Final Summary Overview

Department of Justice, National Institute of Justice Grant Number: 2014-DN-BX-K021
Title*: Body fluid analysis by surface enhanced Raman spectroscopy for forensic applications*
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Executive summary: Surface enhanced Raman spectroscopy, combined with statistical analyses, provides rapid (~minutes) detection and identification of trace amounts of blood, semen, vaginal fluid and saliva and their mixtures. Donor variability is less than the difference between body fluid signatures. The signatures are robust over time (> months) and the molecular components of the body fluid spectra are identified. Trace blood stains can be identified following luminol based location protocols and distinguished from household agents. Animal blood species can also be identified and blood detection sensitivities exceed existing standard methodologies. This nondestructive, portable platform uses minimal sample material amounts allowing for subsequent additional tests of suspected body fluid stains.

Purpose of the project:

There are already a number of specific tests for both presumptive and confirmatory identification of trace amounts of body fluids found at crime scenes based on a wide variety of experimental techniques. Currently, the forensic identification of biological stains is performed one body fluid at a time and furthermore, many tests cannot be performed on-scene as they require extraction or incubation periods of several hours, or the use of laboratory-based, non-portable equipment.

The major goal of this project is to determine and develop the capabilities of an optically based laser light scattering technique, surface enhanced Raman spectroscopy (SERS), for the detection, identification, and characterization of trace amounts of blood, semen, vaginal fluid, and saliva for forensic purposes. The successful development of this methodology will lead to *a single optical platform for the rapid, sensitive, easy-to-use, cost-effective, on-site, detection and identification of human body fluids at a crime scene.* Currently, there is no such single technology to accomplish this, aside from the speed and on-site identification capabilities that SERS can provide for trace amounts of body fluids.

Placing a portable all-in-one identification platform like SERS in the hands of forensic investigators will allow for more comprehensive, conclusive testing to be performed on each stain without the need for multiple, time-consuming assays, increase the likelihood that probative evidence is collected and submitted for laboratory DNA testing, and reduce the time and resources spent documenting, collecting and packaging routine non-biological stains (detergents, cosmetics and household chemicals) which may be identified by alternate light sources or for potential bloodstains, located through the application of luminol.

Although the portability of the SERS instrument makes it ideal for field use, it need not be limited to crime scenes. SERS body fluid testing in forensic laboratories could also be utilized to aid in selecting the most informative stains for costly DNA testing. Because minimal amounts of sample are needed for SERS testing, this leaves sufficient material or material patterns available for other tests.

Project design and methods:

Raman spectroscopy provides many advantages for biomedical applications. It is a label-free, rapid, easy-to-use, light scattering technique that can identify the components in a complex mixture since each component offers it's own unique spectral signature. However, Raman is a relatively weak phenomenon, thus typically hundreds of spectra excited by tens of mW are needed to acquire Raman spectra of pure body fluids. SERS is well-known to effectively enhance Raman cross-section by as much as 10^8 for molecules in the proximity of

nanostructured metal (Au or Ag) surfaces. The signal size amplification of SERS offers several important advantages for forensic analyses, including: (1) greatly improved sensitivity resulting in the identification of trace amounts of diluted body fluids that may elude visual recognition and are not detectable by normal Raman, (2) SERS offers sensitivity to different molecular species in a complex mixture than found for normal Raman and thus improving discriminations via SERS as compared to normal Raman, (3) broad background fluorescence, often observed in Raman spectra of biological materials and cited as a challenge and complication for normal Raman identification methodologies of forensic body fluids is quenched and hence absent in SERS spectra, (4) signal acquisition can be accomplished much faster and at much lower laser excitation power enabling more rapid identification and portability. All of these attributes are realized by the results of this NIJ study.

Fig. 1. SERS Au nanoparticle covered chip (2 mm) on a glass slide compared with dime.

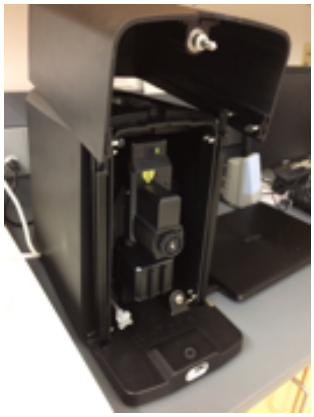

Fig. 2. Portable, high performance Raman microscope (50x objective, 25 lb,, battery powered).

SERS spectra are obtained by placing ~1 μL of sample solution on the gold or silver nanoparticle-covered "chip", shown in Fig. 1, and then placing this chip at the focus of a Raman microscope. Either a research grade laboratory instrument (Renishaw, Inc.RM-2000) or a portable Raman microscope, shown in Fig .2, equipped with a 785 nm excitation laser diode, were used for these studies. The SERS substrates (Au or Ag "chips"), portable microscope and statistical software procedures for spectral classification and identification had already been developed in our laboratory for previous SERS biomedical efforts, and thus we are perfectly positioned to bring this optically based technology to the forensic science community.

Summary of project major findings:

Ability to use SERS to distinguish stains of major body fluids.

