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Dating Bloodstains with Fluorescence Lifetime Measurements

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In May 1871, a young woman was found severely injured in the Eltham district of London, her head brutally smashed with a plaster's hammer. One of the wounds divided the temporal artery, resulting in a large discharge of blood. Several days later, a 20-year old man, her friend and alleged lover, was arrested. Numerous small spots of blood were found on his shirt. The examiner of the case, Dr. Letheby, one of the pioneers of forensic spectral analysis of blood, declined to assign a date to the bloodstains on the shirt and connect the defendant to the murder. "I could not tell the date of these stains, I don't profess to do so—I could not fix any time at which the blood might have got upon the things"^[1]. This statement outweighed the other evidence, the man was sent free, and the crime known as the Eltham murder remains a mystery. Long after that, in 2008, a Florida circuit court judge presiding over a criminal case ruled that "there is no scientifically accepted method of determining the age of a bloodstain"^[2]. The judge granted the defendant's motion to bar testimony concerning the "freshness" of blood found at the crime scene and requested to validate the analysis with dog blood.

These two examples illustrate the important role of bloodstain dating in criminal justice. In the last two hundred years, criminal investigators explored a variety of approaches to establish a reliable quantitative method, from the evaluation of bloodstain colour as shown in TOC, microscopic analysis of red corpuscles and a water solubility scale in the middle of the 19th century^[3], to electrochemistry^[4], electron paramagnetic resonance^[5], atomic force microscopy^[6], polymer chain reaction^[7], and optical spectroscopy^[8] in the 21st century. However, despite large efforts and obvious progress in related bloodstain techniques exemplified by computerized blood splatter analysis^[9], ultra-sensitive mass-spectrometry based chemical composition of blood^[10] and DNA/RNA fingerprinting^[11] bloodstain dating remains an unsolved challenge.

Herein, we demonstrate a novel method for bloodstain dating based on the fluorescence lifetime of blood. We show that the intrinsic fluorescence lifetime quickly and reliably reports the blood age. Our approach is based on a fact that the fluorescence lifetime of tryptophan – the major endogenous fluorophore in blood proteins – is highly sensitive to the protein conformation^[12, 13]. We hypothesized that in the process of blood aging, the major tryptophan bearing proteins, such as albumin and γ -globulins which constitute more than

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95% of the protein mass in blood, undergo structural changes. Consequently, we expected that the time-related degradation of proteins in blood would reflect the blood age.

To verify our hypothesis, we plated fresh blood from different dogs onto plastic Petri dishes. The blood was left exposed to room air and at certain time points, the bloodstain was scraped and dissolved in PBS buffer for lifetime measurements. Each measurement was conducted from three different scratches of blood obtained from four different dogs. For fluorescence lifetime measurements, we utilized a 295 nm subnanosecond pulsed LED in conjunction with the time-resolved single photon counting (TCSPC) technique. Shorter wavelengths potentially excite tyrosine residues leading to energy transfer to tryptophan, while longer wavelengths excite NAD(P)H and other components of blood. These excitations contaminate the tryptophan decay kinetics and thus complicate the lifetime data analysis.

Our results presented in Figure 1, A clearly demonstrate that upon aging, the fluorescence lifetime undergoes a non-linear decrease from ~ 4.0 ns for “fresh” blood almost immediately after drawing to ~ 2.5 ns for a two month old bloodstain, reaching a plateau after ~ 300 hrs. The most drastic change in lifetime occurred in the first 150 hours, or ~ 7 days. Figure 1, B shows the change of the fluorescence decay during the first 7 days after the bloodstain plating, resulting in a decreased slope of the curve, and hence the lowering of the fluorescence lifetime. The two phase exponential modelling of the fluorescence lifetime change with time revealed fast and slow components in the degradation process: fast, (half life ~ 9.7 hrs, 34%) and slow (half life ~ 116 hrs, 66%), $R^2=0.96$. Although the nature of this behaviour is unknown, we attribute the presence of the two phases to the rates of enzymatic degradation of the two major blood proteins: albumin and γ -globulins.

Fluorescence lifetime is an intrinsic property of the fluorophore and refers to the average time the molecule can exist in the excited state prior to emitting a photon. For most of the blood components, the fluorescence lifetime varies from tens of picoseconds to several (up to 15 ns) nanoseconds^[13]. Fluorescence lifetime is largely independent of the wavelength of excitation/emission, fluorophore concentration, and photobleaching^[14]. Since the de-excitation process is associated with an energetically unstable state, fluorescence lifetime is highly sensitive to a great variety of factors. Indeed, the fluorescence lifetime of tryptophan residues in different proteins varies by more than a factor of 100 and is determined by solvent exposure and interactions with other elements of the protein matrix^[15]. Such variance is the reason we are able to monitor blood degradation with fluorescence lifetime.

Serum albumins and γ -globulins comprise more than 95% of the total protein mass in blood. Neat albumin and γ -globulins showed noticeably different fluorescence lifetimes: $\tau_{alb} \sim 6.3 \pm 0.08$ ns and $\tau_{glob} \sim 3.0 \pm 0.07$ ns. The latter was close to the lifetime of free tryptophan with $\tau_{trp} \sim 2.7 \pm 0.15$ ns (see the decay of a free tryptophan in Figure 2, A). Such a difference in fluorescence lifetime is the direct evidence of tryptophan sensitivity to the environment. As expected, albumin (with two Trp residues) and neat tryptophan decays are close to monoexponential, while γ -globulin (with up to ten or even more Trp residues) is multiexponential. Control experiments with aging of neat albumin and γ -globulins, plated in the same manner as the blood, did not present with any fluorescent lifetimes alteration over a 7 day period (Figure 2, B). This suggests that factors other than oxygen and moisture from the air are responsible for lifetime changes in blood. However, under unfolding conditions, the lifetime of albumin experienced gradual decrease with the increase of the urea concentration from initial 6.3 ns to 4.0 ± 0.14 ns in 6M urea solution. In contrast, similar conditions did not affect the lifetime of γ -globulins, indicating its conformation stability (Figure 2, C).

