



B184 Recent Progress in the Development of a Surface-Enhanced Raman Spectroscopy (SERS) Platform for Rapid Identification of Trace Amounts of Human Body Fluids

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After attending this presentation, attendees will gain an appreciation for the developing advances in a SERS-based platform that may result in a new capability for forensics investigators at crime scenes to detect and identify trace amounts of human body fluids with a rapid, easy-to-use single instrument.

This presentation will impact the forensic science community by providing quantitative results of the use of a SERS-based methodology for the identification of dried blood, semen, saliva, vaginal fluid, menstrual blood, and urine from a single portable platform. No such capabilities currently exist in the forensic science community.

The identification of trace amounts of human body fluids, such as blood, semen, vaginal fluid, saliva, and urine, is often central to understanding events at a crime scene. Currently, a variety of tests for detection and confirmatory identification for each of the body fluid types may be carried out. For example, Kastle-Meyer, Takayama, or antibody immunochromatographic strip tests for blood, Prostate Specific Antigen (PSA) detection for semen, Lugol's iodine reaction or messenger RNA (mRNA) -based hormone detection for vaginal fluid, and alpha amylase detection of saliva; however, these individual tests have limitations in terms of sensitivity, specificity, and on-site portability in addition to speed. A portable SERS-based methodology offers the possibility of a single-platform instrument for confirmatory identification at crime scenes with higher sensitivity and specificity and is faster than techniques currently commercially available to crime scene forensic investigators. Progress in the development of a portable, universal body fluid-detection platform is described here.

SERS spectra of dried blood, semen, vaginal fluid, saliva, and urine from multiple donors (360 spectra) were collected by a simple sample protocol which uses 1nL-100nL of body fluid and exhibits reproducible SERS spectra on Au nanostructured substrates.¹ A Partial Least Squares Discriminant Analysis (PLSDA) -based classification procedure results in 98.0% sensitivity and 99.5% specificity. The novel aspect of this classification procedure that permits this robust performance is that the inputs to the PLSDA are barcode representations based on the second derivative of the SERS spectra.² In a further test of methodology, 60 SERS spectra of semen from multiple donors that were not used to build the model were used to challenge this identification procedure. The PLSDA correctly identifies 58/60 SERS spectra, and the resulting set of 360 spectra shows (cross-validation) 96.7% sensitivity and 99.2% specificity.

When menstrual blood is added to this developing SERS library, it is identified as a separate body fluid and the resulting six body fluid PLSDA-classification model results in 96.1% sensitivity and 99.2% specificity. Acquisition of each spectrum requires about 10sec of data collection time and illumination by ~1mW of 785nm laser power, thus enabling the rapid and readily portable nature of this technique.

By expanding the size of the data base, spectral variation due to donor variability was found to be limited sufficiently to allow robust identification of human body fluid types. Several key components of each body fluid have been identified. Molecular vibrational signatures of uric acid, hypoxanthine, protein, and hemoglobin are seen in dried blood SERS spectra; protein, hypoxanthine, and adenine are seen in vaginal fluid spectra; protein, hypoxanthine, and xanthine are seen in semen; and phenylalanine and protein are seen in saliva. These studies intend to show that the SERS spectra of dried blood, vaginal fluid, and semen on various materials such as fabrics, carpet, or glass will have minimal variation and allow identification via the barcode-based PLSDA procedure. Mixture resolution of interest to sexual assault cases (i.e., semen and vaginal fluid, menstrual blood and semen, saliva and vaginal fluid) will be demonstrated.

The quantitative results presented will demonstrate that these most recent advances in the development of SERS for confirmatory identification of trace amounts of body fluids at crime scenes has the potential to be a rapid, sensitive, easy-to-use, portable tool for forensic investigators in the near future.



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Reference(s):

1. Fore J.L., Mei Z., Zegarelli K., Irvine J., Scott B.L., Lemler P., Premasiri W.R., Brodeur A.N., and Ziegler L.D. Body Fluid Analysis by Surface Enhanced Raman Spectroscopy for Forensic Applications. (in preparation for *For. Sci Int.*).
 2. Patel I.S., Premasiri W.R., Moir D.T., Ziegler L.D. Barcoding bacterial cells: A SERS based methodology for pathogen identification. *J Raman Spectrosc* 2008;39:1660.
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