Probably the most valuable result of this project is the finding that SERS spectra can be used to quantitatively identify and distinguish different human body fluids stains. The effectiveness of this technology to identify different dried body fluids by their SERS signature is shown in Figs. 3 - 6. SERS spectra of 24 hour dried strains of human blood, vaginal fluid, semen, saliva and urine are shown in Fig. 3. The solid lines correspond to the averaged spectra from two donors each (30 spectra). Spectral acquisition time for each spectrum is about 10 seconds excited by < 1mW (low laser power) at 785 nm of a laser diode. In order to acquire these spectra 1 μL of water or saline solution are dropped on the dried stain and then pulled back into the pipet and placed on the SERS substrate (Fig. 1). (Some variations of this simple procedure are described below.) No special training is required for this dried sample "extraction" protocol. The shaded regions of the body fluid spectra (Fig. 3) are the standard deviations at each wavenumber for the averaged normalized spectra and indicate the excellent degree of reproducibility obtained for the SERS

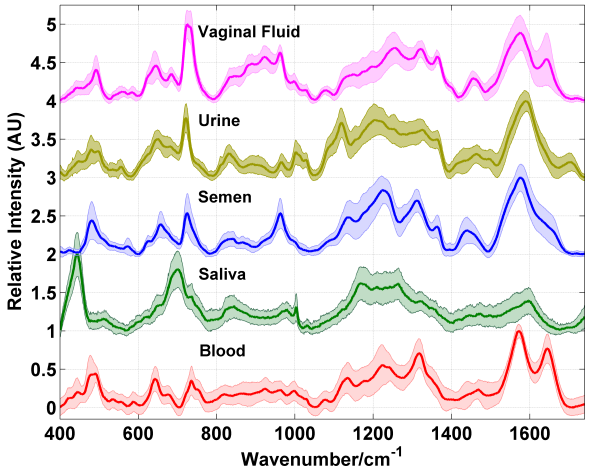

Fig. 3. SERS spectra of dried human body fluids. Shaded regions correspond to standard deviation of 60 spectra (2 donors each body fluid type).

spectra of these dried body fluids. In addition to the high quality of this data, no fluorescence backgrounds are observed unlike normal Raman, and spectral difference are clearly evident for these different body fluid types.

The results of quantitative procedure for body fluid classification and identification is illustrated in Fig. 4. SERS spectra are subject to our "barcode" procedure, i.e. spectra are converted to a series of "ones" or "zeros" based on the sign of the second derivative at each wavenumber and then a partial least squares discriminant analysis (PLS-DA) is carried for classification. PLS-DA is a well established statistical methodology, and when combined with our patented barcode procedure, results in highly successful identification of body fluids via SERS as shown in Fig. 4. The 300 spectra that contribute to the averaged spectra in Fig. 3 are classified by this PLS-DA treatment with 98% sensitivity and 99.5% specificity as given by standard cross-validation procedures.

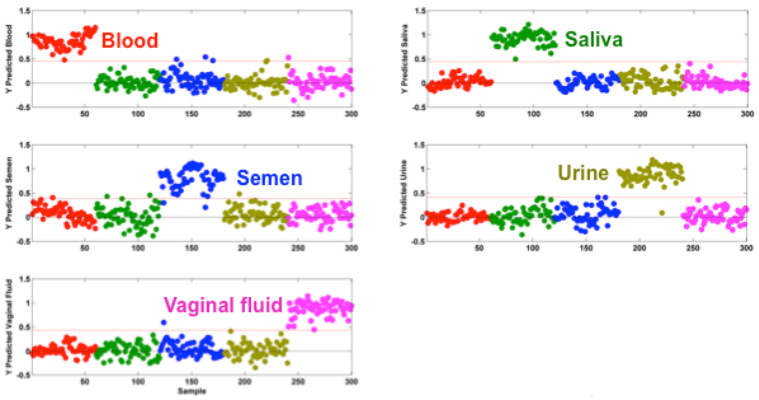

Fig. 4. Barcode PLS-DA classification of 300 SERS spectra of human body fluids (2 donors each); blood, semen, saliva, vaginal fluid and urine. Cross validation results are excellent: 98.0% sensitivity and 99.5% specificity.

Identification of an unknown body fluid is determined by group membership via this PLS-DA classification procedure in the body fluid library. To illustrate, we challenged the classification model with 60 spectra of dried semen stains from donors *not used* to create the classification model. The PLA-DA results are shown in Fig .5 where the "unknown" spectra are the blue data points on the right hand side in the classification panels and are found to match the library semen SERS spectra. 58/60 spectra were correctly identified and the resulting analysis of 360 SERS spectra exhibited analytical sensitivity of 96.7% and specificity of 99.2%. These excellent results are achieved nearly instantaneously after spectral acquisition.

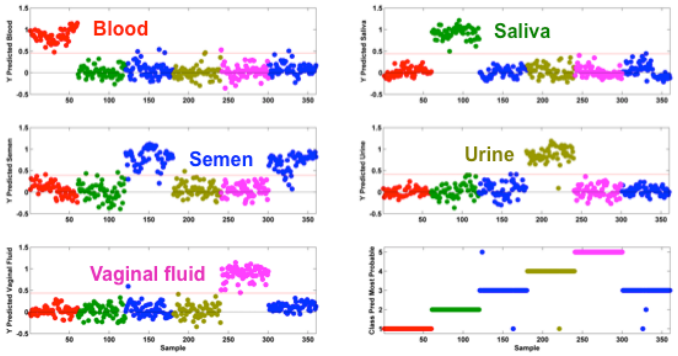

Figure 5. PLS-DA classification model tested the ability of this SERS platform to correctly identify dried semen stains. 58/60 SERS spectra were correctly identified by this procedure and represented by the blue group of spectra on the far right hand side of these panels.

