Spectral analysis of blood stains at the crime scene

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9 - DISCUSSION

This chapter reflects on the contents of this thesis. It summarizes the proposed spectroscopic techniques for the detection, identification and age estimation of blood stains. We discuss the physical light-transport model used to analyse reflectance spectra of blood stains, and compare it with a statistical and empirical method described in literature. The main advantage of a physical approach is the ability to correct for background interferences, which is important in forensic applications, due to the wide range of substrates possibly encountered at crime scenes. We describe several complimentary techniques, which may be combined to gather a maximum amount of information from a sample. To conclude, several practical challenges are discussed, which need to be addressed before the described methods for the analysis of blood stains can routinely be used in criminal investigations.

9.1. INTRODUCTION

Blood stains are often encountered in crime scene investigations and can provide investigators with interesting information regarding the donor of the blood stain (using DNA analysis) or the activities needed to create the stain (by bloodstain pattern analysis). To be able to analyse blood stains however, they first need to be detected. Next, the nature of the stain must be identified to distinguish blood from other substances. Furthermore, blood stains would be even more informative if their age could be measured. This brings us to the three main challenges addressed in this thesis:

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

Our aim was to develop methods to tackle the above described challenges for the analysis of blood stains, which can be applied at the crime scene, so traces can be judged and interpreted in the original context, without the need to wait for results from a remote laboratory. Techniques used for crime scene investigations ideally are non-destructive, portable and require no sample treatment. Several optical spectroscopic techniques fulfil these requirements and were explored in this thesis. Because traces are generally not found on ideal backgrounds used in laboratories, but many different backgrounds can be encountered at the crime scene, we address the problem of background interference and suggest a correction method.

9.2. DETECTION OF BLOOD STAINS

To detect blood stains on black backgrounds crime scene investigators conventionally use chemicals which react with amino acids, proteins, or heme, and induce chemiluminescence or fluorescence in contact with blood. These methods are invasive and require a darkened room for the detection. We explored two non-destructive optical techniques for this purpose: visible hyperspectral imaging and mid infrared imaging.

9.2.a VISIBLE HYPERSPECTRAL IMAGING

Hyperspectral imaging is a promising technique for the detection of latent blood stains. As described in **Chapter 1**, hyperspectral imaging combines spectroscopy with digital imaging and can be used for the non-destructive detection and chemical analysis of forensic traces, e.g. fingerprints. In **Chapter 6**, we demonstrated that visible hyperspectral imaging can be valuable for the detection of latent blood stains on black fabrics. Using this technique combined with data processing methods we successfully enhanced the contrast between blood stains and their backgrounds compared to white light photography.

9.2.b MID INFRARED IMAGING

Another non-destructive technique, which can reveal information invisible to the naked eye is mid infrared imaging, as described in **Chapter 8**. Both temperature differences and differences in thermal material properties can induce contrast in mid infrared images. Apart from measuring post mortem temperatures and the detection of heat traces caused by human contact at the crime scene, mid infrared imaging was used to detect latent blood stains. Blood stains hardly visible to the naked eye became evident using mid infrared imaging, while conventional photography combined with forensic light sources did not generate much contrast between the stain and its background.

9.3. BLOOD STAIN IDENTIFICATION

Most chemical methods used to detect latent blood stains can also be used for identification purposes. Two commonly used tests are tetrabase[75] and Kastle-Meyer, which employ peroxidase activity of haemoglobin molecules, causing a colour change. Again, the main disadvantage of these techniques is their invasiveness. We used near infrared and visible spectroscopy and hyperspectral imaging to identify blood and distinguish it from other substances.

