Non-contact spectroscopic age determination of bloodstains
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INTRODUCTION
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Accurate determination of the time a crime was committed is one of the holy grails in forensic investigations. However, until now, using bloodstains to determine the time elapsed since the crime was committed is still not possible. From a criminalistic point of view, an accurate estimation of when the crime was committed enables to verify witnesses’ statements, limits the number of suspects and assesses alibis. Despite several attempts and exploration of many technologies over the last century, no method has been materialized into forensic practice.

Techniques that have been explored for bloodstain age determination include: atomic force microscopy, electron spin resonance, oxygen electrodes, high performance liquid chromatography and RNA analysis. All these techniques will be discussed in detail in chapter 1. Two important properties of these techniques, the sensitivity to short term or long term ageing and the applicability on crime scenes are visualized in figure 1. It shows that the topic of this thesis, reflectance spectroscopy, is positioned at the left bottom corner of figure 1. Reflectance spectroscopy is most sensitive for short term ageing, i.e. up to an age of 20 days. Moreover, reflectance spectroscopy is a portable and non-invasive technique. No physical contact with the bloodstain occurs when measuring the reflectance spectrum of the bloodstain. This non-invasiveness property makes reflectance spectroscopy a very interesting technique for age determination of bloodstains.

![Figure 1](image.png)

*Figure 1. Overview of techniques suitable for age determination of bloodstains. Reflectance spectroscopy is a technique to measure the color. Since the color of a bloodstain changes with time from bright red to dark brown, reflectance spectroscopy seems to be the suited technique to relate the changing color to the age of the bloodstain and perhaps to understand the chemical reaction responsible for this color change.*
SCAPE OF THIS THESIS

The scope of this thesis is to use spectral analysis of the reflectance spectrum of a bloodstain over a large spectral window (450 - 800 nm) and to use multi-component fitting algorithm to determine amount of hemoglobin derivatives as a function of the age of the bloodstain. Multi-component fitting analysis of reflectance spectra of tissue is routinely used in medicine to determine the oxygen saturation of blood. Yet this study is the first attempt to analyze the reflectance spectra of a bloodstain with a multi-component fit. This thesis describes the challenges and opportunities of the multi-component fitting of bloodstains.

Chapter 1 shows that reflectance spectroscopy for monitoring ageing bloodstains is not a new idea [1-5] and also not the only technique for determining the age of a bloodstain. Some techniques are based on hemoglobin, others on proteins or RNA. This chapter summarizes an extensive literature search and compares the discussed techniques on sensitivity and applicability on a crime scene.

Chapter 2 describes the first attempt to monitor ageing bloodstains, and reveal a transition of HbO₂ to met-Hb and HC. The spectral analysis is appreciated by Kubelka Munk’s approximation to the transport theory of light. At the end of this chapter, a method is described to use hemoglobin derivatives for age estimation of the bloodstains.

Chapter 3 shows the possibility to use multi-component analysis of bloodstains to discriminate between blood and non-blood samples. The correlation coefficient between the reflectance spectrum and the multi-component fit is an excellent parameter to distinguish blood (high correlation) from non-blood (low correlation).

Chapter 4 shows a quantitative method for analysis of non-contact reflectance spectra. By using an empirical photon path length model the amount of hemoglobin derivatives can be determined quantitatively. The method is validated on phantom measurements.

Chapter 5 shows the use of the method of chapter 4 on bloodstains. The quantitative analysis reveals a biphasic oxidation of the oxy-hemoglobin in bloodstains. The oxidation rate is temperature depended, but humidity independent. The transition of met-Hb to HC is also biphasic, and does depend on humidity.

Chapter 6 shows that the reflectance spectrum as a function of absorption is completely determined by the photon path length distribution at zero absorption. The probe specific photon path length distribution is determined by Monte Carlo simulations.

Chapter 7 describes the phantom measurement from chapter 4 in terms of the diffusion approximation of light. Three approaches are explored for the diffusion theory, two with physical model parameters and one with empirical determined, fitted parameters.