A4 Substrate Interference and the Spectroscopic Identification of Body Fluids

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After attending this presentation, attendees will better understand recent methodological advancements toward the non-destructive, on-field detection of body fluids using Raman spectroscopy. Specifically, the issue of substrate interference will be addressed. Attendees learn the research goal and will be presented with the data treatment and instrumental factors, which would be a benefit in this regard. This work has specific implications toward the development of a specialized, automatic instrument, which could be developed to identify body fluids at a crime scene.

This presentation will impact the forensic science community by noting the presence of Raman spectroscopy to have ample applications to forensic sciences, especially in the area of crime scene investigation.

Raman spectroscopy has been found to have ample applications to forensic sciences, especially in the area of crime scene investigation. The technique is based on the detection of scattered light by a sample upon irradiation by a laser light source. This approach is typically rapid, non-destructive, field applicable and confirmatory in the identification of unknowns. The identification of body fluids using this technique would represent a significant improvement over the current methodology, which encompasses a range of destructive chemical assays that provide only presumptive identification. However, the issue of substrate interference is a major hurdle inherent in this approach. With Raman spectroscopy, body fluids exhibit a weak-to-moderate signal while the signal from the material directly underneath can be quite strong. Eliminating the substrates' interference is a prerequisite to confident identification.

Although substrate interference is studied here in the scope of body fluid identification, it is an ubiquitous issue in rapid spectroscopic analysis methods. Until now, the solution to the problem of substrate interference has been to collect the sample for later analysis on a preferential, non-interfering surface. However, the requirements of the forensic community considerably favor analysis *in situ*.

Using blood and semen as model fluids, substrate interference is explored using common materials expected to be present at a crime scene (fabrics, glass, tile, skin, etc.). A Renishaw Raman microscope with variable laser excitation was used in conjunction with a motorized automatic stage. Matlab and WiRE software was used for data interpretation and analysis. A variety of experimental parameters were evaluated with simulated evidence to identify the best conditions to simplify identification. Such parameters evaluated were objective strength, laser excitation, simple spectral subtraction, advanced automatic subtraction, and statistical modeling.

The fluid in question and the nature of the substrate were found to be determinative of the most favorable experimental conditions. In the simplest example, the interference from glass beneath a bloodstain is negligible with careful selection of laser excitation. An example of a complex scenario would be detecting semen on a blended dyed fabric. For this type of evidence, statistical modeling was required to find areas of high fluid concentration before spectral subtraction. It was also discovered that blood could be easily identified from a cotton collection swab after mild data treatment. Identification is possible using auto correlation and previously developed Raman spectroscopic signatures, which provide a statistical measure of certainty.

Substrate interference is one of the final analytical obstacles impeding real-world Raman spectroscopic identification of body fluids. This work demonstrates that a non-specialized common Raman instrument and modest data treatment are capable of overcoming this challenge.

Spectroscopy, Serology, Biological Evidence

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