DOI: 10.32703/2415-7422--2025-15-1-26-46

UDC 340.624.41:343.132.1 343.98.06:611.08

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Bloodstain in forensics: From visual inspections to AI-assisted pattern analysis and age estimation

Abstract. *Bloodstains have long served as critical evidence in forensic investigations, providing insights into the timing and nature of violent crimes. This article traces the historical evolution of bloodstain analysis, from early visual inspection to the adoption of modern methods and technologies. Blood pattern analysis has now advanced into a systematic science and incorporated artificial intelligence technology, offering quantitative insights into the mechanisms of blood spatter. For age estimation of bloodstains, DNA analysis extracts temporal changes in genetic materials from degraded bloodstains. High-performance liquid chromatography further complemented bloodstain investigations by quantifying biochemical markers indicative of time since deposition. Spectroscopic methods, including Raman and infrared spectroscopy, have identified specific molecular vibrations associated with the temporal degradation of blood components, while optical techniques based on photon reflection, absorption, and fluorescence provide alternative pathways for estimating bloodstain age. Smartphone-based colorimetry has emerged as a cost-effective and portable solution, tracking the visible progression of blood color from bright red to dark brown over time. Moreover, hyperspectral imaging integrates imaging and spectroscopy, allowing spatially resolved age estimation by analyzing spectral data at the pixel level. This article highlights the historical progression and technological advancements that have shaped bloodstain analysis in forensic*

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discipline. By integrating modern instrumentation with artificial intelligence technologies, the field continues to move closer to reliable on-site analysis. However, challenges such as environmental variability, substrate effects, and standardization remain. Continued research and validation are imperative to refine these methods and establish standardized protocols for forensic applications. This historical and technical overview underscores the transformative impact of interdisciplinary innovation on the evolution of bloodstain analysis, bridging the gap between laboratory research and practical forensic settings.
Keywords: *bloodstain pattern analysis; age estimation; spectroscopy; hyperspectral imaging; smartphone colorimetry*

Introduction.

Blood comprises approximately 45% of the solid components, i.e., platelets, white blood cells, and red blood cells, suspended in plasma. Its physical properties, such as density, viscosity, and surface tension, are governed by its composition. Upon leaving the human body, blood notably undergoes coagulation and eventually drying. The transformation into a dry stain on the substrate is often accompanied by crack propagation. In forensic investigations, blood has long been regarded as vital evidence, aiding in crime scene reconstruction and including or excluding suspects or victims (Brodbeck, 2012; Das, Harshey, Nigam, Yadav, & Srivastava, 2020; Giulietti, Discepolo, Castellini, & Martarelli, 2023; Seki, Hsiao, Ishizawa, Sugano, &Takahashi, 2024; Stojanović, Stojanović, Šorgić, & Čipev, 2020; Stotesbury, Cossette, Newell-Bell, & Shafer, 2021).

The scientific blood detection in Figure 1 dates back to Ludwig Karol Teichmann's pioneering hemin crystal test in 1853 (Kerr & Mason 1926). The human bloodstains were verified by shape of hemin crystals extracted from a specimen under test. At the beginning of the 20th century, significant breakthroughs for forensic applications included the discovery of blood groups based on different types of red blood cells by Landsteiner (1901) and the introduction of the Kastle-Meyer test, which identifies bloods by the reaction between phenolphthalein hydrogen peroxide in the presence of hemoglobin (Glaister, 1926).

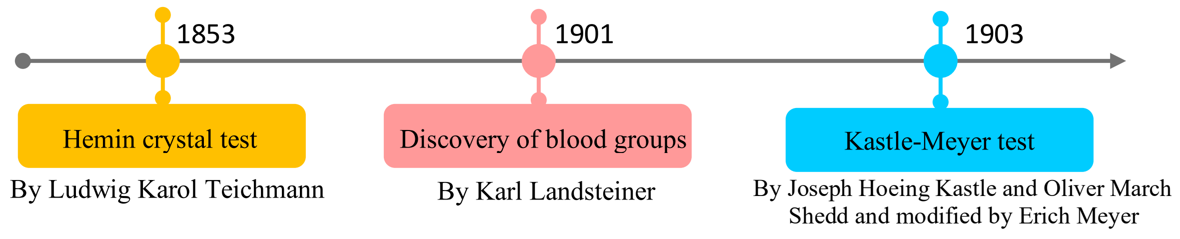

Figure 1. Significant milestones in the pioneering of blood detection methods (Authors' source).

The detection of blood initially relied on visual inspection. At crime scenes, the distinctive bright red blood and reddish-brown bloodstain are often visible, while residual blood, even when attempts have been made to remove it, can be revealed using Luminol sprays. Rudimentary chemical tests can distinguish blood from other reddish substances, also found at the place of crime (Edelman, Manti, van Ruth, van Leeuwen, & Aalders, 2012a). In the laboratory, more advanced tests can verify whether it is human blood and determine the blood group. Blood evidence's physical appearance is recorded in images and documents. For subsequent laboratory analysis, liquid blood is typically collected using a syringe and stored in tubes with anticoagulants. Alternatively, a fabric may be used to absorb blood. Dried blood samples are collected using tape, cotton-tipped applicators, or scrape-off blades. To prevent degradation, blood samples are refrigerated, and stained samples are dried before storage. After collection and preservation, forensic analysis plays a crucial role in extracting meaningful evidence. The introduction of forensic DNA analysis in the 1980s revolutionized criminal investigations, enabling precise identification of individuals. Since the early 2000s, techniques such as Polymerase Chain Reaction (PCR) and Short Tandem Repeat (STR) analysis have significantly expanded the use of DNA profiling in forensic science, supported by databases like the UK's National DNA Database and the USA's CODIS. More recently, advancements in artificial intelligence (AI) have been leveraged to automate forensic DNA profiling, enhancing efficiency and reducing human error.

Beyond blood detection, bloodstain pattern analysis and age estimation provide deeper insights into crime scene reconstruction. The shape, size, and distribution of bloodstains vary based on the nature of the incident, environmental conditions, and the substrate on which they are found (Das, Harshey, Nigam, Yadav, & Srivastava, 2020). For instance, analyzing bloodstains on porous fabric can be challenging, while outdoor stains are often significantly altered by environmental factors. Early investigations relied on visual inspection, categorizing stains as fresh, dry, or old based on their spatial distribution. Accurately estimating bloodstain age is crucial for reconstructing crime scenes (Weber & Lednev 2020). Various methods, including RNA degradation, electron paramagnetic resonance (EPR), oxygen electrode analysis, and chromatography, have been employed for precise age determination (Giulietti, Discepolo, Castellini, & Martarelli, 2023; Seki, Hsiao, Ishizawa, Sugano, &Takahashi, 2024). These techniques reveal that blood undergoes chemical and physical changes over time (Bremmer, Nadort, van Leeuwen, van Gemert, & Aalders, 2011; Cadd, Li, Beveridge, O'Hare, & Islam, 2018). However, despite their high accuracy, they require extensive sample preparation and time-consuming laboratory analysis (Li, Beveridge, O'Hare, & Islam, 2013). As alternatives, spectroscopic and colorimetric methods have gained prominence in bloodstain analysis. These non-contact methods allow for rapid, in-situ examination, making them highly valuable for forensic investigations (Bremmer, Nadort, van Leeuwen, van Gemert, & Aalders, 2011; Weber & Lednev 2020).

