] recommends an overall reaction volume of 50μL. In this research, all amplifications were carried out using 12.5μL reaction volumes. This was due to financial restrictions on the research. To run at the recommended volume, only a quarter of the DNA profiles generated in this study would have been undertaken.

#### 2.1.1.3 Capillary Electrophoresis and DNA Profiling

Following amplification, the PCR products were separated by capillary electrophoresis using an ABI 3100-Avant Genetic Analyser (Applied Biosystems, Warrington, Cheshire). This is a four capillary electrophoresis system [24, 27] which interfaces with the software package (GeneMapper) on the computer system.

For electrophoresis, 2μL PCR product was added to each well containing Hi-Di™ formamide (15.52μL) (Applied Biosystems, Warrington, Cheshire) and GeneScan™ 500 Rox™ size standard (0.48μL) (Applied Biosystems, Warrington, Cheshire, cat no. 401734) on a 96-well half skirt PCR micro plate (Thistle Scientific, Uddingston, Glasgow). An allelic ladder was run with each batch of samples. This was supplied with the AMP*Fl*STR® SGM Plus® kit and contained amplified alleles for all the STR loci included in the kit. PCR products were denatured at 95ºC for 5 minutes on a Hybaid Thermal Reactor (Hybaid Limited, Teddington, Middlesex), before snap cooling on ice for 2 minutes.

The parameters for running SGM Plus® on the ABI 3100-Avant Genetic Analyser are given in table 2-2.

Table 2-2 ABI 3100-Avant Genetic Analyser run parameters for PCR amplified samples

| 3100 Run Module Parameters | AMP*FlSTR*® SGM Plus® |
|---|---|
| Run temperature | 60ºC |
| Cap fill volume | 184mL |
| Current tolerance | 100mA |
| Run current | 100mA |
| Voltage tolerance | 0.6kV |
| Prerun voltage | 15kV |
| Prerun time | 180secs |
| Injection voltage | 1kV |
| Injection time | 12secs |
| Run voltage | 15kV |
| Number of steps | 10 |
| Voltage step interval | 60secs |
| Data time delay | 1sec |
| Run time | 1500secs |

The separation was carried out using 3100 optimised polymer 4 (Applied Biosystems, Warrington, Cheshire) (POP-4) with CE Running Buffer (Web Scientific, Crewe, Cheshire).

The software used to analyse the raw data was GeneMapper® ID version 3.2 (Applied Biosystems, Warrington, Cheshire). The parameters used for analysing the data are given in table 2-3.

Table 2-3 GeneMapper® ID parameters for DNA analysis

| DNA Analysis Parameters | GeneMapper® ID v3.2 |
|---|---|
| Size Calling Method | Local Southern Method |
| Analysis Range | 2500-7000 base pairs |
| Peak Amplitude Detection Threshold | 75 RFU |
| Homozygous Minimum Peak Height | 150 RFU |
| Heterozygous Minimum Peak Height | 75 RFU |
| Maximum Expected Alleles | 2 |

If any DNA Control 007 PCR positive control samples did not give a full DNA profile once the electropherograms had been analysed, all the samples that had been amplified during that particular PCR run were prepared and amplified again until the positive control gave a full DNA profile.

### 2.1.2 Quantification of DNA from Fingerprints

It was important to establish whether a relationship existed between the mass of DNA that could be retrieved from fingerprints and the success in obtaining a DNA profile. Therefore, using four donors (two female, two male) a series of fingerprints were deposited onto UV irradiated glass. Glass was chosen as the deposition substrate as the fingerprint deposits were visible to the eye on the glass without using any fingerprint enhancement method.

Each donor provided fingerprints which would be swabbed. Fingerprints were deposited only when a minimum of 30 minutes had passed since handwashing or gloves had been worn. Donors deposited one fingerprint per finger. After deposition, a specific number of fingerprints were swabbed (from one fingerprint only to ten fingerprints in total) using the wet/dry swab combination swabbing technique, selected due to the results obtained from the experiment undertaken in section 2.1.1. For example, one fingerprint was swabbed using a single wet swab followed by a single dry swab and these combined for the DNA extraction procedure, then two fingerprints were swabbed using a single wet swab followed by a single dry swab and these combined for the DNA extraction procedure, and so on.

These swabs were then extracted, amplified (three replicates), and profiled using the procedures in sections 2.1.1.1 – 2.1.1.3. This allowed the number of reportable alleles (alleles which had been duplicated within the triplicate amplification electropherograms which matched the donor's DNA profile) to be determined. A maximum of 22 reportable alleles would result in a 100% DNA profile.

To determine the amount of DNA in each of the fingerprint extracts, a qPCR reaction was performed on the extracted samples. Each sample was amplified in duplicate. This was achieved using the Plexor® HY system (Promega Corporation, Madison, Wisconsin, product no. DC1001). The qPCR instrument used was the Stratagene Mx3005P™ (Stratagene, La Jolla, California, product no. 401458). The thermal cycling programme is given in table 2-4.

Table 2-4 qPCR thermal cycling programme for the Qiagen extracted samples

| Step | Temperature | Time | No. of Cycles |
|---|---|---|---|
| Initial denaturation | 95˚C | 2 min | 1 |
| Denaturation | 95˚C | 5 sec | 38 |
| Annealing and extension | 60˚C | 40 sec | |
| Melt temperature curve | 65˚C initial temperature with 0.6˚C increase each cycle, 40 sec hold each cycle | | 48 |

For qPCR, 2µL of sample extract was added to 0.25mL PCR plate which included Plexor® HY 2X Master Mix (10µL), Amplification Grade Water (7µL), and Plexor® HY 20X Primer/IPC Mix (1µL) supplied with the kit. A duplicate series of DNA dilution standards were also prepared (from 50ng/µL to 0.0032ng/µL) according to the Plexor® HY Technical Manual in order to generate a standard curve that can be used to identify the concentration of the unknown samples [136].

The quantitative analysis was undertaken using Plexor® Analysis Software, as the Plexor® HY Technical Manual [136] stated that analysis was to be exported to this program and not for the results to be analysed on the software associated with the Stratagene Mx3005P™. The analysis parameters used for quantifying the DNA are given in table 2-5.

Table 2-5 Plexor® Analysis Software run parameters for quantifying DNA

| DNA Quantification Parameters | Plexor® Analysis Software |
|---|---|
| Autosomal STR Dye | FAM |
| Y-STR Dye | CO560 |
| Internal PCR Control (IPC) Dye | CR610 |
| IPC Ct Threshold | 2 cycles |
| Preferred/Target DNA Quantity | 0.75ng/reaction |
| Minimum Input Volume | 0.25µL/reaction |
| Maximum Input Volume | 0.5µL/reaction |
| Minimum Quantity of Sample DNA | 0.10µg/reaction |
| Maximum Quantity of Sample DNA | 1.00µg/reaction |
| Concentration to which Over Concentrated Samples should be Diluted | 0.300ng/µL |

The analysis method used for quantifying this data was a standard curve that is prepared from a dilution series of template DNA of known concentration. Ideally, a standard curve will consist of at least 4 points, and each concentration should be run

at least in duplicate (the more points the better) [137]. This study conformed to these guidelines from the manual. An average mass of DNA was calculated from the duplicated samples.

### 2.1.3 Examining the Relationship between Fingerprint Donation and DNA Shedding

DNA shedding is a term put forward by Lowe *et al.,* [66] as an indication of the propensity of donors to deposit DNA. Eight donors participated in this study. To assess DNA shedding, 'touch' DNA tubes were prepared as indicated in section 2.1.1.

The experiment described in section 2.1.1 was undertaken before this experiment in order to establish the retrieval method which should be used for recovering the DNA from the tubes. The full results are given in section 2.2.1, but for the purposes of this experiment, the results indicated that the optimum DNA retrieval method was to use cotton swabs and a wet/dry combination swabbing technique. This was employed in this investigation and all further DNA recoveries. The DNA extraction, amplification, and electrophoresis are described in sections 2.1.1.1 – 2.1.1.3.

To assess fingerprint donation, the 8 donors were given 10 depletion grids on glass (example shown in figure 2-1), previously cleaned with detergent and ethanol, to donate fingerprints (one for each finger). The same finger was used for each depletion grid moving systematically through the grid. Seven depleted fingerprints were made on each grid for each finger in turn such that the amount of material deposited should decrease along the grid.

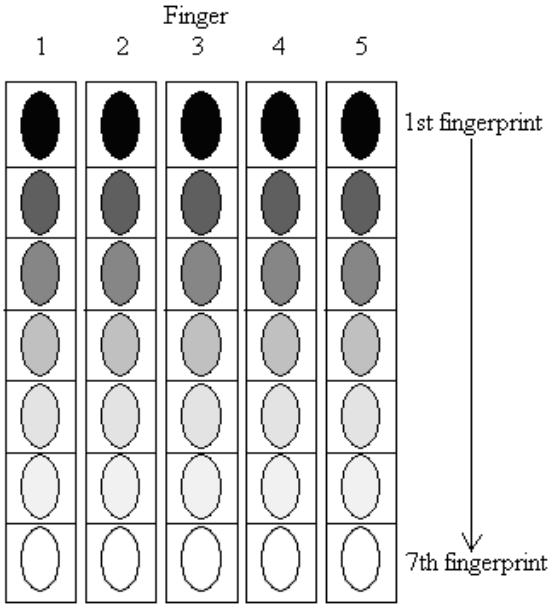



Figure 2-1 Example of a depletion grid

The fingerprints must be assessed in order for the success of visualising a fingerprint from that particular donor to be compared. The best way of assessing a fingerprint is a matter for debate but the assessment should bear close relation to the needs of a fingerprint examiner, which is primarily to see unambiguous ridge detail. In this case, the assessment method employed estimated the proportion of the developed fingerprint's clear ridge detail, with a score assigned to each fingerprint of 0 to 4. This was a much quicker and simpler method for a non-expert to use rather than the counting of minutiae. Fingerprints were enhanced using aluminium powder (Tetra Scene of Crime, Billericay, Essex) and given a score according to the amount of enhanced ridge detail. The scoring system is shown in table 2-6 below:

Table 2-6 Fingerprint scoring system

| Score | Level of Detail |
|---|---|
| 0 | No evidence of print |
| 1 | 0 -1/3 ridge detail |
| 2 | 1/3 – 2/3 ridge detail |
| 3 | 2/3 – 1 ridge detail |
| 4 | Ridge detail over every point of contact visible |

Finally, a resultant score was calculated by taking an average score for each finger's depletion series. This procedure follows the recommended methods suggested by the

Home Office Scientific Development Branch (HOSDB) [138]. Obtaining a score of 3 or 4 would result in a potentially identifiable fingerprint.

### 2.1.4 Effects of Heat Exposure on DNA

Tests were conducted to establish the range of temperatures and exposure times at which DNA was still recoverable. The effect on the resulting DNA profile obtained was also examined. DNA from a single donor was used in this study. Anti-contamination procedures were taken at all times; wearing gloves and a facemask. No non-heated samples were analysed in this experiment. This was due to the financial constraints of the research but also that it is well known that DNA can be recovered and profiled successfully from paper and glass at room temperature [139-140].

#### 2.1.4.1 Substrate – Paper

Paper was taken from an unopened packet of white 80g/$m^2$ A4 recycled paper manufactured by Niceday (Andover, Hampshire). A buccal swab taken from the donor, whose DNA profile was known, was rubbed on the paper.

These DNA samples were subjected to different temperatures for different periods of time, and then recovered using the wet/dry combination swabbing technique. A summary of the experiments is given in table 2-7. Three repeats were undertaken for each temperature and time combination.

Table 2-7 Summary of exposure to heat experiments

| Substrate | Temperature (˚C) | Exposure Time (min) |
|---|---|---|
| Paper | 50 | 10, 20, 40, 80, 160, 320 |
| | 100 | |
| | 150 | |
| | 200 | |

The recovered DNA was then extracted from the saliva, amplified (in triplicate), and then separated using the techniques specified in sections 2.1.1.1 – 2.1.1.3.

### 2.1.4.2 Substrate - Glass

Glass microscope slides were taken from an unopened box, manufactured by VWR (Lutterworth, Leicestershire, product code 631-0117). The glass was decontaminated for DNA by using DNA Away (Molecular BioProducts, San Diego, California). A buccal swab taken from the donor was rubbed on the test slides.

These DNA samples were subjected to different temperatures for different periods of time, and then recovered using the wet/dry combination swabbing technique. A summary of the experiments is given in table 2-8. Three repeats were undertaken for each temperature and time combination.

Table 2-8 Summary of exposure to heat experiments

| Substrate | Temperature (˚C) | Exposure Time (min) |
|---|---|---|
| Glass | 50 | 10, 20, 40, 80, 160, 320 |
| | 100 | |
| | 150 | |
| | 200 | |
| | 250 | |
| | 300 | |
| | 350 | |
| | 400 | |
| | 450 | |
| | 500 | |

The recovered DNA was then extracted from the saliva, amplified (in triplicate), and then separated using the techniques specified in sections 2.1.1.1 – 2.1.1.3.

## 2.2 Results and Discussion

### 2.2.1 Determination of the Most Efficient DNA Retrieval Method

Section 2.1.1 described that three different types of swab (with four different swabbing techniques) were compared with minitapes in order to determine the DNA retrieval method that would give the greatest percentage profile (% profile) which matched the donor's DNA profile. The DNA profiles generated were reported

according to Gill *et al.*, interpretation guidelines for DNA less than 100pg [65]. These interpretation guidelines are:

- Duplication of every allele is demonstrated before reporting
- If the negative controls show duplicated alleles that correspond to those in the samples, the corresponding sample alleles are not reported
- If alleles are found in the negatives, which do not correspond to those in the samples, then this is inconsequential.

When presenting these results, as the donor's profile was known, the DNA profiles from the different retrieval method samples were compared to the known profile. As such, results were reported as a percentage of the number of alleles which matched the donors DNA profile, with 22 alleles giving a 100% profile (homozygote peaks were counted twice). The percentage DNA profile was used to assess how much of the donor's profile could be recovered using the various recovery techniques. The technique which provided the highest percentage profile would be deemed as the most efficient recovery method. This result is an average of three repeats for each of the retrieval methods. The results are shown in figure 2-2.

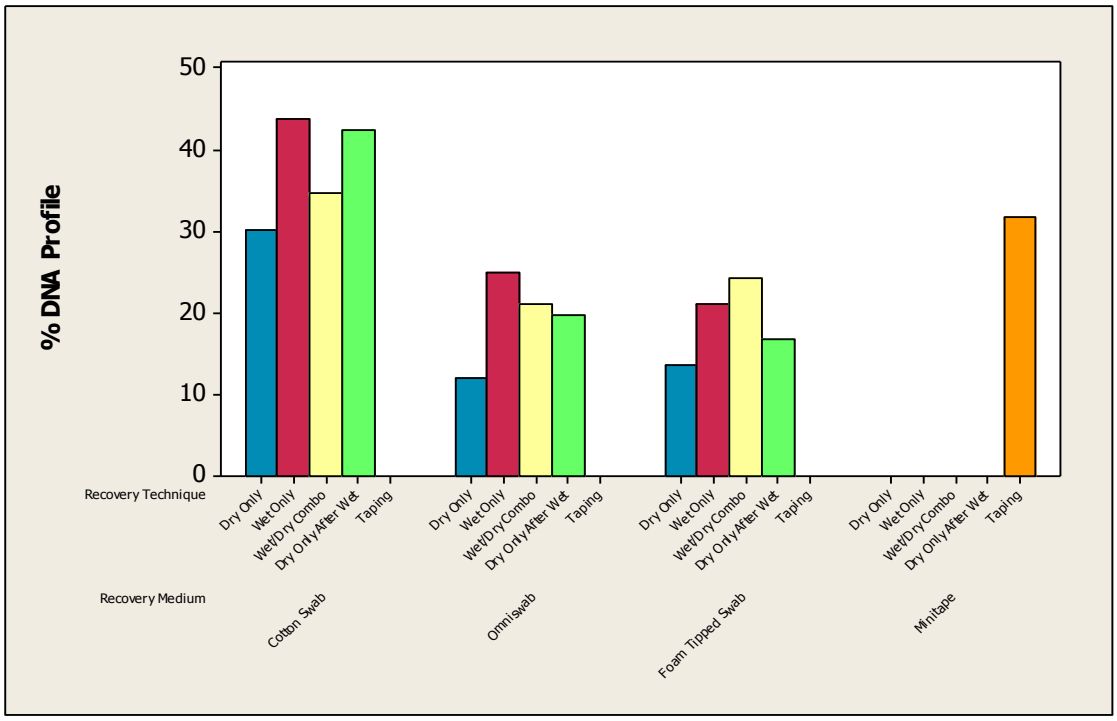



Figure 2-2 Comparison of DNA retrieval methods

The results indicate that the swab which gave the greatest matching percentage profile was the ‘wet only’ cotton swab technique. Although this did give the largest average, the technique which has was chosen for use in all subsequent DNA experiments was the ‘wet/dry combination’ swabbing technique. This decision was made based on the ‘dry only after wet swab’ results. This swab sample was collected from the surface of the same tube as the ‘wet only’ technique swab, after it had been used. The wet and dry swabs were extracted, amplified, and profiled separately, generating two electropherograms (an example is given in figure 2-3, which is the ‘wet only’ swab, and figure 2-4, which was the ‘dry only after wet swab’ which was the dry swab used after the wet swab on the same tube but profiled separately). This indicates that the entire amount of DNA is not being collected by the ‘wet only’ swab (since DNA was also recovered by the subsequently applied ‘dry swab’ as the electropherogram in figure 2-4 shows). Since DNA was being left behind after the ‘wet swab’ and was collected by the application of a ‘dry swab’ the combination of these two techniques was deemed the most efficient.

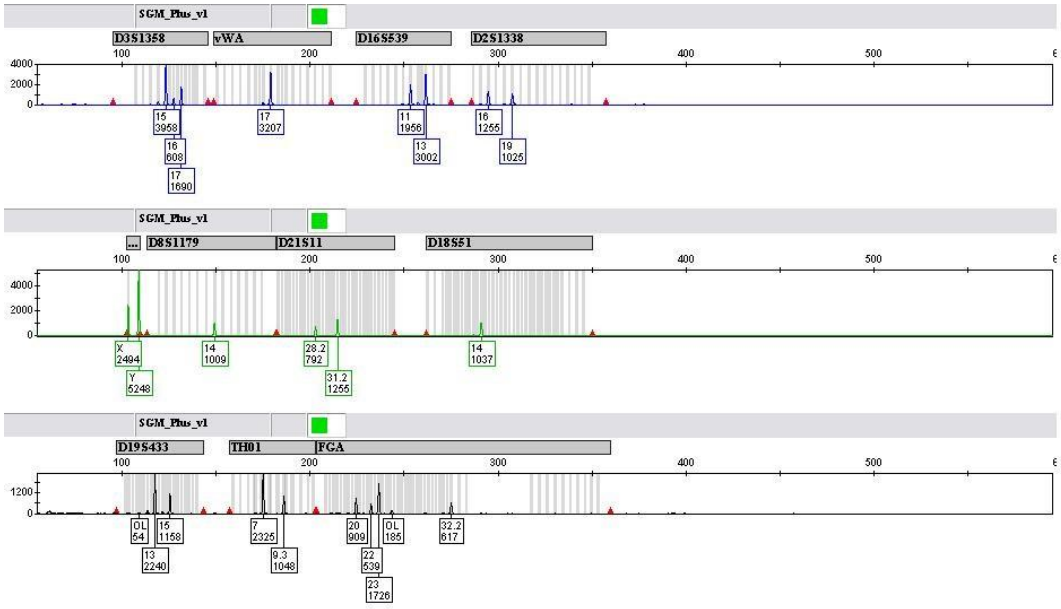



Figure 2-3 Electropherogram of ‘wet cotton swab’ sample

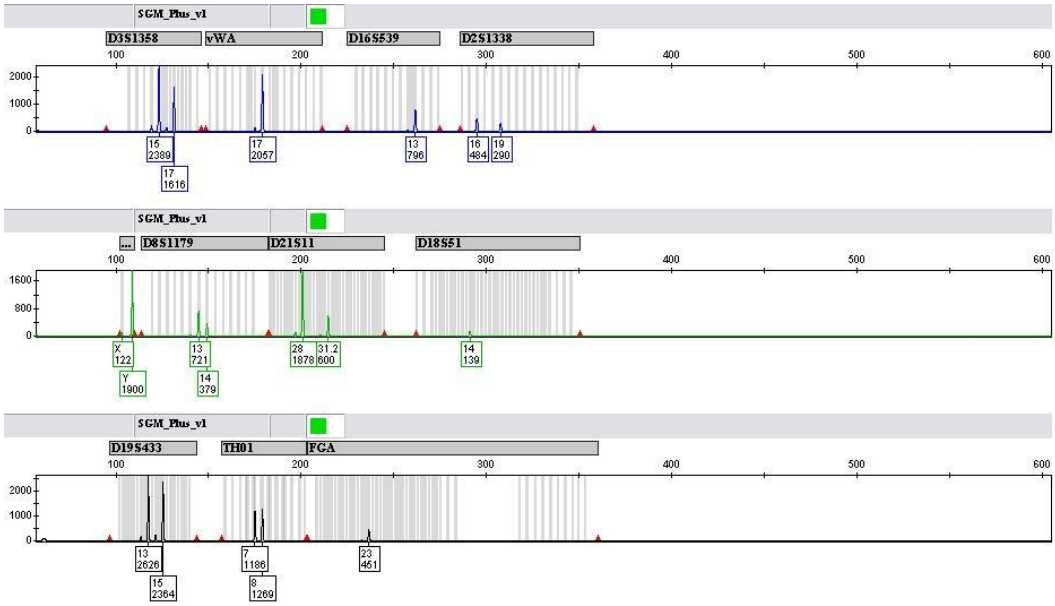



Figure 2-4 Electropherogram of 'dry only after wet swab' sample taken from the same tube

These results have also been found by Sweet *et al.,* [41] and van Oorschot *et al.,* [132] with both papers indicating that the 'double swab' technique, as they refer to it, is an improvement over single swabbing techniques.

## 2.2.2 Quantification of DNA from Fingerprints

As discussed in section 2.1.2, the number of reportable alleles from each fingerprint sample was investigated. The DNA profiles generated were reported according to Gill *et al.*, interpretation guidelines for DNA less than 100pg [65].

When presenting these results, as each donor's profile was known, the DNA profiles from the fingerprint samples were compared to the known profile. As such, results were reported as the number of alleles which matched the donor's DNA profile, with 22 alleles resulting in a 100% profile. This was to give an indication of how likely the possible identification of a person may be if their fingerprint was recovered.

An electropherogram of one of donor 1's fingerprint samples is given in figure 2-5. The electropherogram shows a full genetic complement for donor 1. It also shows pull up in all three dyes at the X chromosome (indicated by the pink stripe) showing that there was too much template DNA in the initial extract.

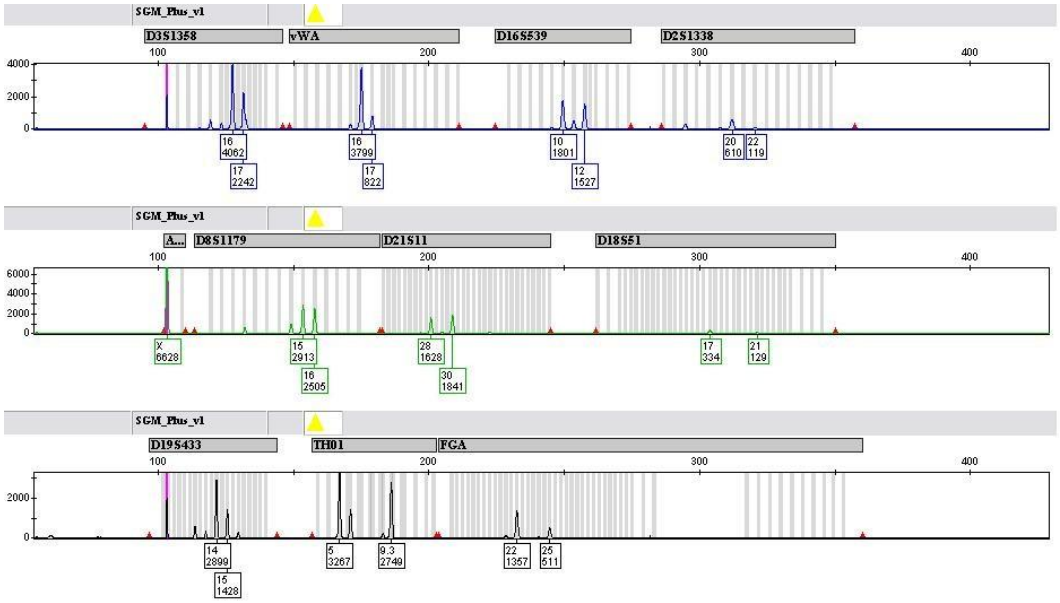



Figure 2-5 Electropherogram of one of donor 1's 10 fingerprint samples

Other alleles are also present in the electropherogram, present due to contamination. At locus D19, extra peaks are observed and are above the 75 RFU minimum peak height used for designating alleles, however their labels have been removed in order to show only the donor's profile in the figure.

The graph indicating the number of reportable alleles obtained against number of fingerprints is shown in the figure 2-6.

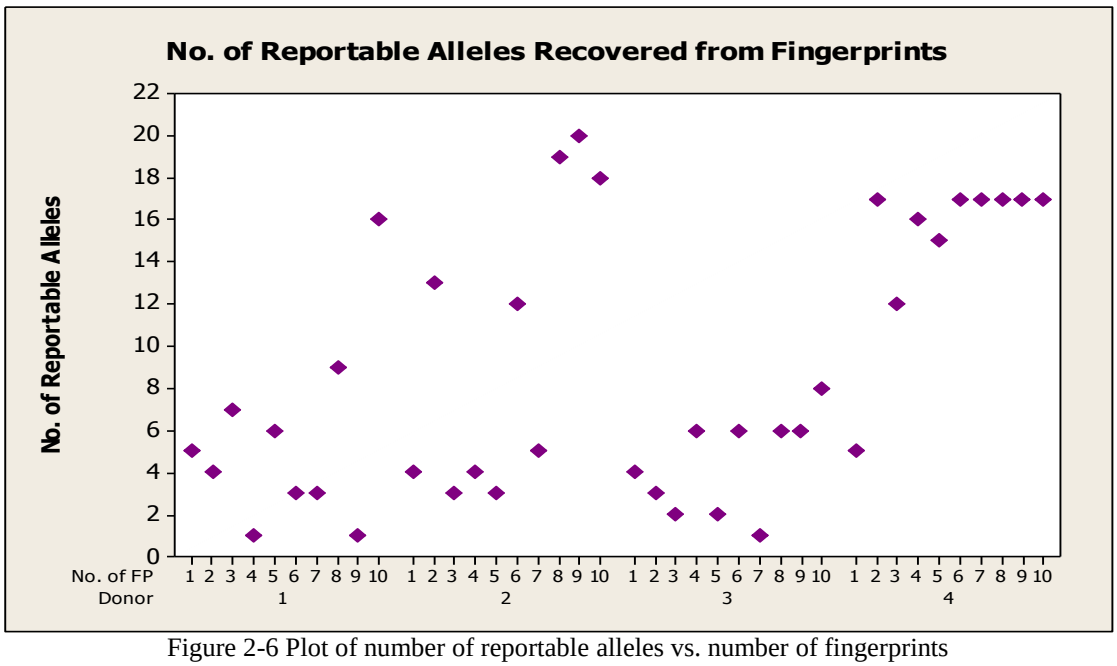



Figure 2-6 Plot of number of reportable alleles vs. number of fingerprints

Figure 2-6 indicates that as the total number of fingerprints swabbed increased, it does not always result in an increase in the number of reportable alleles obtained. It also shows that the number of alleles obtained is variable between each donor; for example, donor 1 has one allele matching from nine fingerprints rather than five alleles matching from one fingerprint. The difference in shedability between each donor can be seen in the graph, as the mass of DNA is variable for each donor and for each sum of fingerprints.

These variations were investigated further using Minitab. The distribution of the data was examined using the Kolmogorov-Smirnov normality test for the number of reportable alleles for each donor. The number of alleles is normally distributed for each donor as P values of 0.139, >0.150, >0.150, and >0.150 for donor's 1, 2, 3, and 4 respectively were higher than the 0.05 test statistic. Therefore, a balanced ANOVA test was conducted which investigates the factors which were varied during the experiment in order to determine their effect on the response variable. Various calculations are required to generate the critical (P) value which indicated whether there were significant differences between the variables under investigation.

Firstly, the sum of squares (SS in the table) is calculated and provides a measure of the variability in the data. The mean squares (MS in the table) are also calculated. This is the estimate of the variance in the data left over after the differences in the mean have been accounted for, and this is calculated by dividing the sum of squares by the degrees of freedom (DF in the table). Degrees of freedom are the number of levels in each factor minus 1. The F value in the table is the comparison of the mean squares for each effect to the mean square error value. This value is used to determine which of the effects in the model are statistically significant, by generating the P value.

This P value is compared to the $\alpha$ value of 0.05, with $P < 0.05$ indicating the variable has a significant effect on the response, or $P > 0.05$ indicating no significant effect on the response [141]. Table 2-9 is the ANOVA table generated by Minitab.

Table 2-9 ANOVA table for number of alleles

Analysis of Variance for No. of Alleles

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Donor | 3 | 703.70 | 234.57 | 14.89 | 0.000 |
| No. of FP | 9 | 390.50 | 43.39 | 2.75 | 0.020 |
| Error | 27 | 425.30 | 15.75 | | |
| Total | 39 | 1519.50 | | | |

Table 2-9 shows that there is a significant difference between each donor, and when comparing the number of fingerprints swabbed. This shows that, in terms of the number of alleles in the profiles, there is a significant difference between each donor and a significant difference between the numbers of fingerprints from which the DNA was recovered. The amount of DNA deposited was also investigated and shown graphically in figure 2-7.

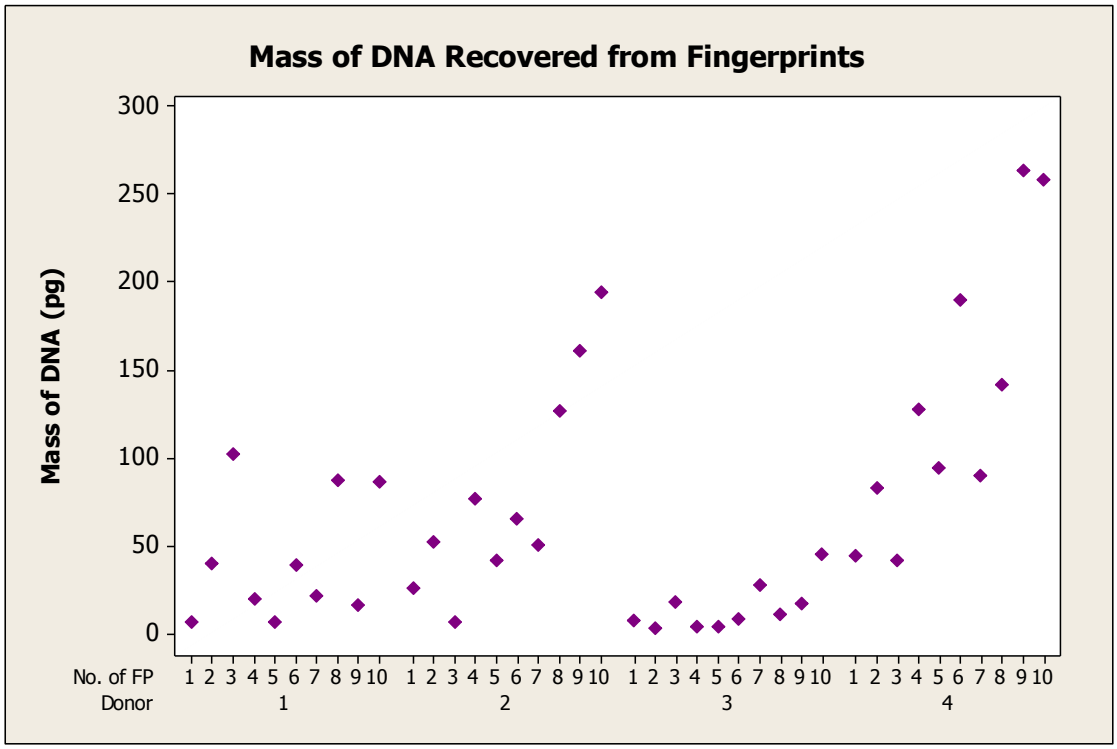



Figure 2-7 Plot of mass of DNA recovered from fingerprints

Figure 2-7 indicates that there is a difference in the mass of DNA retrieved per donor and also per number of fingerprints. For example, donor 3 consistently donated less than 50pg of DNA no matter how many fingerprints they had deposited, whereas donor 4's minimum deposits are around 50pg. This variability could be due to the variation in fingerprint deposition. Fingerprint deposition is dependent on factors such as environmental conditions, the type of surface on which fingerprints are deposited,

the ability of the donor to deposit fingerprints, contact time, and force of contact with the object, to name a few, and will all contribute to the resultant deposited fingerprint [142]. The shedding ability of donors (discussed more in the following section) may also be an influence on the results. This is similar to the findings when comparing number of alleles to number of fingerprints that there is a significant difference between donors and number of fingerprints. This is confirmed by the following ANOVA table (table 2-10).

Table 2-10 ANOVA table for mass of DNA

Analysis of Variance for Mass of DNA

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Donor | 3 | 79375 | 26458 | 14.37 | 0.000 |
| No. of FP | 9 | 55455 | 6162 | 3.35 | 0.007 |
| Error | 27 | 49703 | 1841 | | |
| Total | 39 | 184533 | | | |

The table shows that there is a significant difference between each donor and between numbers of fingerprints in terms of mass of DNA recovered.

In order to assess the possible relationship between the number of reportable alleles obtained and the mass of DNA recovered, these results where plotted on figure 2-8.

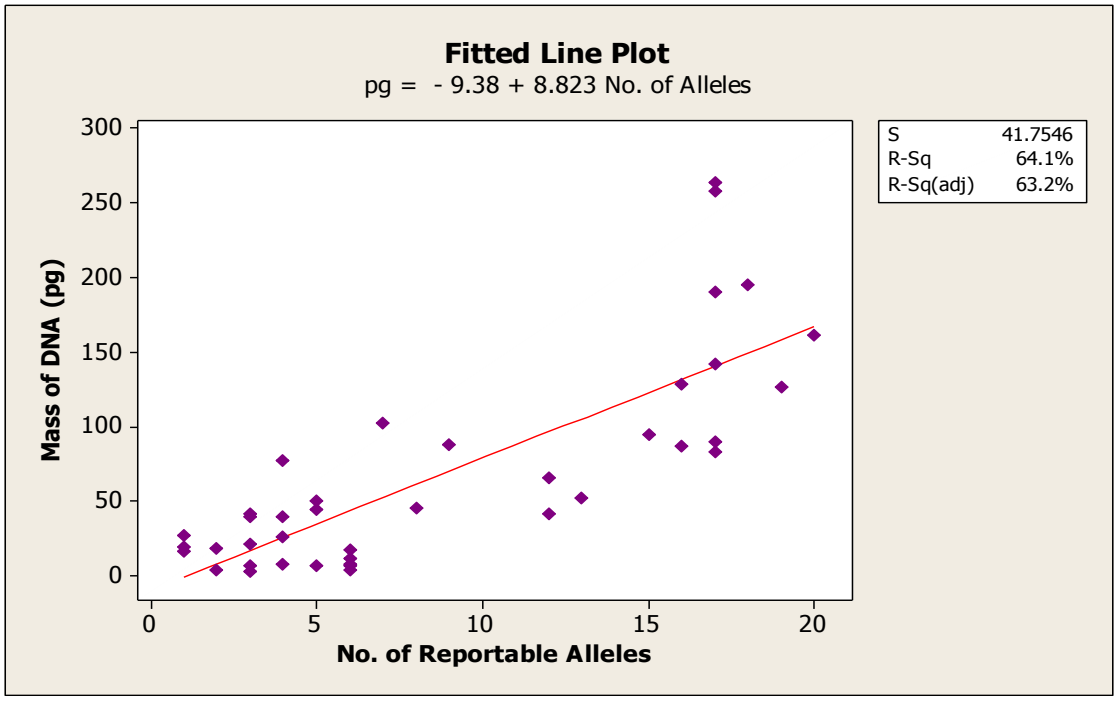



Figure 2-8 Plot of number of reportable alleles vs. mass of DNA

Figure 2-8 shows the linear fitted line plot between the number of reportable alleles obtained and mass of DNA retrieved. The $R^2$ value is a measure of the linearity of the fitted line. Ideally, $R^2$ values should be a minimum of 95% but this is dependent on the purpose of the fitted line. The $R^2$ value generated for figure 2-5 is 64.1%. This is not ideal and is reflected in the graph by the number of samples which deviated from the fitted line. This could be explained by the variability in the nature of fingerprints. Also, for the alleles to be reported, they must be duplicated within the amplification step of the DNA analysis, and that allele must not be duplicated in the amplification in the negative control sample (which there was in this study). If this occurs then the allele must not be reported. For example, in figure 2-9, it can be seen that a 100% DNA profile has been produced from the DNA analysis of one of donor three's ten fingerprint samples. However, this could only be reported as 11 alleles as in the negative control for this donor, 11 alleles were duplicated in the triplicate amplifications of the negative control.

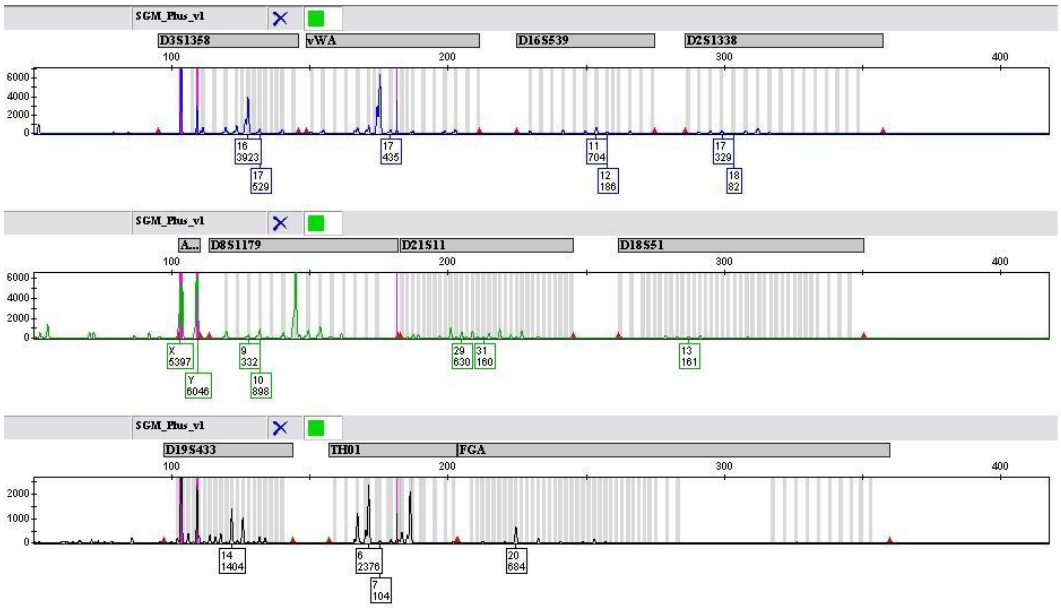



Figure 2-9 Electropherogram of one of donor 3's 10 fingerprint samples

The interpretation of this electropherogram without knowing the DNA profile of the donor would be difficult. This is particularly clear at locus D8S1179, where the donor's alleles of 9, 10 are above the 75 RFU minimum peak height designation but neither of them are the largest peaks at this particular site.

The nature of low level DNA has been discussed by Gill *et al.*, [65], Whitaker *et al.*, [64], and Gill [63]. They state that the analysis of LT-DNA (which is generally termed as <200pg, of which 95% of the samples in this study are) suffers from many disadvantages that are primarily derived from stochastic variation. These unavoidable consequences are:

1. Allele drop out – one allele of a heterozygote locus can be preferentially amplified
2. Stutters – may be preferentially amplified (sometimes known as false alleles)
3. Sporadic contamination – method is prone to amplifying alleles that are not associated with the sample.

All of these consequences were experienced when interpreting the resultant DNA profiles generated in this study. Also, Gill *et al.*, states that 'it is unlikely that a full genetic complement is present below 25-50 pg' [65] which was confirmed by this study. Although Gill *et al*., also state that a full genetic complement (22 alleles in this case) should be seen with a mass of DNA of 250pg, and this was not seen in this study. This may be due to the low volume of the SGM Plus kit used in this research. Ideally, a 50µL resultant volume of SGM Plus master mix and DNA extract should be used in the amplification step of the DNA analysis but due to financial constraints, only 12.5µL was used to replicate the DNA. This could account for the lack of profile at 250pg. It could also certainly be a factor in the resultant profiles for all DNA experiments undertaken in this research. What the results do show is that with a 12.5µL amplification reaction volume partial DNA profiles have been found, indicating that it is possible to obtain at least a partial DNA profile from a single fingerprint.

The overall results of these experiments (using a 12.5µL amplification reaction volume) suggest that the mass of DNA present in up to ten fingerprints may not provide a full DNA profile from the donor.

### 2.2.3 Examining the Relationship between Fingerprint Donation and DNA Shedding

Section 2.1.3 described the experiment conducted to determine whether there was any relationship between the amount of DNA shed by an individual and the level of ridge detail in a fingerprint deposited by that individual.

The fingerprints were analysed first. Each donor deposited 70 fingerprints over ten sets of depletion grids (see figure 2-1). Table 2-11 shows the score for each donor's finger, achieved by calculating an average score for each finger's depletion series.

Table 2-11 Fingerprint score

| | | Finger Score | | | | |
|---|---|---|---|---|---|---|
| | **Donor** | **Thumb** | **Index** | **Middle** | **Ring** | **Little** |
| **Left Hand** | 1 | 4 | 3.4 | 3.7 | 3.6 | 2.9 |
| | 2 | 4 | 3.9 | 3.9 | 3.9 | 3.7 |
| | 3 | 4 | 3 | 3 | 3.1 | 3 |
| | 4 | 4 | 4 | 4 | 3.9 | 3.4 |
| | 5 | 4 | 3.4 | 3 | 3 | 2.7 |
| | 6 | 3 | 2.4 | 2.3 | 2.9 | 3 |
| | 7 | 3.1 | 2.6 | 3 | 2 | 3 |
| | 8 | 3.6 | 3 | 3 | 3.6 | 3.4 |
| **Right Hand** | 1 | 3 | 3.7 | 3.6 | 3 | 3 |
| | 2 | 4 | 4 | 3.7 | 4 | 3.9 |
| | 3 | 3.3 | 3.3 | 3.7 | 3.3 | 4 |
| | 4 | 4 | 4 | 3.9 | 3.4 | 3.7 |
| | 5 | 3.4 | 3 | 3.1 | 4 | 3.1 |
| | 6 | 3 | 3 | 3 | 3 | 3 |
| | 7 | 2.9 | 3 | 2.9 | 3 | 3 |
| | 8 | 4 | 3.6 | 3.4 | 4 | 3.1 |

The fingerprint scores obtained were entered into Minitab for statistical analysis to be undertaken. The Kolmogorov-Smirnov normality test was undertaken and found that the data was not normally distributed. A Kruskal-Wallis non parametric test was undertaken which generated a P value of 0.000. As this is less than the α value of 0.05, there is a statistical difference between the donors. Kruskal-Wallis tests were undertaken for each donor to assess whether there was a statistical difference between the fingerprint scores recorded for each hand. Table 2-12 is the P values generated by Minitab for each donor.

Table 2-12 Table of P values for each donor in terms of fingerprint score

| Donor | Kruskal-Wallis P Value Per Hand |
|---|---|
| 1 | 0.465 |
| 2 | 0.403 |
| 3 | 0.095 |
| 4 | 0.602 |
| 5 | 0.465 |
| 6 | 0.117 |
| 7 | 0.917 |
| 8 | 0.076 |

The Kruskal-Wallis P values show that there are no statistical differences in comparing all of the fingerprint scores by hand for each donor. Therefore there are no observed differences between the scores obtained for each donor by hand.

As previously discussed in section 2.1.3, each DNA extract was amplified in triplicate. Table 2-13 shows the number of alleles that matched each donor's profile for each PCR replicate.

Table 2-13 Number of matching alleles per donor

| | Donor | PCR Replicate - No. Of Matching Alleles | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Left Hand | 1 | 1 | 8 | 7 |
| | 2 | 1 | 6 | 9 |
| | 3 | 2 | 4 | 4 |
| | 4 | 2 | 2 | 2 |
| | 5 | 16 | 10 | 12 |
| | 6 | 3 | 2 | 2 |
| | 7 | 6 | 6 | 6 |
| | 8 | 5 | 3 | 4 |
| Right Hand | 1 | 6 | 3 | 3 |
| | 2 | 2 | 6 | 1 |
| | 3 | 10 | 10 | 2 |
| | 4 | 2 | 2 | 2 |
| | 5 | 2 | 5 | 2 |
| | 6 | 1 | 2 | 2 |
| | 7 | 22 | 22 | 22 |
| | 8 | 0 | 0 | 0 |

The Kolmogorov-Smirnov normality test was also undertaken for the allele data and also found to be non normal. Therefore Kruskal-Wallis tests were undertaken to

assess whether there was a statistical difference in the allele number per hand for each donor. This is shown in table 2-14.

Table 2-14 Table of P values for each donor in terms of number of matching alleles

| Donor | Kruskal-Wallis P Values Per Hand |
|---|---|
| 1 | 0.513 |
| 2 | 0.513 |
| 3 | 0.383 |
| 4 | 1.000 |
| 5 | 0.050 |
| 6 | 0.275 |
| 7 | 0.050 |
| 8 | 0.050 |

The Kruskal-Wallis P values (from table 2-14) showed that five of the eight donors had no significant difference in the number of alleles recovered by hand. Three donor's (5, 7, and 8) had a P value of 0.050 which is equal to the α value. This means that the data interpretation is difficult as it is neither lower nor higher than the α value. This could indicate a slight difference in the number of matching alleles recovered per hand for donor's 5, 7, and 8.

Hand shedding difference has been found by Phipps and Petricevic [143]. This result may be explained by the fact that people use one hand more than the other hand, and as a result of increased contact with items, this dominant hand will generate more loose skin cells than the lesser used hand via the mechanism suggested by Wickenheiser [68].

Alleles were reported in accordance with Gill *et al.*, trace DNA interpretation guidelines [65]. The guidelines had previously been outlined in section 2.2.1. This meant that the PCR replicates would be compared to each other and only duplicated alleles would be reported.

Results from tables 2-11 was averaged for each hand; combined with the actual number of 'reportable' alleles per hand for each donor, and plotted in a scatterplot (figure 2-10) generated by Minitab (the two points indicated on the graph are for left and right hand scores per donor).

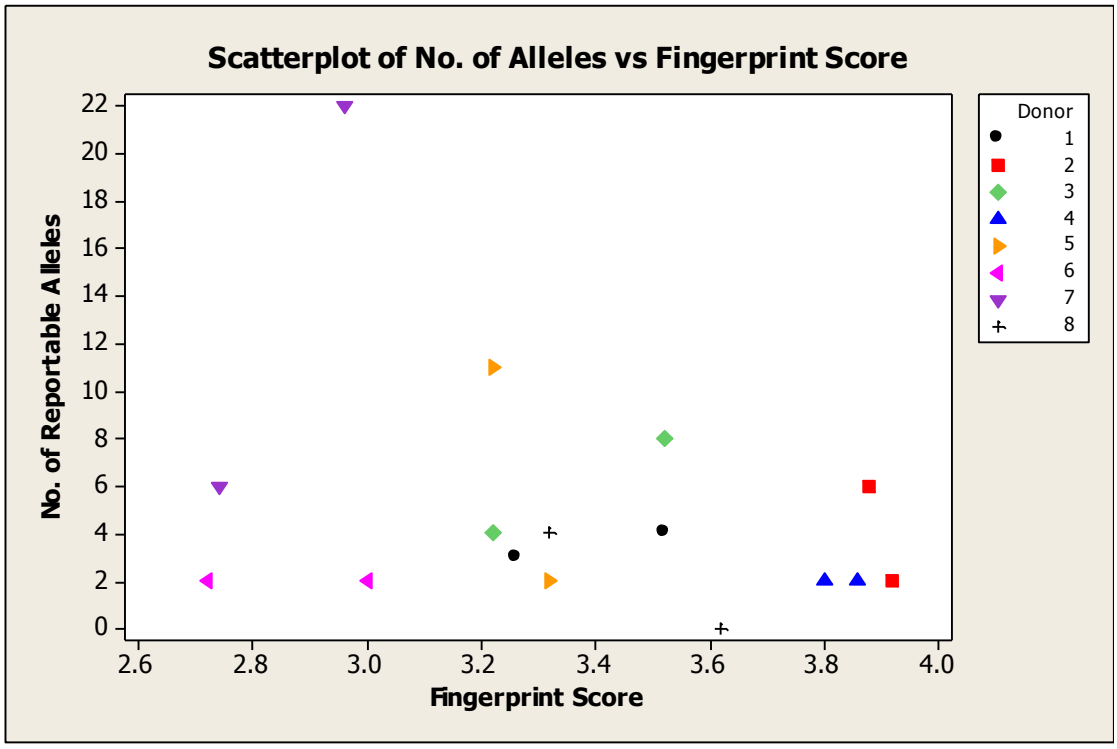


Figure 2-10 Scatterplot of number of alleles vs. fingerprint score

The graph clearly shows that each donor's fingerprint scores and the number of reportable alleles appears to have no relationship. DNA shedding is a measure of how much 'touch' DNA is transferred, with good donors providing a full DNA profile (in this investigation it would equate to 22 alleles). Only donor 7 provided a full complement of DNA for one of their hands. There is no correlation in the fingerprint score and number of reportable alleles therefore, this suggests that there is no correlation between fingerprint deposition and DNA shedding [144].

### 2.2.4 Effects of Heat Exposure on DNA

Section 2.2.2 showed that fingerprints have very low levels of DNA present and could not be relied upon to give any significant amount of DNA. As such, to test the survivability of DNA after exposure to elevated temperatures, buccal swabs taken from a donor were rubbed onto paper and glass.

The DNA profiles generated were reported according to Gill *et al.*, interpretation rules for DNA less than 100pg [65]. The mass of DNA for each sample in this experiment were not quantified but as DNA damage was expected upon heating the samples, it

was necessary to follow specific guidelines when interpreting the results. It also allowed all DNA profiles throughout this whole chapter to be assessed against the same criteria. When presenting these results, as the donor's profile was known, the DNA profiles from the heated samples were compared to the known profile. As such, results were reported in terms of 'number of alleles which match donor's DNA profile', with 22 alleles giving a full profile. This was to give an indication of the possibility of obtaining a 100% profile which could subsequently lead to the identification of the perpetrator. Partial DNA profiles could be used for intelligence purposes.

### 2.2.4.1 Substrate – Paper

The buccal swab rubbed papers were subjected to the various temperatures and exposure times as listed in section 2.1.4.1, with three repeats taken at each temperature and exposure time. On removal from the oven, the papers were swabbed using the wet/dry combination method and these samples frozen immediately. The samples were defrosted on the morning of extraction before following the protocols in sections 2.1.1.1 – 2.1.1.3. An example of an electropherogram generated by one of the paper samples is given in figure 2-11. The electropherogram shows only amplification at the low molecular weight loci, and also shows spurious alleles present at locus D3S1358.

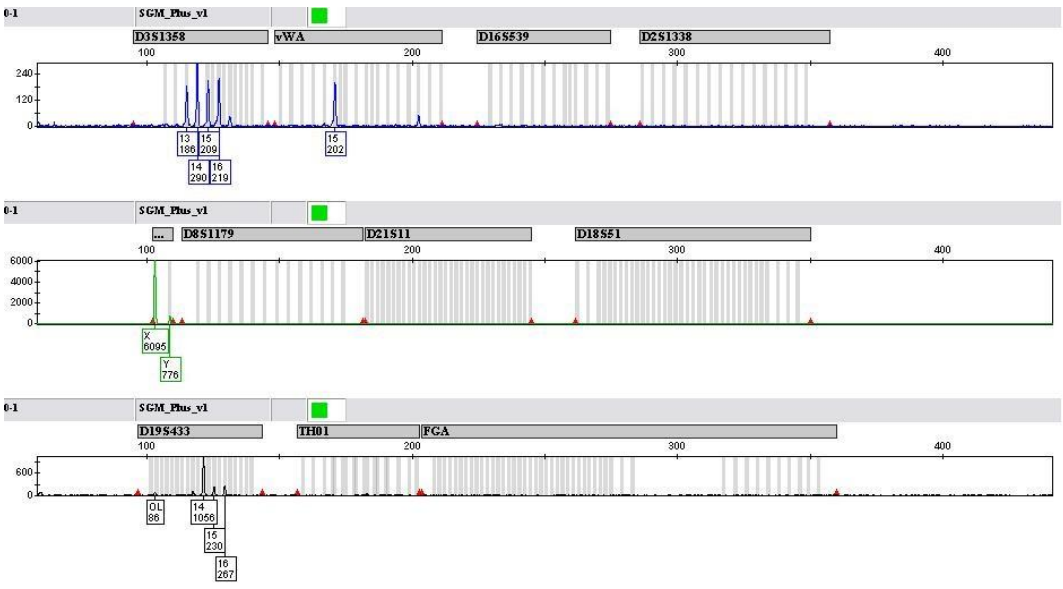



Figure 2-11 Electropherogram of a DNA sample on paper exposed to 150˚C for 20min

Figure 2-12 gives an interval plot indicating the mean number of alleles present which matched the donor's profile against the temperature and exposure time variables from the three repeats and also the variability in the data at a 95% confidence interval.

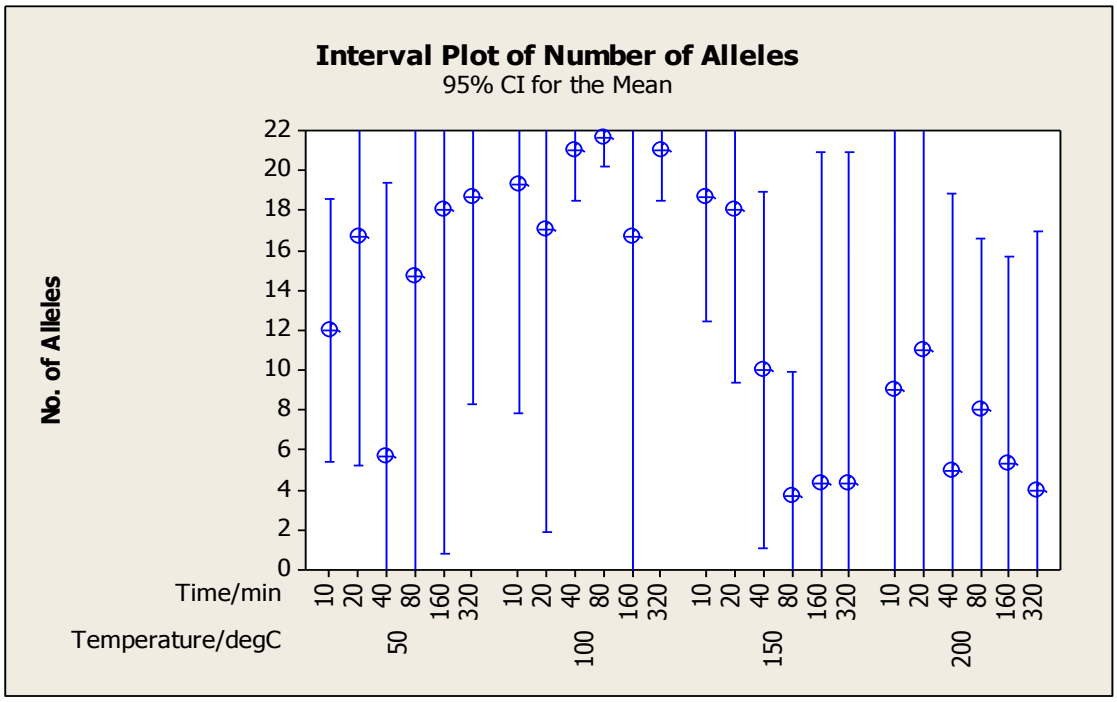



Figure 2-12 Plot of number of alleles which match donor's DNA profile recovered from heated paper

The graph indicates that the mean number of alleles is higher at 50˚C and 100˚C across all exposure times than the results at 150˚C and 200˚C. This indicates that a larger proportion of the DNA profile is recoverable at lower temperatures. The 95% confidence interval lines on the graph indicate a large variability in the data. The large variation could be due to the experimental method. As the buccal swabs were rubbed onto the paper, it was unknown how much DNA was transferred and therefore the number of alleles recovered could also be affected by the amount of DNA initially transferred.