Another figure, demonstrating how this SERS library of body fluid spectra would be employed for dried stain identification is Fig. 6. The SERS spectrum of an unknown dried body fluid was identified by including the spectrum of the "unknown" in the barcode PLS-DA procedure. As seen in Fig. 6, this spectrum classified with the semen spectra already in the library thus establishing the correct identity of this

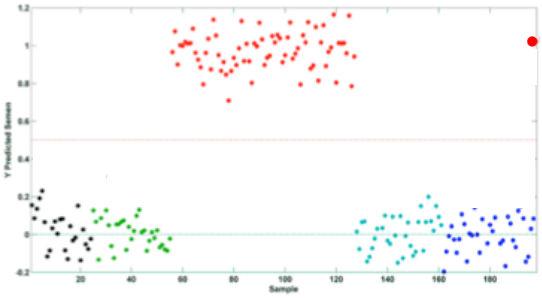

Fig. 6. The PLS-DA and SERS body fluid library are shown to successfully identify an unknown dried body fluid (red dot) as semen.

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dried stain in minutes.

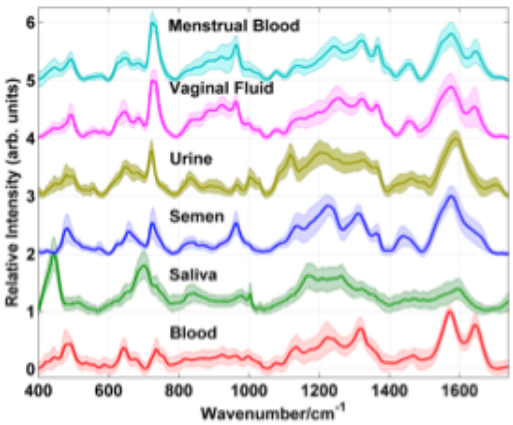

Fig. 7. SERS spectra of six dried body fluid stains including menstrual blood.

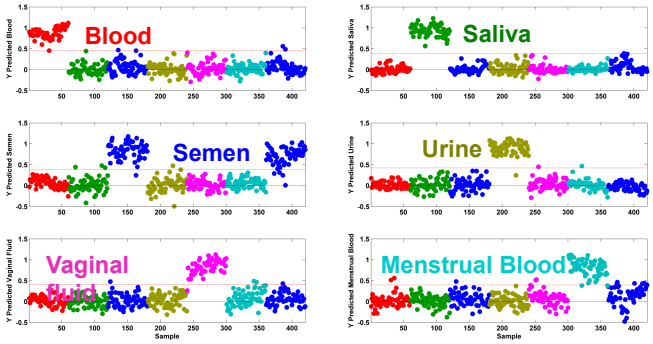

Fig. 8. Barcode PLS-DA classification of SERS spectra of; blood, semen, saliva, vaginal fluid, urine and menstrual blood. 96.5% sensitivity and 99.4% specificity achieved and unknown dried semen identified correctly and distinguished from vaginal fluid and menstrual blood.

Although not part of the original experimental plan, we pushed the methodology further and included SERS spectra of dried stains of menstrual blood in this classification procedure. The resulting SERS spectra are shown in Fig. 7 and corresponding PLS-DA results in 96.1% sensitivity and 99.1% specificity for this six-body fluid library. When the 60 SERS spectra of semen from donors not used to create the SERS library are included they are correctly identified with 96.5% sensitivity and 99.4% specificity as shown in Fig. 8. The ability to unequivocally and rapidly distinguish dried stains of vaginal fluid, semen and menstrual blood is an important outcome for the use of this SERS methodology for the investigation of sexual assault cases.

B. SERS sensitivity and limits of detection

The specificity shown above shows how readily trace amounts of neat body fluid samples can be distinguished on a single platform. This procedure is faster than normal Raman and may offer a more robust statistical analysis procedure since broad fluorescent background are absent and spectral differences are more readily evident. However, one key additional attribute of SERS is sample sensitivity that further distinguishes this approach from normal Raman. In one demonstration of this sensitivity, SERS spectra of a 1 µL diluted whole blood dried (24 hrs) stain were obtained as a function of dilution (Fig. 9). This sample type mimics a crime scene "clean up". SERS spectra, dominated by the characteristic hemoglobin SERS signature, are readily observed for dried blood sample diluted by more than 10,000 times. At the highest dilutions no red color can be seen by eye but such samples result in strong SERS spectra as evident in Fig. 9. Furthermore, at these dilutions *no normal Raman spectrum can be observed*. RSID strip test (immuno-chromatographic assay) can detect 1 µL whole blood (http://www.ifi-test.com/pdf/Blood_Tech_Insert.pdf). These results demonstrate that SERS can produce an identifiable signature with < 100 pL blood (total volume) or in other words a volume of blood that is 10^4 times less than one of the most sensitive current forensic techniques for blood identification.