The purpose of this article is to trace the evolution of bloodstain analysis, from its early foundations to the emergence of artificial intelligence (AI)-assisted techniques for reliable on-site forensic investigations. By bridging laboratory research with practical forensic applications, this interdisciplinary review highlights key advancements shaping the field. The methodology for collecting and reviewing relevant literature are described in the next section. Section 3 examines the historical development and forensic importance of bloodstain pattern analysis. Section 4 examines key advancements in laboratory-based bloodstain age estimation techniques. Meanwhile, portable spectroscopic and colorimetric methods have emerged as promising tools for on-site applications. Section 5 focuses on portable optical and vibrational spectroscopy, colorimetry, and hyperspectral imaging, highlighting their recent advancements and ongoing developments in on-site bloodstain age estimation methodologies. Finally, Section 6 summarizes the discussed methodologies and provides an outlook on AI integration in forensic bloodstain analysis.

Methodology.

To investigate the historical development and current state of bloodstain pattern analysis and age estimation, a bibliometric analysis was conducted. A search was performed in the Scopus database using key terms: "bloodstain," "blood pattern analysis," and "blood age estimation." This search aimed to evaluate the dynamics of the scientific community's interest and the evolution of research trends in various aspects of forensic bloodstain analysis. After excluding irrelevant publications, 64 research articles were categorized into two main groups: bloodstain pattern analysis (Section 3) and bloodstain age estimation. The latter was further divided into laboratory-based methods (Section 4) and on-site spectroscopy and colorimetry (Section 5). Additionally, key references outside the Scopus database (e.g., books, websites) were cited for their relevance to the Scopus-indexed articles. The literature review placed particular emphasis on the advancing roles of smartphone colorimetry, hyperspectral imaging, and emerging AI-assisted methods in forensic investigations. The integration of these technologies was analyzed to provide a comprehensive overview of past developments, current challenges, and potential future directions in the field.

Bloodstain Pattern Analysis.

Bloodstain pattern analysis involves examining the shapes, sizes, orientations, locations, and distribution of blood residues at a crime investigation to reconstruct the physical occurrences that could have taken place (Bergman, Klöden, Dreßler, & Labudde, 2022; James, Kish, & Sutton, 2005; Zou & Stern, 2022). As shown in Figure 2, the first systematic approach was pioneered by Piotrowski (1895), where he explored the influence of various forces on the formation and distribution of bloodstains. Another landmark study was bloodstain trajectory analysis by Balthazard, Piedelievre, DeSoille, & DeRobert (1939). Bloodstain pattern analysis began to gain prominence in the 1950s, with the expert testimony of Paul L. Kirk on bloodstains being accepted as evidence in a US court for the first time in 1957 (EngagedScholarship @ Cleveland State University, 2024). The widespread adoption of these techniques was championed by Herbert Leon MacDonell, who wrote the book (MacDonell, 1972) and organized the first formal bloodstain training course in 1973. In 1983, a group of bloodstain analysts established the International Association of Bloodstain Pattern Analysts to further advance the field (International Association of Bloodstain Pattern Analysts, 2024). Over time, the technique has evolved into an essential forensic tool, though its accuracy and reliability have been under more scrutiny in the last decade (Smith, 2018).

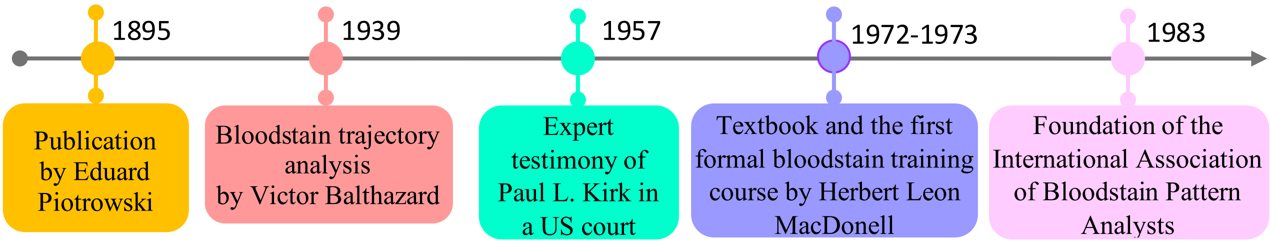

Figure 2. Significant milestones in the pioneering of bloodstain pattern analysis (Authors' source).

Bloodstains are typically classified into three groups, namely passive patterns, spatter patterns, and altered patterns (Singh, Gupta, & Rathi, 2021). Passive patterns are created by gravity and include blood pools, drip stains, flow stains, and serum stains (Singh, Gupta, & Rathi, 2021). Drip stains form as blood falls undisturbed, often taking a spherical shape unless affected by external forces. Depending on the angle of impact, these stains may appear as circular drops or elongated marks (SWGSTAIN, 2009). Spatter patterns consist of small, elliptical, or circular bloodstains created when blood is forcefully ejected, such as when a victim is struck by a hard object (James, Kish, & Sutton, 2005; Rough, Batchelor, Green, & Bainbridge-Smith, 2024). These patterns provide vital details regarding direction, velocity, and impact's origin. Altered patterns arise when bloodstains undergo physical or environmental changes, including diffusion, dilution, insect activity, or physical actions such as smearing (James, Kish, & Sutton, 2005). These patterns can obscure or modify the original blood evidence, complicating forensic interpretation.

Bloodstain patterns dispersed throughout a crime scene can be instrumental in event reconstruction, providing investigators with a comprehensive understanding of the events leading to the bloodshed. By systematically distinguishing and analyzing bloodstain patterns, forensic experts can identify informative features that shed light on the sequence of events (Comiskey, Yarin, Kim, & Attinger, 2016; James, Kish, & Sutton, 2005; Latham, 2024). Blood pattern analysis has been extensively applied to criminal investigations, leveraging common features such as stain morphology and pattern characteristics to uncover critical insights (Rough, 2024). For instance, a study of UK case files from 2012 to 2020 revealed prevalent attack techniques, weapons used, and the frequency of various bloodstain pattern classifications (Home, Norman, Palmer, Field, & Williams, 2022).

Blood pattern analysis methods commonly rely heavily on practical training acquired through workshops, trial-and-error approaches, and practitioner experience. However, empirical research has contributed significantly to refining and understanding the principles behind bloodstain pattern formation. For example, de Bruin, Stoel, and Limborgh (2011) highlighted the importance of larger, elliptical drops when determining the area of origin of bloodstains. Similarly, Comiskey, Yarin, Kim, and Attinger (2016) used a blood-soaked sponge to simulate back-spatter formation, accounting for factors such as air drag and gravity in their analysis of blood velocities. In another study, Singh, Gupta, and Rathi, (2021) utilized artificial bloodstains made with Awlata, an Indian dye, to investigate the correlations between stain properties and blood drop height. Their findings demonstrated that spine formation was inversely proportional to the height of the falling blood, while the distance of satellite stains increased with the drop height.

The classification of bloodstains often relies on grouping samples based on their morphological features. Statistical approaches, such as the likelihood ratio method, play a crucial role in quantifying how much more likely observed data is to support one hypothesis over another. For example, Hook, Fieldhouse, Flatman-Fairs, and Williams (2024) reviewed ten classification techniques from 14 sources to systematically classify bloodstain patterns. Zou and Stern (2022) utilized a bivariate Gaussian model to estimate the feature distributions under particular hypotheses and applied the likelihood ratio to distinguish between impact and gunshot patterns. In subsequent research, Zou and Stern (2025) proposed a semi-projected normal distribution mixture model to describe bloodstain patterns. This model outperformed the semi-wrapped Gaussian model in clustering synthetic data, highlighting its potential for more accurate classification.

With emerging AI technologies, machine learning and deep learning can enhance classification accuracy, decrease human subjectivity in bloodstain analysis, and minimize computation time, all of which would allow for high-throughput analysis. For instance, Jung, Jo, Ahn, Jeong, and Lim (2024) explored the use of machine learning frameworks, such as Convolutional Neural Networks (CNNs), Decision Trees, and Random Forests, to classify bloodstain patterns. Their findings demonstrated that the CNN Inception v3 model could automatically classify drip stains and blood spatters, significantly accelerating investigative processes (Bergman et al., 2022). Similarly, Rough, Batchelor, Green, and Bainbridge-Smith (2024) employed computer vision techniques to detect bloodstains in digital images on plain backgrounds, underscoring the potential of automated approaches in forensic investigations. This automated bloodstain pattern analysis represents a valuable tool for analysts and researchers exploring quantitative methods. Furthermore, integrating blood pattern analysis with the 3D imaging and characterization techniques discussed in subsequent sections offers immense potential for on-site investigations. Such interdisciplinary efforts promise to enhance bloodstain analysis's accuracy, precision, and interpretability, further advancing its forensic applications.

