Neural Network based Hyperspectral imaging for substrate independent bloodstain age estimation

Nicola Giulietti[a], Silvia Discepolo[b], Paolo Castellini[b], Milena Martarelli[b]

[a]*Department of Mechanical Engineering, Politecnico di Milano, Via La Masa 1, Milan, 60131, Italy*
[b]*Department of Industrial Engineering and Mathematical Science, Università Politecnica delle Marche, Via Brecce Bianche 12, Ancona, 20156, Italy, Country Two*

Abstract

Being able to determine the age of a bloodstain can be a key element in a crime scene investigation. Many techniques exploit reflectance spectroscopy because it is very versatile and can be used in the field with ease. However, there are no methods for estimating bloodstain age with adequate uncertainty, and the problem of substrate influence is not yet fully resolved. We develop a hyperspectral imaging based technique for the substrate-independent age estimation of a bloodstain. Once the hyperspectral image is acquired, a neural network model recognizes the pixels belonging to the bloodstain. The reflectance spectra belonging to the bloodstain are then processed by an artificial intelligence model that removes the effect of the substrate on the bloodstain and then estimates its age. The method is trained on bloodstains deposited on 9 different substrates over a time period of 0-385 hours obtaining an absolute mean error of 6.9 hours over the period considered. Within two days of age, the method achieves a mean absolute error of 1.1 hours. The method is finally tested on a new material (i.e., red cardboard) never used to test or validate the neural network models. Also in this case the bloodstain age is identified with the same accuracy.

Keywords: Bloodstain age measurement, Neural Networks, Hyperspectral imaging, reflectance spectra measurement

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Keywords: Bloodstain age measurement, Neural Networks, Hyperspectral imaging, reflectance spectra measurement

1. Introduction

Identifying blood traces and estimating its age over a generic substrate is becoming increasingly relevant in forensics for reliable crime scene reconstruction. The first necessary step in forensic practice is to discriminate blood from other liquid substances or materials that could be confused in terms of color and texture. In this case, the most used techniques are the chemical ones, such as the tetrabase test and the Kastle-Meyer method, that have the drawback of contaminating the bloodstain itself with the consequent modification of its color, fluorescence or luminescence [1, 2]. With new emerging techniques, like RNA degradation, HPLC, EPR, oxygen electrode and spectroscopy, it is possible also to estimate bloodstain deposition age. These techniques have revealed that the physical and chemical components that characterize blood undergo changes with the course of time [3, 4, 5]. Although these techniques have a high level of accuracy, they have a limitation in that they require a long time for sample preparation and laboratory analysis [6]. Recently, optical methods based on the analysis of the bloodstains spectra (e.g., reflectance spectroscopy, hyperspectral imaging, ...) have become widespread. These techniques, besides being rapid, are non-contact and therefore allow immediate in-situ analysis [2, 7].

The bloodstain spectra is the expression of its main chromophore, the hemoglobin. The component of hemoglobin saturated with oxygen (HbO_2) in contact with the external environment oxidizes into met−Hb (met-hemoglobin) and subsequently denatures into HC (hemichrome) [8, 4, 5]. HbO_2, met−Hb, and HC are the derivatives of hemoglobin and their respective changes generate the hemoglobin reaction kinetics [4]. The bloodstain absorbance spectra is featured by specific absorption bands at 415 nm, 542 nm, and 576 nm known as the Soret peak, the β and α peaks, respectively [6, 2]. The temporal variation that these peaks undergo is correlated to the progressive variation of hemoglobin fractions. In fact, as the age of blood increases, the β and α peaks decrease and appears a new peak at 630 nm, corresponding to met−Hb absorption [3, 9].

To estimate the bloodstain age, many studies trace back to the variation that hemoglobin derivative fractions undergo as the time passes, other research refers to the specific wavelengths shift. Bremmer et al. [4] estimates the bloodstain age based on the evolution of hemoglobin fractions. HbO_2, met−Hb, and HC fractions are determined by Linear Least Square (LLS) fitting algorithm. This study is conducted on a white substrate (i.e., white cotton), exploiting a Visible and Near-Infrared (VNIR) system using a tungsten-halogen illumination setup, analysing bloodstain reflectance signal between 450 and 800 nm for a time period of 60 days. As result, at 3 days the estimated ages vary between 1.5 and 6 days; at 12 days, the evaluated age ranges from 6 and 16 days; and at 35 days the estimated age is within 25 and 55 days. Edelman et al [8] employ a model similar to Ref. [4]. Using a Non-Linear Least Squares fitting it estimates the fractions of hemoglobin present in the bloodstain deposited on a white cotton. The study is performed in controlled environment exploiting a hyperspectral camera operating in the visible and near infrared wavelength range and analysing bloodstain reflectance signal between 500 and 800 nm for a time period of 200 days. A halogen broadband white lamp is used as light source. The absolute error of the estimated age increases as the time progresses, with a relative error of 13.4 % of the true age. At 2 days, the Mean Absolute Error (MAE) is 2.7 days, at 15 days MAE is of 4.3 days, at 200 days MAE is of 2.1 days. Li et al. [6], using a Linear Discriminant Analysis (LDA), predict blood age analysing the evolution of the reflectance spectra at specific wavelengths. The bloodstain is deposited on a white paper and it is examined by a hyperspectral camera operating in the visible range. The bloodstain reflectance signal is analysed between 505 - 600 nm for a time period of 30 days under controlled conditions. The accuracy of this method is very high in the early period, with MAE of 0.27 days for the first 7 days. The accuracy of this method decreases after 14 days with a MAE of 1.62 days between 15 and 30 days.

