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Multidimensional Raman Spectroscopic Signatures as a Tool for Forensic Identification of Body Fluid Traces: A Review

The analysis of body fluid traces during forensic investigations is a critical step in determining the key details of a crime. Several confirmatory and presumptive biochemical tests are currently utilized. However, these tests are all destructive, and no single method can be used to analyze all body fluids. This review outlines recent progress in the development of a novel universal approach for the nondestructive, confirmatory identification of body fluid traces using Raman spectroscopy. The method is based on the use of multidimensional spectroscopic signatures of body fluids and accounts for the intrinsic heterogeneity of dry traces and donor variation. The results presented here demonstrate that Raman spectroscopy has potential for identifying traces of semen, blood, saliva, sweat, and vaginal fluid with high confidence.

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INTRODUCTION

The intersection of science and law has given birth to the field of forensics. This discipline is an applied science that integrates knowledge from several areas to develop efficient methods for crime scene investigation.[1–3] The collection and examination of body fluid traces is one of the most important parts of modern crime-scene investigation because it provides information about individuals using DNA analysis.[1–3] A recent comprehensive review of the methods currently used for body fluid identification concluded that although it is most desirable for body fluid tests to be confirmatory, nondestructive, and applicable to multiple fluids, no methods currently fit this description.[4,5] Conventional methods used for body fluid detection are typically destructive and require hazardous chemicals, and their analysis typically involves extensive sample pretreatment by individuals with highly specialized skills.[6–14]

Raman spectroscopy has potential for the rapid, nondestructive, and confirmatory identification of body fluids at crime scenes.[4] No sample preparation is required, and the signal can be collected from samples as small as a few femtoliters of liquid or picograms of solid.[15] Raman microspectroscopy has approximately 10-fold better spatial resolution ($\sim$1 μm or less) than mid-infrared (mid-IR) spectroscopy.[16] Mapping and imaging of the evidence can be performed relatively easily using the variety of automated stages that are currently available. The development of portable

Raman instruments has made it possible to analyze forensic evidence directly at crime scenes.[17–21] The incontestable advantages of Raman spectroscopy have made this technology applicable to the analysis of drugs,[22–25] paints,[26,27] explosives,[19,28,29] pigments,[30] condom lubricants,[31,32] bones,[33] minerals,[34–36] art objects,[37] fingerprints,[23,25,38] and body fluids.[4,5,39–46]

We recently proposed combining Raman spectroscopy with advanced statistical methods as a solution to the challenging problem of identifying body fluid stains.[40–43,46] The novelty of our approach lies in the application of multidimensional Raman spectroscopic signatures to the purpose of specimen identification. Standard spectroscopic methods used for the identification of unknown samples are usually based on the detection of characteristic Raman bands in the recorded experimental spectrum or comparison of the experimental spectrum, as a whole, with spectra from a reference database. Such approaches have been successfully used in forensics,[47] mineralogy,[48] pharmacology,[49,50] the food industry, the production of polymers[16] and biomaterials,[51] biological species identification,[52] and explosives.[29,53,54] However, our studies have revealed a high level of intrinsic heterogeneity of body fluid stains, with multiple overlapping of Raman bands and variable fluorescent backgrounds. Contamination and substrate contribution may also obscure the analysis of unknown samples. The concept of multidimensional spectroscopic signatures was introduced to overcome these complications and minimize the probability of false negative and false positive results.

A multidimensional spectroscopic signature is a set of several characteristic spectra that represent the most important and characteristic spectral variations of the object under study.[4,44] An experimental spectrum can be presented as a linear combination of these spectral components, that is, as a point in multidimensional space at which the coordinates are equal to the contributions of the corresponding spectral components. The identification of an unknown object consists of searching for the best match between the available multidimensional signatures and the experimental data. Each multidimensional signature has been designed such that it covers the intrinsic variability of heterogeneous samples and the variability between samples. In this way, the probability of false negative results is minimized. The probability of false positive identification can be controlled using statistical parameters obtained from multidimensional spectroscopic signature fitting. If the residuals of such a fitting are too large, then an unknown sample will be interpreted as being yet unidentified.

Here, we review recent progress in body fluid trace characterization using Raman spectroscopy and the development of multidimensional signatures. We discuss the strengths and weaknesses of the proposed approach and the potential of using Raman spectroscopy as a method for crime scene investigation. Prospective applications of multidimensional signatures are not limited to forensic science but could potentially be extended to the characterization of any heterogeneous sample. Disease monitoring, differentiation between nonhomogeneous pharmaceutical forms, quality control, studies of developing human tissues, and control of cell culture growth are among the potential areas where the use of multidimensional signatures would be very beneficial.