Laboratory-Based Bloodstain Age Estimation.

After exiting the human body, blood undergoes a series of distinct physical and chemical transformations over time, driven by the degradation of DNA, RNA, proteins, and other biomolecules. These temporal changes are critical in forensic investigations, providing clues about the time since blood deposition. Initially, fresh blood appears bright red due to the presence of oxygenated hemoglobin (oxy-hemoglobin), which reflects red light. The oxidation of hemoglobin causes methemoglobin to develop over time, giving bloodstains a darker brown color. Eventually, hemoglobin is further degraded into hemichrome, contributing to a dark brown to black coloration. These changes are primarily driven by chemical reactions with oxygen in the air and the breakdown of hemoglobin derivatives.

Forensic scientists leverage these biochemical changes to approximate the time since blood deposition, a key factor in reconstructing timelines, identifying suspects, or ruling out individuals in violent crimes. Achieving precise measurements of bloodstain age in real-world scenarios is challenging due to the influence of numerous environmental variables, including light exposure, temperature, humidity, microbial activity, contamination, the kind of substrate the blood is deposited, and the bloodstain's thickness. These factors can accelerate or delay the degradation processes, introducing variability in the interpretation of results. Bloodstain age estimation has been the subject of significant research since 1907 (Das, Harshey, Nigam, Yadav, & Srivastava, 2020). Since the beginning of the last decade of the twentieth century, several researchers have been studying the effects of time on the aging of different pieces of evidence to ascertain the exact or relative time of their occurrence. Despite the importance of bloodstain age estimation, due to these complexities, no single method has been universally adopted for routine use in crime scene investigations.

Since 2020, forensic analyses of bloodstains have increasingly focused on molecular biology techniques, with DNA and RNA analysis at the forefront. DNA-based methods have been used to examine changes in specific sequences (He et al., 2022; Yang et al., 2023), while RNA-based approaches have explored messenger RNA (mRNA) and microRNA (miRNA) degradation as indicators of bloodstain age. For example, RNA degradation rates can ascertain the estimated period since deposition, as RNA is less stable than DNA and degrades at a predictable rate under specific conditions (Elliott, Stotesbury, & Shafer, 2022; Fang et al., 2020; Heneghan, Fu, Pritchard, Payton, & Allen, 2021; Manasatienkij & Nimnual, 2021; Wei, Wang, Wang, Cong, & Li, 2022).

Other biochemical techniques focus on studying hemoglobin and protein modifications. High-performance liquid chromatography (HPLC) has been used to measure levels of hemoglobin A1c, a form of hemoglobin that undergoes glycation, as a function of time (Acar et al., 2020). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has made it possible to identify specific protein modifications, such as oxidation, deamidation, and glycation, which provide valuable indicators for bloodstain age estimation (Heo et al., 2022; Kim et al., 2022; Lee, Lee, Kwon, Lee, & Kang, 2022; Schneider, Roschitzki, Grossmann, Kraemer, & Steuer, 2022; Schneider, Kraemer, & Steuer, 2023).

Morphological and mechanical changes in red blood cells over time have also been investigated using advanced imaging techniques. Atomic Force Microscopy (AFM) has been employed to monitor the elasticity and surface morphology of red blood cells as they age, revealing distinct degradation patterns (Cavalcanti & Silva, 2019; Strasser et al., 2007). Scanning Electrochemical Microscopy (SECM), a technique that maps the electrochemical activity of bloodstains, was employed by Tian, Chen, Ma, and Zhang (2022) to track changes in cell membranes and protein interactions over time.

While these laboratory-based methods provide high precision and detailed insights, they are often impractical for on-site forensic investigations. The reliance on sophisticated instruments, chemical reagents, and trained specialists makes these methods time-intensive and resource-heavy (Seki, Hsiao, Ishizawa, Sugano, &Takahashi, 2024). Furthermore, the need for controlled conditions in laboratory environments limits their applicability in real-world crime scenes. Mobile DNA labs have been developed since the 2010s to enable rapid on-site analysis, particularly in mass disaster scenarios. However, high equipment maintenance and operational costs remain significant drawbacks. Concerns also persist regarding their accuracy and the risk of contamination compared to traditional lab-based methods, raising questions about their scientific rigor and reliability for use in court. As a result, the field has seen a growing interest in portable spectroscopic and colorimetric techniques to close the gap between laboratory precision and practical usability in on-site investigations.

On-Site Bloodstain Age Estimation.

Modern spectroscopic and colorimetric techniques offer the capability to estimate bloodstain age with compact instrumentation (Figure 3). The absorption and reflection of electromagnetic waves at specific wavelengths by the hemoglobin derivatives provide the basis for spectroscopic and colorimetric analyses of bloodstains (Bremmer, Nadort, van Leeuwen, van Gemert, & Aalders, 2011; Bremmer, de Bruin, van Gemert, van Leeuwen, & Aalders, 2012). By measuring the spectral properties of bloodstains in the laboratory, researchers can estimate their age, as the progression of hemoglobin degradation follows a predictable timeline under controlled conditions. However, the practical application of such methods is often limited due to the variability introduced by external factors. Despite challenges posed by environmental factors, substrate variability, and measurement conditions (Zadora & Menżyk, 2018), substantial progress in on-site analysis has been made to mitigate these limitations.

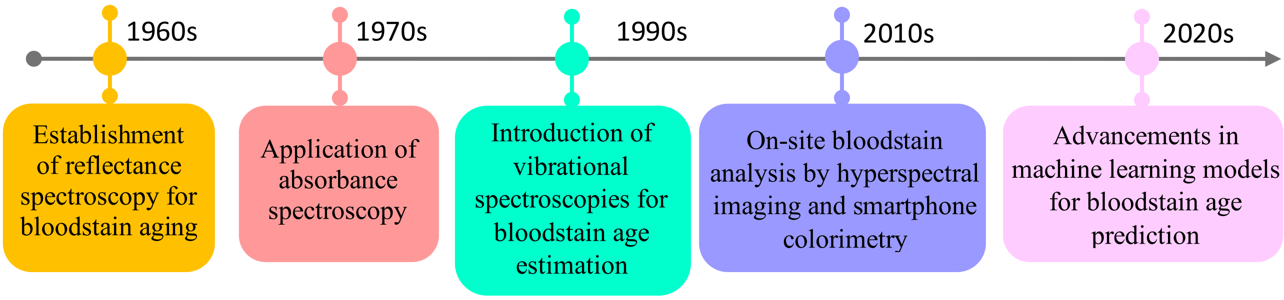

Figure 3. Evolution of on-site bloodstain age estimation (Authors' source).

Reflectance, Absorbance, and Fluorescence Spectroscopies.

In 1960, Patterson proposed that temporal changes in molecular and structural characteristics of the bloodstain can be detected in reflectance spectra and correlated with the bloodstain age (Patterson, 1960). Reflectance spectroscopy measures the sample's reflection of IR or visible light to gather information about its composition. By illuminating the sample's surface, the spectrometer collects the reflected light intensity in relation to wavelength, creating a reflectance spectrum. Alternatively, an early work by Kind, Patterson, and Owen (1972) suggested that the absorbance spectra could be used in estimating the age of bloodstains.

