A universal method for the identification of body fluid traces for forensic purposes based on Raman spectroscopy

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Scenes of violent crimes including homicides or physical and sexual assaults can contain tens to hundreds of stains. These stains vary in nature and can be a combination of fresh body fluids, old body fluids (common in cases of repeated domestic abuse), or environmental contaminates [1]. Crime scene investigators are trained to collect samples from all seemingly relevant stains. They will collect either swabs or cutting of stained areas to submit to evidence. This can lead to an overwhelming number of samples being submitted to the crime lab with the goal of obtaining a DNA profile. However, the time commitment and cost to perform DNA analysis is high thus, it is important to conclusively identify which body fluids are relevant to the current investigation. Due to the large forensic case load, the analysis of body fluids must be done efficiently. Presumptive and confirmatory tests have been developed over the years for the identification of body fluid traces. Colorimetric reactions are the primary method used in presumptive testing assays, while immunochromatographic cards are commonly used for confirmatory identification assays. In forensic labs, phenolphthalein (blood), acid phosphatase and Christmas tree staining (semen), and Phadebas (saliva) are the most frequently used tests to identify these fluids [2]. However, currently, there are no reliable methods for identifying vaginal fluid, urine, or sweat. The majority of current methods have been utilised for many years despite that they have certain disadvantages. For example, they can be destructive to the sample, and each assay can only identify one body fluid. Moreover, most of the tests used by forensic examiners provide presumptive results, meaning that a positive result only suggests the possible presence of a body fluid.

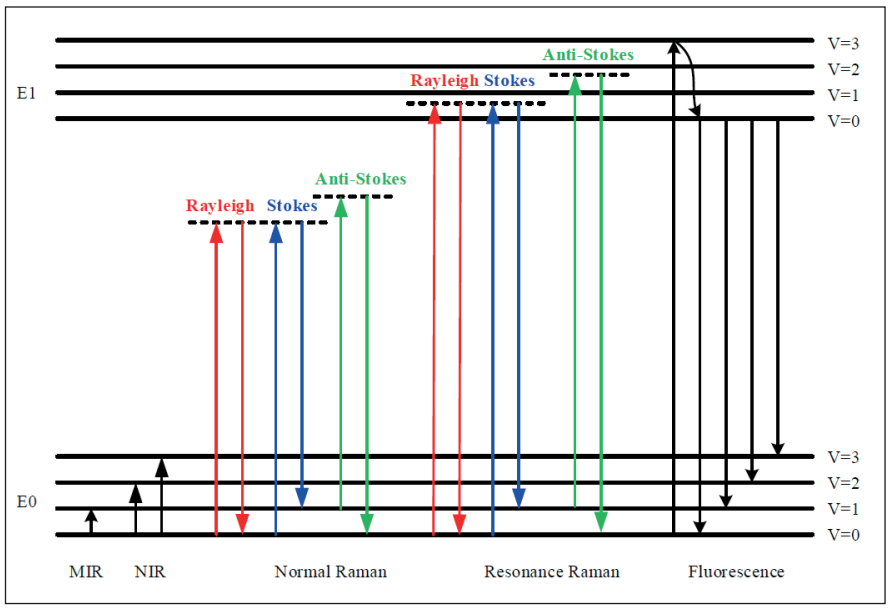

Figure 1. Energy level diagram related to IR absorption, Raman scattering and fluorescence emission. Reprinted from Li, Zhiyun, M. Jamal Deen, Shiva Kumar, and P. Ravi Selvaganapathy. "Raman spectroscopy for in-line water quality monitoring—Instrumentation and potential." Sensors 14, no. 9 (2014): 17275-17303., Copyright 2014, with permission from open access MDPI.

An emerging method that has shown the most promise for identifying biological samples has been vibrational spectroscopy. Vibrational spectroscopy is a non-invasive analytical technique that evaluates the vibrational energy of a molecule upon excitation [3]. It is utilised to identify compounds by their distinct vibrational energy levels, which reflect the specific bonding patterns within the molecule. The two principal forms of vibrational spectroscopy are Raman spectroscopy and Infrared (IR) spectroscopy, with Raman spectroscopy having the most success. This method works by illuminating a sample with a monochromatic laser light. Most of the incident photons are scattered elastically by the sample, meaning they do not change their. However, a small fraction of photons is scattered inelastically, meaning they lose or gain energy in the process. These photons are referred to as Raman scattered photons. The energy changes or Raman shifts of the scattered photons correspond to the vibrational energy levels of the molecules in the sample. By analysing the energy shift of the Raman scattered photons compared to the incident photons, information about the vibrational modes of the sample can be obtained [3].

