Forensic Science International 233 (2013) 288–297

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Age estimation of bloodstains using smartphones and digital image analysis

Phuvadol Thanakiatkrai *, Alisa Yaodam, Thitika Kitpipit

Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

Article history:
Received 27 July 2013
Received in revised form 26 September 2013
Accepted 30 September 2013
Available online 9 October 2013

Keywords:
Forensic science
Hemoglobin
Color analysis
Digital image analysis
Random Forests

ABSTRACT

Recent studies on bloodstains have focused on determining the time since deposition of bloodstains, which can provide useful temporal information to forensic investigations. This study is the first to use smartphone cameras in combination with a truly low-cost illumination system as a tool to estimate the age of bloodstains. Bloodstains were deposited on various substrates and photographed with a smartphone camera. Three smartphones (Samsung Galaxy S Plus, Apple iPhone 4, and Apple iPad 2) were compared. The environmental effects – temperature, humidity, light exposure, and anticoagulant – on the bloodstain age estimation process were explored. The color values from the digital images were extracted and correlated with time since deposition. Magenta had the highest correlation ($R^2 = 0.966$) and was used in subsequent experiments. The Samsung Galaxy S Plus was the most suitable smartphone as its magenta decreased exponentially with increasing time and had highest repeatability (low variation within and between pictures). The quantifiable color change observed is consistent with well-established hemoglobin denaturation process. Using a statistical classification technique called Random Forests™, we could predict bloodstain age accurately up to 42 days with an error rate of 12%. Additionally, the age of forty blind stains were all correctly predicted, and 83% of mock casework samples were correctly classified. No within- and between-person variations were observed ($p > 0.05$), while smartphone camera, temperature, humidity, and substrate color influenced the age determination process in different ways. Our technique provides a cheap, rapid, easy-to-use, and truly portable alternative to more complicated analysis using specialized equipment, e.g. spectroscopy and HPLC. No training is necessary with our method, and we envision a smartphone application that could take user inputs of environmental factors and provide an accurate estimate of bloodstain age.

1. Introduction

Blood is commonly found in violent crimes such as homicides and assaults. Forensic scientists can acquire diverse information from bloodstains, e.g. sequence of events using blood pattern analysis and DNA profiles for individualization. Knowing the time since deposition of bloodstains can provide additional information to the investigators, as this can corroborate an eyewitness's account; narrow the time window for a missing person inquiry, kidnapping, and crimes without any eyewitness; and exclude bloodstains that are irrelevant to the crime.

Bloodstain age estimation dates back to over 50 years ago, and was based on spectrophotometric observation of bloodstain's color change from red to dark brown [1]. Since then researchers have tried numerous techniques to estimate time since deposition of bloodstains, including RNA analysis [2], high performance liquid chromatography [3], force spectroscopy [4], near-infrared spectroscopy [5], UV–vis spectroscopy [6,7], reflectance spectroscopy [8,9], and hyperspectral imaging [10,11]. Despite the revival of research interest in this area in recent years, estimating bloodstain age has not been implemented in routine crime scene investigations. The problem lies in the complex procedures requiring specialist knowledge, low accuracy and precision, and sophisticated, expensive machines. Spectroscopy-based techniques and hyperspectral imaging are the closest to being implemented due to their widespread availability and portability [7].

Once blood leaves the body, hemoglobin (the iron-containing oxygen transport protein in red blood cells) undergoes a non-reversible decay process: (1) rapid saturation of oxy- and deoxy-hemoglobin with oxygen in the atmosphere to form oxy-hemoglobin (2) oxy-hemoglobin auto-oxidizes into met-hemoglobin in the absence of cytochrome *b5* reductase (3) met-hemoglobin then denatures into hemichrome [12]. This is accompanied by the change of color from red to dark brown. The rate of change depends on environmental factors such as exposure to light, temperature,

* Corresponding author. Tel.: +66 74288565; fax: +66 74446681.
E-mail addresses: pthanakiatkrai@gmail.com, phuvadol.t@psu.ac.th (P. Thanakiatkrai).

