B90 Accurately Estimating the Time Since Deposition (TSD) of Bloodstains Aged for More Than Two Years

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After attending this presentation, attendees will better understand how Raman spectroscopy, coupled with multivariate statistical analysis, can be used to analyze bloodstains aged for more than two years and subsequently: (1) confirm that the stains are blood and not another body fluid; as well as, (2) accurately predict the TSD.

This presentation will impact the forensic science community by demonstrating the ability to expand upon a unique non-destructive approach for the confirmatory identification of blood over time and the accurate estimation of bloodstain age, or TSD. Additionally, this presentation can significantly help with crime scene reconstruction by providing more clues about the time of a crime, or order of events, particularly when a body is not available for physical examination.

The identification of a body fluid stain is an important and necessary aspect of many forensic investigations. For blood in particular, knowing the TSD is highly desired in forensics, but it can be extremely complicated to accurately determine in practice. Although there have been numerous attempts to solve this problem using a variety of different techniques, currently no established well-accepted method exists. Since the amount of suspected blood evidence may be miniscule, it needs to be preserved and analyzed efficiently. Therefore, a non-destructive method to competently identify human blood and predict the TSD would be highly valuable. Raman spectroscopy is a technique that has the potential for both non-destructive confirmatory identification of blood and for detecting (bio) molecular changes over time that can be associated with the age of a bloodstain.

Raman spectroscopy has proven to be a versatile and effective analytical technique for numerous forensic applications, including the identification of drugs, explosives, gunshot residue, inks, and paints. Raman analysis often requires no sample preparation, is considered non-destructive, and has the ability to analyze microscopic amounts of sample. This technique is based on the detection of light that is inelastically scattered by a sample upon irradiation from a monochromatic light source. A Raman spectrum contains numerous distinctive bands that correspond to specific molecular vibrational modes. For blood in particular, Raman spectra provide rich detail and it has already been the subject of analysis in many forensic studies.

For this work, a previously developed Raman spectroscopic methodology for determining the TSD of bloodstains was tested.[1] Raman spectroscopy and chemometric modeling were used to analyze fresh bloodstains stored under ambient conditions for more than two years. A recently developed Support Vector Machines Discriminant Analysis (SVMDA) model was used for blood identification.[2] This model allowed for confirming a fluid's identity as blood through differentiation of blood from four other body fluids (i.e., saliva, semen, sweat, and vaginal fluid).

To provide quantitative predictions of the TSD, Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR) models were built on spectra collected at 14 time points. Both models were internally Cross-Validated (CV) and externally validated. The PLSR and PCR models had a CV Root Mean Squared Error (RMSE) of 0.17 and 0.18, respectively. They both showed a high degree of linearity with an R^2 of 0.98. Also, both models demonstrated similar external TSD prediction abilities with an RMSE of prediction of 0.29 and 0.31 for PLSR and PCR, respectively.

These results demonstrate that Raman spectroscopy can be used as a non-destructive analytical tool for confirming the identity of blood over time and discriminating between bloodstains on the scale of hours to days to years. This is very important for forensic science in helping to reconstruct a crime scene, as well as in establishing the relevant association of multiple bloodstains. This approach shows potential for practical use in the field to predict the TSD with a high degree of accuracy, especially since portable Raman spectrometers are now available. In the future, more work will be conducted regarding analyzing bloodstains left to age under different environmental conditions as well as on different substrates.

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Reference(s):

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2. Muro C.K., Doty K.C., de Souza Fernandes L., Lednev I.K. Forensic body fluid identification and differentiation by Raman spectroscopy. *Forensic Chemistry*, in press.

Bloodstains, Raman Spectroscopy, Chemometrics