To assess the normality of the data, a Kolmogorov-Smirnov normality test was undertaken. The distribution of the allele numbers is given in figure 2-13 and also shows that a P value of <0.010 was generated as part of the test. This is less than the α value of 0.05, indicating the data is not normally distributed (this is also shown on the probability plot with a deviation from the normal distribution line, especially when 22 alleles are recovered).

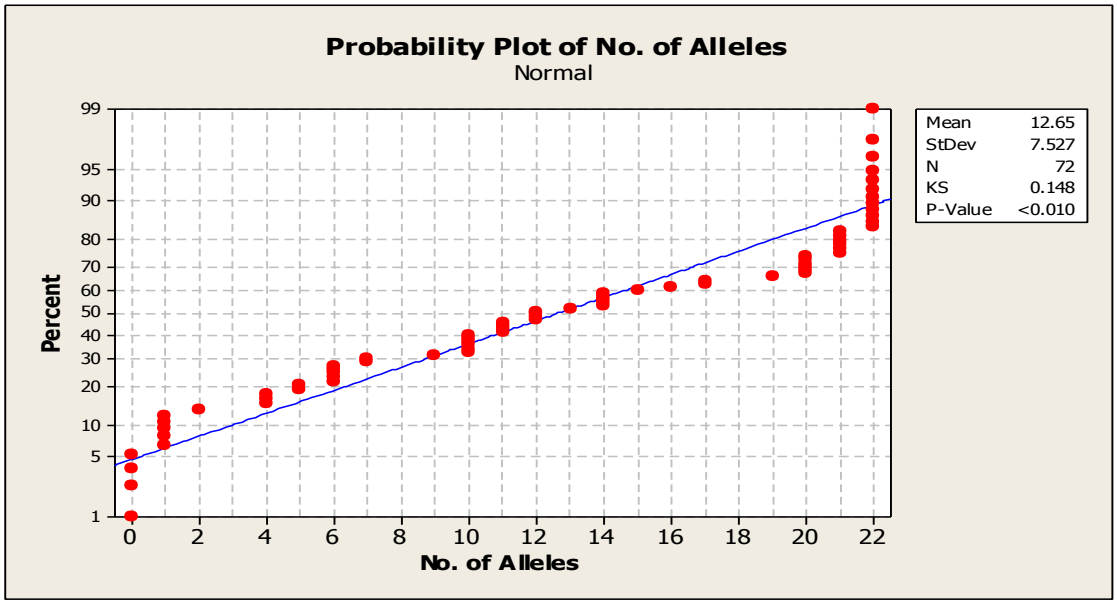



Figure 2-13 Probability plot of number of alleles recovered from heated paper

Therefore, a non parametric Kruskal-Wallis test was undertaken for investigating whether temperature or exposure time is significant to the number of alleles recovered from the heated paper. The Kruskal-Wallis tests, which are also compared to the α value of 0.05, generated P values of 0.000 and 0.618 respectively. Therefore exposure temperature has a significant effect on the number of alleles recovered from the exposed paper, whereas exposure time does not have a significant effect. A main effects plot has been generated (figure 2-14) to compare the average number of alleles recovered for each temperature.

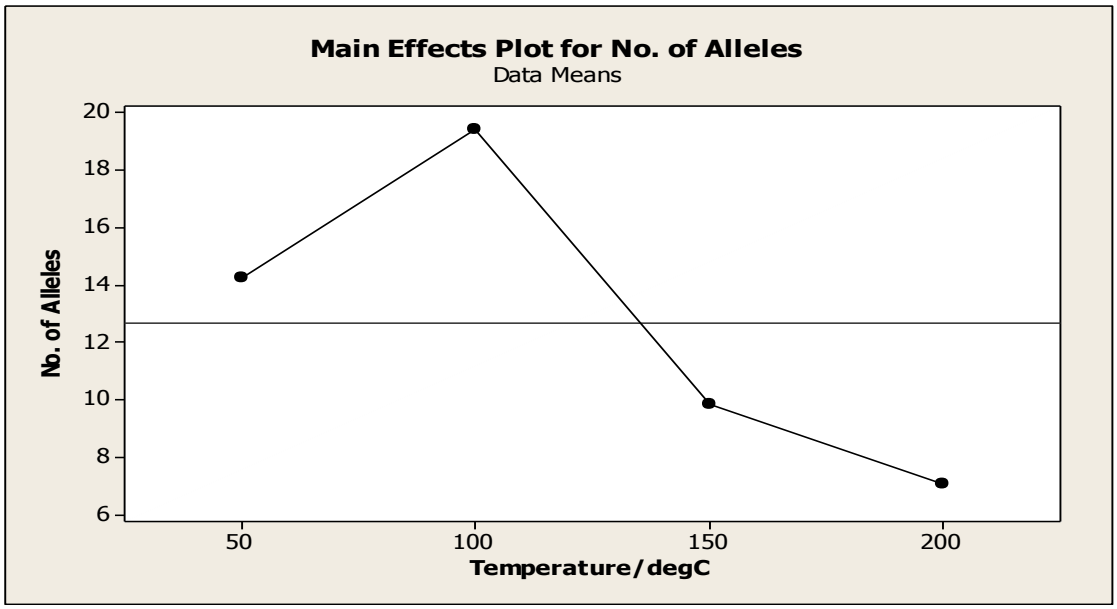



Figure 2-14 Main effects plot of number of alleles on paper exposed to elevated temperatures

The number of alleles recovered at 50˚C is above the mean result for all alleles recovered and a larger increase in matching alleles was observed at 100˚C. This is not surprising as part of the DNA extraction process requires incubating the samples at 56˚C and 70˚C. At higher temperatures, the number of alleles decreases, resulting in partial DNA profiles, and as such cannot as readily be used for identification of a suspect. It may be useful for investigative rather than evidentiary purposes.

### 2.2.4.2 Substrate – Glass

The same process was repeated for depositing DNA onto glass slides. An electropherogram from one of the glass samples is given in figure 2-15.

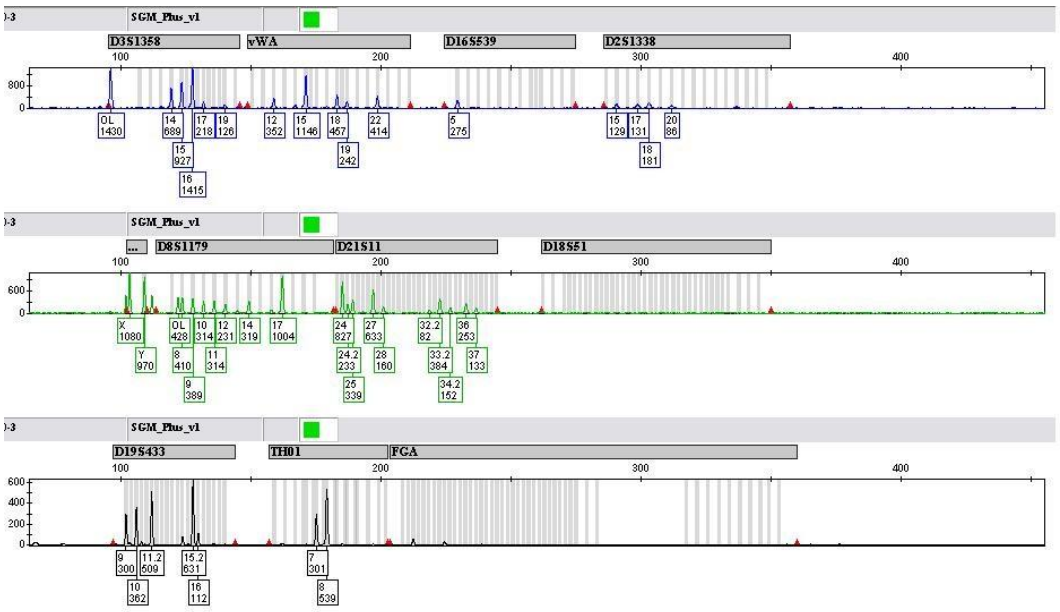



Figure 2-15 Electropherogram of DNA sample on glass exposed to 300˚C for 160min

This electropherogram shows a large amount of allele drop in. The 75 RFU minimum peak height designation level has designated many alleles, especially at the lower molecular weight loci. No alleles are present at two of the higher molecular weight loci (D18S51 and FGA). Having electropherograms with this many spurious alleles makes interpretation very difficult, especially if this was a crime scene sample.

The results for recovering DNA from heat exposed glass were more variable than those obtained for paper. This can be seen by the mean values in the interval plot in

figure 2-16 which shows that there is no discernable pattern, either on the results obtained across the temperatures, or across the same temperature but at different exposure times.

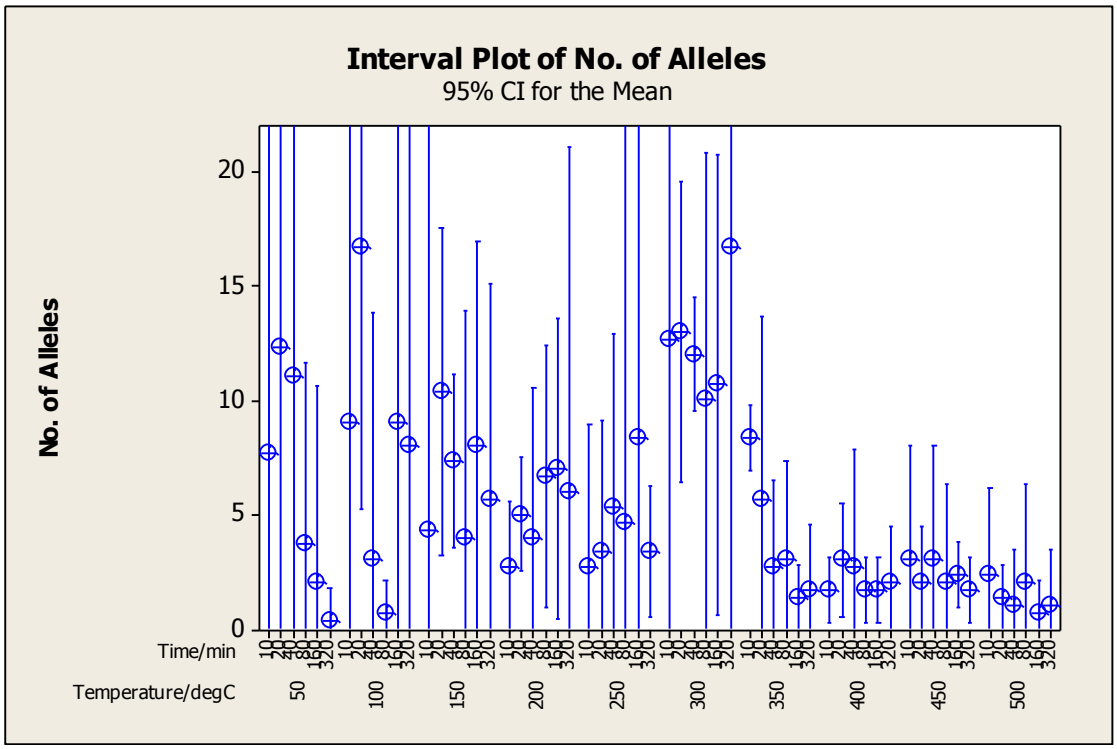



Figure 2-16 Plot of number of alleles which match donor's DNA profile recovered from heated glass

The interval bars on the plot also indicate a wider variation in the number of alleles recovered at the lower temperatures compared to the higher temperatures. The difference observed between the two substrates may be due to the non porous nature of the glass.

A Kolmogorov-Smirnov plot was generated which produced similar results to the plot generated for DNA deposited on paper. The data was not normally distributed and deviated to a greater extent from the straight line when no matching alleles were recovered. This is given in figure 2-17.

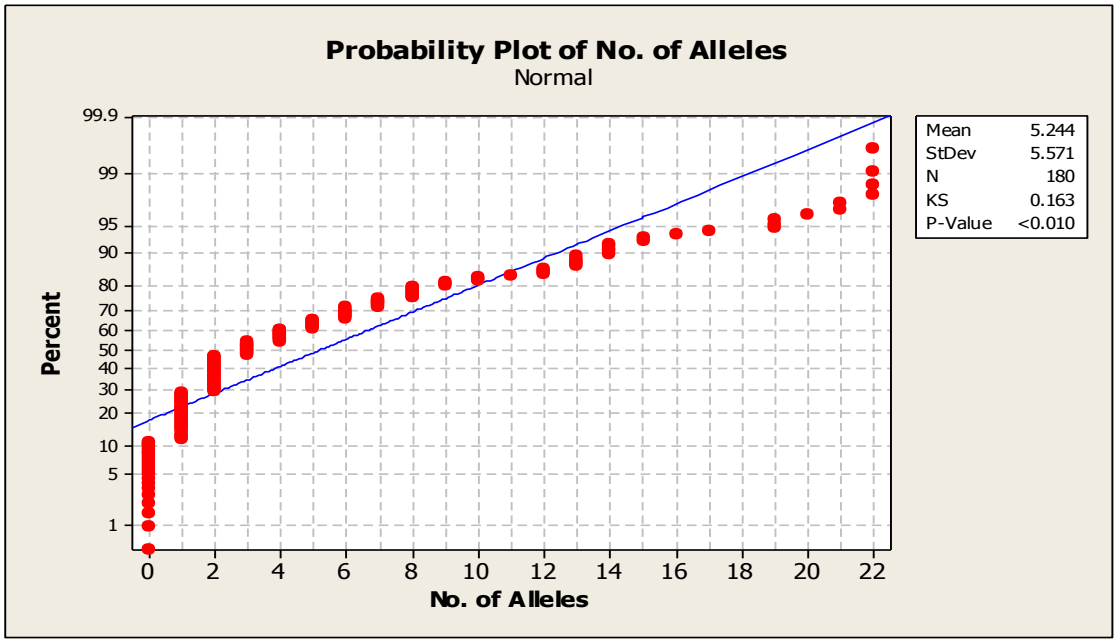



Figure 2-17 Probability plot of number of alleles recovered from heated glass

The probability plot also shows that a P value of <0.010 was calculated therefore Kruskal-Wallis non parametric tests were undertaken to assess the significance of temperature and time on the number of matching alleles. The P values from the Kruskal-Wallis tests were 0.000 and 0.198 for testing temperature and exposure time respectively. Therefore, the exposure temperature was significant to the allele number whereas exposure time was not. A main effects plot was generated using Minitab and this is given in figure 2-18.

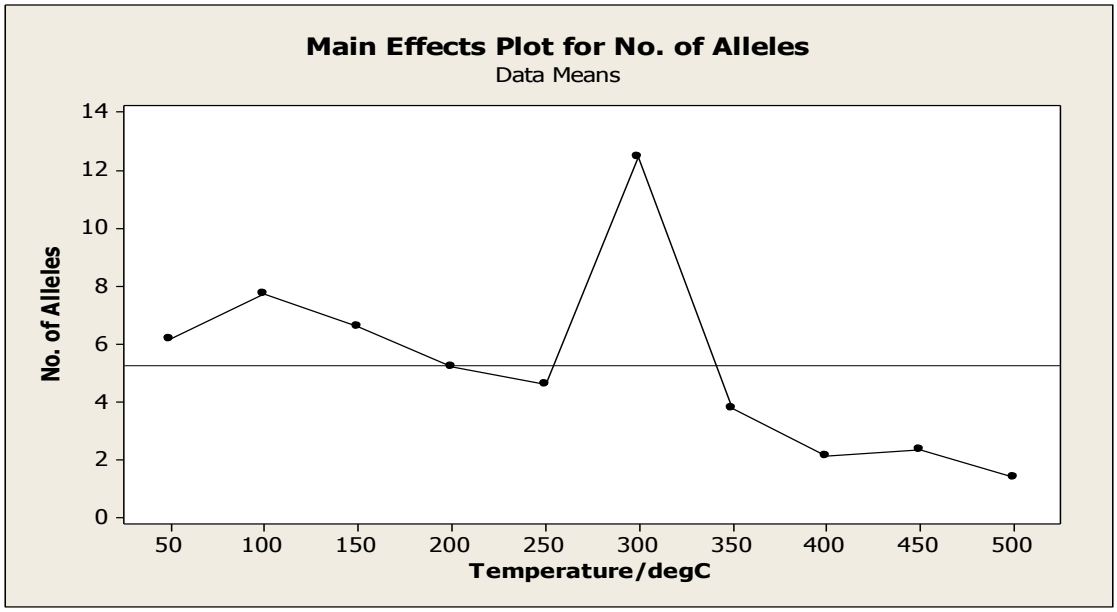



Figure 2-18 Main effects plot of number of alleles on glass exposed to elevated temperatures

The main effects plot shows that (apart from the rise at 100˚C which was mirrored in the paper samples) the number of matching alleles recovered decreases gradually as the exposure temperature increases. There is a sharp increase at 300˚C in the data which is unexplained.

### 2.2.4.3 Substrate Comparison

A comparison of the results obtained by each substrate is shown in the interaction plot in figure 2-19 and described afterwards.

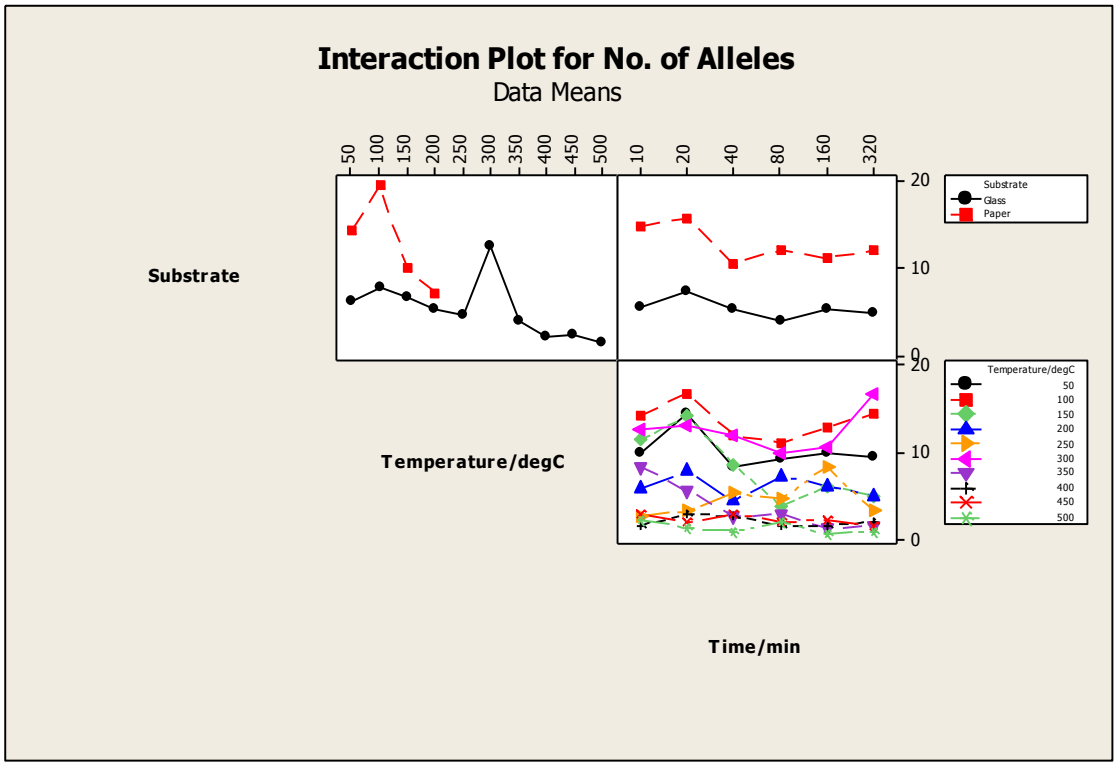



Figure 2-19 Interaction plot of alleles which match donor's DNA profile for both substrates (y-axis = number of alleles)

Figure 2-19 illustrates the effects of the interactions of different variables on the number of alleles which matched the reference profiles. When examining the interaction between *substrate* and *temperature*, the scores obtained from both substrates follow the same trend with an increase at 100˚C. The results demonstrate that higher numbers of alleles were obtained from paper than from glass. Glass has an unexplained peak at 300˚C. For the interaction between *substrate* and *time*, the number of alleles reported is reasonably consistent across all times with paper and

glass each having a slight peak at 20min. No clear relationship is seen between *temperature* and *time* where the interaction results are very erratic.

The interval plots for each substrate show that the number of alleles recovered which matched the donor's profile is subject to a high degree of variability. This is due in part to the variability expected in the experimental design (with an unknown amount of DNA transferred to the substrate) and also due to the 12.5µL PCR reaction volume. However some general conclusions can be made. In general, better results were obtained for paper rather than glass in terms of the number of alleles matching the known profile. This could be due to the porous nature of the paper where the DNA could be being absorbed into the matrix of the paper, and as such is being protected to a certain degree from the heat. This is in agreement with Wickenheiser [68] and Raymond *et al.,* [145] who state DNA recovery is better on porous than non porous surfaces.

Tontarski *et al.,* [81] have published research on the recovery of DNA from bloodstains that have been exposed to fire conditions. Their research was conducted using the organic extraction procedure and the PowerPlex® 16 system for amplification. They reported that they could obtain full DNA profiles using this procedure for temperatures up to 300˚C, although this was dependent on the location of the original bloodstain within the compartment where the fire occurred. No DNA profiles were obtained on samples that had experienced temperatures greater than 300˚C. In the present study only partial DNA profiles were obtained at samples exposed to 300˚C and above. These differences may be to do with the source of DNA used in both cases.

# CHAPTER 3: FINGERPRINTS ON POROUS SURFACES

## 3.1 Experimental

A number of systematic experiments were undertaken in order to establish the range of temperatures and the exposure times at these temperatures that latent fingerprints can withstand and still be recoverable. Three types of fingerprints (eccrine, sebaceous and natural) were examined in each case and statistical analysis carried out on the resultant data. For each specific combination of temperature, time of exposure and age of fingerprint a total of 70 fingerprints were examined consisting of 10 eccrine deposits, 10 sebaceous deposits and 50 natural deposits. An example of the depletion grid used for each variable is given in figure 3-1.

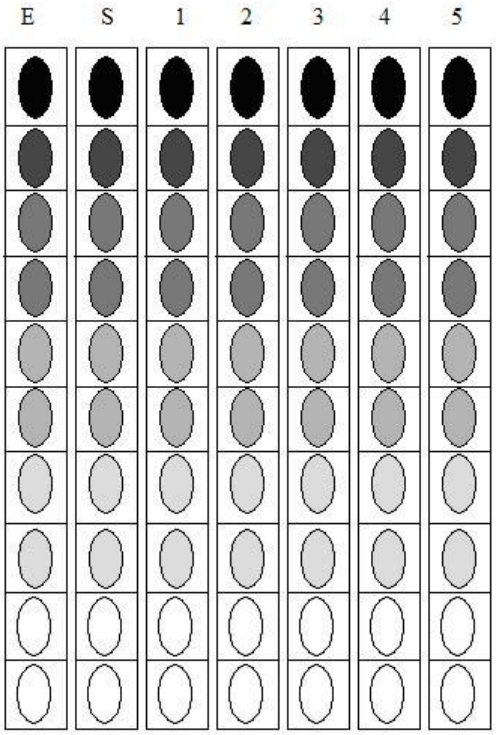



Figure 3-1 Illustration of the depletion grid used for all porous and non porous fingerprint experiments

In figure 3-1, the eccrine fingerprints are shown in column 'E', and these are fingerprints which had been donated only after the donor had worn nitrile gloves for a minimum of 30 minutes prior to deposition. The sebaceous fingerprints are shown in column 'S' and these were obtained from male donors or females who did not wear cosmetics so that when the fingertips were rubbed on the forehead and nose, cosmetic

contamination could be avoided. Columns ‘1, 2, 3, 4, and 5’ on the depletion grid were designated for natural fingerprints, where the donor deposited latent fingerprints ‘as is’. Natural fingerprints were only donated by persons who had not worn gloves or washed their hands for a minimum of 30 minutes prior to deposition. Each column on the deposition grid relates to one donor, who deposited 10 fingerprints down the column in total.

Different donors were used throughout the study due to the number of fingerprints required and the availability of donors. However, when donors were asked to donate, they provided fingerprints on six separate deposition grids so that the same donors were used for the six time variables for each temperature, age, and technique study. For example, the same donors would have deposited fingerprints for variables 10min, 20min, 40min, 80min, 160min, and 320min when investigating the enhancement of ninhydrin on 1 day old fingerprints exposed to 100˚C, but different donors would have been used for fingerprints exposed to 150˚C, and this was continued throughout the study.

Only one column each was used for eccrine and sebaceous fingerprints as the fingerprints were forced into becoming one of the fingerprint deposits, therefore the enhancement of these fingerprints would be irrespective of the donor as the fingerprints were ‘charged’ with eccrine or sebaceous component. For the natural fingerprints, five columns were used to represent a cross section of the general public. This method was used to obtain a general performance indicator of the enhancement technique without the need to undertake repeat experiments. This will affect the variability of the data, and ideally, the same donors would have been used for all experiments described in this chapter and in chapter 4, however the logistics involved made this impossible to achieve. Notwithstanding this some repeatability of the same donors was built into the experimental set up as previously described.

The depletion grid experimental procedure allowed for a general overview of the performance of the fingerprint enhancement techniques. Factors such as environmental conditions, the type of surface on which fingerprints are deposited, the ability of the donor to deposit fingerprints, contact time, and force of contact with the object will all contribute to the resultant deposited fingerprint [142].

The fingerprint score used in the subsequent data analysis was the average score obtained from each of these sets of enhanced deposits. The objective was to be in a position to propose an optimum development technique at the various temperatures examined. The temperatures and duration of exposure were chosen in order to mimic those experienced under conditions commonly experienced in fires.

### 3.1.1 Fingerprints on Paper

The paper used was taken from an unopened packet of white 80g/m$^2$ A4 recycled paper manufactured by Niceday (Andover, Hampshire). A depletion grid was drawn onto the paper surface as described in section 3.1 (figure 3-1).

Three different fingerprint deposits were made. These were an eccrine deposit (achieved by washing hands then wearing nitrile gloves for half an hour), a sebaceous deposit (achieved by rubbing the nose or forehead before donation), and natural deposits (non-groomed fingerprints). Fingerprints were obtained by donors rubbing their hands together to evenly distribute the perspiration immediately before deposition. Fingerprints were deposited a minimum of 30 minutes after handwashing.

The deposited fingerprints were subjected to different ageing times before being heated at different temperatures for different periods of time, and finally enhanced by different methods. A summary of the experiments is given in table 3-1.

Table 3-1 Summary of exposure to heat experiments

| Substrate | Ageing Time | Temperature (˚C) | Exposure Time (min) | Treatment |
|---|---|---|---|---|
| Paper | 1 hour, 1 day, 1 week, 1 month | 50 | 10, 20, 40, 80, 160, 320 | 1. Ninhydrin 2. DFO 3. PD |
| | | 100 | | |
| | | 150 | | |
| | | 200 | | |

Paper was heated to 200˚C due to its relatively low autoignition temperature at approximately 233˚C (although this depends on the type of pulp used, chemical content, paper thickness, and a variety of other characteristics).

**Ninhydrin**

Ninhydrin was prepared as a concentrated solution using ninhydrin (25g) (Sigma-Aldrich, Gillingham, Dorset) and adding 100% ethanol (225mL) (Hayman, Witham, Essex), ethyl acetate (10mL) (Sigma-Aldrich, Gillingham, Dorset), and acetic acid (25mL) (BDH, Poole, Dorset) in that order whilst stirring. The working solution consisted of the concentrated solution (52mL) which was added to 1-methoxynonafluorobutane (1L) (commonly known as HFE7100) (Severn Biotech Ltd, Kidderminster, Worcestershire) whilst stirring. The paper samples were pulled through the working solution until fully immersed, removed, and then left to dry. The paper was then placed in a humid oven and heated for 4 min at 80˚C and nominal 65% humidity. The paper was examined approximately 2 weeks after enhancement as ninhydrin is known to produce fingerprints a few weeks after treatment [146].

**DFO**

1,9-diazafluorene-9-one (DFO) was prepared using DFO (25g) (Sigma-Aldrich, Gillingham, Dorset) and adding methanol (30mL) (Sigma-Aldrich, Gillingham, Dorset), acetic acid (20mL) (BDH, Poole, Dorset), 1-methoxynonafluorobutane/trans-1,2-dichloroethylene mixture (275mL) (commonly known as HFE71DE) (3M Novec, Bracknell, Berkshire), and HFE7100 (725mL) (Severn Biotech Ltd, Kidderminster, Worcestershire) sequentially whilst stirring. The paper samples were pulled through the working solution until fully immersed, removed, and then left to dry before being placed in a dry oven for 20 minutes at 100˚C. The treated paper was examined under light of 473-548nm using a 549nm viewing filter [146].

**Physical Developer (PD)**

Physical developer (PD) working solution was made from a stock detergent solution. The stock detergent solution was prepared by adding n-dodecylamine acetate (2.8g) (ICN, Irvine, California) to distilled water (1L) whilst stirring. To prepare the working solution, silver nitrate (10g) (BDH, Poole, Dorset) was added to distilled water (50mL) whilst stirring. This was then stored in a cool, dark place until required. To

distilled water (900mL), iron (III) nitrate (30g) (Fisher Scientific, Loughborough, Leicestershire); ammonium iron (II) sulphate (80g) (BDH, Poole, Dorset) and citric acid (20g) (BDH, Poole, Dorset) were added whilst stirring until dissolved. The stock detergent solution (40mL) was added to this solution and this was added to the working solution. A maleic acid solution was also prepared. The maleic acid solution was prepared by adding maleic acid (25g) (Merck, Hoddesdon, Hertfordshire) to distilled water (1L) whilst stirring. Five clean dishes were used for the enhancement – one filled with the maleic acid solution, one filled with working solution, and the other three filled with distilled water. The paper was soaked in the maleic acid solution for 10 minutes and then submerged in the working solution for 20 minutes while the dish was gently rocked. The paper was then rinsed in each of the three dishes of distilled water for 5 minutes each and for a further 20 minutes using distilled water in a photographic print washer. The paper was left to dry overnight before being examined [146].

#### 3.1.1.1 Fluorescence of Fingerprints on Paper – An Additional Study

Section 3.1.1 discussed the experiments which were carried out to investigate the recoverability of fingerprints on paper which had been exposed to elevated temperatures by comparing various chemical enhancement techniques (ninhydrin, 1,8-diazafluoren-9-one (DFO), and physical developer (PD)). During that study, it became apparent, as a consequence of observations made [147], that fingerprints on paper subjected to 150˚C fluoresced under examination with green light of waveband 473-548nm with a 549nm viewing filter. Three separate tests were carried out to investigate this phenomenon.

**Eccrine, Sebaceous and Natural Fingerprints on Recycled Paper**

The paper used was the same paper described in section 3.1.1.

As the fingerprints fluoresced at 150˚C, and not at 100˚C and 200˚C (as discussed in section 3.2.1), heating experiments were conducted between 110˚C and 190˚C. A deliberately eccrine deposit, a deliberately sebaceous deposit, and a natural deposit were donated (also described in section 3.1.1). These fingerprints were placed on the

same piece of paper and heated to temperatures in the range of 110˚C to 190˚C in 10˚C increments. The exposure time for each sample was 20 minutes.

**Eccrine Fingerprints on Filter Paper**

The eccrine fingerprint placed on paper at 150˚C was placed into a Shimadzu RF-5301 spectrofluorophotometer (Milton Keynes, Buckinghamshire, UK) in order for the optimal excitation and emission wavelengths to be determined [148].

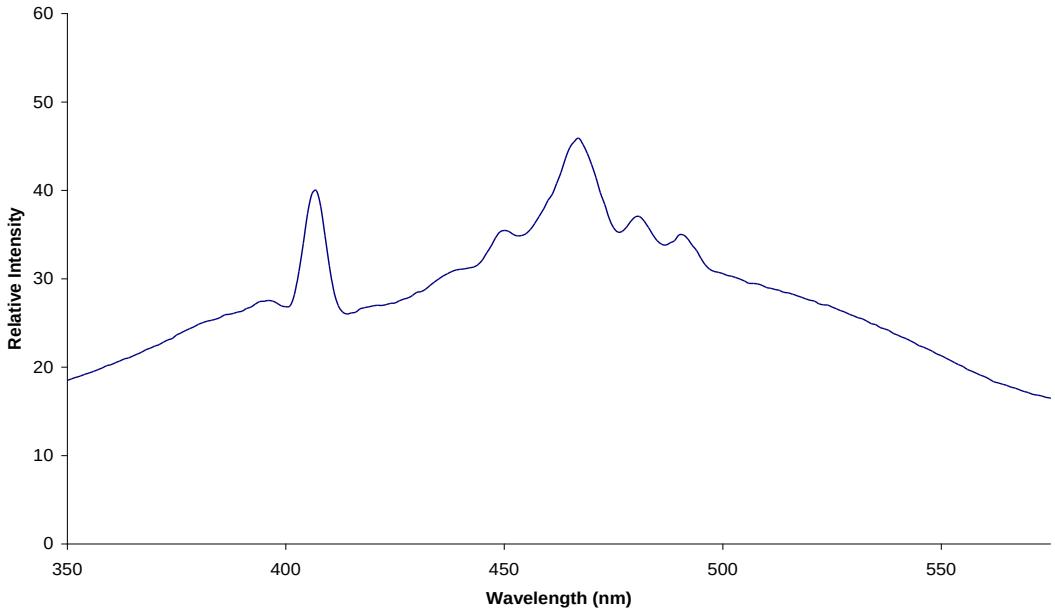



Figure 3-2 Excitation spectrum of an eccrine fingerprint on paper heated at 150˚C

Figure 3-2 shows the excitation spectrum generated by the spectrofluorophotometer for the eccrine fingerprint at 600nm emission.

As the principal excitation peaks were detected at approximately 405nm and 475nm, the fingerprint was excited at these wavelengths and the resultant emission spectra are shown in figure 3-3. The actual excitation spectrum observed for the eccrine fingerprint is very broad.

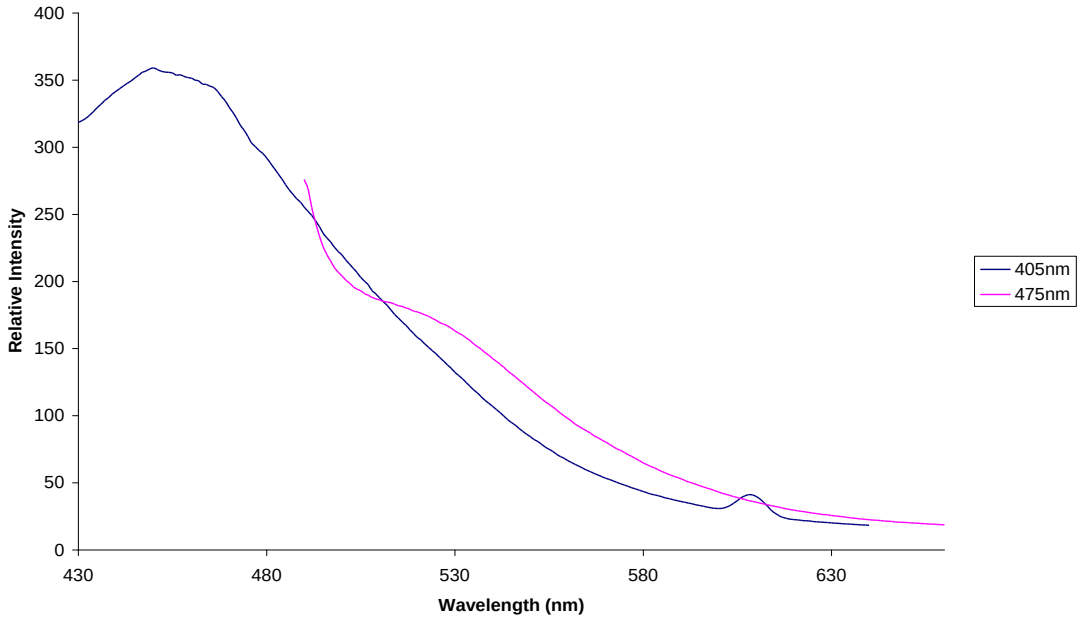



Figure 3-3 Emission spectrum of an eccrine fingerprint on paper heated at 150˚C

Eccrine fingerprints were then deposited onto filter paper which was 110mm in diameter, in hardened, ashless circles (Whatman Ltd, Maidstone, Kent, UK) and only handled whilst wearing gloves. These fingerprints were exposed to the temperatures in the same range of 110˚C to 190˚C and examined under violet-blue light between 350-469nm with a 476nm viewing filter, blue light between 352-509nm with a 510nm viewing filter, and green light between 473-548nm and a 549nm viewing filter provided by a Quaser 40 (Foster + Freeman, Evesham, UK), as the spectrofluorophotometer results showed that fluorescence would be observed within these three bandwidths.

The luminance of the fingerprints was measured using a Konica Minolta LS-100 luminance meter, with the Quaser 180mm away from the paper and the luminance meter 400mm away from the paper (shown in figure 3-4). These were fixed in position for all the luminance measurements. These results indicated that blue light will provide the brightest fluorescence, followed by violet-blue, and finally green.

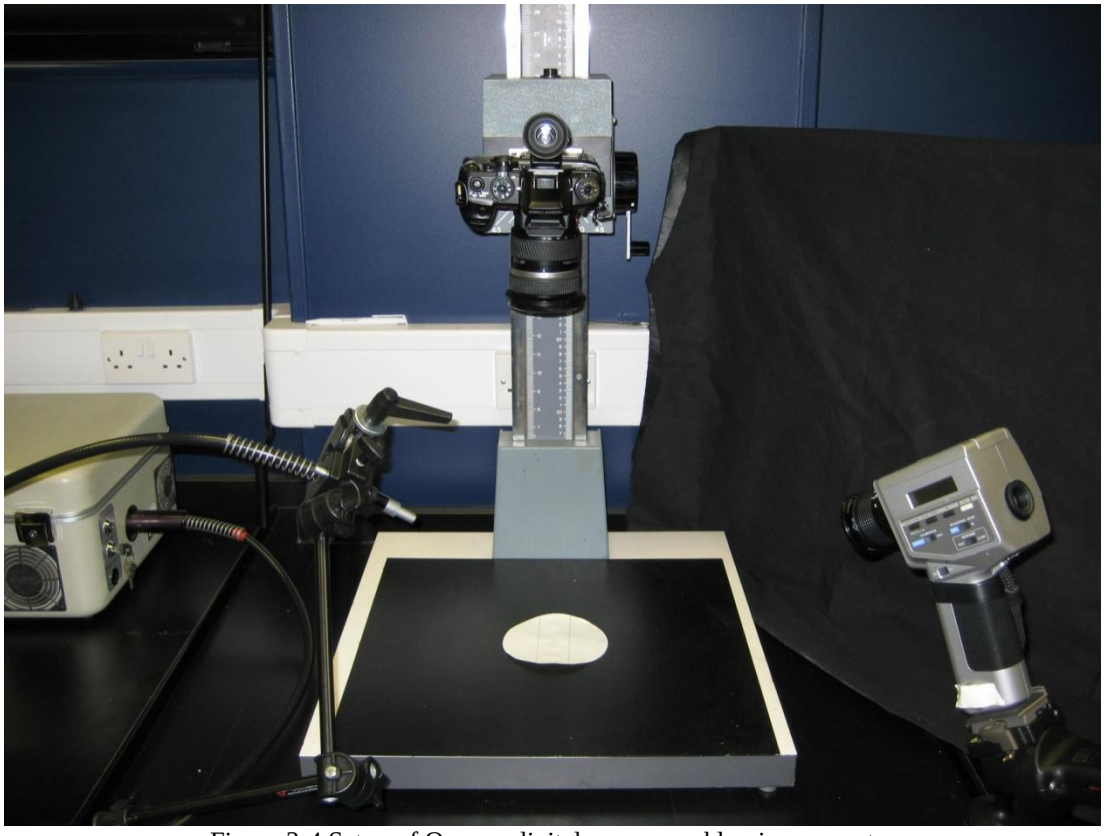


Figure 3-4 Setup of Quaser, digital camera and luminance meter

**Exposure of Eccrine Sweat Constituents to Elevated Temperatures**

Five of the most prevalent amino acids present in fingerprint deposits [149] were subjected to the same temperature conditions as before. 10µL of 1mg/mL of L-alanine, L-aspartic acid, L-lysine hydrochloride, glycine, and L-serine (Fluka BioChemika, Sigma-Aldrich, Gillingham, Dorset, UK) was deposited onto filter paper, as was 10µL of 1mg/mL of sodium chloride and urea (Sigma-Aldrich, Gillingham, Dorset, UK). These were examined under green light as this was first bandwidth found to fluoresce these heated prints. Also, the green NdYAG 532nm laser is a common search laser used for examining crime scenes in the UK and as such, is appropriate for speculatively searching large areas for this type of fluorescent prints.

## 3.2 Results and Discussion

In this section the results of the efficacy of the different enhancement techniques are explored for fingerprints exposed to elevated temperatures on paper. Three types of

fingerprint deposits were made on each sheet (eccrine, sebaceous and 'natural' (fingerprints donated 'as is' with no grooming involved)). This was used to test the effectiveness of the enhancement techniques but also to investigate how the eccrine or sebaceous content of the fingerprint deposits were behaving under elevated temperatures. Natural fingerprints are the closest to 'real' fingerprints.

The effect of changing the variables under examination such as temperature, time, and age, and the interaction between these variables is best achieved using a balanced ANOVA analysis and the associated interaction plots which can be generated (section 2.2.2). A requirement of ANOVA is that the data must be normally distributed. As such, the Kolmogorov-Smirnov normality test was conducted on a subset of the data using Minitab. A probability plot and a P value are generated for each test. If the α value to be tested (in this case 0.05) is less than the P value generated by the normality test then the data can be considered to be normally distributed. A subset of the data, for fingerprints enhanced using ninhydrin, is shown in figures 3-5 (eccrine), 3-6 (sebaceous), and 3-7 (natural).

The P values presented in both figure 3-5 (eccrine) and 3-6 (sebaceous) are both greater than the α value (0.05), indicating normally distributed data. As the fingerprints are being 'forced' to be eccrine, at the start of the depletion series the amount of eccrine deposit transferred onto the paper will be high. As the depletion series continues, the transfer should be less and less, which should give normally distributed data. The Kolmogorov-Smirnov test showed a normal distribution. The sebaceous fingerprints are also being 'forced' to be sebaceous, which would result in normally distributed data. The Kolmogorov-Smirnov result indicated that the sebaceous fingerprints were also normally distributed. From figure 3-7, the P value is <0.01, indicating that the data is not normally distributed. Looking at figure 3-7, the data is approximating a normal distribution in that the data values have only a slight deviation from the blue line on the graphs. In general the data reflected a normal distribution in two of the three fingerprint classes, and approximated a normal distribution for the other class.

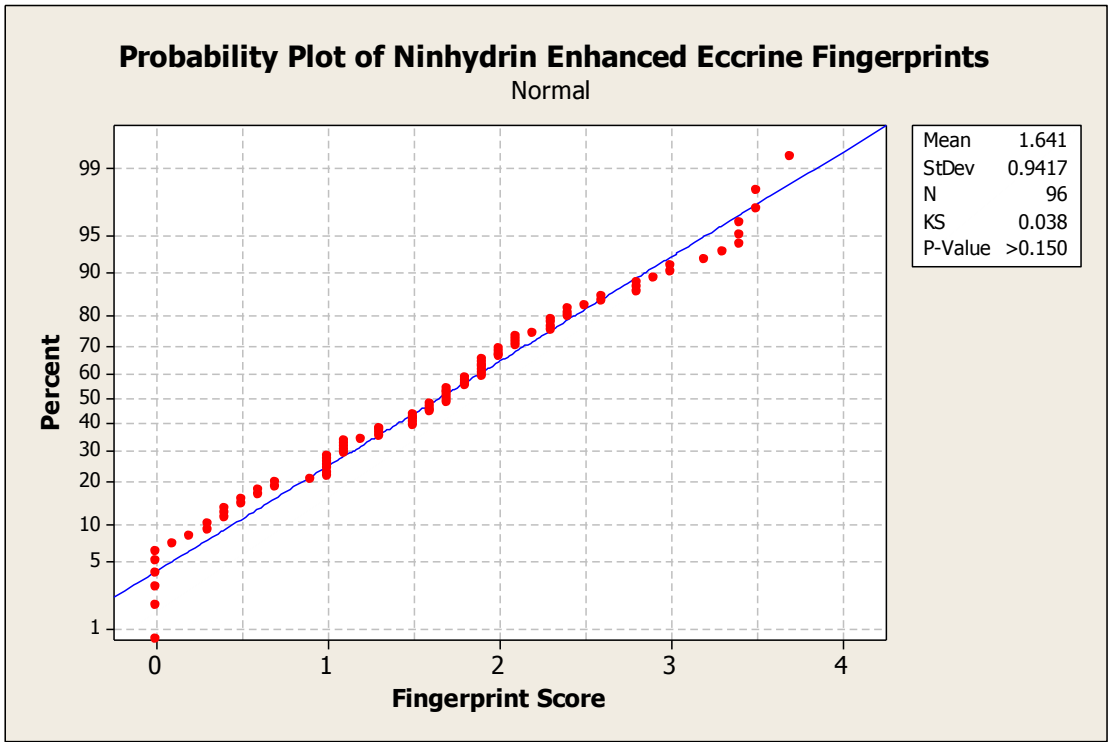



Figure 3-5 Probability plot of ninhydrin enhanced eccrine fingerprints

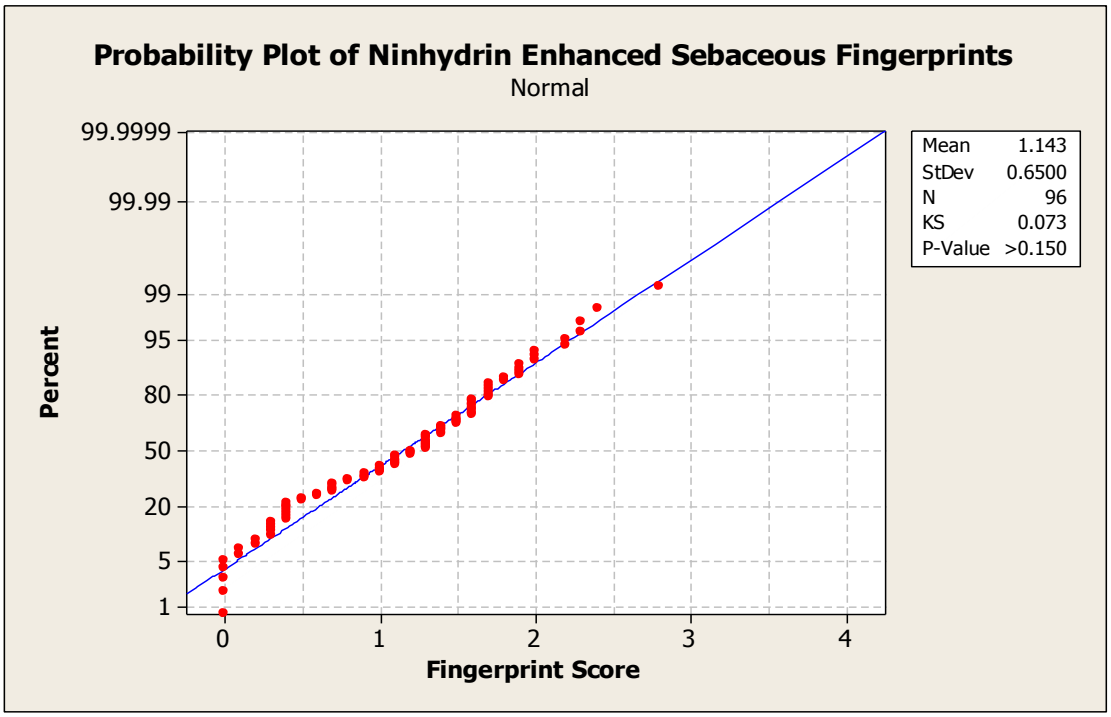



Figure 3-6 Probability plot of ninhydrin enhanced sebaceous fingerprints

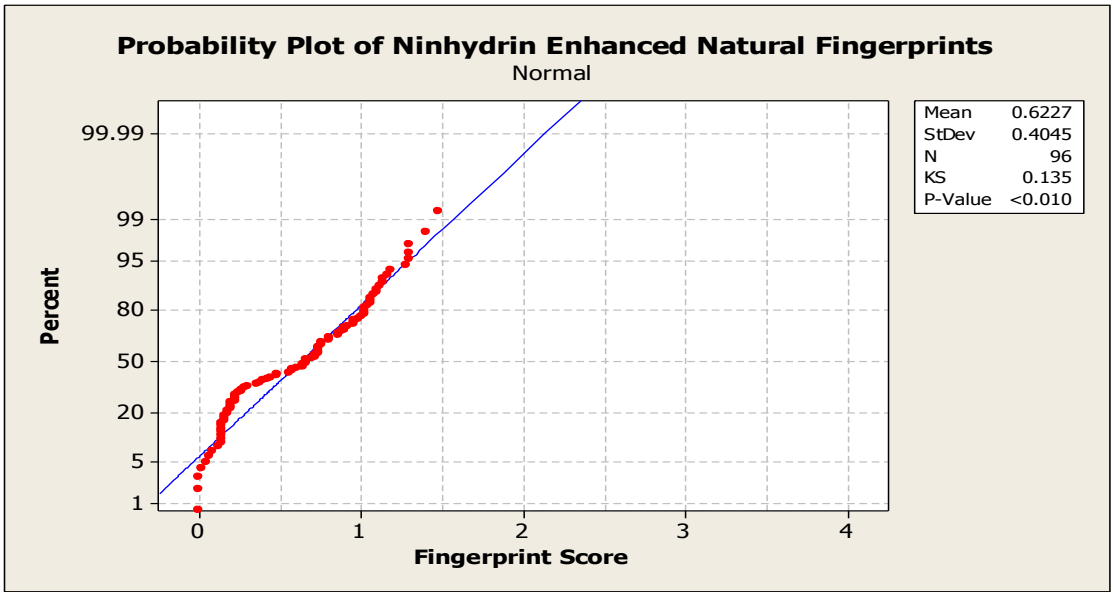



Figure 3-7 Probability plot of ninhydrin enhanced natural fingerprints

The Kolmogorov-Smirnov test will be applied to all data sets for each fingerprint enhancement technique, but only the P values will be given rather than probability plots for each data set.

### 3.2.1 Fingerprints on Paper

The paper used in this study was standard office photocopy paper. This was chosen as it is the most common type of paper submitted to forensic science laboratories for fingerprint enhancement [150]. It is found in most domestic houses, but also in many offices in the business sector. A deliberately eccrine, a deliberately sebaceous, and five depletions of natural fingerprints (from five separate donors) were deposited on each sheet of paper. In total each sample sheet contained 70 fingerprints. These were aged for a set period of time and then exposed to a specific temperature for a set time period according to the experimental protocol previously described. While the samples were being aged, they were placed face up upon shelves in order that the fingerprints deposited onto the substrate were exposed to the atmosphere and other environmental conditions such as heat and light. The scoring method described in section 2.1.3 was used and was split into eccrine, sebaceous, and natural fingerprints for scoring. The techniques used to enhance the fingerprints on the paper were ninhydrin, DFO, and PD.

When the paper was placed in the oven at both 150˚C and 200˚C, even for the minimum exposure time of 10 minutes and aged for one month prior to heat exposure some of the deposited fingerprints were observed to undergo natural enhancement, that is to say the fingerprint was enhanced without the requirement of any chemical or physical development method. This is shown in figure 3-8, which also illustrates the colour change exhibited by the paper at 200˚C at different exposure times. This enhancement has been referred to as the charring process (charring is the resultant residue of the incomplete combustion of a solid when exposed to heat) [151-152].

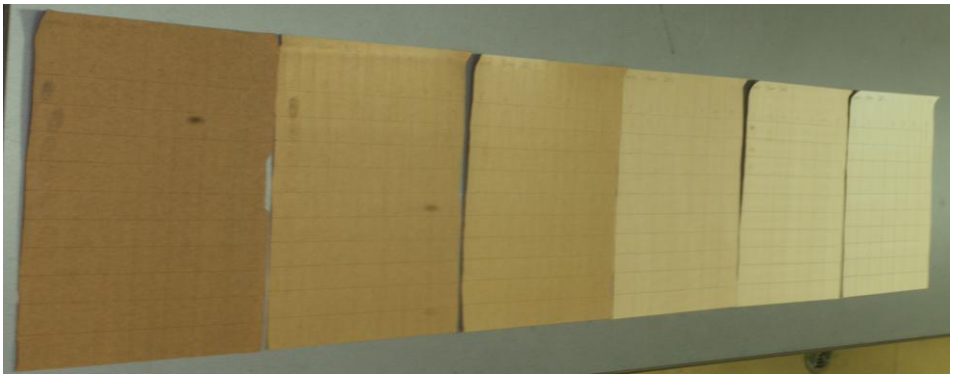
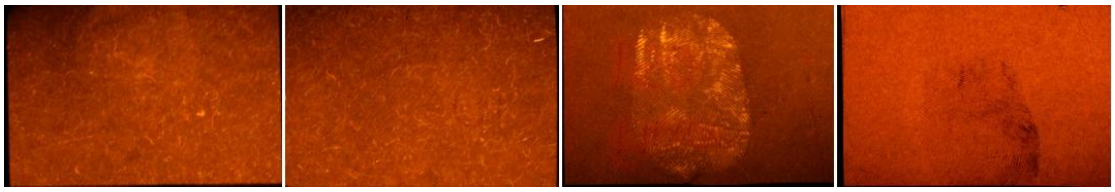


Figure 3-8 Photograph of paper subjected to 200˚C - aged for 1 month.
Fingerprints are visible on the sheets, especially clear on the furthest left two sheets of paper

As a result of observations reported by Townley [147] all of the paper samples were also examined under fluorescent lighting conditions similar to those used with DFO enhanced fingerprints

### 3.2.1.1 Fluorescence

It was found that fingerprints subjected to 150˚C heat naturally fluoresced on exposure to 473-548nm using a 549nm viewing filter. There was no fluorescence of fingerprints at any other temperature. This is shown in figure 3-9.



50˚C 100˚C 150˚C 200˚C

Figure 3-9 Photographs of natural fingerprints on paper subjected to heat for 20min - aged for 1 day

The Kolmogorov-Smirnov test for normality was undertaken for each of the three fingerprint deposits enhanced by fluorescence. As fluorescence did not occur at 50˚C and 100˚C, these negative results have been removed from both the normality tests and the ANOVA tests in order that it would not skew the data. The P values from the Kolmogorov-Smirnov tests were >0.15, >0.15, and 0.134 for eccrine, sebaceous, and natural fingerprint deposits respectively. Therefore, the results that could only be obtained at 150˚C and 200˚C were normally distributed for all three deposits.

The fingerprints exposed to 150˚C and 200˚C, at the various exposure times, ages and classification (eccrine, sebaceous, and natural) were scored and these scores were inputted and analysed using Minitab. A balanced ANOVA test was conducted (as in section 2.2.2) which investigated the factors which were varied during the experiment. The ANOVA analysis was used to determine their effect on the response variable (the fingerprint score). The ANOVA table generated by Minitab is illustrated in table 3-2.