Subsequent studies in the 2010s sought to enhance the accuracy of these methods. To manage complex spectral datasets, chemometric methods, including partial least squares regression (PLSR) and principal component analysis (PCA), have been used in bloodstain analysis to derive valuable data and facilitate more accurate interpretations with statistical confidence (Sharma & Kumar, 2018). Bremmer et al. (2011; 2012) used reflectance spectroscopy to identify the hemoglobin derivatives and determine the age of blood stains. Li, Beveridge, O'Hare and Islam (2011) employed spectral pre-processing and linear discriminant analysis to develop a predictive model for age estimation up to 37 days based on spectral properties of equine blood on a glazed white tile. Sun et al. (2017) applied 3 chemometric methods to develop a prediction model for bloodstained aged 2–24 h, 1–7 days, and 7–45 days based on reflectance spectroscopy. The least-squares-support vector machines (LS-SVM) outperformed the PLSR and PCA.

Hanson and Ballantyne (2010) demonstrated the use of a Ultraviolet-visible (UV-vis) spectrophotometer to monitor the blue shift attributed to the modifications of hemoglobin and its derivatives in bloodstains aged up to 1 year. The effects of temperature and humidity were also investigated. Bergmann, Heinke and Labudde (2017) studied bloodstain ageing for up to 3 weeks on different substances and subsequently studied the effect of the coagulation agent from UV-vis absorbance spectra of bloodstains (Bergmann, Leberecht, & Labudde, 2021).

On the contrary, fluorescence spectroscopy measures the amount of fluorescent light that is emitted from a sample after it has been energized by a light source. As a result of photon absorption, a molecule can enter an excited state and subsequently emit fluorescence as it returns to its ground state. The technique, therefore, provides details regarding the molecule structure, energy levels, and chemical environment of the fluorescent molecules in the sample. In the context of bloodstain age estimation, fluorescence spectroscopy is employed to examine the temporal changes of fluorophores in blood, especially tryptophan. Weber, Wójtowicz, and Lednev (2021) found that the fluorescence intensity significantly decreases within 24 hours, and the emission spectra exhibit shifts in peak positions. To determine the age of bloodstains, mathematical models were created using the fluorescence data obtained from blood samples with known ages.

Vibrational Spectroscopy.

Since 1990s, Fourier Transform Infrared (FTIR) and Raman spectroscopies have been developed to provide more accurate and detailed analysis of bloodstains. Both spectra are derived from characteristic vibrational modes and frequencies within molecules. Because each sample has a unique set of molecular bond vibrations, these two vibrational spectroscopies can be complemented to characterize different functional groups (Das, Harshey, Nigam, Yadav, & Srivastava, 2020). The effects of interfering substances and substrates may also be discriminated (Kistenev, Borisov, Samarinova, Colón-Rodríguez, & Lednev, 2023; Sharma, Chophi, Jossan, & Singh, 2021). For FTIR spectroscopy, the frequency of absorbed IR light equal to the vibration frequency of the functional groups in bloodstains was identified, and PLSR was often used in the analysis. The linear correlation between FTIR parameters from characteristic vibration bands and the blood deposition time could be established and further developed for forensic investigation. Edelman, van Leeuwen, and Aalders (2012b) compared ageing bloodstains with non-blood controls on cotton fabrics of different colors. From the Near Infrared (NIR) spectra, the time since deposition up to 1 month was correctly predicted but the uncertainty was significantly increased after 10 days. The Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) enhances the sensitivity in determining the functional groups by incorporating a reflective crystal in the set-up (Alkhuder, 2022). ATR-FTIR with PLSR was used to compare indoor and outdoor bloodstains ranging in age from 1 to 85 days (Lin et al., 2017). From ATR-FTIR spectroscopy coupled with chemometric techniques, Kumar, Sharma, and Sharma (2020) obtained the estimation model for up to 175 days.

In Raman spectroscopy, photons are inelastically scattered by molecules in a sample. When a laser beam is directed at a bloodstain, the interaction between photons and the molecules found in the sample causes a shift in the energy levels of the photons, resulting in scattered light with different wavelengths. This scattered light contains information on the molecular composition and structure of the sample. Raman spectroscopy is at the forefront of research and allows for single-point observations. However, its practical application is currently limited by several factors, including the high cost of equipment, variability in results depending on the substrate, and the need for specialized expertise to ensure consistent and reliable measurements (Doty, McLaughlin, & Lednev, 2016; Seki, Hsiao, Ishizawa, Sugano, & Takahashi, 2024). In the context of bloodstain age estimation, Raman spectroscopy analyzes the changes in the molecular components of blood over time. As blood ages, the transformation of hemoglobin molecules alters their Raman spectra. By comparing the Raman spectra of bloodstains with known ages to those of unknown stains, the age of the bloodstains can be estimated based on the degree of spectral similarity or dissimilarity. Various Raman spectroscopic parameters, such as the intensity ratios of specific Raman bands or the presence of characteristic peaks, have been explored to develop models for estimating bloodstain age. These models are typically created using multivariate analysis or machine learning algorithms to correlate the Raman spectral data with the known ages of bloodstains. Doty, McLaughlin, and Lednev (2016) used PLSR calibration curves to predict the bloodstains aged up to 1 week and, in a subsequent work, estimated the bloodstain age of up to 2 years based on Raman spectra (Doty, Muro, & Lednev, 2017). More recently, datasets from Raman spectra of bloodstains under varying temperatures and humidity levels were used to build prediction models using Support Vector Machine Regression (SVR), Kernel Ridge Regression (KRR), and CNN (Zhang, Wang, Chen, Tian, & Gao, 2023).

Colorimetry.

Fresh bloodstains typically exhibit a bright red color, while older stains appear darker and brownish. This color change is attributed to iron oxidation and hemoglobin breakdown after leaving the blood vessel. Accurately determining the time since deposition based solely on color remains challenging due to its sensitivity to aforementioned environmental factors and lighting conditions during measurement. However, significant progress in estimating bloodstain age based on color has been achieved over the past decade. For instance, Thanakiatkrai, Yaodam, and Kitpipit (2013) captured digital images of bloodstains under controlled lighting conditions using a light box and analyzed the images with the ImageJ program on a personal computer. They examined time-dependent colorimetric characteristics in CMYK, HSV, HSL, and RGB color spaces and recommended monitoring the M value, which exhibits a logarithmic decrease over time with the highest R^2 for estimating bloodstain age. Several parameters, including anticoagulants, humidity, light exposure, temperature, and substrate type, which significantly affect the rate of color change in bloodstains, were also explored in this study (Thanakiatkrai, Yaodam, & Kitpipit, 2013).

A spectrophotometric colorimeter can measure the transmittance and reflectance of the continuous spectrum to determine the color tone, enabling the explicit estimation of bloodstain color changes over time (Seki, Hsiao, Ishizawa, Sugano, & Takahashi, 2024). In the 1950s, when spectrophotometry was the primary method of study, obtaining the reflectance spectrum of the visible light region took almost an hour, making the process time-consuming (van Kampen & Zijlstra, 1961). Despite its simplicity and relatively low cost (Kind, Patterson, & Owen, 1972), spectrophotometry holds high accuracy potential but faces reproducibility challenges, particularly due to its reliance on broad spectra within the visible light range (Sun et al., 2017). Marrone et al. (2021) employed a spectrophotometric colorimeter to capture CIELAB/CIELCh color values over time as blood transitioned from bright red to dark brown. By correlating these color values with bloodstain aging, they developed predictive models to estimate the time since deposition. With advancing AI technology, Seki, Hsiao, Ishizawa, Sugano, and Takahashi (2024) applied machine learning techniques to estimate bloodstain age by analyzing time-dependent color changes using a spectrophotometric colorimeter. They quantified the color tone of each bloodstain in the CIELAB color space and developed a predictive model for bloodstain age using a random forest regression approach.

