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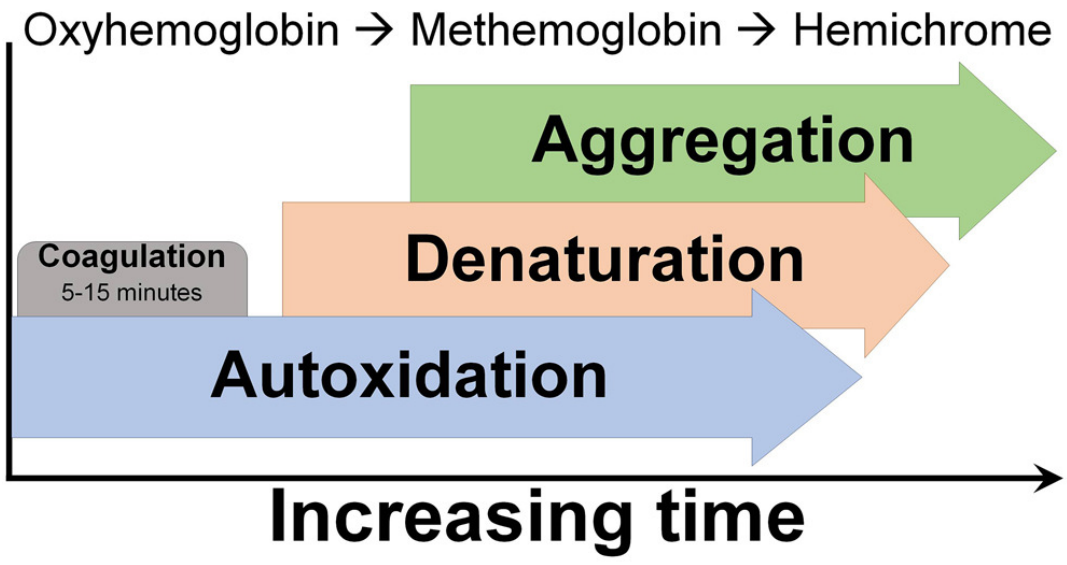
An innovative forensic "clock" for determining the time of a crime

Determining the time since deposition, or age, of bloodstains found at crime scenes is a highly important and integral part of forensic investigations. Knowing this crucial information can help forensic investigators determine the order of events and provide clues for more accurate crime scene reconstruction. For years researchers have attempted to develop reliable ways to predict the age of bloodstains, all with varying success. Currently, no technique is being used as an accepted standard that can provide high precision and accuracy for bloodstain age estimation, which is necessary for a method to be approved and established as admissible in a court of law.

In this work we utilized Raman spectroscopy to analyze bloodstains aged from 1 hour to 168 hours (1 week). Raman spectroscopy, based on inelastic light scattering, has the potential to confirm the identity of bloodstains and detect their (bio)molecular changes over time, all in a nondestructive manner. Light scattering occurs when a sample (i.e., blood) is irradiated by laser light, and some scattered photons change their energy due to excitation of molecular vibrations in blood. We analyze the energy distribution of these photons, which informs us about the (bio)chemical composition of blood samples.

Fresh human peripheral blood was collected by pinprick of a fingertip of healthy consenting adult (18+ years) volunteers. Bloodstains were created by immediately depositing a small amount (~30 µL) of fresh blood, without the addition of preservatives or anticoagulants, onto aluminum foil covered microscope slides and leaving them to age on a laboratory bench top; the temperature or humidity were not controlled. Using fresh whole blood was imperative for the real-world forensic and biomedical relevance of the study since this is how bloodstains would be found naturally at crime scenes.



Scheme 1. A scheme demonstrating the order of changes occurring naturally to a bloodstain as time progresses.

Scheme 1 includes the three main forms of hemoglobin and the changes that occur to bloodstains as they age naturally; starting with coagulation and autoxidation, and followed later on by denaturation and aggregation. Some peaks in the Raman spectra increase in their intensity over time while others decrease or do not change much at all (not shown). Those changes can be attributed to the denaturation and aggregation of hemoglobin in the blood, which was confirmed by two-dimensional correlation spectroscopic analysis (2D CoS).