Table 3-2 ANOVA table for fluorescence in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Deposit | 2 | 2.95458 | 1.47729 | 56.46 | 0.000 |
| Age | 3 | 0.11570 | 0.03857 | 1.47 | 0.241 |
| Temp | 1 | 0.03098 | 0.03098 | 1.18 | 0.285 |
| Time | 5 | 1.27712 | 0.25542 | 9.76 | 0.000 |
| Deposit*Age | 6 | 0.14889 | 0.02482 | 0.95 | 0.476 |
| Deposit*Temp | 2 | 0.10167 | 0.05084 | 1.94 | 0.161 |
| Deposit*Time | 10 | 0.45390 | 0.04539 | 1.73 | 0.118 |
| Age*Temp | 3 | 0.44294 | 0.14765 | 5.64 | 0.003 |
| Age*Time | 15 | 0.32594 | 0.02173 | 0.83 | 0.639 |
| Temp*Time | 5 | 0.06376 | 0.01275 | 0.49 | 0.783 |
| Deposit*Age*Temp | 6 | 0.85000 | 0.14167 | 5.41 | 0.001 |
| Deposit*Age*Time | 30 | 0.55447 | 0.01848 | 0.71 | 0.827 |
| Deposit*Temp*Time | 10 | 0.15793 | 0.01579 | 0.60 | 0.798 |
| Age*Temp*Time | 15 | 0.38452 | 0.02563 | 0.98 | 0.498 |
| Error | 30 | 0.78494 | 0.02616 | | |
| Total | 143 | 8.64734 | | | |

The P values in table 3-2 which are in red indicate that these independent factors (*type of deposit* and *time*) have a significant effect on the fingerprint score obtained while the values in green indicate that the interaction between factors taken in combination with each other (*age* and *temperature;* and *type of deposit* and *age* and *temperature*) are also causing a significant effect on the score. All other independent factors or interaction factors were deemed to be insignificant to the fingerprint scored obtained,

i.e. changing the age of the fingerprint or the combination of varying the type of deposit and exposure temperature, did not affect a change in the fingerprint score.

ANOVA analysis within Minitab also has a facility which allows the means of the main effects to be graphically displayed. This is a way of visually demonstrating the means of the independent factors from the ANOVA test. As *type of deposit* and *time* were significant to the response, a main effects plot has been generated in Minitab. This plot is given in figure 3-10. An interaction plot was also generated using Minitab. This interaction plot is shown in figure 3-11.

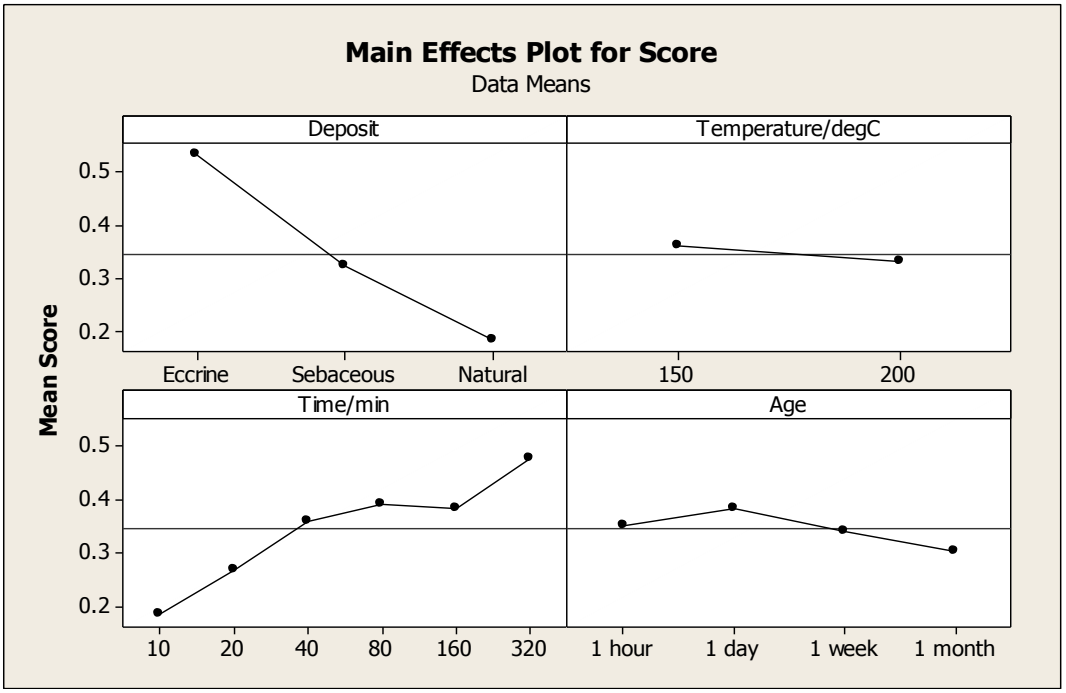



Figure 3-10 Main effects plot for enhancement by fluorescence

The main effects plot shows that the results for each deposit vary greatly from the mean of all scores, with eccrine fingerprints producing greater scores. It also shows the increased enhancement achieved with increased exposure times (except for the dip in result at 160min exposure). The scores separated by either temperature or age show that across all variables, the scores stay relatively close to the mean response, illustrating their lack of effect on influencing the score. However, with the naturally varying fingerprints, the differences between variables could be lessened or even more pronounced than suggested by this work.

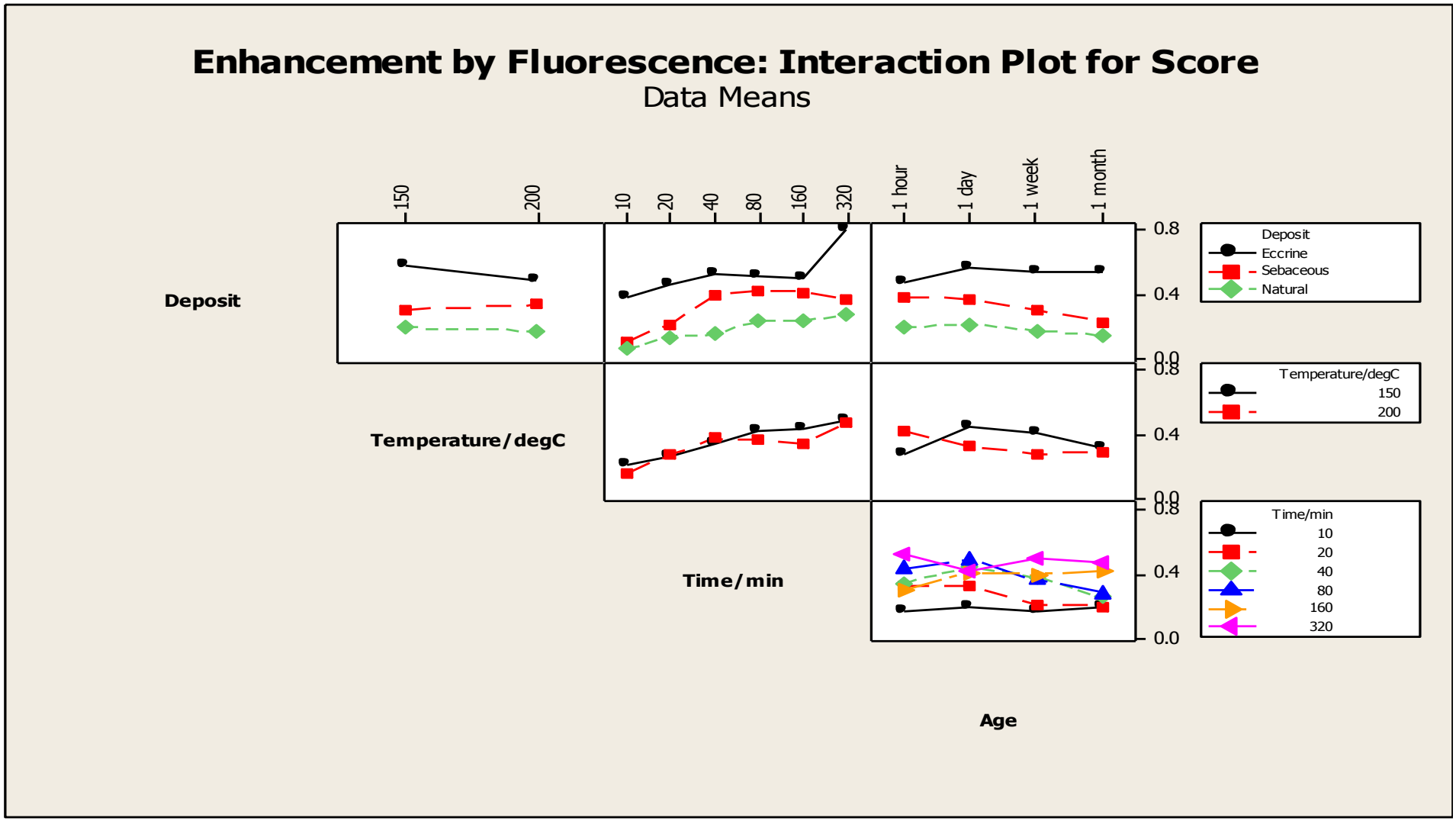



Figure 3-11 Interaction plot for fluorescence enhancement on paper (y-axis = score)

Interaction plots are used to study the change in the mean result by examining the interaction between two variables. In figure 3-11, the *type of deposit* combined with *temperature* demonstrated little change in the scores observed by varying the temperature. The highest scores were achieved by the eccrine deposits compared to the other two, corroborating the results seen in the main effects plot in figure 3-6. The interactions involving *age* on the interaction plot see an increase in score observed with age, again supporting the results shown in the main effects plot. All other interactions showed generally linear responses. Overall, eccrine deposits gave the highest scores across all temperatures, exposure times, and ages. For *temperature* combined with *time,* no results were observed at 50˚C and 100˚C with slightly better results shown at 150˚C and above. This was mirrored with *temperature* combined with *age* which again gave no results at 50˚C and 100˚C and improved enhancement at temperatures at 150˚C and above.

#### 3.2.1.1.1 Fluorescence of Fingerprints on Paper – An Additional Study

As discussed in section 3.1.1.1, three tests were undertaken to investigate the fluorescence phenomenon. The results of these small tests are discussed below.

**Eccrine, Sebaceous and Natural Fingerprints on Recycled Paper**

A preliminary test was conducted to investigate whether the type of fingerprint deposit influenced the resulting natural fluorescence. This was achieved by comparing an eccrine, a sebaceous, and a natural fingerprint over a temperature range of 110˚C to 190˚C in 10˚C increments. These fingerprints were then examined under green light between 473-548nm with a 549nm viewing filter in order to determine which of the three deposits provided the greater fluorescence intensity. The following figure shows the photographs taken of the eccrine, sebaceous, and natural deposits across the temperature ranges. The photographs were taken at various exposure times in order for the photographs produced to be to the best of the camera's ability and are shown in figure 3-12.

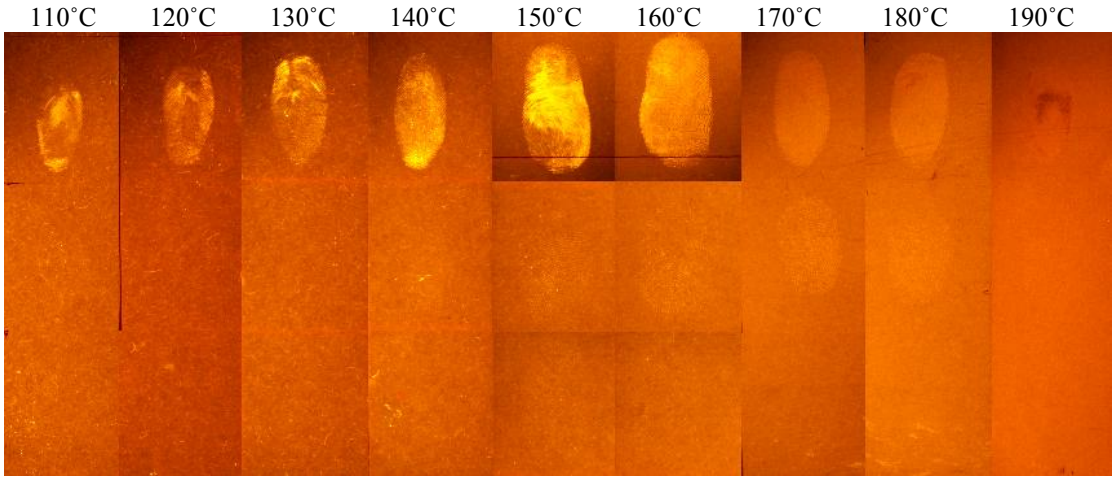



Figure 3-12 Photographs of fingerprints exposed to various temperatures for 20min
(Top to bottom: eccrine, sebaceous, and natural)

By visualisation alone, the eccrine fingerprints produced the brightest fluorescence. These brightness levels were measured using a luminance meter (with a 549nm filter over the lens) and the results are displayed in figure 3-13 (a scatterplot outputted using Minitab).

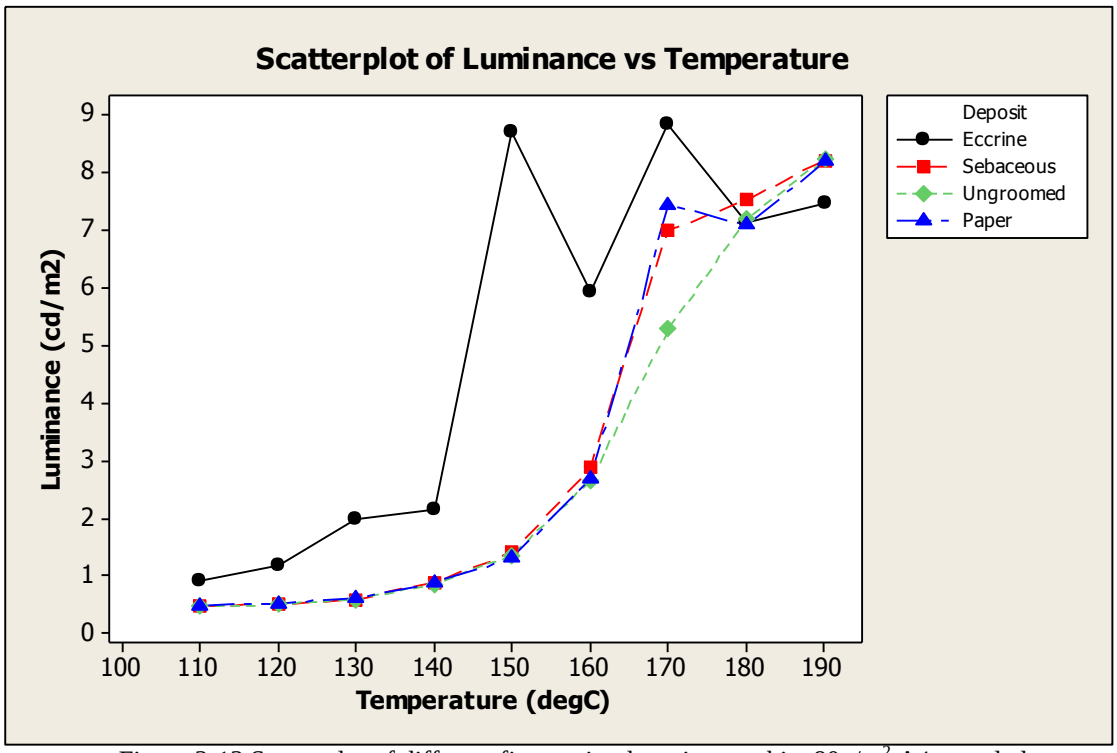



Figure 3-13 Scatterplot of different fingerprint deposits on white 80g/m$^2$ A4 recycled paper

The graph shows that the eccrine fingerprints' luminance is greater than that of the other two deposits and that of the background paper (up to 170˚C). An anomalous result was also observed at 160˚C for the eccrine deposit. The luminance measurements of the sebaceous and natural fingerprints are very similar to the paper fluorescence until 170˚C. These results coincide with the visual interpretation of the fingerprints, indicating it is one or more components of the eccrine fingerprint deposit which are thermally degrading to produce this fluorescence.

**Eccrine Fingerprints on Filter Paper**

Eccrine fingerprints were deposited onto filter paper to examine the effects of different wavebands of light on the resultant fluorescence. Filter paper was used for this part of the experiment because it contains fewer additives that may degrade and add to background fluorescence. These eccrine fingerprints were excited by violet-blue light between 350-469nm with a 476nm viewing filter, blue light between 352-509nm with a 510nm viewing filter, and green light between 473-548nm and a 549nm viewing filter provided by a Quaser 40. This fluorescence was photographed at various exposure times and shown in figure 3-14.

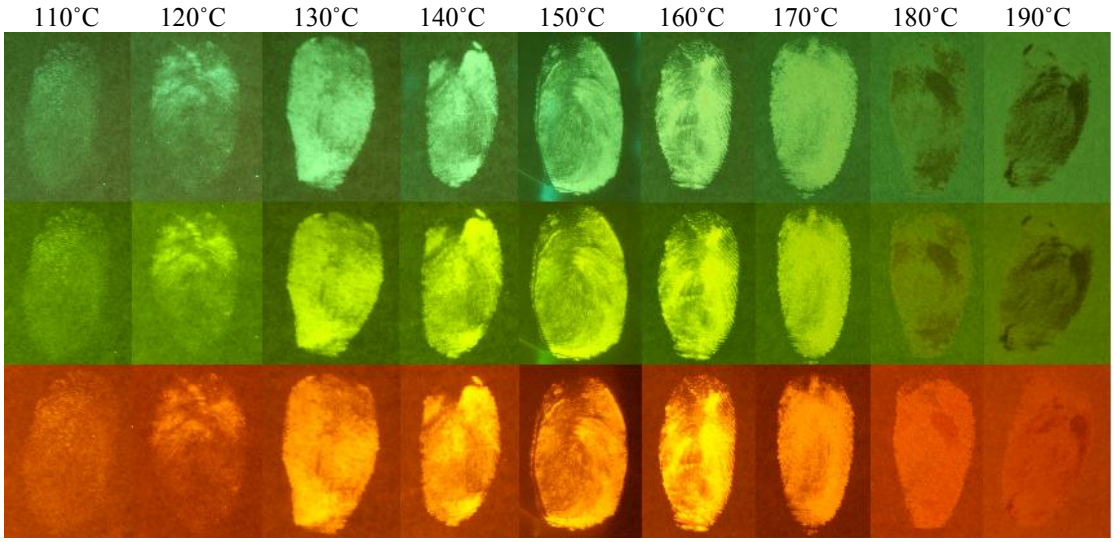



Figure 3-14 Photographs of the eccrine fingerprints exposed to various temperatures for 20min
(Top to Bottom: illumination bands of violet-blue, blue, and green)

The fluorescence for each bandwidth of light was measured using the luminance meter and compared in figure 3-15.

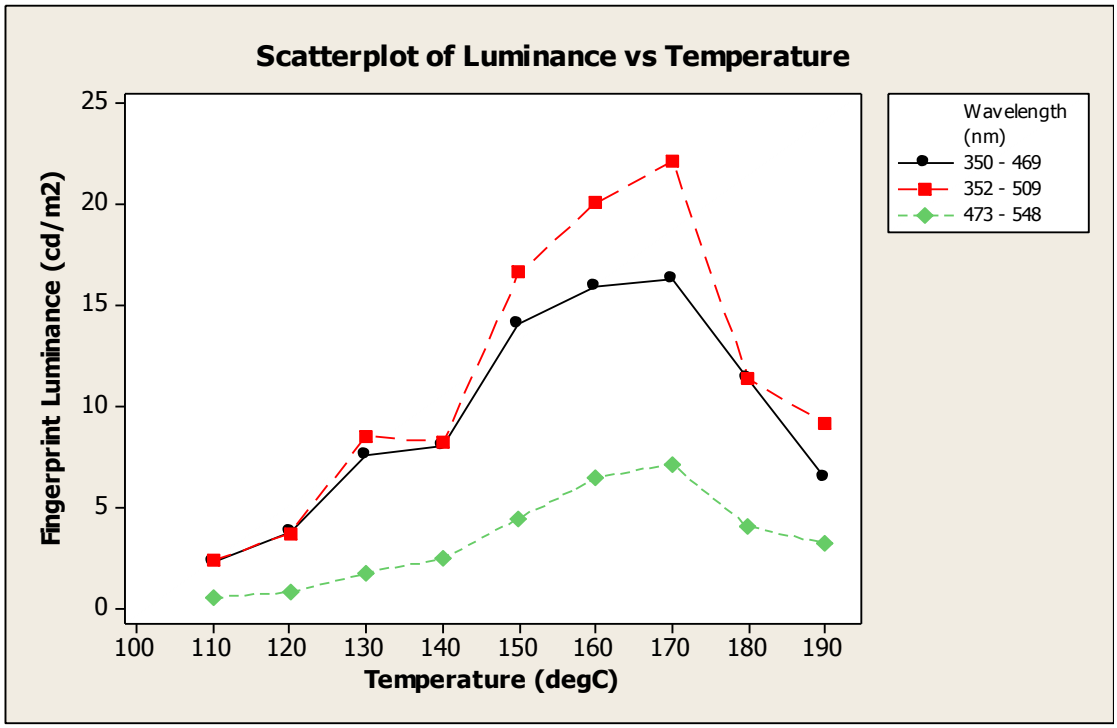



Figure 3-15 Scatterplot of eccrine fingerprint deposits excited by different bandwidths of light on 110mm diameter, hardened, ashless filter paper

The photographs and the graph indicate that light of blue wavelength is producing a brighter fluorescence, followed by violet-blue, and finally green. The photographs were taken at various exposure times automatically selected by the camera. As light of green wavelength was producing the dullest luminance measurements, it was selected for examination of the amino acids, sodium chloride, and urea as if luminance is found with green light, then violet-blue and blue would have to be brighter. Also, the green NdYAG 532nm laser is a common search laser used for examining crime scenes in the UK and as such, is appropriate for speculatively searching large areas for this type of fluorescent fingerprints.

**Exposure of Eccrine Sweat Constituents to Elevated Temperatures**

Eccrine fingerprints consist of a very high percentage of water. The remainder is a complex mixture of organic (amino acids, proteins, and lactate) and inorganic material ($Na^+$, $K^+$, $Cl^-$, and trace metal ions) [90]. This small study concentrated on investigating the effects of the elevated temperatures on the amino acids, sodium chloride, and urea of which the total amounts present in a print is reported to be between 0.3-2.59mg/L, 0.52-7mg/mL, and 10-15mM respectively [153].

Filter paper was used again for this part of the experiment due to its fewer additives and also due to its absorbency in allowing the solutions to dry faster on this substrate than the white 80g/m$^2$ A4 recycled paper. Therefore 10µL of each of the solutions was deposited onto the filter paper and subjected to the same temperatures range.

Figure 3-16 shows the photographs for each amino acid, sodium chloride and urea taken with excitation from green light between 473-548nm and using a 549nm viewing filter.

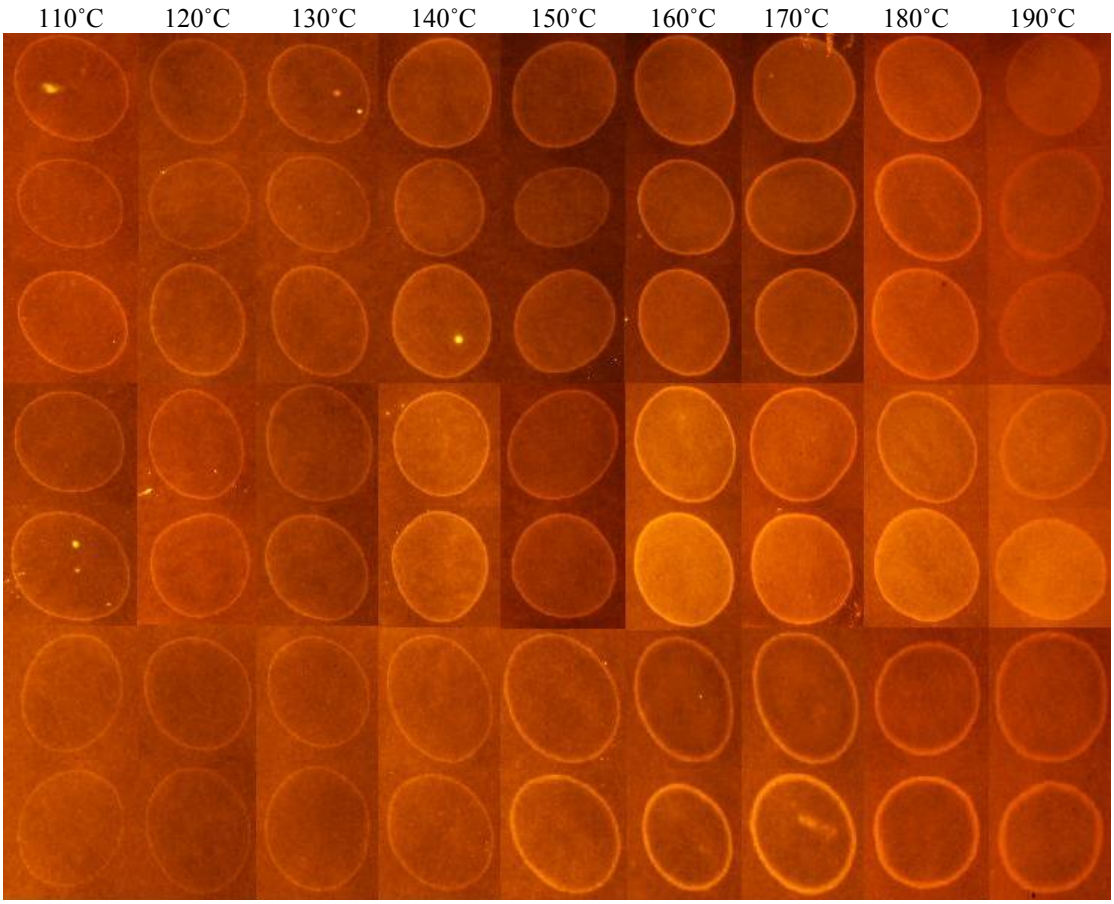



Figure 3-16 Photographs of the solutions exposed to various temperatures for 20min
(Top to Bottom: alanine, aspartic acid, glycine, lysine, serine, sodium chloride, and urea)

Figure 3-17 shows the luminance measurements for the eccrine fingerprint, each amino acid, and the filter paper.

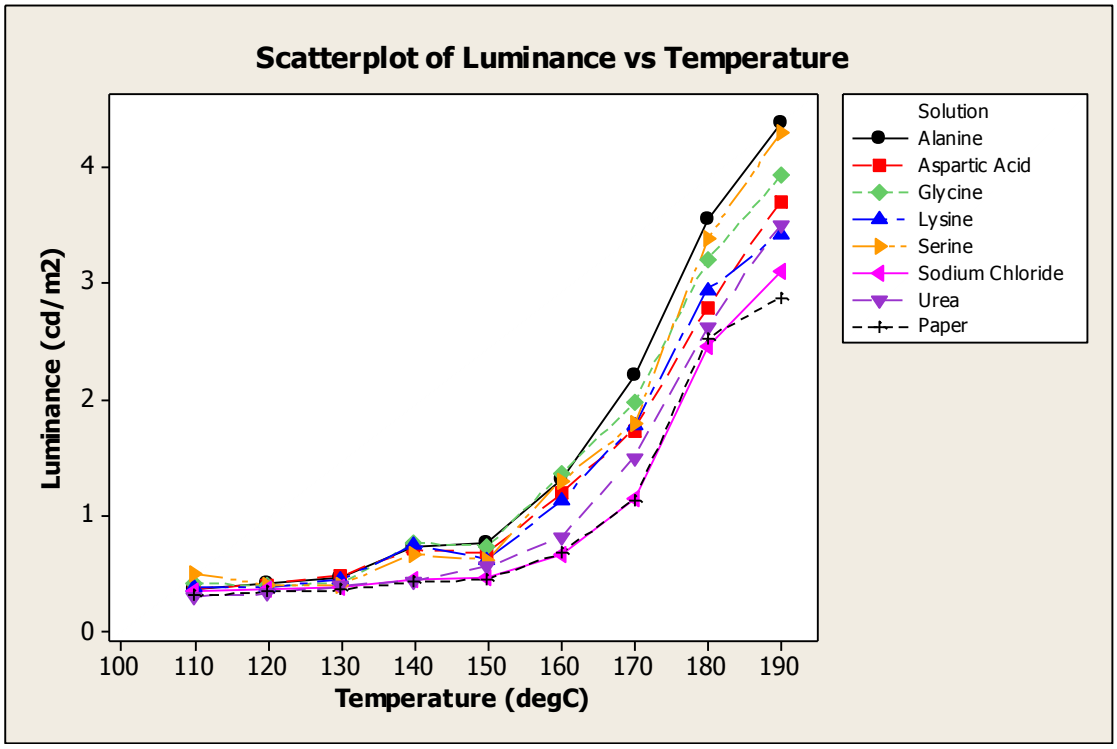


Figure 3-17 Scatterplot of each solution on 110mm diameter, hardened, ashless filter paper

All five amino acids appear to be behaving in a similar manner to each other until 160˚C. They deviate away from the background fluorescence of the paper at 140˚C, where a slight shoulder on the graph is observed before the larger increase observed at 160˚C. Alanine shows the greatest fluorescence, followed by the other four. Urea is also showing a slight fluorescence but the shoulder at 140˚C is not shown, indicating that this is a feature of the amino acid degradation only. Sodium chloride does show a slight fluorescence visually but it is not detected by the luminance meter until 190˚C. The level of fluorescence is used to measure the difference between the amino acids, sodium chloride, and urea, not to obtain an absolute luminance measurement. This would be difficult to ascertain. Firstly, the solutions used in this study were 1mg/mL which is approximately 1000 times more concentrated than reported amino acids concentrations (the difference in concentrations was unavoidable due to limitations in the available equipment in the laboratory this work was carried out in). It has also been reported that amino acid concentrations in fingerprints can vary themselves, in as much as 2 to 20 times depending on collection methods [154].

Richmond-Aylor *et al.*, [155] have undertaken work into identifying the thermal decomposition products of several amino acids using pyrolysis gas

chromatography/mass spectrometry. This work indicated that the decompositions in figure 3-18 were taking place.

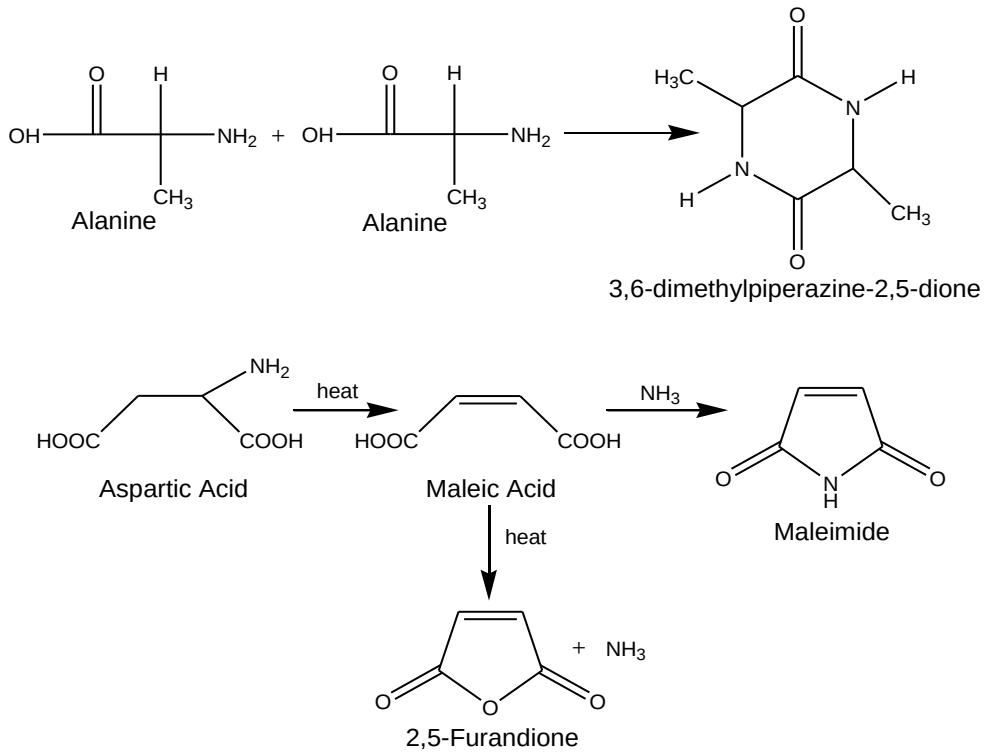



Figure 3-18 Decomposition of amino acids

From the above structures, it is unlikely 3,6-dimethylpiperazine-2,5-dione would fluoresce. Although it is cyclic in nature, it is not planar and also is not completely conjugated [156]. Alternatively, there is a possibility that 2,5-furandione and maleimide could fluoresce. Richmond-Aylor *et al.*, study involved pyrolysis parameters of 50˚C to 500˚C, which is 2.5 times greater than the temperatures in this work. Therefore as the 3,6-dimethylpiperazine-2,5-dione structure does not represent an aromatic molecule, the molecule which is fluorescing may be an intermediate and not the resultant product suggested by Richmond-Aylor *et al.* [155]. Thermal decomposition products of urea could not be found in the literature.

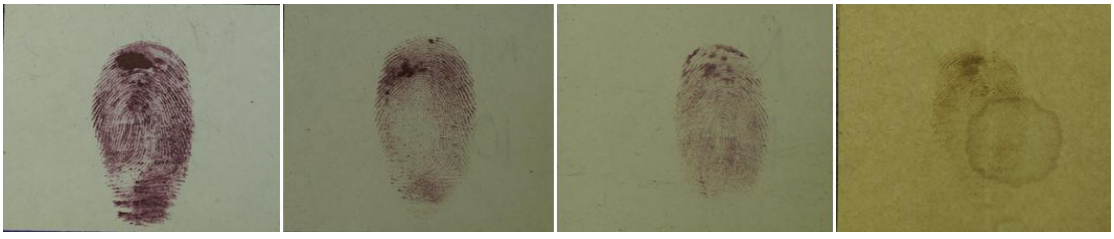
It has been shown that fingerprints will fluorescence under excitation from violet-blue light between 350-469nm with a 476nm viewing filter, blue light between 352-509nm with a 510nm viewing filter, and green light between 473-548nm and a 549nm viewing filter. The study showed that it is the eccrine component of the latent fingerprint which is causing the fluorescence of the fingerprints after exposure to

temperatures in excess of 100˚C, with the maximum luminance observed for fingerprints heated in the range of 160-180˚C. Thermal decomposition of amino acids has been investigated by Richmond-Aylor *et al.,* [155] and has suggested three decomposition reaction products, of which two have the molecular structure which could account for this fluorescence. Sodium chloride and urea are also accountable for the fluorescence in the mark but other eccrine sweat constituents could also be contributing to the fluorescence.

This contradicts the work of Brown *et al.,* [157] who did not observe fluorescence at exposure temperatures below 220°C. However the exposure times used in their study were shorter than those reported here and may account for the discrepancy. Brown *et al.,* also report that the fluorescence was independent of the type of secretion, and suggest that the reason for the observed contrast is that the presence of the fingerprint constituents causes the paper to heat more rapidly than it otherwise would, accelerating charring in these regions. This small study showed that amino acids are contributing to the fluorescence of the fingerprints deposited on paper after heating, and it is not simply preferential charring as suggested by Brown *et al*.

#### 3.2.1.2 Ninhydrin

Ninhydrin is a colour change technique, which requires no subsequent light source exposure for enhancement. Figure 3-18 shows that as it is heated, the white copy paper which carries the fingerprint deposit changes colour. This causes difficulties with contrast of the enhanced fingerprint with the background paper surface on paper exposed to 200˚C. The effect became more pronounced over longer exposure times. The colour of the ninhydrin enhanced mark also changes from purple to brown on heat exposed fingerprints. Ninhydrin enhanced fingerprints are shown in figure 3-19.



50˚C 100˚C 150˚C 200˚C

Figure 3-19 Photographs of natural fingerprints on paper subjected to heat for 20min - aged for 1 day

DeHaan has suggested that 'the proteins in latent deposits can be denatured by high temperatures so that they no longer react, but if the paper has not been charred by the fire it may be worth testing' [5]. This denaturing could explain the change from purple development to brown development for those fingerprints which have experienced higher temperature environments. The fluorescence study showed that a change is occurring to the amino acids upon heating; therefore the brown development of fingerprints on paper exposed to higher temperatures by ninhydrin could be a result of this change. The normality tests for the data had previously been shown in figures 3-5, 3-6, and 3-7. The Kolmogorov-Smirnov test gave P values of >0.15, >0.15, and <0.01 for eccrine, sebaceous, and natural deposits respectively. Although the natural deposit does not indicate normally distributed data when compared to the α value of 0.05, the graph showed that it was approximating a normal distribution. Therefore, ANOVA analysis was used for examining the fingerprints that were enhanced using ninhydrin.

The scores obtained by grading the fingerprints enhanced by ninhydrin were analysed by ANOVA using Minitab. The data is shown in table 3-3.

Table 3-3 ANOVA table for ninhydrin in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Deposit | 2 | 49.7432 | 24.8716 | 308.88 | 0.000 |
| Age | 3 | 3.3402 | 1.1134 | 13.83 | 0.000 |
| Temp | 3 | 70.6955 | 23.5652 | 292.65 | 0.000 |
| Time | 5 | 3.0511 | 0.6102 | 7.58 | 0.000 |
| Deposit*Age | 6 | 1.9498 | 0.3250 | 4.04 | 0.001 |
| Deposit*Temp | 6 | 8.3767 | 1.3961 | 17.34 | 0.000 |
| Deposit*Time | 10 | 1.9761 | 0.1976 | 2.45 | 0.012 |
| Age*Temp | 9 | 8.9032 | 0.9892 | 12.29 | 0.000 |
| Age*Time | 15 | 3.4844 | 0.2323 | 2.88 | 0.001 |
| Temp*Time | 15 | 7.0201 | 0.4680 | 5.81 | 0.000 |
| Deposit*Age*Temp | 18 | 13.2132 | 0.7341 | 9.12 | 0.000 |
| Deposit*Age*Time | 30 | 2.5838 | 0.0861 | 1.07 | 0.391 |
| Deposit*Temp*Time | 30 | 3.0442 | 0.1015 | 1.26 | 0.202 |
| Age*Temp*Time | 45 | 5.0466 | 0.1121 | 1.39 | 0.092 |
| Error | 90 | 7.2470 | 0.0805 | | |
| Total | 287 | 189.6750 | | | |

Using the significance rule outlined, all four independent variables (in red) are significant to the outcome of the fingerprint score, and almost all the interaction effects (in green) except for *type of deposit* combined with *age* and *time* and *type of deposit* combined with the *temperature* and the *time* are significant to the ninhydrin

response. A main effects plot was generated using Minitab and this is given in figure 3-20.

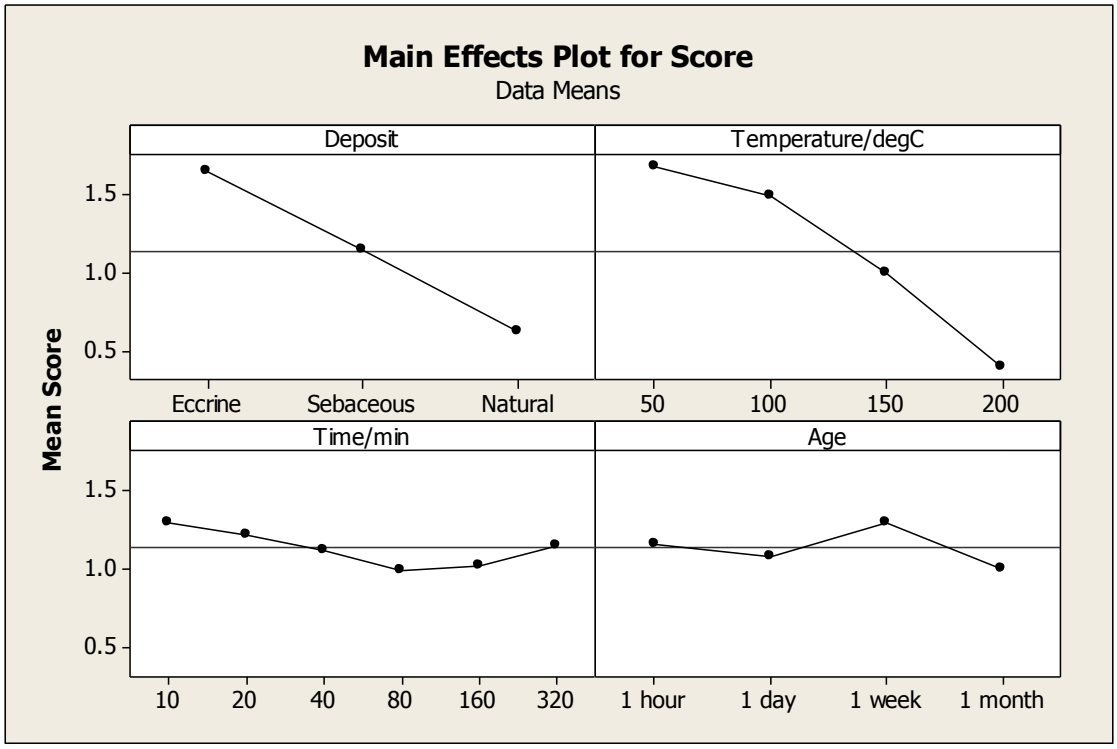



Figure 3-20 Main effects plot for enhancement by ninhydrin

The main effects plot shows that of the four independent factors, it is in fact the *type of deposit* and *temperature* which is influencing the resultant fingerprint score more than the other two variables. This is shown by the larger deviation in the scores from the mean response line. Although *time* and *age* are closer to the mean response, the deviations from the line, although smaller than the other two factors, is still significant to the response, as shown in table 3-3 with all four independent variables being significant to the fingerprint score. These results, both for the main effects plot and the interaction plot cannot be assumed to be absolute values. The natural variation is fingerprints has been well documented, and as such, results given in these enhancements could result in a slight increase or even decrease in difference between each factor.

An interaction plot was also generated on Minitab and this is shown in figure 3-21.

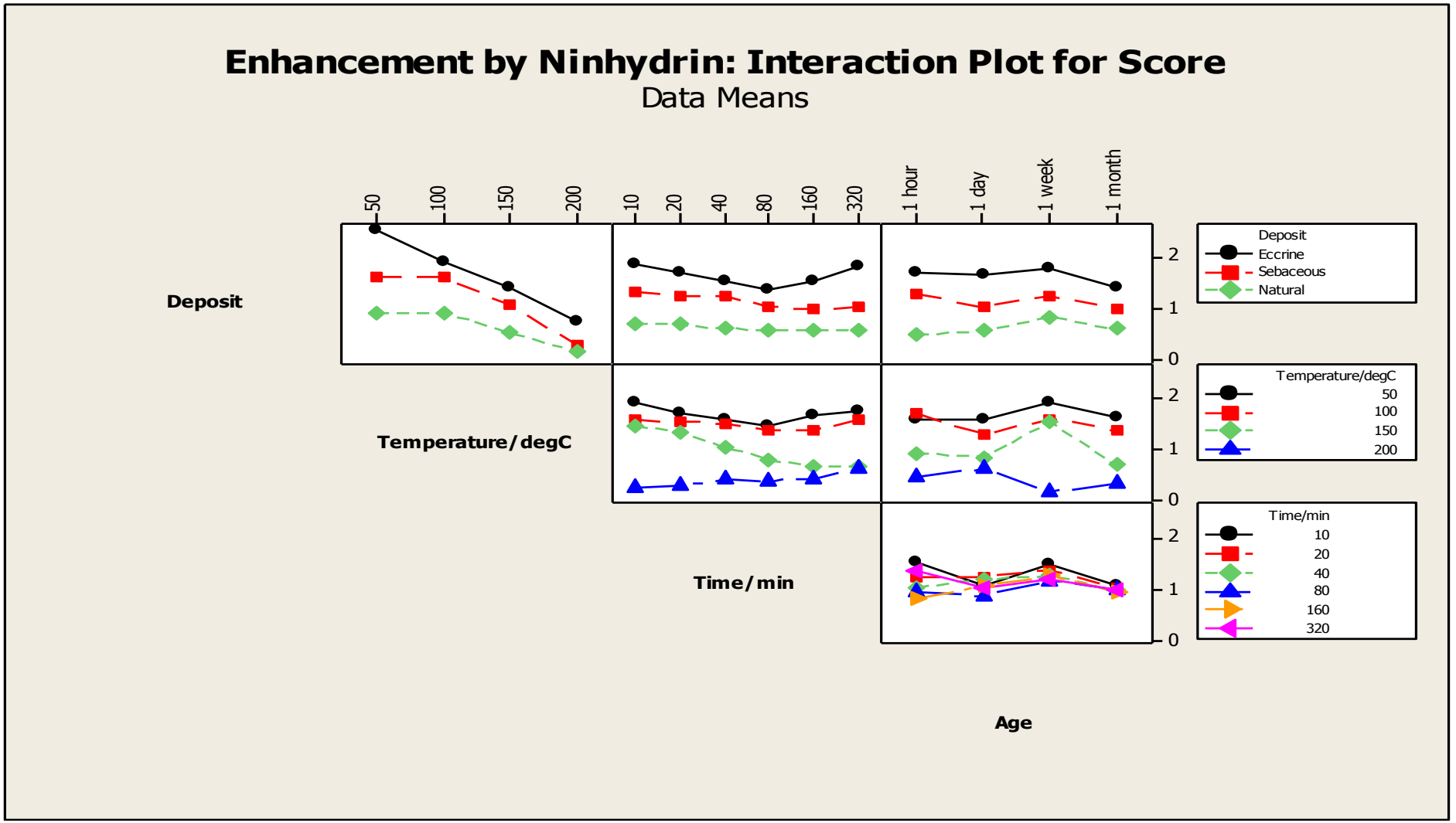


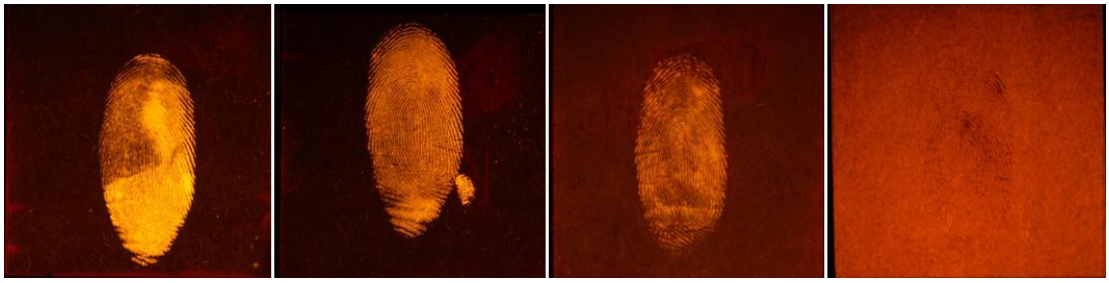

Figure 3-21 Interaction plot for ninhydrin enhancement on paper (y-axis = score)

Investigating the means for *type of deposit* combined with *temperature*, all three types of deposits are decreasing their score as the temperature increases and eccrine deposits produce the highest scores. For *type of deposit* combined with *time* and *age*, the reported scores are the same across all times (except a slight dip at 80min exposure time) and ages. Again the eccrine deposits are giving the highest scores. Analysing *temperature* combined with *time* and *age*, the highest scores are observed at the lower temperatures and the scores are reasonably linear across all times and ages. The fingerprint scores associated with *time* combined with *age* indicated that the fingerprints were enhanced to the same degree irrespective of age and exposure time.

The results of the ANOVA analysis and the main effects and interaction plots demonstrated that eccrine deposits scored better over all of the variables than sebaceous deposits. This is unsurprising as ninhydrin reacts specifically with the amino acids present in the eccrine component of a latent fingerprint. Ninhydrin performs better than fluorescence alone at 200˚C but it is limited when the heat exposed paper is very dark and the fingerprint is exposed to high temperatures for longer times.

### 3.2.1.3 DFO

DFO is an enhancement reagent that reacts with the amino acids present in the fingerprint deposits and enhanced fingerprints are only visible when viewed under specific lighting conditions (between 473-548nm and using a 549nm viewing filter). It was found that as the paper changed colour, the contrast between the fluorescent fingerprint and the background changed. An example of the changes in this contrast is shown in figure 3-22.



50˚C 100˚C 150˚C 200˚C

Figure 3-22 Photographs of natural fingerprints on paper subjected to heat for 20min - aged for 1 day

Initially the fingerprints fluoresce but as the temperature increased, the paper substrate also started to fluoresce and as a consequence contrast became an issue. This explains the poorer scores observed at 150˚C and 200˚C.

The Kolmogorov-Smirnov normality test gave P values for eccrine deposits of 0.045, and for sebaceous and natural deposits to be <0.010. Although all three results indicated nonparametric data, as they still approximated a normal distribution in their graphs, ANOVA testing was continued.

The grading scores for the DFO enhanced fingerprints were analysed using ANOVA. Table 3-4 is the data generated by the statistical package.

Table 3-4 ANOVA table for DFO in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Deposit | 2 | 34.7184 | 17.3592 | 167.01 | 0.000 |
| Age | 3 | 0.8961 | 0.2987 | 2.87 | 0.041 |
| Temp | 3 | 272.6040 | 90.8680 | 874.25 | 0.000 |
| Time | 5 | 1.5232 | 0.3046 | 2.93 | 0.017 |
| Deposit*Age | 6 | 8.8863 | 1.4810 | 14.25 | 0.000 |
| Deposit*Temp | 6 | 7.8349 | 1.3058 | 12.56 | 0.000 |
| Deposit*Time | 10 | 7.0237 | 0.7024 | 6.76 | 0.000 |
| Age*Temp | 9 | 20.6931 | 2.2992 | 22.12 | 0.000 |
| Age*Time | 15 | 3.1608 | 0.2107 | 2.03 | 0.021 |
| Temp*Time | 15 | 17.5267 | 1.1684 | 11.24 | 0.000 |
| Deposit*Age*Temp | 18 | 14.9040 | 0.8280 | 7.97 | 0.000 |
| Deposit*Age*Time | 30 | 5.4280 | 0.1809 | 1.74 | 0.024 |
| Deposit*Temp*Time | 30 | 4.7380 | 0.1579 | 1.52 | 0.068 |
| Age*Temp*Time | 45 | 7.1453 | 0.1588 | 1.53 | 0.045 |
| Error | 90 | 9.3545 | 0.1039 | | |
| Total | 287 | 416.4370 | | | |

All four independent variables are significantly different to each other and all except one interaction (for *deposit* combined with *temperatur*e and combined with *time*) are statistically significant also. Of the four independent variables, two have a P-value of 0.000 whereas two have P values which are greater than zero. Therefore, in thinking about the output on the main effects plot, the deviations from the mean response line will be larger for *type of deposit* and *temperature* than for the other two factors. The ANOVA values for *age* and *time* are closer to the α value of 0.05, indicating that although the results are significant to the response, they are only marginally significant using the ANOVA test parameters.

The main effects plot for the fingerprints enhanced by DFO is given in figure 3-23.

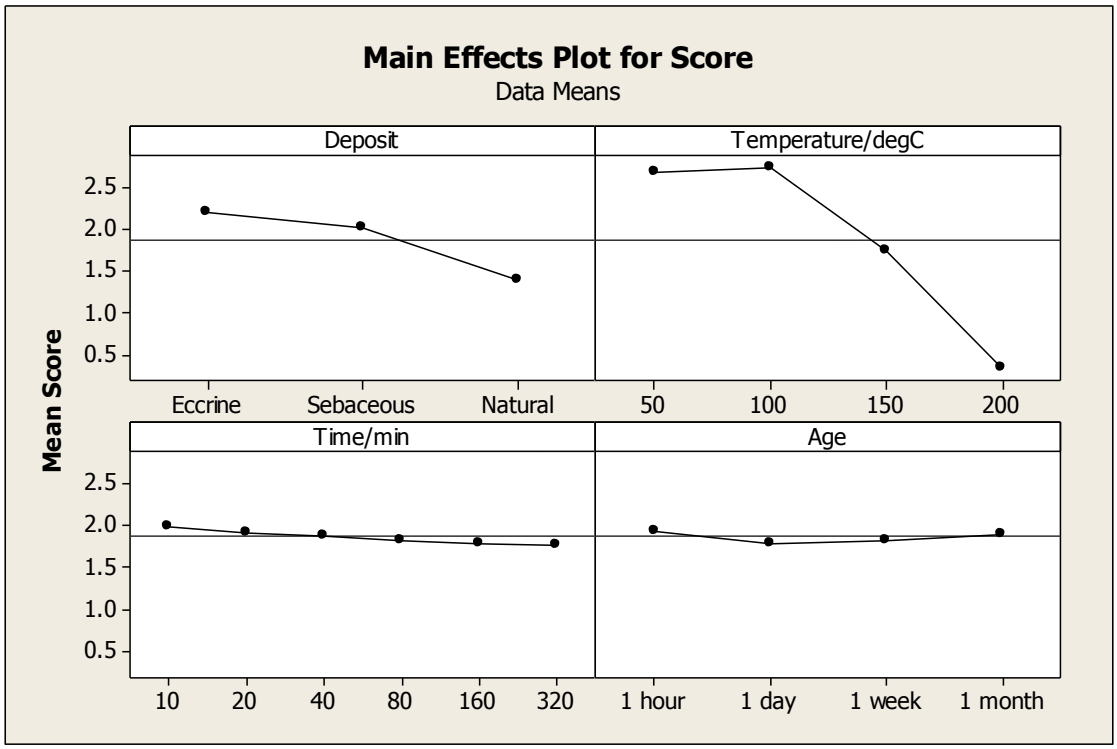



Figure 3-23 Main effects plot of enhancement by DFO

The main effects plot in figure 3-23 shows that the most significant factor to the response factor is *temperature* closely followed by *type of deposit*. Although by comparison to the main effects plot for fluorescence enhancement (figure 3-10), which displayed two factors which were not significant to the response, the lines appeared to deviate more from the mean response line than in this main effects plot where the two factors with the least deviation from the mean response line were significant. This is due to the scales on the y-axis. Interestingly, the mean score for each of the three deposits is higher than the mean scores obtained for ninhydrin enhancement.

The interaction plot for the scores obtained by enhancing fingerprints by DFO on paper is given in figure 3-24.