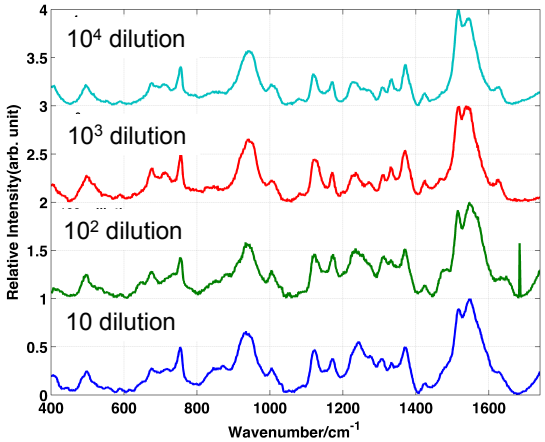

Fig. 9. SERS spectra of dried diluted human blood as a function of dilution. The consistent SERS spectrum is that of hemoglobin. A normal Raman spectrum cannot be observed from these dried stains of diluted blood.

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Similarly diluted semen, vaginal fluid and saliva can also be detected and identified by SERS. Components of saliva and semen can be detected for dilutions on the order of 10^3. See Figs. 10 for the SERS spectra of stains of dried diluted semen stains for up to 10^3 volume diluions Vaginal fluid samples were collected from swabs from volunteers and then extracted from a portion of the sample swab placed in 10μL of water. 1μL of this solution is placed on the SERS substrate. Thus, this optimized sample protocol already sampled a vaginal fluid at relatively low concentration. Normal Raman spectrum *could not* be obtained for this diluted vaginal fluid sample preparation or for the 10^3 diluted semen and saliva samples.

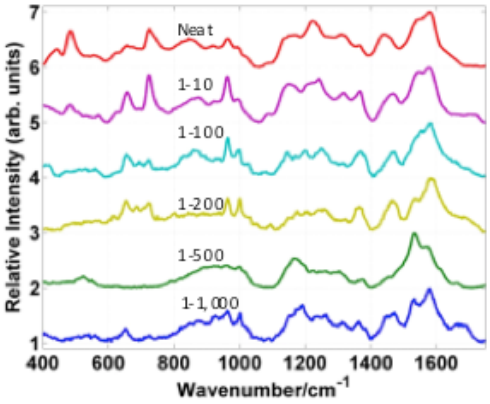

Fig. 10. Dilution effects on dried semen. Dilution by 1000 still results in a characteristic SERS spectrum.

Blood identification after Luminol location

A standard current methodology for presumptive identification of human blood is the characteristic blue emission following application of luminol to suspected or potential blood stains at a crime scene. However, the problem with this rapid and convenient crime scene methodology is that many household items, for example household cleaners such as bleach, can cause this same bluish emission. Another very significant outcome of this work is the discovery that after location of a *potential* blood stain by luminol SERS can provide confirmatory identification. For example, the SERS spectrum of a 100-fold dilution of a dried blood stain treated with luminol is readily recognized as the SERS hemoglobin spectrum of a 10^3 diluted dried blood stain as shown in Fig. 11. The corresponding SERS spectrum of diluted bleach stain which also gives the characteristic blue glow shows a very different signature making positive identification of blood, via the unique hemoglobin signal, trivial to distinguish from household cleaners. This development will transform the luminol test from presumptive to confirmatory when combined with SERS detection capabilities. We recently developed an even better protocol for the SERS detection of dried blood stains (see below) and we are currently working on incorporating this simple improvement into the luminol/SERS procedure and anticipate even greater blood sensitivity levels as a result.

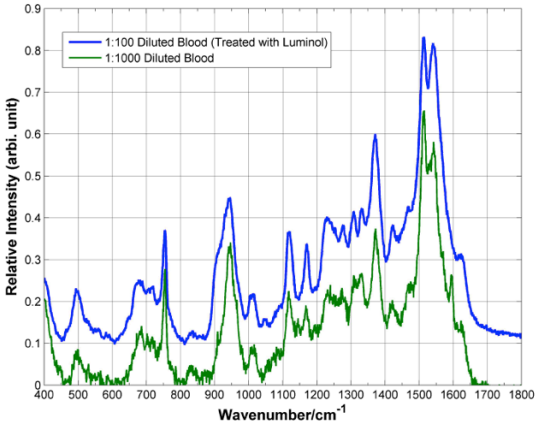

Fig. 11. SERS spectrum of dried diluted human blood compared with dried diluted blood sprayed by luminol. The SERS signature is dominated by hemoglobin. No SERS signature of bleach, which gives the same blue emission as blood, cannot be detected in SERS.

Au vs. Ag nanoparticle effects on SERS signatures of human body fluids.

Blood: We can produce both Au and Ag nanoparticle covered substrates. Originally, we had envisioned using only Au substrates for this project but this introduces another degree of freedom for potentially enhanced body fluid identification. For most complex biological samples, 785 nm excited SERS spectra are very comparable (although not completely identical due to metal dependent molecular interactions) on these two nanostructured metal surfaces. However, for two forensically relevant biological compounds, significant spectral differences are observed on these two metal substrates. This is a useful result because: (1) it provides additional confirmatory signals for body fluid identification and (2) for comparisons with other SERS workers. Both hemoglobin and proteins have very different SERS signatures on Ag and Au

substrates. For example, SERS spectra of dried *neat* blood samples on Au and Ag substrates are shown in Fig. 12. The Au spectrum via our standard 1μL drop and pipette transfer procedure is due to a variety of biological components (see below). However, the SERS spectrum on Ag is dominated by a product of an instantaneous redox reaction involving hemoglobin and yields a unique and extremely strong SERS signature that only results from blood. These results are compared with hematin, a hydroxide substituent Fe porphyrin compound in Fig. 12, to illustrate that the redox product appears on the Ag but not the Au SERS substrate, and it is very similar to hematin SERS spectrum. This additional SERS based test for hemoglobin, offers another simple procedure for confirmatory blood identification.

