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A comparison of visible wavelength reflectance hyperspectral imaging and Acid Black 1 for the detection and identification of blood stained fingerprints

Samuel Cadd, Bo Li, Peter Beveridge, William T. O'Hare, Andrew Campbell, Meez Islam *

School of Science and Engineering, Teesside University, Borough Road, Middlesbrough, TS1 3BA, United Kingdom

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ABSTRACT

Bloodstains are often encountered at scenes of violent crime and have significant forensic value for criminal investigations.
Blood is one of the most commonly encountered types of biological evidence and is the most commonly observed fingerprint contaminant. Presumptive tests are used to test blood stain and blood stained fingerprints are targeted with chemical enhancement methods, such as acid stains, including Acid Black 1, Acid Violet 17 or Acid Yellow 7. Although these techniques 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 hyperspectral imaging (HSI) is used for the non-contact, non-destructive detection and identification of blood stained fingerprints on white tiles both before and after wet chemical enhancement using Acid Black 1. The identification was obtained in a non-contact and non-destructive manner, based on the unique visible absorption spectrum of haemoglobin between 400 and 500 nm. Results from the exploration of the selectivity of the setup to detect blood against ten other non-blood protein contaminants are also presented. A direct comparison of the effectiveness of HSI with chemical enhancement using Acid Black 1 on white tiles is also shown.
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1. Introduction

Bloodstains are often encountered at scenes of violent crime and have significant forensic value [1]. Blood is one of the most commonly encountered types of biological evidence [2]. Analysis of the blood spatter pattern can help with reconstructing events and DNA profiling can be carried out, which may assist to establish the identity of the suspect.

The current forensic workflow for the examination of suspected blood stains starts with visual examination, including the use of alternate light sources, to confirm the identity of the substance before further analysis [3]. This is followed by analysis using chemical presumptive tests to provide an indication as to the presence of blood [4]. Presumptive tests are however not specific to blood and can generate false positives [4]. Contamination of the stain may also occur, such as altering the shape of the stain or dilution, which may affect subsequent DNA analysis [5].

The examination of fingerprint evidence also routinely involves the analysis and identification of biological samples, as blood is the most commonly observed fingerprint contaminant [4]. Fingerprints contaminated with blood are subject to chemical enhancement methods depending on the substrate, including acid stains such as Acid Black 1, Acid Violet 17 or Acid Yellow 7 [4]. These chemical enhancement techniques, although successful, have several disadvantages. The most significant is their potentially destructive nature, as incorrect application of the enhancement process will result in a loss of ridge detail [4]. There is also a risk of background staining obscuring any enhanced ridge detail, which can be affected by the substrate nature. Porous surfaces are less suitable for enhancement with Acid black, as considerable background staining can occur [6]. The enhancement methods are also purely presumptive, as successful enhancement of a blood stained fingerprint does not conclusively confirm the presence of blood, due to the potential for false positives from other non-blood proteins. Additionally the staining process may interfere with subsequent recovery of DNA material from the fingerprint.

Considerable previous research has explored the detection and identification of blood stains using a variety of techniques, including

Abbreviations: AB, Acid Black 1; DS, Detect stain; DSLR, Digital single lens reflex; HSI, Hyperspectral imaging; PRD, Partial ridge detail; WRD, Whole ridge detail.

* Corresponding author.
E-mail addresses: cadd.sam@gmail.com (S. Cadd), tianyufang1958@googlemail.com (B. Li), p.beveridge@tees.ac.uk (P. Beveridge), w.t.ohare@tees.ac.uk (W.T. O'Hare), a.w.campbell@tees.ac.uk (A. Campbell), m.islam@tees.ac.uk (M. Islam).

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RNA analysis [7,8]; microscopy techniques [9], and spectroscopic methods [10–13].

Recent research has explored the use of non-contact reflectance spectroscopy to detect blood stains with high levels of specificity [1]. This led to the use of visible wavelength hyperspectral imaging (HSI), which was first reported for the detection and age determination of horse blood stains between 442 nm and 585 nm as proof of concept research [14]. A similar approach was presented by another research group for the identification of blood stains [15,16], detailing a presumptive method for the rapid non-destructive detection of blood stains.