9.3.a VISIBLE SPECTROSCOPY AND HYPERSPECTRAL IMAGING

In **Chapter 3** we demonstrated that blood stains can be identified based on their visible reflectance spectra, by deducing the presence of haemoglobin and its oxidation products oxyhaemoglobin, methaemoglobin, and hemichrome. We started using a spectroscopy setup in the laboratory and moved on to hyperspectral imaging in a simulated crime scene. Using hyperspectral imaging for the analysis of blood stains has several advantages compared to conventional spectroscopy. First of all, reflectance spectra of many blood stains can be measured in one scan, which makes the analysis of a complete crime scene far less time-consuming. Additional information is acquired, because the spatial information of the blood stain distribution is recorded simultaneously, showing the location and context of each stain and thus reducing the amount of documentation needed. Being able to view the spectral and spatial information side by side may also improve bloodstain pattern analysis, by enabling to group stains from individual events based on their age.

9.3.b NEAR INFRARED SPECTROSCOPY

When the absorption of visible light is dominated by the background, we recommend to move beyond the visible wavelength range for the identification of blood stains. In **Chapter 7** we showed that blood stains can be identified using near infrared (NIR) spectroscopy, even on black backgrounds. NIR spectra of blood stains show absorption peaks characteristic for several components of human blood, e.g. haemoglobin, albumin, globulin. Based on these peaks, blood stains can be distinguished from other samples visually mimicking blood.

9.4. BLOOD STAIN AGE ESTIMATION

In many criminal investigations there is a lack of temporal information. Only few techniques are routinely used in forensic investigations, e.g. determination of the time of death[169]. Because of the importance to situate events in time, age estimation techniques are one of the holy grails in forensic science. In this thesis, we described how blood stains can be dated using visible and NIR spectroscopy and hyperspectral imaging.

9.4.a VISIBLE SPECTROSCOPY AND HYPERSPECTRAL IMAGING

In **Chapter 4** we described how the age of blood stains on white backgrounds can be estimated by the analysis of their visible reflectance spectra, which can either be measured using spectroscopy or hyperspectral imaging. From these spectra, we not only derived the presence of the haemoglobin derivatives used for identification purposes, but were able to deduce their relative concentrations. This way we gained insight in the chemical reactions taking place in the blood stains. By comparison with a reference dataset, the measured concentrations were used to estimate the age of a questioned blood stain.

In **Chapter 5** we adapted the light transport model used to be able to correct for background absorptions and demonstrated the applicability of this technique for blood stain age estimation in casework. In a recent case we estimated the age of blood stains using reflectance spectroscopy. A statistical approach was introduced to calculate an age interval. At the scene of a presumed double homicide followed by suicide, we analyzed blood stains on several backgrounds at two distinct locations: downstairs where the victims were found, and upstairs. The results indicated that the group of blood stains found upstairs was older than the blood stains found in the vicinity of the bodies downstairs, suggesting that the blood stains found upstairs were not related to the crime. DNA evidence showed that the blood stains upstairs all belonged to one of the victims, who lived in the residence. The time interval estimated for the creation of the blood stains downstairs included the moment gun shots were heard by a witness. Combined with other evidence, the gathered information could lead to a better reconstruction of the timeline of events.

9.4.b NEAR INFRARED SPECTROSCOPY

In **Chapter 7** we successfully explored NIR spectroscopy for the age estimation of blood stains on dark backgrounds. While visible spectroscopy provides information about chromophores, NIR spectroscopy gives insight in molecular vibrations induced by the interaction with light. As a result, NIR spectroscopy gives more insight in the chemical structure of samples compared to visible spectroscopy. However, while only a few clearly distinguishable chromophores, i.e. the haemoglobin derivatives, absorb visible light, it is less clear which components present in blood stains contribute to the absorption of NIR light, because the molecular vibrations are not specific for one certain molecule. The large number of possibly absorbing components, among which haemoglobin, albumin, and globulin, complicated the analysis and compelled us to use a different approach for the data analysis. We chose Partial Least Squares (PLS) regression for the age estimation task. PLS is a useful statistical tool for the analysis of spectroscopic data, as it can handle datasets with more variables than measurements, and the data may contain highly correlated predictor variables[131]. PLS makes linear combinations of the original predictor variables to construct new variables, which are the most relevant for estimating the age. We built a PLS model using a training set of blood stains on black cotton and tested it on blood stains on red, green and blue cotton. The results demonstrated that we were able to estimate the age of blood stains independent of the background colour. Different background materials however may introduce new absorption peaks, which can disturb the age estimation task, demanding a new training set.

