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# The non-contact detection and identification of blood stained fingerprints using visible wavelength reflectance hyperspectral imaging: Part 1

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ABSTRACT

Blood is one of the most commonly encountered types of biological evidence found at scenes of violent crime and one of the most commonly observed fingerprint contaminants. Current visualisation methods rely on presumptive tests or chemical enhancement methods. Although these can successfully visualise ridge detail, they are destructive, do not confirm the presence of blood and can have a negative impact on DNA sampling.

A novel application of visible wavelength reflectance hyperspectral imaging (HSI) has been used for the detection and positive identification of blood stained fingerprints in a non-contact and non-destructive manner on white ceramic tiles. The identification of blood was based on the unique visible absorption spectrum of haemoglobin between 400 and 500 nm.

HSI has been used to successfully visualise ridge detail in blood stained fingerprints to the ninth depletion. Ridge detail was still detectable with diluted blood to 20-fold dilutions. Latent blood stains were detectable to 15,000-fold dilutions. Ridge detail was detectable for fingerprints up to 6 months old. HSI was also able to conclusively distinguish blood stained fingerprints from fingerprints in six paints and eleven other red/brown media with zero false positives.



## 1. Introduction

Blood is one of the most commonly encountered types of biological evidence found at scenes of violent crime [1] and is the most commonly observed fingerprint contaminant [2].

The first challenge for examiners when dealing with blood evidence is to establish that the substance is blood before performing further analysis [3]. Even for experienced examiners some blood stains can be missed or confused with other substances. The misidentification of blood can lead to lost time, mislead investigations and wasted resources in carrying out potentially expensive follow up analyses, such as DNA analysis. The current forensic workflow involves visual examination followed by chemical presumptive tests to indicate the presence of blood [2]. Whilst these chemical tests can be very sensitive, they are not specific to blood and can generate false positives [2]. Additionally these chemical tests may contaminate the stain, leading to dilution and alteration of the shape of the stain as well as potentially affecting subsequent DNA analysis [4]. Because of these issues examiners normally test areas only when they suspect blood evidence is present but cannot be visually identified, due to problems detecting latent (not visible) stains or stains on dark backgrounds.

Fingerprints contaminated with blood are subject to chemical enhancement methods, such as acid stains, including Acid Black 1, Acid Violet 17 or Acid Yellow 7 [2]. Although these techniques can successfully visualise ridge detail, they have a number of drawbacks. They can be destructive, as incorrect application of the enhancement process will result in a loss of ridge detail [2] and there is a risk of background staining of the substrate obscuring any enhanced ridge detail. Also, successful enhancement of a blood stained fingerprint does not conclusively confirm the presence of blood and the staining process may interfere with subsequent recovery of DNA material from the fingerprint.

Previous studies exploring blood stain detection originally focused on blood typing [5] or the use of alternate light sources, such as ultraviolet light [6]; or high intensity light sources [7]. However the former requires considerable sample preparation and is destructive; and the latter both rely on minimal background interference. UV light could also affect subsequent DNA recovery, as DNA in biological samples can be degraded by ultraviolet light [8]. Other methods such as immuno-chromatographic analysis [9] or RNA analysis [10,11] have been successfully used to identify blood stains. These methodologies are destructive however, which is not suitable for blood stained fingerprints, where the aim is to confirm the contaminant is blood whilst preserving ridge detail.

*Abbreviations:* DS, Detect stain; DSLR, Digital single lens reflex; HSI, Hyperspectral imaging; PRD, Partial ridge detail; UV, Ultraviolet; WRD, Whole ridge detail.

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Optical techniques, such as microscopic analysis have been used to confirm the presence of blood [12], although this approach has so far only been explored on lifts of microscopic blood stains. Spectroscopic methods have been used to determine the presence of blood through compositional analysis. Raman spectroscopy has been used with some success in controlled lab conditions, but there remain substrate interference challenges [13]. X-ray fluorescence spectroscopy was explored in terms of gunshot residue through a collaborative piece of research between the National Institute of Justice (NIJ) and the National Aeronautics and Space Administration's (NASAs) Goddard Space Flight Centre (GSFC) [14]. Blood was successfully identified through the presence of iron from haemoglobin, although this could be vulnerable to false positives with other substances with high iron concentrations.

More recently research has explored the use of non-contact reflectance spectroscopy to detect blood stains with high levels of specificity [15]. Blood was identified based on the spectral shape of the α and β bands in haemoglobin between 500 nm and 600 nm, and these bands were also used to estimate the age of the blood stains. Our group also reported a similar contemporaneous approach for the age estimation of blood stains using a microspectrophotometer [16].

Recent exploration of blood stain identification using near-infrared demonstrated significant advantages over visible light, particularly on dark coloured substrates [17,18], although the spectral property of other protein-containing substances can be similar to blood resulting in false positives [3]. Other work has explored the visible region using forensic photography. One study successfully used a background correction technique to improve the detection of blood stains on coloured and patterned substrates [19], although the sensitivity was not as high in comparison to existing presumptive tests.