Raman spectroscopy can be used to identify and characterise molecules in a variety of samples, including solids, liquids, and gases. By collecting multiple spectra from a sample, the resulting signatures account for the intrinsic heterogeneity of dry traces of body fluids and variations among individuals [3]. It is a non-destructive technique that does not require sample preparation, making it a useful tool in fields such as chemistry, biology, and forensic science. Multidimensional spectroscopic signatures for complex biological substances are produced and able to be analysed using advanced statistical methods, called chemometrics. Chemometrics involves the use of mathematical and statistical techniques to extract meaningful information from multivariate chemical datasets. Spectral outputs, although appearing as a single line, are made up of hundreds or thousands of data points. Consequently, spectral results require more advanced analysis techniques, as compared to univariate data that involve only a single variable. To process, quantify, and classify vibrational spectroscopic data, multivariate statistical analyses are essential.

Work conducted by the Lednev laboratory at the University at Albany, SUNY has developed and validated many aspects of the novel methodology. Specifically, an automatic statistical model for the identification of all main body fluids including blood, semen, vaginal fluid, sweat, saliva and urine was created [4-6]. The model provides 100% accuracy of the identification when the stain is found on a noninterfering substrate. A primary concern in forensic science is being able to analyse samples that are on substrates that interfere with analysis [7]. In response, a statistical approach, which allows for 'ignoring' the substrate interference was developed. The latter approach has been validated for fresh (10-hour old) blood and semen stains on various substrates, which exhibited strong Raman and fluorescence emission overwhelming the signal from semen [8, 9]. In addition, it was shown that Raman spectroscopy allows for differentiating peripheral and menstrual blood [10], human and animal blood [11], and offers information about the donor phenotype profile including sex, race, and age [12-14] and time since deposition of bloodstains [15-17]. Despite this success, more investigation is required before the proposed methodology can be utilised in practical forensic applications. This comes in the form of SupreMEtric commercialising the discussed technology for forensic scientists.

Previous research has established that these signatures are unique and distinguishable, allowing for identification of all main bodily fluids. To advance that work, SupreMEtric is developing the first universal method for non-destructive, confirmatory identification of bodily fluids in trace biological stains revealed at a crime scene, overcoming the limitations of standard presumptive and confirmatory biochemical tests. With this technology, crime scene investigators can collect samples from a crime scene under investigation using standard approaches already in use; once in the laboratory, samples are analysed a patented digital method used for determining spectroscopic signatures developed for each body fluid type. SupreMEtric's advanced, proprietary algorithm recognises trace signals on interfering substrates, and can confirm the presence or absence of each body fluid type in the sample, without altering or destroying the evidence.

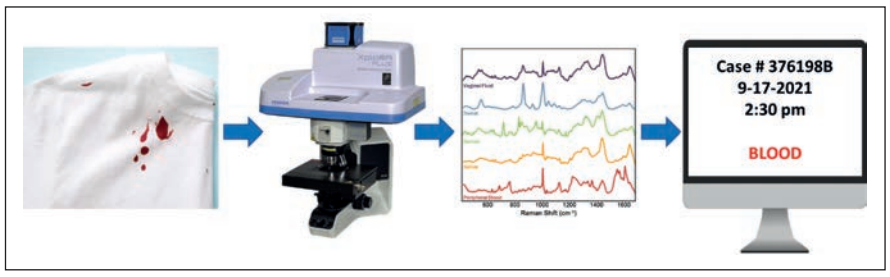

Figure 2. Workflow for body fluid identification

This solution has the potential to significantly advance crime scene analysis by replacing the plethora of single body fluid-specific chemical tests, which are inaccurate and destructive, with a single statistical analysis software able to positively identify all bodily fluids, be it in the forms of stains, solids, or liquid traces on interfering substrates. This will allow crime labs to speed up decisions regarding sample content and further analyses from days to minutes. The application of a statistical method removes the subjectiveness of reading the results that occurs with standard colorimetric tests, and produces results with an error/uncertainty rate, which is important when presented in judicial proceedings.