9.5. SPECTRAL PRE-PROCESSING

When moving a laboratory technique to the crime scene, the measurement setup is typically less controlled, e.g. the distance from different samples to the detector may vary. To reduce spectral variability caused by these variations in the measurement geometry, we needed advanced pre-processing of the data. By using the standard normal variate correction algorithm, we were able to correct for spectral differences which were due to physical variability such as illumination intensity differences rather than chemical variability, as shown in Figure 9. The introduction of this correction algorithm was an important step needed for the practical applicability of the technique. Without this correction, blood stains of the same age show big spectral differences, e.g. the offset, which makes it difficult to distinguish blood stains of different ages. After correcting the spectra, the physical differences between blood stains of the same age disappear and the chemical differences between blood stains of different ages remain clearly distinguishable. Several other pre-processing techniques have been explored, e.g. multiplicative scatter correction, derivation, and smoothing (see **Chapter 2** for a description of these techniques), but have not proven their added value. The main risk when using such techniques is the loss of informative peaks and the creation of artefacts.

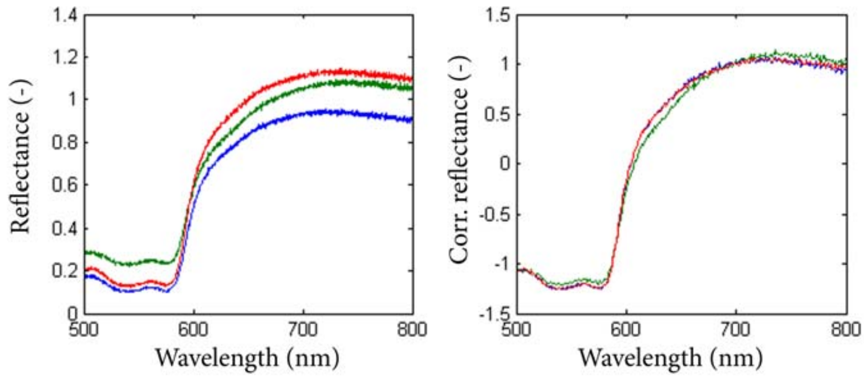

Figure 9.1. Reflectance spectra of blood stains without correction (left) and with standard normal variate correction (right). After correction the spectra of blood stains with the same age overlap (red and blue lines, 2 hours old) and are clearly separated from a spectrum of a blood stain with a different age (green line, 3 hours old).

9.6. DATA ANALYSIS

After pre-processing of the data, the main challenge was to extract the chemical changes from the reflectance spectra and relate them to the age of blood stains. We used a physical approach to gain insight in the concentration of oxyhaemoglobin, methaemoglobin and hemichrome present in blood stains, which in turn can be used to estimate the age. Other research groups used a statistical or an empirical approach to analyse similar data, on which we elaborate below.

9.6.a PHYSICAL APPROACH

Although the changing absorption properties are clearly notable in the corrected reflectance spectra of blood stains, more factors influence the shape of the spectra. The difference between emitted and detected light not only results from light absorption, but photons are moreover lost by other interactions with a blood stain, e.g. scattering, transmission and specular reflections. The blood stain substrate complicates the situation even more, by likewise reflecting, transmitting, scattering and absorbing light.