The emerging field of smartphone colorimetry harnesses the power of both smartphone cameras and colorimetric applications (apps) to quantify colors. The “Smart Forensic Phone,” an Android-based smartphone app developed by researchers at Yonsei University, was a significant advancement in crime scene analysis (Shin et al., 2017). This app utilized colorimetric analysis in the HSV color space of the V value to rapidly determine the time since blood deposition on five different substrates. However, it should be noted that the colorimetry approach may not be as effective beyond 42 hours, as bloodstain colors tend to exhibit minimal changes after this time. Furthermore, the Yonsei research group has expanded their work by incorporating classification and pattern recognition capabilities for cracks and blood pools in smartphone images to determine bloodstain age while still relying on the V value from the colorimetric study (Choi, Shin, Hyun, Song, & Jung, 2019). Dinmeung, Sirisathitkul, and Sirisathitkul (2023) used the iPhone application called "Colorimeter X" to analyze the bloodstains left on tissue papers and cotton fabrics over one to eleven days, corresponding to the CMYK, HSV, and RGB color schemes. They observed linear decreases in R, G, B, and V values as the temporal transitions from bright red to dark brown. The V value has the highest R2 based on linear least square fitting. The increase in the K value during the blood aging process was identified as an additional effective metric to support the B, R, and V values in estimating the bloodstain age. Besides the colorimetry, Li et al. (2023) demonstrated that images of bloodstains could be analyzed by deep learning-based BloodNet and correlated their pattern to the time since deposition.

Hyperspectral Imaging.

Since 2012, hyperspectral imaging has been utilized to assess the age of bloodstains, offering a cutting-edge approach to forensic analysis. This technique captures images across multiple electromagnetic spectrum wavelengths, typically spanning visible and nearinfrared regions, using advanced portable devices. The resulting data, known as a hypercube, gives a spectrum for each pixel (x, y) and an image for each wavelength, enabling detailed characterization of the reflectance or absorption of various substances within the samples (de Cassia Mariotti, Ortiz, & Ferrao, 2023). Edelman, van Leeuwen, and Aalders (2012b) correlated the spectral characteristics with the relative amount of hemichrome, methemoglobin, and oxyhemoglobin in bloodstains, leading to a non-destructive approach for estimating age up to 200 days. Li, Beveridge, O'Hare, and Islam (2013) investigated spectral variations in bloodstains over time and developed a model for estimating age up to 30 days based on the collected hyperspectral images and linear discriminant analysis. Their model was particularly accurate within the first 7 days but had a higher error rate, particularly after 14 days. Cadd, Li, Beveridge, O'Hare, and Islam (2018) focused on the bloodstained fingerprints and observed the variation in visible light absorption by hemoglobin derivatives with bloodstain age. With Forenscope-Mobile Multispectral UV-VIS-IR Imaging Systems, Kaya, Karadayi, Karadayi, and Çetin (2023) studied bloodstains of varying ages on textiles under various washing circumstances. Giulietti, Discepolo, Castellini, and Martarelli (2023) utilized hyperspectral images of bloodstains deposited on nine different substrates, varying in color and composition, to estimate the age of bloodstains using a multilayer perceptron regression model. Unlike other methods based on hemoglobin kinetics, this approach overcomes the limitations posed by environmental factors such as temperature, humidity, and others.

Conclusions and Outlook.

Since its inception, the forensic science of bloodstain analysis has undergone significant evolution, with its roots tracing back to early visual approximations of bloodstain characteristics. The pattern of bloodstains has always been crucial in forensic investigations. Moreover, blood pattern analysis provides valuable insights into the mechanics of bloodshed at crime locations. By examining the size, shape, and distribution of bloodstains, investigators can infer key details, including the angle of impact, the velocity of blood spatter, and the nature of the force involved. Historical advancements in this field, from early qualitative assessments to modern computational modeling, have transformed it into a powerful tool for reconstructing events.

Over time, advancements in technology have expanded the scope of bloodstain analysis to include age estimation alongside blood pattern analysis, providing critical insights into the timing of criminal events. In its earliest days, investigators relied on simple observations of color changes in drying bloodstains, which could only offer rudimentary estimations. Integrating spectroscopy and colorimetric techniques has significantly improved the accuracy and applicability of bloodstain age estimation. Raman Scattering and FTIR spectroscopy have emerged as non-destructive methods for characterizing molecular vibrations in blood components, allowing for detailed assessments of chemical changes that occur as bloodstains age. Similarly, UV-visible spectroscopies, including reflectance, absorbance, and fluorescence, exploit the interaction of light with blood to extract valuable information about hemoglobin degradation and other biochemical markers over time. These advancements in optical methods have moved forensic science closer to reliable, on-site applications for age determination.

Alongside spectroscopy, hyperspectral imaging has proven particularly promising by combining imaging with spectral analysis to comprehensively assess bloodstains across a wide range of wavelengths. This approach not only aids in age estimation but can also offer spatial context for interpreting bloodstain patterns, bridging the gap between quantitative analysis and visual interpretation. Additionally, smartphone-based colorimetry has emerged as a cost-effective and portable alternative, enabling investigators to track colorimetric changes in bloodstains over time with high precision. However, environmental factors such as temperature, humidity, substrates, and stain thickness remain challenges, potentially influencing the accuracy of these methods.

Integrating AI technologies into bloodstain pattern analysis and age estimation represents a transformative advancement in forensic science, offering a more precise, objective, and high-throughput approach to interpreting bloodstains. Machine learning and deep learning frameworks, such as CNNs, decision trees, random forests, support vector machines, and multilayer perceptron models, have demonstrated remarkable potential in classifying bloodstain patterns, estimating bloodstain age, and minimizing the impact of environmental variables. The combination of spectroscopy, hyperspectral imaging, and colorimetric analysis with AI-assisted predictive modeling is enhancing the accuracy and applicability of bloodstain analysis across forensic investigations.

Despite these advancements, several challenges remain. The lack of standardized protocols and the influence of external factors such as temperature, humidity, and substrate properties continue to affect model generalizability. Future research must prioritize the expansion of large-scale, high-quality datasets encompassing a wider range of conditions to improve model robustness. The development of more adaptive and interpretable AI models, capable of integrating multimodal data sources, including spectral, colorimetric, and spatial pattern information, could significantly enhance forensic reliability.

Moreover, interdisciplinary collaboration between forensic scientists, AI researchers, and law enforcement agencies will be critical in translating these AI-driven methodologies into practical forensic applications. Efforts to validate AI models through repeated experiments, cross-laboratory testing, and calibration refinements will be essential to ensure their credibility in judicial proceedings. Additionally, the integration of automated 3D imaging, real-time computer vision systems, and explainable AI could further refine the forensic workflow, making on-site bloodstain analysis more accessible and interpretable for investigators. By addressing these challenges, the integration of AI in forensic bloodstain analysis will continue to push the boundaries of forensic science, enabling more reliable, rapid, and scientifically rigorous bloodstain interpretation that withstands scrutiny in legal contexts.

Funding.
This research received no external funding.

Conflicts of interest.
The authors declare no conflict of interest.

References

Acar, K., Kurtulus Dereli, A., Avci, E., Zeybek, V., Kutlu, E., Demir, S., & Senol, H. (2020). Determination of haemoglobin A1c levels using high-performance liquid chromatography of bloodstains. *Medicine, Science and the Law, 60*(1), 19–25. https://doi.org/10.1177/0025802419879272

Alkhuder, K. (2022). Attenuated total refection-Fourier transform infrared spectroscopy: A universal analytical technique with promising applications in forensic analyses. *International Journal of Legal Medicine, 136*, 1717–1736. https://doi.org/10.1007/s00414-022-02882-2

Balthazard, V., Piedelievre, R., DeSoille, H., & DeRobert, L. (1939). Etude des Gouttes de Sang Projecte. *Presented at the 22nd Congress of Forensic Medicine, Paris, France* [in French].

