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The age estimation of blood stains up to 30 days old using visible wavelength hyperspectral image analysis and linear discriminant analysis

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ABSTRACT

A novel application of visible wavelength hyperspectral image analysis has been applied to determine the age of blood stains up to 30 days old. Reflectance spectra from selected locations within the hyperspectral image, obtained from a portable instrument, were subjected to spectral pre-processing. This was followed by the application of a linear discriminant classification model, making estimations possible with an average error of ±0.27 days for the first 7 days and an overall average error of ±1.17 days up to 30 days. This is also the first reported study of the determination of the age of fresh blood stains (less than one day old) with an error of ±0.09 h. The studies have been made under controlled conditions and represent, at this stage, proof of concept results but also are the most accurate age estimation results for measurements between 0 and 30 days reported to date. The results are consistent with well-established kinetic processes suggesting that the pre-processing stages described are revealing spectroscopic changes which are reliably following the time dependent oxidation of HbO_2. The potential for parameterisation of environmental factors to make the method generally applicable at crime scenes is discussed, along with the developments required to further improve classification and to make the instrument genuinely portable.

1. Introduction

Bloodstain evidence is often encountered at the scene of a violent crime. The pattern of stains may provide evidence on the sequence of events and biological evidence may help in identification of victims or xsuspects [1,2]. For more than 100 years, there has also been an interest in using blood stain evidence to determine the time of a violent event [3].

Many techniques have been applied to determination of the age of blood stains [4–15] but these techniques are mostly complex and expensive and do not yet offer obvious potential for development of robust at-the-scene analysis. On the other hand, a simple technique might be based on the noticeable colour change from red to brown as blood ages [4]. In a fresh blood stain haemoglobin exists as oxyhaemoglobin (HbO_2) [16]. The visible spectrum of HbO_2 consists of 3 main peaks [17]. The strongest peak at ~415 nm is called the Soret band (or γ band), whilst the two weaker peaks at ~540 and 576 nm are labelled the β and α bands respectively. As the blood stain ages, the biggest change in the spectrum occurs in the region of the α and β bands due to the oxidation of HbO_2 to first met-haemoglobin (met-Hb) and then hemichrome (HC). Potentially, this well understood kinetic process could be monitored using visible spectroscopy, which might be developed into a non-contact, non-destructive means of determining the age of a blood stain instantly and at the scene. Moreover, it might also be possible to combine this with an imaging technique that also provides spatial resolution to aid in blood pattern analysis at scenes where blood staining occurred over an extended period.

Despite the apparently obvious relationship between colour and age, no effective technique is yet available [18]. Possibly due to the considerable variability found in the relationship between absorbance or reflectance and the age of blood stains, pioneering publications [3] did not lead on to substantial further work until recently. Studies published in 1986 [19] and in 2008 [12] noted changes in the absorption spectrum of blood stains as a function of time, but both groups were mainly interested in enhanced detection of minute blood stains and thus did not investigate this in a quantitative manner. Renewed interest in the application of visible spectroscopy methods to age determination of blood stains begins in 2010 with Hanson and Ballantyne [20,21] who attempted to use the blue shift in the peak wavelength of the Soret band at ~415 nm to determine the age of blood stains over the period of 1 year. However, although this group re-established the potential for this approach, they also met with considerable variation, probably due to environmental factors, indicating that further development was still required. If the colour change of blood stains is to be useful as the basis of forensic analysis, it will be necessary to deal with environmental factors and the consequent lack of precision.

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Recent studies [14,22] have begun to rigorously consider the precision of measurements based on the underlying kinetic processes. Bremmer et al. [22] described the use of diffuse reflectance spectroscopy to follow the ageing of blood stains deposited on white cotton under controlled conditions. They used a kinetic model of the two stage conversion of HbO_2 to HC to fit their experimental spectra. Concurrently with this study, Li et al. [14] followed the ageing of blood stains deposited on a white tile, under controlled conditions, using a Microspectrophotometer (MSP) to record visible wavelength reflectance spectra. In this study spectra were subjected to spectral pre-processing (to minimise the effect of baseline shifts and sample scattering) and feature selection (to identify regions of the spectra where the biggest change occurred over the timescale of measurement) followed by linear discriminant analysis (LDA). Both studies found that it was possible to make significant improvements over previous spectroscopic methods. The method of Bremmer et al. predicted ages of between 25 and 55 days for a sample with an actual age of 35 days. Over 37 days the method of Li et al. demonstrated an average error of ± 2.45 days, with an error of less than one day in just over 30% of measurements. Both of these studies attempted to control potential sources of variation, so it is likely that the estimates based on blood stains at real scenes would be less precise.

Most previous studies have followed the ageing of blood stains over time periods greater than one month. However, a recent study by Bremmer et al. [33] has shown that the biggest change in the visible reflectance spectra of blood occurs over the 1st day of ageing. This suggests that the methodology previously used by Li et al. could be applied for the accurate age determination of blood stains less than 1 day old. If this could be demonstrated, then it may provide an alternative or complementary method for the determination of the time of violent incidents where blood stains were present, alongside existing methods for determination of time of death.