All the methods described above have proven to be very accurate in estimating the age of blood, but they have only been applied to cooperative substrates (i.e., white paper and white cotton) and under controlled conditions, far from what might be a crime scene. The absorption capacity of a substrate can have a significant impact on the quality of data in HSI. When a substrate has a high absorption capacity at certain wavelengths, it means that the substrate absorbs more light at those wavelengths, reducing the amount of light available to be reflected back to the HSI sensor. This can result in a lower signal-to-noise ratio and reduced spectral contrast between different materials or substances, making them more difficult to be detected and distinguished. Some recent works have addressed the issue of the influence of the substrate where the bloodstain is deposited on the blood spectra. Bergmann et al. [9] proposed an absorption spectroscopy-based method for predicting the age of bloodstains regardless of the substrate in which they were deposited. This approach is based on the use of absorbance spectra acquired from the bloodstain dissolved with distilled water. The bloodstain is deposited on three different substrates (cotton, polyester and glass) and is examined by a spectrophotometer operating in the visible range between 400 - 640 nm for a time period of 21 days. A halogen-tungsten lamp is used as light source and test is made under controlled conditions. The data are first reduced through PCA, then are classified through conventional classification methods (i.e., k-nearest neighbor). The MAE within three weeks amounts to

2.19 days. Despite the promising results in all the three different substrates, the method proved to be feasible only in laboratory environments, due to the conditions under which the tests were conducted (i.e., use of distilled water as solvent and controlled environment). Edelman et al. analyze bloodstains in non-cooperative, non-ideal colored substrates (i.e., red, green, blue and dark cotton) by conducting spectroscopic analysis in the infrared range between 800–2778 nm [1]. In fact, when bloodstains are deposited on colored materials, it becomes a difficult challenge to estimate their age in the visible range because of the absorption of visible light by the substrate [8]. In [1], Edelman examined bloodstains for 28 days, and predicts their age regardless of the color of the cotton where the bloodstain was deposited by using a Partial Least Squares (PLS) Regression model generated for each colored background. The age of bloodstains up to 28 days is estimated with a Root Mean Square Error of prediction of 8.9%. However, the method has been applied only on cotton substrates of different colors and it is not straightforward that it can work for different materials. In this paper we propose a neural network based hyperspectral imaging method for the age prediction of substrate-independent bloodstains. The proposed method, starting from the acquisition of a single hyperspectral image, enables the detection of blood traces (i.e., discrimination of blood from other substance stains) and the estimation of their age, regardless of the test environmental conditions and of the substrate where the bloodstain is deposited. The network is trained on hyperspectral images of bloodstains deposited on 9 different substrates different in color and material, having different liquid absorption capacity. The stains have been observed over a time range of 0 to 16 days (i.e., 385 hours). To prove that the method works is any kind of substrate, it has been also applied to a bloodstain deposited on a red cardboard which was never used for either training or neural network model validation.

2. Materials and methods

2.1. Sample preparation

Blood given by a healthy female volunteer is collected in acidic vacutainers and it is deposited with her consent, through a pipette, directly on diverse substrates. As shown in figure 1, bloodstains were deposited on 10 different substrates (i.e., jeans tissue, napkin, white paper, yellow paper, white tile, green sponge, red fabric, cardboard and wood for training phase and red cardboard for testing phase), following the same procedure described in detail in Ref [10]. The size of the resulting blood spot on each substrate is kept to approximately one centimetre in diameter. However, due to variations in the absorption of blood in different substrates, it is not always possible to achieve this exact size, although the procedure is performed with the utmost precision. To avoid introducing any potential method-related biases in the process of blood deposition, the use of precise instruments was intentionally avoided in the stain deposition procedure. The substrates chosen differ in material, color, and liquid absorption capacity, and are materials that could be found within a crime scene. The model was trained on all substrates except red cardboard, which was used as a test substrate to measure the model's performance in a substrate not included in the training set. This type of surface, as seen in Ref. [10], is very absorbent, and because of its color greatly distorts the reflectance spectra of the bloodstain (i.e., α and β peaks disappear) and thus constitutes a challenging task for the age estimator (Figure 2).

