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### **B193 A Raman “Spectroscopic Clock” for Bloodstain Age Determination: The First Week After Deposition**

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After attending this presentation, attendees will better understand how Raman spectroscopy, coupled with 2D Correlation Spectroscopy (2D CoS) and chemometrics, can be used to analyze bloodstains, link known molecular changes with spectral variations, and predict the Time Since Deposition (TSD) for up to one week.

This presentation will impact the forensic science community by introducing a unique non-destructive method for confirmatory blood identification and bloodstain age prediction, accompanied by a statistical level of confidence, all of which could potentially be performed at the crime scene.

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 molecular changes over time.

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, paints, and dyes. Raman analysis often requires no sample preparation, is typically 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 has been the subject of analysis in many forensic studies already. Popularity of Raman spectroscopy has been growing in forensic science, especially due to recent advancements in portable instrumentation and the breadth of both current and potential applications.

For this study, a Raman spectroscopic approach was developed for determining the age of bloodstains up to one week old. Raman spectroscopy, along with 2D CoS and statistical modeling, was used to analyze fresh bloodstains at ten time points under ambient conditions. The results of the 2D CoS indicate a high correlation between multiple Raman bands and the age of a bloodstain. A regression model was built to provide quantitative predictions of the TSD, with a cross-validated root mean squared error of 0.13 and an  $R^2$  of 0.97. It was determined that a “new” (one hour old) bloodstain could be easily distinguished from bloodstains at other ages, which is very important for forensic science in helping to establish the relevant association of multiple bloodstains. Additionally, all bloodstains were identified as blood by comparing the measured spectra to multidimensional body fluid spectroscopic signatures. These results demonstrate that Raman spectroscopy can be used as a non-destructive analytical tool for discriminating between bloodstains on the scale of hours to days. This approach shows promise for immediate practical use in the field to predict the time since deposition with a high degree of accuracy.

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#### **Raman Spectroscopy, Bloodstain Age, Chemometrics**