In addition to improving the precision of age estimates, further work is also required to provide spatial as well as spectral resolution. Hyperspectral imaging is a well-established technique which is able to record a spatially resolved image with spectral information from several contiguous spectral bands. There are several methods of performing hyperspectral imaging, but conceptually the simplest involves placing a continuously tuneable wavelength selective device in front of an imaging sensor. Historically, hyperspectral imaging was developed for satellite imaging [23] but recently its use has spread to many disciplines including the food industry, agriculture and medicine [24–26]. Photographic image enhancement through the use of wavelength selective filters is well established and has, for example, been applied to blood stain detection and to finger-mark and shoe-mark detection [27]. Very recently, during the course of preparation of this manuscript, Edelman et al. [15] published the first study on the use of visible wavelength hyperspectral imaging for the age estimation of blood stains between 0 and 200 days old. Their approach essentially uses the methodology developed earlier by their group in the study of Bremmer et al. [22] combined with some of the spectral pre-processing techniques described by Li et al. [14] on hyperspectral images obtained with a state-of-the art experimental setup.

The aims and novelty of the current study were twofold. Firstly, to further develop the spectral processing and feature selection methods adopted by Li et al. [14] to improve the precision of age estimation, in particular for fresh blood stains. Secondly, to apply the methodology to a newly developed portable hyperspectral imaging instrument which could potentially be used at a crime scene by an officer or a Crime Scene Investigator. This would address a major limitation of the study of Li et al. as the MSP instrumentation was not portable and could not be used at a crime scene. With portability and cost in mind, the aim is to develop a hyperspectral imaging instrument which would be significantly simpler than that used by Edelman et al. by applying methodology for age estimation of blood stains that would not require high spectral resolution (<5 nm).

2. Material and methods

2.1. The hyperspectral imaging system

A simple low cost prototype hyperspectral imaging system (Fig. 1) was constructed to record the images of blood stains. The system was based around a CRi VariSpec liquid crystal tuneable filter (LCTF) (CRi Inc, USA) which operated at visible wavelengths from 400 nm to 720 nm, with a full width half maximum (FWHM) spectral bandwidth of 20 nm, and a working aperture of 20 mm. The LCTF was coupled to a 1280×1024 pixel, complementary metal oxide semiconductor (CMOS) camera (Thorlabs DCC1545M, UK) using a 12 mm focal length C-mount lens. Samples were illuminated using a 230 W solid state plasma light source (Thorlabs HPLS-30-02, UK) which output ~10 W of radiation between 350 nm and 700 nm over an area of ~100 cm^2. Control of the LCTF and image capture was performed using custom developed software written in C++ (Microsoft, USA). Images were captured between 505 nm and 600 nm with spectral sub sampling at 5 nm intervals, resulting in an image cube at 20 wavelengths for each scan. Spectra from the image cube were subsequently pre-processed and analysed using custom routines developed in MATLAB 2009a (MathWorks, USA) and subjected to statistical analysis using SPSS (IBM, USA).

2.2. Ageing study (30 days)

The spectral characteristics of ageing blood probably depend on a number of factors in addition to time. It was decided to control some of these factors to avoid confounding the analysis of the underlying process.

2.2.1. Environmental variables

2.2.1.1. Type of blood. In order to fulfil local ethics committee requirements based on the UK Human Tissue Act 2004, human blood stains could not be stored for more than 72 h and thus could not be used for long term tests. Consequently, screened equine blood was used to create the blood stains used in this study. Given that human and equine haemoglobin are very similar, it is reasonable to assume that the kinetic behaviour of the blood stains over 1 month timescales would also be very similar and that equine blood could be used as a suitable surrogate for human blood in age estimation studies.

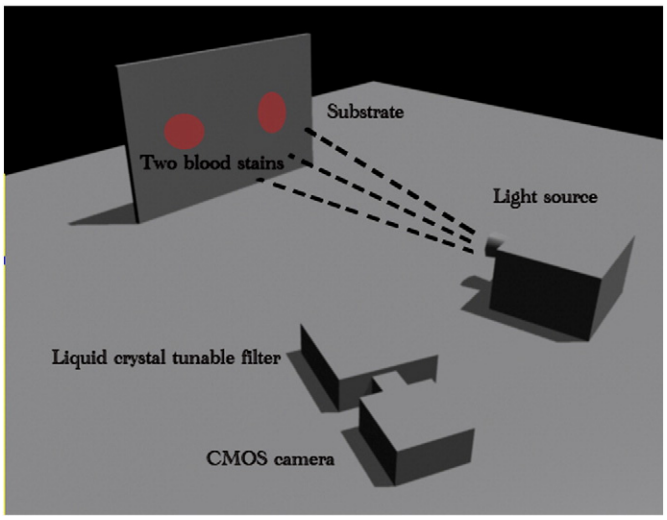

Fig. 1. A schematic of the hyperspectral imaging system.