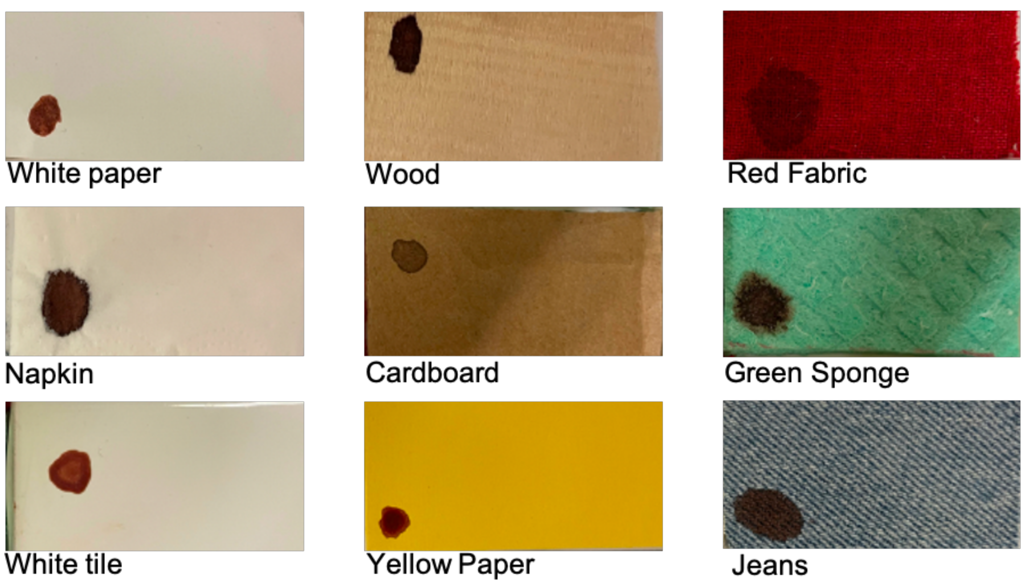

Figure 1: Bloodstain samples on 9 different substrates used for model training.

2.2. Hyperspectral imaging system

Measurement were performed using the Hinalea 4250 (TruTag Technologies, Inc.) hyperspectral camera system, based on front-staring Fabry–Pérot

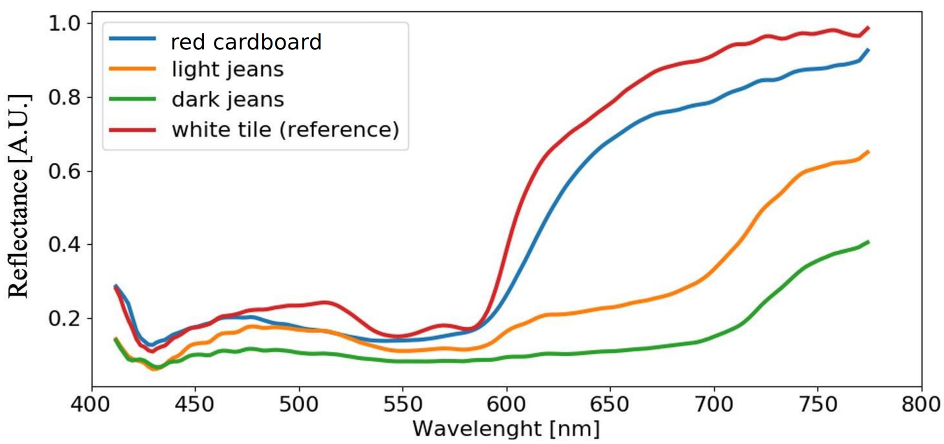

Figure 2: Reflectance spectra comparison on diverse substrates acquired at $t = 0.5$ h. [10]

technology. The camera is equipped with a tunable filter, allowing sequential selection of the visible and near-infrared spectral bands, in the range between 412 nm and 1000 nm, with a step size of 2 nm (i.e., 300 nominal spectral bands). The Hyperspectral camera generates a hyperspectral cube characterized by two spatial dimensions (i.e., sensor height and width) and one spectral dimension (i.e., number of spectral bands).

2.3. Data Collection

The camera was fixed at a height of 30 cm from the sample surface (i.e., 0.06 mm/pixel of spatial resolution). Two halogen lamps, symmetrically mounted on either side of the hyperspectral camera, were used as the lighting system avoiding illumination gradients (Figure 3). This cost-effective and readily available type of illumination provides good light uniformity in the 450-800 nm range and is used in several studies [4, 8, 11, 9].

Bloodstain Hyperspectral image acquisition was conducted for a period of 16 days, with a daily acquisition frequency, except for the first day, when the blood reflectance spectra rapidly changes in relation to its progressive drying. For this reason, on the first day, the acquisition was carried out every hour from hour 1 to hour 8. In total, data were acquired at 1, 2, 3, 4, 5, 6, 7, 8, 24, 25, 26, 29, 31, 48, 50, 98, 123, 149, 169, 223, 288, 312, 363, 385 hours of ageing. The high frequency of acquisition in the early hours aims to capture the dynamic spectral changes due to blood drying. After the first few days,

