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PII: S0165-9936(17)30431-4

DOI: [10.1016/j.trac.2017.12.003](https://doi.org/10.1016/j.trac.2017.12.003)

Reference: TRAC 15066

To appear in: Trends in Analytical Chemistry

Received Date: 25 October 2017

Revised Date: 1 December 2017

Accepted Date: 3 December 2017

Please cite this article as: K.C. Doty, I.K. Lednev, Raman spectroscopy for forensic purposes: recent applications for serology and gunshot residue analysis, *Trends in Analytical Chemistry* (2018), doi: 10.1016/j.trac.2017.12.003.

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## **Raman spectroscopy for forensic purposes: recent applications for serology and gunshot residue analysis**

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## **ABSTRACT**

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ding author. Tel.: +1 518 591 8863; fax: +1 518 442 3462. E-mail address:<br>
bany, New Y The realm of forensics is scientifically complex with multiple disciplines utilizing a plethora of analytical techniques to identify, detect, and differentiate between countless types of evidence for solving crimes. The rapid, highly-selective, and nondestructive method of Raman spectroscopy (RS) has shown continued promise for analysis of many types of forensic samples. The incorporation of chemometrics further enhances the specificity of the RS, and offers the opportunity of automatic data analysis and estimation of error rates, which are important requirements for modern forensic tools. Applications of RS in forensic serology and for the analysis of gunshot residue (GSR) were chosen for this review since RS promises significant advancement of these areas for practical forensics. The studies included here, particularly with the utilization of portable instrumentation, support how crucial RS is to the field of forensic science, and should help facilitate its incorporation for routine sample analysis in the near future.

**Keywords**: Forensic science, Chemometrics, Trace evidence, Body fluids, Serology, Gunshot residue, Surface-enhanced Raman spectroscopy

### **1. INTRODUCTION**

Forensic science is a continually evolving component of the criminal justice system. With each subsequent year, new discoveries are made and advancements of old technologies come to light. Some are in the form of a proof-of-concept study while others may be a fully-validated technique ready for implementation in an accredited forensic laboratory. It is pertinent to stay at the forefront of emerging and established technologies, as well as establish guidelines to follow. In support of this, a relatively new endeavor by the National Institute of Standards and Technology (NIST) was the establishment of the Organization of Scientific Area Committees (OSAC) for forensic science, which targets the streamlining of forensic disciplines to have more structured and cohesive standards, rules, and regulations [1]. This is an extremely important initiative for the advancement of forensic science.

Ethion. Reamary spectroscopy. From analysis of boany linuts and inks to incret, explosive<br>idue, Raman spectroscopy has proven to be an extraordinarily powerful technique<br>plications. The advantages of Raman spectroscopy are One versatile analytical technique that has found an important niche as a developing forensic method is Raman spectroscopy. From analysis of bodily fluids and inks to fibers, explosives, and gunshot residue, Raman spectroscopy has proven to be an extraordinarily powerful technique for forensic applications. The advantages of Raman spectroscopy are extensive and transcend techniques that attempt to accomplish the same goals. For instance, Raman spectroscopic analysis is highly selective where samples on the order of picogram quantities or femtoliter volumes can produce a signal. Unlike many techniques, Raman analysis is nondestructive and does not require sample preparation, which allows for preservation of the sample, administration of subsequent analyses such as DNA extraction, and *in situ* testing methodologies; all of which are extremely important from a forensic perspective. Furthermore, the technique is not limited by sample type since solids, liquids, and gases of any kind can be analyzed via Raman spectroscopy. More importantly, with the availability of hand-held and portable instrumentation, samples can be analyzed, *in situ*, directly at a crime scene, police station, or other location where evidence may be found or require analysis.

