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Towards substrate-independent age estimation of blood stains based on dimensionality reduction and k-nearest neighbor classification of absorbance spectroscopic data

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ABSTRACT

The age determination of blood traces provides important hints for the chronological assessment of criminal events and their reconstruction. Current methods are often expensive, involve significant experimental complexity and often fail to perform when being applied to aged blood samples taken from different substrates. In this work an absorption spectroscopy-based blood stain age estimation method is presented, which utilizes 400–640 nm absorption spectra in computation. Spectral data from 72 differently aged pig blood stains (2 h to three weeks) dried on three different substrate surfaces (cotton, polyester and glass) were acquired and the turnover-time correlations were utilized to develop a straightforward age estimation scheme. More precisely, data processing includes data dimensionality reduction, upon which classic k-nearest neighbor classifiers are employed. This strategy shows good agreement between observed and predicted blood stain age ($r > 0.9$) in cross-validation. The presented estimation strategy utilizes spectral data from dissolved blood samples to bypass spectral artifacts which are well known to interfere with other spectral methods such as reflection spectroscopy. Results indicate that age estimations can be drawn from such absorbance spectroscopic data independent from substrate the blood dried on. Since data in this study was acquired under laboratory conditions, future work has to consider perturbing environmental conditions in order to assess real-life applicability.

1. Introduction

The investigation of crimes is as old as crime itself. While perpetrators develop increasingly sophisticated schemes on the one hand, numerous methods originated from the fight against felony on the other hand. Today all scientific and technical aspects of crime investigation, such as ballistics, toxicology and parts of psychiatry as well as psychology, are being covered by the field of “forensic science”. One well studied, old and yet still evolving forensic application is the analyses of blood patterns. By examining the size and shape of blood trails, specialists gather important information about the position and motion of suspects and victims. Moreover, the complex composition of blood can also be analysed to gain information about stain age. Since the early 20th century various techniques have been established to estimate the age of blood stains in order to make knowledge-based statements regarding the circumstances of a crime. Furthermore, the age estimation of blood samples can be applied to analyse multiple blood traces found on a crime scene with respect to their time of origin. As a result, such analyses provide the means to identify non-relevant traces which can then be excluded from the investigation process. In this study a new computer-aided approach to address the problem of blood age estimation is presented. The authors show that a combination of dimensionality reduction and statistical evaluation of obtained time-related features can lead to a good estimation accuracy within a time range of three weeks.

Numerous methods to estimate the age of blood stains were published until today. Unfortunately there is still no “standard method” due to the practical limitations or accuracy of the publicized methods. The foundation of scientific blood age estimation was laid by Ziemke in 1901, who studied the different absorption bands of hemoglobin derivatives [1]. Six years later Tomellini designed a colour scale to contrast different blood age stages [2]. Leers then postulated a significant change in the visible spectral range of blood caused by hemoglobin turnover over time [3]. A coherence between the solubility of blood in water and its age *ex vivo* was first identified by Schwarzacher [4], where a

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decrease in solubility with increasing stain age was demonstrated. The first spectrometry-based study of blood was presented by Patterson who measured the CIE chromaticity coordinate of blood stains on filter paper by means of colorimetry. This method was refined by Kleihauer et al. [5,6]. Until this time different substrate concentrations and additives still caused varying and incomparable results. Addressing this problem Kind et al. presented and discussed the so-called α-*ratio* as a time descriptive feature, which corresponds to the ratio of the α-peak (at 576 nm) and the absorbance plateau between the α- and β-peak [7].

Current methods for blood age estimation can be distinguished by the considered blood compounds (erythrocytes, leukocytes and blood plasma). There is one approach which utilizes the change of the mRNA/tRNA ratio in white blood cells [8] to calculate stain age within a specified time window starting from a month since deposition. Furthermore different constituents of the blood serum can be used as the base to estimate blood age. Common approaches are the measurement of the α, β, and γ globulin concentrations [9], the degradation rate of aspartic acid racemization [10], and the activity of different enzymes, such as lactate dehydrogenase or glutamate-oxaloacetate transaminase [11]. Not a specific age but the time of day when the trace originated can be calculated through the ratio of the hormones melatonin and cortisol [12].