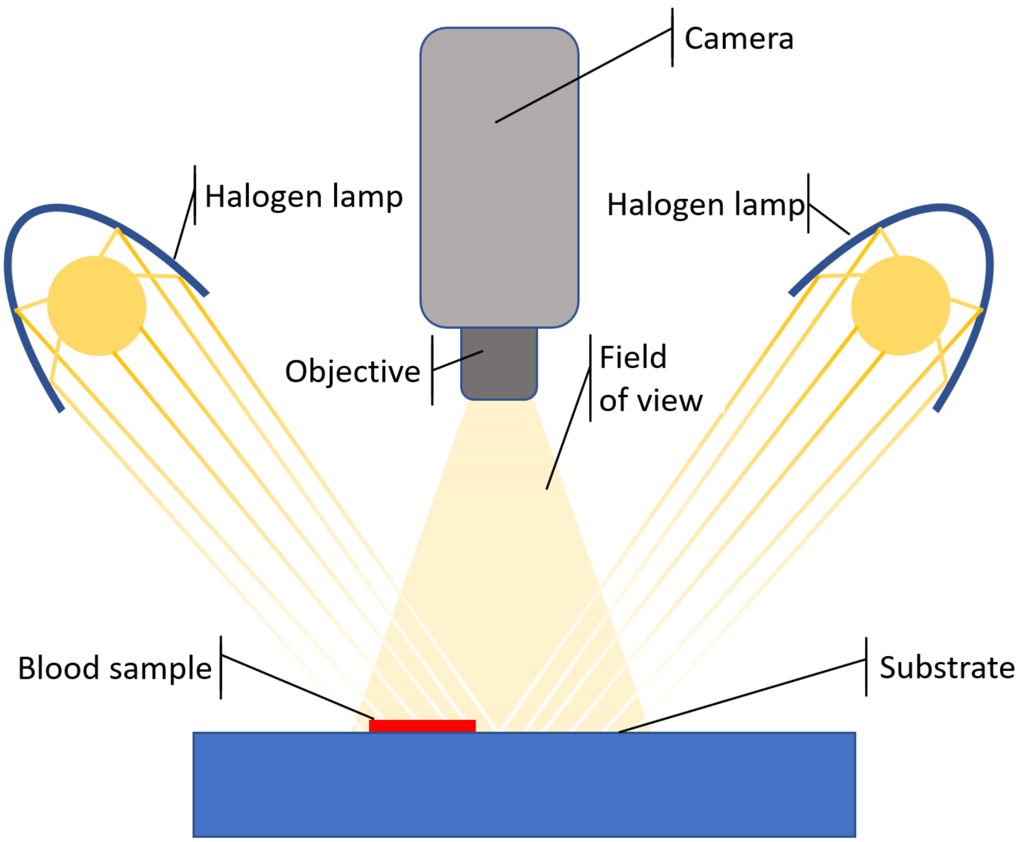

Figure 3: Camera and lighting setup.

however, it is only the effect of oxidation of the blood that dominates, which is a much slower process.

2.4. Data Pre-Processing

Hyperspectral image analysis was limited to the range between 412 - 774 nm (i.e., 185 nominal spectral bands) for two different reasons. First, in this range, all the characteristic peaks of hemoglobin are present; second, as explained in Ref. [10, 8], halogen illumination ensures high signal to noise ratio only in this range. Dark frame and flat field calibration was performed before each acquisition. The procedure developed in Ref. [10] was exploited in order to automatically distinguish bloodstains pixels and to correct the substrate spectral distortion. The acquired reflectance spectra were then corrected by exploiting the Standard Normal Variate (SNV) in order to mitigate spectral distortions and non-linearity due to scattering [6, 8, 4] according to equation 1 such that each spectra has zero mean and unit standard deviation. Spectra standardization does not alter its shape and is proven to be effective as a data pre-processing technique for training regression models [12, 13].

$$\bar{R}_{\lambda s} = \frac{\bar{R}_{\lambda} - mean(\bar{R}_{\lambda})}{standard\ deviation(\bar{R}_{\lambda})} \tag{1}$$

2.5. Bloodstain age estimation neural networks model

A Multi Layer Perceptron (MLP) regression model was developed in order to estimate the age of the target bloodstain. The model takes as input the reflectance spectra pre-processed as described in section 2.4 and returns as output the predicted age (Figure 4). The pre-processed bloodstain reflectance spectra collected in 9 substrates at different ages (Section 2.3 and 2.4), split into train, validation and test with a split ratio of 8:1:1, were used for model training.

The Bayesian optimization method explained in Ref. [10, 14, 15] is exploited in order to select the proper number of layers, neuron per layer and model hyper-parameters that minimize the target objective function. Given the nature of the problem, the MAE was chosen as loss function; the formula is given by Eq. 3, where N is the number of total observation, y_i is the ground truth (i.e., actual bloodstain age) of target input x_i (i.e., input bloodstain reflectance spectra) and $p(x_i)$ is the model output (i.e., predicted bloodstain

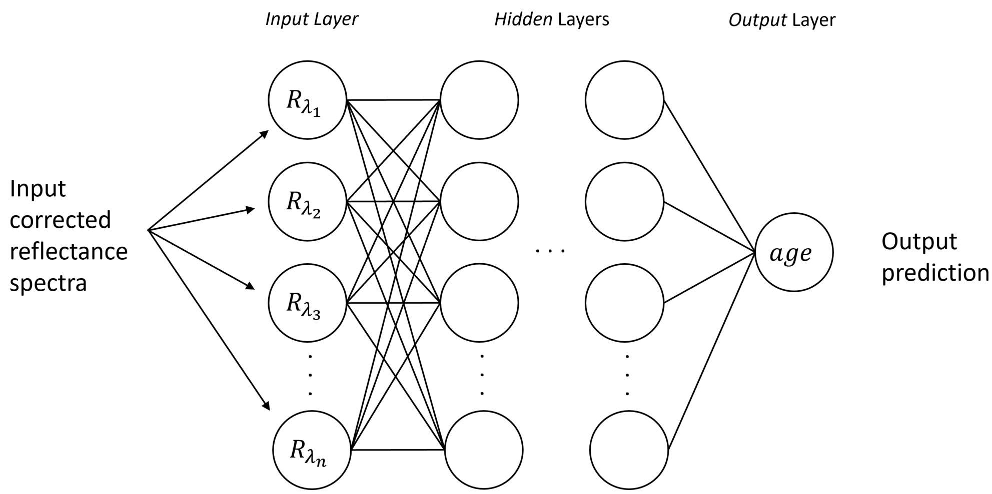

Figure 4: MLP regression model for bloodstain age estimation [10]

age). The objective function to be optimized is given by equation 3. All parameters that were employed in the Bayesian optimization process are shown in equation 2. The parameters and their ranges of variability, chosen based on our prior knowledge of the range of reasonable values for each parameter and the empirical observations obtained from preliminary experiments, are given in Table 1:

- Batch size bs: a hyper-parameter that specifies the number of training samples propagated through the network in one forward/backward pass.