Bergman, T., Klöden, M., Dreßler, J., & Labudde, D. (2022). Automatic classification of bloodstains with deep learning methods. *KI - Künstliche Intelligenz*, *36*, 135–141. https://doi.org/10.1007/s13218-022-00760-y

Bergmann, T., Heinke, F., & Labudde, D. (2017). Towards substrate-independent age estimation of blood stains based on dimensionality reduction and k-nearest neighbor classification of absorbance spectroscopic data. *Forensic Science International*, *278*, 1–8. https://doi.org/10.1016/j.forsciint.2017.05.023

Bergmann, T., Leberecht, C., & Labudde, D. (2021). Analysis of the influence of EDTA-treated reference samples on forensic bloodstain age estimation. *Forensic Science International*, *325,* 110876. https://doi.org/10.1016/j.forsciint.2021.110876

Bremmer, R. H., de Bruin, K. G., van Gemert, M. J., van Leeuwen, T. G., & Aalders, M. C. (2012). Forensic quest for age determination of bloodstains. *Forensic Science International*, *216*(1–3), 1–11. https://doi.org/10.1016/j.forsciint.2011.07.027

Bremmer, R. H., Nadort, A., van Leeuwen, T. G., van Gemert, M. J., & Aalders, M. C. (2011). Age estimation of blood stains by hemoglobin derivative determination using reflectance spectroscopy. *Forensic Science International*, *206*(1–3), 166–171. https://doi.org/10.1016/j.forsciint.2010.07.034

Brodbeck, S. (2012). Introduction to bloodstain pattern analysis. *Journal for Police Science and Practice, 2*, 51–57. https://doi.org/10.7396/IE_2012_E

Cadd, S., Li, B., Beveridge, P., O'Hare, W. T., & Islam, M. (2018). Age determination of blood-stained fingerprints using visible wavelength reflectance hyperspectral imaging. *Journal of Imaging, 4*(12), 141. https://doi.org/10.3390/jimaging4120141

Cavalcanti, D. R., & Silva, L. P. (2019). Application of atomic force microscopy in the analysis of time since deposition (TSD) of red blood cells in bloodstains: A forensic analysis. *Forensic Science International*, *301*, 254–262. https://doi.org/10.1016/j.forsciint.2019.05.048

Choi, W., Shin, J., Hyun, K. A., Song, J., & Jung, H. I. (2019). Highly sensitive and accurate estimation of bloodstain age using smartphone. *Biosensors and Bioelectronics, 130*, 414–419. https://doi.org/10.1016/j.bios.2018.09.017

Comiskey, P., Yarin, A., Kim, S., & Attinger, D. (2016). Prediction of blood back spatter from a gunshot in bloodstain pattern analysis. *Physical Review Fluids*, *1*(4), 043201. https://doi.org/10.1103/physrevfluids.1.043201

Das, T., Harshey, A., Nigam, K., Yadav, V. K., & Srivastava, A. (2020). Analytical approaches for bloodstain aging by vibrational spectroscopy: Current trends and future perspectives. *Microchemical Journal*, *158*, 105278. https://doi.org/10.1016/j.microc.2020.105278

de Bruin, K., Stoel, R., & Limborgh, J. (2011). Improving the point of origin determination in bloodstain pattern analysis. *Journal of Forensic Sciences, 56*(6), 1476–1482. https://doi.org/10.1111/j.1556-4029.2011.01841.x

de Cassia Mariotti, K., Ortiz, R. S., & Ferrao, M. F. (2023). Hyperspectral imaging in forensic science: An overview of major application areas. *Science & Justice, 63*, 387–395. https://doi.org/10.1016/j.scijus.2023.04.003

Dinmeung, N., Sirisathitkul, Y., & Sirisathitkul, C. (2023). Colorimetric parameters for bloodstain characterization by smartphone. *Arab Journal of Basic and Applied Sciences, 30*(1), 197–207. https://doi.org/10.1080/25765299.2023.2194129

Doty, K. C., McLaughlin, G., & Lednev, I. K. (2016). A Raman "spectroscopic clock" for bloodstain age determination: The first week after deposition. *Analytical and Bioanalytical Chemistry*, *408*(15), 3993–4001. https://doi.org/10.1007/s00216-016-9486-z

Doty, K. C., Muro, C. K., & Lednev, I. K. (2017). Predicting the time of the crime: Bloodstain aging estimation for up to two years. *Forensic Chemistry, 5*, 1–7. https://doi.org/10.1016/J.FORC.2017.05.002

Edelman, G., Manti, V., van Ruth, S. M., van Leeuwen, T., & Aalders, M. (2012a). Identification and age estimation of blood stains on colored backgrounds by near infrared spectroscopy. *Forensic Science International, 220*, 239–244. https://doi.org/10.1016/j.forsciint.2012.03.009

Edelman, G., van Leeuwen, T. G., & Aalders, M. C. G. (2012b). Hyperspectral imaging for the age estimation of blood stains at the crime scene. *Forensic Science International, 223*(1–3), 72–77. https://doi.org/10.1016/j.forsciint.2012.08.003

Elliott, C. I., Stotesbury, T. E., & Shafer, A. B. A. (2022). Using total RNA quality metrics for time since deposition estimates in degrading bloodstains. *Journal of Forensic Sciences, 67*(5), 1776–1785. https://doi.org/10.1111/1556-4029.15072

EngagedScholarship @ Cleveland State University. (2024). Retrieved from https://engagedscholarship.csuohio.edu/kirk_photos/

Fang, C., Liu, X., Zhao, J., Bingbing, X., Jialin, Q., Wenli, I., … Yan, J. (2020). Age estimation using bloodstain miRNAs based on massive parallel sequencing and machine learning: A pilot study. *Forensic Science International: Genetics, 47*, 102300. https://doi.org/10.1016/j.fsigen.2020.102300

Giulietti, N., Discepolo, S., Castellini, P., & Martarelli, M. (2023). Neural network based hyperspectral imaging for substrate independent bloodstain age estimation. *Forensic Science International*, *349*, 111742. https://doi.org/10.1016/j.forsciint.2023.111742

Glaister, J. (1926). The Kastle-Meyer test for the detection of blood: Considered from the medico-legal aspect. *The British Medical Journal, 1*(3406), 650–652. Retrieved from https://pmc.ncbi.nlm.nih.gov/articles/PMC2523691/pdf/brmedj08322-0010.pdf

Hanson, E. K., & Ballantyne, J. (2010). A blue spectral shift of the hemoglobin soret band correlates with the age (time since deposition) of dried bloodstains. *PLoS One*, *5*(9), e12830. https://doi.org/10.1371/journal.pone.0012830

He, H., Zhang, Q., Niu, Q., Li, Y., Sun, Q., & Zhao, D. (2022). Estimating bloodstain formation time by quantitative analysis of mtDNA degradation. *Forensic Science International, 339*, 111411. https://doi.org/10.1016/j.forsciint.2022.111411

Heneghan, N., Fu, J., Pritchard, J., Payton, M., & Allen, R. W. (2021). The effect of environmental conditions on the rate of RNA degradation in dried blood stains. *Forensic Science International: Genetics*, *51*, 102456. https://doi.org/10.1016/j.fsigen.2020.102456

Heo, T. M., Gwon, S. Y., Yang, J. H., Hyun, S. H., Kang, H. G., & Sung, H. J. (2022). Hemoglobin subunit beta protein as a novel marker for time since deposition of bloodstains at crime scenes. *Forensic Science International, 336*, 111348. https://doi.org/10.1016/j.forsciint.2022.111348

Home, P. H., Norman, D. G., Palmer, A., Field, P., & Williams, M. A. (2022). Quantifying forensic investigations involving bloodstain pattern analysis within the UK. *Forensic Science International, 339*, 111424. https://doi.org/10.1016/j.forsciint.2022.111424