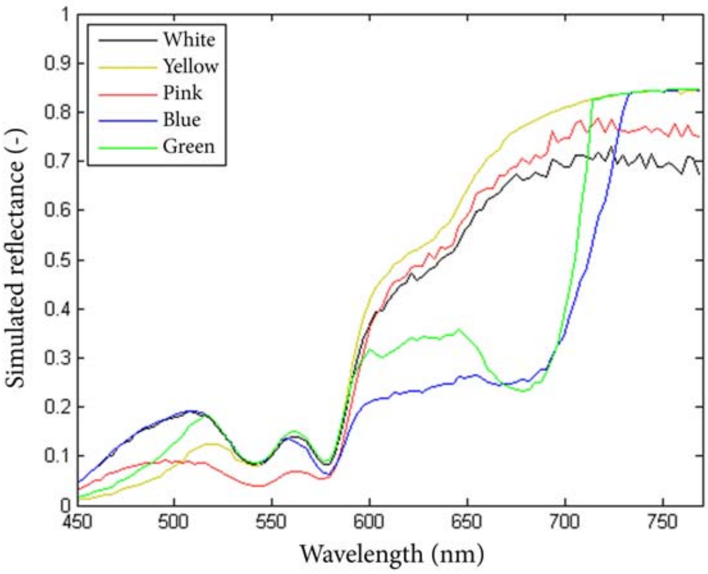

Figure 9.2. Simulated reflectance spectra for blood stains of 30 μm on white, yellow, pink, blue and green backgrounds.

To demonstrate the effect of the background colour on the reflectance spectra we simulated a two-layered sample, consisting of a layer of blood and a background layer using the Monte Carlo technique[170] (Figure 9.2). Reflectance spectra of blood stains with green and blue backgrounds show dips around 680 nm, whereas spectra of yellow and pink backgrounds mainly deviate from the white background in the short wavelength region. These background interferences have to be taken into account to deduce solely the contribution of absorption by the blood stain, from which the chemical composition of the blood stain can be deduced.

The complex interaction of the incoming light with a blood stain and its background can be approximated using a two flux model, in which an upward and downward flux of light are transported. This model leads to a system of differential equations, which can be solved analytically to give a relationship between the reflectance and the absorption and scattering properties of a sample, as described in **Chapter 5**:

$$R = \frac{1 - R_b \cdot (a - b \cdot \coth(bST))}{a - R_b + b \cdot \coth(bST)}, \text{ with } a = \frac{S+K}{S} \text{ and } b = \sqrt{a^2 - 1}, \qquad (9.1)$$

where R is the reflectance, R_b the reflectance of the background, S is a scattering coefficient, K an absorption coefficient and T is the blood stain thickness[86]. As a first approximation, the presence of a background was disregarded in **Chapter 4**, by assuming a blood layer of infinite thickness interacting with the incoming light, which reduces the formula:

$$R = 1 - \frac{K}{S}\left(\sqrt{1 + \frac{2S}{K}} - 1\right). \qquad (9.2)$$

By fitting this equation with the measured reflectance spectra, we successfully calculated the haemoglobin derivative fractions for blood stains on white cotton. In **Chapter 5** we extended this method, by taking the contribution of the background into account, as described in equation 9.1. In theory, this method works for each background, as the reflectance of the clean background can be measured and incorporated in the model.

9.6.b STATISTICAL APPROACH

Recently, more research groups have studied the use of hyperspectral imaging either for blood stain identification or age estimation purposes. While the data acquisition methods of these groups were similar, the main difference lies in the approach of the data analysis. Li et al proposed a statistical method for blood stain identification and age estimation based on linear discriminant analysis (LDA)[67, 68]. LDA is a multivariate classification algorithm Li et al used to separate a class of blood stains from other stains (the identification task) and to categorize the blood stains in predefined age classes (the age estimation task). Using a training set, the LDA algorithm selects wavelengths with a minimal within class variability and a maximum between class variability, whereupon a linear combination of these wavelengths is used for classification. For this approach to be successful, it is important that the training set used is representative for the questioned blood stain. In forensic practice, if blood stains are found on other backgrounds or in other environmental circumstances, a new training set is thus required, representative for the situation at the crime scene.