- Learning rate lr: a hyper-parameter that determines how much the model weights are updated in response to the estimated loss during training.

- Number of epochs e: a hyper-parameter that defines the number of times the entire training dataset is passed through the network during training.

- Activation function af: a hyper-parameter that determines the output of a neural network node given its input. The activation function can be chosen from among linear, Sigmoid, ReLU, and Tanh [16].

Table 1: Hyper-parameters involved in the Bayesian Optimization technique and their corresponding bounds

		from	to
bs	Batch Size	16	256
lr	Learning Rate	0.0001	0.1
e	Number of epochs	20	1000
nl	Number of layers	1	12
nn	Neuron per layers	10	400

- Optimizer o: a hyper-parameter that updates the model in response to the loss function during training. The optimizer can be chosen from among SGD, Adam, RMSprop, Adadelta, and Adagrad [17].
- Number of layers nl: a hyper-parameter that specifies the number of layers in the neural network model.
- Number of neurons per layer nn: a hyper-parameter that specifies the number of neurons in each layer of the neural network model.

$$MAE = f(x, y, bs, lr, e, af, o, nl, nn) \tag{2}$$

$$MAE = \frac{\sum_{i=1}^{N} |y_i - p(x_i))|}{N} \tag{3}$$

2.6. Substrate-independent bloodstain age estimation workflow

The proposed procedure workflow for the substrate-independent bloodstain age estimation is reported in Figure 5 and can be described as follows.

a The job starts (1) with the acquisition of the hyperspectral image containing the target bloodstain (2).

b The first MLP model developed in Ref. [10] (4) is applied to all the acquired reflectance spectra in order to perform the autonomous selection of bloodstains reflectance spectra (3).

c The second MLP model developed in Ref. [10] (5) is applied to all selected bloodstains reflectance spectra in order to remove the substrate spectral distortion (6).

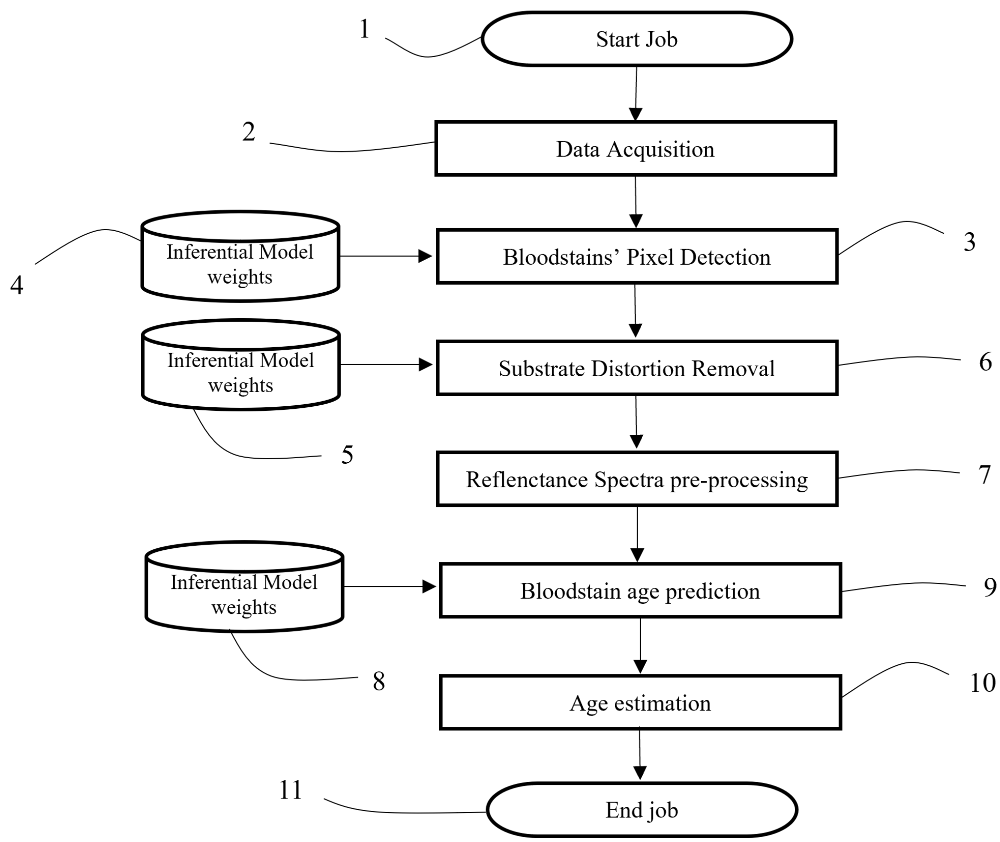

Figure 5: Proposed procedure workflow for the substrate-independent bloodstain age estimation

d The corrected reflectance spectra are pre-processed according to the procedure explained in 2.4 (i.e., SNV) (7).

e The developed MLP model (8) is applied to the pre-processed reflectance spectra obtaining a predicted age for each spectra given as input (9).

f All predicted ages are averaged by removing their outliers and the mean predicted age is obtained (10).