Hook, E., Fieldhouse, S., Flatman-Fairs, D., & Williams, G. (2024). Bloodstain classification methods: A critical review and a look to the future. *Science and Justice, 64*(4), 408–420. https://doi.org/10.1016/j.scijus.2024.06.004
https://doi.org/10.1016/j.forsciint.2022.111512

International Association of Bloodstain Pattern Analysts. (2024). *International Association of Bloodstain Pattern Analysts.* Retrieved from https://www.iabpa.org/about_the_iabpa.php

James, S., Kish, P., & Sutton, T. (2005). *Principles of bloodstain analysis*. Boca Raton, Florida: CRC. https://doi.org/10.1201/9781420039467

Jung, H., Jo, Y. S., Ahn, Y. J., Jeong, J. P., & Lim, S. K. (2024). A first step towards a machine learning-based framework for bloodstain classification in forensic science. *Forensic Science International, 365*, 112278. https://doi.org/10.1016/j.forsciint.2024.112278

Kaya, D. O., Karadayi, S., Karadayi, B., & Çetin, G. (2023). Evaluation of the detectability of different ages of bloodstains on fabrics in different washing conditions and at various wavelengths. *Journal of Forensic and Legal Medicine, 94*, 102486. https://doi.org/10.1016/j.jflm.2023.102486

Kerr, D. J. A., & Mason, V. H. (1926). The haemochromogen crystal test for blood. *The British Medical Journal, 1*(3395), 134–136.

Kim, H. J., Lee, Y. R., Lee, S., Kwon, S., Chun, Y. T., Hyun, S. H., … Kang, H. G. (2022). Discovery of donor age markers from bloodstain by LC-MS/MS using a metabolic approach. *International Journal of Legal Medicine, 136*, 297–308. https://doi.org/10.1007/s00414-021-02640-w

Kind, S. S., Patterson, D., & Owen, G. W. (1972). Estimation of the age of dried blood stains by a spectrophotometric method. *Forensic Science*, *1*, 27–54. https://doi.org/10.1016/0300-9432(72)90146-x

Kistenev, Y. V., Borisov, A. V., Samarinova, A. A., Colón-Rodríguez, S., & Lednev, I. K. (2023). A novel Raman spectroscopic method for detecting traces of blood on an interfering substrate. *Scientific Reports, 13*, 5384. https://doi.org/10.1038/s41598-023-31918-9

Kumar, R., Sharma, K., & Sharma, V. (2020). Bloodstain age estimation through infrared spectroscopy and chemometric models. *Science & Justice, 60*(6), 538–546. https://doi.org/10.1016/j.scijus.2020.07.004

Landsteiner, K. (1901). Über Agglutinationserscheinungen normalen menschlichen Blutes [On Agglutination Phenomena of Normal Human Blood]. *Wiener klinische Wochenschrift – The Central European Journal of Medicine, 14,* 1132–1134 [in German].

Latham, H. (2024). Bloodstain pattern analysis methodology. In T. L. Wolson (Ed.), *Handbook of bloodstain pattern analysis* (pp. 327–331). Boca Raton, Florida, United States: CRC Press. https://doi.org/10.4324/9781003163695-21

Lee, Y. R., Lee, S., Kwon, S., Lee, J., & Kang, H. G. (2022). Bloodstain metabolite markers: Discovery and validation for estimating age of bloodstain within 7 days. *Analytical Chemistry, 94*, 13377–13384. https://doi.org/10.1021/acs.analchem.2c01903

Li, B., Beveridge, P., O'Hare, W. T., & Islam, M. (2011). The estimation of the age of a blood stain using reflectance spectroscopy with a microspectrophotometer, spectral pre-processing and linear discriminant analysis. *Forensic Science International*, *212*(1–3), 198–204. https://doi.org/10.1016/j.forsciint.2011.05.031

Li, B., Beveridge, P., O'Hare, W. T., & Islam, M. (2013). The age estimation of blood stains up to 30 days old using visible wavelength hyperspectral image analysis and linear discriminant analysis. *Science & Justice, 53*(3), 270–277. https://doi.org/10.1016/j. scijus.2013.04.004

Li, H., Shen, C., Wang, G., Sun, Q., Yu, K., Li, Z., … Lian, C. (2023). BloodNet: An attention-based deep network for accurate, efficient, and costless bloodstain time since deposition inference. *Briefings in Bioinformatics, 24*(1), bbac557. https://doi.org/10.1093/bib/bbac557

Lin, H., Zhang, Y., Wang, Q., Li, B., Huang, P., & Wang, Z. (2017). Estimation of the age of human bloodstains under the simulated indoor and outdoor crime scene conditions by ATR-FTIR spectroscopy. *Scientific Reports*, *7*, 13254. https://doi.org/10.1038/s41598-017-13725-1

MacDonell, H. L. (1972). *Flight characteristics and stain patterns of human blood*. Washington: National Institute of Law Enforcement and Criminal Justice.

Manasatienkij, C., & Nimnual, A. (2021). Forensic blood stain aging using reverse transcription real-time PCR. *Forensic Science International: Reports, 3*, 100205. https://doi.org/10.1016/j.fsir.2021.100205

Marrone, A., La Russa, D., Montesanto, A., Lagani, V., La Russa, M. F., & Pellegrino, D. (2021). Short and long time bloodstains age determination by colorimetric analysis: A pilot study. *Molecules*, *26*, 6272. https://doi.org/10.3390/molecules26206272

Patterson, D. (1960). Use of reflectance measurements in assessing the colour changes of ageing bloodstains. *Nature*, *187*, 688–689. https://doi.org/10.1038/187688a0

Piotrowski, E. (1895). *Ueber entstehung, form, richtung und ausbreitung der blutspuren nach hiebwunden des kopfes [On the formation, form, direction, and spreading of blood stains after blunt trauma to the head].* Vienna, Austria: University of Vienna [in German].

Rough, R. (2024). Dripping blood: Stains, patterns, and trails. In T. L. Wolson (Ed.), *Handbook of bloodstain pattern analysis* (pp. 158–169). Boca Raton, Florida, United States: CRC Press. https://doi.org/10.4324/9781003163695-8

Rough, R., Batchelor, O., Green, R., & Bainbridge-Smith, A. (2024). An automated method for the generation of bloodstain pattern metrics from images of blood spatter patterns. *Forensic Science International, 363*, 112200. https://doi.org/10.1016/j.forsciint.2024.112200

Schneider, T. D., Kraemer, T., & Steuer, A. E. (2023). Determination of the time since deposition of blood-traces in a forensic context: Application of untargeted LC-HR-MS/MS metabolomics profiling. *Drug Testing and Analysis, 15*(8), 840–852. https://doi.org/10.1002/dta.3480

Schneider, T. D., Roschitzki, B., Grossmann, J., Kraemer, T., & Steuer, A. E. (2022). Determination of the time since deposition of blood traces utilizing a liquid chromatography–mass spectrometry-based proteomics approach. *Analytical Chemistry*, *94*, 10695–10704. https://doi.org/10.1021/acs.analchem.2c01009

Seki, T., Hsiao, Y. Y., Ishizawa, F., Sugano, Y., & Takahashi, Y. (2024). Establishment of a random forest regression model to estimate the age of bloodstains based on temporal colorimetric analysis. *Legal Medicine, 69*, 102343. https://doi.org/10.1016/j.legalmed.2023.102343

Sharma, S., Chophi, R., Jossan, J. K., & Singh, R. (2021). Detection of bloodstains using attenuated total reflectance-Fourier transform infrared spectroscopy supported with PCA and PCA–LDA. *Medicine, Science and the Law*, *61*(4), 292–301. https://doi.org/10.1177/00258024211010926