A plethora of research has been carried out using Raman spectroscopy and other analyses for a variety of forensic applications. This review will focus on recent studies, published primarily from 2015 and onward, for forensic purposes in serology and the analysis of gunshot residue (GSR). The included studies specifically utilize Raman spectroscopy for analysis since the technique demonstrates unique suitability and promises significant advancement of these areas for practical forensics. Two recent review articles have covered many important studies up to 2014 involving the use of Raman (and infrared (IR)) spectroscopy to analyze trace evidence such as hairs, fibers, GSR, paints, and inks, bodily fluids, bones, drugs, pharmaceuticals, explosives, and chemical and biological threat agents [2, 3]. However, since that time, a variety of new studies have been published and many of those will be included here. This review demonstrates that both serology and GSR analysis have particular interest in forensic operations whether it be for crime scene investigation, trade, import/export, or (inter)national security. Since only a few specific topics are covered in detail for this review, readers are encouraged to learn more from other reviews about how Raman spectroscopy has been applied in forensic disciplines such as nuclear forensics [4], drug analysis [5, 6], ink analysis [7, 8], and the analysis of explosives [9].

## **1.1. RAMAN SPECTROSCOPY**

**EXECTROSCOPY**<br>
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since being discovered almost 90 years ago. One of the more recent areas of in<br>
since being discovered almost 90 y Raman spectroscopy has shown great prominence as a useful analytical tool for many different applications since being discovered almost 90 years ago. One of the more recent areas of interest for using Raman spectroscopy is forensic science, and, in particular, for security purposes. The technique uses monochromatic light to interact with and analyze samples. It works by irradiating a sample and collecting the inelastically scattered light, which is sent to a detector to generate a Raman spectrum. The spectrum consists of a series of peaks of varying intensities, which are directly attributed to the vibrational modes of certain chemical species as identified by their Raman shift (cm-1). This highly selective technique provides fingerprint-like spectra allowing for differentiation of nearly every compound.

In Raman spectroscopy, excitation laser light with different wavelengths have certain advantages and disadvantages. UV-visible laser lines such as 415-nm and 532-nm can typically result in resonance enhancement of the Raman signal from molecules with electronic transitions in this spectral range. Longer wavelengths, such as 785-nm and 1064-nm laser lines, are helpful since they minimize the fluorescence interference that Raman spectroscopy can suffer from, and are more common wavelengths for use in portable or handheld Raman spectrometers. These types of instruments are particularly useful for security applications since they can be used directly at the scene of a crime or at an entry/exit point such as a country's border.

Raman spectroscopy is a highly selective technique, but tends to lack sensitivity. More specifically, the ability to identify diluted solutions or where the concentration of the analyte of interest is extremely small can be difficult. As is discussed here, surface-enhanced Raman spectroscopy (SERS) has been implemented for some applications to obtain signal enhancement leading towards detection and identification of a substance where conventional Raman spectroscopy failed due insufficient sensitivity. For example, Izake reviewed a variety of homeland security applications where portable SERS and spatially offset Raman spectroscopy (SORS) instruments were utilized [10]. More recently, in 2015, a thorough review was published

for SERS analysis of questioned documents (inks), fibers, paint, explosives, fingerprints, body fluids, drugs, fibers, and more [11]. The combination of SERS with other techniques such as thin layer chromatography (TLC) and various extraction techniques, has been reported in a 2017 TrAC review by Zang et al. [12]. Also, reproducibility and repeatability validation of SERS for forensic science has been discussed [13], as well as using SERS for art and archeological applications [14], detecting pathogens in food [15], and fingerprint identification [16, 17].

w oy range cut. (1-3). Thus, reportantioning university and repetationing valuation of our<br>anticology and discussed [13], as well as using SERS for art and areheology<br>spiral and the spiral and the spiral and the spiral in The extremely important and useful technology, SORS, was introduced by Pavel Matousek for security applications [18]. This technique involves the collection of Raman scattered light from a different point than the focal point of the excitation laser beam, and can be used for the noninvasive identification of substances that may be concealed in a (plastic or paper) container, such as pharmaceutical materials [19]. In 2016, Guicheteau and Hopkins reviewed some of the security and defense applications that incorporated SORS as the sample analysis technique [20]. SORS has also been used to analyze bones to identify disease-related biochemical differences [21], which could be expanded to other forensic applications such as determining the postmortem interval (PMI) after death. Furthermore, with portable capabilities, SORS has many highly important uses and applications for enforcing security measures [22].

