Non-contact spectroscopic age determination of bloodstains

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SUMMARY

Bloodstains are among the most encountered traces on crime scenes. Estimation of the age of a bloodstain can be a first indication for forensic investigators to establish when the crime was committed. Or, if the time of perpetration is evidenced otherwise, bloodstain age determination may either confirm or exclude a bloodstain as being relevant to the crime. Age determination of bloodstain can be measured by reflectance spectroscopy and its forensic relevance is huge. Reflectance spectroscopy is a technique to measure color quantitatively. This technique is routinely used in clinic to determine oxygen saturation in blood, and is being explored to differentiate between healthy tissue and tumors. Already in the 1960s this technique was suggested as a candidate for measuring the age of a bloodstain. So far, analysis of reflectance measurements has been limited to a single wavelength. However, the key to success of this thesis proved to be analyzing the reflectance spectrum over a large spectral window (450-800 nm) simultaneously. Large spectral window analysis of the reflectance spectrum of a bloodstain allows for determination of relative amounts of hemoglobin derivatives: oxy-hemoglobin (HbO_2), met-hemoglobin (met-Hb) and hemichrome (HC) in bloodstains. Accordingly, these hemoglobin amounts can be related to the age of the bloodstain.

Chapter 1 summarizes an extensive literature search and describes research on age determination of bloodstains in general. This chapter starts with an overview of the physical and chemical properties of red and white blood cells during the ageing process in a bloodstain. The main part is a summary of all the techniques that have been explored to determine the age of a bloodstain. For instance, with atomic force microscopy an increase in elasticity of the red blood cell after deposition has been observed. Another technique, Electron Spin Resonance has been used to map the magnetic properties of hemoglobin in bloodstains during ageing. And RNA analysis has successfully been explored to measure changes in RNA proteins, up to 15 years after deposition of the bloodstain. All these techniques are compared with reflectance spectroscopy, which measured changing color of a bloodstain. The color of a bloodstain changes because of an oxidation process of the hemoglobin present. It is found that reflectance spectroscopy is most accurate, especially for bloodstains up to one month old. Additional advantage of reflectance spectroscopy is the non-destructive nature of this technique and, as a result, its great applicability on the crime scene.

Chapter 2 describes reflectance spectroscopic measurements on 40 bloodstains by analyzing the reflectance spectrum over the spectral range of 450-800 nm simultaneously. The relative amounts of the hemoglobin derivatives are determined by comparing the reflectance of a bloodstain with the spectra of HbO_2, met-Hb and HC with a non-linear least square fit. Intially in a bloodstain, all hemoglobin is HbO_2, but thereafter $HbO_2 \rightarrow$ met-Hb $\rightarrow$ HC. This transition has been determined for 20 bloodstains on cotton at room temperature during 60 days. The determined transition rate is employed for age determination on a second group of 20 bloodstains. Age

determination is possible: for one-day-old bloodstains with accuracy of a few hours; for ten-days-old bloodstains with accuracy of a few days.

An important step in age determination of bloodstains proved a quality check of the linear squares fit algorithm. In case of low agreement between reflectance of the bloodstain and hemoglobin fit, the outcome of the fit will become unreliable. The amount of agreement can be expressed in a correlation coefficient, r^2. The minimal correlation for a reliable fit analysis has (arbitrarily) been determined to be $r^2=0.98$. In **chapter 3**, the correlation coefficient is used to discriminate bloodstains from non-bloodstains. The reflectance spectrum of 35 blood-mimicking stains have been measured, including, wine, ketchup and fake blood. All 35 stains had an r^2 lower than 0.97, while 98% of 2000 measurements on 40 bloodstain showed an r^2 higher than 0.97. Fitting of hemoglobin spectra to the reflectance of bloodstains appears to be an almost perfect method to discriminate blood from non-blood on white cotton. A remarkable result was found that, from all non-blood stains, the stain created with lip gloss from a local pharmacy showed the highest correlation coefficient.

In chapter 2, the Kubelka Munk model for light transport has been used to translate absorption spectra into reflectance spectra. It appeared impossible to relate Kubelka Munk's parameters to the optical properties, when measuring on phantoms. The relation between optical properties – scattering and absorption – is necessary for quantitative reflectance measurements. For that reason, the effective photon path length approach is explored in **chapter 4**. It proved possible to determine the relation between reflectance and absorption could be determined for a large range of scattering and absorption coefficients by measuring the reflectance signal on phantoms containing a controlled amount of water, fat and a chromophore. The wide range of absorption and scattering coefficients represents the large variety of optical properties in bloodstains. It was found that the effective photon path length decreases with absorption, whereas it remains constant with scattering, if scattering is high – which is the case for cotton. At the end of this chapter it is shown that the effective photon path length model can also been applied for stains on cotton.

Chapter 5 describes that the effective photon path length model can also be used for measuring the blood volume fraction of diluted bloodstains on cotton. Additional evidence for the effective photon path length approach is given by optical coherence tomography analysis at 1300 nm, a non-absorbing wavelength. When analyzing the reflectance spectrum of a bloodstain in terms of the hemoglobin derivatives, it is observed that the transition of HbO_2 into met-Hb is biphasic. Initially, the transition rate is high, but after a few hours, the rate drops dramatically. The biphasic nature of the transition was already observed for hemoglobin in an aqueous solution by Tsuruga *et al.*, but never before in bloodstains. Additionally, the transition rate as a function of temperature and humidity has been measured. The transition rate strongly depends on temperature, but only partly so on humidity. The first part of the transition, HbO_2 → met-Hb is humidity independent, but, on the contrary, the second part met-Hb

→ HC depends on humidity. The discovery of a humidity independent transition of HbO$_2$ to met-Hb is important for translating laboratory results into forensic practice. Lack of information on the humidity on the crime scene needs no longer hamper age determination of the bloodstain.

At the end of this thesis an analysis of the reflectance spectrum of blood mimicking phantoms is presented in great detail. In **chapter 6**, the photon transport has been simulated by Monte Carlo computations. It was found that the reflectance signal as a function of absorption is completely determined by the photon path length distribution at zero absorption. In **chapter 7**, the reflectance signal is analyzed by the diffusion approximation. It was possible to describe phantom measurements, as presented in earlier chapters, by this approximation even for measurements that do not meet the conditions this approximation – yet only when model parameters were fitted.