0379-0738/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2013.09.027

and humidity [12,13]. Since this change is temporally dependent, the amount of these derivatives has been used for estimating time since deposition of bloodstains [9–11].

As the phenomenon is observable with the naked eyes, we expected the color change to be quantitatively detectable in a digital image of the bloodstains. A digital image is formed when an image sensor converts reflected light that has passes through three color filters – red, green, and blue (RGB) – into digital signals. The intensity of the RGB values determines the final color of each pixel (the smallest element in a display device). We hypothesized that these values and their counterparts, e.g. cyan, magenta, yellow, and key (CMYK), can be plotted against time since deposition to generate a calibration curve; subsequently, the time since deposition of unknown samples can be determined by comparing their color values to the calibration curve. A similar process has been used to correlate the color change of chicory to storage time [14], as well as to determine the concentration of amphetamine, methylamphetamine [15], and trinitrotoluene (TNT) [16,17].

In this study, we used digital image analysis of bloodstains to estimate the time since deposition and evaluated the effects of smartphone camera, person-to-person variation, temperature, humidity, light exposure, anticoagulant, and substrate on the estimation process. Our proposed technique requires only a digital camera and a computer, both of which are readily available in any forensic laboratory. A smartphone application can be developed to carry out the technique proposed at crime scenes, making this method low-cost, simple, rapid, and truly portable.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from four volunteers to assess person-to-person variation and blind test and only from one volunteer for all other experiments (as recommended by Bremmer et al. [13]) using procedures approved by the Prince of Songkla University Ethical Committee (ethical approval no. 56-293-19-2). Informed written consents were obtained from all volunteers. Three female and one male volunteers donated blood. All volunteers were Asian, healthy, non-smoker, and ate a normal diet. None of the females were menstruating at the time of blood collection. The mean age was 23.5 ± 2.4 years. Venous blood was collected from venipuncture into an additive-free microcentrifuge tube.

All bloodstains were made with 50 μl of blood and kept in the dark at 25 °C except for the light exposure study, temperature study, and mock casework test. The time between blood collection and deposition onto substrates was less than 30 s. Five bloodstains were deposited for each study unless otherwise stated:

- Color value selection and within-/between-person variation: bloodstains from four individuals on filter paper.
- Smartphone: bloodstains on filter paper taken with three smartphone cameras (iPhone 4, iPad 2, and Samsung Galaxy S Plus).
- Temperature: bloodstains on filter paper kept at −20 °C, 4 °C and 25 °C.
- Humidity: bloodstains on filter paper at room temperature with 30% and 50% relative humidity.
- Light exposure: bloodstains on filter paper kept under direct sunlight, fluorescent lamp, and in the dark.
- Anticoagulant: blood samples collected in 1.5 mg ethylene diamine tetra acetic acid (EDTA), 0.2 mg of heparin, and no anticoagulant on filter paper.
- Substrate: bloodstains on denim, filter paper, glass, gypsum board, leather, and white cotton.
- Blind test: 40 bloodstains deposited on filter paper. Four stains were randomly chosen, assigned a random five-digit identifier code, and frozen at −80 °C at 15 min, 30 min, 1 h, 6 h, 1 day, 3 days, 7 days, 14 days, 28 days, and 42 days.
- Mock casework: 24 bloodstains deposited randomly on household objects – white A4 paper, gray stone slab floor, white plastic dish, white sneakers, white t-shirt, and cream leather handbag. Temperature and humidity were not controlled in this study. Eight samples were randomly chosen and frozen at −80 °C at the age of less than one day, another eight between one day and one week, and the last eight between one week and one month.

2.2. Photographic system

We set up a simple, low-cost photographic system consisted of a white foam light box (2666 cm^2 inner surface area) illuminated evenly with a Sylvania Osram DULUX S 9-Watt Cool White bulb (G32-2 pin base, 600 lumens, 4100K color temperature, Sylvania Osram, Thailand). Fig. 1 shows a schematic drawing of the light box. The fluorescent lamp output all wavelengths of the visible spectrum, from 350 nm to 750 nm [18]. On top of the box was a hole just big enough for a smartphone camera lens to fit.