Although not yet widely used in forensic science research, due to the complexity of the technique, which requires advanced user experience and expensive equipment, tip-enhanced Raman spectroscopy (TERS) has proven to be very powerful. In 2016 Kurouski reviewed a wide variety of applications using TERS, some of which demonstrated nanometer resolution and single-molecule detection [23]. From that review one study in particular was forensically relevant, which used TERS to identify iron gall ink and indigo dye, *in situ*, on Kinwashi paper [24]. In the future, TERS may be more widely applied to forensic applications, but for now the research is limited.

Some of the current applications of Raman spectroscopy for security and forensic applications involve the use of a library to search for (illegal) compounds of interest, which is very similar to what is done with a mass-spectrometry (MS) search/comparison (for forensic drug analysis). However, these Raman spectral libraries are by no means as comprehensive nor as widely available as some of the libraries used for MS analysis. This is a major shortcoming that should

be addressed for future applications. Before discussing any research studies in-depth, a brief overview of chemometrics is provided since this utilization of (advanced) statistical analysis for the chemical data generated is becoming more and more common, particularly for Raman spectral datasets.

## **1.2. CHEMOMETRICS**

Collecting a Raman spectral map, a method known as hyperspectroscopy, is uniquely informative for heterogeneous samples. With the collection of larger and more complex datasets, conventional and simple data analysis procedures tend to become less reliable. To circumvent this problem, chemometrics, or multivariate statistical methods, can be utilized for the analysis of large Raman spectral datasets. At each wavenumber  $(cm<sup>-1</sup>)$ , a spectrum will have an intensity value, which can differ based on your sample, from being zero/non-existent (absence of a peak) to being a relatively a high value (large/pronounced peak). For these purposes, the wavenumbers are referred to as the variables, and, depending on specific applications, supervised or unsupervised statistical approaches are used.

**IOMETRICS**<br>g a Raman spectral map, a method known as hyperspectroscopy, is united for the foregreeneous samples. With the collection of larger and more complex dat and simple data analysis procedures tend to become less r An unsupervised model is one that does not include user-defined labels or values so only the data collected is used for constructing models. Some common examples of unsupervised modelling are principal component analysis (PCA) and hierarchical clustering analysis (HCA). Supervised modelling involves the use of user-defined data labels or values in addition to the data collected to provide better separation and differentiation capabilities. Some common examples of supervised modelling include linear discriminant analysis (LDA), partial least squares discriminant analysis (PLSDA), support vector machines discriminant analysis (SVMDA), and artificial neural networks (ANN). The application of variable selection technique, such as a genetic algorithm (GA), can further assist with classification capabilities. For brevity, this section represents a basic overview of chemometrics, and readers are encouraged to explore other, more detailed, sources if interested in learning more about the subject [2, 3, 25, 26].

## **2. DISCUSSION**

as worthwinne in forensic science over the last couple decades. One of the several and series the development of portable and hand-held Raman spectrom of or for dat collection and substance identification directly in the f Although the analytical technique of Raman spectroscopy has been established as a useful tool for analyzing a variety of samples for almost one hundred years, it has only been seriously recognized as worthwhile in forensic science over the last couple decades. One of the major "game changers" has been the development of portable and hand-held Raman spectrometers, which allow for data collection and substance identification directly in the field such as a crime scene or ports of entry/exit such as an airport or a country's border. However, many of the techniques discussed in this review have unfortunately not yet been developed enough for advancement to the stage of portability. One of the issues with this is the lower sensitivity of the portable/hand-held instruments as compared to benchtop instruments. Also, specifically related to forensics, the developed methodology needs to be critically validated, which requires a significant amount of time and very explicit protocols; particularly if a forensic lab is accredited. Regardless, the studies included here are worthwhile and important to forensic science, and thus deserve proper discussion.

