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Feasibility of a handheld near infrared device for the qualitative analysis of bloodstains

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ABSTRACT

One of the most common tasks in criminal investigation is to determine from which tissue source a biological fluid stain originates. As a result, there are many tests that are frequently used to determine if a stain is blood, semen or saliva by exploiting the properties of certain molecules present within the fluids themselves. These include chemical reagents such as the Kastle-Meyer or Acid Phosphatase tests, as well as other techniques like the use of alternative light sources. However, most of the tests currently available have some major drawbacks. In this study, a handheld near-infrared spectrometer is investigated for the specific identification of deposited bloodstains. First, a calibration was carried out by scanning over 500 positive (blood present) and negative (blood absent) samples to train several predictive models based on machine learning principles. These models were then tested on over 100 new positive and negative samples to evaluate their performance. All models tested were able to correctly classify deposited stains as blood in at least 81% of tested samples, with some models allowing for even higher classification accuracy at over 94%. This suggests that handheld near infrared devices could offer great opportunity for the rapid, low cost and non-destructive screening of body fluids at scenes of crime.

1. Introduction

According to the Office for National Statistics, over 1.1 million violent offences occurred within the UK in 2016 [1]. Forensic investigation of these types of offences is likely to involve the identification and analysis of stains from biological fluids, such as blood, semen or saliva, which may be present on a variety of surfaces (both porous and non-porous). The presence or absence of these stains can have a significant effect on the outcome of an investigation, both by elucidating the sequence of events involved in the offence and by linking individuals to an offence via the analysis of DNA. The availability of quick, inexpensive, facile and non-destructive tests that can be carried out at the scene of crime is thus of crucial importance for the timely and accurate identification of body fluids.

Currently, there is a wide range of 'in-field' techniques that help forensic investigators determine the identity of crime scene stains. However, most of these techniques are presumptive and may only be used to indicate what the possible identity of a stain may be. A confirmatory test is often required to avoid the risk of false positive or negative results [2]. One well-known example of a presumptive technique is the use of alternate light sources (ALS) to enhance body fluid stains. This technique is particularly useful in visualising stains invisible to the naked eye (or those deposited on dark surfaces) and is based on the fluorescence emission of certain molecules within these fluids after excitation at a particular wavelength [3,4]. Although fast and simple, this technique is largely non-specific, as many and background substrates can fluoresce at the same wavelength [2]. In addition, blood does not fluoresce upon light source illumination and is therefore incompatible with ALS testing [3,4]. Another disadvantage of this technique is that certain types of radiation (i.e. ultra-violet) may cause damage to DNA present within the sample [5,6].

Other presumptive tests for body fluids include various chemical reagents, such as the Kastle-Meyer (KM) test or luminol for blood [7–10], the acid phosphatase (AP) test for semen [11,12], or the Phadebas® test for saliva. Most of these are based on specific chemical properties of molecules present in the target body fluid, such as the peroxidase-like activity of haemoglobin (KM test), or the action of salivary amylase on starch (the Phadebas® test) [13,14]. Such activity is then used to generate a change of colour or luminescence (as in the case of luminol) to indicate a positive result. While in some cases a surface stain will be analysed by first rubbing a piece of filter paper on it and then applying the chosen reagent to the paper, when the amount of sample is limited the reagent will be applied directly to the stain on its original surface. This in turn may prevent the stain from being used for

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subsequent DNA testing. In addition, some chemical presumptive tests require total darkness for proper visualisation (i.e. Luminol) [9,10] and most of such reagents may only identify one type of body fluid per test.

More recently, body fluid identification has been performed using immunological chromatographic testing cartridges, which exploit specific antibody-antigen interactions to differentiate between different fluids. One commercially available example of this technology is the Hematrace® device used for the identification of bloodstains [15]. Although quick and specific, the main disadvantage of such devices is that they are sample destructive (i.e. fluid samples cannot be recovered from the cartridge after testing), an important issue in situations where the amount of sample available is small.

In view of these challenges, it has become necessary to develop new techniques that can be used to identify biological fluid stains directly at the scene of a crime. Recent technological progress has allowed manufacturers to miniaturize a number of analytical instrumentation, resulting in the production of portable and handheld devices that enable scientists to carry out analysis at any location [16–20]. The development of devices for the examination of evidence at crime scenes, without the need for transportation to a specialist laboratory (which is often costly and time-consuming) is therefore of great interest to the forensic body fluid identification community.