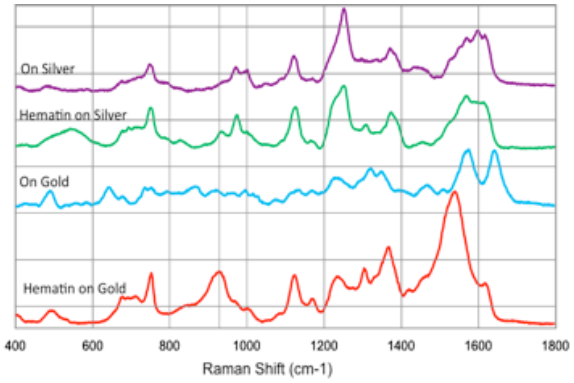

Fig. 12. SERS spectra of dried blood on Ag and Au substrates compared to SERS spectra of hematin. The similarity of the SERS spectra of dried blood and hematin on Ag

Vaginal fluid: We also recently discovered that for vaginal fluid, SERS spectra on Au and Ag are different. We've determined that this is due to the different inherent signatures that proteins display on our Ag and Au SERS substrates and proteins make a recognizable contribution to the SERS vaginal fluid signature. Note how the broad features in the vaginal fluid spectra on Au (Fig. 3 and upper panel in Fig. 13) are no longer present in the same fluid on Ag (lower panel Fig. 13). We attribute this to the more structurally perturbing effects of Ag ions as compared to Au ions. Again, this effect may be exploited to improve detection and identification of vaginal fluid.

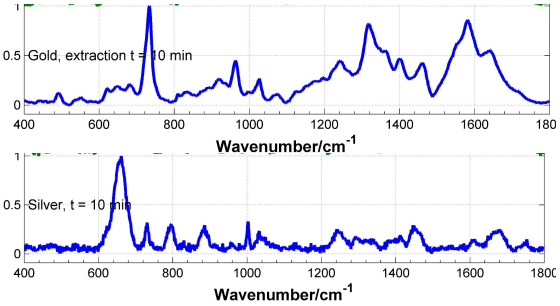

Fig. 13. Comparison of SERS spectra of same dried vaginal fluid sample on Au (upper) and Ag (lower) nanoparticle substrates. Signatures of aggregated proteins vanishes in the Ag spectrum.

Acetic acid effect on blood: We discovered that when a dried blood stain is extracted for SERS signal acquisition with 1 μL of 50% acetic acid (a very weak acid), a highly robust SERS spectrum of hemoglobin is obtained. (See Fig. 14.) In contrast, when 1 μL water or saline are used for this purpose a distinct but characteristic spectrum is observed as show in Fig. 3. While dried blood is properly identified by this spectrum, use of the acetic acid protocol may further improve analytical sensitivity and specificity, and the resolution of blood containing mixtures. The weak acid facilitates the heme prophyrin escaping from both the protein and red blood cell resulting in an enhanced, i.e. more intense hemoglobin signature. Sensitivities of > 1 part blood in 10^4 dilution in blood are readily observed with excellent S/N when the acetic acid solution is used. Given the S/N and larger signal size for the acetic acid procedure, we believe even higher sensitivities could be achievable with additional efforts.

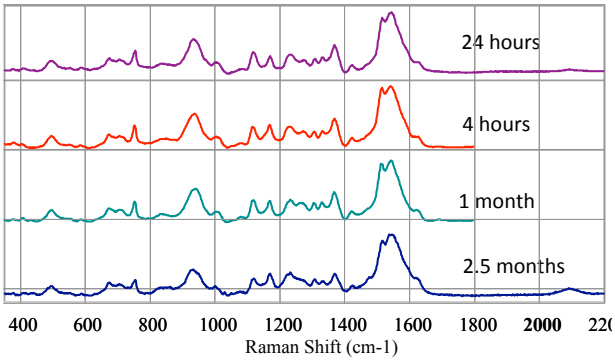

Fig. 14. SERS spectrum of dried human blood obtained by extraction with 1 μL of 50% acetic acid. The spectrum is very robust and does not appear to change with time, at least over several months. Great S/N is obtained with this protocol.

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SERS spectral molecular components

Molecular components contributing to the SERS spectra of human body fluids have been partially identified. In brief, the principal molecular components that we have identified contributing to the SERS spectra of the four key body fluids of interest on Au nanoparticle covered substrates, prepared by the water or saline solution extraction protocol, are:

Blood – uric acid, hypoxanthine, HSA, hemoglobin
Semen – protein, hypoxanthine, xanthine
Saliva – phenylalanine, protein, thiocyanate (SCN^-)
Vaginal fluid – protein (HSA), hypoxanthine, adenine

For example, the contributions of the key molecular components to the SERS spectrum of vaginal fluids are shown in Fig. 15. As mentioned above, on Ag substrates the SERS spectrum of blood is just that of a redox product of the heme group and when extracted by acetic acid solution, the spectrum of dried blood is exclusively that of hemoglobin. Assignment of all the vaginal fluid on Ag spectral features has not been completed. However, vibrational bands associate with aromatic residues of proteins are clearly identifiable.