## **2.1. FORENSIC SEROLOGICAL ANALYSIS**

The analysis of bodily fluids is a very important part of a variety of disciplines, including the medical field, veterinary science, and forensic science. Serology, from a forensic standpoint, involves the analysis of bodily fluid traces, which is a foundational aspect of forensic investigations, particularly for sexual assaults, homicides, and other violent crimes. Ultimately, a DNA profile can be generated from biological evidence, but there are other types of analyses that can be utilized prior to, or in concurrence with, DNA profile comparisons in support of claims or conclusions being made. In 2015, Zapata et al. reviewed various studies for the forensic analysis of body fluids using spectrometric techniques [27, 28], some of which will be discussed here.

Lednev and coworkers pioneered the analysis of bodily fluids with Raman spectroscopy, first by demonstrating the differentiation of the five major bodily fluids including peripheral blood, saliva, semen, sweat, and vaginal fluid [29]. The differentiation of these bodily fluids was further advanced in 2016 through more sophisticated statistical analyses incorporating variable selection methods (i.e. interval partial least squares discriminant analysis (iPLSDA) and genetic algorithms (GA)) to obtain nearly complete differentiation with the lowest prediction accuracy of 96.4% for external validation [30]. Other types of multivariate statistical analyses have been utilized for generating spectroscopic signatures of peripheral blood [31], saliva [32], semen [33], sweat [34], and vaginal fluid [35], which were later compared in a review [36].

and regnal riants [cor], which vertect and complexe matrices [cor].<br>The determination and differentiating those five bodily fluids, the Lednev rese<br>demonstrated their ability to distinguish between human and animal blood [ In addition to identifying and differentiating those five bodily fluids, the Lednev research group also demonstrated their ability to distinguish between human and animal blood [37-39] as well as menstrual blood from peripheral blood [40]. They have also been able to identify bodily fluids that had been contaminated with dust, sand, and soil [41] and overcome interferences from various substrates that blood [42] or semen [43] could be deposited on, as well as mixtures of blood and semen [44]. Crime scenes are not pristine environments, and therefore contaminations are common. In one study three everyday substances, including dust, sand, and soil, were used to contaminate bloodstains to mimic somewhat real-world forensic samples. Figure 1 shows averaged Raman spectra of pure blood, dust, sand, and soil, as well as blood in the presence of each of the three contaminants. For each type of contaminant, two representative spectra are shown to demonstrate sample heterogeneity and that at certain mapping points the spectra may be dominated by peaks from the contaminant (Figure 1E, G, and I) whereas at other points the spectra resemble an uncontaminated blood stain (Figure 1F, H, and J). Automatic mapping allows for selecting spots on the contaminated sample where the contribution from blood dominates the spectrum. As is evident from this figure, and explained in detail in the paper, a multidimensional Raman spectroscopic signature of blood was used to reconstruct a new spectrum, which allowed for successful identification of blood even when the stain was contaminated.



**Figure 1**. Selected Raman spectra of pure blood (A), sand (B), dust (C), soil (D), and blood contaminated by sand (E, F), dust (G, H), and soil (I, J). The black lines on plots (A–J) represent the result of fitting using the multidimensional Raman signature of blood. Reprinted from A. Sikirzhytskaya, V. Sikirzhytski, G. McLaughlin, I.K. Lednev, Forensic Identification of Blood in the Presence of Contaminations Using Raman Microspectroscopy Coupled with Advanced Statistics: Effect of Sand, Dust, and Soil, Journal of Forensic Sciences, 58 (2013) 1141-1148 [41]. Copyright 2017, with permission from Wiley.