9.6.c EMPIRICAL APPROACH

Apart from the physical and statistical approaches described above, reflectance spectra can be analysed using an empirical approach. In an attempt to develop a method for the identification of blood stains applicable for many different backgrounds, Janchaysang et al acquired several empirical criteria for the detection and identification of blood stains based on their reflectance spectra or mathematical transformations of these spectra, e.g. the absorbance at a certain wavelength should not exceed a certain threshold[171]. Each questioned stain was tested upon all criteria using Boolean logic, and was excluded or included accordingly. Although criteria were selected based on empirically collected data from blood stains on a variety of substrates, it remains difficult to extrapolate this method to the infinite amount of backgrounds possibly encountered at the crime scene.

9.7. MULTIDISCIPLINARY APPROACH

As demonstrated in this thesis, one of the main challenges when performing spectral measurements at the crime scene is how to correct for background interferences. These interferences are a general problem encountered in forensic applications, where traces are typically not found on ideal neutrally reflecting backgrounds used in laboratories, but all possible backgrounds can be encountered (e.g. different materials, porous, non-porous, coloured, patterned, etc.). Although all spectroscopic techniques are hampered by certain backgrounds, different techniques can be complementary. Therefore, a multidisciplinary approach is advised in which several techniques are combined to optimize the results for each specific background.

We introduced a method to correct for coloured backgrounds which cause interference in visible reflectance spectra (**Chapter 5**). On dark backgrounds however, light absorption of the background is dominant and hampers the use of visible reflectance spectroscopy or hyperspectral imaging for the identification and age estimation of blood stains. NIR spectroscopy can be used instead (**Chapter 6**), but is on its turn hampered by dominating water absorptions when blood stains are wet.

9.7.a OTHER SPECTROSCOPIC TECHNIQUES

Apart from the techniques explored in this thesis, several other non-destructive spectroscopic techniques may provide information useful for the analysis of blood stains. Raman spectroscopy is a vibrational technique complementary to NIR spectroscopy. Raman spectra generally have sharper and better resolved peaks than NIR spectra, and can provide more chemical information of unknown samples. Recently, several research groups have studied the use of Raman for the identification of blood stains[84, 103, 147]. It was demonstrated that blood stains can be identified based on their Raman spectra, and that human blood can be distinguished from blood from other species[104]. Because quantitative information about haemoglobin derivatives can be derived from Raman spectra, it may also be possible to use them for age estimation of blood stains in the future[172, 173]. However, Raman spectroscopy is complicated by strongly fluorescing backgrounds, like fabrics[105]. Furthermore, Raman spectra can be very complex and can contain broad and superimposed bands, especially for biological samples, which often include spectral features of multiple constituents: proteins, lipids, DNA, RNA, individual amino acids, biological chromophores (haemoglobin), and other metabolites[147]. The extraction of useful information requires advanced data analysis.

Even more chemical information about blood stains can be obtained by means of the mid infrared (mid IR) wavelength region. In **Chapter 8** we used Mid IR imaging for the detection of blood stains. It would be interesting to extend this to Mid IR hyperspectral imaging or spectroscopy. Mid IR reflectance spectroscopy provides information about fundamental molecular vibrations, of which overtones and combination bands are visible in the NIR region[138, 142]. Although no forensic applications of mid IR spectroscopy for the analysis of blood stains are described, it may be worthwhile to explore. On the other side of the electromagnetic spectrum, with wavelengths shorter than the visible region, ultraviolet (UV) spectra may provide useful information as well. Using UV-Vis spectroscopy Hanson and Ballantyne revealed a blue shift in the so-called Soret band of haemoglobin. The peak of this band starts at 412 nm in fresh blood stains and shifts to shorter wavelengths as the age of the stain increases[174]. Because of its high absorption intensity, using the Soret band is expected to be highly effective for the identification and age estimation of blood stains. Further research in this wavelength range is recommended. If we move to even shorter wavelengths, we arrive in the region of X-ray radiation. Trombka et al explored the use of X-ray fluorescence (XRF) for the identification of blood stains[175]. XRF is an analytical method which can identify the elemental composition of a sample. Trombka et al showed that XRF allows the identification of blood by the detection of iron present in the haemoglobin molecule. To test the practical value of this technique, the specificity and sensitivity of this technique must be studied. Also, possible background interferences are yet unknown.