Current efforts to achieve this goal have largely focused on spectroscopic techniques, widely used in many other analytical disciplines [21,22]. A number of studies have already been performed to determine the effectiveness of various spectroscopic techniques for the identification of biological fluids, including ultraviolet-visible (UV–vis) [21], infrared (IR) [23–25] and Raman spectroscopy [26–29]. While UV radiation may damage DNA contained within a fluid sample [5,6], IR spectroscopy has proven to be an interesting means to 'fingerprint' body fluids as this technique can determine the presence of specific classes of molecules in body fluids with sample volumes as small as 10–20 μL [23–25]. Nevertheless, most of the IR studies carried out so far employ benchtop instruments that cannot be taken to the crime scene. There is also the additional disadvantage that the presence of any water in the sample could interfere with the detection of certain types of analytes [25]. There have been successful attempts to identify the signature of different body fluids using Raman spectroscopy, coupled with near-infrared (NIR) excitation [26–28]. However, when not coupled to NIR, Raman spectroscopy presents some drawbacks, such as the strong fluorescence generated by some substrates (such as glass), which can mask signals produced from body fluid samples [26,27].

Within the context of forensic analysis, NIR spectroscopy can offer several advantages including:

- Little or no sample preparation, which may allow users to analyse evidence in situ i.e. at the scene of crime [30].
- Rapid sample scanning time (completed within a few seconds), which could significantly reduce the amount of time spent on a case [30].
- Non-destructive analysis, preventing the loss of samples needed for downstream DNA processing [30].
- Ability to scan samples inside glass or plastic containers, which may be potentially useful in applications such as the analysis of seized drugs of abuse, or the analysis of evidence at the scene of crime without unwrapping it or taking it out of its container [31–34].
- Minimal masking of analytes bands due to the presence of water molecules are well known and localised [35,36].
- No issues with fluorescence from background substrates, which could potentially mask the presence of biological samples [26–28].

This study therefore investigates the use of a handheld NIR device, the SCiO® made by Consumer Physics®, for the identification of body fluid stains. SCiO® is a handheld NIR spectrometer, able to scan solid and liquid samples in the range of 700–1100 nm, the region of the third overtone in the NIR spectrum [30]. The detector of this device measures attenuated reflectance from the sample scanned, which means the device allows the user to scan samples on opaque surfaces. The device is connected to a mobile phone by Bluetooth and controlled through the mobile app SCiO® Lab. Through this app the device sends the data obtained from the sample and displays it in a user-friendly format while, at the same time, uploads the data to an online 'cloud' database so it can be studied on any other device.

As blood is one of the body fluids most commonly found at the scene of crime, bloodstains were selected as the focus of this investigation. First, a number of classification models were created using online software tools provided by Consumer Physics® (https://www.consumerphysics.com/, Israel) after scanning samples of blood deposited on different surfaces. Although full details are proprietary, these tools utilise a "powerful cloud-based machine learning" method which is based on Partial Least Square Regression (PLSR), a multivariate often used as an alternative to Principal Component Analysis (PCA) [37]. After data processing, the performance of each model was tested by using them to identify new samples on glass. Only a few studies have investigated the use of NIR spectroscopy for the characterization of biological evidence in a forensic context [38,39]. Edelman *et al.* [38] showed that NIR spectroscopy has the potential to be employed not only for the identification of body fluids but also to estimate the time since deposition of a stain. The study however employed a benchtop instrument.

Pereira *et* al. [38] recently sought to identify bloodstains for forensic purposes utilising the MicroNIR™ handheld NIR spectrometer produced by US company Viavi. Whilst successfully able to differentiate human blood from several animal blood and non-blood samples, this specific device is unlikely to be amenable to current crime scene testing processes. First, it may be challenging for scenes of crime officers and police responders (who are likely to have limited spectroscopy training) to operate the specialized chemometric software required for data analysis. Conversely, SCiO® Lab software allows the development of dedicated applications for the testing of specific sample types, which allow personnel with no background in NIR analysis to use the device "as is" with minimal user input. Second, unlike the SCiO® device which is currently priced at around £225, the MicroNIR™ may cost between £16–20,000 depending upon application. Purchasing an array of such devices for crime scene deployment is likely to be unfavourable with forensic service providers in a time where analysis budgets are increasingly restricted. Lastly, the MicroNIR™ device does not support wireless operation of 'cloud'-based data storage.

All this considered, the success of this study would mean that a handheld device might have the potential to be used as a fluid identification technique directly at the scene of crime with no need of sample preparation, thus, reducing the cost and time needed for forensic examinations.

2. Materials and methods

2.1. Device and software

All scans were carried out using the SCiO® NIR scanner from Consumer Physics (Tel Aviv, Israel) as illustrated in Fig. 1. Samples were scanned on a flat benchtop, with the device placed over the sample using the shade provided in order to protect the sample from any external source of light. All samples on glass slides or thin surface materials were kept at a height of 5 cm, preventing the device from inadvertently scanning any surface below the samples. Data collection was controlled via the mobile app SCiO Lab® for Android.

2.2. Instrument calibration

2.2.1. Blood on glass slides

Prior to the deposition of samples, all slides were cleaned with ethanol (Sigma-Aldrich, Dorset, UK). Blood samples were taken by