Currently the most widely applied age estimation approaches use the time-correlated alteration of the red blood cells (erythrocytes). Methods established to this day are high performance liquid chromatography [13], electron spin resonance spectroscopy [14], Clark electrode measurements of the oxygen concentration [15], bioaffinity-based assays [16] and atomic force microscopy [17]. In addition, many scientists rely on spectroscopy- or spectrometry-based techniques. Some works have focused on the infrared- [18,19] and the visible light range. The latter was examined in a wide spectral range by Bremmer et al. and [20] and Li et al. [21] and in sub-ranges in order to investigate shifts of characteristic spectral absorbance peaks. These are the oxygen peak [7] and the Soret peak [22] associated with hemoglobin derivatives. In 2012 Bremmer et al. compared the most noted methods regarding their estimation inaccuracy and their flexibility with respect to prediction parameters [23]. They showed that every single approach is only reliable in a certain parameter range and that the inaccuracies of all investigated methods are quite high with increasing blood age. A novel orthogonal approach is to digitally process the information from examined blood patterns. Thanakiatkrai et al. published a portable and rapid estimation method based on the utilization of multiple smartphone cameras in order to record color values from digital images and produce assertions about blood age with an error rate of 12% within 42 days by means of Random Forest classifiers [24].

However, reflection spectroscopic sample analyses based on other substrates than absorbing white substrates and the crucial influence of different surfaces is rarely discussed in related literature. A promising approach provided Edelman et al. who utilized near infrared spectral features for an estimation method which is independent from the color of the cotton samples [19]. In a recent practical evaluation of the color independence of blood samples green and blue backgrounds as well as non-homogenic blood stains and different stain thickness cause estimation errors due to unpredictable reflectance behavior [25]. Beside that this approach shows the strengths of spectral features on the field of substrate independence which are just limited by the spectroscopic data acquisition itself.

In order to address this problem the authors present a statistical approach to evaluate spectral changes of blood stains over time associated to *ex vivo* denaturation of hemoglobin. Based on the preprocessed absorbance spectra from acquired data, a set of feature-based classifiers are demonstrated for blood-age estimation. The aim of this study is to investigate the potential to reach independence from substrate by considering spectra of dissolved blood samples as input data.

In addition, by using only a minimal number of chemicals, the proposed experimental setup is aimed towards efficient and flexible utilization at crime scenes. In fact only distilled water is required.

In the following sections a brief summary about the role and structure of hemoglobin as well as its observable chemical turnover processes *in vivo* and *ex vivo* are provided; both are of great importance to time-correlated spectral changes in aging blood. Further, experimental setup and data acquisition processes are described, followed by elucidations on computational statistical analyses. A prediction scheme for spectra-based blood age estimation is proposed followed by a demonstration and discussion of its predictive power. Concluding thoughts with respect to prospects on future work are finally given.

2. Background

One of the major roles of blood is to ensure oxygen supply to living cells. The transport of oxygen is carried out by hemoglobin, a chromoprotein located on the cell surface of the red blood bodies (erythrocytes). Each hemoglobin molecule consists of two α and two β polypeptide chains forming four symmetrically arranged globular subunits. The oxygen fixation of hemoglobin is realized by an iron-II-complex, the prosthetic heme group. Four of these complexes are non-covalently bound to the four hemoglobin subunits. An illustration of the hemoglobin molecule and bound heme groups is shown in Fig. 1. Non-covalent oxygen binding is

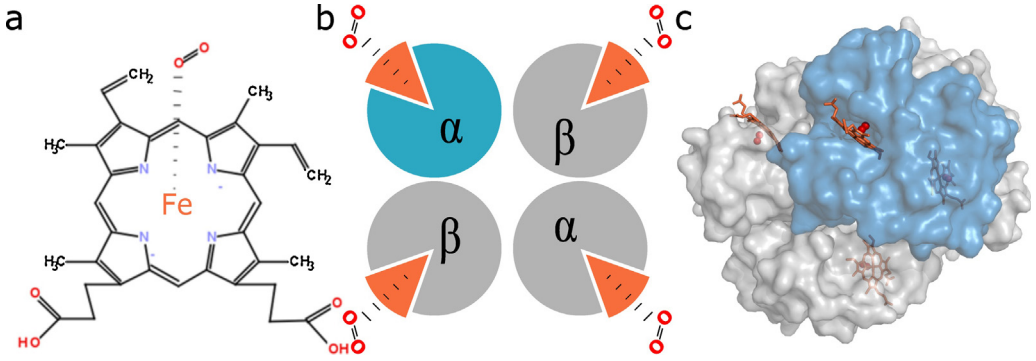

Fig. 1. Structure of the heme group and hemoglobin. (a) Skeletal formula of a heme group consisting of a porphyrin and a central iron atom loaded with an oxygen molecule. (b) Schematic representation of the hemoglobin subunit arrangement. Four heme groups (depicted as orange segments) are bound to four peptide chains (two α and two β chains) comprising the hemoglobin molecule. (c) Experimental structure of hemoglobin (PDB Id: 2h35) with four bound heme groups (shown as sticks). Color highlighting is analogous to (b). One of the peptide chains in (b) and (c) is highlighted in blue for visual clarity.