9.7.b OTHER TECHNIQUES

Apart from the optical spectroscopic methods described above, other methods for age estimation have been explored in the past, as reviewed by Bremmer et al, e.g. high performance liquid chromatography, electron paramagnetic resonance, atomic force microscopy and RNA degradation measurements[94]. These methods are more invasive and can only be performed in the laboratory.

Recently, Gas Chromatography- Mass Spectrometry (GC-MS) has been explored to study the composition and degradation of fingerprints[176]. Similarly, MS has the potential to become an important tool for the age estimation of blood stains, due to its simplicity, sensitivity and effectiveness in separating and identifying chemical components. Portable GC-MS systems enable the application of mass spectrometry in non-laboratory environments, but they still require extensive sample preparation due to the constraints of chromatography, thus increasing total analysis time. Ambient MS techniques have greatly simplified and increased the speed of MS analysis, and can be performed directly on samples, including complex matrices such as biological fluids in their natural environment[177]. One of the simplest ambient MS methods reported is paper spray MS, which generates ions by applying a high voltage to a paper triangle wetted with a small volume of a solution. Samples can be transferred from surfaces using the paper as a swab. Paper spray MS has proven to be highly useful for drug monitoring from whole blood spots[178]. The applicability of this relatively new technique in forensic casework however, remains largely uninvestigated.

The well-known expression "Absence of evidence is not evidence of absence" can be used to demonstrate the limitations of forensic techniques. No single technique is capable to detect and identify all types of traces at the crime scene without the need of further confirmation. By combining several (spectroscopic) techniques and selecting the optimal technique for each background, the applicability of methods for detection, identification and age estimation of blood stains in forensic practice can be extended. All information gained with different techniques can be useful for a crime scene investigator, provided they are correctly interpreted. To determine which technique is favourable in certain circumstances, it is important to study the boundaries of each technique.

9.8. TOWARDS IMPLEMENTATION

Before the new method for age estimation of blood stains described in **Chapter 5** can be applied in standard forensic practice, key steps in the research process are refining and validating the data to meet the needs of the legal and scientific communities. It is important to explore the boundaries of the technique and to describe the limitations, e.g. minimal blood stain size, minimal and maximum blood stain thickness, and requirements of the background colour, porosity etc. Furthermore, the effect of different aging circumstances should be known.

9.8.a ENVIRONMENTAL INFLUENCES

The described approach of splitting the spectra into the different chemical components (**Chapter 4, 5**) has the advantage that the influence of temperature, humidity or other environmental circumstances can be studied. As described by Bremmer et al, the temperature increases the speed of the chemical reactions in a blood stain, whereas humidity only influences the denaturation of methaemoglobin into hemichrome[56]. The influence of environmental factors at the crime scene will make precise estimation of the absolute age of blood stains challenging. If the environmental factors can be reconstructed we are able to study the kinetics of the haemoglobin derivatives, which can be used to estimate the absolute ages of blood stains, as shown in the case example in **Chapter 5**. Additionally, even in unknown circumstances, we demonstrated the possibility to determine the relative age of different blood stains, under the assumption that they were exposed to similar environmental conditions. Because the fraction of HbO_2 decreases in time, this fraction can be used to determine the order of formation of different blood stains, as demonstrated by the analysis of the simulated crime scene in **Chapter 4**.