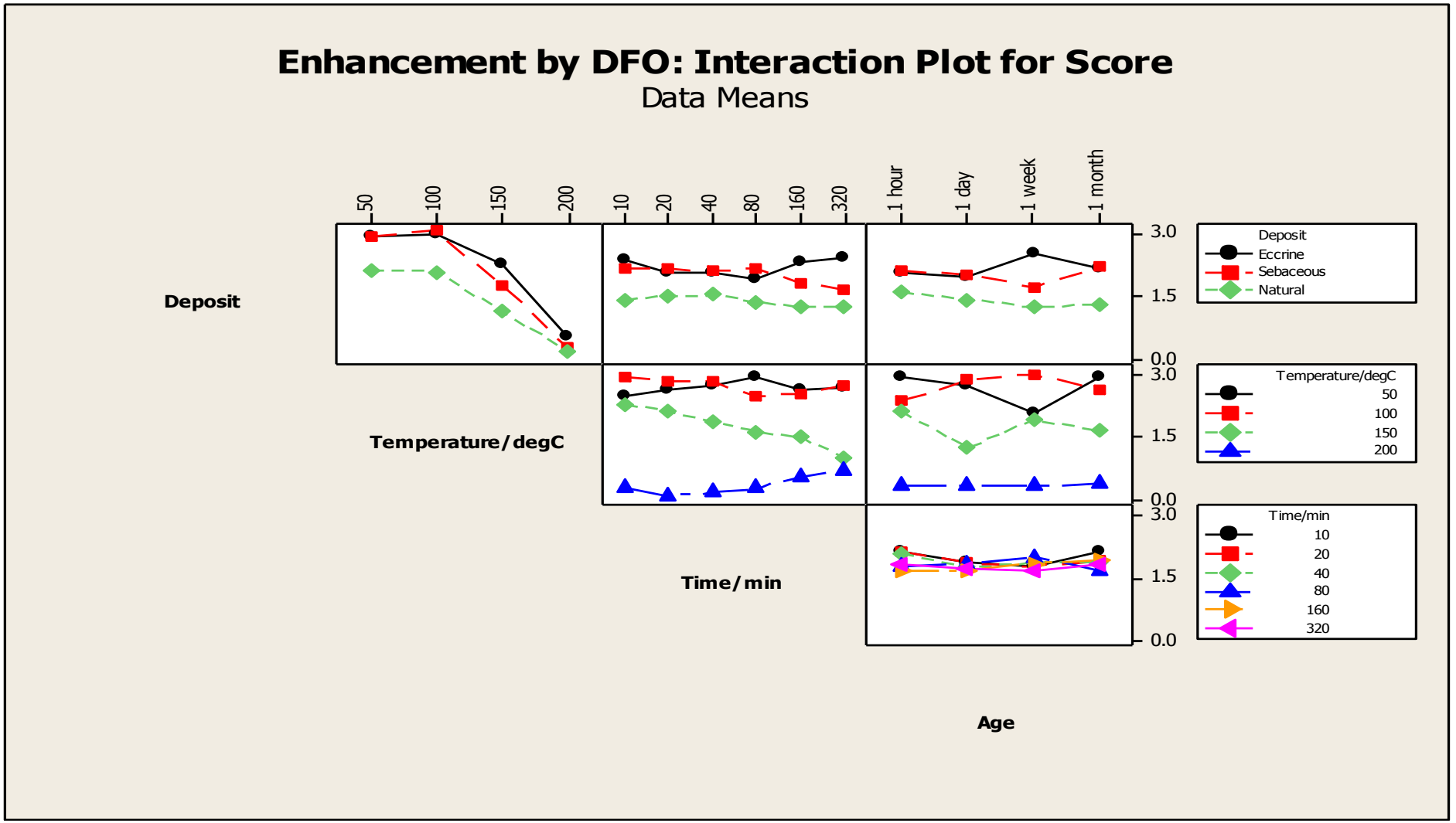


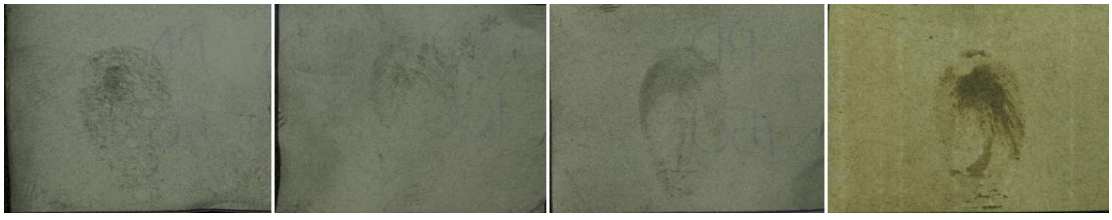

Figure 3-24 Interaction plot for DFO enhancement on paper (y-axis = score)

Figure 3-24 indicates that when the *type of deposit* interacts with *temperature*, the scores associated with all three types of deposits decrease as the temperature increases with eccrine deposits demonstrating the highest scores. The examination of the *type of deposit* combined with *time* or *age* on the chart shows only slight differences between scores obtained with varying the exposure time and age, with eccrine deposits giving consistently higher scores. For *temperature* combined with *time,* greater scores are produced at the lower exposure temperatures. Combining *temperature* and *age* provides erratic results, with samples exposed at 200˚C performing poorly. The interaction between *time* and *age* produced similar scores across all four ages of fingerprints.

As DFO preferentially enhances fingerprints containing amino acids, the higher scores obtained for eccrine deposits are understandable. However, this does not explain the increase in score observed for the sebaceous deposit. A reasonable explanation for this anomaly could be due to the natural variation in fingerprints that as the donor 'charged' their fingertip with sebaceous material they inadvertently picked up a large amount of eccrine material too. The results indicate that DFO is a fairly successful process at all ages of deposit and heat exposure times, but when the paper was subjected to 200˚C, as with ninhydrin, the enhancement technique performs poorly.

### 3.2.1.4 Physical Developer (PD)

PD is the only fingerprint technique which is known to enhance fingerprints on wetted paper. In practical terms, it is important to know whether PD will enhance fingerprints which have been exposed to elevated temperatures as wetted paper is expected from fire scenes as a result of normal suppression activities. Photographs of PD enhanced fingerprints at various temperatures are shown in figure 3-25.



50˚C 100˚C 150˚C 200˚C

Figure 3-25 Photographs of natural fingerprints on paper subjected to heat for 20min - aged for 1 day

Kolmogorov-Smirnov normality tests were also undertaken on the PD enhancement data for each deposit. The P values obtained were 0.043, <0.010, and >0.15 for the eccrine, sebaceous and natural deposits respectively. From this test, it can be seen that the eccrine data is almost normally distributed; the sebaceous data is not although its graph shows that it is approximating a normal distribution, and the natural data is normally distributed. As such, ANOVA analysis of the scores of the PD enhanced fingerprints was undertaken. The following table (table 3-5) shows the results of this analysis.

Table 3-5 ANOVA table for PD in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Deposit | 2 | 6.1880 | 3.0940 | 26.23 | 0.000 |
| Age | 3 | 8.5189 | 2.8396 | 24.07 | 0.000 |
| Temp | 3 | 7.7137 | 2.5712 | 21.80 | 0.000 |
| Time | 5 | 6.7436 | 1.3487 | 11.43 | 0.000 |
| Deposit*Age | 6 | 13.8656 | 2.3109 | 19.59 | 0.000 |
| Deposit*Temp | 6 | 9.4176 | 1.5696 | 13.31 | 0.000 |
| Deposit*Time | 10 | 1.2346 | 0.1235 | 1.05 | 0.412 |
| Age*Temp | 9 | 23.9355 | 2.6595 | 22.54 | 0.000 |
| Age*Time | 15 | 20.1582 | 1.3439 | 11.39 | 0.000 |
| Temp*Time | 15 | 8.8293 | 0.5886 | 4.99 | 0.000 |
| Deposit*Age*Temp | 18 | 15.5400 | 0.8633 | 7.32 | 0.000 |
| Deposit*Age*Time | 30 | 3.6359 | 0.1212 | 1.03 | 0.444 |
| Deposit*Temp*Time | 30 | 3.5708 | 0.1190 | 1.01 | 0.468 |
| Age*Temp*Time | 45 | 18.9047 | 0.4201 | 3.56 | 0.000 |
| Error | 90 | 10.6172 | 0.1180 | | |
| Total | 287 | 158.8736 | | | |

The ANOVA analysis shows that all independent variables are significant to the outcome of the fingerprint score. It also shows that all but three interaction effects (*type of deposit* combined with *time*, *type of deposit* interacting with *age* and *time*, and *type of deposit* interacting *temperature* and *time*) have a significant effect on the response.

As all independent factors are significant to the fingerprint score, and all P values are zero, the main effects plot should indicate variability in each factor distinctly away from the mean response line. The main effects plot is shown in figure 3-26.

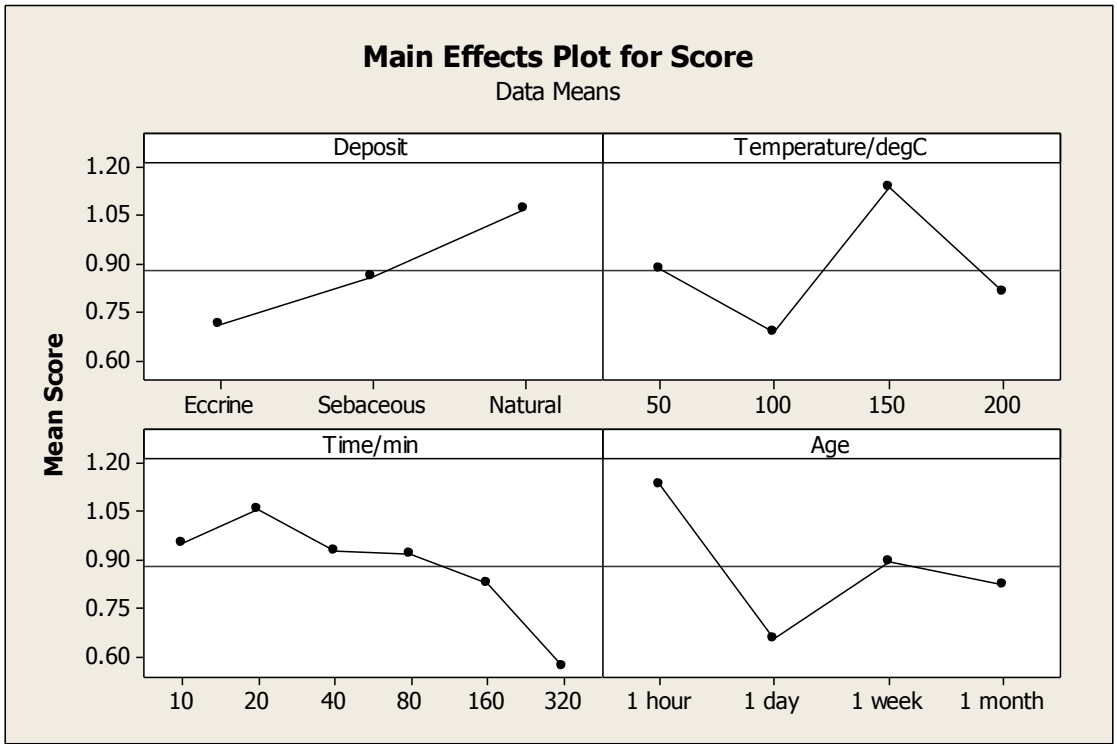


Figure 3-26 Main effects plot for enhancement by PD

The main effects plot does show wide variation in each factor from its mean response. In comparison to the other enhancement methods, this is the only technique which shows natural fingerprint scores to be higher than the other two deposits. There is no trend visible for varying the exposure *temperature* and likewise for the *age* of the fingerprints. By varying the exposure *time*, the fingerprint scores generally decrease with overall increased exposure time. Therefore, overall, based on these results, PD is a technique in which the results cannot be predicted and perhaps, highlights the variability in fingerprints more than the other techniques.

The interaction plot for this technique is shown in figure 3-27. Even though these variables have a significant effect, no clear trends were in evidence for any specific type of deposit.

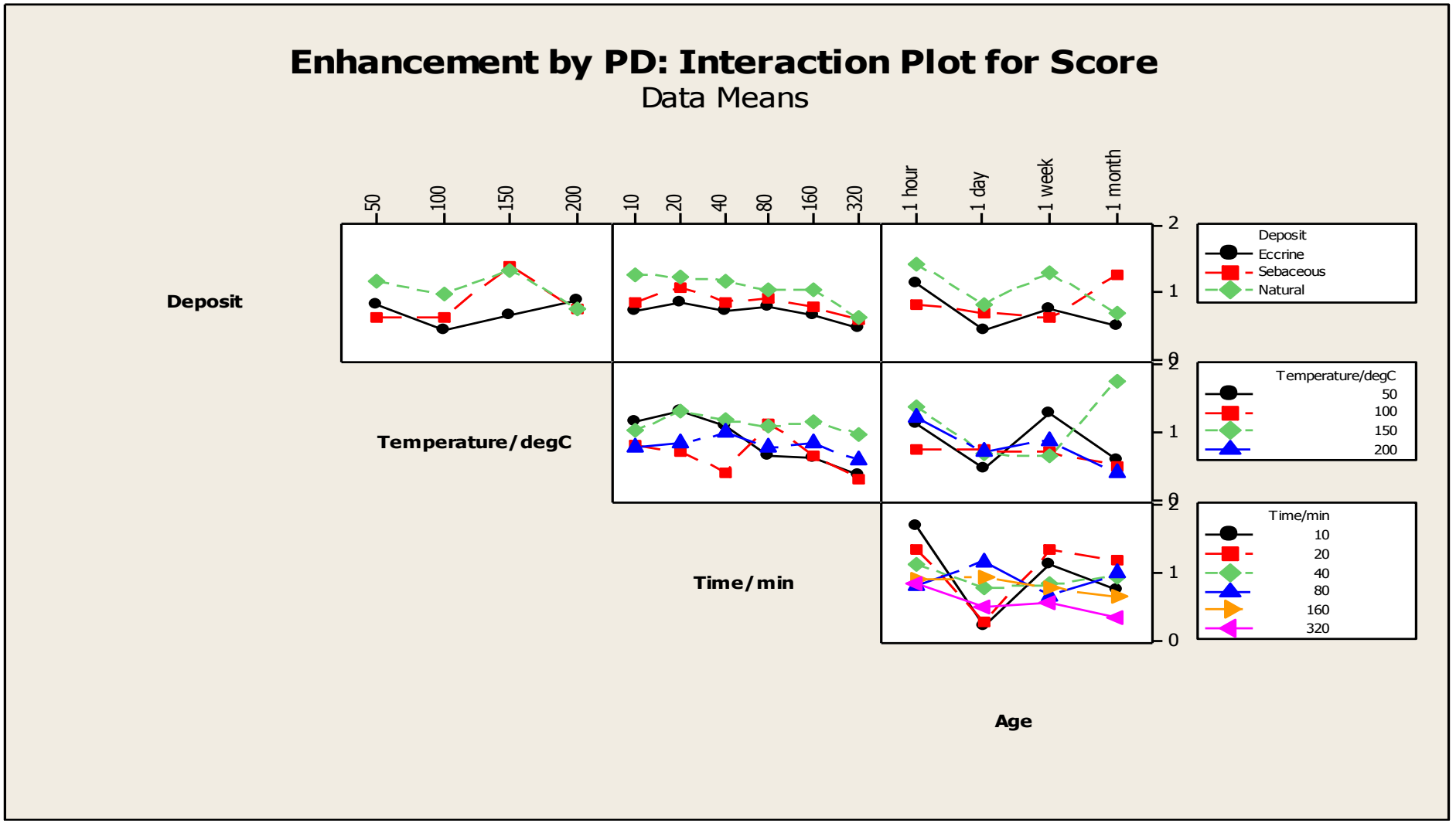



Figure 3-27 Interaction plot for PD enhancement on paper (y-axis = score)

Mimicking the main effects plot, no clear trends are shown in the interaction data and the results are quite erratic. It is worthy to note that PD enhanced the natural fingerprint deposits best out of the three deposits.

The results from the ANOVA test and the main effects and interaction plots indicate that PD behaves in a much more erratic fashion and there are no trends within the results. The two plots also demonstrated that natural fingerprints produced higher fingerprint scores than eccrine or sebaceous deposits with this enhancement technique. This is a good result in terms of the practical application of the technique. The result obtained was surprising as PD is proposed to react with the sebaceous components of the fingerprint [110]. This suggests that the PD reaction is more complicated than originally proposed in the literature.

#### 3.2.1.5 Enhancement Technique Comparison

A comparison of the fingerprint enhancement techniques for the fingerprints exposed to elevated temperatures that had been deposited on paper was also undertaken. As with each enhancement technique independently, fingerprint variability may sway the results. This cannot be measured, and as such, conclusions based on fingerprint variability cannot be exact.

As the results obtained from each of the enhancement techniques were either normally distributed or approximated normal distribution, a one-way ANOVA test was conducted to compare the overall scores of the techniques. This measured the effectiveness of the enhancement technique as a whole. One-way ANOVA is calculated in the same way as a balanced ANOVA test, except that only one factor is tested. It is tested against the α value of 0.05. Table 3-6 gives the results of the one-way ANOVA test.

Table 3-6 One-way ANOVA test for score

**One-way ANOVA: Score versus Technique**

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Technique | 3 | 424.909 | 141.636 | 207.86 | 0.000 |
| Error | 1148 | 782.244 | 0.681 | | |
| Total | 1151 | 1207.153 | | | |

The P-value of zero is less than the α value, therefore there is a significant difference between the techniques. Figure 3-28 gives the main effects plot which compares the scores for each of the enhancement techniques.

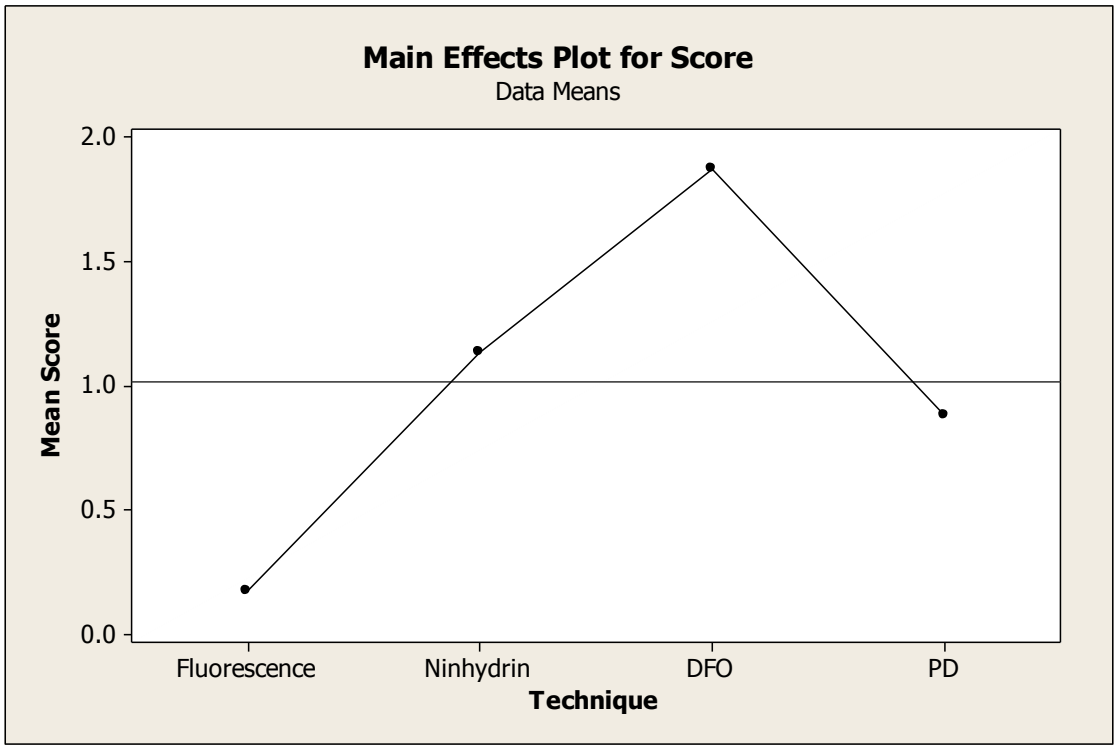



Figure 3-28 Main effects plot comparing enhancement techniques

The main effects plot shows that overall, the fingerprints enhanced by DFO have significantly higher scores than the other enhancement *techniques*. On average, ninhydrin is the second highest scoring technique, followed by PD, and finally fluorescence. It is unsurprising that the DFO results are superior to the scores obtained from the ninhydrin enhancement; the sensitivity of DFO is greater than ninhydrin in that it can react with smaller traces of amino acids [108], as such providing an increase in enhancement scores. This confirmed the work of Bleay *et al.,* [119] and Bradshaw *et al.,* [117, 120] where better results are obtained on paper with DFO compared with ninhydrin.

It would also be advantageous to compare the results obtained for each enhancement technique with respect to type of deposit, to evaluate the enhancement techniques especially for natural fingerprints since these are closest to 'real' fingerprints in this work. An interaction plot has been generated only for this interaction, with the plot given in figure 3-29.

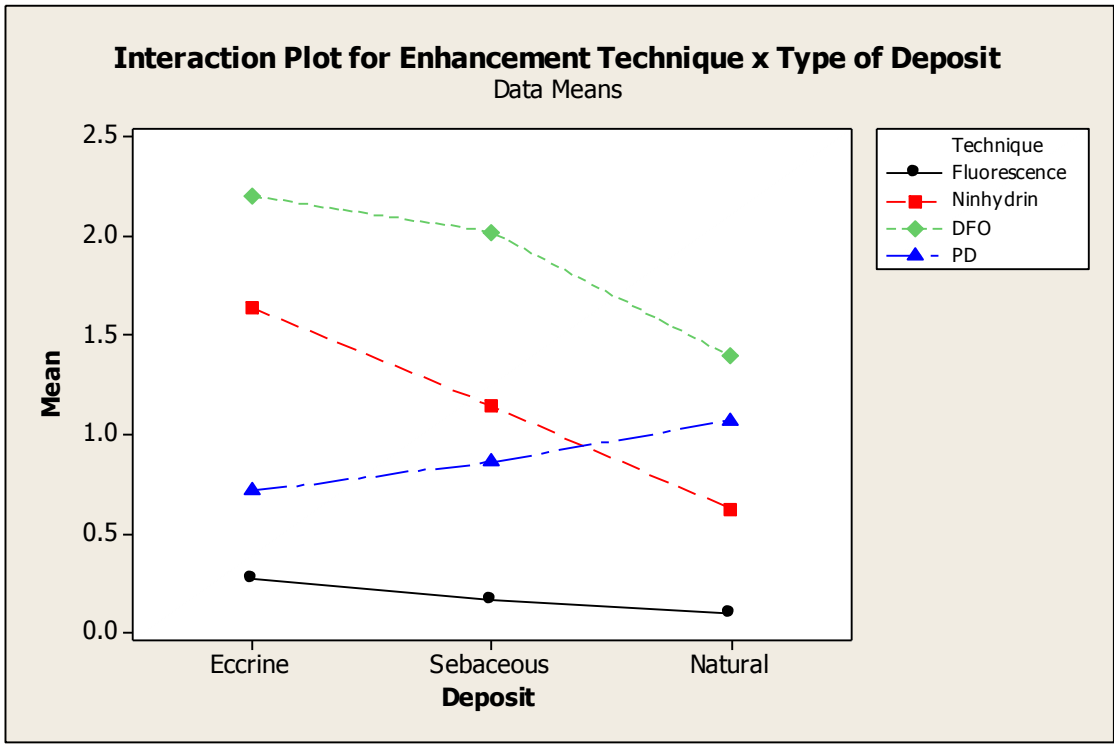



Figure 3-29 Interaction plot comparing enhancement technique and type of deposit (y-axis = score)

Interesting results were observed in the interaction plots generated for comparing the enhancement techniques. When examining the *technique* combined with the *type of deposit*, the fingerprint scores enhanced by DFO were greater over all three deposits, but PD replaced ninhydrin as the next placed technique for natural fingerprints only. As natural fingerprints are the closest representation to 'real' fingerprints, it was important to reflect that PD was generally a more successful technique than ninhydrin, although DFO is superior to both.

Fluorescence is generally used before any chemical or physical enhancement technique in operational circumstances; however it gave the poorest results in the experiments undertaken. It would appear that on dry paper DFO produced better results compared with the other technique. When examining the results for natural fingerprints (which mimics fingerprints encountered in operational scenarios), PD is the second most successful technique. It also has the advantage that it can be used on wet paper. Ninhydrin results were poorer than those fingerprints enhanced by DFO, but were better than the scores recorded for PD enhanced fingerprints for two of the deposit types.

Due to issues surrounding the contrast of ninhydrin especially on badly charred paper, it is recommended to be used only as part of a sequential process on dry paper. The temperature to which the paper was exposed was also a factor when comparisons were made within each enhancement technique, with generally lower recoverability of fingerprints exposed to higher temperatures. The length of time the paper was exposed and the age of the fingerprints did not influence the results obtained as much as the temperature change.

# CHAPTER 4: FINGERPRINTS ON NON POROUS SURFACES

## 4.1 Experimental

Similarly to the experiments performed on the porous surface, a series of systematic experiments were undertaken in order to establish the range of temperatures and the exposure times at these temperatures that latent fingerprints can withstand and still be recoverable on uPVC, glass and ceramic. Three types of fingerprints (eccrine, sebaceous and natural) were repetitively examined and graphically interpreted in each case. As before, the objective was to be in a position to propose an optimum development technique at the various temperatures examined.

### 4.1.1 Fingerprints on uPVC

uPVC was purchased from Wickes (St. Albans, Hertfordshire) and cut to A4 size. It was cleaned using Fairy washing-up liquid (Proctor and Gamble, Brooklands, Weybridge), air dried, washed with ethanol (Hayman Limited, Witham, Essex), and air dried once again. A grid was drawn onto the uPVC surface (figure 3-1) and the fingerprint samples were obtained as in section 3.1.1.

Fingerprints were subjected to different environmental exposure times before being subjected to different temperatures for different periods of time, prior to enhancement. A summary of the experiments is given in table 4-1.

Table 4-1 Summary of exposure to heat experiments

| Substrate | Ageing Time | Temperature (˚C) | Exposure Time (min) | Treatment |
|---|---|---|---|---|
| uPVC | 1 hour, 1 day, 1 week, 1 month | 50 | 10, 20, 40, 80, 160, 320 | 1. Black Magnetic Powder<br>2. Black Magnetic Powder → Powder Suspension<br>3. Superglue - BY40 |
| | | 100 | | |
| | | 150 | 10, 20 | |

Due to the thermal decomposition of uPVC at approximately 140˚C [158], the uPVC was only heated to 150˚C for a maximum of 20min to minimise human exposure to decomposition products.

**Superglue followed by BY40**

Superglue fuming was achieved by using Cyanobloom (3g) (Foster + Freeman, Evesham, Worcestershire). The uPVC was placed in the superglue cabinet (Mason Vactron MVC5000, Evesham, Worcestershire) and closed. The superglue fuming programme is given in table 4-2.

Table 4-2 Superglue fuming cycle programme

| Superglue Programme | Time (min) | Temperature (˚C) | Relative Humidity (%RH) |
|---|---|---|---|
| Initialising | 1 | 24 | 66 |
| Humidity | 15 | 25 | 70 |
| Glue | 20 | 120 | 80 |
| Initialising | 1 | 110 | 80 |
| Purge | 40 | reducing to 29 | reducing to room humidity |

Once completed, the uPVC was dyed using a fluorescent dye by dipping each plate in BY40 (2g in 1L of ethanol) (Keystone, Huddersfield, West Yorkshire), rinsed in cold water and left to dry overnight. The uPVC was examined under light of 385-469nm and viewed using a 476nm viewing filter [146].

**Black Magnetic Powder**

Black magnetic powder was selected for enhancement out of all the powders based on HOSDB guidelines [159]. The magnetic applicator was dipped into the black magnetic powder (CSI Equipment Ltd, Northampton, Northamptonshire). The applicator was drawn over the uPVC without the head of the applicator making contact with the plastic. Once this was completed, the powder was removed from the applicator and the clean applicator was drawn over the surface to remove any loose powder before examining the substrate [146].

**Powder Suspension**

After the powder enhancement had been examined, the uPVC was treated with black powder suspension and also examined. The powder suspension used was the HOSDB formulation of magnetic iron oxide (20g) (Fisher Scientific, Loughborough, Leicestershire) suspended in a 1:1 ratio of Kodak Photo-Flo Wetting Agent (10mL) (Kodak, Paris, France) and distilled water (10mL). The powder suspension was applied to the glass using a small brush and then once the surface had been coated, it was washed off using tap water. The substrate was left to dry overnight prior to examination [114].

#### 4.1.1.1 Identification of Heat Distorted Fingerprints on uPVC – An Additional Study

The results of the study described in section 4.1.1 and discussed in section 4.2.1, showed that upon heating the uPVC, due to the nature of the substrate, the uPVC softened at approximately 85˚C, stretched under direct heat, and hardened once removed from the heat source. Fingerprints which were subsequently enhanced had distorted in shape also. This raised the question as to whether the enhanced, distorted fingerprints would be identifiable using a fingerprint identification system (AFIS).

The fingerprint identification system has three fundamental stages: (a) data acquisition, where the fingerprint to be recognised is sensed; (b) feature extraction, where a machine representation (pattern) is extracted from the sensed image; and (c) matching, the comparison of the representations derived from the sensed image with representations stored in the system. The comparison typically yields a matching score, quantifying the similarity between the stored fingerprint and searched fingerprint both of which are displayed to an expert for visual comparison. One challenge with the matching aspect of the system is its need to deal with displacement of the minutiae from their 'true locations' by elastic distortion of the fingerprint skin. Such elastic distortions can account for minutiae matching errors [160]. As such, the algorithms in the systems which undertake the feature extraction and matching processes have counter measures incorporated to deal with elastic distortion [161].

For fingerprints recovered from heat exposed surfaces, identification may be impeded by conditions and distortions that the fingerprint may experience.

The study investigated whether five separate donor's full set of ten fingerprints could be identified using an AFIS (Metamorpho™ AFIS (Sagem)). The system and associated database used for this experiment was available for research purposes only and was not connected to any law enforcement AFIS. The system had previously been loaded with 24,110 fingerprint images taken from the NIST Image Groups Special Database 14 (NIST Mated Fingerprint Card Pairs 2). The five donors' inked fingerprint impressions had also been scanned into the system prior to analysis.

'Natural' fingerprints were obtained by each donor rubbing their hands together to evenly distribute residues immediately before deposition. The volunteers were given two sheets of cleaned uPVC, one for each hand (the uPVC was purchased and cleaned in accordance with section 4.1.1). On each sheet was drawn a five box grid (figure 4-1), and each box was labelled with thumb, index, middle, ring or little finger. The fingerprints donated were enhanced using powder suspension as previously described in section 4.1.1. The uPVC was left to dry overnight before photographs of the fingerprints were taken using the Integrated Rapid Imaging System (IRIS) designed and produced by the Home Office Scientific Development Branch (HOSDB) [162] before being loaded onto the AFIS system.

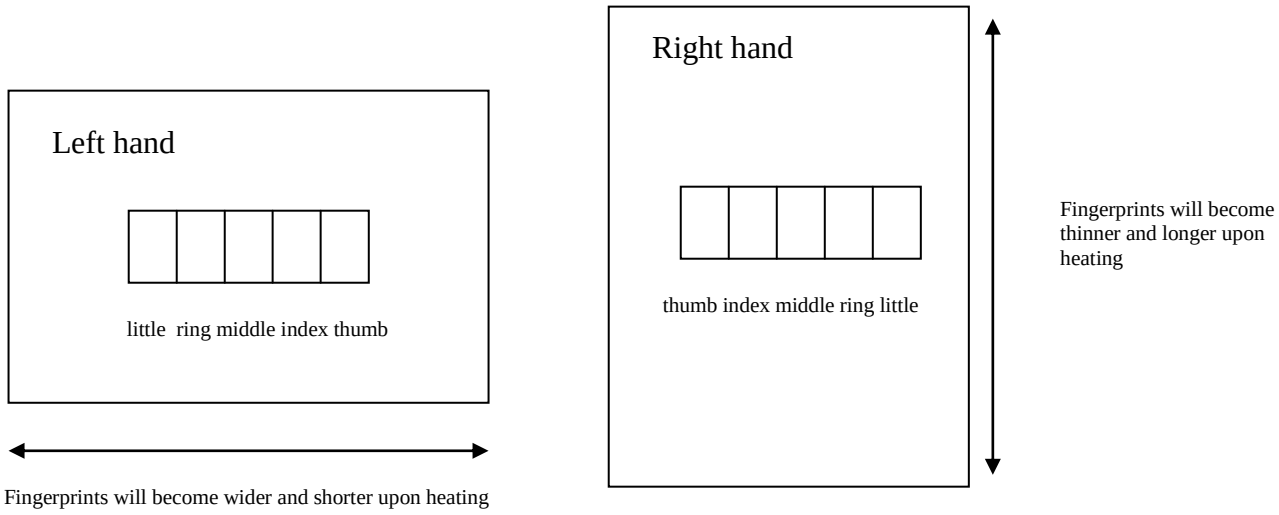



Figure 4-1 Layout of uPVC sheets and grids

The uPVC sheets containing the fingerprints were heated in order to obtain different degrees of distortion. This was achieved by placing the uPVC sheets (after fingerprint enhancement and photography) in a dry oven at 150˚C for 20 minutes. This caused the enhanced fingerprints on the uPVC to distort also, both horizontally and vertically, due to the orientation and anisotropic flow properties of the uPVC sheets.

### 4.1.2 Fingerprints on Glass

Glass was supplied by Stevenage Glass (Stevenage, Hertfordshire) and cut to A4 size. It was cleaned using Fairy washing-up liquid (Proctor & Gamble, Brooklands, Weybridge), air dried, washed with ethanol (Hayman Limited, Witham, Essex), and then air dried once again. A grid was drawn onto the glass surface (figure 3-1) and the fingerprint deposits made were the same as in section 3.1.1.

Fingerprints were subjected to different ageing times before being subjected to different temperatures for different time periods. Each set of aged fingerprints were enhanced by different methods. An experimental summary is given in table 4-3.

Table 4-3 Summary of exposure to heat experiments

| Substrate | Ageing Time | Temperature (˚C) | Exposure Time (min) | Treatment |
|---|---|---|---|---|
| Glass | 1 hour, 1 day, 1 week, 1 month | 50 | 10, 20, 40, 80, 160, 320 | 1. Powder Suspension 2. Superglue - BY40 |
| | | 100 | | |
| | | 150 | | |
| | | 200 | | |
| | | 250 | | |
| | | 300 | | |
| | | 350 | | |
| | | 400 | | |
| | | 450 | | |
| | | 500 | | |

The superglue method and powder suspension methods used were the same as described in section 4.1.1.

### 4.1.3 Fingerprints on Ceramic

White ceramic tiles (20cm x 25cm) were purchased from Wickes (St. Albans, Hertfordshire). The tiles were prepared as in section 4.1.2 and samples were deposited as in section 4.1.1.

Fingerprints were subjected to different ageing times before being heated to different temperatures and for different periods of time. They were enhanced using different methods. A summary of the experiments is given in table 4-4.

Table 4-4 Summary of exposure to heat experiments

| Substrate | Ageing Time | Temperature (˚C) | Exposure Time (min) | Treatment |
|---|---|---|---|---|
| Ceramic | 1 hour, 1 day, 1 week, 1 month | 500 | 10, 20, 40, 80, 160, 320 | 1. Powder Suspension<br>2. Superglue - BY40<br>3. VMD (short study) |
| | | 550 | | |
| | | 600 | | |
| | | 650 | | |
| | | 700 | | |
| | | 750 | | |
| | | 800 | | |

Powder suspension and superglue methods used were the same as described in section 4.1.1.

**VMD**

Vacuum Metal Deposition (VMD) was carried out using a West Technology Systems Ltd metal deposition machine (WTSL, Yate, Gloucestershire). The tiles were attached to the workholder using string to minimise the amount of contact on the surface. The chamber was pumped down to a vacuum of $2x10^{-4}$ torr. Firstly, gold (Manesty, Knowsley, Merseyside) and then zinc (Sigma-Aldrich, Gillingham, Dorset) was evaporated before bringing the chamber back to atmospheric pressure for removal of the tile [146].

### 4.1.4 The Effects of Heat on Fingerprint Deposits on Metal – An Additional Study

This study was conducted to investigate the change occurring in fingerprints exposed to elevated temperatures such as those experienced at arson scenes. In this study, scanning electron microscopy (SEM) had been used to obtain compositional and morphological information on fingerprint deposits. In this technique, an electrically conductive sample is loaded into an evacuated sample chamber, and a beam of high energy electrons is focused onto the sample surface. Images are obtained by scanning the beam spot over the area of interest in a raster pattern, whilst recording one or more signals at each point in the scan. These signals can result from various beam-sample interaction processes, but this study focuses on two of them: secondary electron emission and characteristic X-ray fluorescence. The examination and visualisation of fingerprints by SEM is not a new concept [163-165]. Scruton *et al.,* investigated the deposition of the latent fingerprint onto a surface, determining that eccrine deposits form droplets and sebaceous deposits leave continuous films [163]. Garner *et al.,* investigated the use of SEM as an alternative to photography. They had aged and heated some of their samples at 260˚C for 1 hour but no spectrochemical results were generated [164].

SEM sample stubs were manufactured from a 25mm diameter stainless steel rod. This rod was cut into discs (stubs) of 5mm depth and the faces polished to a high shine using grit paper P800 with light oil (WD40).

Natural fingerprints were deposited onto these stubs (one fingerprint per stub) 10 minutes before exposure to elevated temperatures in a furnace (B&T chamber furnace, Carbolite Company Ltd, Hope Valley, England), with three repeats (three stubs) per temperature. Only one donor was used and was not allowed to deposit fingerprints until more than 30 minutes after handwashing had elapsed.

Samples were placed on a stainless steel tray and covered by another stainless steel tray, in order to create a barrier against the radiant heat and to also ease the removal of the samples from the furnace. All samples were exposed for a duration of 20 minutes.

The temperature range investigated was 100-900˚C, increasing in 100˚C increments. Room temperature exposed samples were also prepared.

One week after heat exposure (due to instrument availability), secondary and backscattered electron images were captured and elemental analysis of the thirty samples was undertaken using SEM-WDX (SX100 Electron Probe Microanalyser, Cameca, Gennevilliers, France) by placing the stainless steel stubs straight into the instrument. 10kV voltage and 1nA current was used for image collection, with the current increasing to 10nA for spectrum collection and elemental mapping. Three WDX spectrometers were used, with diffracting crystals of thallium acid phthalate (TAP, 2d=25.75Å), penta-erythritol (PET, 2d=8.75Å) and W–Si multilayer pseudocrystal (PC1, 2d=60.04Å).

## 4.2 Results and Discussion

### 4.2.1 Fingerprints on uPVC

Fingerprints deposited on uPVC were subjected to the temperature conditions described in section 4.1.1. The enhancement methods employed were chosen as black magnetic powder which has been suggested as the most effective powdering technique on uPVC [159], a sequential process of black magnetic powder followed by powder suspension which allows for the enhancement of samples which have been exposed to water, and superglue followed by BY40 which is a process employed on dry, non porous surfaces.

Figure 4-2 shows the change undertaken by the substrate after exposure to 50˚C, 100˚C and 150˚C for a period of 20 minutes.

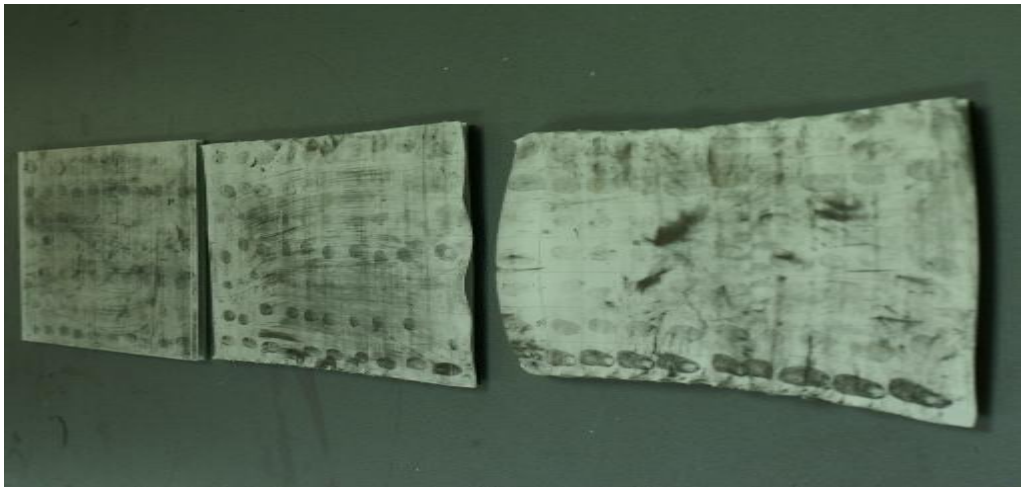


Figure 4-2 uPVC at 50˚C (left), 100˚C (middle), and 150˚C (right)

#### 4.2.1.1 Black Magnetic Powder

Black magnetic powder is easy to apply to non porous surfaces; however it can only be used on dry surfaces, which is its limitation. In general, like other fingerprint powders, black magnetic powder will preferentially enhance fresh fingerprints rather than older fingerprints [166].

When applying black magnetic powder to the uPVC, most of the enhancement achieved was negative enhancement. This means that the background substrate was enhanced by the powder and the fingerprint ridges were left clear, rather than the powder adhering to the deposit. Also, as predicted, the black magnetic powder produced better results on 1 hour and 1 day old fingerprints compared with 1 week and 1 month old fingerprints.

There were various health and safety factors which had to be taken into account in generating the uPVC samples and in particular the gaseous products derived during thermal decomposition of the uPVC. As a consequence the uPVC was only heated to 150˚C for 10min and 20min therefore not all of the exposure times were experienced by the substrate at this temperature. The result of this was that it was not possible to carry out the ANOVA tests for any of the scores obtained using the various enhancement techniques for uPVC as the dataset was unbalanced.

Main effects and interaction plots can still be generated, however it will not be possible to assess whether any of the independent factors or interaction between the factors are significant to the resultant fingerprint score. As such the main effects plot is shown in figure 4-3.

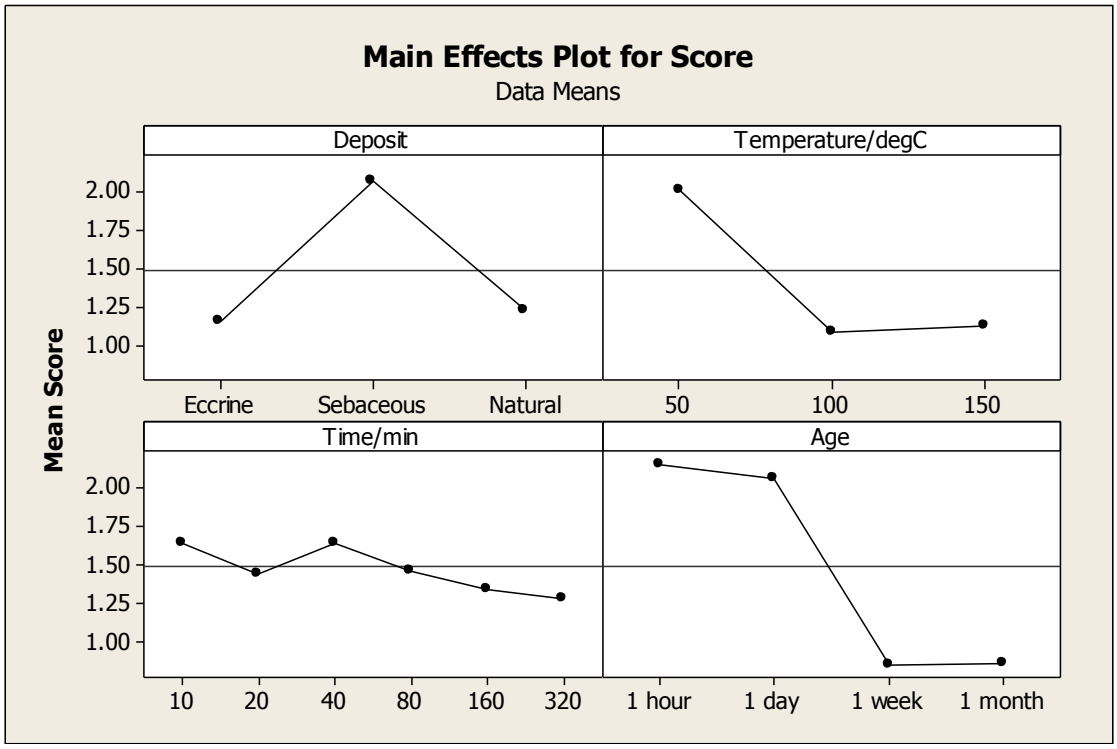



Figure 4-3 Main effects plot for enhancement by black magnetic powder

The main effects plot indicates that the scores observed for the sebaceous fingerprint *deposits* are superior to both the eccrine and natural fingerprint scores. In this case, the mean results observed for the eccrine and natural deposits are approximately the same. Also, when examining the effect of *temperature*, it can be seen that greater results are obtained at 50˚C exposure. Generally, the *time* scores are close to the mean but decrease from 40min onwards. There is also a large decrease in the successful enhancement of fingerprints which are 1 week and 1 month old in *age*. This was expected as a previous study by Bandey *et al.*, had shown that when comparing 1 day and 1 week old fingerprints on uPVC, the number and quality of fingerprints had decreased by approximately 20% [159].

The interaction between the factors is assessed in an interaction plot, which is given in figure 4-4.

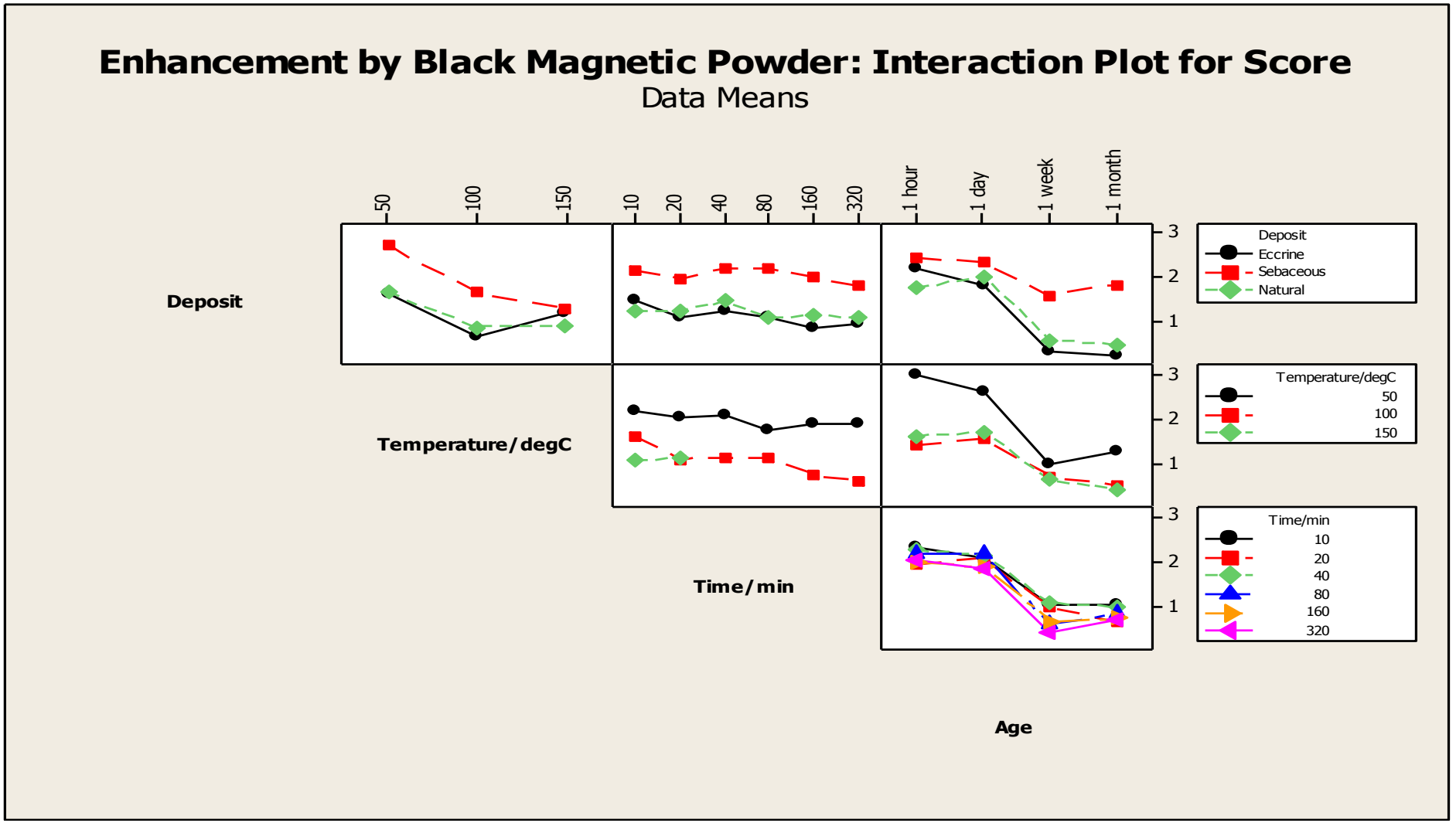



Figure 4-4 Interaction plot for black magnetic powder enhancement on uPVC (y-axis = score)

Examining the interaction between the *type of deposit* and *temperature* indicates that the best scores were observed at 50˚C and that the sebaceous deposits gave the highest scores. The scores observed for the *type of deposit* combined with *time* were generally the same across all exposure times but, again, the sebaceous deposits gave better results. As expected, the interaction between the *type of deposit* and *age* showed the scores dropping as the age of the deposits increased, although this was less pronounced in the enhancement of the sebaceous fingerprint deposit. Investigating the interactions between *temperature*, *time* and *age*, the scores obtained at 50˚C were greater than the other exposure temperatures. Another expected interaction was the higher scores observed at 1 hour and 1 day ageing for *time* combined with *age*. As with all of the results, fingerprint variability may have an effect on the scores obtained by the selected enhancement techniques used in this research.

The results from the interaction plot indicate that black magnetic powder, as expected, was useful for enhancing 1 hour and 1 day old fingerprints, and it was more effective on sebaceous fingerprints than other types. Overall, black magnetic powder is a relatively poor enhancement technique for uPVC based on the scores achieved but it is a relatively quick, easy, and cheap technique to use. It could be used before powder suspension (which will be discussed in the following section) if the surface is dry as part of a sequential process.

#### 4.2.1.2 Black Magnetic Powder followed by Powder Suspension

The previous section illustrated that black magnetic powder on its own gives poor results on uPVC. Current research is being undertaken by HOSDB into the sequential use of black magnetic powder followed by powder suspension on a variety of non porous surfaces [167]. This approach was used in this research in two ways – firstly to investigate if the powder suspension would enhance any additional fingerprints after using black magnetic powder, and secondly, as powder suspension is suitable for use on items that have been exposed to moisture, to investigate whether it is suitable for use on uPVC. It was found that powder suspension was suitable for enhancement on uPVC, and that it did provide many additional fingerprints that black magnetic powder alone had not enhanced, including fingerprints which were aged for 1 week and 1 month.

To investigate the independent factors on the resultant fingerprint score, a main effects plot was generated. This plot is given in figure 4-5.

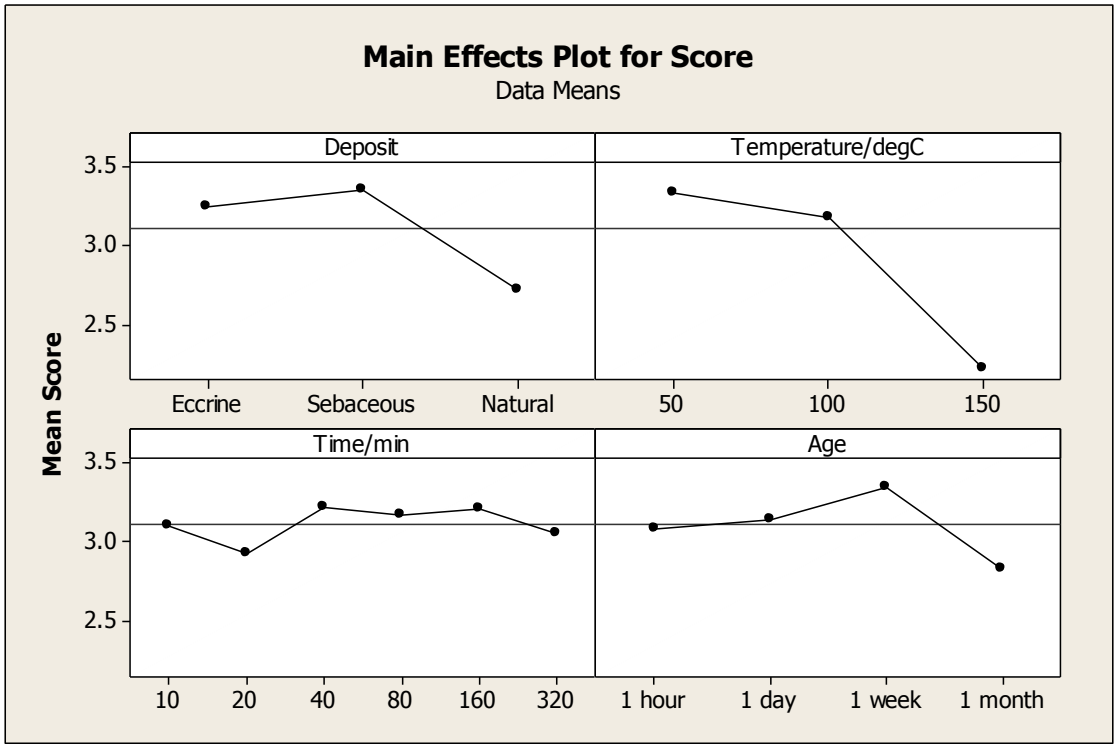



Figure 4-5 Main effects plot for enhancement by black magnetic powder – powder suspension

In comparison with the main effects plot for black magnetic powder only (figure 4-3), the difference in mean scores between eccrine and sebaceous is not as large. Therefore, the eccrine component of the fingerprint residue is being successfully enhanced by the powder suspension, rather than just the sebaceous components. Scores are also increased at 100˚C exposure compared to that of black magnetic powder only. Again, *time* is less of a factor when it comes to influencing the score due to its relatively straight line and increased scores are observed at 1 week and 1 month are improved by subsequent enhancement by powder suspension.

The interaction plot for this sequential process is given in figure 4-6.

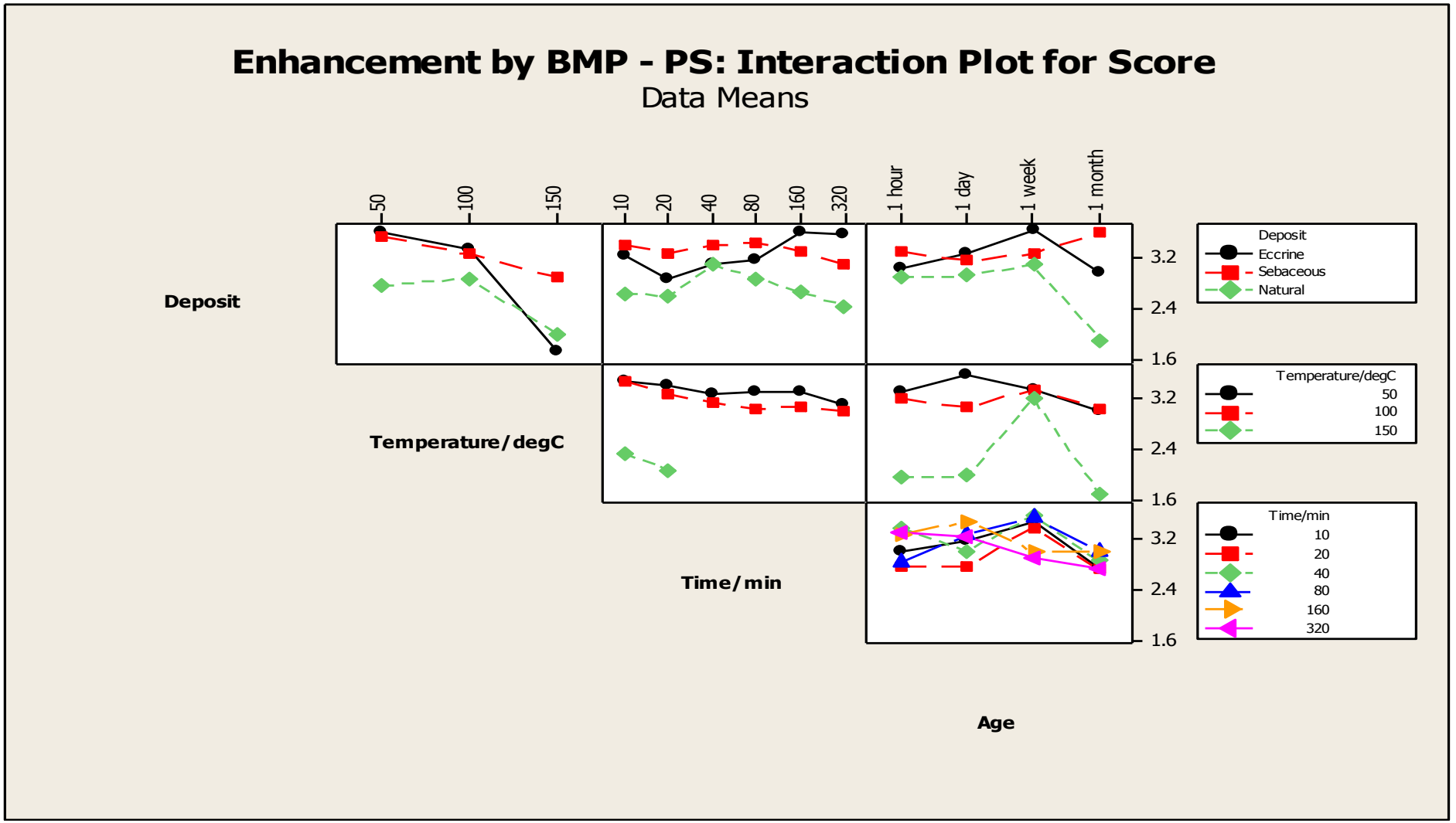



Figure 4-6 Interaction plot for black magnetic powder – powder suspension enhancement on uPVC (y-axis = score)

The scores obtained from the combination of *type of deposit* and *temperature* illustrate that eccrine and sebaceous fingerprints are behaving the same until 150˚C when eccrine scores decrease sharply. Natural fingerprints in this interaction increase slightly before a sharp fall at 150˚C. Erratic results were observed with the *type of deposit* combined with *time*. In the interaction plot for *type of deposit* combined with *age* in figure 4-3 (black magnetic only enhancement), the scores decreased sharply for 1 week and 1 month old fingerprints, but in figure 4-4, the decrease does not occur until fingerprints are 1 month old. This shows a clear increase in the ability of the powder suspension over the black magnetic powder enhancement alone. For *temperature* and *time*, the scores for fingerprints exposed to 50˚C and 100˚C are similar, with 150˚C exposure giving much poorer results. This is mirrored in the interaction between *temperature* and *age* where the 50˚C and 100˚C results are similar except at 1 day old, and 150˚C results poorer (except for an anomalous peak at 1 week). These anomalies could be explained by the fingerprint deposition variability. Erratic results were observed between *time* and *age*. Using powder suspension after black magnetic powder increases the scores and number of fingerprints enhanced. Powder suspension can also be used as a stand-alone technique.