BACKGROUND

Raman Spectroscopy of Biological Samples. Raman spectroscopy is a type of vibrational spectroscopy that is based on the inelastic scattering of laser light through its interaction with vibrating molecules.[55] Inelastic (Raman) scattering occurs when incident photons with wavenumber $\bar{\nu}$ ($\lambda = c/\nu$ is the excitation wavelength) interact with molecules yielding scattered photons with shifted frequencies ($\nu \pm \Delta\nu$). Raman scattering is different from Rayleigh (elastic) scattering, in which the incident and scattered photons have the same frequencies (Fig. 1). The Raman shift ($\Delta\bar{\nu}$), i.e., the difference between the wavenumbers of the incident and scattered photons, reports on the energy of molecular vibrations within samples. Ultimately, Raman spectra provide information regarding molecular chemical structure, molecular conformation, interactions between molecules and the surrounding environment, and the physical state and condition of matter. Raman spectra can be very complex and contain broad and superimposed Raman bands.[56–59] This is especially true for the Raman spectra of biological samples, which often include spectral features of multiple constituents: proteins, lipids, DNA, RNA, individual amino acids, biological chromophores (heme, carotenoids, and melanin) and other metabolites. In such cases, the extraction of useful information requires significant effort, such as the application of comprehensive chemometric methods.

A variety of laser sources providing excitation in a wide ultraviolet–visible–infrared (UV-VIS-NIR) spectral range is utilized in modern Raman spectroscopy. Recent studies have demonstrated that under certain conditions (resonance and surface enhancement), Raman spectroscopy can be performed at the single-molecule level.[60] Raman spectroscopy is not limited by physical state and can be performed on gaseous, solid, liquid, gelatinous, nontransparent, and heterogeneous samples with complex chemical compositions. The main disadvantages of Raman spectroscopy are the weakness of the Raman effect in the absence of resonance and surface enhancement and the potential for fluorescence interference. The probability of the Raman effect is approximately only 10^{-6}–10^{-9} per incident photon, which imposes the need for sensitive and optimized light detectors. Fluorescent contributions can be minimized using time- or space-resolved Raman spectroscopy or by using numerical methods to eliminate fluorescent background from previously recorded Raman spectra.

Raman Microspectroscopy. Two- and/or three-dimensional spectroscopic mapping is an efficient way to collect information about intrinsically heterogeneous samples. Typically, Raman spectral acquisition is accomplished by moving the sample in a stepwise manner until the entire region of interest is characterized. Modern equipment allows various settings for mapping measurements, such as spectral acquisition from a rectangular or circular area (filled or

