Science and Justice xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Science and Justice

journal homepage: www.elsevier.com/locate/scijus

The application of visible wavelength reflectance hyperspectral imaging for the detection and identification of blood stains

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ARTICLE INFO

Article history:
Received 18 January 2014
Received in revised form 10 May 2014
Accepted 13 May 2014
Available online xxxx

Keywords:
Forensic science
Blood stain detection
Hyperspectral imaging
Liquid crystal tuneable filter

ABSTRACT

Current methods of detection and identification of blood stains rely largely on visual examination followed by presumptive tests such as Kastle–Meyer, Leuco-malachite green or luminol. Although these tests are useful, they can produce false positives and can also have a negative impact on subsequent DNA tests. A novel application of visible wavelength reflectance hyperspectral imaging has been used for the detection and positive identification of blood stains in a non contact and non destructive manner on a range of coloured substrates. The identification of blood staining was based on the unique visible absorption spectrum of haemoglobin between 400 and 500 nm. Images illustrating successful discrimination of blood stains from nine red substances are included. It has also been possible to distinguish between blood and approximately 40 other reddish stains. The technique was also successfully used to detect latent blood stains deposited on white filter paper at dilutions of up to 1 in 512 folds and on red tissue at dilutions of up to 1 in 32 folds. Finally, in a blind trial, the method successfully detected and identified a total of 9 blood stains on a red T-shirt.

1. Introduction

The first challenge for crime scene examiners when dealing with blood evidence is to establish that the substance in question is blood, before the stains are further analysed, as the colour and appearance can be confused with other substances depending on the colour and pattern present on the substrate [1]. Usually visual examination followed by a presumptive test is carried out, for example the Kastle–Meyer (KM), the Leuco-malachite green (LMG) and the luminol tests [2,3]. The luminol test was first used for the identification of blood stains by Specht in 1937 [4]. The test is very sensitive and works well when the blood stains are located on absorbent substrates such as fabric and carpeting, however it can produce false positives and there have been suggestions that it can interfere with subsequent tests including DNA analysis [4–6]. In addition, spraying luminol onto a suspected stain can lead to dilution of the stain and alter its shape. Likewise the LMG and KM tests, although sensitive, are also known to produce false positives. In recent years forensic research has focussed on non-destructive methods of identification, including the detection of latent fingerprints [7–9] and foot marks in soil [10] as well as the identification of the blood stain using imaging techniques. Edelman et al. [11] published recent work on the identification of the blood stains in the near-infrared region The advantage of near-infrared detection is that the measurement is not affected by dark coloured substrates, but the spectral property of other substances, especially those which contain protein, can be similar to blood and thus may lead to false positive results. The measurement is also complicated for wet blood stains as the absorption of water dominates the spectra in the near-infrared region.

Other work has been done in the visible region, In 2003, Wagner et al. [12] proposed a background correction technique in forensic photography, using a high intensity light source (Polilight) and a digital single lens reflex camera and making measurements at 395 nm, 415 and 435 nm. This technique improved the detection of blood stains on coloured and patterned substrates. Although the sensitivity of this technique was not as high as the presumptive tests, the non-destructive nature was seen as being valuable for the preservation of crime scene evidence. Recently, Janchaysang et al. [13,14] described the first use of visible wavelength hyperspectral imaging for the detection and identification of blood stains. They appear to have extended the spectral fitting spectroscopic approach previously reported by Bremmer et al. [15] based on the spectral shape of the α and β bands in haemoglobin between 500 nm and 600 nm.

In this study a novel application of visible wavelength hyperspectral imaging based on the Soret γ band absorption in haemoglobin between 400 and 500 nm is used for the non-contact detection and identification of blood stains. Possible advantages of utilising the γ band absorption over the α and β band absorptions which have been used in previous studies are higher sensitivity and specificity for the detection and identification of blood stains.

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http://dx.doi.org/10.1016/j.scijus.2014.05.003
1355-0306/

2. Material and methods

2.1. The hyperspectral imaging system

The hyperspectral imaging system used in this study, consisted of a liquid crystal tuneable filter coupled to a CMOS camera and a light source for scene illumination. A CRi Varispec liquid crystal tuneable filter (LCTF) (CRi Inc. USA), capable of operation over a visible wavelength range of 400 nm and 720 nm, and a full width half maximum (FWHM) spectral bandwidth of 20 nm was used. The camera was a 1280 × 1024 pixel complementary metal oxide semiconductor (CMOS) device (Thorlabs DCC1545M, UK), with 8 bit A/D conversion, and a maximum frame rate of 25 frames per second. This was attached to a 12 mm focal length C-mount lens resulting in a field of view of approximately 30°. A typical sample instrument working distance of approximately 30 cm was used. A 230 W solid plasma light source (Thorlabs HPLS-30-02, UK) was used to provide the incident light. For this study, the radiant power output of the light source was approximately 10 W between 400 and 700 nm covering an area of approximately 100 cm^2 giving a power density of approximately 100 mW/cm^2. Crucially there was a significant output at approximately 400 nm which was needed to record the peak of the Soret band absorption, unlike most tungsten-halogen light sources. 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 500 nm with spectral sub sampling at 5 nm intervals, resulting in an image cube at 21 wavelengths for each scan. Spectra from the image cube were subsequently pre-processed and analysed using custom routines developed in MATLAB 2009a (MathWorks, USA). The time required to acquire and process an image was approximately 20 s.