Over the last couple of years visible wavelength hyperspectral imaging has been reported for firstly the age determination of blood stains [16,20] and also the detection of blood stains using the α and β bands between 500 nm and 600 nm [21–23]. The methods proposed allowed for rapid, non-destructive presumptive blood stain detection. Most recently our group has proposed a new blood stain identification approach based on hyperspectral imaging and the use of the Soret γ band absorption in haemoglobin [3]. This was shown to provide a higher sensitivity and specificity for the detection and identification of blood stains over previously proposed methods.

There is to date a definite gap in previous research concerning the detection of blood stained fingerprints using non-contact and non-destructive methods. An ideal method should be highly sensitive and effective even with blood diluted to latent (not visible by the human eye) levels in both stains and fingerprints and should be highly specific to avoid false positives. The visible wavelength hyperspectral imaging method proposed in this paper meets all these requirements. In this study we present a novel application of visible wavelength hyperspectral imaging (HSI) based on the Soret γ band absorption in haemoglobin between 400 and 500 nm for the non-contact, non-destructive detection and identification of both blood stains and ridge detail in blood stained fingerprints on white tiles. This is the first time that the detection of blood stained fingerprints has been explored using hyperspectral imaging and is potentially a significant step towards a reliable method for both non-destructive blood identification and the detection of ridge detail in blood stained fingerprints.

## 2. Material and methods

### 2.1. Contamination of digit

Both human and horse bloods were used as contaminants in this study. Human blood from a consenting healthy volunteer was used where the blood stains were to be analysed and disposed of within 1 day (trial 1). A sterile lancet (FinePoint, USA) in a Penlet Plus lancing device (LifeScan, USA) was used to pierce the left middle finger. The finger was gently squeezed to encourage blood flow and the resulting blood drops were evenly spread over the ridge detail on the right middle finger. In trials 2, 3 and 4 screened horse blood was used. This was deposited into a Petri dish containing a small sponge. The right middle finger was pressed against the sponge to evenly coat the digit and the blood stained fingerprint then deposited onto the substrate. For standard samples human and horse blood stained fingerprints were allowed to dry for approximately 1 h before analysis. Wet samples were analysed within 20 min of deposition and dry samples were left to dry for 2 h before analysis. Application of paints and other contaminants was carried out by applying a small quantity to a gloved left middle finger. The contaminant was then evenly spread over the ridge detail on the right middle finger and the fingerprint deposited. All white ceramic tiles (B&Q, UK) were cleaned with distilled water and thoroughly dried before labelling and fingerprint deposition.

### 2.2. HSI system — Version 1

The HSI system (*version* 1) used in this study was similar to that detailed in [3], consisting of a liquid crystal tuneable filter (LCTF) coupled to a 2.3 megapixel Point Grey camera and a light source for scene illumination. The light source was comprised of two 40 W LEDs; one violet giving an output at 410 nm and one white, giving an output between 450 and 700 nm. Control of the LCTF and image capture was performed using custom developed software written in C++ (Microsoft, USA). Images were captured between 400 nm and 680 nm with spectral sub sampling at 5 nm intervals, resulting in an image cube at 56 wavelengths for each scan. Spectra from the image cube were subsequently analysed using custom routines developed in Visual Studio (Microsoft, USA) and Spyder (Python, USA). The time required to acquire and process an image was approximately 30 s.

### 2.3. HSI system — Version 2

An improved version of the HSI system (*version* 2) was create using an upgraded camera and an improved liquid crystal tuneable filter (LCTF). The camera was a 5.5 megapixel CMOS camera (PCO Edge, Germany) coupled to a Varispec CRI LCTF. The same lighting setup was used as detailed above in Section 2.2. Control of the LCTF and image capture was again performed using custom developed software written in C++ (Microsoft, USA), as detailed in Section 2.2.

### 2.4. Hyperspectral reflectance image acquisition and pre-processing

The hyperspectral reflectance measurements were made following the method detailed in [3]. A reference image ($R_0$) was obtained using a blank ceramic tile. This image was recorded in a 5 nm series of 56 discrete wavelengths between 400 nm and 680 nm. The sample image ($R_s$) was recorded at the same wavelengths under the same illumination conditions and integration time settings on the camera. The hyperspectral reflectance image (R) consisted of a data cube of 1920 × 1200 pixel values at 56 discrete wavelengths. Additional information regarding sample image processing can be found in [3].

### 2.5. Criteria for the identification of blood stains

The presence of haemoglobin in blood dominates the blood reflectance spectrum in the visible region [16,20]. The spectrum contains a strong narrow absorption at 415 nm called the Soret or γ band with two weaker and broader absorptions between 500 and 600 nm known as the β and α bands [3]. Due to the absorption in the blue part of the visible spectrum, the Soret band is responsible for giving blood its distinctive red colour. Other red substances also absorb in the blue region of the visible spectrum between 400 and 500 nm. However, the width of these absorption features is typically much broader and also not centred at 415 nm. This forms the basis of the methodology to identify and discriminate blood stains from