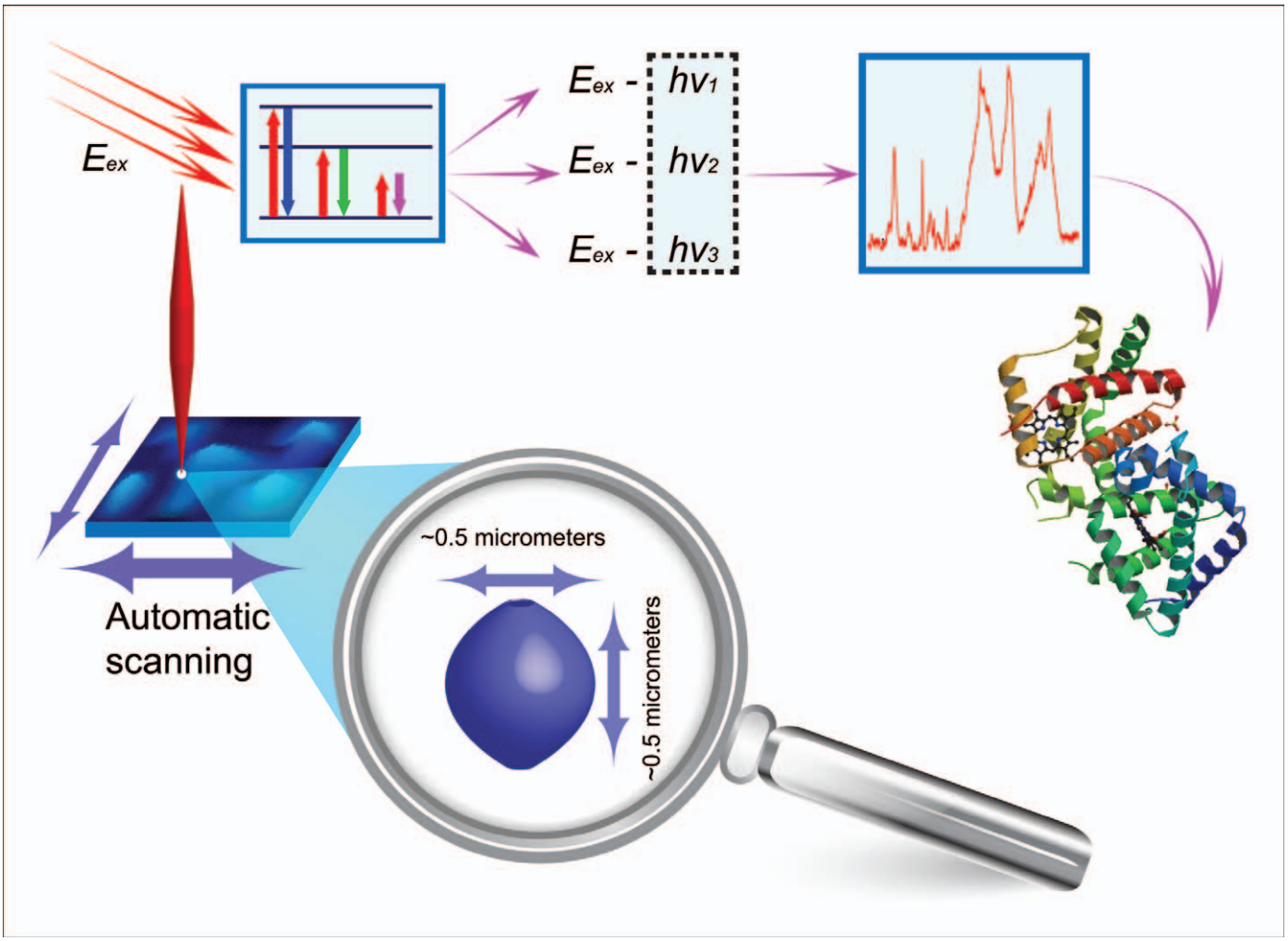

FIG. 1. Theory of Raman spectroscopy and experimental design. Raman spectroscopy is based on the inelastic scattering of laser light through its interaction with vibrating molecules. Scattered photons with shifted frequencies provide information regarding molecular structure and physical state. Raman imaging is accomplished by moving the sample in a stepwise manner and acquiring spectral information from each spot.

outlined), a line, a point or a depth slice. Therefore, each spectrum represents a specific area/portion of the sample.

Several approaches have been recently developed for treating such spectroscopic data sets. Various types of maps can be created using direct analysis of the data, curve fitting, pretreatment, or developing topology maps, component maps, principal component anaylsis (PCA) maps, and multivariate curve analysis maps.[16,61,62]

Identification of Unknown Samples. One of the more common statistical problems in spectroscopic data analysis is the proper identification of unknown species. A variety of statistical methods can be applied to this problem. The identification of unknown samples is typically performed using database search methods. Commonly, a feature extraction step anticipates the database search by reducing the dimensionality of raw instrumental output by the selection of relevant peaks or regions.[63] Various automatic approaches for spectral identification exist. The majority of selection algorithms can be classified as filters, wrappers, or embedded methods. Filters (*t*-tests) select one feature at a time and rank features on their classification power.[64] Wrapped methods (genetic algorithms) search for subsets of features. Decision trees, mimetic algorithms, sparse regressions, networks with bottleneck-layers, and many other machine-learning methods are all types of embedded methods.[65] All of the methods listed above are applied during the analysis of complex chemical mixtures.

Quantitative analyses of multicomponent mixtures can be performed by fitting their spectral profiles to reference spectra of pure components.[66] The application of multidimensional signatures, described here, shares certain similarities with the problem of mixture analysis. The local composition of a heterogeneous stain can be treated as a mixture of several basic components. Relative concentrations of such components may vary significantly within the sample, but the list of components under

consideration is limited. A multidimensional spectroscopic signature of a body fluid must consider both the heterogeneous nature of dry traces and donor variation. The identification process entails simply determining which predefined set of spectra (signature) best fits the unknown experimental data. It is important to set criteria for "the best fit". In our studies, three statistical criteria were successfully tested, including the sum of squares due to error (SSE), the coefficient of determination (R^2), and the root mean squared error (RMSE):

$$SSE = \sum (y_i - \hat{y}_i)^2 \quad (1)$$

$$R^2 = 1 - \sum (y_i - \hat{y}_i)^2 / \sum (y - \bar{y})^2 \quad (2)$$

$$RMSE = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n}} \quad (3)$$

where y_i, $\hat{y}_i$, and $\bar{y}$ indicate actual, fit, and mean values, respectively. Extensive fitting and cross-fitting of the developed signatures supported the validity of this approach (see Raman Spectroscopic Signatures of Body Fluids subsection below).

EXPERIMENTAL PROCEDURES

Samples. A variety of human and animal body fluids were studied in our laboratory using Raman microspectroscopy, including human blood, semen, saliva, sweat, and vaginal fluid; canine blood and semen; and feline blood. The samples of human body fluid were obtained from anonymous donors and volunteers. The samples of animal body fluid were provided by local veterinary clinics. All samples were tested using an automatic mapping technique with two different stages. A 10 μL drop of each body fluid sample was placed on a microscope slide covered with aluminum foil to reduce fluorescent interference from the glass.

Raman Measurements. A Renishaw inVia confocal Raman spectrometer equipped with a research-grade Leica microscope, 20–50× long-range objectives, and WiRE 3.2 software was used to collect spectra over the range of 100–3200 cm^{-1}. A 785 nm laser light was utilized for excitation. The laser power was carried between 11 and 120 mW on the dried samples. The spot size of the excitation beam on the sample was typically ~5 μm using the standard confocal mode. The spectral resolution was about 3 cm^{-1}. A silicon standard was used for calibration. The lower plate of a Nanonics AFM MultiView 1000 system was used to analyze human and animal blood and semen and human saliva during the early stages of the project. The measurements were taken using Quartz II and QuartzSpec software (Cavendish Instruments Ltd, UK). All samples were allowed to dry completely and were subjected to automatic mapping that scanned a sample area of 75 × 75 μm and measured Raman spectra from 16–36 random points in the area.

Recent instrumentation developments in our laboratory include the integration of a Renishaw PRIOR automatic stage system, which was used for automatic mapping of sweat and vaginal fluid samples. For each sample, the automatic mapping was performed on three areas of 60 μm × 40 μm (32 points each) for sweat and 3.5 mm × 2.5 mm (108 points each) for vaginal fluid.