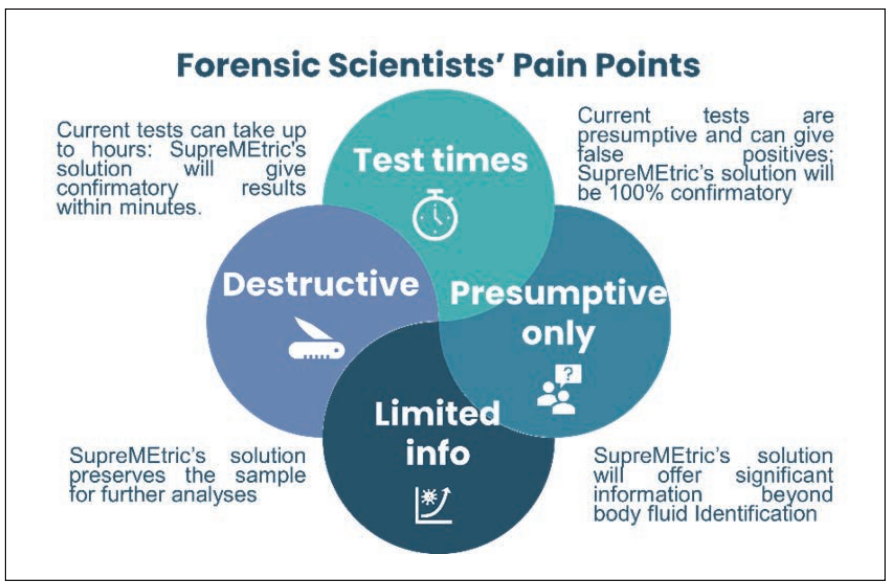

Figure 3. Major pain points in forensic science that SupreMEtric's technology aims to address.

The highly sensitive technology does not require any sample preparation, which complements the non-destructive analyses and ensures the preservation of forensic evidence. The first-generation technology will be able to be integrated with benchtop Raman instruments for easy and convenient applications in crime laboratories (federal, state, or private). Raman spectroscopy is the most selective spectroscopic technique and a technology of choice for in-field trace analysis for other forms of forensic evidence, which can be adapted for analysis of biological samples. In the future, SupreMEtric plans to integrate the technology with portable Raman instruments, which are already used in a variety of field applications, to enable real-time identification of bodily fluids at the crime scene.

Recent advances in forensic DNA analysis have significantly improved accuracy of suspect identification. However, solving crimes, particularly violent ones, relies heavily on forensic evidence collected at a crime scene. Biological stains from bodily fluids such as blood, saliva, sweat, urine, semen, and vaginal fluid are the most sought-after pieces of evidence, as they can not only provide DNA evidence, but also help identify the perpetrator and uncover crime events. Currently only a small fraction of bodily fluid analyses is admitted as probative evidence in court, and even fewer can withstand scrutiny of criminal defence, which demands admissions that are 'beyond reasonable doubt'. This is because many of the analytical methods used today to identify bodily fluids are presumptive (i.e., can be either inconclusive or produce false-positive results) rather than confirmatory (i.e., 100% positive identification). In addition, most in-field methods rely on enzymatic or immunologic testing, which are often destructive or consumptive in nature, preventing further analyses. The process of running multiple fluid-specific tests further complicates analytical workflow, adding time and cost to forensic lab operation.

Since forensic scientists today must conduct several individual tests for different body fluids, this runs the risk of damaging or destroying samples. This is especially challenging when only trace amounts of bodily fluids are present, or when bodily fluids are found in mixed samples, as this may prevent accurate identification of these fluids, and limit or even prevent the extraction and/or identification of DNA. Without being able to identify which type of bodily fluid the DNA was derived from, it is not always possible to conclusively recreate a full crime scene and the actions of those involved. A 2017 NIJ report on sexual assault cases indicated that non-DNA evidence (such as bodily fluids) was a key to conviction in 80% of cases [18]. Currently, in cases when multiple DNA and fluids are present in a sample, DNA analysis cannot be linked to a single individual. In addition, only half of the biological stains collected at crime scenes contain DNA evidence, of which only 38% is eligible to be entered into the Combined DNA Index System (CODIS) database. As such, the absence of testing capabilities that can provide confirmatory identification of biological evidence from crime scenes critically prevents ongoing investigations. Due to the nondestructive and noncontact nature of this technique, practitioners will be able to use Raman spectroscopy to analyse body fluids in situ when brought to the laboratory. The preservation of DNA evidence, as well as the reduction of non-biological samples sent for DNA analysis, will have beneficial overall effect on multiple sections of forensic laboratories.

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