9.8.b HUMAN VARIABILITY

Apart from the environmental circumstances, more research is needed to gain knowledge about the biological variability between and within humans. In a prior study of Bremmer et al[1], no significant differences were found between or within donors. Because we analysed blood of healthy non-smoking volunteers who usually have only a small percentage (<4%) of carboxyhaemoglobin[99], the possible presence of this haemoglobin derivative was not taken into account in this thesis. The increased level of carboxyhaemoglobin expected for smokers, people suffering from sickle cell disease, fire victims or in cases of fatal carbon monoxide poisoning, may influence the results in forensic practice[100-102]. However, because our model does not include the absorption properties of carboxyhaemoglobin, we expect a bad correlation between the measured reflectance spectrum and the theoretical fit. By embedding a threshold for this correlation as a quality check for the fit (**Chapter 4, 5**), results will be inconclusive in these cases, rather than giving a wrong age estimation.

9.8.c BLOOD STAIN THICKNESS

Another source of spectral variation is the blood stain thickness. Monte Carlo simulations of reflectance spectra of blood stains with various thicknesses on a white background are shown in Figure 9.3. As expected, this figure shows that the overall reflectance is higher for thinner blood stains. For thicker blood stains, the characteristic absorption features of oxyhaemoglobin (dips at 540 and 576 nm) and methaemoglobin (dip at 630 nm) are less pronounced[82]. The influence of the blood stain thickness on the identification (**Chapter 3**) and age estimation (**Chapter 4, 5**) tasks should be studied to gain insight in the limitations of the technique. Thick blood stains are expected to complicate the analysis due to their high absorption and thus low signal to noise ratios, whereas thin blood stains will be greatly influenced by the background.

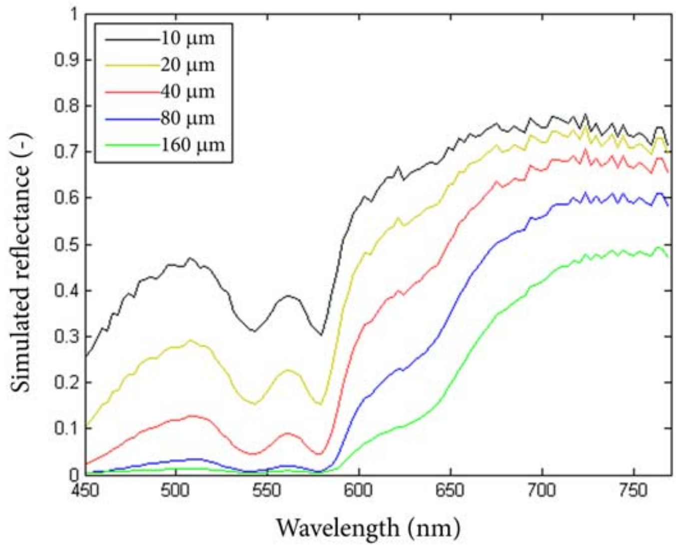

Figure 9.3. Simulated reflectance spectra for blood stains of 10, 20, 40, 80 and 160 µm on a white background.

9.8.d COLLECTION OF BLOOD STAINS

In case the background interference is too dominant to correct for, no matter which technique is used, blood stains can be extracted from the background to be applied on an ideal non-interfering background (e.g. white cotton when visible spectroscopy is used). This practical approach enables investigators to bring a blood stain to the laboratory for further analysis. The challenge in this case, however, lies in the fact that the chemical composition of the blood stain may not be changed while transferring it to another background. Traditional collection of blood stains with moistened cotton swabs will alter their chemical composition, as the addition of water to a blood stain is known to induce the transition from hemichrome back to methaemoglobin[109]. Further research is needed to invent a collection procedure which does not influence the results of the age estimation task. When successful, stains may be collected from the scene and stored deep-frozen to slow down or even stop any further chemical reactions taking place before the analysis is performed. In that case however, the method is no longer non-destructive.