Data Treatment. Raman spectra were pretreated using GRAMS/AI (Thermo Galactic, Salem, NH) software, which eliminated cosmic ray contributions from each spectrum before baseline correction. The spectra were then imported into MATLAB 7.4.0 (The Mathworks, Natick, MA) for statistical analysis and normalized to adjust for the varying amount of background interference in each spectrum. Significant factor analysis (SFA)[67] and principal component analysis (PCA) were performed to determine the number of principal components in the data set obtained for a single basis sample chosen randomly for each body fluid. The data were then cross-validated to confirm the number of principal components that best described the spectral data. The alternate least squares (ALS) algorithm was used to extract the individual spectral components for each body fluid. For human blood, semen, saliva and vaginal fluid, the components found for the basis sample were fitted to the average spectrum obtained from the remaining samples. The Curve Fitting Toolbox in MATLAB was used to perform the residual analysis of the difference between the fitted and experimental spectra. Liquid blood spectra were recorded for comparison and treated with GRAMS/AI 7.01 to remove cosmic rays and correct for background interference. Characteristic spectroscopic features were assigned to the biochemical constituents of human body fluids (for example, molecules of hemoglobin in dried blood samples).

For human sweat, experimental Raman spectra obtained from all samples were used to determine the multidimensional signature to overcome the effect of significant spectral variations between donors. The residual analysis was similar to that used for other body fluid data.

A modified approach was tested for sweat and vaginal fluid Raman spectroscopic data. Two data sets were formed for each of these body fluids. The first data set contained all Raman spectra after baseline correction using the adaptive and iteratively reweighted penalized least-squares baseline correction algorithm (AIRPLS).[68] The second data set included the fluorescent background only. For each body fluid, both data sets were treated separately to extract virtually pure Raman and fluorescent components of multidimensional signatures. A quantitative statistical analysis using SSE, R^2, and RMSE was performed to confirm a satisfactory fitting of all experimental spectra.[40–43]

Raman Spectroscopic Signatures of Body Fluids. Dry traces of body fluids are heterogeneous in nature, and their chemical composition also varies between donors. Therefore, no single Raman spectrum can adequately represent a particular body fluid. We recently proposed a new approach for body fluid identification based on the unique multidimensional Raman signatures of fluids. Usually, Raman spectra of biological samples have a significant fluorescent contribution, and two strategies based on the concept of spectroscopic signatures can be used to identify unknown stains. The first strategy is to extend the multidimensional signatures with the fluorescent background and a horizontal line.[42] The horizontal line takes the offset variation into account. In this case, raw experimental spectra can

be fitted by components of the signature without any treatment. The second strategy is to perform a baseline correction of the raw spectra before the statistical analysis and determination of the multidimensional signatures, which contain only Raman components.[46] Both strategies have their advantages. Prior baseline correction leaves only a highly informative Raman signal, whereas the incorporation of the less informative but still characteristic fluorescent backgrounds may increase the specificity of the multidimensional signature. Spectral components of human blood, semen, and saliva multidimensional signatures present only the Raman contribution, while the contribution of wider fluorescent features is excluded by prior baseline correction. Full multidimensional signatures of blood, saliva, and semen include averaged fluorescent backgrounds in addition to the Raman spectral components, whereas both Raman and fluorescent components were calculated chemometrically to obtain the multidimensional signatures of sweat and vaginal fluid. We found that these body fluids have varying fluorescent backgrounds and should be represented as a linear combination of several spectral profiles (two profiles for sweat and three for vaginal fluid). The fact that all body fluids have different fluorescent components allows us to speculate that fluorescence should not be viewed as undesirable because it can be used as a significant tool in the process of characterizing particular body fluids. The use of fluorescent components was tested with multidimensional signatures of sweat and vaginal fluid (see Identification of an Unknown Body Fluid Stain Using Multidimensional Signatures subsection).

All calculated Raman signatures were successfully fitted to experimental data collected from multiple donors of different races, genders, and ages. The high goodness-of-fit of the statistical results (see Table I) demonstrates the high specificity of the developed signatures.

Blood.[42] The experimental Raman spectrum of blood (Fig. 2) has strong spectral features of two proteins: hemoglobin and fibrin, which are the main building blocks of red blood cells and plasma, respectively.[69] The presence of two spectral components dominated by these proteins was also revealed by advanced statistical analysis of Raman spectra recorded from multiple spots of a single dry sample of blood (Figure 3A).[70,71]