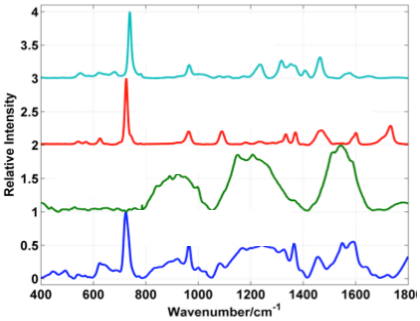

Fig. 15. Identified components of vaginal fluid that account for many features of the SERS spectrum on Au substrates. (HAS, human serum albumin).

Body fluid mixtures.

Since each body fluid offers a unique spectrum resolution of body fluid mixtures can be accomplished via SERS. For example, considering mixtures of semen and vaginal fluid which can be critical body fluids mixtures for identification in sexual assault cases, we were able to show with 96.4% sensitivity and 96.3% specificity via a PLS-DA analysis, that a specific semen-vaginal fluid mixture (1:1) could be distinguished form the pure vaginal fluid and pure semen SERS spectra, as shown in Fig. 16. Although a PLS-DA classification model was used here, further work is needed to determine if this is the best statistical procedure for mixture resolution.

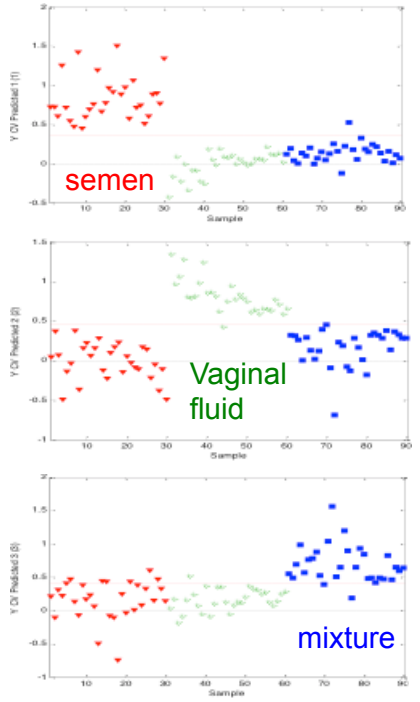

Fig. 16. PLS-DA analysis of the SERS spectra of pure semen, pure vaginal fluid (green) and the 1:1 vaginal fluid-semen mixture (blue). 96.4% sensitivity and 96.3% specificity is achieved for this classification procedure.

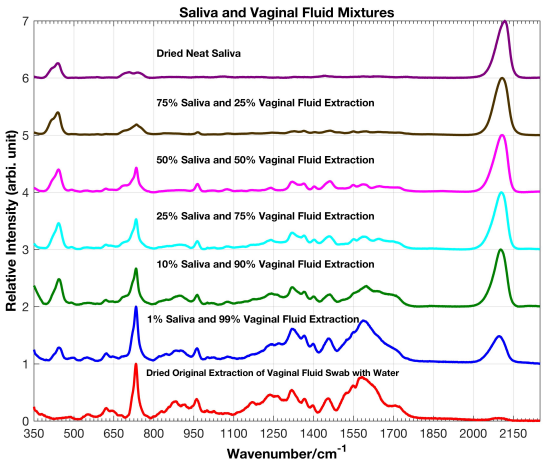

Fig. 17. SERS spectra of vaginal fluid and saliva mixtures. The SCN^- band at ~2100 is a clear indicator of saliva in this mixture down to < 1% saliva (blue spectrum).

SERS analysis of other binary body fluid mixtures were also obtained including saliva and blood, and vaginal fluid and saliva (shown in Fig. 17) and at least empirically show that the two body fluid mixture could be identified. Due to the distinct and characteristic SCN^- component of saliva, 1% of saliva in vaginal fluid can be detected by SERS as evident in Fig. 17 in just a matter of

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minutes. Again, such an identification, in terms of speed and sensitivity, could not be accomplished by normal Raman.

Body fluid aging.

Another goal of this project was to determine if the SERS spectrum of the dried body fluid stain could determine how long the sample had been outside the body in order to date a crime event. The down side of an identification procedure that was sensitive to *in vitro* degradation processes is that identification of body fluid may not be robust due to the changing molecular signatures. For the most part, the SERS signatures of blood, vaginal fluid, semen or saliva are robust with environmental exposure and do not show clear evidence of aging effects. For example, the SERS signature of saliva was stable and unchanged over a 20 month period as shown in Fig. 18. Similarly, SERS spectra of a dried human blood stain obtained by our 50% acetic acid protocol shows no significant changes with time over a 2.5 month period.

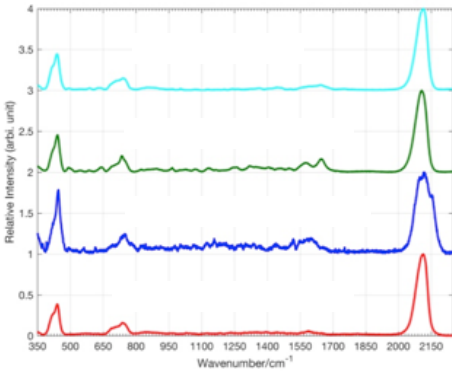

Fig. 18. SERS spectrum of a dried saliva sample from a single donor over a period of 20 months is essentially unchanged.