Sharma, V., & Kumar, R. (2018). Trends of chemometrics in bloodstain investigations. *TrAC Trends in Analytical Chemistry, 107*, 181–195. https://doi.org/10.1016/j.trac.2018.08.006

Shin, J., Choi, S., Yang, J. S., Song, J., Choi, J.S., & Jung, H. I. (2017). Smart forensic phone: Colorimetric analysis of a bloodstain for age estimation using a smartphone. *Sensors and Actuators B: Chemical, 243*, 221–225. https://doi.org/10.1016/j.snb.2016.11.142

Singh, P., Gupta, N., & Rathi, R. (2021). Blood pattern analysis-a review and new findings. *Egyptian Journal of Forensic Sciences*, *11*(9), 1–7. https://doi.org/10.1186/s41935-021-00224-8

Smith, L. (2018, December 13). How a dubious forensic science spread like a virus. *ProPublica*. Retrieved from https://features.propublica.org/blood-spatter-analysis/herbert-macdonell-forensic-evidence-judges-and-courts/

Stojanović, I., Stojanović, J., Šorgić, D., & Čipev, A. (2020). Effect of incomplete sampling description in DNA reports on bloodstain pattern analysis and reconstruction of a crime scene. *Medicine, Science and the Law*, *60*(4), 301–304. https://doi.org/10.1177/0025802420926876

Stotesbury, T., Cossette, M. L., Newell-Bell, T., & Shafer, A. B. A. (2021). An exploratory time since deposition analysis of whole blood using metrics of DNA degradation and visible absorbance spectroscopy. *Pure and Applied Geophysics*, *178*(3), 735–743. https://doi.org/10.1007/s00024-020-02494-0

Strasser, S., Zink, A., Kada, G., Hinterdorfer, P., Peschel, O., Heckl, W. M., Nerlich, A. G., & Thalhammer, S. (2007). Age determination of blood spots in forensic medicine by force spectroscopy. *Forensic Science International, 170*(1), 8–14. https://doi.org/10.1016/j.forsciint.2006.08.023

Sun, H., Dong, Y., Zhang, P., Meng, Y., Wen, W., Li, N., & Guo, Z. (2017). Accurate age estimation of bloodstains based on visible reflectance spectroscopy and chemometrics methods. *IEEE Photonics Journal, 9*(1), 6500614. https://doi.org/10.1109/JPHOT.2017.2651580

SWGSTAIN. (2009). Scientific working group on bloodstain pattern analysis: Recommended terminology. *Forensic Science Communications*, *11*(2), 14–17. Retrieved from https://theiai.org/docs/SWGSTAIN_Terminology.pdf

Thanakiatkrai, P., Yaodam, A., & Kitpipit, T. (2013). Age estimation of bloodstains using smartphones and digital image analysis. *Forensic Science International, 233*, 288–297. https://doi.org/10.1016/j.forsciint.2013.09.027

Tian, L., Chen, H., Ma, R., & Zhang, M. (2022). Estimating time since deposition of bloodstains by scanning electrochemical microscopy. *ChemElectroChem, 9*(17), e202200434. https://doi.org/10.1002/celc.202200434

van Kampen, E. J., & Zijlstra, W. G. (1961). Standardization of hemoglobinometry: II. The hemiglobincyanide method. *Clinica Chimica Acta*, *6*(4), 538–544. https://doi.org/10.1016/0009-8981(61)90145-0

Weber, A. R., & Lednev, I. K. (2020). Crime clock: Analytical studies for approximating time since deposition of bloodstains. *Forensic Chemistry, 19*, 100248. https://doi.org/10.1016/j.forc.2020.100248

Weber, A., Wójtowicz, A., & Lednev, I. K. (2021). Post deposition aging of bloodstains probed by steady-state fluorescence spectroscopy. *Journal of Photochemistry and Photobiology B: Biology, 221*, 112251. https://doi.org/10.1016/j.jphotobiol.2021.112251

Wei, Y., Wang, J., Wang, Q., Cong, B., & Li, S. (2022). The estimation of bloodstain age utilizing circRNAs and mRNAs biomarkers. *Forensic Science International, 338*, 111408. https://doi.org/10.1016/j.forsciint.2022.111408

Yang, F., Qian, J., Qu, H., Ji, Z., Li, J., Hu, W., … Yan, J. (2023). DNA methylation-based age prediction with bloodstains using pyrosequencing and random forest regression. *Electrophoresis*, *44*(9–10), 835–844. https://doi.org/10.1002/elps.202200250

Zadora, G., & Menżyk, A. (2018). In the pursuit of the holy grail of forensic science: Spectroscopic studies on the estimation of time since deposition of bloodstains. *TrAC Trends in Analytical Chemistry, 105*, 137–165. https://doi.org/10.1016/j.trac.2018.04.009

Zhang, R., Wang, P., Chen, J., Tian, Y., & Gao, J. (2023). Age estimation of bloodstains based on Raman spectroscopy and chemometrics. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, *290*, 122284. https://doi.org/10.1016/j.saa.2022.122284

Zou, T., & Stern, H. S. (2022). Towards a likelihood ratio approach for bloodstain pattern analysis. *Forensic Science International, 341*, 111512.

Zou, T., & Stern, H. S. (2025). A Dirichlet process model for directional-linear data with application to bloodstain pattern analysis. *Computational Statistics and Data Analysis, 204*, 108093. https://doi.org/10.1016/j.csda.2024.108093

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Плями крові в судово-медичній експертизі: Від візуального огляду до аналізу зразків та оцінки часу за допомогою ШІ

Анотація. *Кров'яні плями давно служать важливим доказом у судово-медичних розслідуваннях, надаючи відомості про час та природу насильницьких злочинів. У статті прослідковується історичний розвиток аналізу кров'яних плям, від початкової візуальної інспекції до впровадження сучасних методів та технологій. Аналіз кров'яних плям тепер став системною наукою і інтегрував технології штучного інтелекту, надаючи кількісні відомості про механізми розбризкування крові. Для оцінки часу кров'яних плям використовується аналіз ДНК, який дозволяє виявити тимчасові зміни в генетичних матеріалах із деградованих плям крові. Високопродуктивна рідинна хроматографія доповнила дослідження кров'яних плям, кількісно визначаючи біохімічні маркери, що вказують на час з моменту їх відкладення. Спектроскопічні методи, зокрема раманівська та інфрачервона спектроскопія, виявили специфічні молекулярні вібрації, пов'язані з тимчасовою деградацією компонентів крові, тоді як оптичні методи на основі відбиття фотонів, абсорбції та флуоресценції пропонують альтернативні шляхи для оцінки віку кров'яних плям. Колориметрія на базі смартфонів стала економічно ефективним і портативним рішенням, відстежуючи видиму зміну кольору крові від яскраво червоного до темно-коричневого з часом. Більше того, гіперспектральна візуалізація поєднує візуалізацію і спектроскопію, дозволяючи оцінювати вік з просторовою роздільною здатністю, аналізуючи спектральні дані на рівні пікселів. У статті підкреслюється історичний розвиток та технологічні досягнення, які сформували аналіз кров'яних плям у судово-медичній дисципліні. Інтегруючи сучасне обладнання з технологіями ШІ, ця галузь наближається до надійного аналізу на місці. Проте існують проблеми, такі як варіативність середовища, вплив субстратів та стандартизація. Продовження досліджень та валідація є необхідними для вдосконалення цих методів і встановлення стандартизованих протоколів для судових застосувань. Цей історичний та технічний огляд підкреслює трансформуючий вплив міждисциплінарних інновацій на розвиток аналізу кров'яних плям, що поєднує дослідження в лабораторії та практичне застосування в судово-медичних умовах.*

Ключові слова: *аналіз плям крові; оцінка часу; спектроскопія; гіперспектральна візуалізація; колориметрія на смартфоні*

Received 16.12.2024
Received in revised form 25.02.2025
Accepted 27.02.2025