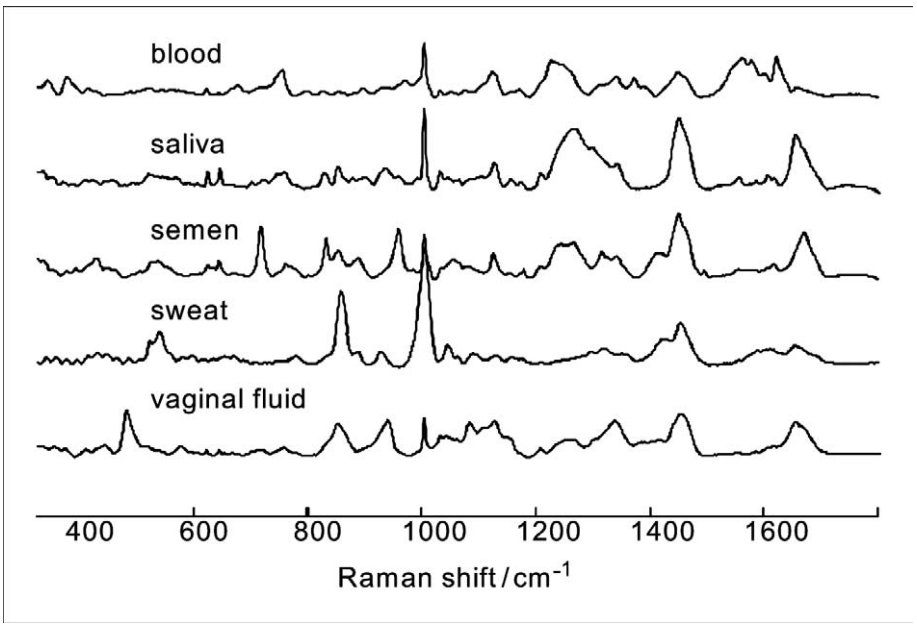

Fig. 2. Raman spectra of blood, semen, saliva, sweat, and vaginal fluid after fluorescent background subtraction.

Component 1 has characteristic peaks of hemoglobin and its derivatives at 1000, 1368, 1542, and 1620 cm^{-1} (Fig. 3A).[39,69] The peaks at 967, 1248, and 1342 cm^{-1} of component 2 were attributed to pure fibrin, one of the components of coagulated blood.[72] The amide III vibrational mode of the protein backbone, which consists of C–N stretching, N–H bending, and C–C stretching,[42,69] is evident at 1247 cm^{-1}. This assignment was also supported by observation of the coagulation process when blood dries. We found that liquid blood is dominated by the hemoglobin spectral component, whereas dried blood has a noticeable contribution from fibrin.[73]

We also found that all samples showed the same two principal components, regardless of the donors. The multidimensional spectroscopic signature of blood was based on calculated Raman spectral components (Fig. 3A) and one fluorescent background extracted from the average spectrum. This signature was fitted to all experimental Raman spectra of the dried blood samples with high goodness-of-fit. This result demonstrates that the spectroscopic signature can be applied to any human blood sample to potentially identify the sample and can be used to distinguish blood from other body fluids or red substances of an artificial nature found at crime scenes.

Semen.[40] Along with blood, semen is one of the most prevalent body fluids found during criminal investigations, especially in cases involving sexual assault.[74] The spectrum of pure semen (Fig. 2) is complex and contains contributions from multiple biochemical species, such as the amino acid tyrosine (641, 798, 829, 848, 983, 1179, 1200, 1213, 1265, 1327, and 1616 cm^{-1}),[75,76] proteins (1668 and 1240 cm^{-1}, and peaks characteristic of albumin at 759, 1003, 1336, and 1448 cm^{-1}),[56,71,77,78] possibly choline (715 cm^{-1}),[56] and spermine phosphate hexahydrate (888, 958, 1011, 1055, 1065, 1125, 1317, 1461, and 1494 cm^{-1}).[57,58]

The multidimensional signature of semen contains three Raman spectral components that were obtained by statistical analysis and one fluorescent background extracted from the average spectrum (Fig. 3B). The Raman components of semen are complex and cannot