3. Results

3.1. Substrate-independent bloodstain age estimation MLP model training

From the data acquired in section 2.3, 282616 reflectance spectra are collected with known age (i.e., ground truth) and substrate deposition. The whole dataset is divided into train, validation and test dataset according to the scheme 8:1:1, considered effective in most problems with large dataset [18]. Each reflectance spectra is treated as a single observation. These data are used for training the MLP model and the model architecture and hyper-parameters are tuned following the Bayesian optimization technique described in section 2.5. The parameters that minimize the loss function are:

- Batch size $bs = 81$.
- Learning rate $lr = 0.0345$.
- Number of epochs $e = 251$.
- Activation function $af = linear$.
- Optimizer $o = Adadelta$.
- Number of layers $nl = 3$.
- Number of neurons per layers $nn = [152, 70, 15]$.

MAE performance in the training and test set shows no evidence of overfitting. The model is cross-validated with a k-fold validation method (5 folds) obtaining an average MAE of 15.7 hours with a standard deviation of 0.3 hours [19] (i.e., Standard deviation of the training loss obtained in 5 trainings performed with the k-fold cross-validation technique).

3.2. Age prediction on Test dataset

The previously trained model is applied to the test dataset (i.e., the dataset not used in the training phase) following the workflow proposed in section 2.6 and the age of target bloodstains are estimated from 0 to 385 hours. Each bloodstain acquired at different times is represented by a hyperspectral image. Consequently, for a bloodstain acquired at a certain time, there are a number of spectra related to the number of pixels belonging to that bloodstain. The age of the blood is calculated for each spectrum and then averaged over the number of spectra available (i.e. the number of pixels belonging to the bloodstain). This average value represents the age of the blood stain. The standard deviation is also calculated to assess the consistency of the estimated age between the different spectra. Then the MAE is computed with respect to the actual age which is plotted in Figure 6 in relation to the deposition time (actual age) and the different substrates. For completeness also the standard deviation is plotted in relation to the actual age and the substrates (Figure 7).

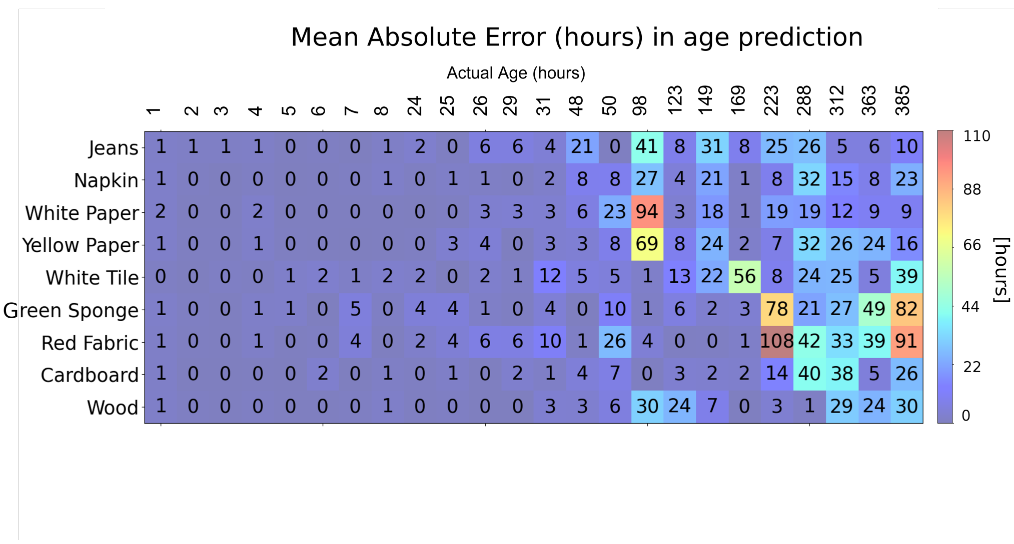

Figure 6: MAE of bloodstain age estimation over actual age on the 9 substrates. The results have been rounded to the nearest integer.

The age estimated for the blood spectra with the same age but belonging to different substrates is averaged and plotted with respect to the actual age in Figure 8. The black dots represents the average value and the vertical lines the standard deviation. The values of MAE and standard deviation are

