Spectral analysis of blood stains at the crime scene

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1 - INTRODUCTION

This introductory chapter emphasizes the need for innovative techniques which aid crime scene investigations. Techniques used at the crime scene are ideally portable, rapid, and non-destructive. Because existing spectroscopic techniques meet these criteria, they are highly suitable for crime scene analysis. In this thesis, we propose the use of several optical techniques for the detection, identification, and age estimation of blood stains. We explored the visible, near infrared, and mid infrared wavelength range for this purpose, as outlined in this chapter.

1.1. CRIME SCENE INNOVATION

Crime scene investigators play a vital role in most criminal cases. They have the responsibility to detect, document and analyze evidence, and to select, collect and package traces for further analysis in the laboratory. All further forensic investigations depend on the quality of the initial investigation at the crime scene. If traces are overlooked or destructed, they will not be analyzed and therefore will not be used as evidence in the court of law. This problem denotes the importance of techniques which aid crime scene investigators in their search for relevant traces.

Due to technological improvements, techniques traditionally used in laboratories are getting faster, more portable and more informative. These innovations induce new possibilities for the detection and analysis of traces at a scene of crime, within their original context. Techniques already used in forensic laboratories may be explored for this purpose. Likewise, exploration of technologies developed in other disciplines may also lead to interesting forensic innovations. Existing techniques from outside the forensic world, e.g. the spectral analysis of blood, can possibly be adapted for crime scene investigation purposes. Recently, Bremmer et al[1] demonstrated how a method routinely used in medicine for blood oxygen saturation measurements could be adapted to a forensic application. They were able to relate the concentration change of oxyhaemoglobin, methaemoglobin and hemichrome – all reaction products of haemoglobin – to the age of blood stains in a laboratory setup, based on diffuse reflectance spectroscopy. Spectroscopic age estimation of blood stains is an innovative technique giving an estimation of the moment blood was shed, information currently not available to the investigator. Just like medicine, forensic science benefits from non-invasive techniques like optical spectroscopy. Ideally, diagnostic techniques in medicine are applied in vivo, rather than in the laboratory, which requires the tissue to be removed from the body. Similarly, it is profitable to analyse forensic traces non-invasively within their original context at the crime scene.

The transition from laboratory measurements to crime scene analysis, however, brings some challenges, typical for forensic casework. While in a laboratory setup ideal substrates are used, many different and far from ideal substrates can be encountered at a crime scene. Also, measurements are preferably performed without touching the trace. To deal with these circumstances, we can use techniques developed for remote sensing applications, or for the pharmaceutical and food industries. The need for high speed online processing in these industries has driven the development of fast methods for both data acquisition and analysis, which can also be valuable at the crime scene.

1.2. AIM

In this thesis, we describe several methods for the analysis of blood stains. Blood stains are among the most important types of evidence in forensic investigations. Analysis of blood stains can provide insight in *what* happened, as blood stain patterns inform investigators about the activities needed to create the stains, *who* was involved, by subsequent DNA-analysis, and spectral analysis may even indicate *when* the blood was shed. At a crime scene, however, some blood stains may be visible with the naked eye, while others are indiscernible, thus motivating the need for technology increasing the contrast between a stain and its background. After the detection of a stain, an identification test is needed to indicate the nature of the stain. When a blood stain identification test is positive, it is interesting to know when the stain was created in order to create a timeline of events. Our aim was to develop methods for:

1. the detection of latent blood stains;
2. the identification of blood stains;
3. the age estimation of blood stains,

which can be applied at the crime scene. Driven by the importance of non-destructive analysis we evaluated several optical techniques for these purposes. We explored the visible wavelength range and beyond.

1.3. OUTLINE

In **Chapter 2**, we describe the advantages and challenges of hyperspectral imaging for forensic applications. Because hyperspectral imaging integrates conventional imaging and spectroscopy to obtain both spatial and spectral information from a sample, it enables investigators to analyze the chemical composition of traces and simultaneously visualize their spatial distribution. The technique therefore offers significant potential for the detection, visualization, identification and age estimation of forensic traces. The application of this technique for the visualization and chemical analysis of forensic traces is reviewed.