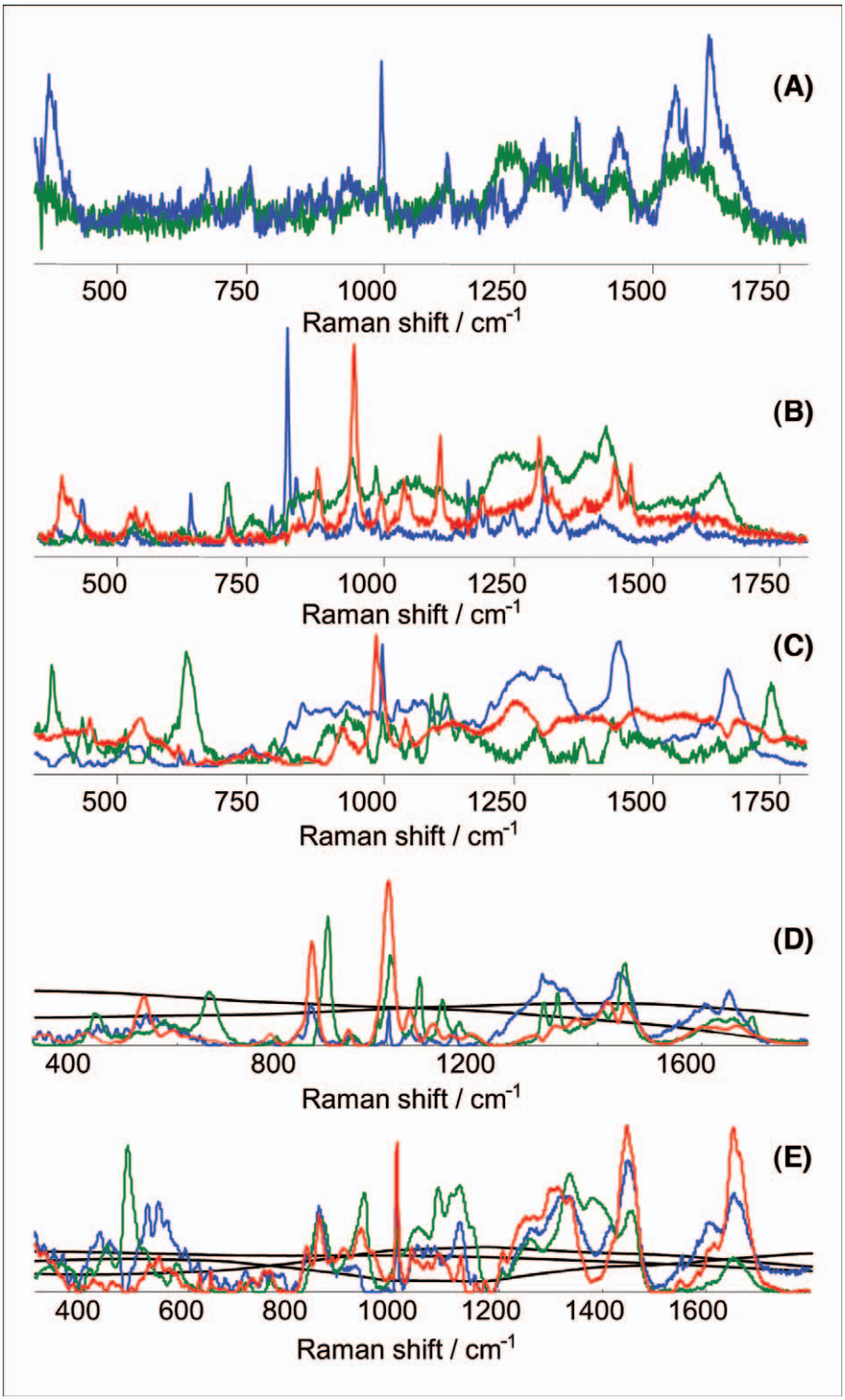

FIG. 3. Spectroscopic signatures of (A) semen, (B) blood, (C) saliva, (D) sweat, and (E) vaginal fluid. Blue, green, and red spectra correspond to the first, second, and third Raman components, respectively. Black lines are statistically obtained fluorescent components of sweat and vaginal fluid.

be assigned to pure biochemical species. Spectral features of component 1 are consistent with the characteristic peaks of tyrosine, one of the most abundant free amino acids in semen. Other peaks in spectral component 1 are residual and more prominent than in the other two spectral components. Component 2 is dominated by the strong peaks at 1668 and 1240 cm^{-1} that are associated with the amide I and amide III vibrations of the protein backbone and peaks that presumably originate from albumin and choline (see above for the peak positions). The spectrum for component 3 shares obvious similarities with previously reported spectra of spermine phosphate hexahydrate,[57,58] which is present at a high concentration in human semen (0.2–2.5 mg/mL).

Saliva.[41] Variations in Raman spectra of saliva associated with multiple donors were significantly larger than variations found for other body fluids (Fig. 2). To accommodate these variations, we assessed Raman spectra that were recorded for all samples to obtain the multidimensional signature, as discussed in the Background section. Three spectral components and one fluorescent background extracted from an average spectrum characterize the spectroscopic signature of saliva (Fig. 3C). Spectral component 1 contributes most significantly to the overall spectra of saliva and corresponds to proteins and glycoproteins (1002, 1444, and 1653 cm^{-1}).[71,75,79–81] The presence of saccharides (323 and 521 cm^{-1})[75,82] and acetates (632, 1295, 1434, and 1744 cm^{-1})[59,83] is indicated in component 2. Spectral component 3 accounts for a smaller part of the spectral variation in saliva, and the peaks at 544, 919, and 991 cm^{-1} were tentatively assigned to arginine.[83]

Sweat.[46] Dried human sweat appeared to be the most heterogeneous object in our study. We found that the Raman and fluorescent spectral features of sweat are highly variable. The fluorescent background of human sweat cannot be simplified; because of the variation of a single profile, the multidimensional signature requires two fluorescent and three Raman components (Fig. 3D). The fluorescent and Raman components were determined independently by mul-

tivariate curve resolution MCR (alternating least squares, ALS) analysis of experimental data split by the AIRPLS algorithm. A quantitative statistical analysis using SSE, R^2, and RMSE confirmed a satisfactory fitting of all experimental spectra using the resulting sweat signature.[43] The major constituents of human sweat are water (99%), lactate (lactic acid), urea (uric acid), diethylene glycol, alanine, valine, and leucine. Spectral component 1 was characterized by an 856/1003 cm^{-1} doublet and a strong Raman band at 1003 cm^{-1}, which was assigned to the CN stretching of urea molecules.[84] The 856 cm^{-1} band was tentatively assigned to lactic acid.[85,86] Raman peaks at 552, 800, 1150, 1310, 1445, 1608, and 1651 cm^{-1} that were found in component 2 are indicative of urea, proteins, and lactic acid. Amino acids and protein significantly contribute to component 3 (662, 886, 1062, and 1297 cm^{-1}).[87]