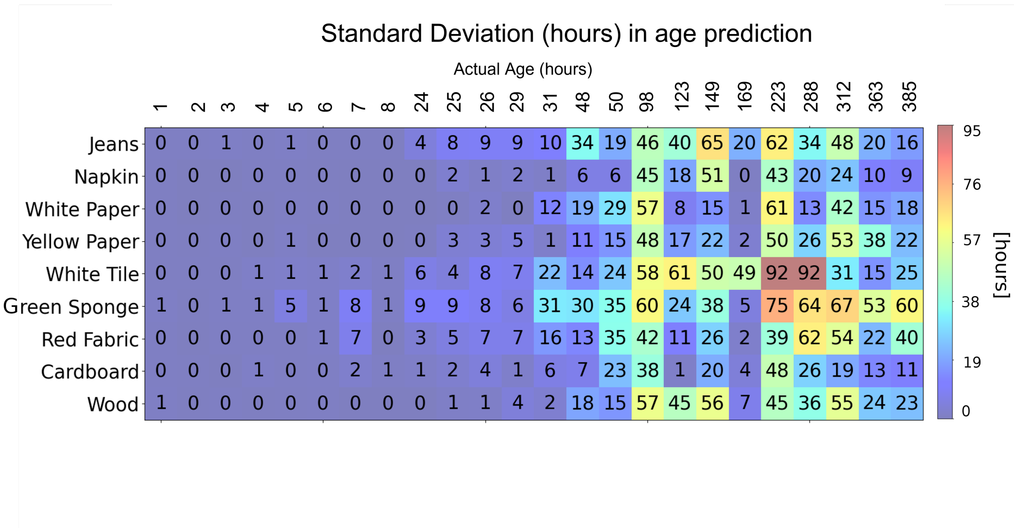

Figure 7: Standard deviation of bloodstain age estimation over actual age on the 9 substrates. The results are reported as integers.

listed in table 2.

3.3. Age prediction on new substrate

The proposed methodology was finally tested on bloodstains deposited on a new substrate, never before seen by the model developed in this work (i.e., red cardboard). The results, in terms of MAE and standard deviation, are reported in Figure 9.

4. Discussion and Conclusions

This paper proposes a new methodology for estimating the age of a bloodstain in a crime scene based on the use of hyperspectral imaging, regardless of the substrate on which the bloodstain was deposited. The method turns out to be fully automatic and devoid of subjectivity: it first identifies the pixels belonging to the bloodstains, pre-processes the corresponding reflectance spectra, and finally performs age prediction thanks to a developed MLP model. The MLP network provides computational speed and can achieve competitive results in bloodstain classification even compared to more modern and complex deep neural networks [20]. This could ensure, for future developments, the possibility of integrating this application into portable

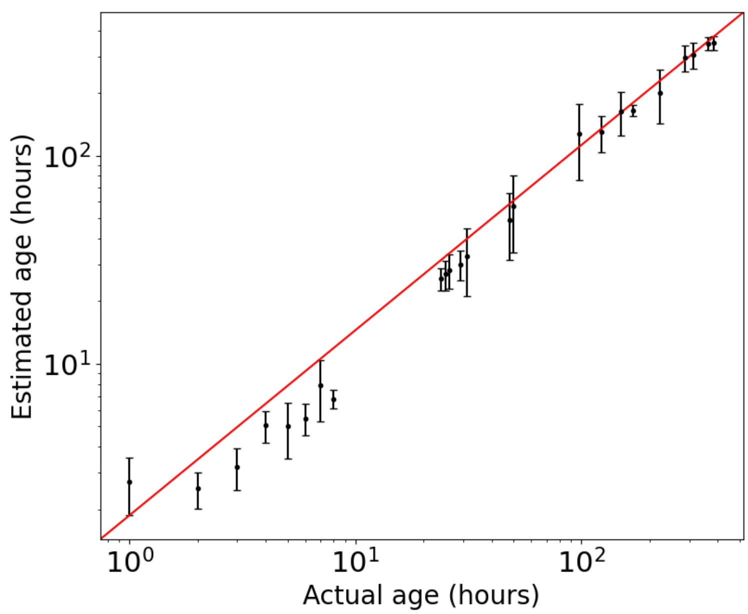

Figure 8: Average estimated age of bloodstains over actual age (black dots). Error bars represent the standard deviation of the estimated age of the blood spectra with the same age but belonging to different substrates. The red line depicts the ideal condition where estimated and actual age are equal.

Mean absolute Error (hours) in age prediction

Actual Age (hours)	1	2	3	4	5	6	7	8	24	25	26	29	31	48	50	98	123	149	169	223	288	312	363	385
Red Cardboard	2	0	0	1	0	0	0	0	0	0	2	3	8	0	3	40	3	15	2	72	17	3	13	26

Standard Deviation (hours) in age prediction

Actual Age (hours)	1	2	3	4	5	6	7	8	24	25	26	29	31	48	50	98	123	149	169	223	288	312	363	385
Red Cardboard	0	0	0	0	0	0	0	0	0	0	1	0	6	4	8	14	1	10	0	25	15	34	11	11

Figure 9: MAE of bloodstain age estimation (top plot) and standard deviation (bottom plot) over actual age. The results have been rounded to the nearest integer.

Table 2: MAE and standard deviation of bloodstain age prediction exploiting proposed workflow at different ageing time

Actual Age (hours)	**MAE (hours)**	**STD (hours)**
1	1.7	0.8
2	0.5	0.5
3	0.2	0.7
4	1.1	0.9
5	0.0	1.5
6	0.5	0.9
7	0.9	2.6
8	1.2	0.7
24	1.6	3.0
25	1.9	4.3
26	2.2	5.2
29	1.1	4.9
31	2.0	11.8
48	0.8	17.3
50	7.3	22.9
98	29.1	50.6
123	6.9	25.6
149	14.1	38.5
169	3.9	10.4
223	22.2	57.6
288	6.8	42.1
312	6.2	44.3
363	16.7	23.8
385	36.7	25.5