9.8.e ACCURACY

In forensic practice, many factors contribute to spectral differences which increase the calculated confidence intervals for age estimations, e.g. detector noise, biological variations in blood stains, environmental differences, etcetera. Additionally, there will be errors in the estimated haemoglobin derivative concentrations due to imperfect corrections for blood stain thickness, and background optical properties. To culminate small age intervals, we recommend to perform spectroscopic measurements as soon as possible. The chemical reactions taking place in the blood stains are rapid in the beginning, but slower in a later stage[56]. As a result, the accuracy of age estimations decreases with the age of the blood stain.

In the described case example (**Chapter 5**), it would have been interesting to know which person was shot first and what the time difference was between the different shots**.** Li et al demonstrated in a laboratory hyperspectral imaging setup that the age of blood stains can be determined with an accuracy of 1 hour within the first day after bleeding[68]. If our measurements were performed immediately after discovery of the crime scene, it may have been possible to indicate the sequence of different blood stain patterns created around the victims (different blood stains found downstairs).

In forensic practice, the accuracy will depend on the actual age of the blood stain and knowledge of the environmental conditions. How this influences the evidential value depends on the hypotheses relevant to the case. Confidence intervals in casework are generally expected to be larger than intervals resulting from measurements performed in a laboratory setup.

9.8.f MOBILE ANALYSIS AT THE CRIME SCENE

The equipment used by crime scene investigators is ideally portable, wireless, waterproof, easily decontaminated and user-friendly. Recent technological advances enabled the development of fast, wireless, high resolution hyperspectral imaging systems, facilitating the transfer from the laboratory to the crime scene, as demonstrated in this thesis (Figure 9.4). The development of portable equipment enables the analysis of an unknown stain at the crime scene without waiting for results from the laboratory.

Figure 9.4. Photographs of a hyperspectral imaging system at a simulated crime scene.

Due to current developments hyperspectral imaging systems become smaller, more sensitive, user-friendly and cost effective. A next generation of snapshot systems is expected in the near future, consisting of a novel multispectral sensor integrating a tiled filter and a CMOS sensor. Similar to conventional Bayer filters used in colour imaging, a set of Fabry-Pérot filters can be chosen, filtering specific wavelengths needed for a certain application. While in the past spectral imaging technology developed from multispectral imaging using several broad wavelength bands, to hyperspectral imaging using many narrow wavelength bands, the new tiled filter based technique reduces the amount of wavelength bands again, but keeps the ability to use narrow wavelength bands. This evolution encourages the selection of wavelengths which are most relevant and informative to observe spectral differences. Prior knowledge about absorption properties, stepwise multiple linear regression, or multivariate techniques such as Principal Component Analysis and Partial Least Squares can be explored for an optimal wavelength selection[179].

Another technology expected in the future is the use of adapted smartphones with additional filters, which can be used for the analysis of blood stains at the crime scene. Thanakiatkrai et al recently proposed to use smart phone cameras only for the age estimation of blood stains. Using an optimally controlled light box they successfully classified blood stains in broad age intervals. However, the described technique is expected to be highly sensitive to minimal changes in this setup, e.g. the camera used, the intensity of the light source, or the distance to the blood stain. Nevertheless, it is worthwhile to explore these new technologies for forensic analysis.

9.8.g CONCLUSION

In this thesis, we demonstrated several spectroscopic techniques for the non-destructive detection, identification and age estimation of blood stains at the crime scene. The non-destructive detection of latent blood stains, either by visible hyperspectral imaging or mid infrared imaging, facilitates bloodstain pattern analysis and the selection of relevant traces for DNA-analysis. Both visible and near infrared reflectance spectra show typical absorption properties of blood stain constituents, and can thus be used to discriminate blood from other substances. Subsequently, the age of blood stains can be estimated using these innovative techniques, providing investigators with new information which can be used to determine the moment a crime was committed, or whether certain blood stains are crime related. For more than a century, many research has been devoted to the development of techniques for age estimation of blood stains. However, to our knowledge, none of them have yet been implemented in standard forensic practice. We demonstrated a case example in which we successfully estimated the age of several blood stains. When introduced in criminal investigations, results can be used for incrimination or exclusion purposes and for elucidating criminal events.