Powder suspension is a relatively new fingerprint enhancement technique, compared with the longevity of techniques such as powdering and superglue enhancement. The mechanisms behind powder suspension and the components of the fingerprint residue are unknown. HOSDB, in partnership with Brunel University and the University of Lincoln, are currently investigating the enhancement mechanisms of powder suspension using microscopy techniques (at Brunel University) and gas chromatography mass spectrometry (at the University of Lincoln). This study has been ongoing for a number of years and the outcome of the results are expected to be published within the next year.

HOSDB's MoFDT non porous fingerprint charts are also being revised to include powder suspension at its most appropriate place in the chart sequences. Again, these revised charts are due to be released within the next year.

#### 4.2.1.3 Superglue followed by BY40

Superglue followed by BY40 is a technique used for enhancing fingerprints on non porous surfaces that have not been wetted [100]. It is the most commonly used fingerprint enhancement technique for non porous surfaces and is used by police fingerprint laboratories both in the UK and abroad [75]. It is expected to produce better results on uPVC than using fingerprint powders (such as black magnetic powder) alone as it is not as readily affected by the age of the fingerprint. This is an advantage as the amount of time which has passed between the collection of the item and its subsequent chemical processing is of no consequence with this technique.

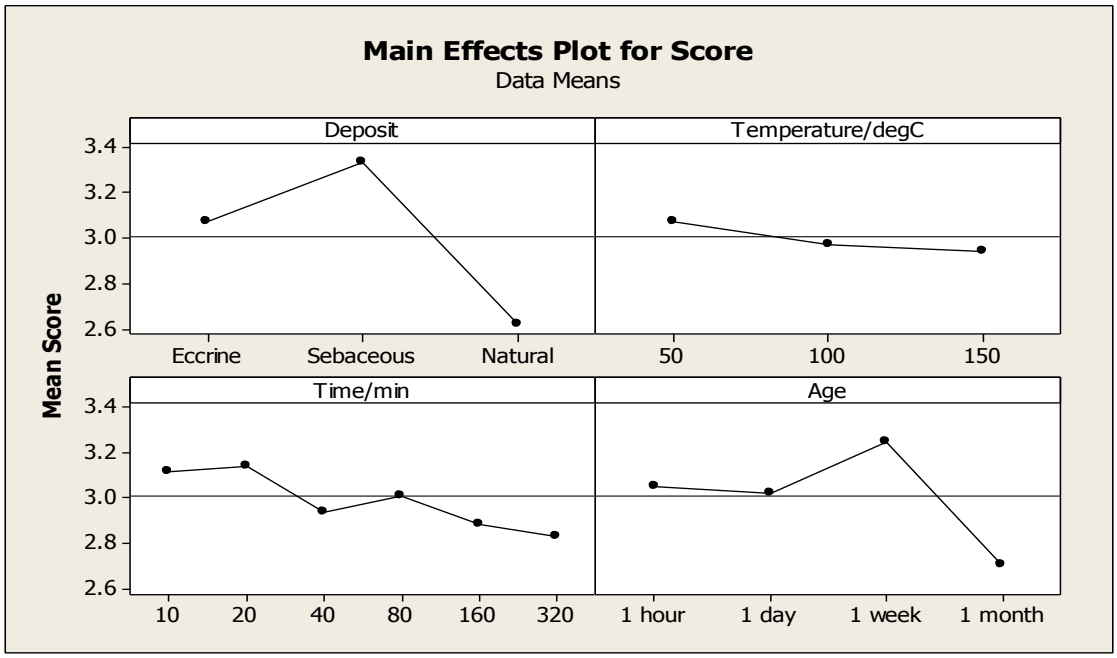



Figure 4-7 Main effects plot for enhancement by superglue followed by BY40

Similarly for the other techniques, sebaceous deposits have had the more successful enhancement but the effectiveness of enhancement across all three *temperatures* tested has improved, especially for 150˚C. The superglue enhancement seems more dependent on exposure time than in comparison to the other two techniques and gives unpredictable results for fingerprint *age*, with an increased enhancement for 1 week old fingerprints and a decreased enhancement for 1 month old fingerprints. This may be due to variability in the fingerprint deposition.

The interaction plot for the data is shown in figure 4-8.

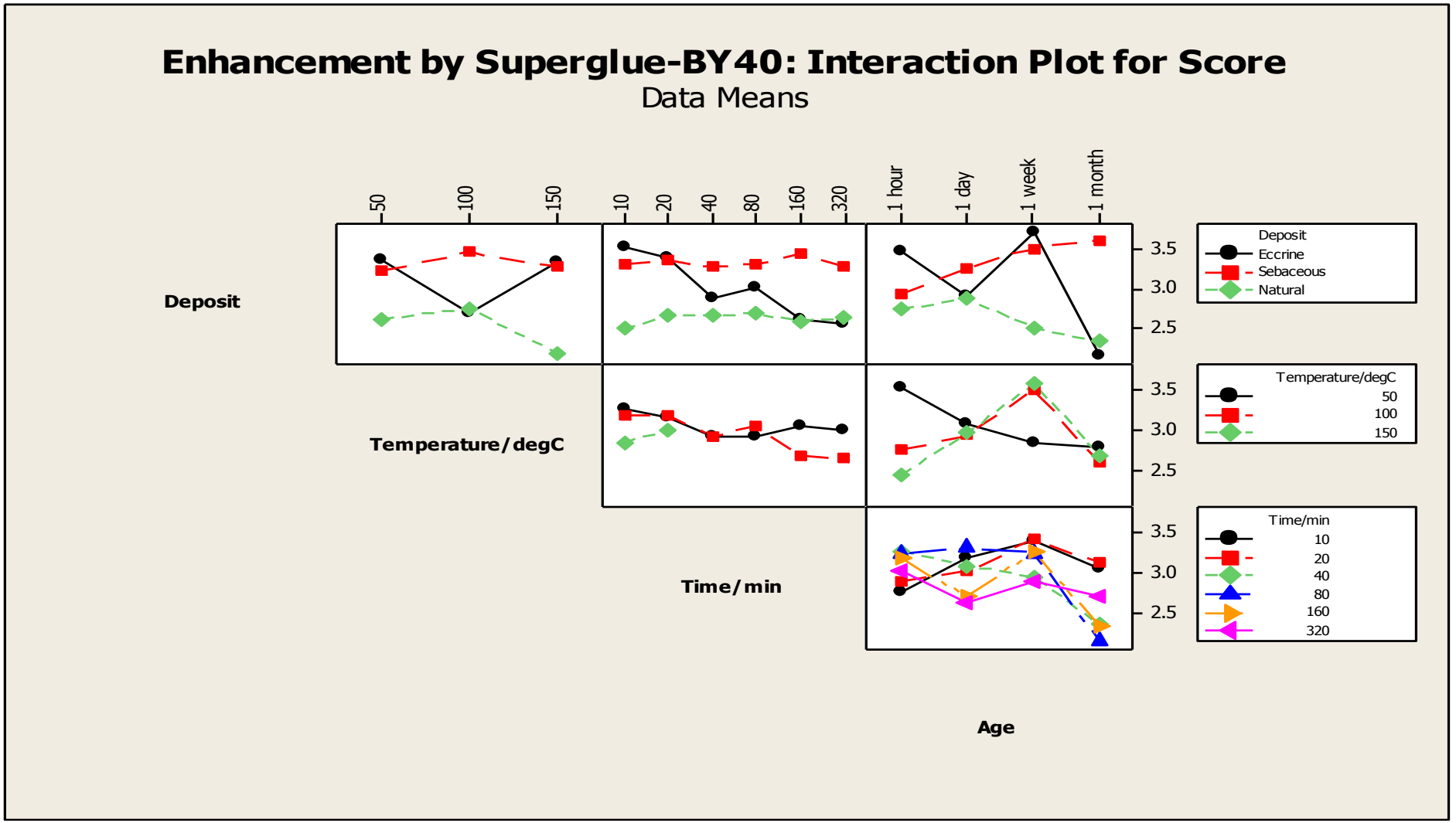



Figure 4-8 Interaction plot for superglue followed by BY40 enhancement on uPVC (y-axis = score)

The results of the interaction plot for the *type of deposit* and *temperature* were unusual as eccrine fingerprint deposits showed a dramatic decrease at 100˚C but then increased again at 150˚C, whereas the sebaceous fingerprints maintain high scores across all temperatures. Lower scores were observed overall for natural fingerprints. The *type of deposit* combined with *time* showed that the scores obtained from eccrine enhanced fingerprints were decreasing as the exposure time increased, whereas sebaceous and natural deposits performed generally the same across all times. The results of the *type of deposit* and time of exposure combined with *age* showed varying results for all three deposits.

#### 4.2.1.4 Enhancement Technique Comparison

A comparison of the fingerprint enhancement techniques for the fingerprints exposed to elevated temperatures that had been deposited on uPVC must be undertaken. As with each enhancement technique independently, fingerprint variability may sway the results. This cannot be measured, and as such, conclusions based on fingerprint variability cannot be exact. Figure 4-9 gives the main effects plot for comparing the fingerprint enhancement techniques on uPVC.

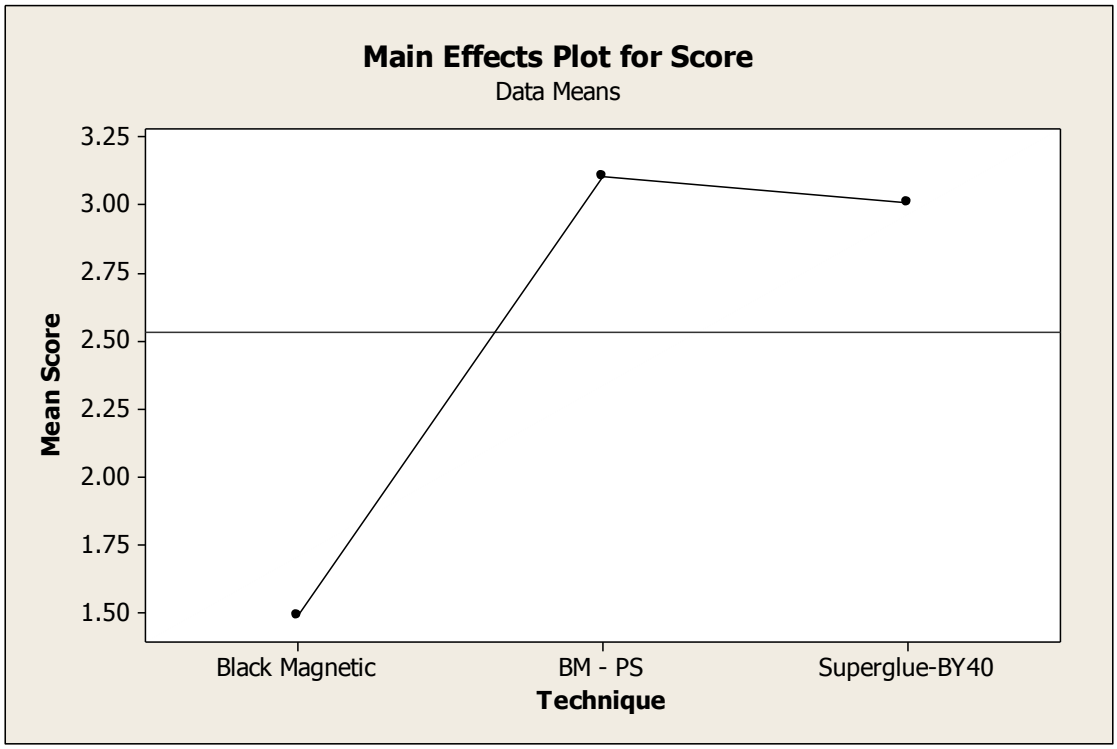



Figure 4-9 Main effects plot for comparing enhancement techniques

As the main effects plot shows, the black magnetic powder enhancement method alone is giving a very poor mean response. Therefore, this enhancement technique is poor compared to the other two methods. It is assumed that even taking variability into account, black magnetic powder would always be a poor performing technique. However, this work demonstrated that the mean responses for black magnetic powder followed by powder suspension, and superglue are very close. Therefore, either one would be a suitable enhancement technique.

As natural fingerprints are the closest to 'real' fingerprints in this research, an interaction plot has been generated to examine the effectiveness of each enhancement method on each deposit type. Figure 4-10 is the interaction plot generated by Minitab and is described afterwards.

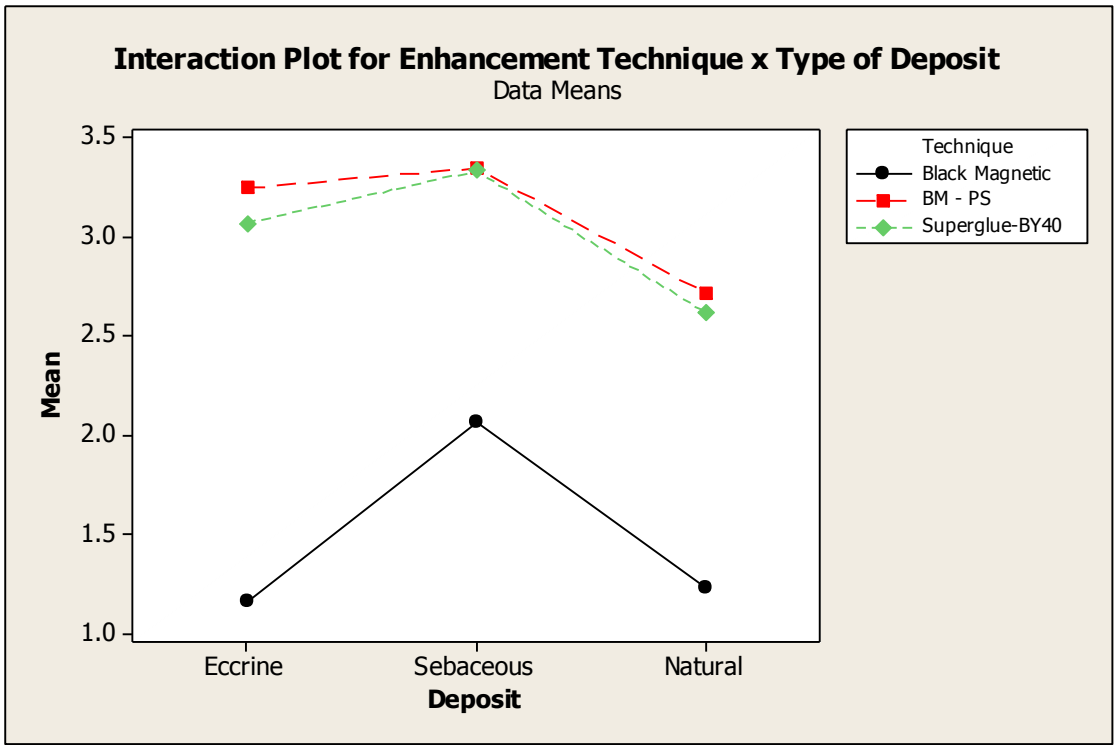


Figure 4-10 Interaction plot comparing enhancement technique and type of deposit (y-axis = score)

As the interaction plot shows, the enhancement score means for black magnetic powder alone are much lower across all three deposits. The results for superglue and black magnetic powder followed by powder suspension follow the same pattern. Acknowledging the variability in deposited fingerprints, it is not possible to say which technique is superior in the enhancement of fingerprints deposited on uPVC.

As natural fingerprints are the closest representation to the fingerprints seen in an operational environment, it is encouraging that the experiments carried out demonstrate that the scores for two of the techniques are almost identical across all of the variables. This means that for a dry uPVC surface, both techniques are available for use, and that on a wet surface, using powder suspension on its own should be theoretically as good when treating uPVC for fingerprints.

#### 4.2.1.5 Identification of Heat Distorted Fingerprints on uPVC – An Additional Study

Section 4.1.1.1 described the experimental protocol undertaken to investigate whether fingerprints deposited on uPVC, heated at 150˚C for 20 minutes to cause distortion, and enhanced using powder suspension could be identified using Sagem Metamorpho™ AFIS. The AFIS system uses MetaMatcher algorithms in order to accurately match fingerprints by using a fusion of independent matching algorithms. The exact algorithms are unavailable for commercial reasons. When the system has matched a fingerprint, it provides a candidate list which gives the identity of the depositor, what finger it has matched with, and a matching score. The most closely matching fingerprint is placed first in the candidate list. The score is a way of quantifying the similarity between the fingerprint being searched and the reference fingerprint stored in the database. In this experiment, the score is higher if the quality of the fingerprint is greater (more minutiae to match against). Each fingerprint in the candidate list also has a score assigned to it. This study will use the score for the 2$^{nd}$ placed fingerprint in the candidate list (referred to as the alternative fingerprint) to examine the difference between these two fingerprints. A ratio of the score obtained for the matched fingerprint (1$^{st}$ place) and the score for the alternative fingerprint (2$^{nd}$ place) can be calculated. A high ratio would indicate a strong identification, as the alternative fingerprint would have a low number of minutiae matching the original fingerprint, and the matching and alternative fingerprints would be very different from each other. A low ratio (close to 1) would indicate a weak identification, with a larger number of matching minutiae in the alternative fingerprint. A ratio equal to 1 would indicate that the matching fingerprint could not be distinguished from its alternative match. These ratios were compared and plotted on the following graph (figure 4-11).

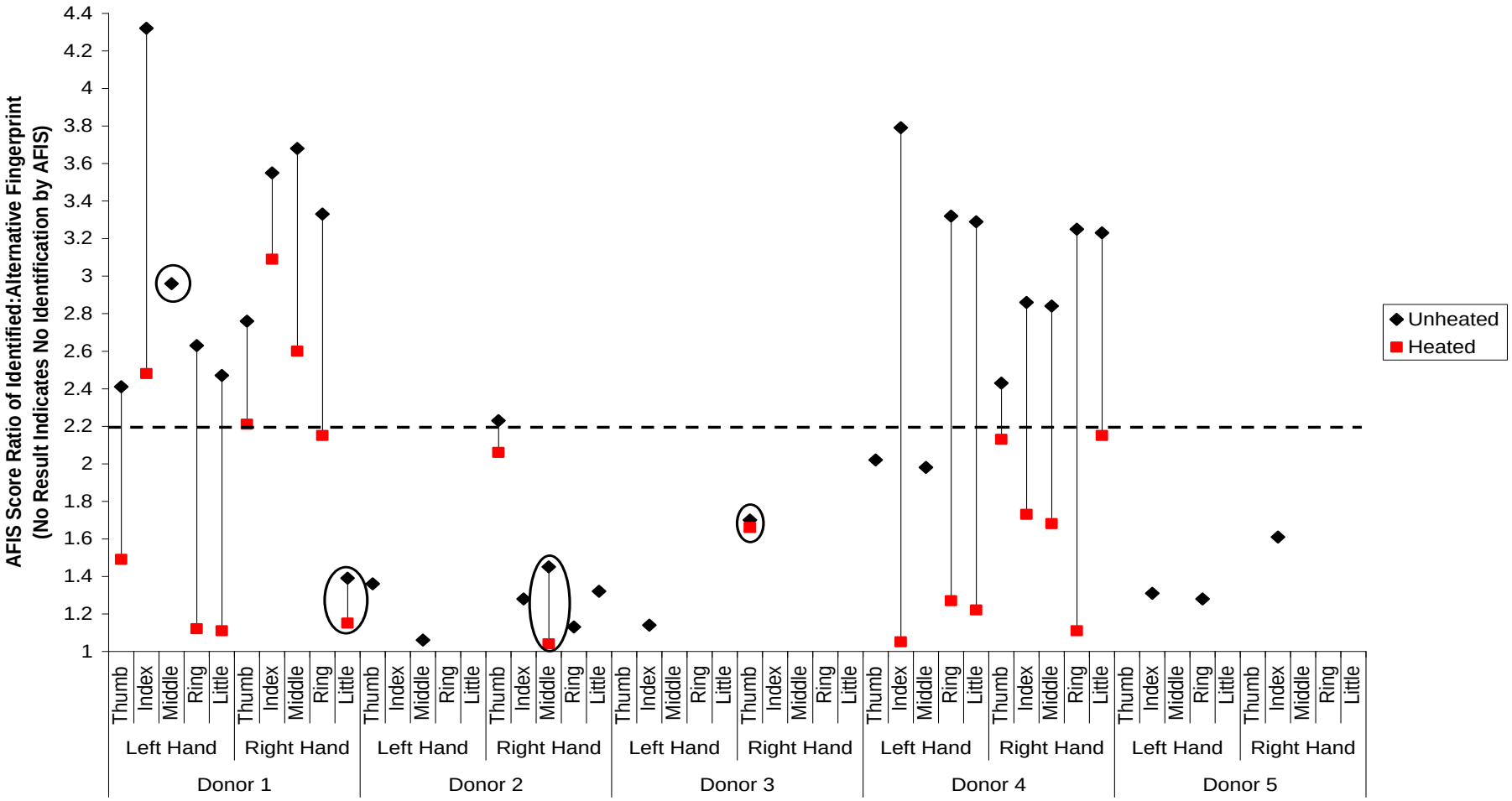



Figure 4-11 Comparison of AFIS results for unheated and heated fingerprints

The graph shows that donors 1 and 4 are 'good' fingerprint donors (all ten pre-distorted fingerprints were identified), donors 3 and 5 were 'poor' donors (less than five identifications were made from the pre-distorted marks), and donor 2 was intermediate (between five and ten identifications were made).

The graph also shows that out of a total of 50 fingerprints, 32 unheated (undistorted) fingerprints were identified (matched by AFIS to the correct finger of the donor) and 20 of the heated (distorted) fingerprints were identified. No identifications were made on distorted fingerprints which were from previously unidentified fingerprints. This indicates that distortion due to heating did not improve the quality of the mark.

The score ratios obtained for the distorted fingerprints were always less than those for the corresponding fingerprints prior to distortion. The data indicates a trend; if the score ratio of the unheated fingerprint was above 2.2 (this cut-off is shown by a dotted line on figure 4-11), it is likely that the distorted fingerprint will also be identified. There are only four exceptions to this rule (ringed in figure 4-11) – there was one initial identification that did not give a corresponding distorted identification, and three 'pairs' of identifications below this 2.2 cut-off point. This is a suggested cut off point based on examination of the data in figure 4-11.

**Effect of fingerprint donors on the score ratio**

The ratio scores had all been inputted in Minitab for analysis. A boxplot was generated on Minitab to look at the variation in the data between each donor. Figure 4-7 showed that donor's 1 and 4 had deposited a better quality of fingerprint based on their ratio's, therefore, it is anticipated that donor's 1 and 4 will have higher median results than the other donor's in the boxplot. The boxplot for donor comparison is shown in figure 4-12.

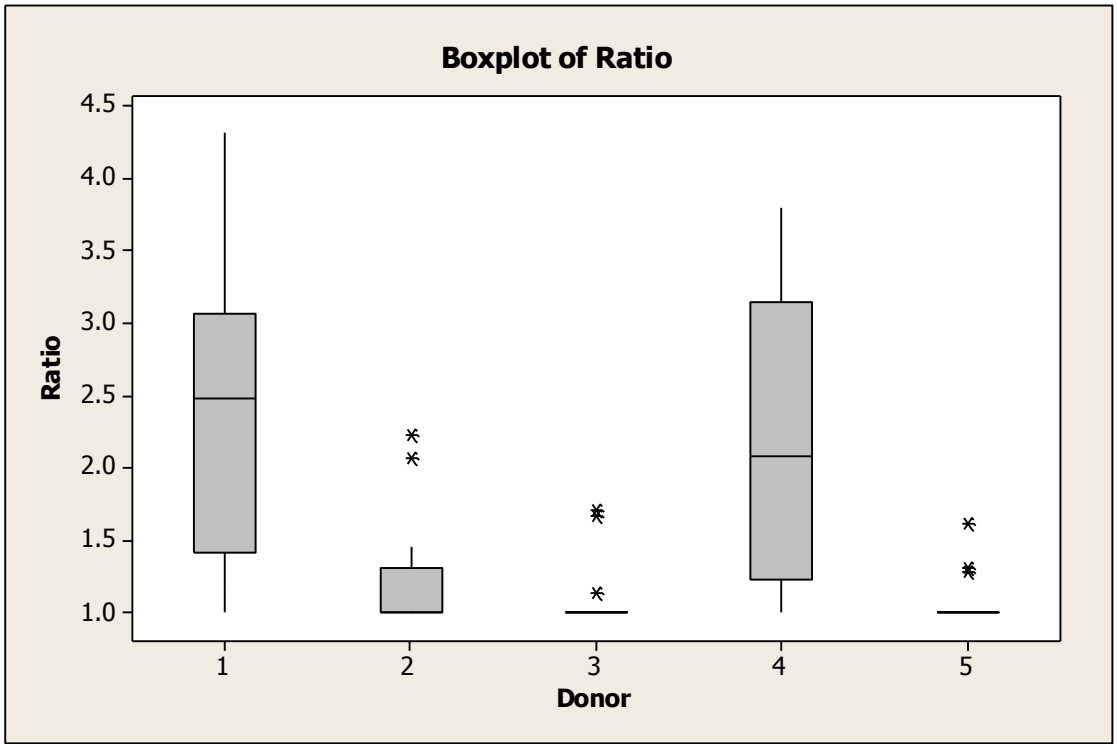


Figure 4-12 Boxplot of ratio for each donor

The boxplot shows that donor's 1 and 4 have a larger range in their ratios than the results from the other three donors. Also, there are no outliers in the data in donor's 1 and 4, whereas, the other three all have outliers indicating results that deviate from the normal pattern of the data. Median values are approximately 2.5, 1.0, 1.0, 2.0, and 1.0 for donors 1, 2, 3, 4, and 5 respectively. This shows that the middle values in the data for donor's 1 and 4 are indicating a difference between the fingerprint and the alternative fingerprint, whereas the median ratio's for donor's 2, 3, and 5 shows that AFIS cannot identify the fingerprint from the alternative fingerprint. The boxplot shows that the donor will have an effect on the ability for the AFIS to identify the fingerprint.

**Effect of horizontal and vertical distortion on the score ratio**

Figure 4-1 illustrated that the fingerprints were being distorted in two different directions because of the different orientations of the test panels – left handed prints were becoming wider and shorter upon heating (stretched horizontally), and right handed prints were becoming thinner and longer (stretched vertically) upon heating. An example of this is shown in figure 4-13.

(i) (ii) (iii) (iv)

Figure 4-13 Photographic images of Donor 1's (i) undistorted left index fingerprint, (ii) distorted left index fingerprint which shows horizontal distortion of the fingerprint, (iii) undistorted right index fingerprint, and (iv) distorted right index fingerprint which shows vertical distortion of the fingerprint

A boxplot was generated to examine the score ratios for the distorted fingerprints within each donor, to determine whether the direction of distortion had a significant effect. These results are shown in figure 4-14.

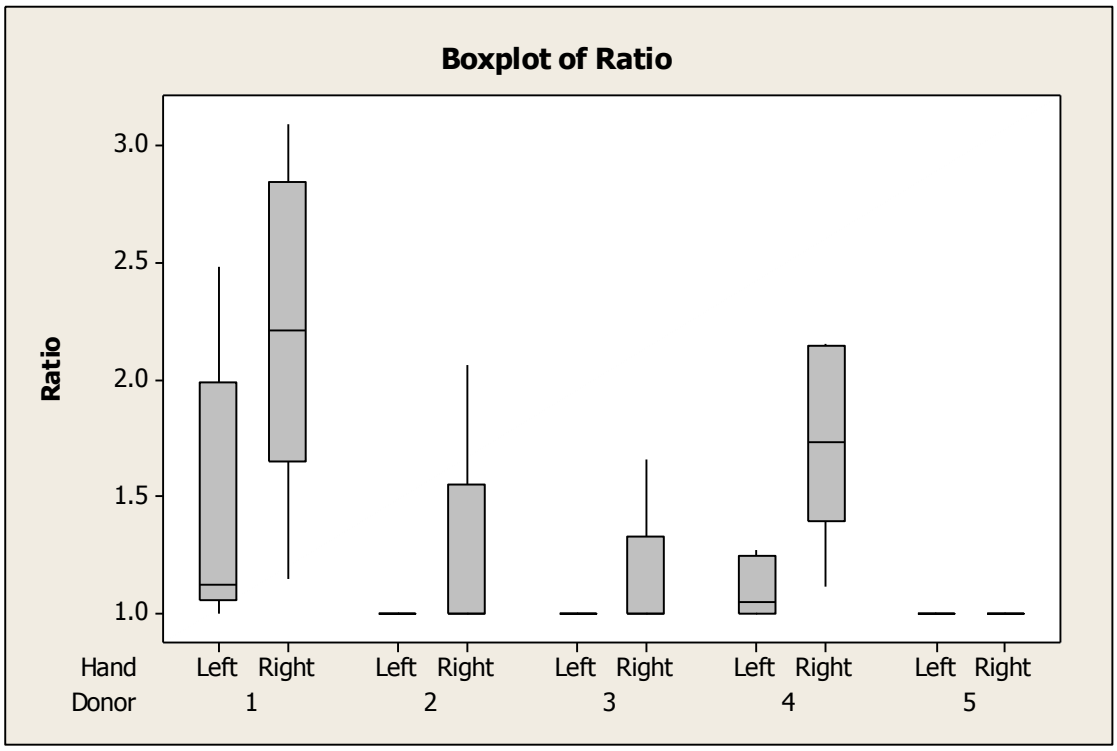



Figure 4-14 Boxplot of ratio for each donor's distorted fingerprints

This boxplot shows that for donor's 1 and 4, the median in boxplot for the right hands in greater than the left, indicating increased ratios and as such an increased difference observed between the fingerprint and the alternative fingerprint. For donor's 2 and 3,

the median is still approximately 1.0 but the data range is spread more for the right hands than the left, which shows a greater ratio observed in the right hands. No difference was observed for donor 5. As figure 4-1 illustrated, the left hand uPVC sheets were distorted horizontally and the right hand uPVC sheets were distorted vertically. Therefore, greater ratios were observed for 4 out of the 5 donors for the right hands, the vertically distorted fingerprints. As such, the AFIS system will identify vertically distorted fingerprints more readily than horizontally distorted ones.

It is not a surprise that the Sagem Metamorpho™ AFIS was able to identify many of the distorted fingerprints and match them to the corresponding undistorted image. Most fingerprint matching algorithms incorporate ad hoc counter measures to deal with distortion. In general this is achieved by relaxing the spatial relationships between minutiae [161]. This relaxing can be achieved in various ways; tolerance boxes [168-169], local triangulation matching [170-171], warping [172-173], thin-plate spline [174-175], normalisation [176-177], and radial basis function [178], each with its advantages and disadvantages. The counter measure in the Sagem Metamorpho™ AFIS is unknown.

Cappelli *et al*., [179] introduced a plastic distortion model. This was to cope with the non-linear deformed fingerprints obtained from online acquisition sensors. This model indicated that low distortion was observed on the apex of the fingerprint, with greater distortion observed radiating out from the apex. This model can be adopted by the deposition of a fingerprint on a substrate, and incorporates 3D to 2D mapping of fingerprint skin. This model, and other research into distortion and countering distortion on AFIS, concentrates on the stretching and distortion of a fingerprint as it is deposited and as such, is limited to how stretched or distorted the mark can be. The distortion observed in this study is greater than that which would be expected through the normal maximum elastic distortion fingerprint skin can experience.

Whatever type of counter measure is in place within the Sagem Metamorpho™ AFIS in order to deal with the distortion aspect of fingerprints it may not be optimised for abnormally distorted fingerprints such as those encountered in this study. Notwithstanding this, the algorithm was reasonably successful at identifying the donor finger, although, it is significantly better at coping with vertically stretched

fingerprints than horizontally stretched fingerprints. The identification was also dependent on the quality of the fingerprint deposited before distortion.

This work was undertaken to assess the effectiveness of the Sagem Metamorpho™ AFIS at identifying the donor finger of a fingerprint before and after it had been distorted due to the action of heat onto the receiving surface. The results indicated that, if the level of detail in the original deposited fingerprint was sufficient, then a fingerprint exposed to elevated temperatures sufficient to cause distortion of the receiving surface (and by consequence the fingerprint deposit) could, in 40% of cases, be identified by Sagem Metamorpho™ AFIS once it was enhanced, imaged and analysed. These results are also dependent on the direction of distortion, with vertical distortion providing greater discrimination. The results from this research should be advantageous to scene examiners and fingerprint examiners alike who encounter the difficulties of recovering and examining fingerprints which have been exposed to fire scenes on a daily basis.

### 4.2.2 Fingerprints on Glass

Fingerprints were deposited and subjected to the conditions described in section 4.1.2 (see table 4-3). The enhancement methods used for the visualisation of the fingerprints were powder suspension, as it can be used on both dry and wet non porous surfaces, and superglue followed by BY40 as it can be used on dry surfaces. Other fingerprint development techniques have been investigated on non porous surfaces with VMD the most effective technique, but expensive [119]. Black powder suspension and superglue followed by BY40 were assessed across more variables in this study, with a comparison to VMD at higher temperatures on ceramic only (section 4.2.2.4).

It is important to note here that fingerprints which were exposed to direct heat and air flow over the glass surface from within the furnace did not survive at temperatures of 350˚C and over. Once they were protected from these conditions, fingerprints did survive. Therefore the results mentioned in the following sections from 350˚C upwards are from fingerprints which were shielded from the direct heat and air flow in the furnace. The samples were shielded by placing them within a stainless steel tray

and placing another stainless steel tray directly above it. This would protect the sample from direct heat and air flow during the heat exposure in the furnace. In real fire environments, it is possible for items to become shielded during the fire. Parts of the item's surface would be protected by its location in the room, i.e. within a cupboard. The underneath of objects would also be protected from direct heat and air flow by the surface it is sitting on i.e. the underneath of a telephone would be protected by being placed on a table. Fire damage to a compartment or items within a compartment can also shield other items from direct fire and heat as it is common for plaster to come away from walls, ceiling tiles to fall down, etc. which subsequently protects the items underneath.

#### 4.2.2.1 Powder Suspension

The following images show that the powder suspension is enhancing components in both the sweaty eccrine and fatty sebaceous fingerprints (figure 4-15).

Figure 4-15 Photograph of eccrine (left – which was scored as grade 3) and sebaceous (right – which was scored as grade 4) fingerprints on glass subjected to 250˚C for 10 min - aged for 1 day

The Kolmogorov-Smirnov normality tests were conducted for fingerprints deposited on glass and enhanced using powder suspension. The P values for all three types of deposits were <0.010. Unlike the normality graphs generated for fingerprints deposited on paper, the data does not approximate a normal distribution, as the results deviate from the normal distribution line much more than on the porous surface. The normality graphs generated for each deposit enhanced by powder suspension are given in figures 4-16, 4-17, and 4-18. A possible explanation for the data either not

being normally distributed, or at least approximating a normal distribution, could be the substrate. The nature of non porous surfaces means that the fingerprint deposit will sit on top of the surface, rather than be absorbed into the matrix of the porous surface, leaving the fingerprint more susceptible to damage which could account for its larger number of zero results.

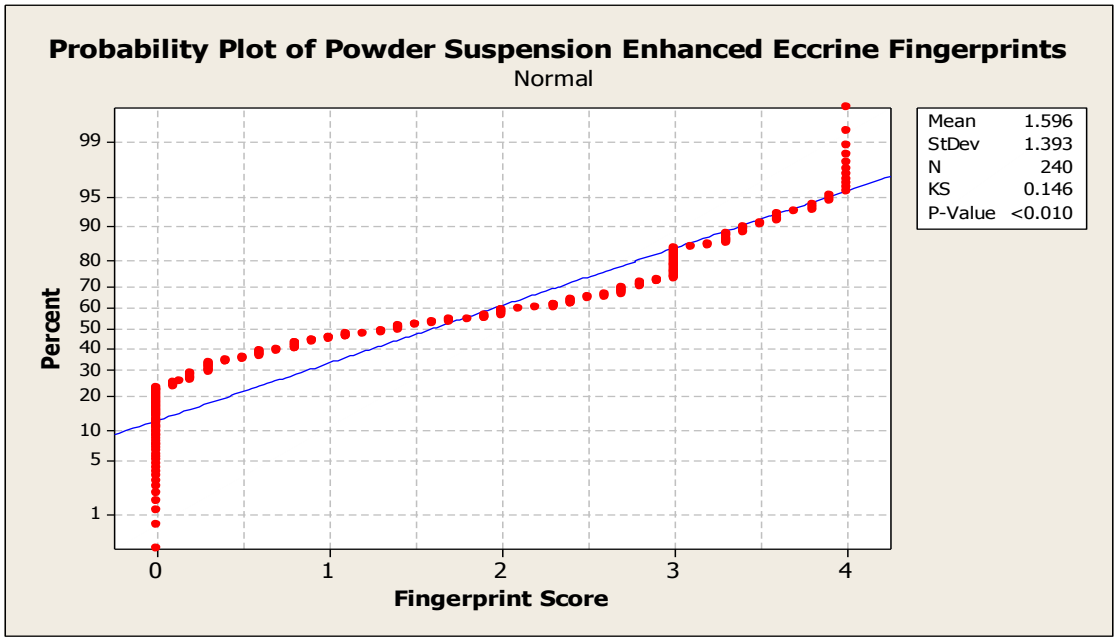



Figure 4-16 Probability plot of powder suspension enhanced eccrine fingerprints

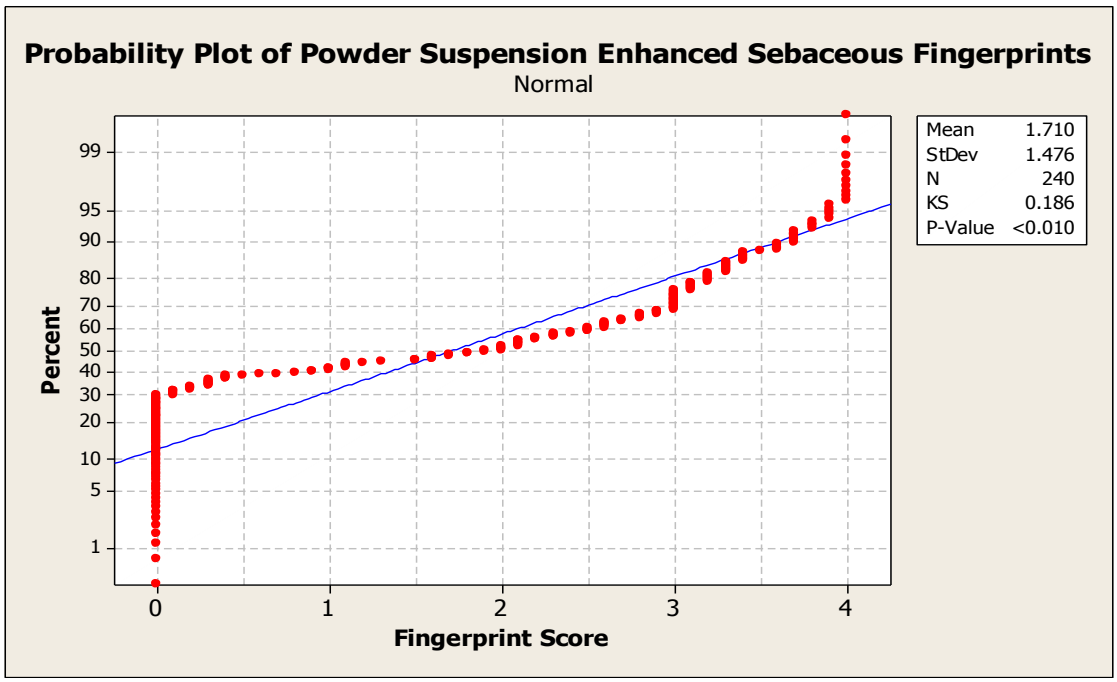



Figure 4-17 Probability plot of powder suspension enhanced sebaceous fingerprints

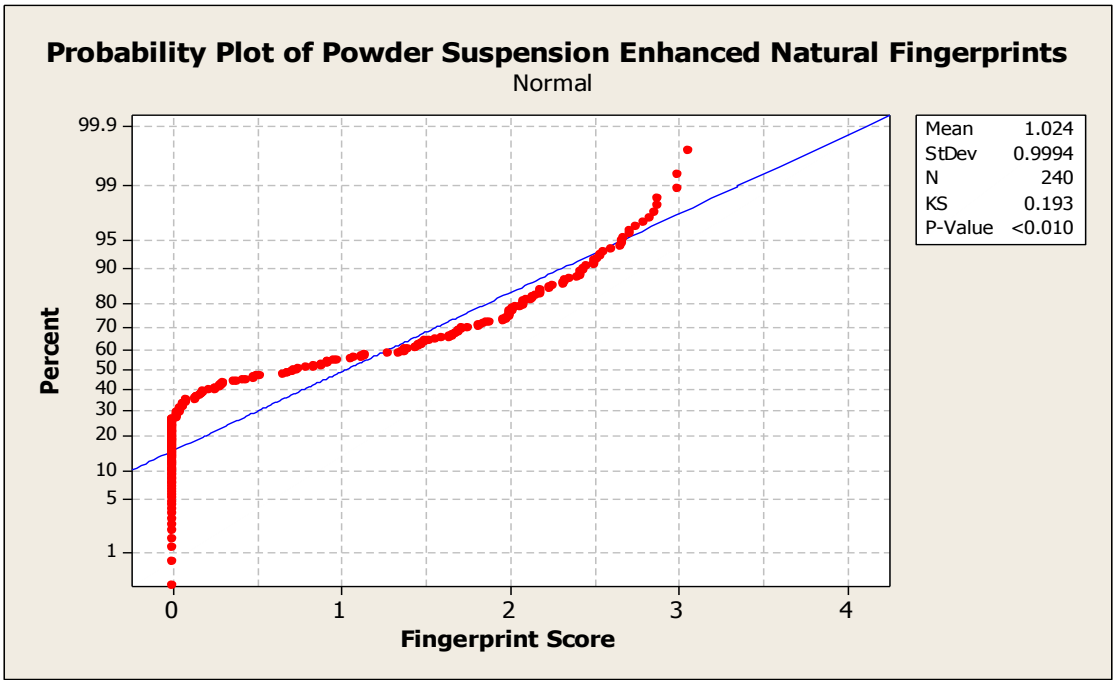



Figure 4-18 Probability plot of powder suspension enhanced natural fingerprints

From all three probability plots, it can be seen that the majority of results scored zero, therefore the score data is not normally distributed. As such, balanced ANOVA testing cannot be undertaken and the non parametric Kruskal-Wallis test was performed instead. This statistical test is similar to the one-way ANOVA test that was used to compare the enhancement technique for fingerprints deposited on paper however the data does not have to be normally distributed for this test to be used. Kruskal-Wallis performs hypothesis testing on whether the medians of the data tested are equal or not. The hypothesis is tested against the same α value of 0.05. If the P value in the Kruskal-Wallis test is less than the α value, the null hypothesis (medians are equal) is rejected in favour of the alternative hypothesis. As the Kruskal-Wallis test can only be tested against one factor, no significance testing of the interactions between the factors was possible. Table 4-5 gives the P values for each factor tested using the Kruskal-Wallis test.

Table 4-5 Kruskal-Wallis test results for fingerprints on glass enhanced by powder suspension

| Factor | P Value |
|---|---|
| Type of Deposit | 0.000 |
| Temperature | 0.000 |
| Time | 0.000 |
| Age | 0.509 |

The Kruskal-Wallis P values for each of the four independent factors indicate that *type of deposit*, *temperature*, and *time* are significant to the resultant fingerprint score. *Age* is not a significant factor. Although ANOVA testing cannot be carried out due to the data, main effects and interaction plots can still be generated as these simply show the means of the data for each variable and are not used for significance testing. Figure 4-19 plot is the main effects plot for powder suspension enhanced fingerprints.

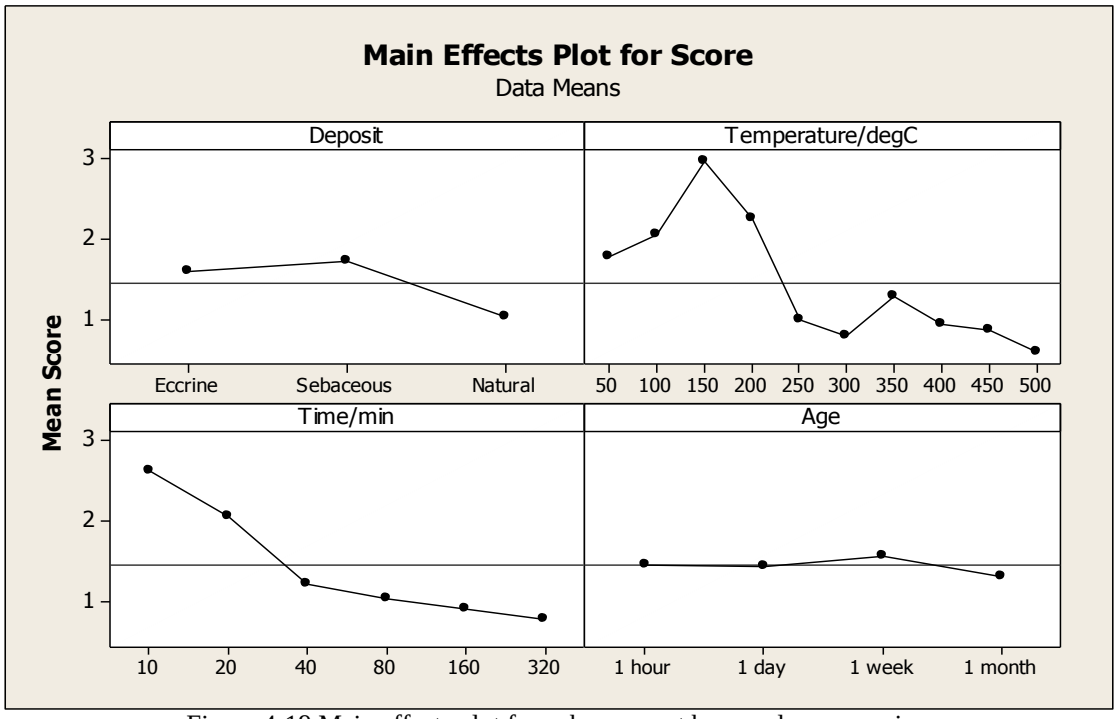



Figure 4-19 Main effects plot for enhancement by powder suspension

For *type of deposit*, eccrine and sebaceous are providing almost equal average scores whereas natural scores are lower in the plot. Analysis of *temperature* shows an increase in scores at 150˚C before decreasing, and another slight increase at 350˚C. This may be due to the introduction of the stainless steel 'shield' at this temperature, showing that protection will shelter the fingerprints from the direct heat. A decrease in score is observed with an increase in exposure *time*, and the results for 1 hour and 1 day old fingerprints remain on the mean line in the graph with slight deviations at 1 week and 1 month old fingerprints. This matches the result of the Kruskal-Wallis test, that the *age* of the fingerprint is not significant to the score, as the mean results remain close to the mean score line on the graph.

The interaction plot is given in figure 4-20.

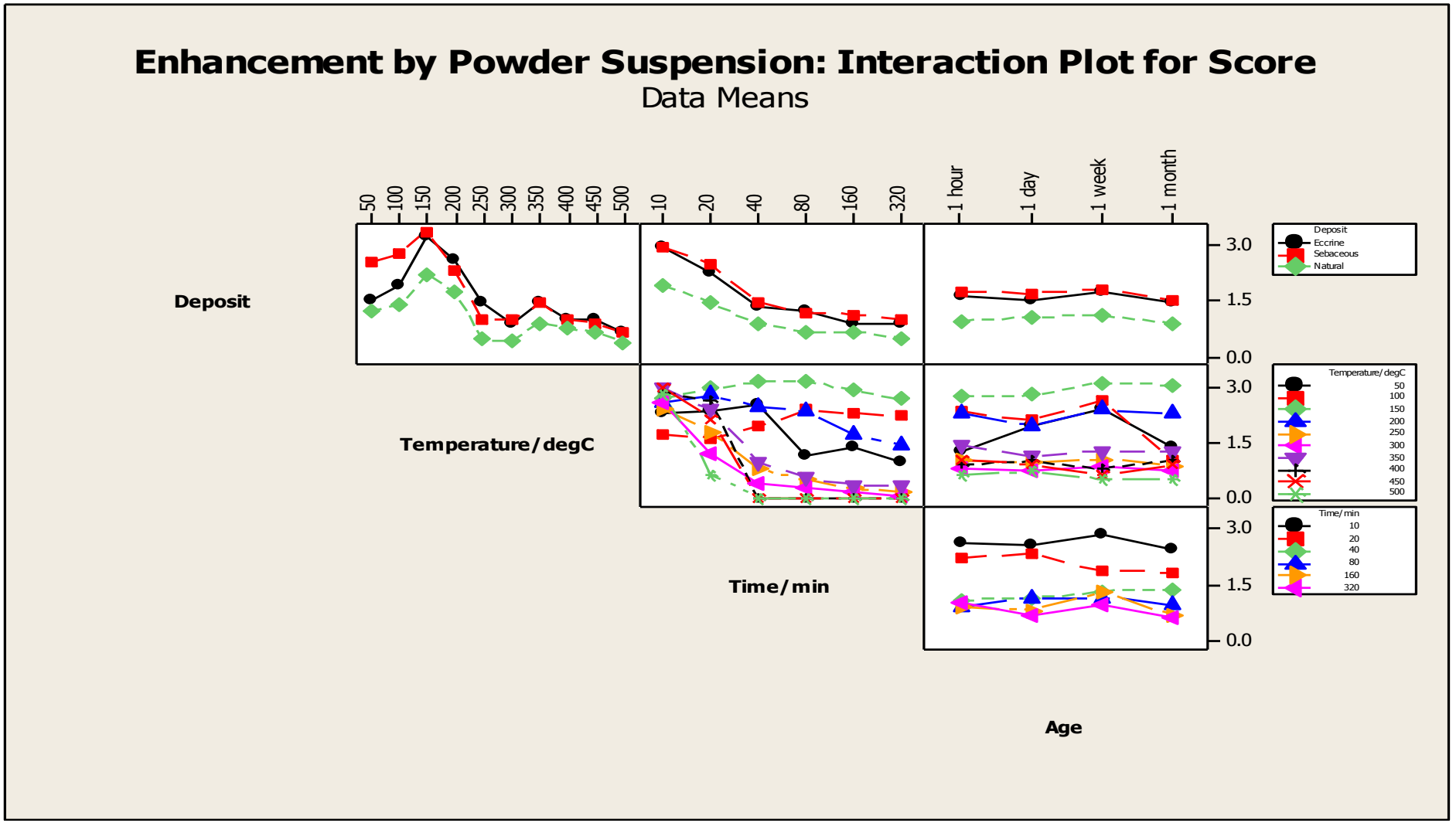



Figure 4-20 Interaction plot for powder suspension enhancement on glass (y-axis = score)

The interaction plot shows that when combining the *type of deposit* and *temperature* the trends for the three types of deposits are similar with a peak in scores observed at 150˚C. As the components of the fingerprint deposit which react with powder suspension are unknown, it is difficult to provide a definitive explanation as to the improvement in the fingerprints enhanced at 150˚C. One possible solution is the degradation of the components of the residue being more reactive to the powder suspension than the unchanged components. The sebaceous deposits gave the highest scores at 50˚C and 100˚C before eccrine deposits scored higher. Similar results were observed when the *type of deposit* was combined with *time* of exposure and also the *age* of the deposit, where the eccrine and sebaceous enhanced fingerprints produced similar scores, with poorer scores from the natural fingerprints. Interesting results were obtained from the *temperature* and *time* interaction with the quality of the fingerprints exposed to 50˚C decreasing as the exposure time increased whereas at 100˚C and 150˚C all of the deposits performed the same across the exposure times where increases in the quality of the deposit are observed. At 200˚C a steady decrease in quality is observed after about 20min exposure but from 250˚C and upwards, very poor results were recorded from approximately 20min or 40min exposure onwards depending on the temperature. This suggests a significant degradation of the deposits irrespective of deposit type are occurring at elevated temperatures; however it is significant that even at high temperatures (500˚C) good quality prints can be recovered if the exposure to the heat flux is of short duration (less than 10min). The interaction between the *temperature* and the *age* of the fingerprint indicates that fingerprints exposed to temperatures of up to 200˚C, have been enhanced and graded to give similar scores across all ages of deposit. At temperatures of 200˚C and over, the quality of the enhanced fingerprints are consistently poor. The *time* of exposure combined with the *age* of the deposit provided consistent scores across the age of the deposit, and deposits exposed to the temperatures for less that 20 minutes generally produced better quality fingerprints.

The results from the Kruskal-Wallis tests, and the main effects and interaction plots indicate that powder suspension appears to be targeting both the eccrine and sebaceous components of the fingerprint due to the high scores observed for each. It is not possible to say which type of deposit is targeted more as the results for eccrine and sebaceous deposits are very similar and the variation in fingerprint deposition

must be taken into account when analysing these figures although, at lower temperatures the sebaceous component exhibits slightly better enhancement. From a practical aspect, it is valuable that powder suspension is an effective technique for the enhancement of fingerprints on glass as the technique has the advantage of being effective on both wet and dry non porous surfaces.

#### 4.2.2.2 Superglue followed by BY40

When superglue was used to enhance fingerprints on the glass samples, enhancement was still possible for samples exposed to 500˚C at all exposure times. The samples were then dyed using BY40. The scores obtained by grading the fingerprints enhanced by superglue followed by BY40 were tested for normality. Similar graphs as to those shown for powder suspension in section 4.2.2.1 were generated and all P values were <0.010, indicating non parametric data. As such, Kruskal-Wallis tests were undertaken for each of the four independent factors. The following table (table 4-6) is the results of the analysis.

Table 4-6 Kruskal-Wallis test results for fingerprints on glass enhanced by superglue-BY40

| Factor | P Value |
|---|---|
| Type of Deposit | 0.000 |
| Temperature | 0.000 |
| Time | 0.000 |
| Age | 0.340 |

The results of the Kruskal-Wallis tests was the same as that of the powder suspension enhanced fingerprints in that it was only the age of the fingerprints which did not have a significant effect on the fingerprint score. Again, the explanation for this could be the results of the nature of the substrate.

As with the powder suspension enhanced fingerprints, a main effects plot and an interaction plot was derived on Minitab. The main effects plot is shown in figure 4-21.