Where the blood stains were analysed and disposed of within one day (trials 1–3), human blood from a consenting healthy volunteer was used. In other trials screened horse blood was used. Blood stains were allowed to dry for approximately 1 h before measurement.

2.2. Hyperspectral reflectance image acquisition and pre-processing

The hyperspectral reflectance measurements were made by first recording a reference image (R_0) of a blank piece of the substrate on which the blood sample was deposited. This image was recorded at a series of 21 discrete wavelengths from 400 nm to 500 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) which consisted of a data cube of 1280 × 1024 pixel values at 21 discrete wavelengths, was then calculated using Eq. (1):

$$R = \frac{R_s}{R_0} \times 100. \quad (1)$$

The factor of 100 was introduced so that the calculated reflectance values covered a larger portion of the 8 bit greyscale used to display the intensity of the image at each pixel. Historically the Kubelka–Munk equation has been applied to diffuse reflectance spectra to compensate for the effects of sample scattering [16]. In this study a modified version of the Kubelka–Munk equation was used to correct the reflectance image as shown in Eq. (2):

$$f(R) = \frac{\left(1-\left(\frac{R}{100}\right)\right)^2}{2\left(\frac{R}{100}\right)} \times 200 = \frac{k}{s} \quad (2)$$

where R is the reflectance image, k is the absorption coefficient of the sample, s is the scattering coefficient of the sample, and f(R) is the resulting reflectance image after correction. The factors of 100 and 200 are again introduced to provide a better match of the values of the reflectance image to the 8 bit greyscale values.

2.3. Trials

Four trials were designed.

1) Undiluted blood stains were deposited on substrates with dark colour or a colour similar to blood stain, including dark blue card, blue jeans and red paper. The amount of blood deposited on the substrate was controlled so that the stain was visible.
2) Undiluted blood stains were deposited on white photocopier paper alongside other substances including lip sticks, red ink and coffee.
3) Serial dilutions of blood with deionised water were tested on white filter paper (2 fold to 1024 fold dilutions) and red tissue (2 fold to 64 fold dilutions).
4) A blind trial was conducted using a red T-shirt with blood stains on it, provided by the Metropolitan Police Service.

2.4. The criteria for the identification of blood stains

The reflectance spectrum of blood stains in the visible region is dominated by the spectrum of haemoglobin present in blood [17,18]. It mainly consists of a strong narrow absorption centred at approximately 415 nm sometimes called the Soret or γ band with two weaker and broader absorptions between 500 and 600 nm known as the β and α bands. The Soret band is responsible for giving blood stains their distinctive red colour due to the absorption in the blue part of the visible spectrum. Other red substances also absorb in the blue region of the visible spectrum between 400 and 500 nm. However, the width of the 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 other similarly coloured substances. The reflectance spectra of suspected blood stains were statistically compared against a set of reference blood stains using the coefficient of determination (R^2) [11] It was expected that real blood stains would produce an R^2 value close to 1 whilst other stains would produce a value significantly less than 1. A threshold R^2 value could be used as the criteria for identification of blood stain. The reference blood stains library included aged blood stains between 1 day and 1 month old. The library of non blood stains contained approximately 50 reddish coloured substances which could be visually confused with blood. These included reddish coloured paints, nail varnishes, lipsticks and stains such as chocolate and jam.

For most substrates each pixel in the reflectance image was automatically analysed using the threshold R^2 value. The pixels which satisfied the criterion were marked as white, whilst all other pixels were marked as black. This allowed regions of the image where blood stain was present to be identified.

3. Results

3.1. The discrimination of blood stain from other red substances

Fig. 1 shows the normalised absorption spectra of 9 substances which could be confused with blood stains, recorded using the hyperspectral imaging system. It can be seen that there are significant differences in spectral peak and shape between blood stain and the other substances.

Fig. 2 is a box plot showing the coefficients of determination (R^2) for the reference blood stains and the other blood stain like substances. As there are substantial differences between the two groups of spectra, a threshold R^2 value of 0.9 was sufficient to discriminate blood stains from the other substances.

Fig. 3 shows colour photographs of blood stains and various other stains. Images 3a and 3b were obtained using substances which were separately deposited on white paper, cut out and mounted on a glass

Please cite this article as: B. Li, et al., The application of visible wavelength reflectance hyperspectral imaging for the detection and identification of blood stains, Sci. Justice (2014), http://dx.doi.org/10.1016/j.scijus.2014.05.003