Chapter 3 demonstrates the applicability of spectroscopy and hyperspectral imaging as a non-destructive indicative test for the identification of blood. By deducing the presence of haemoglobin oxidation products from reflectance spectra of blood stains, blood can be distinguished from other red and brown samples visually mimicking blood. Measurements were first performed using a fibre optic probe and a spectrometer in a controlled laboratory setup. Based on the results, the spectral processing steps were adapted to minimize the influence of non-chemical factors as the measurement distance and angle. With the adapted analysis, the identification method was shown to be suitable for a less-controlled hyperspectral imaging setup. The sensitivity and specificity of the technique were investigated, and the practical applicability was demonstrated in forensic casework.

The spectral analysis of blood stains was expanded to include age estimation in **Chapter 4**. Based on the reflectance spectra of blood stains, not only the presence of haemoglobin oxidation products can be assessed, but their relative concentrations can be calculated simultaneously. As described above, the amount of oxyhaemoglobin, methaemoglobin and hemichrome in a blood stain can be used to estimate the age of the stain. However, the technique described by Bremmer et al using a spectroscopy setup, was highly sensitive to small changes in the geometry of the setup, which complicated the use at the crime scene. We demonstrated that an improved spectral analysis greatly extended the applicability. Using a hyperspectral imaging system we were able to estimate the age of different blood stain patterns at a simulated crime scene. When applied in criminal casework, this new technique provides investigators with information useful to determine the moment a crime was committed, or to select relevant stains for further analysis.

The above described method is hampered by coloured backgrounds, as these absorb visible light, and thereby disturb the measured reflectance spectra of blood stains. **Chapter 5** addresses this problem. We proposed an adapted algorithm to correct for background absorptions. Additionally, we described a statistical approach to calculate an age interval for a questioned blood stain. The applicability of the new technique for blood stain age estimation in forensic casework and its possible value for crime investigations is demonstrated in **Chapter 5**. In a case report we described a shooting incident in which 3 bodies were found dead in a living room. For crime reconstruction purposes, bloodstain pattern analysis was performed at the scene. As an innovative aid to the crime scene analysis, the absolute and relative age of different groups of bloodstains were measured using visible reflectance spectroscopy. Combined with other evidence, the results could lead to a better reconstruction of the timeline of events, useful for the verification of possible scenarios.

On dark objects, the visualization of latent stains is already challenging in forensic practice. **Chapter 6** covers the detection of blood stains on black backgrounds using visible hyperspectral imaging, which is useful for bloodstain pattern analysis, or to aid the collection of traces for further tests, e.g. blood stain identification or DNA analysis. This chapter shows that blood stains can be distinguished from many black fabrics based on the different absorption properties. Several chemometric methods are tested to optimize the contrast.

When the absorption of light is dominated by the background material, visible spectroscopy is hampered. Therefore we explored the wavelength range beyond the visible for the analysis of blood stains. In **Chapter 7** we successfully identified blood stains on coloured and black backgrounds and estimated their age using near infrared (NIR) spectroscopy. Compared to visible spectroscopy, NIR spectroscopy provides more information about the chemical structure of samples, as NIR absorptions are caused by specific molecular vibrations. However, apart from the chromophores oxyhaemoglobin, methaemoglobin and hemichrome, several other components present in blood contribute to the absorption of NIR light, which complicates the analysis. For this reason, we used correlation analysis and partial least squares regression analysis for the identification and age estimation of blood stains using NIR spectroscopy.

A final interesting wavelength range explored in this thesis is the mid infrared, or thermal wavelength range. As a result of improvements in technology and a decrease in cost, mid infrared imaging is an emerging technique for law enforcement and forensic investigators. All objects radiate infrared energy, invisible to the human eye, which can be converted into visible images by mid infrared cameras, thereby visualizing differences in temperature and/or emissivity of objects. The rapid, non-destructive and non-contact features of mid infrared imaging indicate its suitability for a wide range of forensic applications, including the detection of latent bloodstains. **Chapter 8** provides an overview of the principles and instrumentation involved in mid infrared imaging. Difficulties concerning the image interpretation are addressed. Reported forensic applications are reviewed and supported by practical illustrations.

To conclude, all topics described above are discussed from a forensic practical point of view in **Chapter 9**. Emphasis is laid on further steps needed for the actual implementation of the described innovative techniques in standard forensic practice.