***Vaginal Fluid.*[43]** The identification of vaginal fluid stains and semen is very important for investigations of sexual assault cases. Simple, nondestructive methods for the rapid determination of these traces will be extremely valuable to the forensic community. The spectrum of pure vaginal fluid (Fig. 2) appears to have some similarities with that of semen (both fluids have a significant amount of urea and lactate/lactic acid), but substantial differences remain. SFA and PCA were applied to calculate the principal components in a single vaginal fluid sample (basis sample), and the spectral components were extracted using the ALS algorithm. Fluorescent backgrounds, which were obtained using AIRPLS, were treated with the same algorithm. Therefore, the Raman spectroscopic signature of vaginal fluid contains three Raman and three fluorescent components. The major components of vaginal fluid have a composite character and could be assigned to urea,[84,87–89] lactate,[86] lysozyme, acetic acid,[90] and pyridine.[74] Raman components 1 and 2 (Fig. 3E) have similar spectral profiles and consist of the same Raman bands with different relative intensities. These spectral components contain the characteristic features of lactic acid, urea, and proteins. Raman component 3 contains unique bands at 481 and 1380 cm^{-1}, which correspond to the N–C=O bending and CN stretching vibrations of urea, respectively. It was found that the fluorescent background in the case of vaginal fluid is more variable compared to sweat. A linear combination of three spectra is required to compensate for this variation. However, the variations in the Raman portion of the vaginal fluid spectra between donors are much smaller than those in sweat. A quantitative statistical analysis confirmed the satisfactory fitting of all experimental spectra of vaginal fluid using the set of Raman and fluorescent spectral components.[43]

Identification of an Unknown Body Fluid Stain Using Multidimensional Signatures. The values of SSE, R^2, and RMSE were used as quantitative characteristics of the fitting. An ideal fit would yield calculated profiles that completely match the experimental spectra. The SSE, R^2, and RMSE values represent the total deviation of the fitted data points from the experimental data. The SSE and RMSE values will be closer to 0 and R^2 will be closer to 1 if the signature corresponds to a body fluid. In this case, the variations in the goodness-of-fit parameters will be caused mainly by noise contribution, and the residuals from the ALS fitting will resemble the normal distribution. The results of fitting for different body fluids are illustrated in Table I.

Figure 4 demonstrates several examples of Raman signature fitting. The two upper pairs of spectra show fitting using the correct signature, whereas the three lower pairs are illustrations of "improper" fitting. Multidimensional signatures of vaginal fluid and sweat used for fitting contained both Raman and fluorescent components. This approach was preferable relative to the elimination of the underlying fluorescence by a simple baseline correction followed by fitting with Raman components only.

Other statistical criteria such as the F-test and Durbin–Watson statistic can be applied to assess the quality of fitting.[91] The identification problem can be broken down into a simple comparison of parameters of signature fitting to some unknown Raman spectrum with prior established boundaries. More work needs to be done to accommodate effects of contaminations, body fluid mixing, and aging (see Conclusions).

TABLE I. The sum of squares due to error (SSE), coefficient of determination (R^2), and root mean squared error (RMSE) calculated for the blood, sweat, and vaginal fluid signature fittings. Bold values show the results of fitting when the signature is consistent with the type of body fluid stain.

Sample\Signature	SSE	R^2	RMSE
Vaginal fluid\ Vaginal fluid	**0.298**	**0.995**	**0.010**
Sweat\Sweat	**0.365**	**0.984**	**0.018**
Saliva\Saliva	**0.550**	**0.979**	**0.018**
Semen\Semen	**0.055**	**0.998**	**0.0063**
Blood\Blood	**0.049**	**0.998**	**0.0067**
Sweat\Blood	6.250	0.925	0.059
Saliva\Semen	5.100	0.905	0.053
Saliva\Vaginal fluid	2.406	0.952	0.044
Semen\Vaginal fluid	5.410	0.924	0.053
Blood\Vaginal fluid	7.396	0.910	0.070

Nonhuman Body Fluids. Spectroscopic signatures of nonhuman body fluids have not yet been developed, but preliminary studies have demonstrated that their Raman spectra have characteristic features that allow nonhuman and human body fluids to be distinguished, despite the fact that fluids with similar compositions present a significant problem during species identification. Human, canine, and feline blood traces were discriminated within a confidence interval of 99% by Raman spectroscopy analysis coupled with cross-validated PCA.[39] Significant differences were found between human and canine semen. Peaks at 418 and 1248 cm^{-1} are unique to the canine spectrum, whereas peaks at 536, 1004, and 1342 cm^{-1} were observed only in the human samples. In addition, noticeable differences are found in the relative intensities of the common peaks.