SERS spectra of vaginal fluid spectra do not vary significantly as a function of time as well and appear independent of storage time and conditions. For example, as shown in Fig. 19 the SERS spectrum of a vaginal fluid sample from a frozen single donor swab is essentially unchanged over the studied 6 month period. Furthermore, SERS spectra of vaginal fluid are constant throughout the menstrual cycle thus exhibiting additional robustness as shown in Fig. 20.

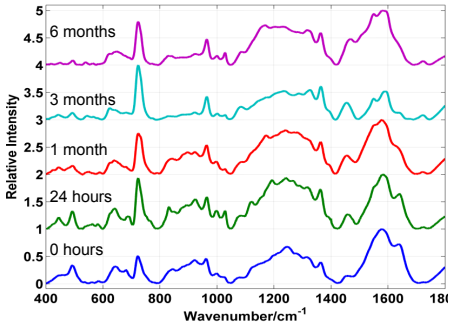

Fig. 19. SERS spectra of vaginal fluid from a frozen single donor swab over a period of 6 months (10x dilution).

Donor variability.

In order to build a spectroscopic platform that can distinguish one body fluid type from another, it is essential that the spectral differences between donors has to be smaller than the differences between the average spectra of the body fluid types. Our data demonstrates the donor variability is indeed less than the differences between the SERS spectra of blood, semen, vaginal fluid and saliva. This is implicit in the results of the statistical treatments displayed in Fig. 4 – 7 and partially evident from comparisons of the body fluid spectra shown in Fig. 3. However, we show this more explicitly for some body fluid types below. SERS spectra of saliva from 12 donors (Fig. 21) and of semen from four donors (Fig. 22) reveal that the donor variability is not significant. When the SERS spectra of dried human blood are analyzed particularly via the 50% acetic acid protocol, the spectrum is exclusively that of hemoglobin and thus no donor variability is evident at all. Furthermore, the "barcode" classification procedure used for this study is not very sensitive to variations in relative intensity of components to a SERS spectrum since it is determined by the sign of the second derivative spectrum, i.e. the relative curvatures and not

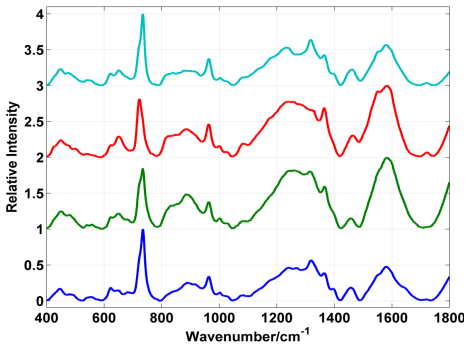

Fig. 20. Spectra of vaginal fluid from weeks 1-4 (from bottom to top) from a single donor demonstrating that the SERS signature does not change during a menstrual cycle.

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the Raman intensities as a function of wavenumbers and this also contributes to a minimization of donor variability to the statistical analysis used in identification procedure.

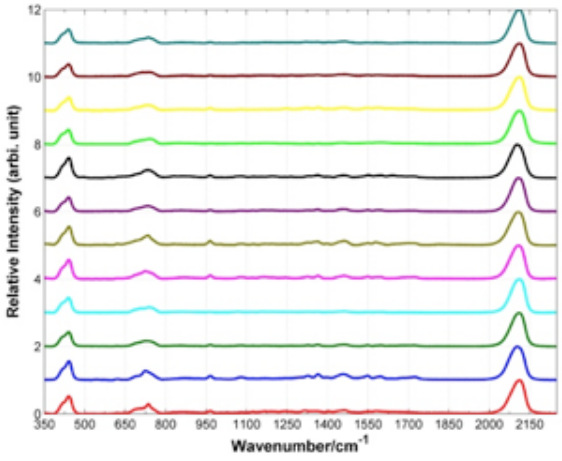

Fig. 21. Averaged SERS spectra of dried neat saliva from 12 donors on Au substrates demonstrates lack of donors variability.

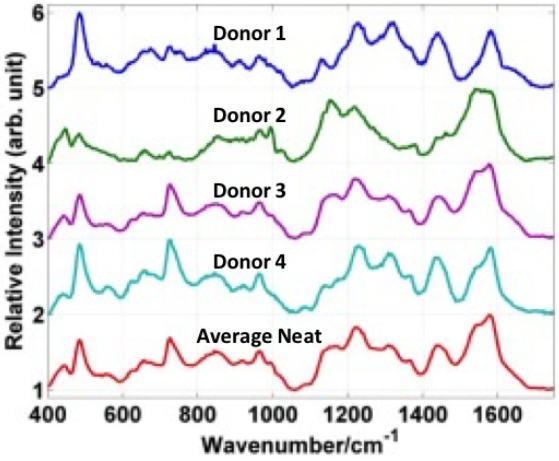

Fig. 22. Averaged SERS spectra of dried semen from four donors show little variation.