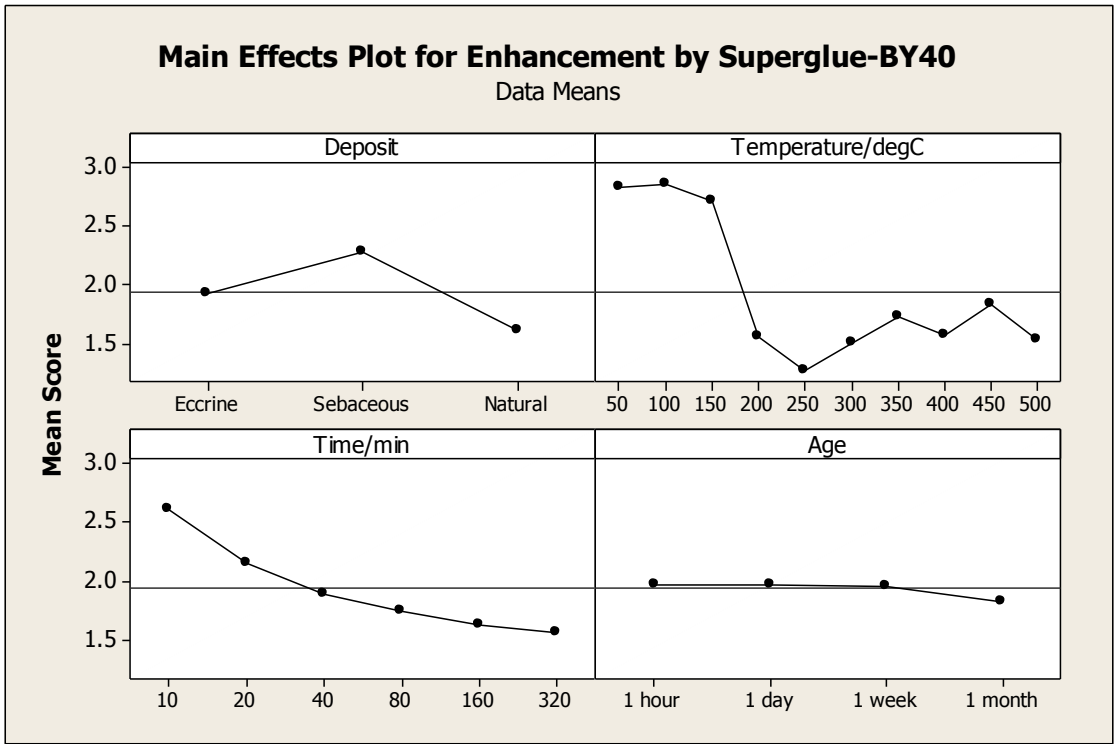


Figure 4-21 Main effects plot for enhancement by superglue-BY40

The main effects plot mirrors the results displayed in table 4-6 from the Kruskal-Wallis tests. A difference can be seen in the *type of deposit* graph with sebaceous fingerprint scores higher than the mean, eccrine scores on the mean, and natural scores below the mean. The variability witnessed in the powder suspension temperature main effects plot is also given in the superglue followed by BY40 *temperature* graph. Although the increase seen at 300˚C suggests that the shielding was not the reason for the increase, as this was introduced at 350˚C. Fingerprint variability could be the explanation. A steady decrease in scores is shown with increased exposure *time* and the only slight deviation in the *age* graph is shown at 1 month old fingerprints, showing that varying the age is not significant to the fingerprint score for fingerprints enhanced by superglue followed by BY40.

The interaction plot is given in figure 4-22.

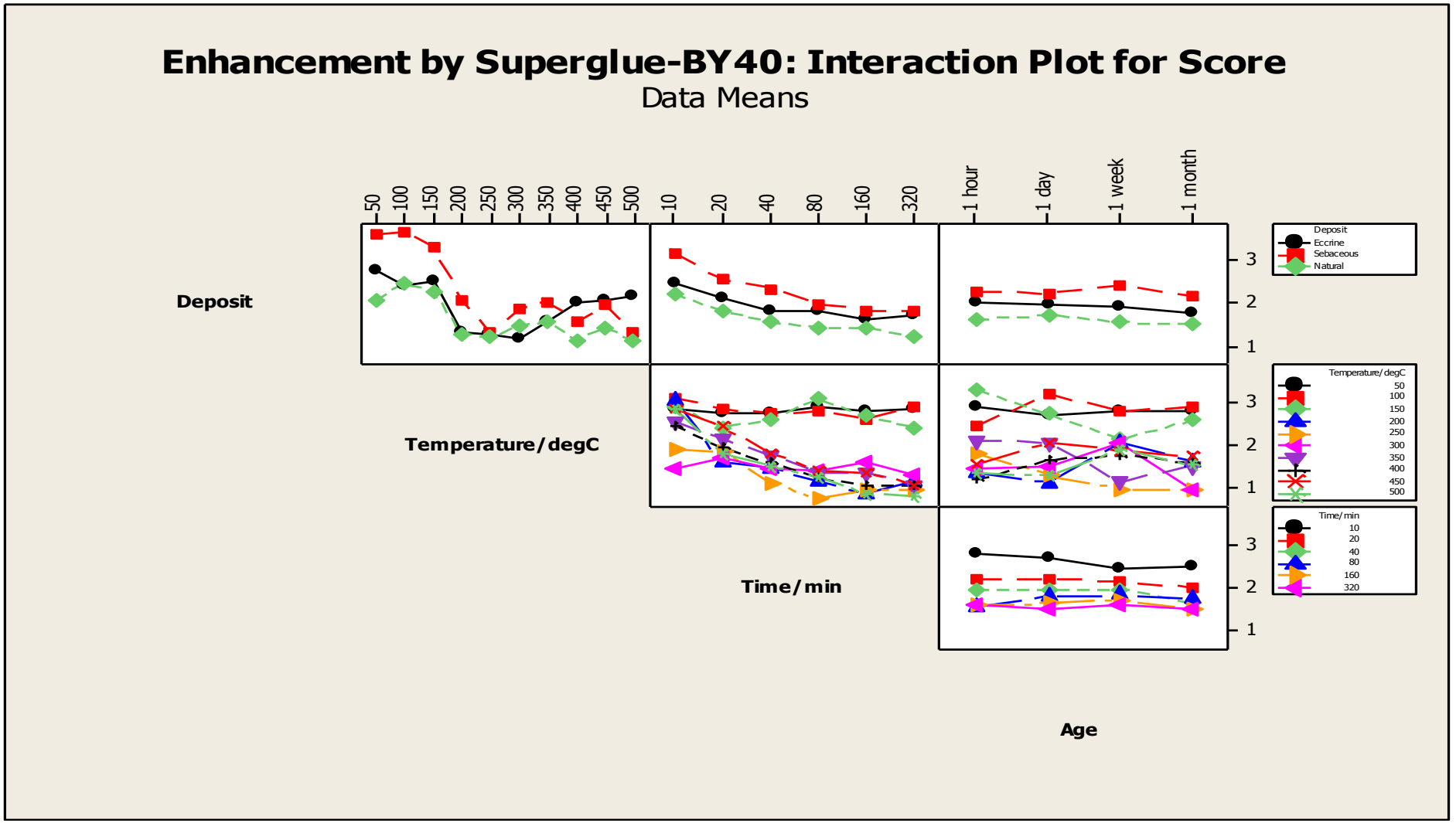



Figure 4-22 Interaction plot for superglue followed by BY40 enhancement on glass (y-axis = score)

Examination of the interaction plot shows that when *type of deposit* is combined with *temperature* all three types of deposits follow approximately the same trend. In terms of fingerprint scores, sebaceous fingerprints gave the highest scores at lower temperatures and the eccrine deposits gave slightly higher scores than sebaceous fingerprints at higher temperatures. The interaction between the *type of deposit* and the *tim*e of exposure, and the interaction with *age* of the deposit shows that sebaceous deposits are again producing the highest scores indicating better quality enhanced fingerprints but the scores are decreasing with increased exposure time and there is little difference in the scores depending on the age of the deposit. These trends are observed with all deposit types. The *temperature* combined with *time* indicates that the scores obtained between 50˚C and 150˚C were approximately the same across the exposure times for all deposit types but from 200˚C upwards, the quality of the enhanced print decreased with increased exposure time. Similar trends were observed for the *temperature* combined with the *age* of the deposit.

Fingerprints were still being enhanced for deposits exposed at temperatures of 500˚C for 320min albeit the scores of the enhanced fingerprints decreases markedly when exposed to temperatures above 150˚C. Superglue enhancement targets components of the fingerprint which are water soluble and as such may not be very successful on exhibits recovered from fire scenes since water is used in suppression activities. Notwithstanding this it has been demonstrated that even at very elevated temperatures superglue is an effective and viable fingerprint enhancement technique and as such merits consideration for exhibits which have been protected from excessive heat flux or significant exposure to water.

#### 4.2.2.3 Enhancement Technique Comparison

The purpose of depositing an eccrine fingerprint, a sebaceous fingerprint, and a natural fingerprint was to evaluate if certain components of the fingerprint survived longer under the various heating exposures.

The scores obtained by grading the fingerprints enhanced by powder suspension and superglue followed by BY40 were compared by undertaking a Kruskal-Wallis which

gave a P value of 0.000, indicating that there is a significant difference in the scores recorded from the fingerprints enhanced by the two enhancement techniques.

As with other enhancement technique comparisons, a main effects plot for enhancement technique only, and an interaction plot comparing the interaction between technique and type of deposit have been generated using Minitab. These plots are figures 4-23 and 4-24 respectively.

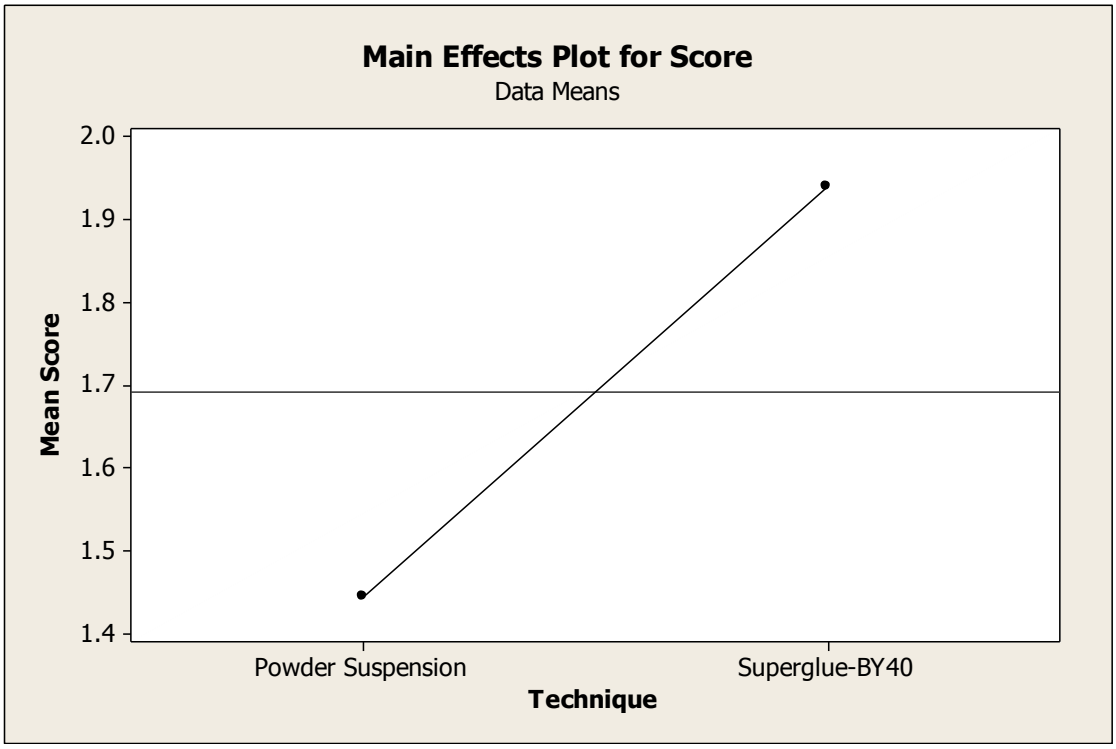



Figure 4-23 Main effects plot comparing enhancement techniques

The main effects plot shows that the Kruskal-Wallis test was correct in indicating that there was a significant difference between the scores for each enhancement technique for fingerprints deposited on glass exposed to temperatures up to 500˚C. The interaction plot in the next figure (figure 4-24) examines the interaction between the type of deposit and the enhancement technique.

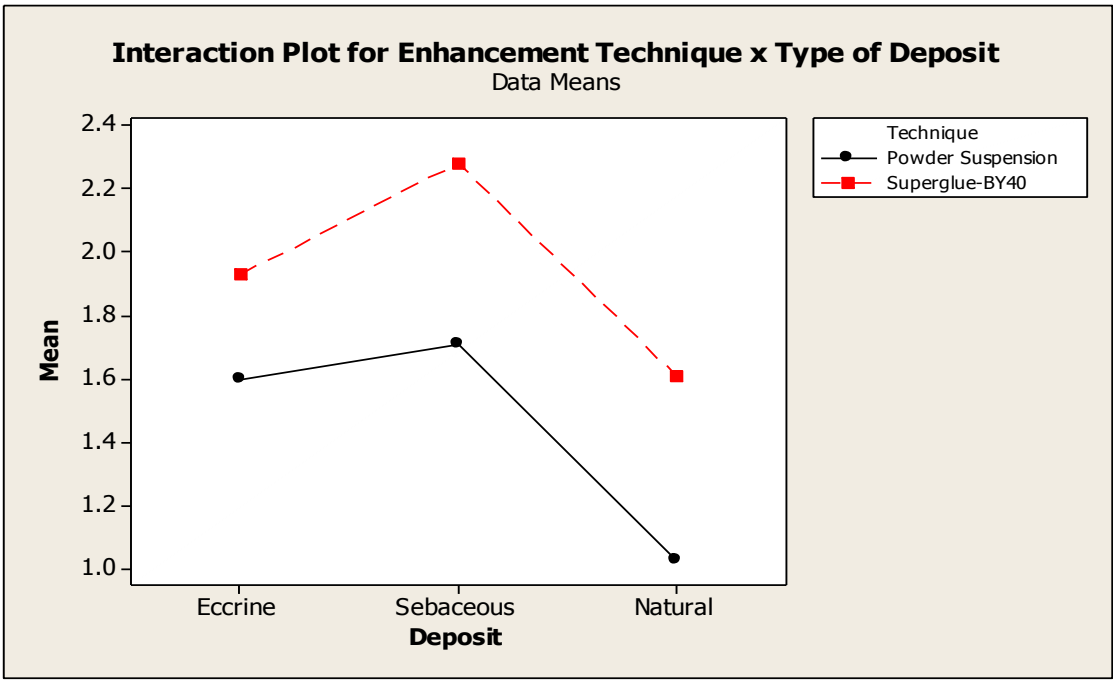


Figure 4-24 Interaction plot comparing enhancement technique and type of deposit (y-axis = score)

The combination of the *technique* and the *type of deposit* illustrates that the two enhancement techniques follow the same general pattern with superglue-BY40 enhancement providing better overall quality fingerprints as evidenced by the increased scores for these samples.

Both enhancement techniques appear to favour the sebaceous component of the fingerprint and have demonstrated the potential of fingerprint enhancement using superglue-BY40, in particular, of deposits exposed for short periods of time up to 500˚C and where the substrate remains dry. If the article is wet, then best results will be obtained using powder suspension. This is different to the findings reported by HOSDB who suggest that black powder suspension is the best performing treatment for non porous surfaces exposed to temperatures up to 200˚C [119]. Also this work has shown that from 350˚C upwards, glass needs to be protected from the radiant heat and air flow for fingerprints to survive.

### 4.2.3 Fingerprints on Ceramic

Glass will start to crack and deform at temperatures above 500˚C and as such it was unsuitable for any higher temperature work. Ceramic was used to enable the fingerprint deposits to be exposed to higher temperatures on a similar chemically inert

surface. Results for fingerprints on ceramic at 500˚C were obtained to check for its similarities to glass at that temperature and to corroborate that the results obtained on both surfaces at the same temperature are comparable. For powder suspension enhancement, a Kolmogorov-Smirnov test was carried out to check the data was normally distributed. The test produced a P value of <0.010, indicating that the scores were not normally distributed. Therefore a Kruskal-Wallis test was undertaken to assess whether there was any difference in the powder enhancement scores at 500˚C on glass and ceramic. The Kruskal-Wallis test produced a P value of 0.291, showing that the results are not significantly different. A normality test was carried out for the superglue enhancement scores but the Kolmogorov-Smirnov P value was 0.056 showing normally distributed data. A one-way ANOVA was conducted to assess the difference in the superglue scores for each surface and this produced a P value of 0.778. This showed there was no significant difference between the superglue scores enhanced on glass and ceramic. Thus it can be assumed that the enhancement by these two techniques of fingerprints deposited on both of these non porous surfaces behave in a similar manner.

#### 4.2.3.1 Powder Suspension

Kolmogorov-Smirnov normality tests were conducted for the scores for each deposit enhanced by powder suspension enhancement. Each deposit's test provided P values of <0.010, indicating scores that were not normally distributed. As such, Kruskal-Wallis tests were performed for each factor with the results given in table 4-7.

Table 4-7 Kruskal-Wallis test results for fingerprints on ceramic enhanced by powder suspension

| Factor | P Value |
|---|---|
| Type of Deposit | 0.681 |
| Temperature | 0.000 |
| Time | 0.000 |
| Age | 0.678 |

Two of the independent factors are significant to the fingerprint score. Similarly to the analysis of the results obtained for glass, main effects plots and interaction plots will be generated on Minitab to graphically display the data. Therefore in the main effects plot, it is expected for *type of deposit* and *age* scores to be close to the mean response

line with *temperature* and *time* scores deviating from the line. The main effects plot is given in figure 4-25.

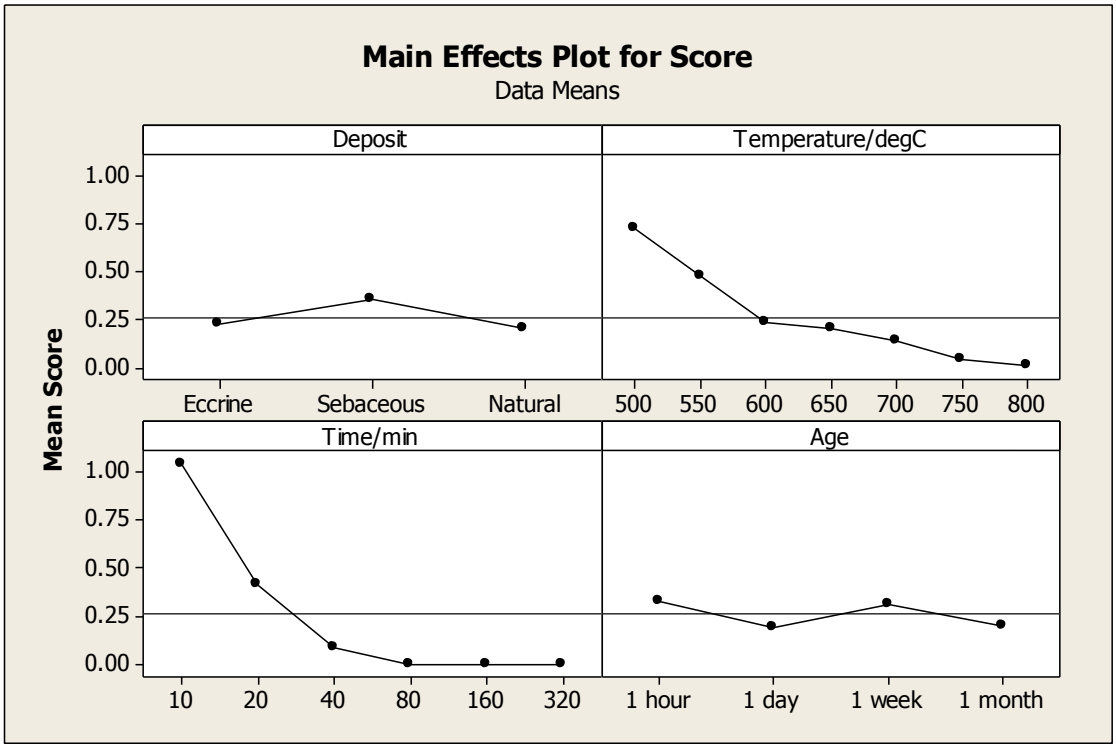



Figure 4-25 Main effects plot for enhancement by powder suspension

The main effects plot shows that the sebaceous fingerprints are marginally higher than for the other two *deposits* but using the Kruskal-Willis test, the differences between the scores is not significant. The scores are decreasing with increased *temperature* and scores are also decreasing, but more gradually, with increased exposure *time*. The Kruskal-Wallis test showed that the fingerprint scores were not significantly different with increased *age* and this is reflected in the graph with the fingerprint scores remaining close to the mean response line for all ages.

The interaction plot is given in figure 4-26.

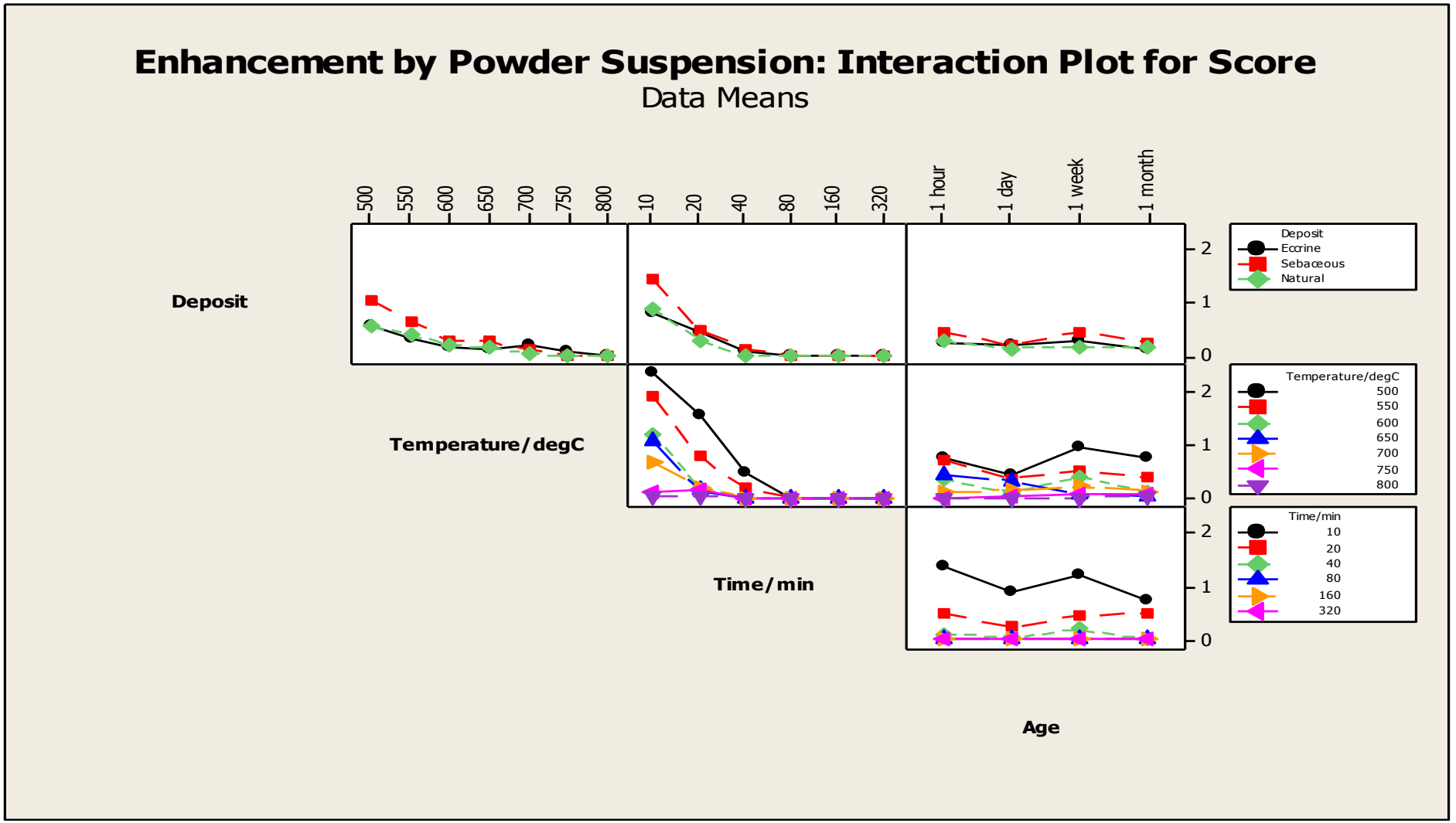



Figure 4-26 Interaction plot for powder suspension enhancement on ceramic (y-axis = score)

From the interaction plot, the combination of the *type of deposit* and *temperature* indicates that all three types of deposits are behaving in the same manner across the temperatures but with sebaceous deposits giving the highest scores at 500˚C and 550˚C. *Type of deposit* combined with the *time* of exposure shows the scores are decreasing across all times. Again sebaceous fingerprints gave the highest scores at 10min, 20min, and 40min exposure. There is no real observable difference in the scores obtained for the interaction between the *type of deposit* and the *age* of the deposit. The quality of the enhanced fingerprints decreases rapidly once the deposits experience temperatures of over 500˚C for greater than 10min.

The results from the Kruskal-Wallis tests and the main effects and interaction plots indicate that powder suspension will enhance fingerprints on samples which have low exposure times (less than 80mins) to temperatures up to 700˚C; however the quality of the enhanced fingerprints remains poor which would indicate that whichever component(s) of the fingerprint deposit which react with powder suspension have been eradicated by exposure to the heat flux. Since powder suspension will enhance fingerprints on wetted surfaces it may be that it is the only technique available to use on exhibits exposed to high heat flux where water has been used to suppress a fire.

#### 4.2.3.2 Superglue followed by BY40

The scores obtained by grading the fingerprints on ceramic enhanced by superglue followed by BY40 were analysed for normality using the Kolmogorov-Smirnov test. The scores examined at 500˚C were normally distributed but when each deposit was tested, the Kolmogorov-Smirnov scores gave P values of 0.010 for each one. This indicated that the scores were not normally distributed, therefore Kruskal-Wallis tests were used for each variable.

It had previously been suggested that the change in the fingerprint scores from normally distributed to not normally distributed may be a result of the substrate, with the porous surface protecting the fingerprint more than the non-porous surface. Another reason for this change could the temperature range. The fingerprints deposited and analysed on paper were stopped at 200˚C due to the autoignition temperature of paper. Therefore the techniques in the paper analysis never reached the

temperature where they stopped enhancing. For the non porous study on ceramic, the temperature range was limited by the effectiveness of the technique, resulting in more zero scores being recorded. This could account for the tailing in the normality plots.

The Kruskal-Wallis test results are given in table 4-8.

Table 4-8 Kruskal-Wallis test results for fingerprints on ceramic enhanced by powder suspension

| Factor | P Value |
|---|---|
| Type of Deposit | 0.000 |
| Temperature | 0.000 |
| Time | 0.000 |
| Age | 0.805 |

The P values indicate that all but *age* are significant to the fingerprint score. The main effects plot is given in figure 4-27.

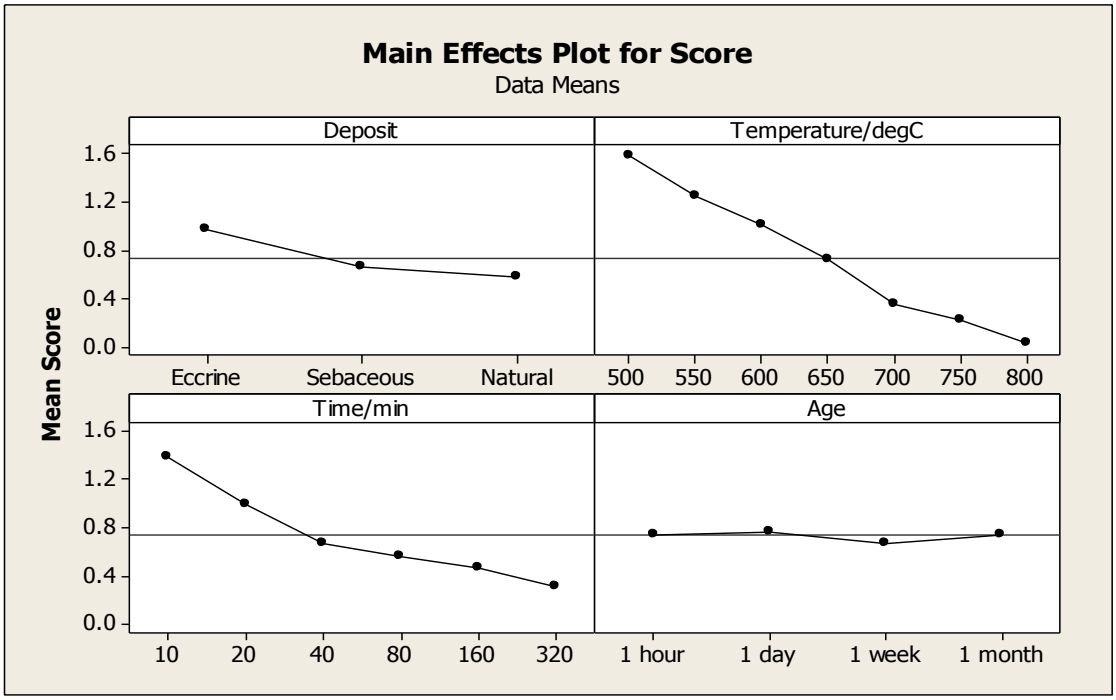



Figure 4-27 Main effects plot for enhancement by superglue-BY40

The main effects plot shows that eccrine *deposits* are producing better scores. Gradual decreases in score are observed with increased *temperature* and exposure *time*. No difference is shown in the scores for each *age*. An interaction plot was also derived on Minitab and this is shown in figure 4-28.

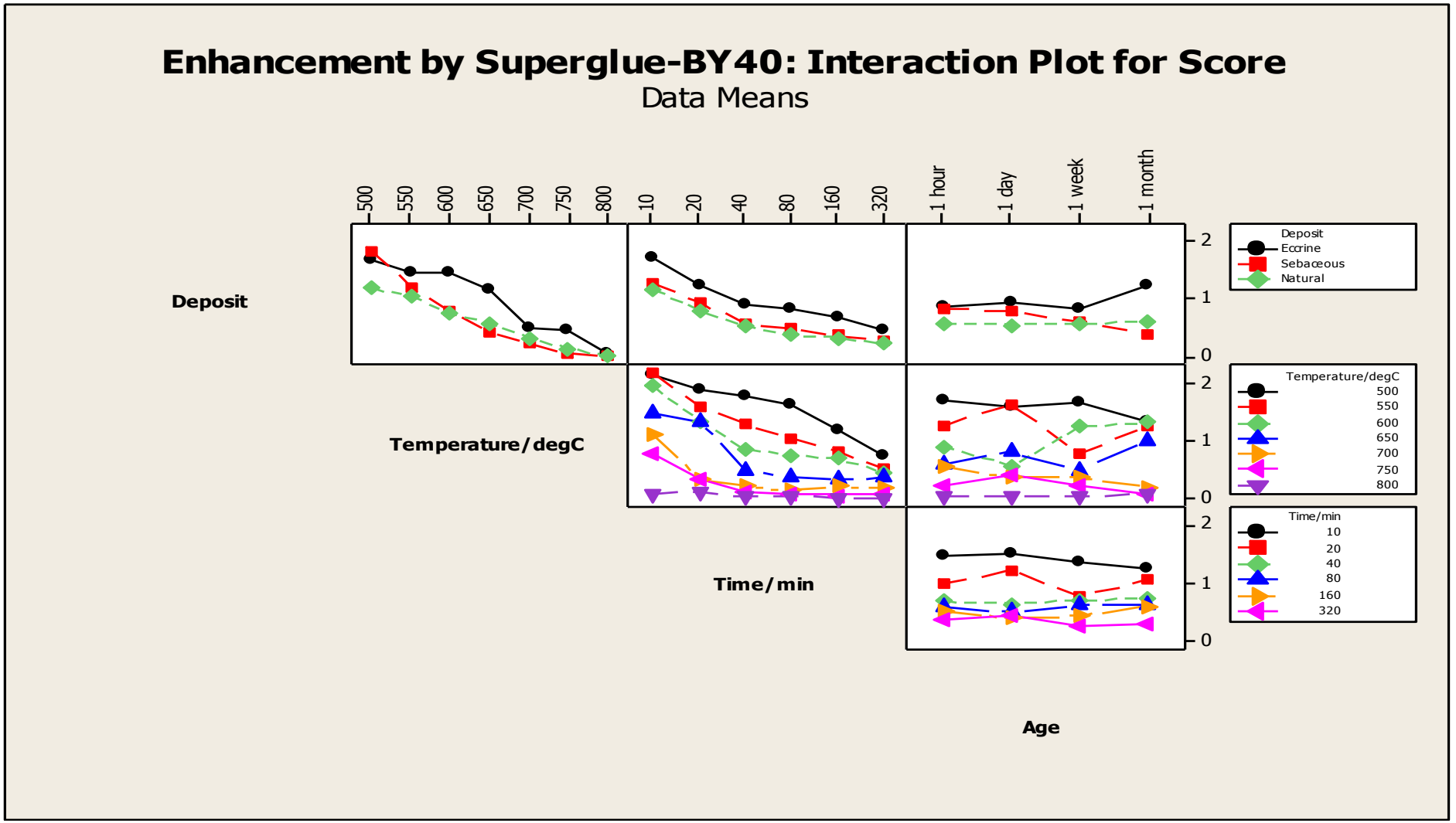



Figure 4-28 Interaction plot for superglue followed by BY40 enhancement on ceramic (y-axis = score)

In general the scores of the enhanced fingerprints were poor particularly once the temperature increased over 650˚C and exposure was for longer than 20 minutes. In this case eccrine deposits generally performed better which continued the trend observed with the various deposits on glass where the eccrine deposits began to outperform sebaceous deposits at the higher temperatures. The overall quality of the fingerprints remain poor. The results from the Kruskal-Wallis tests and the main effects and interaction plots indicate that superglue followed by BY40 is still producing results at all exposure times on dry ceramic at temperatures up to 750˚C for 10 minutes even though the quality of these fingerprints is not high.

#### 4.2.3.3 Enhancement Technique Comparison

As the fingerprint scores for both powder suspension and superglue followed by BY40 were not normally distributed, a Kruskal-Wallis test was employed to test if there was a difference in the scores between the two enhancement techniques. The Kruskal-Wallis test P value was zero, showing a significant difference. A main effect plot was generated to graphically interpret the scores for the two techniques. This is given in figure 4-29.

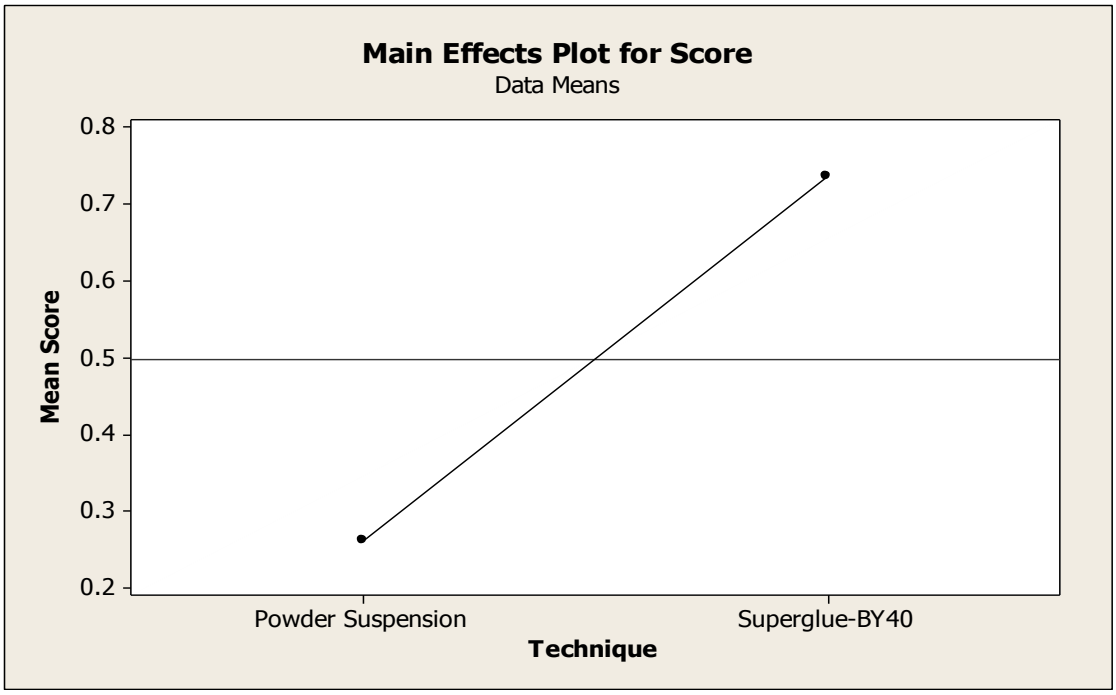



Figure 4-29 Main effects plot for comparing enhancement techniques

The main effects plot shows that there is a difference in the mean scores for each technique and that the superglue followed by BY40 is the superior enhancement technique.

An interaction plot examining the interactions between type of deposit and the enhancement technique was generated using Minitab and is given in figure 4-30.

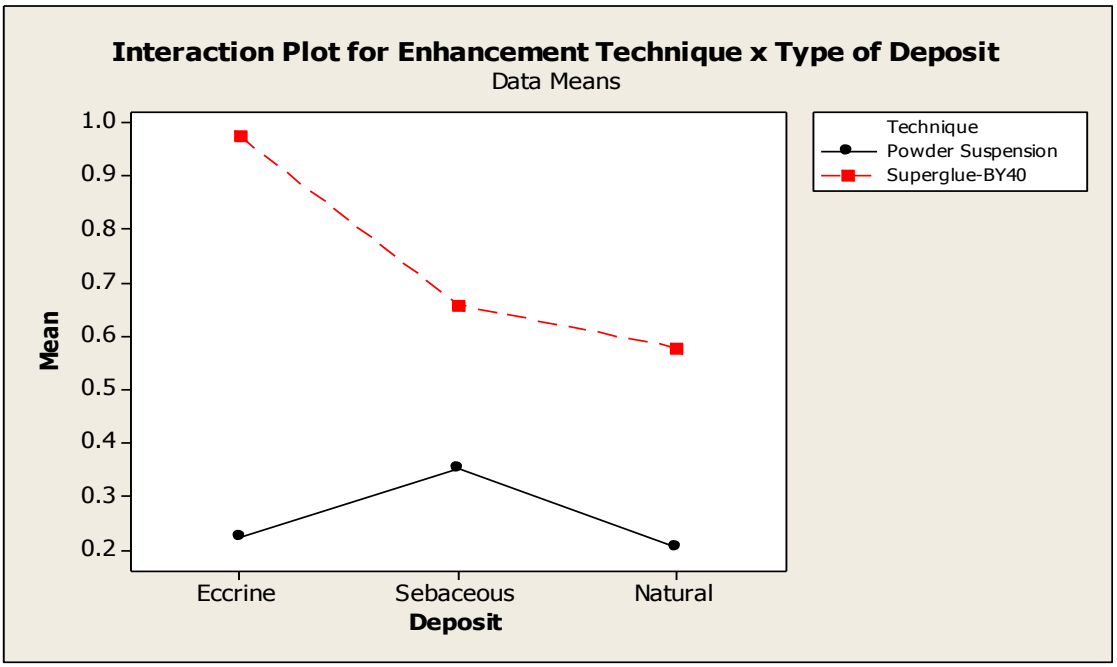



Figure 4-30 Interaction plot comparing enhancement technique and type of deposit (y-axis = score)

In general BY40 dyed superglue continued to outperform powder suspension continuing the trend observed at lower temperatures with the glass study. In terms of powder suspension, sebaceous deposits demonstrated better enhancement than the other deposits, whereas eccrine deposits were enhanced to a greater degree with superglue followed by BY40. Although the scores for natural fingerprints are low, it is reassuring that at least some sort of enhancement is achieved on fingerprints exposed to these elevated temperatures.

This experimental work has shown that superglue followed by BY40 is the most effective technique for enhancing fingerprints on ceramic subjected to temperatures of 500˚C to 800˚C. This contradicts Bradshaw *et al.*, [117] and the HOSDB report [119] which states superglue is effective only up to 500˚C. This data shows that better results are obtained for the enhancement of natural fingerprints by superglue-BY40.

Therefore, as natural fingerprints are the closest representation to fingerprints found at a crime scene, superglue followed by BY40 would be the recommended technique if the article was dry. If the article is wet, results will still be obtained using powder suspension but of a lower quality.

#### 4.2.3.4 VMD

Due to time constraints, VMD was only briefly examined in order to investigate its effectiveness at 10min and 20min exposure times, at 700˚C, 750˚C, and 800˚C, and after 1 hour and 1 day ageing, in comparison with powder suspension and superglue followed by BY40. The literature suggests that VMD can enhance fingerprints exposed to 900˚C but areas in which water has dried will show up during treatment and it may be difficult to resolve fingerprints in practical situations [63]. The Kruskal-Wallis test provided a P value of 0.002, indicating a significant difference in the enhancement techniques. The main effects plot is given in figure 4-31.

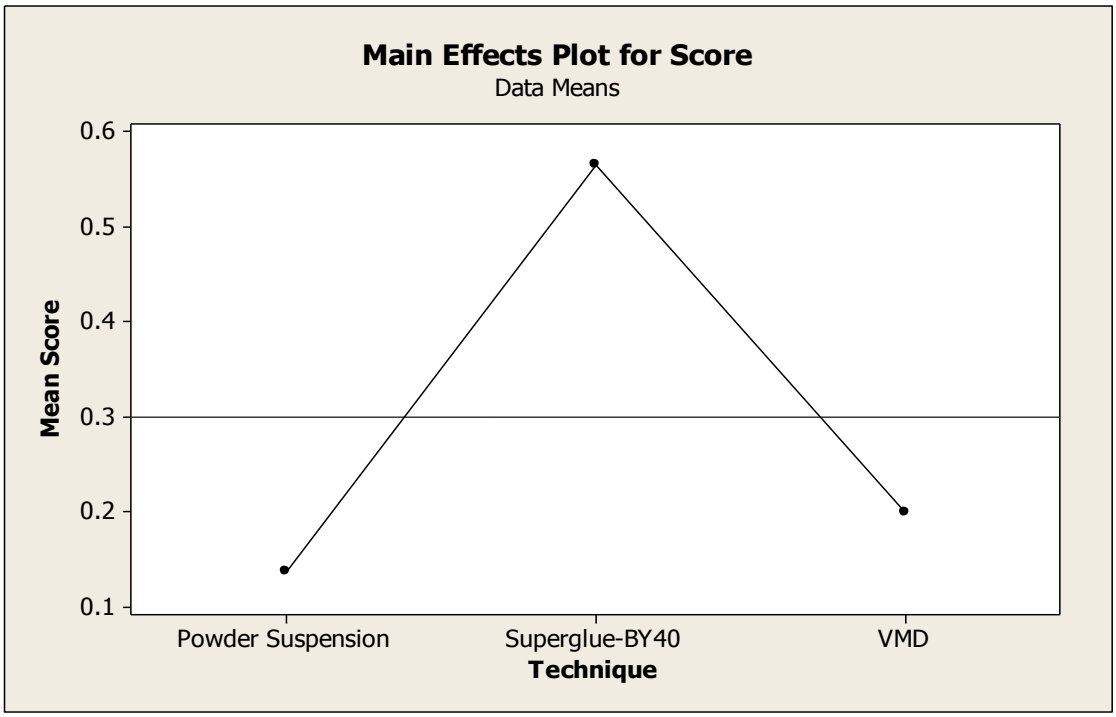



Figure 4-31 Main effects plot for comparing enhancement techniques

The main effects plot shoes that superglue followed by BY40 is the most superior technique with VMD providing a slight improvement in mean scores compared to powder suspension. The interaction plot is given in figure 4-32.

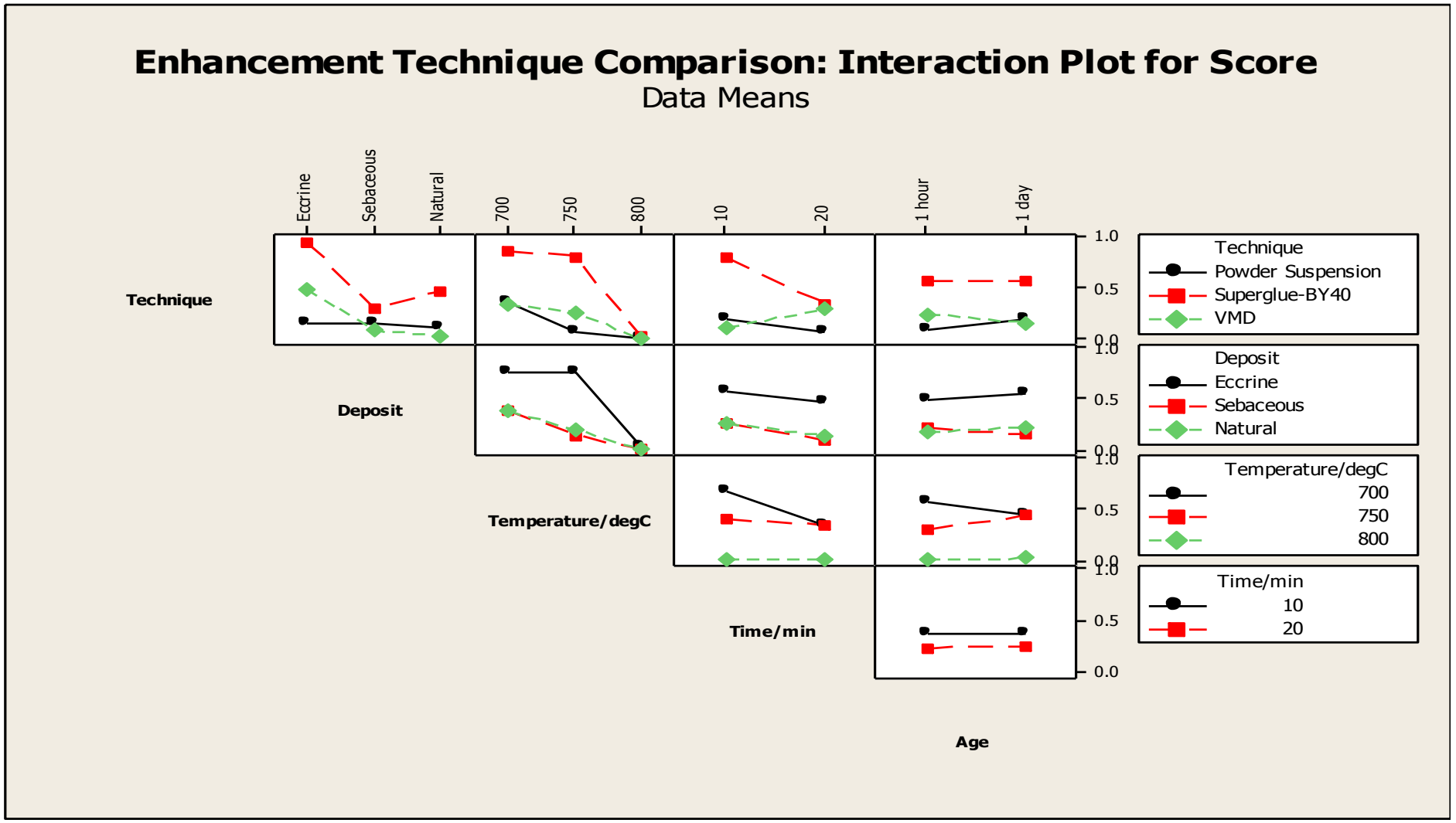



Figure 4-32 Interaction plot for powder suspension, superglue followed by BY40, and VMD comparison on ceramic (y-axis = score)

The results of the interaction plot for the *technique* combined with the *type of deposit* indicated that superglue-BY40 outperformed VMD and powder suspension on all deposit types with the eccrine deposits followed by natural providing the best quality enhanced fingerprints. Again the scores of the enhanced fingerprints were poor across all techniques in comparison to enhancement at temperatures less than 500˚C. The results for the *technique* and *time* on exposure appears to reverse this trend slightly as superglue-BY40 and powder suspension scores decrease with increased exposure time, whereas VMD scores increase. Even though the quality of the enhanced fingerprint is generally poor the results obtained indicate that at some level the fingerprints survive temperatures of up to 750˚C for exposure times of 20min.

### 4.2.4 The Effects of Heat on Fingerprints Deposits on Metal – An Additional Study

The latent fingerprints deposited on the stainless steel that was exposed to room temperature through to 900˚C can be viewed using oblique lighting. Figure 4-33 shows an example of stub at each exposure temperature. The stainless steel remained silver in colour and the fingerprints had also remained translucent for samples exposed at room temperature, 100˚C and 200˚C. After exposure to 300˚C and 400˚C, the surface of the stainless steel had changed to a brass colour, and the fingerprints deposited on these temperature exposed stubs have changed to a dark brown colour. There is still poor contrast between these fingerprints and the background stainless steel. At 500˚C, the stainless steel continues to be brass coloured but the fingerprint deposits have now changed to a dark blue/black colour. After exposure to 600˚C, the colour of the stainless steel surface has now changed from brass to purple with the regions of the fingerprint deposit appearing silver. Similarly, at 700˚C the steel surface has gone from purple to blue but the fingerprint ridges continue to appear silver. The fingerprint to background contrast observed on these samples is much greater than any of the samples from the lower exposure temperatures. After exposure to 800˚C and 900˚C, the stainless steel has returned to its original silver colour but the metal has dulled, instead of the high shine on the surface observed at the lower temperatures. The fingerprints have a ‘burnt on’ appearance, indicated from their colour change of silver to dark brown. All phenomena observed at each temperature were witnessed across all three repeat samples within the test batch.

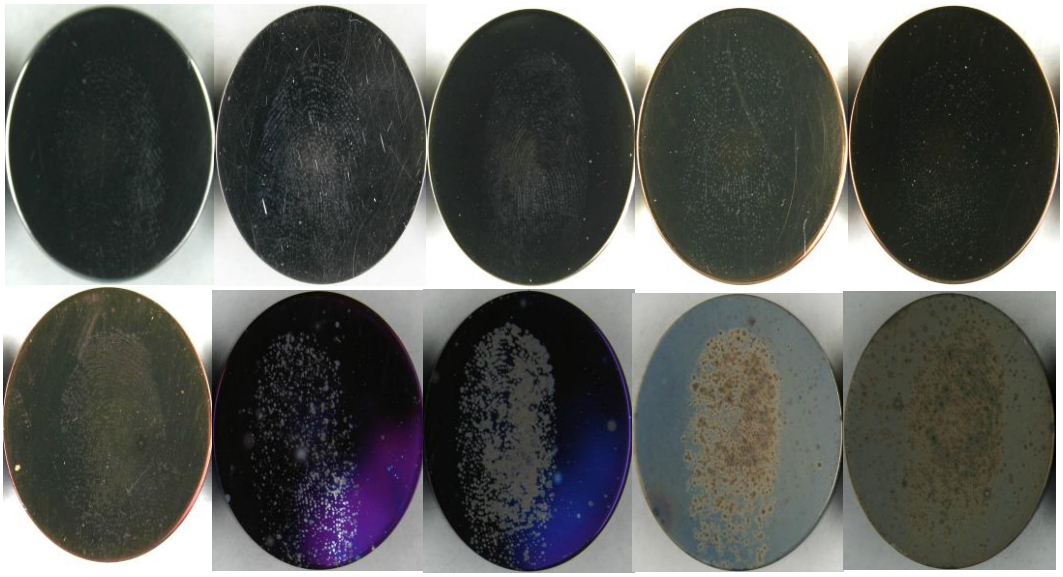


Figure 4-33 Photographs of fingerprints deposited on SEM stubs exposed to, from left, room temperature, 100˚C, 200˚C, 300˚C, 400˚C (top) and 500˚C, 600˚C, 700˚C, 800˚C and 900˚C (bottom)

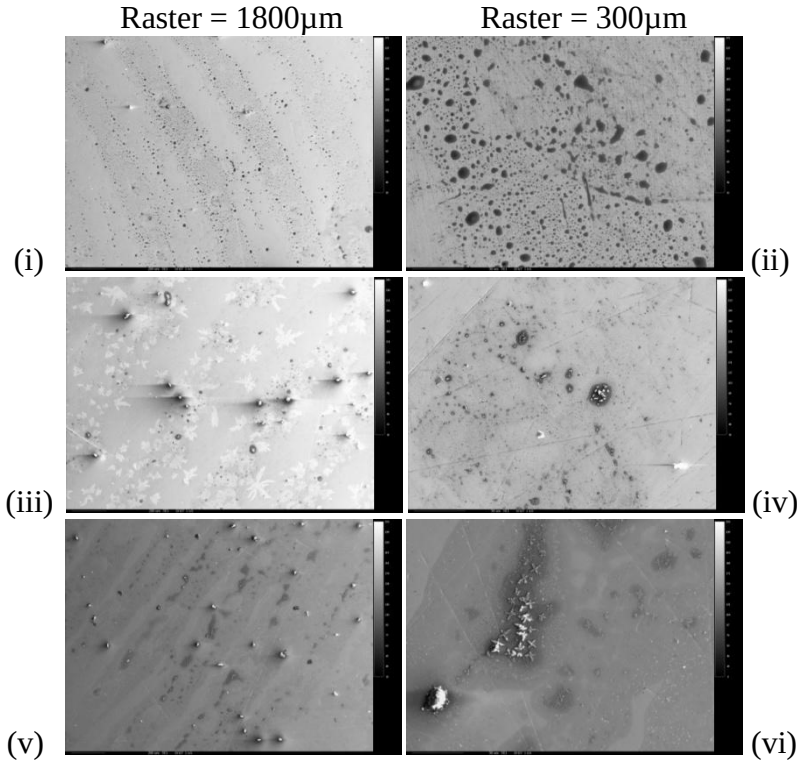
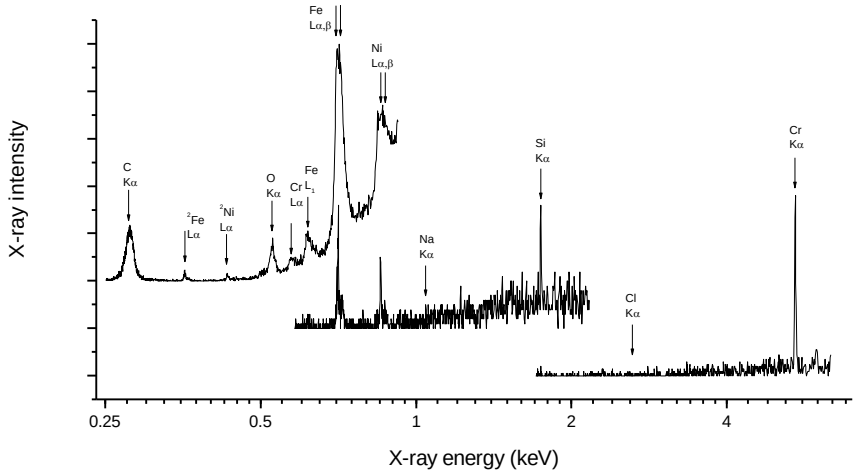


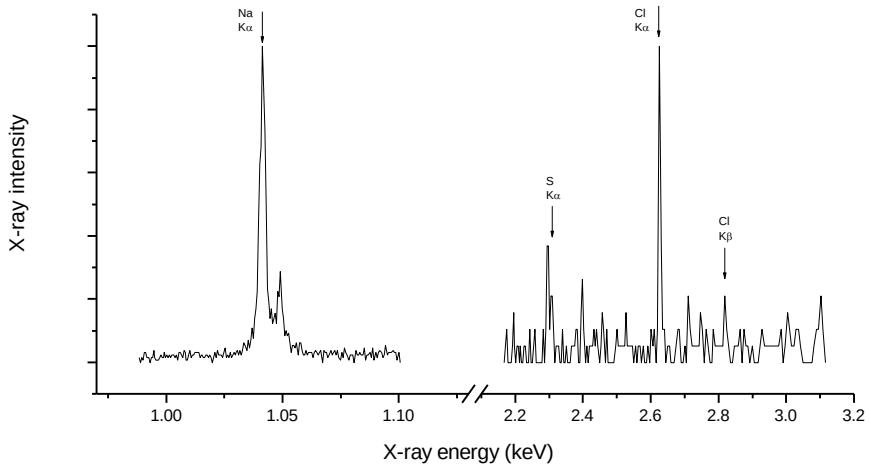

Figure 4-34 Secondary electron images of (i) room temperature sample, (ii) room temperature sample magnified, (iii) 100˚C sample, (iv) 100˚C sample magnified, (v) 200˚C sample, and (vi) 200˚C sample magnified

SEM images were captures for the stubs exposed to room temperature, 100˚C, and 200˚C. Figure 4-34 shows images captured at two different rasters. In the figure, images (i) and (ii) clearly show that at room temperature, the fingerprint deposit is in the form of droplets on the stainless steel surface. At 100˚C, the fingerprint deposit is starting to dry out. At the raster of 1800µm (iii), a 'star like' pattern is observed. This could be the result of the moisture being driven off in the deposit. At the 300µm image (iv), small crystals are found. At 200˚C, the 1800µm image (v) shows a drier deposit, with the magnified image (vi) showing a greater abundance of crystal formation.