Most recently our research group proposed a new blood stain identification approach based on the Soret γ band absorption in haemoglobin [3,17]. Previous work has also investigated changes to the Soret band in the UV–Visible spectrum of haemoglobin [18], although this was purely focused on changes to the wavelength of the Soret band peak over time for age determination only, and did not allow for identification or confirmation of blood. The approach carried out by our research group indicated a higher sensitivity and specificity for the detection and identification of blood stains over previously proposed methods [15,16]. Recent research has since successfully demonstrated the application of this technique for the detection, identification, and visualisation of ridge detail in blood stained fingerprints deposited on white ceramic tiles [19] and a range of other substrates commonly encountered at scenes of crime [20].

To date there has been no comparison between HSI and existing chemical enhancement methods, such as Acid Black. One of the key issues involving the optical analysis of fingerprints is to ensure that sufficient ridge detail is captured to allow for comparison of crime scene marks against fingerprints from a suspect. A comparison of existing enhancement methods against HSI would allow for the sensitivity of the setup to be measured, which is necessary if HSI is to be further developed [20].

In this study a novel application of hyperspectral imaging (HSI) is used for the non-contact, non-destructive detection and identification of blood stained fingerprints on white tiles both before and after wet chemical enhancement using Acid Black 1. Results from the exploration of the selectivity of the setup to detect blood against ten other non-blood protein contaminants are also presented. A direct comparison of the effectiveness of HSI with chemical enhancement using Acid Black 1 on white tiles is also presented. This is the first time a direct comparison between chemical enhancement methods and the detection of blood stained fingerprints using hyperspectral imaging has been explored. Results potentially indicate a significant step towards a reliable method for both non-destructive blood identification and the detection of ridge detail in blood stained fingerprints, both before and after chemical enhancement.

This work is following on from [19,20], where hyperspectral imaging was demonstrated for the first time to detect, identify, and visualise to a sufficient resolution the ridge detail of blood stained fingerprints across a wide range of substrates. The research presented in this paper again demonstrates the potential of HSI, through a comparison of the successful non-destructive detection and identification of blood stained fingerprints against Acid Black chemical enhancement.

2. Materials and methods

2.1. Contamination of digit and substrate preparation

Horse blood were used as the contaminant in this study and 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 white tile. Application of other non-blood protein 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. The hyperspectral imaging (HSI) system

The HSI system used in this study was the same setup detailed in [3,19,20], 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. Hyperspectral reflectance image acquisition and pre-processing

The hyperspectral reflectance measurements were made following the method detailed in [3,19,20]. 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].

2.4. Criteria for the identification of blood stains

Haemoglobin dominates the blood reflectance spectrum in the visible region [14,17]. 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 absorption in the blue part of the visible spectrum, the Soret band results in the distinctive red colour of blood. Other red substances also absorb between 400 and 500 nm in the blue region, although these absorption features tend to not be centred at 415 nm and are much broader. This is the basis of the methodology used in this study to identify and discriminate blood from other similarly coloured substances. Further information is detailed in [3]. From the reflectance images obtained, the pixels which satisfied the criterion were marked as black, whilst all other pixels were marked as white. This allowed regions of the image where the blood stained fingerprint was present to be identified, as well as clear distinction of the ridge detail.

2.5. Acid Black 1 enhancement process

Deposited blood stained fingerprints were enhanced using a 3 stage water-based Acid Black 1 (Amido black 10B) process, as proposed by CAST [4]. The ceramic tiles were submerged in a fixing solution composed of 5-sulphosalicylic acid 2 g/100 mL (%) and distilled water for 5 min and then submerged in a staining solution composed of acid dye 0.2 g/100 mL (%), acetic acid 5% *v/v* and distilled water for 4 min. After staining, the ceramic tiles were rinsed using a washing solution composed of acetic acid 5% *v/v* and distilled water. The enhanced fingerprints were then left to dry at room temperature for at least 30 min before photography or further analysis.

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