CONCLUSIONS

Our studies have demonstrated the possibility of characterizing and identifying the origin of unknown body fluid stains based on their unique Raman signatures. Overall, Raman spectroscopy coupled with advanced statistical analysis demonstrates great potential for the nondestructive and rapid confirmatory identification of body fluids at crime scenes. Raman spectroscopic signatures were tested in multiple samples from a

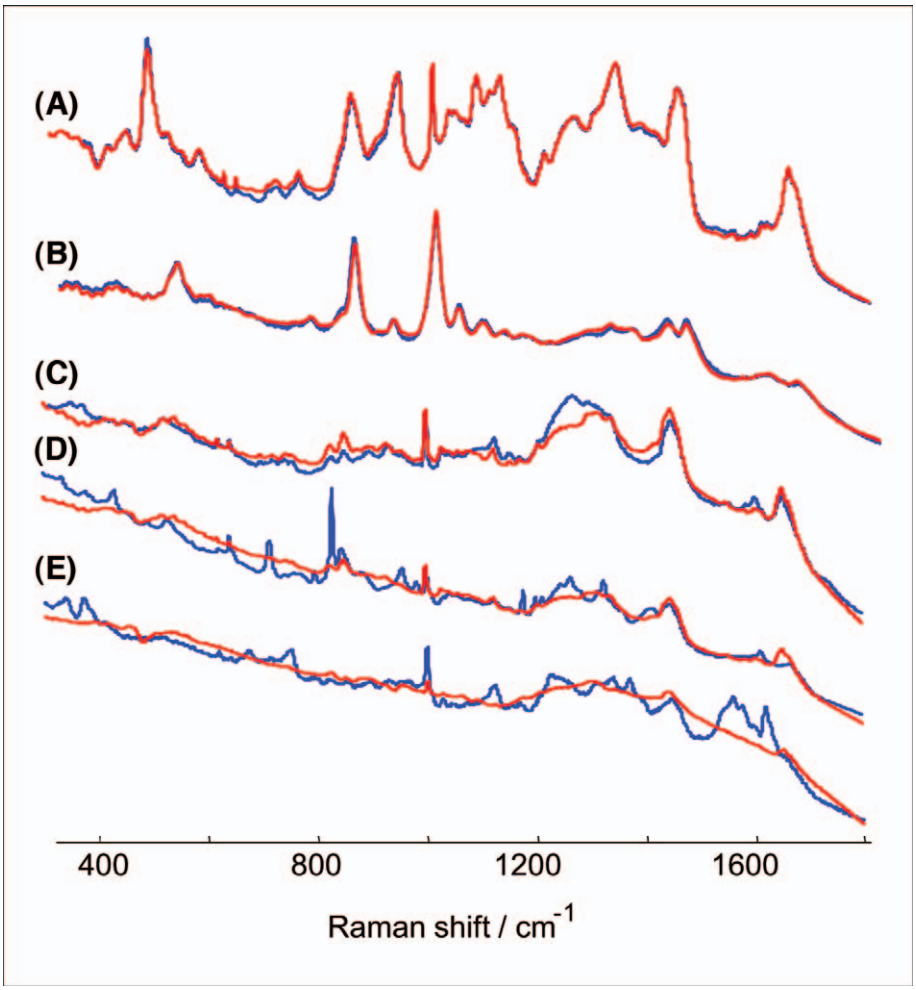

FIG. 4. The average Raman spectra (blue lines) of various body fluid samples are fitted by different Raman signatures. The red lines represent the calculated spectra. (A) Raman spectrum of vaginal fluid fitted using the signature of vaginal fluid. (B) Raman spectrum of sweat fitted using the signature of sweat. (C–E) Raman spectra of saliva, semen, and blood fitted with the spectroscopic signature of vaginal fluid.

variety of pure body fluids obtained from donors of different races, genders, and ages. These signatures were fitted to the experimental Raman spectra of various dried body fluid stains. High goodness-of-fit statistical results (SSE, R^2, and RMSE) were obtained for consistent body fluid–Raman signature pairs. We demonstrated that the use of characteristic spectroscopic signatures could be applied to any human body fluid sample for its potential identification and in distinguishing it from other body fluids and substances found at a crime scene. Spectral features of the Raman spectral component were assigned to individual biochemical constituents. The origin of fluorescent components must still be interpreted with caution because several constituents of human body fluids may have similar fluorescent profiles.

The identification of pure body fluids can also be achieved by such chemometric methods as PCA, linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA), support vector machines (SVM), and artificial neural networks (ANN). For example, we demonstrated that human, feline, and canine blood samples can be distinguished using PCA. We also tested, LDA, SIMCA, and partial least squares discriminant analysis (PLS-DA) as alternative methods for the discrimination of semen, blood, and saliva traces.[44] The application of multivariate classification methods will be significantly impaired in cases of contamination, body fluid mixing, aging, and spectral contributions from substrates. We believe that the effective identification of real-life samples can be achieved using the combination of multidimensional signature fitting with multivariate classification and regression analyses.

This brief article illustrates the great potential of Raman microspectroscopy combined with the use of advanced statistics for the nondestructive and confirmatory on-field identification of body fluid stains. However, more work needs to be conducted before this promising technique can be used by crime scene investigators. Specifically, aging of a biological stain under various environmental conditions, including high and low humidity or photodegradation due to sunlight, should be documented. Our laboratory is expanding this method to include mixtures of body fluids respectively and samples contaminated with nonbiological components. Opportunities also exist to utilize Raman spectroscopy for differentiating body fluid traces of human and animal origins at crime scenes.[39]

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