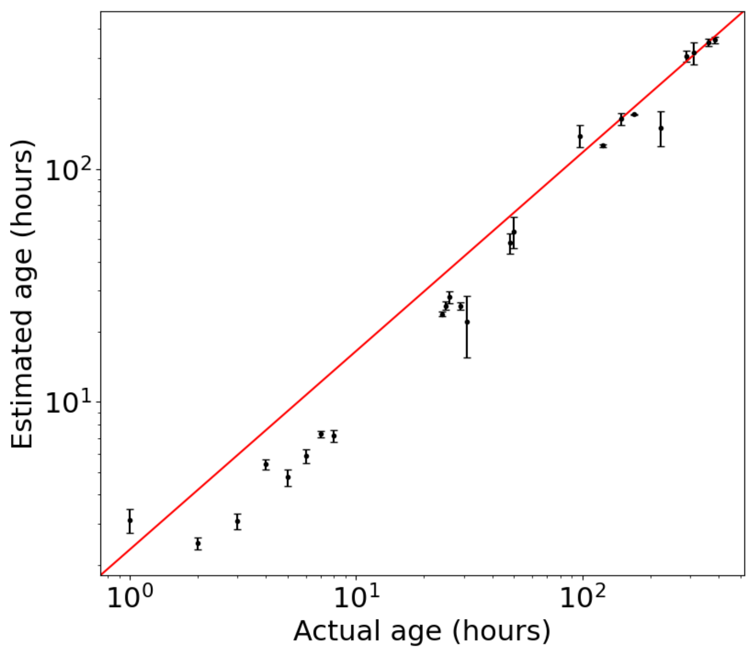

Figure 10: Average estimated age of bloodstains deposited on red cardboard substrate over actual age (black dots). Error bars represent the standard deviation of the estimated age of the blood spectra with the same age but belonging to different substrates. The red line depicts the ideal condition where estimated and actual age are equal.

low-power computing systems for use by operators directly at crime scenes. A current challenge in forensic casework is the substrate-independent bloodstain age estimation. In fact, if the blood stain under analysis is deposited on a coloured or absorbent background, this may compromise the estimation of the age of the blood stain using hyperspectral imaging in the visible range.[8]. In this paper, a substrate-independent bloodstain age estimation technique is proposed that exploits the results obtained in Ref. [10] to minimize the spectral bias introduced by an uncooperative substrate. Using this technique, a neural network model capable of estimating the age of blood deposited on nine different substrates is developed. The proposed method achieves a MAE in blood age estimation of 6.9 hours in the time range 0-385 hours, and 1.1 hours in the range of 0-48 hours. The MAE and standard deviation increases proportionally with aging time and does not appear to be particularly affected by the type of deposition substrate (Figure 6 and Figure 7). The model shows no signs of over-fitting and is cross-validated by the k-fold (5 fold) method obtaining an average MAE of 15.7 hours with a standard deviation of 0.3 hours. It should be emphasised that it is critically important to average the predicted blood age estimate on the single spectra; averaging the prediction over the totality of the spectra of a single bloodstain will change from an MAE of 15.7 hours to an MAE of 6.9 hours. In order to test the ability to generalize, the model was tested on bloodstains deposited on a new colored and absorbent substrate (i.e., red cardboard) obtaining an MAE of 9.2 hours in the time range 0-385 hours, and 1.5 hours in the time range 0-48 hours. This shows that by training the model in nine different materials, it can maintain similar performance on a new deposition substrate. Of key importance was the correction of the substrate effect by the approach developed by Giulietti et al. in Ref. [10]. Without the application of the correction method at the pre-processing stage (i.e., Point 6 in Figure 5), the model gives same results in the test set (i.e., MAE = 9.4 hours) but at the same time was unusable in new materials (MAE = 98.4 hours on red cardboard). Contrary to what might be expected, better results are obtained over time in less cooperative materials (i.e., Napkin, Cardboard, Wood and Jeans). Figures 8 and 10 show that the proposed method tends to underestimate the predicted age, especially at low ages. The proposed use of neural networks makes it possible to overcome the limitation of environmental conditions (i.e., humidity, temperature, and other environmental parameters), unlike other approaches based on hemoglobin kinetics that study the behavior of HbO2, MetHb, and HC fractions within the blood over time [8][11].

This will be achieved through appropriate future new training that also goes to take into account diverse environmental conditions. In future works, the study of the effect of stain thickness in estimating the age of blood will be considered. In particular, the effect of higher velocity impact splashes (i.e. low stain thickness) on the ability to determine the age of the stain compared to a controlled, thicker drop will also be analysed.

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Author Contribution Form:

Term	N.G.	S.D.	P.C.	M.M.
Conceptualization	yes	yes	yes	yes
Methodology / Study design	yes	yes	yes	yes
Software	yes			
Validation	yes			
Formal analysis	yes			
Investigation	yes	yes	yes	yes
Resources	yes	yes	yes	yes
Data curation	yes			
Writing – original draft	yes	yes		
Writing – review and editing	yes		yes	yes
Visualization	yes			
Supervision			yes	yes
Project administration			yes	yes
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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

Neural Network based Hyperspectral imaging for substrate independent bloodstain age estimation

Nicola Giulietti, Silvia Discepolo, Paolo Castellini, Milena Martarelli

- Hyperspectral imaging is used to measure the reflectance spectra of bloodstains
- Neural network models are exploited to automatically recognize blood spots within a hyperspectral image
- Neural network models are exploited for the substrate-independent estimation of the age of a bloodstain