Animal blood identification by SERS

Our results on using SERS for distinguishing different animal blood, while not complete, are exceptionally promising. We have just discovered a simple protocol for blood testing that allows SERS to unequivocally distinguish different species as shown in Fig. 23. SERS spectra of dried blood of four different animal are compared with human blood when 1 μL of a moderately basic bicarbonate buffer is dropped on the stain and then placed on a Au substrate. A PLS-DA model shows perfect ability to distinguish these groups. Our 50% acetic acid protocol will not provide animal blood identification because the resulting SERS spectrum (not shown) is due to essentially hemoglobin only and thus is species independent. On the other hand, the basic buffer results in a range of other compounds from these dried blood samples that serves to provide unique and reproducible SERS signatures that can be used for blood species identification.

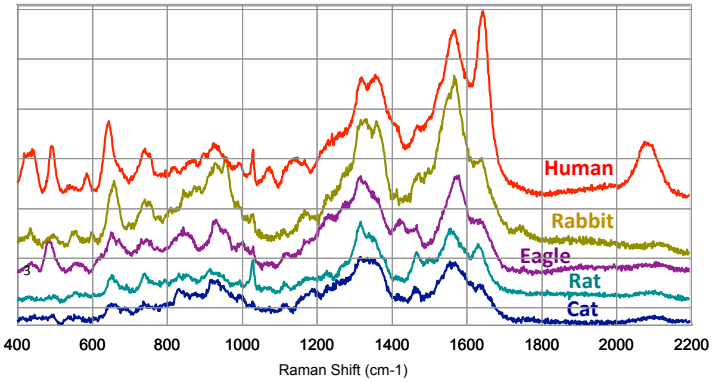

Fig. 23. SERS spectra of animal blood on Au substrates extracted by bicarbonate buffer solution. Cat, rat, rabbit and eagle blood can be readily distinguished from human blood.

Stain substrate dependence:

For the range of materials we observed during the course of this work, the changes to the SERS spectra of semen, vaginal fluid and blood when collected from very different presenting materials have been minimal. (See Fig. 24 for an example.) Some changes to the relative intensities are observed but all the same bands are present, and

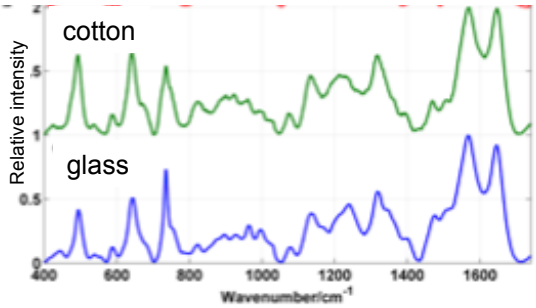

Fig. 24. . SERS spectra of dried blood deposited on jeans, cotton cloth, glass.

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for our second derivative based barcode identification procedure, the relative intensity changes are not an impediment for robust classification.

Implications for criminal justice policy and practice in the United States:

There is a continuing need to exploit new and developing technologies in order to ensure that the best and most accurate outcomes of a criminal investigation are achieved. Body fluids, such as blood, semen, vaginal fluid and saliva are among the most important forensic evidence that can be collected at a crime scene. Rapid detection and identification of these fluids can help identify the perpetrator and victim, and aid in understanding the events, including the timeline of a crime. Ideally, a detection and identification platform for these fluids is confirmatory, rapid, portable and easy-to-use, providing crime scene investigators with maximal time for subsequent law enforcement activities. High levels of sensitivity and specificity with on-site capabilities are clearly important attributes of such a platform. Furthermore, non-destructive and/or highly sensitive techniques are desirable for forensic identification because they leave sufficient amounts of trace evidence for other detection platforms. The results obtained over the course of this two-year study summarized above reveal that the incorporation of a SERS based platform for forensic science investigations, both at crime scenes and in the laboratory, provides improved rapid identification of trace amounts of body fluids with unprecedented sensitivity and specificity.

In the recent (December 2016) NIJ Forensic Science Technology Working Group discussion of operational requirements, we identified approximately fifteen technological needs that a single body fluid SERS platform could readily address. A few of the most relevant needs from this list of high priority technological needs are verbatim quoted here:

- Rapid, affordable and minimally-/non-destructive automated test to detect, locate, and/or confirm the presence of semen/sperm in a dried/aged or degraded stain and swab, and a method to efficiently physically isolate sperm cells/sperm-specific-DNA from non- sperm cells.
- The ability to quickly detect biological materials/fluid at a crime scene or on evidence taken from a crime scene, and simultaneously determine what type of biological fluid/cell type with minimal or no destruction of evidence sample(s).
- A better sexual assault evidence collection kit (to improve rapid rape kit screening and processing).
- The ability to detect/identify biological material (e.g., cell free DNA) that is invisible to the eye or alternate light sources (i.e. material left on touched objects), having sufficient quantity for downstream DNA analysis.
- Rapid, affordable and minimally-/non-destructive automated test to detect, locate, and/or confirm the presence of semen/sperm in a dried/aged or degraded stain and swab.
- Effective, faster, more efficient processes in sample detection, collection, handling, and analysis, data handling and reporting

As a reading of this list above indicates, this grant effort has unequivocally demonstrated that the development of a SERS based platform for body fluid identification and detection can address each of these projected needs in forensic science and we look forward to the continued refinement of this technology and it's implementation in crime scene applications more directly. We are planning a subsequent DOJ proposal to further refine the procedures and results uncovered here, and to help put this enabling technology in the hands of trained forensics scientists as quickly as possible.