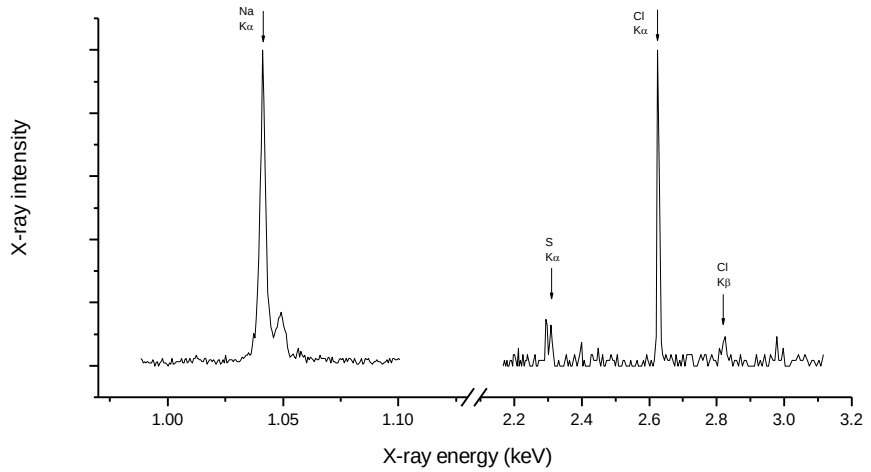
WDX analysis was undertaken on the fingerprints exposed to these three temperatures, resulting in the spectra produced in figure 4-35. Spectrum (i) is a full spectral scan of one of the droplets in the room temperature sample. The spectrum obtained from the room temperature sample was the same as produced for the background metal, indicating that the SEM probe was penetrating through the wet deposit and not producing an individual spectrum of the deposit itself. Spectrum (ii) is a spectrum taken with the probe focussed on one of the crystals in a sample exposed to 100˚C, which indicated that the crystal was sodium chloride, and spectrum (iii) is a spectrum taken again with the probe focussed on a crystal but formed during exposure to 200˚C. This spectrum showed the presence of an increased amount of sodium chloride.




(i)




(ii)




(iii)

Figure 4-35 WDX spectra for (i) room temperature sample, (ii) 100˚C sample, and (iii) 200˚C sample

Figure 4-36 contains elemental maps from samples exposed to room temperature, 100˚C, and 200˚C. These maps also indicate the presence of sodium chloride at 100˚C

and 200˚C, and its absence at room temperature. These maps provide further proof that the crystals formed at these lower temperatures are sodium chloride.

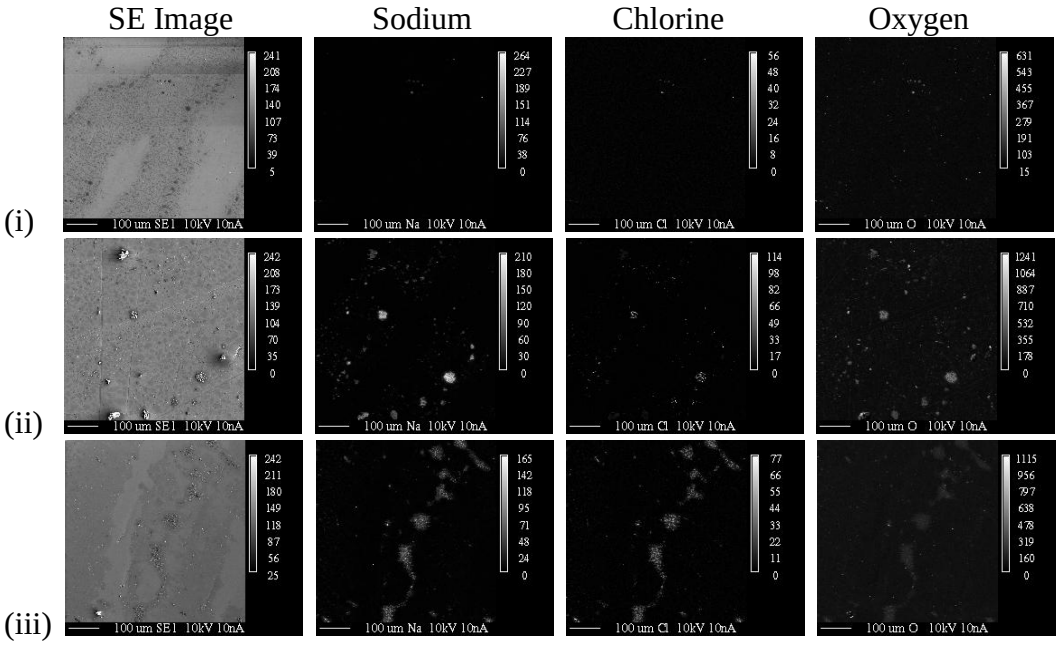



Figure 4-36 Secondary electron images and elemental mapping for (i) room temperature sample, (ii) 100˚C sample, and (iii) 200˚C sample

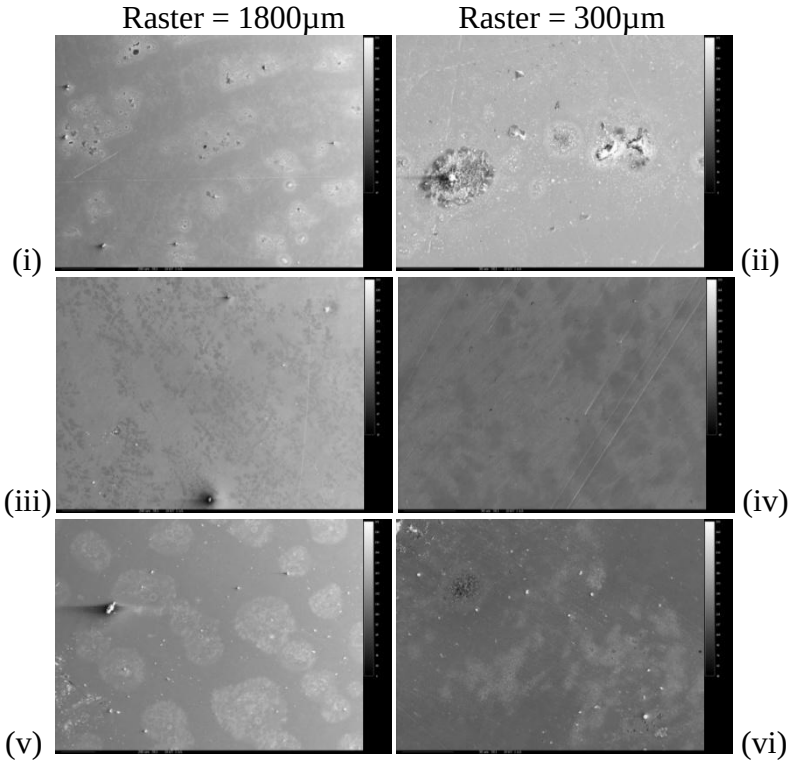



Figure 4-37 Secondary electron images of (i) 300˚C sample, (ii) 300˚C sample magnified, (iii) 400˚C sample, (iv) 400˚C sample magnified, (v) 500˚C sample, and (vi) 500˚C sample magnified

The scanning electron microscope images taken of the samples exposed to 300˚C, 400˚C, and 500˚C are found in figure 4-37. Images (i) and (ii) are of the samples exposed to 300˚C. This shows that the fingerprint deposit has completely dried out but some crystals are still present on the surface. Images (iii) and (iv) shows that after exposure at 400˚C the sodium chloride crystals are absent and this is also witnessed for samples exposed to 500˚C in images (v) and (vi).

Elemental mapping for a sample exposed to 400˚C is given in figure 4-38. This shows that there is a small presence of sodium, an even smaller amount of chlorine, but that oxygen is now present in the fingerprint deposit.

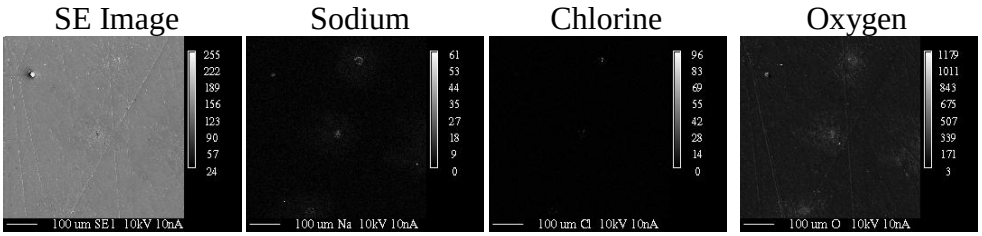


Figure 4-38 Secondary electron image and elemental mapping for 400˚C sample

Figure 4-39 gives the SEM images for exposure to 600˚C and 700˚C. Images (i) and (ii) show a dry irregular residue formed on top of the surface whereas images (iii) and (iv) indicates that the surface is being damaged by the residue.

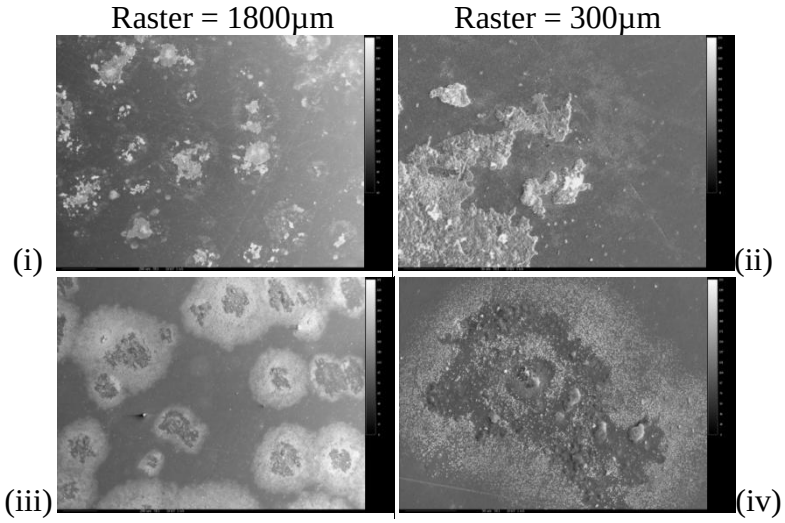
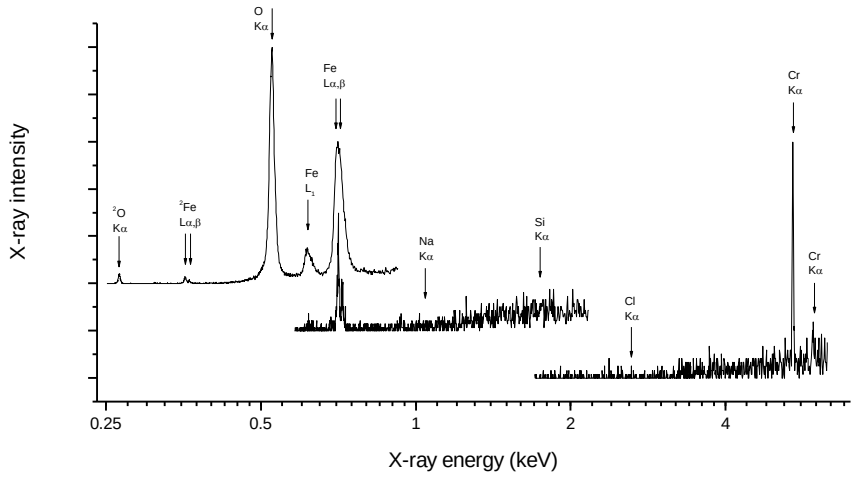
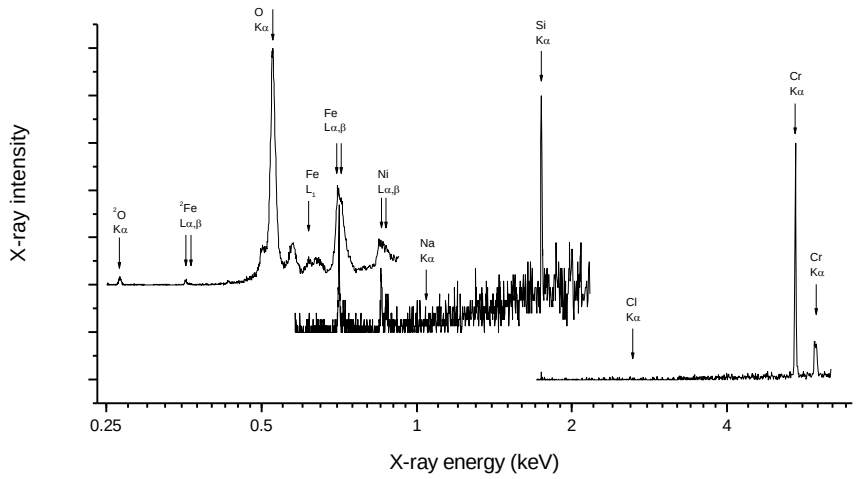


Figure 4-39 Secondary electron images of (i) 600˚C sample, (ii) 600˚C sample magnified, (iii) 700˚C sample, and (iv) 700˚C sample magnified

Figure 4-40 provides the WDX analysis of the samples exposed to 600˚C and 700˚C. Both spectra indicate that sodium and chlorine are no longer present but that there is a large increase in the amount of oxygen present. The spectra also show that carbon is not present in samples, signifying that the fingerprint residue is not charring. Elemental mapping for the 600˚C samples also supports the formation of an oxide in the fingerprint deposit and the absence of sodium chloride (figure 4-41).




(i)




(ii)

Figure 4-40 WDX spectra for (i) 600˚C sample, and (ii) 700˚C sample

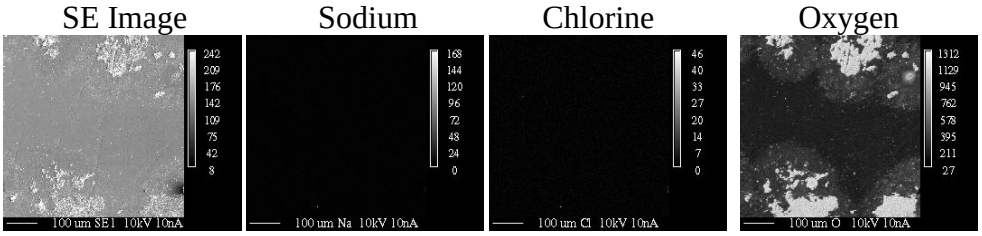


Figure 4-41 Secondary electron image and elemental mapping for 600˚C sample

The SEM images captured for 800˚C and 900˚C samples again indicate the damage (possibly by oxidation) to the surface of the stainless steel (figure 4-42). The only difference between the two temperatures is that at 800˚C (images (i) and (ii)); the fingerprint deposit is still discernable from its background. This is less pronounced at 900˚C (images (iii) and (iv)).

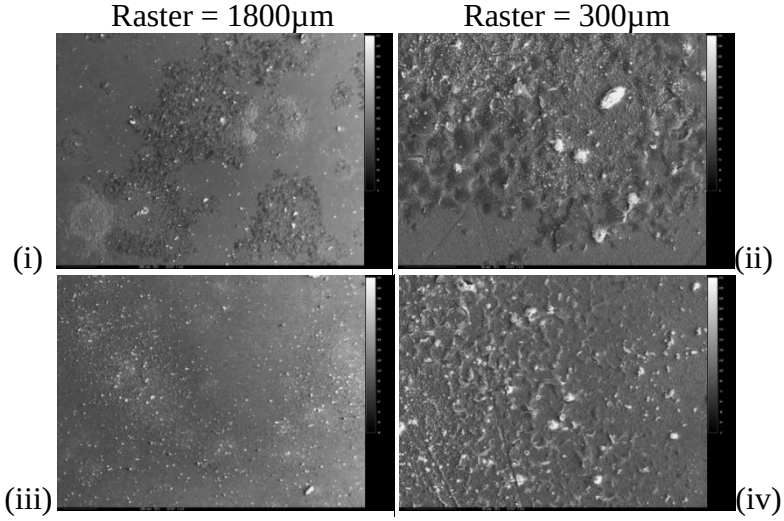


Figure 4-42 Secondary electron images of (i) 800˚C sample, (ii) 800˚C sample magnified, (iii) 900˚C sample, and (iv) 900˚C sample magnified

Figure 4-43 shows the elemental maps obtained from a sample exposed to 900˚C. This shows that the whole surface of the stainless steel has now oxidised.

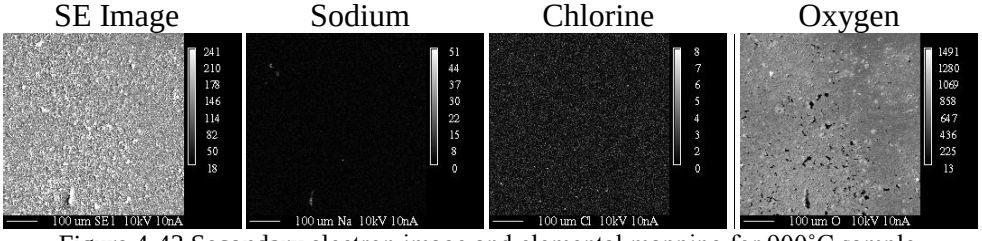


Figure 4-43 Secondary electron image and elemental mapping for 900˚C sample

Bond [180] has recently published research which involved the heating of several different types of metal in order to visualise fingerprints, with the aim of enhancing fingerprints on cartridge cases and metals which had been exposed to elevated temperatures and as such his data is relevant to this study. His research explores the idea that the enhancement of fingerprint deposits on metal is due to corrosion. He suggests that 'an association between chloride salt secretion and metal corrosion because of fingerprint deposition has been known for many years [181] as chemically aggressive chloride ions will combine readily with metals to form metal salts [182], the reaction being accelerated by elevated temperatures [183].' He also asserts that 'the ability to enhance fingerprints appears to be independent of the time interval between deposition and heating, which would support an explanation that the corrosion is determined primarily by the presence of a chemically aggressive element such as chlorine.' These conclusions were based on the results obtained from depositing fingerprints on copper and brass.

Bond also investigated steel as a substrate, but concluded 'fingerprint development on steel produced different results to brass and copper in that the development appeared to be dependent on temperature with higher temperatures producing more development.' This concurs with this study as the contrast between the background metal and the fingerprint increased as the temperature also increased. Bond discussed that all of the fingerprints developed on steel discs had a blue/black appearance (in accordance with the results observed at 500˚C in this investigation) although Bond heated his samples only until the metal samples reached their required temperature and to a maximum temperature of 600˚C. He did not expose his samples to elevated temperatures for a specific period of time as in this investigation. He suggests that the enhancement of the fingerprints on steel is due to a chemical process similar to bluing, rather than corrosion, which is a passivation process in which the substrate is partially protected from corrosion. It is named after the resulting protective film, and is achieved by applying a solution of salts to steel and heating.

This research clearly shows, by SEM images, spectrochemical analysis, and elemental mapping, that sodium chloride salt is not present above 300˚C. Therefore, bluing may be occurring on temperatures up to 300˚C, but it is unlikely that the enhancement achieved upwards of this temperature is due to bluing. Therefore the mechanism for

the enhancement of fingerprints deposited on steel and exposed to elevated temperatures is more complicated that originally reasoned.

To summarise the results of the SEM images, as the exposure temperature increases, the water in the fingerprint deposits is evaporating off. This forms sodium chloride crystals at temperatures up to 300˚C although these crystals are no longer present at higher temperatures. At the uppermost temperatures of the study, the fingerprint deposit appears to be damaging the surface directly beneath the ridges, before the whole surface oxidises due to the heat. The spectral results and elemental maps agree with the conclusions made from the SEM images. At the lower temperatures, the presence of sodium chloride is confirmed, with an increase in oxygen observed as the temperature increases.

The corrosion and bluing suggestions postulated by Bond are certainly viable at temperatures of 300˚C or below but it is unlikely that they are the cause of the enhancement at temperatures above this 'cut-off' temperature due to the lack of sodium chloride salt. These results provide valuable information for the development or selection of fingerprint enhancement reagents on stainless steel that has been exposed to fire conditions, as by identifying the species remaining in the deposits more specific reagents can be employed.

This goes some way to explain the poorer results observed at higher temperatures in the fingerprints on glass and ceramic studies discussed in sections 4.2.2 and 4.2.3.

# CHAPTER 5: FIRE ENVIRONMENT EXPOSURE

## 5.1 Experimental

'Live burn' facilities were made available at the Grampian Fire and Rescue Development Centre in Portlethen, Aberdeenshire. This is a four storey, multi-compartment training facility constructed of metal where real fires can be set and either left to burn out or can be extinguished. A photograph of the facility is shown in figure 5-1.

Figure 5-1 Image of Grampian Fire and Rescue training facility

### 5.1.1 Fire Environment Exposure on DNA

At the facility, fires are initiated using a naked flame to ignite a fire load consisting of wooden pallets and sheets of chipboard.

The compartment used for this test is illustrated in figure 5-2.

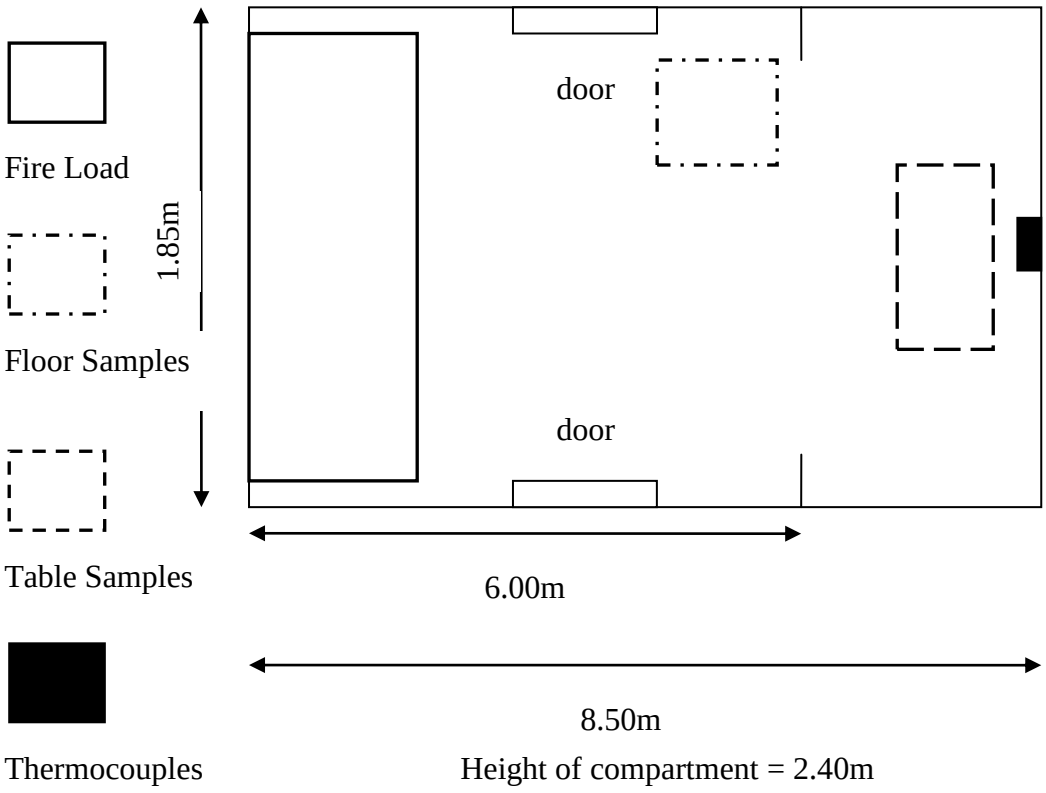



Figure 5-2 Dimensions of fire compartment used

Thermocouples were placed into the compartment as indicated in figure 5-2. Three thermocouples were inserted – one at ground level, one at the height of the table (0.45m), and the highest one at 1.9m. The thermocouples were attached to a USB TC-08 Thermocouple Data Logger (Pico Technology, St. Neots, Cambridgeshire) and the temperatures collected by the PicoLog Data Acquisition software (Pico Technology, St. Neots, Cambridgeshire). The temperature data was written to a text file before exporting into MS Excel for analysis.

To investigate the recoverability of DNA two different types of sample were placed within the compartment. These were chewing gum (Wrigley's Extra Spearmint, Wrigley UK, Plymouth, Devon) and glass bottles (S Pellegrino Sparkling Natural Mineral Water, Nestlé, Vevey, Switzerland). DNA recovery from fingerprints was not included as part of this study as it would be not be possible to determine if a negative result was due to the exposure to the fire environment, or due to the lack of DNA initially transferred.

In total, 13 chewing gum samples were prepared. One donor chewed 13 separate pieces of chewing gum for a total of 20min each prior to storage within UV irradiated Petri dishes. This was undertaken 1 day prior to exposure. One sample was used as a positive control, and the other twelve samples were placed within the fire compartment, on ceramic tiles, with 3 repeats at each placement. The chewing gum samples were placed on ceramic tiles which were inverted so that the gum was to come in to contact with the floor.

13 glass bottle samples were also prepared. One donor drank all 13 bottles of water 1 day prior to exposure. One bottle was used as a positive control, and the other twelve samples were placed similarly to the chewing gum samples, again with 3 repeats at each placement.

Figure 5-2 also shows the two areas where samples were placed into the compartment. These are approx. 6.00m from the fire on the floor (Floor Samples in figure 5-2), and approx. 8.50m from the fire on and below a table (Table Samples in figure 5-2). Sample placement is summarised in table 5-1.

Table 5-1 Summary of sample placement within the compartment

| Sample | Area of Fire | Sample Placement |
|---|---|---|
| Chewing Gum | Floor Samples | Face Up (Exposed) |
| | | Face Down (Not Exposed) |
| | Table Samples | Top of Table, Face Up |
| | | Underneath Table, Face Up |
| Glass Bottles | Floor Samples | With Bottle Cap |
| | | Without Bottle Cap |
| | Table Samples | Top of Table, Without Bottle Cap |
| | | Underneath Table, Without Bottle Cap |

Samples placed on the table were 45cm above the ground.

Figure 5-3 is a photograph taken of inside the compartment looking down from the fuel load. This image shows the sample placement and the thermocouples drilled through a piece of timber at the far end of the compartment.


Figure 5-3 Photograph showing thermocouple and sample placement within compartment

#### 5.1.1.1 DNA Extraction

The DNA was extracted from the chewing gum samples three days after exposure using a QIAGEN QIAamp® DNA Micro Kit. The chewing gum hardened when it had been exposed to the fire conditions and broke apart into small, hard pieces when removed from the fire, therefore when 30mg of the chewing gum was used in the protocol, the 30mg was taken from all areas of the gum (i.e. outer edge exposed to fire, centre of the ball of gum, etc.).

The protocol for extracting DNA from chewing gum varies slightly from the swab protocol given in section 2.1.1.1. Therefore the following protocol [48] was used to extract the DNA from all 13 chewing gum samples.

1. Transfer 30mg of chewing gum into 1.5mL tube.
2. Add 20µL Proteinase K to the tube.
3. Add 300µL Buffer ATL. Vortex immediately for 10sec.
4. Incubate 56$^{o}$C for 3 hour, vortex for 10sec every 10 minutes.
5. Briefly centrifuge after incubation.
6. Add 300µL Buffer AL, spiked with 1µL of 1µg/µL carrier RNA in Buffer AE.
7. Vortex for 15sec.
8. Incubate 70$^{o}$C for 10 minutes, vortex for 10sec every 3 minutes.
9. Briefly centrifuge after incubation.
10. Add 150µL of ethanol. Vortex for 15sec and briefly centrifuge.
11. Transfer entire lysate to Qiagen spin column.
12. Spin for 1 minute at 8000rpm.
13. Add 500µL AW1 to the spin column.
14. Spin for 1 minute at 8000rpm.
15. Remove the spin column to a fresh tube.
16. Add 500µL AW2 to the spin column.
17. Spin for 1 minute at 8000rpm.
18. Remove the spin column to a fresh tube.
19. Spin for 3 minutes at 14000rpm, until membrane is dry.
20. Place spin column in fresh 1.5mL tube.
21. Add 30µL DNA storage buffer to the spin column.
22. Incubate at room temperature for 1 minute.
23. Spin for 1 minute at 14000rpm.
23. Store at 4˚C.[6]

The mouths of the glass bottles were swabbed using the wet/dry combination method and both placed within the same 1.5mL tube 3 days after exposure. On the same day, the DNA was extracted using the same swab protocol as outlined in section 2.1.1.1.

[6] ATL, AL, AE, AW1, and AW2 are solutions provided with the QIAGEN QIAamp® DNA Micro Kit

#### 5.1.1.2 DNA Quantification

Prior to DNA amplification, the samples were quantified using the Plexor® HY system and the Stratagene Mx3005P™. This can be used as a test to check for inhibition within the amplification. The same protocol was used as outlined in section 2.1.2.

The results of the qPCR indicated inhibitors were present within the DNA extracts. Therefore the samples were concentrated and purified using Microcon® YM-100 Centrifugal Filter Units (Millipore, Watford, Hertfordshire) and the protocol outlined within their user guide [184].

Samples were concentrated from their initial 30µL volume down to 15µL.

#### 5.1.1.3 DNA Amplification, Capillary Electrophoresis, and DNA Profiling

After the extraction, quantification, and subsequent concentration and purification of the extracts using Microcon® YM-100 Centrifugal Filter Units, the chewing gum and glass bottle samples followed the same protocols for amplification, electrophoresis and profiling. These were achieved using the same protocols as previously stated in sections 2.1.1.2 and 2.1.1.3.

### 5.1.2 Fire Environment Exposure on Fingerprints

Fingerprints deposited on paper, uPVC, and ceramic were also placed within the compartment both on and under the table, and face up or face down on the floor closer to the fire (as shown in the diagram in figure 5-2). Also, the glass bottles used for the DNA samples had natural fingerprints deposited on them (both on the glass and the porous labels) by the donor whilst he was drinking from the bottles. As such, the glass bottles also underwent fingerprint enhancement treatment. These were used as blind trials for the developed methodology as the location of the fingerprints on the bottles were unknown.

The paper, uPVC, and ceramic samples were all prepared as stated in sections 3.1.1, 4.1.1, and 4.1.3 respectively by placing one depletion set of eccrine fingerprints, one depletion set of sebaceous fingerprints, and five depletions of natural fingerprints. The donors varied from surface to surface but the same donors were used within each surface. Samples were aged for 1 day prior to exposure. The glass bottles were not cleaned prior to passing to the donor for their participation.

#### 5.1.2.1 Fingerprints on Fire Environment Exposed Paper

Table 5-2 provides a summary of the placement of the paper, uPVC, ceramic, and glass bottles within the compartment. Three repeats were placed in each area in order to compare enhancement techniques after exposure.

Table 5-2 Summary of sample placement within the compartment

| Sample | Area of Fire | Sample Placement |
|---|---|---|
| Paper, uPVC, and Ceramic | Floor Samples | Face Up (Exposed) |
| | | Face Down (Not Exposed) |
| | Table Samples | Top of Table, Face Up |
| | | Underneath Table, Face Up |
| Glass Bottles | Floor Samples | With Bottle Cap |
| | | Without Bottle Cap |
| | Table Samples | Top of Table, Without Bottle Cap |
| | | Underneath Table, Without Bottle Cap |

After the fire was starting to decay, water was used to extinguish the flames. This caused a problem as the paper samples began to move around the compartment. As a consequence the use of water was limited to only a few seconds and the fire was allowed to burn out itself. Therefore the majority of the samples remained dry after heat exposure in the compartment. After exposure to the fire the paper samples were treated using the following methods:

Sample 1: Fluorescence, followed by DFO, followed by PD

Sample 2: Paper wetted, followed by fluorescence, followed by DFO, then PD

Sample 3: Paper wetted, followed by fluorescence, followed by PD.

Only one of the paper samples placed on top of the table survived. As such, the only treatment employed for this area was to wet the paper before enhancement by DFO and then PD, to mimic worst case scenario (wetted paper) and to test the full sequence of techniques. Also, one of the face up floor samples was damaged by the fire (only half of it was recovered). This sample was also tested using DFO followed by PD only. DFO and PD were prepared in reference to section 3.1.1.

#### 5.1.2.2 Fingerprints on Fire Environment Exposed uPVC

The uPVC samples had soot deposited on them, although this was very light in most cases. As soot is a potential problem for fingerprint enhancement, lifting tape was used as a potential soot remover [119]. After exposure to the fire environment in the compartment, the dry uPVC samples were enhanced using the following methods:

Sample 1: Lifting tape, followed by superglue, followed by BY40
Sample 2: Lifting tape, followed by powder suspension
Sample 3: Powder suspension only.

Half of the uPVC samples were badly damaged. The three repeats of uPVC placed on top of the table were unrecoverable, and the three samples which were placed face up on the floor were also badly damaged making fingerprint recovery impossible. The superglue followed by BY40 and the powder suspension methods were prepared in reference to section 4.1.1. The lifting tape method is per the HOSDB Arson publication [119].

#### 5.1.2.3 Fingerprints on Fire Environment Exposed Ceramic

The enhancement techniques used in section 5.1.2.2 for uPVC were also used for the ceramic tile samples. All of the ceramic tile samples were recovered from the compartment. All samples suffered from soot deposition but very heavy soot deposits were visible only on the ceramic tiles placed on top of the table.

#### 5.1.2.4 Fingerprints on Fire Environment Exposed Glass Bottles

All of glass bottles were recovered dry from the compartment. The only treatment they had undergone before fingerprint enhancement was that the bottle necks had previously been swabbed for DNA (section 5.1.1). The glass bottles recovered from on top of the table were heavily sooted. Lifting tape was used on these samples in an attempt to remove the soot. Since lifting tape is not recommended for removing soot on porous substrates and the labels on the bottles were porous Absorene Paper and Book Cleaner (Absorene Manufacturing Company Inc, St. Louis, Missouri) was used in accordance with the HOSDB Arson publication [119].

Each glass bottle and label was examined using fluorescence prior to any chemical enhancement. The porous labels had to be treated before attempting enhancement on the glass and were enhanced using ninhydrin. This decision was made purely on the basis that DFO would only work on flat surfaces and since the labels on the curved bottles could not be removed without damaging them, DFO was excluded as an enhancement technique. The ninhydrin was prepared as in section 3.1.1, although rather than pulling the bottle and label through the ninhydrin solution, it was brushed on and left to dry before placing within the humid oven.

Powder suspension and white Wet Wop™ (Armor Forensics, Jacksonville, Florida) were also used on these samples [180, 185] using the following sequence of ninhydrin, followed by powder suspension, followed by Wet Wop™. Wet Wop™ is a white powder suspension technique.

## 5.2 Results and Discussion

### 5.2.1 Effects of Fire Environment Exposure on DNA

Section 5.1.1 discussed how the temperatures within the fire were collected using thermocouples, a data logger, and data acquisition software. Figure 5-4 shows graphically the temperatures recorded using the apparatus and plotting the results using Microsoft Excel XP.

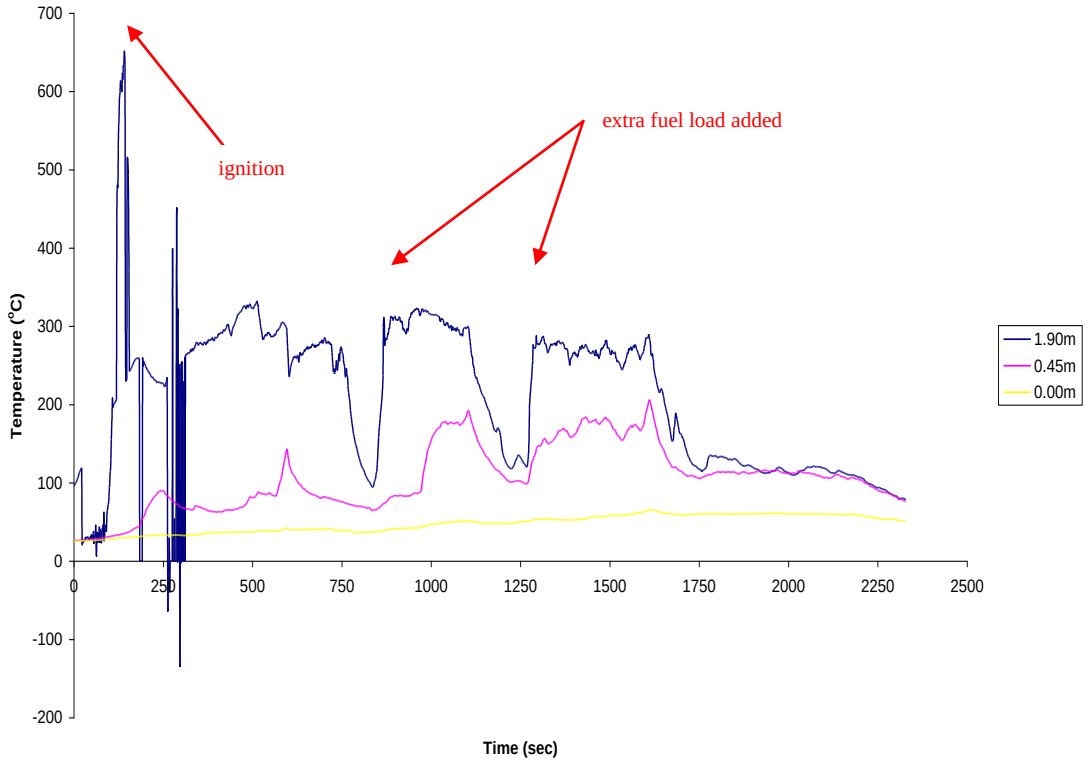



Figure 5-4 Time/temperature graph of inside the compartment

The thermocouples were drilled through a piece of timber to keep them at a constant height throughout the fire. The time/temperature graph shows that the maximum temperature reached at 1.90m (ceiling height) was approximately 650˚C. After this maximum was reached, at approximately 300sec, the thermocouple readings became more erratic and reached a maximum of about 325˚C. Further fuel was added to the fire at approximately 825sec and 1250sec and this can be seen by a corresponding rise in the temperature.

On entering the compartment after the fire, it become apparent that the top piece of timber holding the thermocouples had been damaged due to the heat and as a result the top thermocouple was no longer at 1.90m. Therefore these temperatures were disregarded. Of main interest are the ground level and table height temperatures due to sample placement at these heights.

### 5.2.1.1 Chewing Gum Samples

All chewing gum samples were recovered from the compartment. The samples recovered from on top of the table and face up on the floor were heavily sooted. The other samples (underneath the table and face down on the floor) experienced a slight discolouration.

All samples underwent the DNA procedure outlined in section 5.1.1 and sections 2.1.1.1 - 2.1.1.3, with the chewing gum positive sample providing a full DNA profile which matched the donor in all three replicate PCR's.

The following graph (figure 5-5) shows the number of alleles in the profile which matched the donor categorised by sample placement.

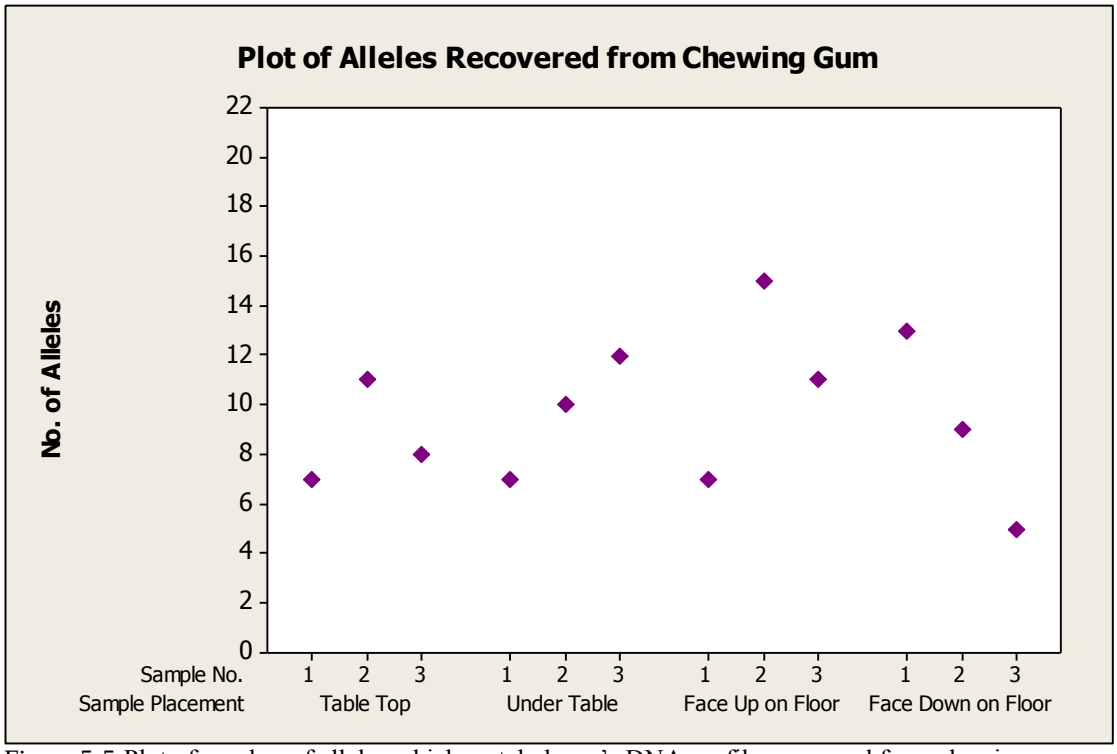



Figure 5-5 Plot of number of alleles which match donor's DNA profile recovered from chewing gum

Figure 5-5 shows that a full DNA profile was not achieved for any of the samples after exposure to the fire environment. All the results are between 7 alleles and 15 alleles, with a wider difference of 8 alleles between the face up or face down floor

samples, than the 5 allele difference between the samples underneath the table and on top of the table.

The Kolmogorov-Smirnov normality test was undertaken to assess the normality of the number of alleles. This gave a P value of >0.150, showing that the data follows a normal distribution. As such, a one-way ANOVA test was conducted on the data for sample placement which provided a P value of 0.825. This is well above the 0.05 significance value. Therefore, based on these results, the area in the fire that chewing gum is placed is not significant to the resultant DNA profile obtained. This was irrespective of the difference in temperature of approximately 100˚C between the samples placed on the table (exposed to approximately 150˚C) and those at ground level (exposed to approximately 50˚C).

An example of an electropherogram from the chewing gum samples is given in figure 5-6.

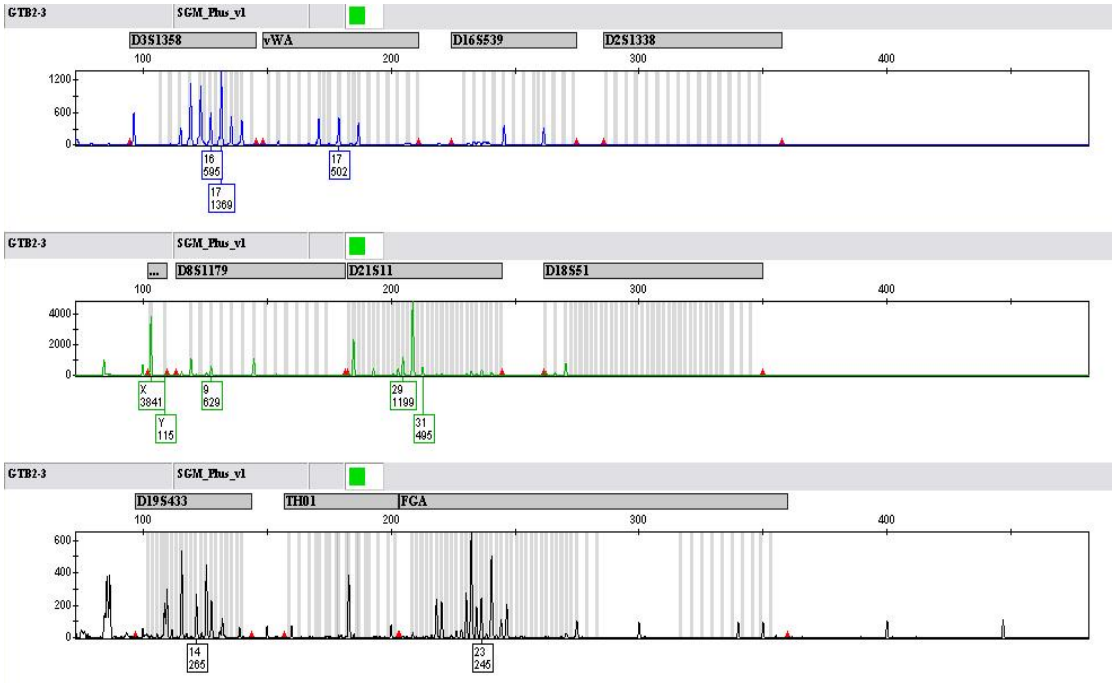



Figure 5-6 Electropherogram of one of the chewing gum samples that was placed underneath the table

The electropherogram shows that at certain loci (D3S1358 and FGA), spurious alleles are present indicating contamination of the samples. It is also difficult to determine which alleles belong to the 'chewer' of the gum i.e. by examining locus vWA three

alleles are present of approximately the same peak height, indicating a mixed DNA profile. As the donor's DNA profile was known in this study, it was possible to specifically look for the presence of the donor in the samples, however if this was a crime scene sample, the mixture interpretation would be difficult, with contamination being an issue in real life circumstances. Locus D16 gave only two alleles however neither of these matched the donor, which highlights the complexity of the interpretation of these electropherograms. The electropherogram above is typical of the low template DNA samples seen in this research, with amplification more prevalent in the lower molecular weight loci.

The results show that DNA transferred onto chewing gum that has been placed within a compartment and exposed to a fire environment, will undergo some degradation as a result of its exposure; however a proportion of the DNA survives and can be recovered and analysed to produce a partial profile. Therefore if a piece of chewing gum is recovered from an operational fire scene, it is worth analysing it for DNA, although interpretation of the electropherogram will be difficult.

#### 5.2.1.2 Glass Bottle Samples

All of the glass bottle samples were recovered from the compartment. Of these samples, only the samples which were placed on top of the table were heavily sooted. The necks of each bottle were swabbed using the wet/dry combination method and analysed according to the protocol outlined in section 5.1.1 and sections 2.1.1.1 – 2.1.1.3. The positive control glass bottle sample was analysed using this protocol also. It provided a full profile which matched the donor for all three PCR replicates.

Figure 5-7 is the graph outputted from Minitab showing the number of alleles in the profile which matched the donor.

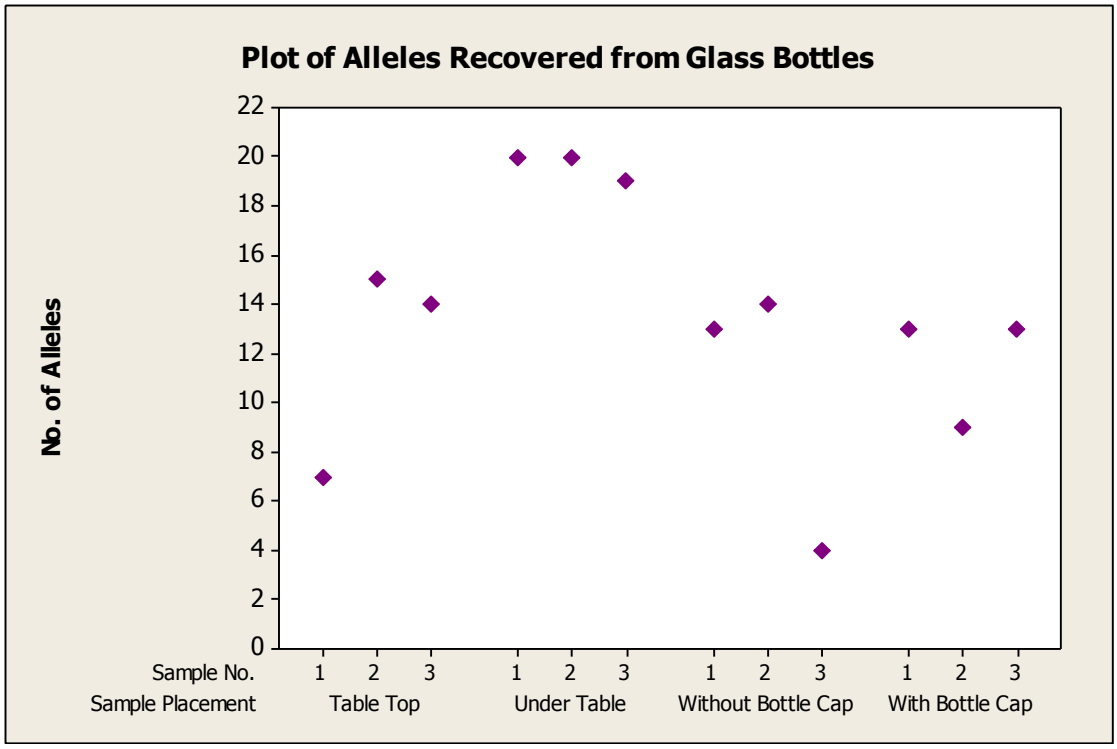



Figure 5-7 Plot of number of alleles which match donor's DNA profile recovered from glass bottles

Figure 5-7 shows that none of the samples yielded a full DNA profile which matched the donor. Of these samples, the glass bottles which were underneath the table without the bottle cap yielded the best DNA profiles. These profiles were also reasonably consistent.

The results obtained from the bottles on top of the table may be due to the non porous nature of the glass offering poor protection from the direct heat, or the soot deposited onto the surface, or a combination of both. The samples with and without the bottle caps which were placed within close proximity to each other (samples placed on the floor) appear to produce the same results irrespective of the protection afforded by the cap.

The graph appears to show that there is a difference in the DNA retrieved from under the table in relation to the other locations within the compartment. However, the Kolmogorov-Smirnov test indicated that the data was normally distributed (P value is >0.150) and a one-way ANOVA test was undertaken. This provided a P value of 0.055 which is marginally above the confidence limit of 0.05 indicating that the

location within the compartment does not have a significant effect. These results are not as clearly defined as for the chewing gum samples.

Figure 5-8 gives an example of one of the electropherograms produced from the bottle samples placed within the fire compartment.

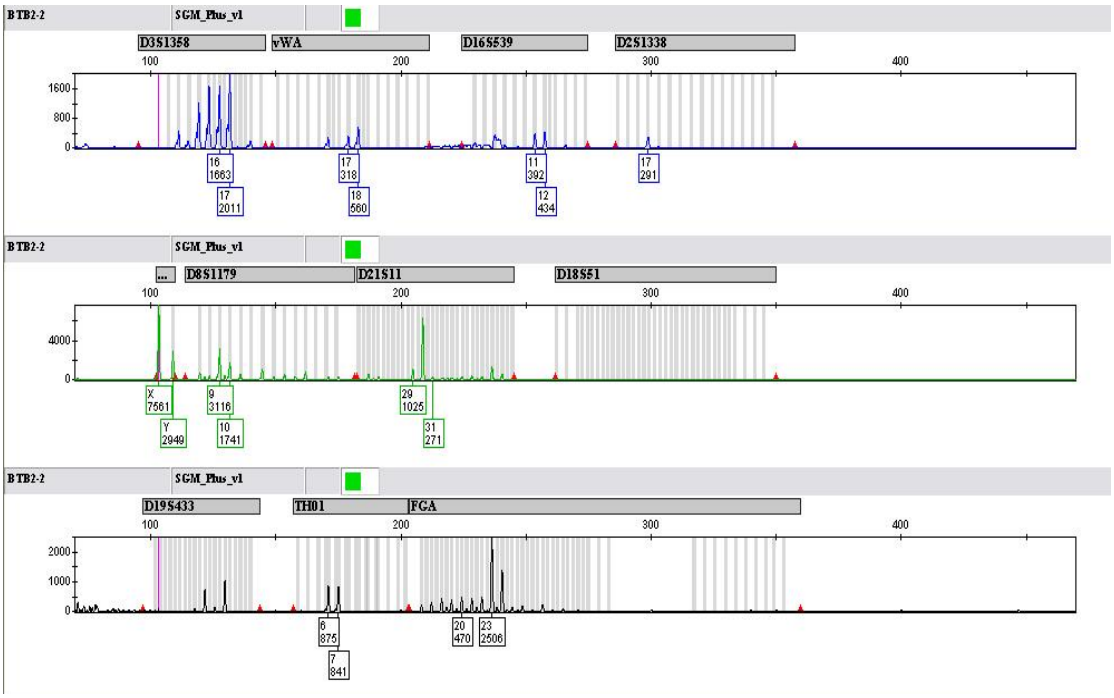



Figure 5-8 Electropherogram of one of the bottle samples that was placed underneath the table

Figure 5-8 shows an example which is similar to the electropherogram for the chewing gum samples. Contamination is present in D3S1358 and FGA in particular. Similar to figure 5-6's D16S539 locus, two alleles with larger peak heights than the others would normally be designated with some degree of certainty, however neither of the two alleles match the donor's designation at that particular locus.

The results for the DNA recovered from the glass bottles show that the recovery is independent of the placement of the samples. This also means it is irrespective of the temperatures experienced (between 50˚C and 150˚C) at these areas of testing within the compartment. Similar to the results obtained for the chewing gum, when examining non porous samples recovered from fire scenes, it is a benefit to analyse for DNA but interpretation of the resulting profiles will be difficult.

### 5.2.2 Fire Environment Exposure on Fingerprints

Paper, uPVC, ceramic, and glass bottles were placed within the test compartment in similar locations to the DNA samples described previously. Once the fire had been extinguished the samples were removed to the laboratory and subjected to the various enhancement techniques as previously described. Different enhancement techniques were used on different samples that had been placed in the same area of the fire for comparison because three fingerprint depletion grid samples were available per location. For each sample, a series of sequential processes were undertaken using the methodology previously described. This replicates the approach that would be taken in an operational fingerprint enhancement laboratory. The fingerprint samples were placed in the compartment according to figure 5-2.

#### 5.2.2.1 Fingerprints on Fire Environment Exposed Paper

As previously discussed (section 3.2.1.1), when the paper was heated to approximately 150˚C the fingerprints on the paper were naturally enhanced. This was also observed on samples that were placed within the fire compartment. Figure 5-9 shows a fingerprint in normal lighting conditions that was enhanced through the action of heating alone.

Figure 5-9 Photograph of naturally enhanced fingerprint on paper (top of table sample)

An example of resultant fingerprints after the enhancement outlined in section 5.1.2.1 is given in figure 5-10.

Sample 1 Sample 2 Sample 3

Figure 5-10 Photographs of fingerprints on paper exposed to fire environment
(Sample 1: Fluor→DFO→PD; Sample 2: Wet→Fluor→DFO→PD; Sample 3: Wet→Fluor→PD)

As the images show, large areas of ridge detail were recovered using all three methods; however, sample 2's enhancement sequence provided a sharper, clearer fingerprint. Fingerprints could also be enhanced on paper that was charred and an example of this is shown in figure 5-11.

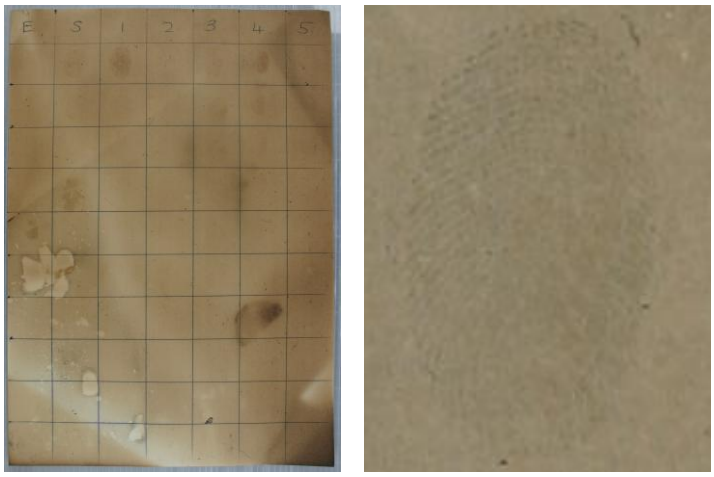


Figure 5-11 Photograph of charred sheet of paper placed 'face up' on the floor (left) and
a fingerprint on the charred paper enhanced by PD (right)

The fingerprints were graded according to the grading system outlined in section 2.1.3. The scores were recorded after each enhancement technique in turn and are presented in table 5-3.