In addition to the proposed change in protein conformation, the release of a variety of organic and inorganic quenchers, such as free amines or unbound Fe-ions, might also contribute to the change of fluorescence lifetime. Despite the extensive studies of the metabolism of blood components in vivo, little is known about their fate outside of the living body. Future studies will delineate a detailed mechanism and reveal the role of enzymes in the blood degradation process. Such information will not only be imperative in understanding blood aging but will also help to take into account a variety of parameters to correctly estimate the date of the bloodstain.

In summary, fluorescence lifetime was shown to be a promising technique to evaluate the age of a bloodstain in a crime scene within the first week. The method is fast (~20 min), reproducible, concentration independent, and requires miniscule amount of the bloodstain with minimal sample preparation. The major improvement of the technique will come from the development of the lifetime imaging technique, where the average fluorescence lifetime is evaluated across the region of interest in the bloodstain. Such an approach will minimize the problems with sampling and provide more accurate results. The measurements can be potentially performed in solid state using a handheld fluorescence lifetime instrument, which could be applied to the bloodstain directly at the crime scene with no additional sample handling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. The Proceedings of the Old Bailey, London's Central Criminal Court, 1674 to 1913. London's CCC; 1871. case t18710710
2. Twelfth Judicial Circuit, 2007-Cf-22337-NC; I.A.B.P.A. News; 2010; p. 29
3. Taylor, AS. The principles and practice of medical jurisprudence. Churchill; London: 1865.
4. Matsuoka T, Taguchi T, Okuda J. Biol Pharm Bull. 1995; 18:1031. [PubMed: 8535389]
5. Fujita Y, Tsuchiya K, Abe S, Takiguchi Y, Kubo S, Sakurai H. Forensic Sci Int. 2005; 152:39. [PubMed: 15939175]
6. Strasser S, Zink A, Kada G, Hinterdorfer P, Peschel O, Heckl WM, Nerlich AG, Thalhammer S. Forensic Sci Int. 2007; 170:8. [PubMed: 17095174]
7. Anderson S, Howard B, Hobbs GR, Bishop CP. Forensic Sci Int. 2005; 148:37. [PubMed: 15607588]
8. Bottonjic-Sehic E, Brown CW, Lamontagne M, Tsaparikos M. Spectroscopy. 2009; 24:42. Bremmer RH, Nadort A, van Leeuwen TG, van Gemert MJ, Aalders MC. Forensic Sci Int. 2010; 206:166. [PubMed: 20729018] Hanson EK, Ballantyne J. PLoS One. 2010; 5:e12830. [PubMed: 20877468]
9. Bevel, T.; Gardner, RM. Bloodstain pattern analysis: with an introduction to crime scene reconstruction. 3rd. CRC Press/Taylor & Francis; Boca Raton: 2008. Peschel O, Kunz S, Rothschild M, Mützel E. Forensic Sci Med Pathol. 2010; 1. [PubMed: 20084553]
10. Li W, Tse FLS. Biomed Chromatogr. 2010; 24:49. [PubMed: 20017122] Maurer HH. Clin Biochem. 2005; 38:310. [PubMed: 15766732]
11. Gill P, Jeffreys AJ, Werrett DJ. Nature. 1985; 318:577. [PubMed: 3840867] Setzer M, Juusola J, Ballantyne J. J Forensic Sci. 2008; 53:296. [PubMed: 18298493]
12. Broos J, Tveen-Jensen K, de Waal E, Hesp BH, Jackson JB, Canters GW, Callis PR. Angew Chem. 2007; 119:5229. Vivian JT, Callis PR. Biophys J. 2001; 80:2093. [PubMed: 11325713]

13. Berezin MY, Achilefu S. Chem Rev. 2010; 110:2641. [PubMed: 20356094]
14. Chen Y, Periasamy A. Microsc Res Tech. 2004; 63:72. [PubMed: 14677136]
15. Alcala JR, Gratton E, Prendergast FG. Biophys J. 1987; 51:597. [PubMed: 3580486] James NG, Ross JA, Mason AB, Jameson DM. Protein Sci. 2010; 19:99. [PubMed: 19916167]

Figure 1.

A: Fluorescence lifetime of blood samples in PBS buffer as function of aging time (only up to 400 hrs is shown). Different points (colours and shapes) represent four different dogs (average from three measurements, error < 4%). The fit (dashed line) was calculated from the two-phase decay modelling. Insert in log scale shows all data up to 1600 hrs. B: Selected decays of blood samples from the same bloodstain in PBS buffer. Top line – day 1, middle line – day 4, lowest line – day 7. (Ex/em 295/350 nm and prompt ex/em 295/320 nm)

Figure 2.

A: Fluorescence decays of γ -globulin, albumin, and tryptophan in PBS buffer suggest varying environments around tryptophan. B: Fluorescence lifetime of albumin (circles) and γ -globulin (squares) plated as blood and treated as bloodstain samples in PBS buffer and at different urea concentrations. Error <4%. (Ex/em 295/350 nm).