In order to predict the age of bloodstains, partial least squares regression (PLSR) analysis was implemented. An externally validated calibration curve, generated from the PLSR model built, is shown in Figure 1. Minor differences in predictions of fresh blood are to be expected since variations will occur during spectral acquisition. Nonetheless, the prediction accuracy demonstrated by the PLSR model was high with cross-validated root mean squared error (RMSE) and coefficient of determination (R^2) values of 0.13 and 0.97, respectively. The RMSE of prediction was 0.34, or 2.19 hours, with an R^2 of 0.97.

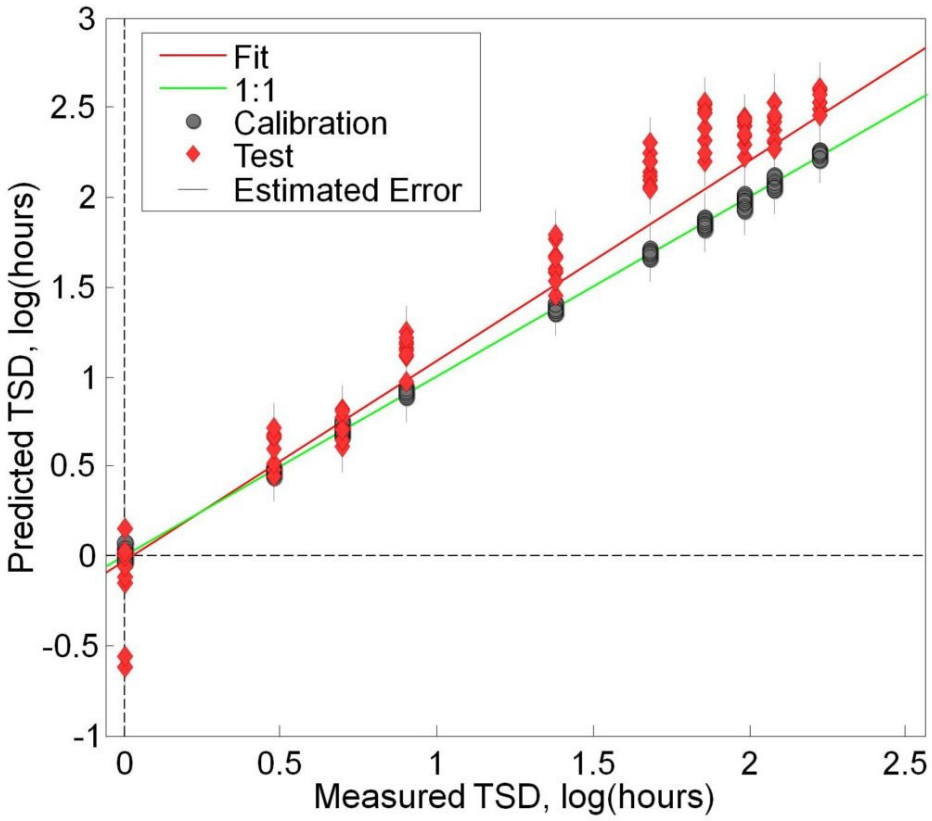


Fig. 1. PLSR plot for blood samples showing age predictions up to 1 week for both internal (grey circles) and external (red diamonds) datasets versus the measured (actual) age, with estimated errors included. The red line demonstrates the actual fit and the green line is the ideal 1:1 fit.

All bloodstains were identified as blood through matching with spectroscopic signatures, and the 2D CoS results indicated a high correlation between several Raman bands and the age of a bloodstain.

Furthermore, our approach demonstrates that Raman spectroscopy can be used as a nondestructive analytical tool for discriminating between bloodstains on the scale of hours to days; 'new' (1 hour) bloodstains were easily distinguished from bloodstains at other ages. This capability is extremely important for forensic investigations to help reconstruct crime scenes and establish the relevant association of multiple bloodstains. The developed methodology shows promise for practical use to predict bloodstain age with a high degree of accuracy. Controlling specific environmental conditions (i.e., humidity and temperature) and testing the methodology with a portable (on-field) instrument, to streamline the integration for use at crime scenes, is part of our future directions.

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