Table 5-3 Sequential fingerprint scores for natural fingerprints on paper after each technique (n/t = not tested; n/r = not recovered)

| Technique Sequence | Sample | After Fluorescence | After DFO | After PD |
|---|---|---|---|---|
| Fluor-DFO-PD | Top of Table | n/r | n/r | n/r |
| Fluor-DFO-PD | Under Table | 0.00 | 0.06 | 0.70 |
| Fluor-DFO-PD | Face Up | 0.00 | 0.00 | 0.32 |
| Fluor-DFO-PD | Face Down | 0.04 | 1.00 | 1.68 |
| Wet-Fluor-DFO-PD | Top of Table | 0.00 | 0.00 | 1.02 |
| Wet-Fluor-DFO-PD | Under Table | 0.00 | 0.00 | 1.96 |
| Wet-Fluor-DFO-PD | Face Up | 0.00 | 0.00 | 1.62 |
| Wet-Fluor-DFO-PD | Face Down | 0.00 | 0.00 | 1.74 |
| Wet-Fluor-PD | Top of Table | n/r | n/t | n/r |
| Wet-Fluor-PD | Under Table | 0.00 | n/t | 1.56 |
| Wet-Fluor-PD | Face Up | 0.00 | n/t | 1.30 |
| Wet-Fluor-PD | Face Down | 0.00 | n/t | 1.62 |

Table 5-3 shows poor results for enhancement by fluorescence and DFO (especially after wetting the sample before chemically treating with DFO where no score was recorded), and that the scores are greatly improved after processing with PD. This was unsurprising as DFO is known to target the water soluble amino acids present in fingerprint deposits. Final scores were recorded after the final treatment of PD, and it is these scores which will be used for statistical analysis. The enhancement of the fingerprints through the application of more than one development technique emphasises the importance of the sequential treatment approach.

Not all of the paper samples were recovered from the compartment. Two of the samples which were on top of the table were destroyed by the fire. As a consequence, it was only possible to use one enhancement sequence on the recovered samples. The sequence chosen was wetting the paper, followed by fluorescence, DFO, and finally PD. This sequence was chosen firstly to mimic worst case scenario of the paper being wet by fire suppression, and secondly to test the capabilities of the full fluorescence-DFO – PD sequence.

Given that the sample numbers were different for each sample location it was not possible to undertake the ANOVA analysis as the sample set was unbalanced. The interaction plot for the samples recovered and enhanced generated using Minitab is given in figure 5-12. In the interaction between the *technique* and the *area within the*

*fire* there are no results for the DFO – PD sequence and wet – PD sequence due to destruction of the paper samples that were placed in this area.

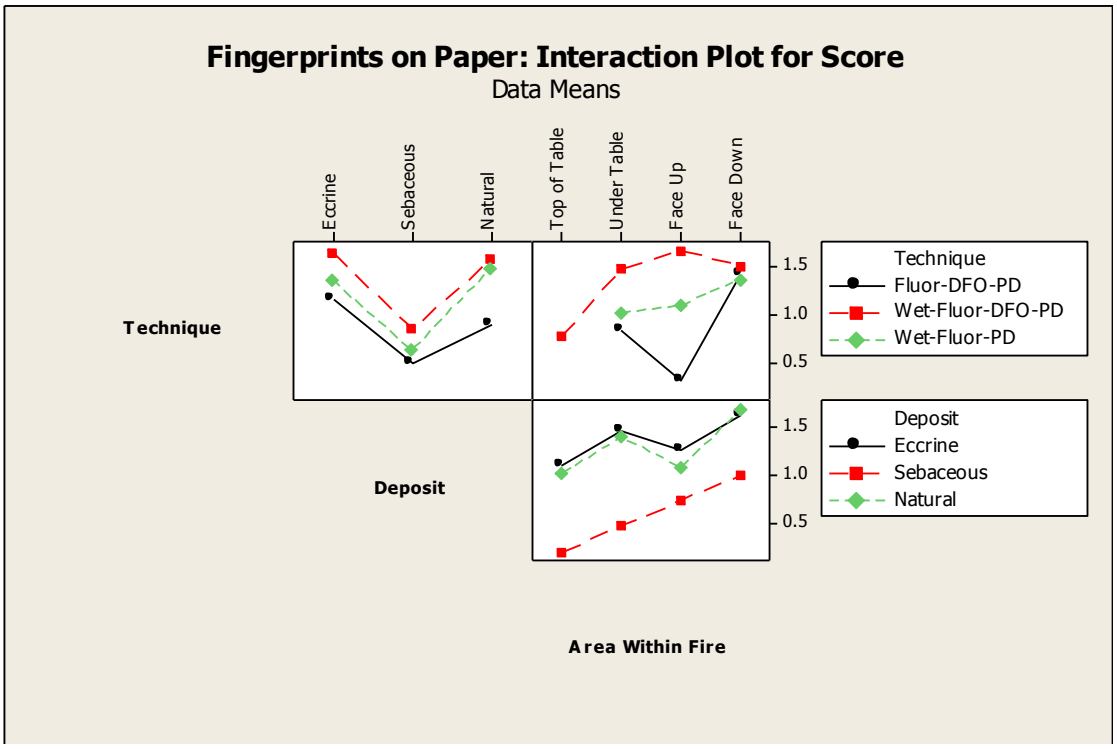



Figure 5-12 Interaction plot for enhancement technique comparison (y-axis = score)

The interaction plot has shown some surprising results. At the interaction between the *technique* and the *type of deposit*, the scores for natural fingerprints (closest to those fingerprints encountered in operational circumstances) are greater than sebaceous fingerprints, and for the wet–fluorescence–PD sequence, natural fingerprints even outperform eccrine fingerprints. The sequence of wet–fluorescence–DFO–PD delivers better scores across all types of deposit. As previously stated, theoretically using DFO on a piece of wet paper should not enhance any fingerprints, and this was witnessed when examining the paper under specific lighting conditions before chemically treating with PD. It appears that the action of DFO treatment on the paper prior to the PD development increases the quality fingerprints obtained after PD treatment across all deposits. This is also shown in the interaction between the *technique* and the *area within the fire* from which the samples were recovered which demonstrates that more protected samples (face down on the ground and underneath the table) provided better quality enhanced fingerprints. Similar results are observed with the interaction

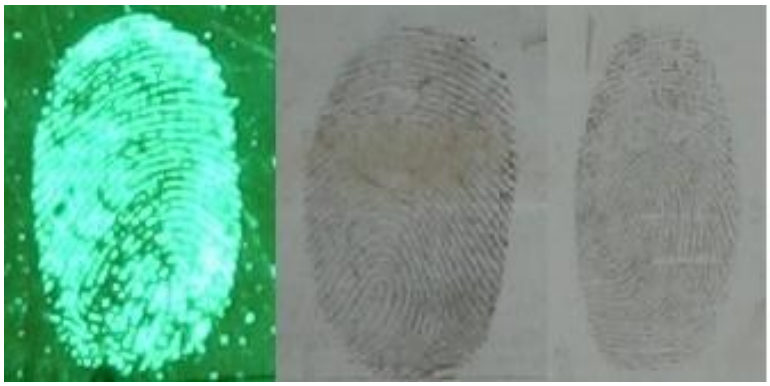
between the *type of deposit* and the *area within the fire*, for the natural and eccrine fingerprints.

The results of the laboratory controlled study (section 3.2) indicated that if the paper was exposed to temperatures up to 100˚C, then DFO maximised the recovery of fingerprints deposited on the paper. For samples exposed to temperatures to approx. 200˚C, PD provided the best quality of enhanced fingerprints. During the fire compartment tests a maximum temperature of 50˚C was recorded at ground level and a maximum temperature of 200˚C for the top of the table upon which some of the samples were placed (0.45m from the floor). Therefore, using the laboratory controlled data only, the ground level samples should all have recorded a high level of enhancement with DFO but this was not the case. The results of this study confirmed the findings of Bradshaw *et al.,* [117], who also found DFO to be highly effective within the laboratory but that its effectiveness diminished once samples were exposed to a real fire environment. This may be a result of the fine layer of soot which had been deposited onto the paper during exposure to the fire. The HOSDB Arson newsletter [119] also states that PD was 'the best reagent for paper exhibits recovered from simulated fire scenes'. Bradshaw *et al.,* [117] did not investigate sequential processing and the results obtained in this work demonstrates that such a process incorporating DFO as part of the sequence prior to application of PD gives added value to the result obtained. Therefore, for fingerprints on paper recovered from a fire scene, the ideal sequence would be (after fluorescence examination) to treat the paper with DFO first followed by PD processing and that this has the advantage of being effective on both dry and wet samples. Weaker results were observed in the development of sebaceous fingerprints when exposed to the fire environment rather than under laboratory controlled conditions. This could be explained by the possible preferential deposition of soot onto the sebaceous fingerprints preventing subsequent chemical enhancement, as more soot was present on the sebaceous fingerprints than on the eccrine or natural fingerprints which had been deposited on the paper.

#### 5.2.2.2 Fingerprints on Fire Environment Exposed uPVC

As previously discussed in section 5.1.2.2, not all of the uPVC samples were recovered from all areas of the compartment. The samples that were placed upon the

table were not recoverable – they had softened and deformed to such an extent that they had stuck to the table – and the samples that were placed face up had the become badly distorted. Therefore, only the samples that were placed underneath the table and the face down samples on the ground were examined and treated with the enhancement technique sequence outlined in 5.1.2.2. Again a sequential process was undertaken. Figure 5-13 shows images of fingerprints that had been enhanced as a result of the sequential processing (LT refers to lifting tape).



Sample 1 Sample 2 Sample 3

Figure 5-13 Photographs of fingerprints on uPVC exposed to fire environment (Sample 1: LT→Superglue→BY40; Sample 2: LT→Powder Suspension; Sample 3: Powder Suspension Only)

Kolmogorov-Smirnov normality tests were undertaken for each deposit type. P values of 0.129, >0.150, and >0.150 were generated for the eccrine, sebaceous, and natural fingerprints respectively. Therefore, as the data was normally distributed and given that three sets of samples were recovered from two areas of the fire compartment the resultant data was balanced and could be analysed using ANOVA. The results are given in Table 5-4.

Table 5-4 ANOVA table for enhancement technique comparison in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Technique | 2 | 0.1142 | 0.0571 | 0.41 | 0.689 |
| Deposit | 2 | 3.0248 | 1.5124 | 10.85 | 0.024 |
| Area Within Fire | 1 | 1.1552 | 1.1552 | 8.29 | 0.045 |
| Technique*Deposit | 4 | 0.8150 | 0.2038 | 1.46 | 0.361 |
| Technique*Area Within Fire | 2 | 0.3028 | 0.1514 | 1.09 | 0.420 |
| Deposit*Area Within Fire | 2 | 1.0044 | 0.5022 | 3.60 | 0.127 |
| Error | 4 | 0.5576 | 0.1394 | | |
| Total | 17 | 6.9740 | | | |

The ANOVA table shows that the *type of deposit* and *area within the fire* are marginally significant to the outcome of the fingerprint score. The sequence of t*echniques* used was not significant to the result. The interaction effects were also not significant to the results obtained.

A main effects plot generated by Minitab is given in figure 5-14.

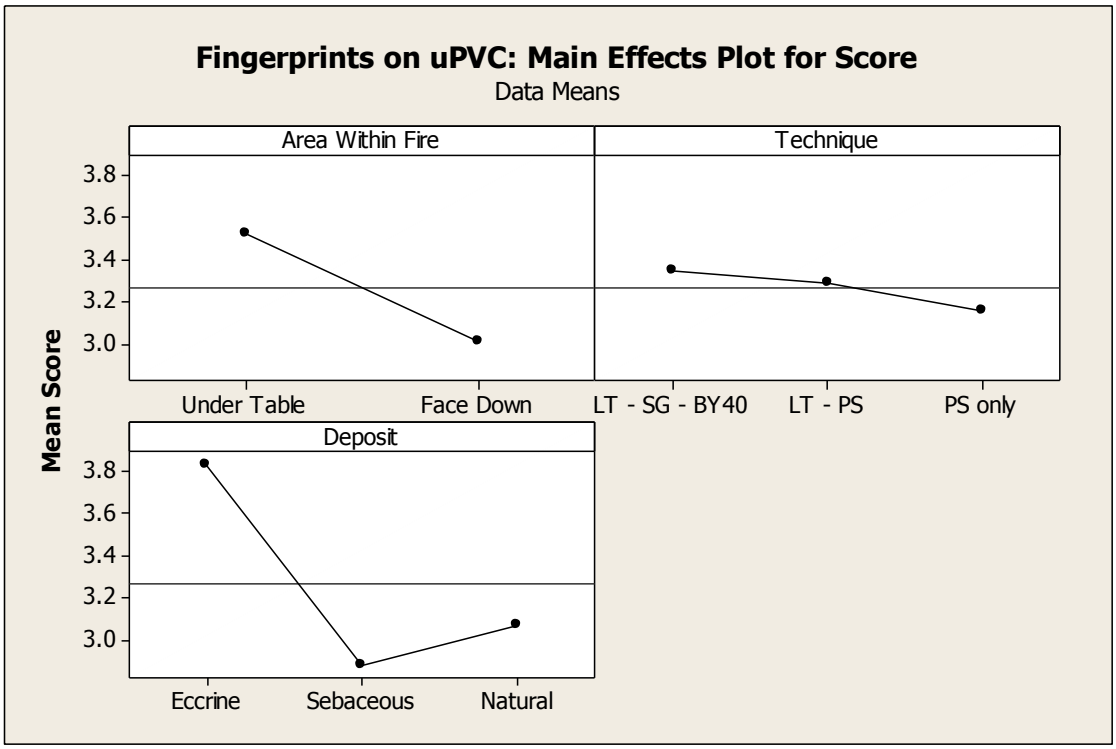



Figure 5-14 Main effects plot for uPVC

The main effects plot examines the effect of each variable independently on the fingerprint score. The ANOVA results in table 5-4 showed that *technique* was not significant to the score and this is also seen in the main effects plot where the three enhancement techniques are close to the mean line (calculated to be 3.26 by Minitab as the mean of all scores). The ANOVA results showed that *area within fire* and types of *deposit* were both significant to the score observed. This is shown from the main effects plot too, with samples placed underneath the table and eccrine fingerprints providing higher fingerprint scores. As the interactions between the variables were not significant to the response, there was no need to analyse an interactions plot.

The results in the laboratory controlled experiments were affected by the type of fingerprint enhancement technique used, and interactions between the variables but in the fire environment experiment, it was the type of deposit and the area that the substrate was placed within the fire that was significant to the fingerprint scores. Therefore, it can be deduced from the main effects plot that although not significant to the outcome, soot removal using lifting tape, followed by superglue and the BY40 dye produces marginally better scores than the other two techniques involving powder suspension. Eccrine fingerprint scores are producing better scores than sebaceous and natural fingerprints and 'protected' areas within the room will improve scores also. Therefore, samples offered some sort of protection that could be recovered from a fire environment should be examined first by enhancing with superglue-BY40 if dry, and powder suspension if wet (employing lifting tape as a soot remover prior to chemical enhancement before either of the techniques).

#### 5.2.2.3 Fingerprints on Fire Environment Exposed Ceramic

The ceramic tiles were all recovered from the fire compartment. Of these, all samples had experienced some degree of soot deposition. The samples which were placed upon the table were the worst affected. Figure 5-15 is a photograph of a full ceramic tile that was retrieved from on top of the table within the compartment. The tile was placed face upwards on the table top and experienced approximately 200˚C. The surface bearing the fingerprint deposits which were placed face down were protected from the soot deposition.

Figure 5-15 Photograph of heavily sooted ceramic sample recovered from top of the table

As can be seen in figure 5-15, the surface has suffered from a very heavy deposit of soot which may limit the ability to enhance any fingerprint deposits which may be present. The soot deposition at floor level (approximately 50˚C) was much less and for the samples placed faced upwards it was noted that the soot deposition preferentially targeted the fingerprint deposits. As such, a certain degree of enhancement was achieved by the soot alone. This was also described by Bradshaw *et al.*, [117], where the soot behaved in a manner similar to fingerprint powders. An example of this enhancement is shown in figure 5-16.

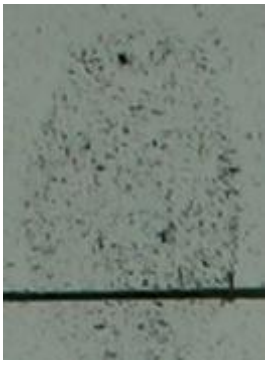
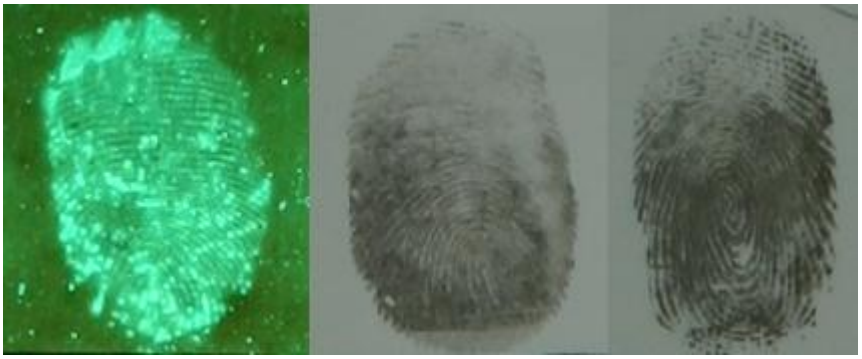


Figure 5-16 Photograph of soot enhanced fingerprint on ceramic recovered from faced up floor sample

The fingerprints placed on the ceramic tiles where enhanced using the same techniques and sequence of techniques as those used on the uPVC. Figure 5-17 gives examples of fingerprints enhanced using the three separate sequences of techniques.



Sample 1 Sample 2 Sample 3

Figure 5-17 Photographs of fingerprints on ceramic exposed to fire environment
(Sample 1: LT→Superglue→BY40; Sample 2: LT→Powder Suspension;
Sample 3: Powder Suspension Only)

Kolmogorov-Smirnov normality tests were undertaken for each deposit type. P values of 0.084, >0.150, and 0.076 were generated for the eccrine, sebaceous, and natural fingerprints respectively. Therefore the fingerprint scores for each deposit are normally distributed and ANOVA testing can be used to analyse the data. The heavily sooted ceramic tiles were also treated using the three sequences of techniques but none of the samples yielded fingerprint ridge detail. As such, the positioning of the sample within the fire scene and the degree of sooting experienced by the fingerprint deposit is key to its successful recovery. This is reflected in the ANOVA results given in table 5-5 where the area of deposit and nature of deposit both had a significant effect on the quality of the enhancement achieved.

Table 5-5 ANOVA table for enhancement technique comparison in terms of score

Analysis of Variance for Score

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Technique | 2 | 0.2016 | 0.1008 | 0.15 | 0.862 |
| Deposit | 2 | 7.2745 | 3.6372 | 5.44 | 0.021 |
| Area Within Fire | 3 | 37.4109 | 12.4703 | 18.67 | 0.000 |
| Technique*Deposit | 4 | 0.2115 | 0.0529 | 0.08 | 0.987 |
| Technique*Area Within Fire | 6 | 2.6541 | 0.4424 | 0.66 | 0.682 |
| Deposit*Area Within Fire | 6 | 4.4389 | 0.7398 | 1.11 | 0.413 |
| Error | 12 | 8.0173 | 0.6681 | | |
| Total | 35 | 60.2088 | | | |

Table 5-5 again provides very similar outcomes to the results obtained from the ANOVA statistical analysis on uPVC. The sequence of the *techniques* used is not significant to the outcome of the fingerprint score, but both the *type of deposit* and the *area within the fire* where significant to the response. All three of the two-way interactions were not a significant influence on the results obtained. This is shown graphically in figure 5-18.

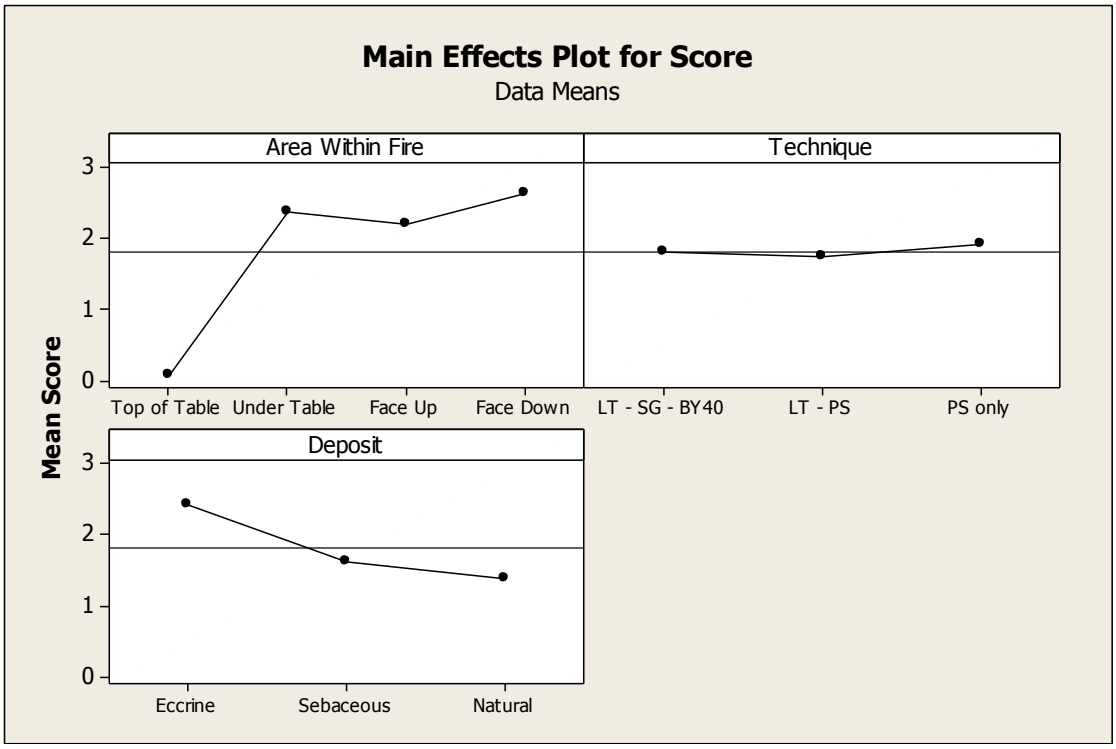


Figure 5-18 Main effects plot for ceramic

The ANOVA results in table 5-5 showed that *technique* was not significant to the score and this is also seen in the main effects plot where the three enhancement techniques remain reasonably close to the mean response line. The ANOVA results showed that *area within fire* and types of *deposit* were both significant to the score observed and this is reflected in the main effects plot too, with samples placed underneath the table and eccrine fingerprints providing higher fingerprint scores. As the interactions between the variables were not significant to the response, no interactions plot was required. This varies slightly to the work of Bradshaw *et al.*, [117] and the recommendations within the Arson newsletter [119], as they claimed that powder suspension was the most effective process in all cases, whereas this work shows that it is the area within the fire and the fingerprint deposit which is significant to the fingerprint score, not the fingerprint enhancement method.

### 5.2.2.4 Fingerprints on Fire Environment Exposed Glass Bottles

The glass bottles which were placed into the compartment for subsequent DNA profiling were also examined for fingerprints. The bottles were placed on top of the table, under the table and on the floor away from the table in the fire compartment as

previously described (section 5.1.1). The position of the fingerprints, if any, on these samples were unknown and all fingerprints deposited were natural depositions rather than deliberately eccrine or sebaceous deposits. As such these samples can be considered as a blind trial.

The bottles that were placed on top of the table were heavily sooted and two soot removal techniques were attempted on the bottles in order to expose any fingerprint deposits to subsequent enhancement techniques. Lifting tape was used on the glass surface and Absorene was used on the labels attached to the bottles. Figure 5-19 shows one of the samples that were placed on top of the table that has been partially treated with lifting tape and Absorene. Little difference was observed on the glass after the use of lifting tape, but the employment of Absorene made a marked difference to the appearance of the paper labels.

Figure 5-19 Photograph of glass bottle – half of which has been treated with lifting tape and Absorene

Prior to any chemical treatment, the bottles were examined using light of 473-548nm using a 549nm viewing filter. No fingerprints were visible after illumination. Previous results had indicated that DFO was a superior enhancement reagent to ninhydrin for fingerprint deposits on paper. DFO can only be used on flat surfaces and as such could not be used on the bottle labels in situ and it would not have been possible to remove the labels from the bottles without damaging them. As a consequence ninhydrin was used on the bottle labels. Only smudges were observed on the labels after enhancement and no ridge detail was visible.

Previous results suggested that there was no significant difference in the sequence of enhancement techniques used for the development of fingerprints on non porous surfaces and as such, for ease of use, powder suspension was chosen to enhance any

fingerprints present on the glass once the lifting tape had been used to remove the soot. No fingerprints or ridge details were observed on the glass bottles.

It was suggested to use white Wet Wop™ (a white powder suspension) after powder suspension if no fingerprints were found [185], as better results had been obtained on operational samples with this sequence of techniques. This was used, on top of the existing powder suspension, and the samples left to dry overnight. The use of the white powder suspension reagent was found to enhance some areas of ridge detail, primarily on the bottom edges of the bottles. These areas of ridge detail were lifted using lifting tape, placed on acetate sheets, and the resultant acetates scanned. An example of these enhanced fingerprints is shown in figure 5-20.

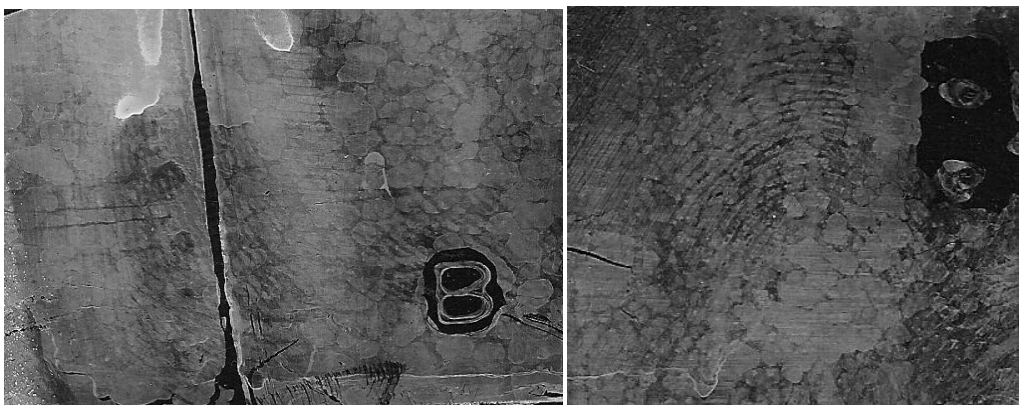


Figure 5-20 Scanned images of recovered ridge detail from the glass bottles within the fire compartment (Left: from bottle placed underneath table; Right: from bottle placed on ground)

The ridge detail was found only on the glass bottles that had either been underneath the table or on the ground and as such had experienced both lower temperatures but more critically less soot deposition. No fingerprints were recovered on the heavily sooted samples that were on top of the table.

# CHAPTER 6: CONCLUSIONS AND FURTHER WORK

## 6.1 Conclusions

In section 1.8, the aims and objectives of the research were clearly outlined within four main areas. The results obtained from chapters 2, 3, 4, and 5 are combined and concluded in reference to these four areas and given below.

### 1. Investigation into the survivability of DNA at elevated temperatures

The survivability of DNA exposed to elevated temperatures was investigated in this work. Laboratory studies showed buccal cells (a rich source of DNA) deposited on paper could yield partial SGM Plus DNA profiles of the donor when exposed to elevated temperatures. More DNA alleles survive at the lower temperatures than at the higher temperatures. The results obtained for buccal cells deposited on glass showed a more gradual decrease in the number of alleles with increased temperature but an unexplained increase in allele numbers occurred at 300˚C. This could be due to the differences in the porosity of the substrate where the paper offered greater protection to the DNA as it was absorbed into the matrix of the substrate and this merits further investigation.

Glass bottles and chewing gum samples containing DNA were placed within the fire test compartment and all of the heat exposed (exposed between 50˚C and 200˚C) samples gave partial SGM Plus DNA profiles suggesting that samples recovered from such scenes were unlikely to provide a full profile of the donor. Therefore, the use of DNA profiling will be limited for items recovered from within compartments exposed to high heat flux in the investigation of fires. Other avenues of evidence should be explored before undertaking DNA profiling of such items, due to its expense and the high likelihood that only a partial profile will be obtained. Swabs of samples should be recovered but may only be of use when a suspect is found and the partial profile could be compared to the suspects DNA profile.

## 2. Investigation into the survivability of latent fingerprints exposed to elevated temperatures

**Porous surfaces – paper**

Previous work demonstrated that fingerprints could be repeatedly obtained from a wide variety of articles retrieved from fire scenes [117-118]. However, none of the previous studies have used sufficient numbers of fingerprint deposits or a range of deposit types to enable a detailed statistical analysis of the results to be robustly undertaken. This research has established that various ages of fingerprints deposited on paper and exposed to various temperatures at various exposure times could survive. Fingerprints that had been deposited on paper were found to enhance naturally once the paper had reached a particular temperature (150˚C). Also fingerprints that had been exposed to temperatures within the range of 110˚C-190˚C would fluoresce under light of certain wavelengths. These laboratory controlled experiments demonstrated that fingerprints could still be enhanced after exposure to 200˚C for 320min. Secondly the most effective technique for the enhancement of the different types of fingerprint deposits (eccrine, sebaceous and natural deposits) in laboratory controlled conditions has been established.

The results indicated that DFO enhanced fingerprints provided better scores on enhancement below 150˚C, but the yield is decreasing once the temperature is elevated above this level. This can be explained by the fluorescence study results, as the amino acids that DFO reacts with are undergoing a change upon heating (with fluorescence a by product of this change). Therefore, the abundance of amino acids present above 150˚C is decreased, resulting in decreased scores observed with this technique.

Ninhydrin performed well on paper that had not yet started to char (up to 150˚C). Once the paper began to char the contrast between the Ruhemann's purple formed as a consequence of the ninhydrin reaction and the charred paper became an issue. DFO was more effective than ninhydrin on white paper and still achieved results on charred paper. Physical developer (PD) performed well across the tests conducted producing good quality enhanced fingerprints. PD also has the added advantage of being

applicable to surfaces which have been exposed to moisture. Therefore, from the laboratory studies, the sequential enhancement methods for porous substrates such as paper exposed to elevated temperature would be DFO followed by PD if the samples had not been exposed to moisture and PD only if the samples had been wet.

The effectiveness of these techniques used in sequence (as they would be under operational conditions) was assessed under actual fire conditions. For the paper samples recovered from the fire test the results were a little different than the laboratory experiments. In this case the most effective sequence of techniques were demonstrated to be fluorescence, followed by DFO, followed by PD for both dry and wet samples. It appeared that for these samples DFO had an important part to play prior to the application of PD on wet samples which was unexpected. It may be that the DFO reagent provided some type of fixing effect on the fingerprint deposit prior to application of PD and future work may be undertaken to develop this further. The suggested flow chart for fingerprints deposited on paper that have been recovered from a fire scene is shown in figure 6-1.

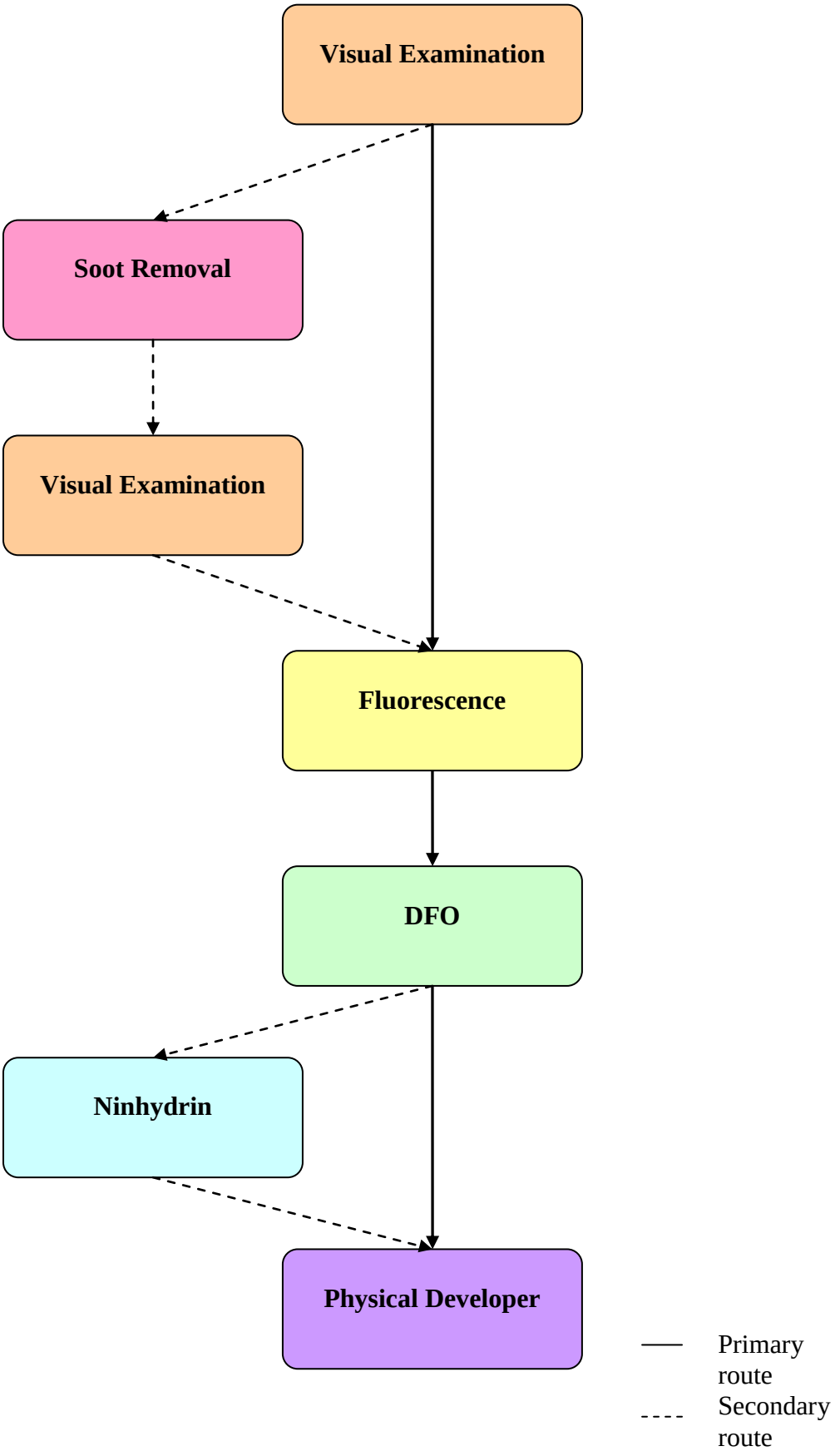



Figure 6-1 Flow chart of fingerprint processes for porous surfaces exposed to fire environment

**Non porous surfaces – uPVC**

For fingerprints that had been deposited on uPVC, it was found that the fingerprints would distort upon heating of the plastic. Black magnetic powder performed very poorly on fingerprints that had been heated under laboratory controlled conditions in comparison to powder suspension and superglue followed by BY40 which performed equally well both on the laboratory samples and the samples recovered from the fire tests. As uPVC is more likely to be found around the door frames or window frames, the ability to recover exhibits and bring them to a fingerprint enhancement laboratory may be limited and as such the recommendations would be that if the sample was recoverable and remained dry, the technique to be selected would be superglue followed by BY40 but if the sample was wet or unrecoverable, powder suspension would be the superior technique because of its effectiveness on wet surfaces and its safe use at scenes.

In a related study, the effect of the distortion experienced by fingerprints deposited on uPVC, due to the thermal degradation of the polymer, to subsequent AFIS analysis was examined. It was demonstrated that if the overall quality of the enhanced mark of fingerprint was high, AFIS could still identify the donor even when significant horizontal and vertical distortion had occurred.

**Non porous surfaces – glass and ceramic**

For fingerprints deposited on glass and ceramic surfaces, it was found that samples exposed to direct heat and air flow at temperatures of 350˚C or greater would not survive without some form of protection. For protected samples, fingerprints could be enhanced by powder suspension up to temperature of 700˚C providing its exposure to the temperature was less than 80min, although the quality of the enhanced fingerprints was poor. Superglue followed by BY40 was a much more effective technique in terms of producing better quality enhanced fingerprints compared to powder suspension. A short study on the effectiveness on VMD was also undertaken. This study showed that the enhancement by superglue followed by BY40 was also more effective than VMD, which in turn outperformed powder suspension. Both VMD and powder suspension could be effectively used on wet surfaces. In samples that were exposed to a fire

environment, only superglue followed by BY40 and powder suspension was tested and both techniques performed equally well. For temperatures above 500˚C the quality of the enhanced fingerprints decreased markedly. VMD can only be applied within the laboratory and is not always easily accessible operationally. Generally the sebaceous fingerprints were poorly enhanced in the fire environment exposed samples when compared to the laboratory controlled samples. This could be due to preferential deposition of soot on these types of deposits preventing any subsequent chemical enhancement.

A study was conducted which investigated the change in fingerprint deposit components after heating using scanning electron microscopy. The results indicated that an oxidation reaction was taking place in the fingerprint deposit due to the large amount of oxygen present in the elemental maps of temperatures approximately 600˚C.

The suggested flowchart for fingerprints deposited on non porous surfaces such as uPVC, glass and ceramic when the sample is known to have remained dry is given in figure 6-2 and when exposed to moisture is given in figure 6-3.

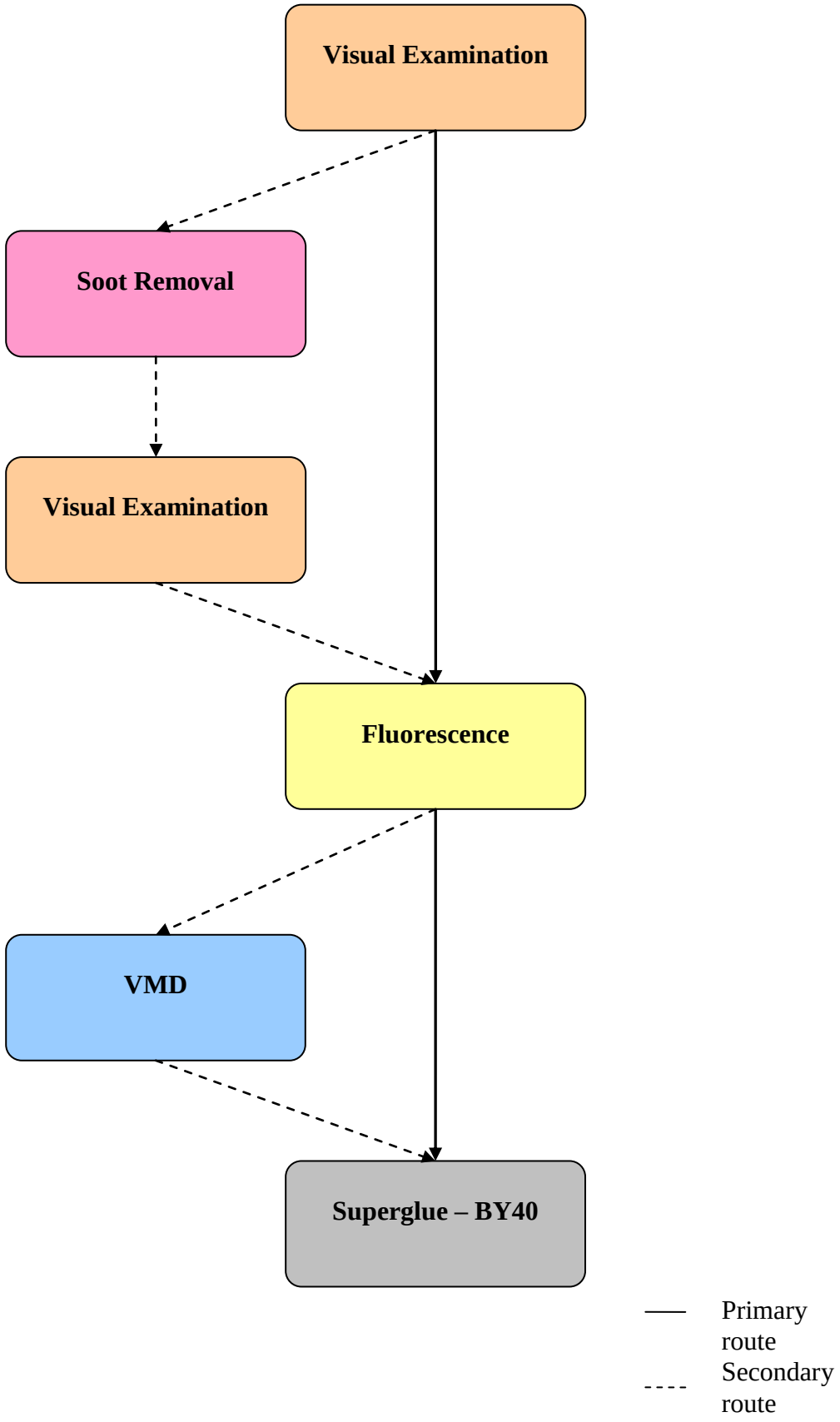



Figure 6-2 Flow chart of fingerprint processes for dry non porous surfaces exposed to fire environment

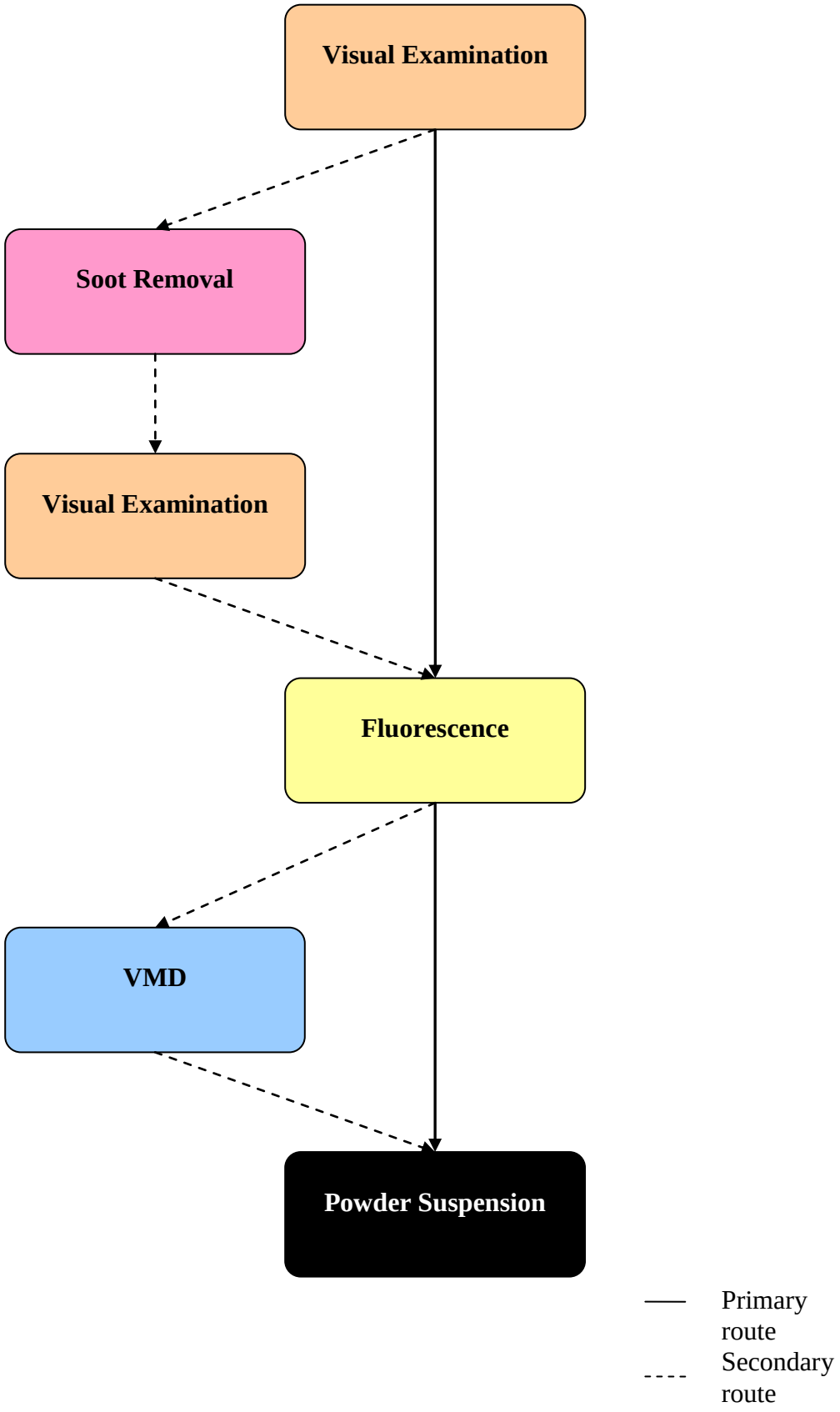



Figure 6-3 Flow chart of fingerprint processes for wet non porous surfaces exposed to fire environment

## 3. Investigation of the recovery of DNA from fingerprints

The relatively new DNA recovery technique called ‘minitaping’ was compared with four different swabbing methods of DNA recovery from fingerprints using cotton swabs, Omniswabs and Foam Tipped Applicators. This comparison showed the optimum recovery method for LT-DNA was the wet/dry combination swabbing method using a sterile cotton swab.

Two small studies were also undertaken with the first study trying to identify whether a relationship existed between DNA shedding and fingerprint donation and the second study, to compare the mass of DNA recovered in relation to the number of fingerprints examined. The results from both studies were donor dependent. It was demonstrated that there is no correlation in the level of detail witnessed in a fingerprint and the amount of DNA a person sheds, and also that, generally, the quantity of DNA will increase with an increase in the number of fingerprints swabbed, although the mass of DNA retrieved was dependent on the donor.

## 4. Production of a best practice guide

As part of this research, a questionnaire was distributed to both scene examiners and members of fire investigation units within the fire service. The purpose of the questionnaire (attached as an appendix) was to determine some of the underlying attitudes and working practices of scene examiners and fire and rescue service fire investigators in relation to the survivability and recovery of fingerprints and DNA from fire scenes encountered under operational circumstances.

The questionnaire was sent to eight police forces (Cumbria Constabulary, Greater Manchester Police, Lancashire Constabulary, Leicestershire Constabulary, Suffolk Constabulary, Surrey Police, Sussex Police, and West Mercia Constabulary) and SPSA Glasgow. Twenty nine questionnaires were returned (51.7% male; 48.3% female). 79.3% of the scene examiners said their particular employer could do more in terms of fire investigation, with 62.1% stating more training would be beneficial and only 10% wanted dedicated fire investigation scene examiners with many citing

the view that they would become deskilled or that shift pattern/annual leave would cause problems due to the size of their department.

A similar questionnaire was also distributed to Strathclyde Fire and Rescue and London Fire Brigade's fire investigators. Nine questionnaires were returned (this was a smaller, all male group). All of the fire investigators had undertaken forensic awareness training of some type. The fire investigators stated that in their view the police could do more in relation to fire scene investigation either by developing dedicated fire scene examiners or through a combination of more budgets and resources, and training.

Interestingly, both want scene examiners to undertake more training in fire investigation; however there was a difference of opinion in the provision of fire dedicated scene examiners.

The awareness of the fire service and scenes of crime officers of the survivability of fingerprints and DNA at fire scenes is illustrated in figure 6-4.

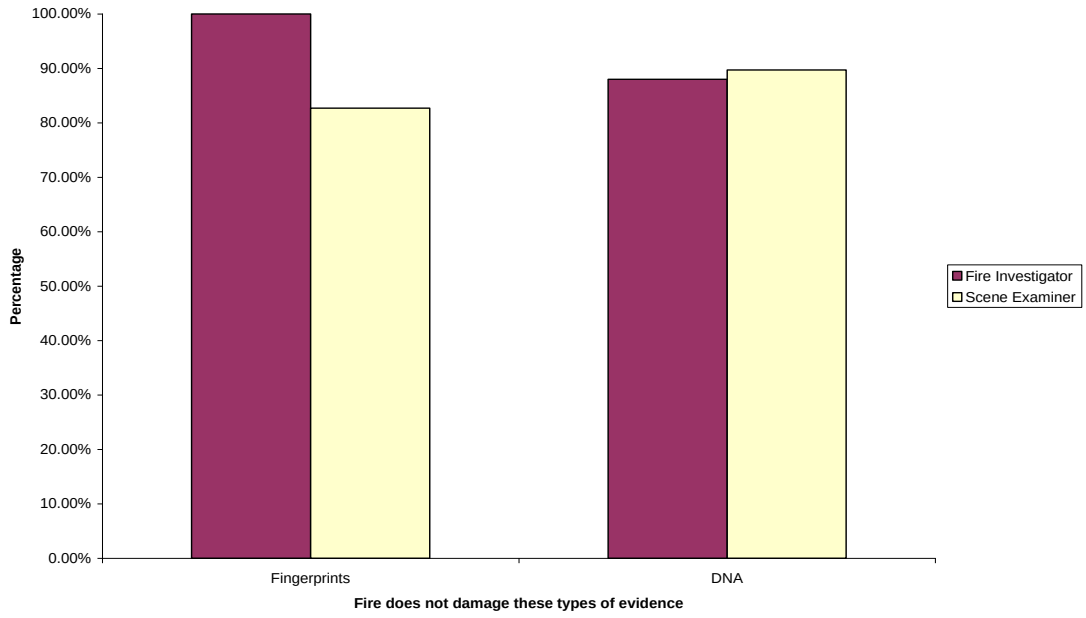



Figure 6-4 Percentage of fire investigators and scene examiners who believe fire **does not** damage fingerprints and DNA

The results indicate that both fire investigators and scene examiners are aware that fingerprints and DNA can survive fire exposure, which has also been demonstrated in this work. The main concern interpreted from the questionnaire responses from the fire service investigators was their perception that the scene examiners were unwilling to investigate fire scenes, especially when almost all scene examiners quizzed stated that fingerprints and DNA could survive exposure to fire environments and as such their attendance would be required to correctly recover these types of evidence.

The results obtained from this work will be used to develop a best practice guide in collaboration with the Home Office Scientific Development Branch for the recovery of fingerprints from fire scenes and heat exposed substrates. It is hoped that as a consequence this work will encourage and advance the forensic examination and investigation of fire scenes for these types of evidence.

## 6.2 Further Work

There are many areas in which this research can be advanced and these as follows:

- Investigate the changes occurring to a fingerprint deposit over time
- Investigate the changes occurring to a fingerprint deposit after exposure to temperature (i.e. component degradation)
- Investigate the effectiveness of white powder suspension (various brands) and compare to magnetic iron oxide formulation for fire environment exposed surfaces
- Investigate the improvement in results for wet paper by undertaking the DFO enhancement process prior to PD chemical treatment
- Investigate the changes occurring in DNA samples when exposed to elevated temperatures
- Investigate other methods of DNA extraction and LT-DNA analysis and compare to Qiagen Mini-kit extraction and 34 cycles PCR method.

Exploring these areas will further enhance the research already conducted in this work.

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# APPENDIX

## Scene Examiner Fire Scene Questionnaire

***PLEASE NOTE: The results from this questionnaire will be used to help identify areas where further work or research might be carried out to help police forces and the fire service in investigating fire/arson scenes. This questionnaire is not a test of a force or an individual. Any published outcomes will be a collation from replies received and will not identify any individual or organisation. Please be honest with your answers and provide as much detail as possible.***

**Please tick the most appropriate answers and/or fill in the comments boxes as required**
If you require more space for your comments, please use a separate sheet of paper and attach securely to this sheet.

**1. About you**

1.1 Name (optional);

1.2 Sex;

1.3 Age;

1.4 Force;

1.5 Job title;

1.6 No. of year's experience;

**2. Your perception/experience to investigating a fire scene**

2.1 Based on your perception/experience of investigating fire/arson scenes, please click on the box and select the option from the drop down list which you believe to be true;

Fire does/does not destroy fingerprints

Fire does/does not destroy DNA

2.2 Based on your answer to question 2.1, why do you think this? E.g. research, operational experience, etc.?

2.3 What specific training are you given to investigate fire scenes, who provided it, and how useful did you find it when investigating these scenes? E.g. degree/qualification, on job training, etc.?

2.4 In your opinion, what is the percentage attendance rate of a forensic scientist to a fatal fire within your force?

☐ 0% ☐ 1-20% ☐ 21-40% ☐ 41-60% ☐ 61-80% ☐ 81-99% ☐ 100%

2.5 Do you believe your force could do more in terms of fire investigation?

☐ Yes ☐ No

If you answered yes, what could they do to improve? E.g. more training, dedicated fire SOCOs, etc.?

**3. Your forces current approach to fire scene investigation <u>in force</u>**

3.1 What approach do your Scene of Crime Officers currently take and what PPE do they wear? E.g. use fingerprint enhancement techniques, swab for DNA, wear face masks, etc.?

3.2 What approach do your laboratory staff currently take? E.g. do they use fingerprint enhancement techniques, swab for DNA, etc.?

**4. Your force's approach to fire scene investigation <u>out of force</u>**

4.1 Which Forensic Service Provider, if any, does your force use most frequently?

4.2 What types of samples are sent to the Forensic Service Provider and for what purpose? E.g. swabs or, actual evidential item, for DNA analysis, etc.?

5. If you have any general comments, please write them in the box below;

# Fire Investigators Questionnaire

***PLEASE NOTE: The results from this questionnaire will be used to help identify areas where further work or research might be carried out to help police forces and the fire service in investigating fire/arson scenes. This questionnaire is not a test of a fire investigation unit or an individual. Please be honest with your answers and provide as much detail as possible.***

**Please tick the most appropriate answers and/or fill in the comments boxes as required**

If you require more space for your comments, please use a separate sheet of paper and attach securely to this sheet.

**1. About you**

1.1 Name (optional);

1.2 Sex;

1.3 Age;

1.4 Fire Service;

1.5 Job title;

1.6 No. of year's experience;

**2. Your perception/experience to investigating a fire scene**

2.1 Based on your perception/experience of investigating fire/arson scenes, please click on the box and select the option from the drop down list which you believe to be true;

Fire does/does not destroy fingerprints

Fire does/does not destroy DNA

2.2 Based on your answer to question 2.1, why do you think this? E.g. research, operational experience, etc.?

2.3 Do you believe that the Police Force associated with your Unit are 100% effective in investigating arson attacks?

☐ Yes ☐ No

If you answered no, what could they do to improve this? E.g. more training, specialised fire SOCOs, larger forensic budgets, etc.?

2.3 In your opinion, what is the percentage attendance rate of a forensic scientist to a fatal fire within your area?

☐ 0% ☐ 1-20% ☐ 21-40% ☐ 41-60% ☐ 61-80% ☐ 81-99% ☐ 100%

2.4 What fire investigation training are you given, who provided it, and how useful did you find it when investigating these scenes? E.g. degree/qualification, on job training, etc.?

2.5 Have you undertaken any forensic awareness training?

☐ Yes ☐ No

If you answered yes, what type of training, who provided it, and how useful did you find it when investigating an fire scene? E.g. degree/qualification, in house training, etc.?

**3. Your Unit's current approach to fire scene investigation**

3.1 What forensic approach do your Fire Investigation Unit take to any fire scene **before** entering the scene and the cause has been determined? E.g. cordon off scene, wear gloves, face masks, etc.?

3.2 If the cause has then been established to be of a criminal nature, do you then take forensic precautions **after** this determination, and if so what precautions? E.g. cordon off scene, wear gloves, face masks, etc.?

4. If you have any general comments, please write them in the box below;