A Samsung Galaxy S Plus was used to capture digital images (five images per stain) of the bloodstains in all studies except in the smartphone study. Five photographs were taken from each stain to account for variations in precision due to experimental errors, such as inhomogeneous lighting and flickering of the fluorescent lamp. Images were taken at 15 min, 30 min, 1 h, 3 h, 6 h, 12 h, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 2 months, 3 months, 4 months, 5 months, and 6 months. For blind testing, we photographed all the previously frozen stains on the 42nd day. All settings were set to automatic (white balance, ISO, focusing mode, and metering mode). The file type selected was 24-bit JPEG and the resolution was 2592 × 1944, 2592 × 1936, and 960 × 720 pixels for the Samsung Galaxy S Plus, iPhone 4, and iPad 2, respectively. The color intensity in four color models (RGB, CMYK, HSV, HSL) of each image were extracted using an ImageJ macro (http://imagej.nih.gov) that we developed. The macro randomly selected ten pixels of the bloodstain and averaged their color values. The color values were then exported to R statistical program (http://cran.r-project.org) for further analysis.

2.3. Statistical analysis

We transformed the time since deposition using base-10 logarithm to linearize the relationship between color values and time (in hours). Outliers were discarded prior to further statistical analysis. We then applied a linear regression to each color value and time and determine the correlation coefficient of each relationship. This was done to determine the best predictor for time since deposition. To estimate the effects of different donors, we used linear mixed modeling appropriate to the data collected.

For the smartphone, temperature, humidity, light exposure, and anticoagulant studies, we plotted the average (calculated from all photographs of all bloodstains unless stated otherwise) and 95% bootstrapped confidence interval of the magenta value at each time-point and fitted a local polynomial regression (LOESS) to reflect the biphasic change of hemoglobin derivatives. We concluded a significant difference when there was no overlap between the bootstrapped confidence intervals of the fit.

To determine the prediction accuracy of the method, we split the bloodstains of the person-to-person experiment into a training set (70% of data) and validation set (30% of data). Calibration curves were constructed using the data from the training set. The magenta values of bloodstains validation set were fitted to the training set calibration curve and their time since depositions were estimated. Moreover, we applied a machine learning process called Random Forests™ to predict time since deposition. The method constructed many decision trees (i.e. forest) from the bootstrapped samples of the training set (2/3 of the data) and used these forests to classify unknown samples in the validation set (1/3 of the data) [19]. In contrast to the calibration curve method, the Random Forests™ classification algorithm used more than one color values measured from the bloodstain images to classify their time since deposition. The out-of-bag (OOB) estimate of error rate represented the prediction error [19].

To further test the prediction accuracy of the Random Forests™ model built from the person-to-person variation study, we performed additional blind tests with 40 bloodstain samples and 24 mock casework samples. The actual ages of the samples and the estimated ages using the Random Forests™ model were compared to determine the prediction accuracy and estimation error. For the blind samples, the time-points used were the same with the person-to-person variation study. For the

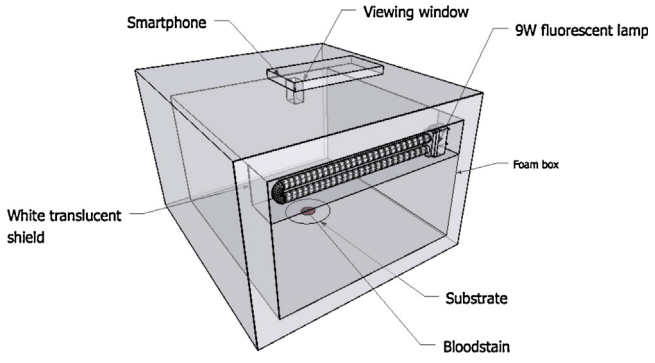

Fig. 1. 3D sketch of the photographic system used in this study. A Sylvania Osram DULUX S 9-Watt Cool White bulb was used to create an even illumination in the light box. Photographs were taken by placing a smartphone camera on the viewing window.