More recently they reported on proof-of-concept studies using Raman spectroscopy for the differentiation of human races for semen [45] and blood [46] donors, and the biological sex for blood [47] and saliva [48] donors, in addition to their ability to predict how long a bloodstain has been left on a surface (i.e., the time since deposition (TSD)) for up to one week [49] and, even

more astonishingly, for up to two years [50]. There are certain spectral changes that occur as bloodstains age, and some of these can be seen in Figure 2. This figure shows averaged preprocessed spectra of bloodstains at various time points from one hour up to 19008 hours (two years) for two blood donors. It is evident that the peaks indicated by dashed or solid lines, and those included in the shaded areas, are changing over time, which correlate to natural biochemical changes such as denaturation and aggregation of hemoglobin. Claire Muro et al. helped to advance the research progress in forensic serology by confirming that the limit of detection for Raman spectroscopic analysis of bloodstains is a single red blood cell, or ~250 fL of blood, which is approximately 5000x less than what is currently needed to obtain a DNA profile from a blood sample [51].



**Figure 2**. The averaged preprocessed spectra for bloodstains from the male (black traces) and female (red traces) donors aged in an ambient environment for up to two years. The most prominent changes are indicated as dotted lines (increasing with time), solid lines (decreasing

with time), and shaded regions. Reprinted from Forensic Chemistry, 5, K.C. Doty, C.K. Muro, I.K. Lednev, Predicting the time of the crime: Bloodstain aging estimation for up to two years, 1- 7 [50]. Copyright (2017), with permission from Elsevier.

the realm of bodily fluid analysis has not only been carried out by the Lednev rest<br>state done similar work such as characterizing human semen and blood<br>etroscopy and ATR-FTIR in the mid-IR region, even when a statin was a Work in the realm of bodily fluid analysis has not only been carried out by the Lednev research group. Others have done similar work such as characterizing human semen and blood using Raman spectroscopy and ATR-FTIR in the mid-IR region, even when a stain was aged [52]. Recently, Atkins et al. reviewed many important studies where Raman spectroscopy was used to analyze blood [53]. Feine, Gafny, and Pinkas have confirmed the presence of semen in seminal fluid, without urine being a problematic false positive, using prostate specific antigen (PSA) detection and Raman spectroscopy [54]. Also, human and animal blood have been differentiated using Raman spectroscopy coupled with PLSDA [55], as well as with a portable Raman instrument [56]. As previously mentioned, it is known that portable Raman spectrometers are less sensitive in comparison to research-grade benchtop instruments. However, the fact that there was still enough variation between human and animal blood samples (based on spectra acquired using a portable instrument) to differentiate between the two types of blood by PCA, demonstrates a high potential of Raman spectroscopy to be used at the scene of a crime for biological stain identification and characterization.

Although this list is quite exhaustive, more work still needs to be done in the area of forensic serology such as analyzing bloodstains for their TSD at increased/decreased temperature and humidity, along with other bodily fluids in all environments. Also, incorporating donors that may have biological conditions that could affect one or more of their bodily fluids and therefore provide spectra that are different from those of a "healthy" donor should be researched. Some work has commenced for genetic profiling of human donors, but, for the most part, extensive studies targeting the differentiation of donors based on sex, age, and possibly race are currently lacking in the field. As is shown in other parts of this review, bodily fluids could be "contaminated" with drugs or other compounds/chemicals, which could provide complications in their identification. These possible discrepancies or interferences should also be addressed in future studies.

## **2.2. GUNSHOT RESIDUE AND EXPLOSIVES**

n documented gun violence [57] is a major concern these days, particularly wi<br>vailability of guns and ease of attainment in certain states of the U.S. The analy<br>due (GSR) has conventionally been carried out using scanning The rise in documented gun violence [57] is a major concern these days, particularly with the increased availability of guns and ease of attainment in certain states of the U.S. The analysis of gunshot residue (GSR) has conventionally been carried out using scanning electron microscopyenergy dispersive X-ray spectroscopy (SEM-EDX), but this technique does have some flaws. Although highly reliable, SEM-EDX cannot detect organic GSR particles. Also, due to the absence of lead, which is required for GSR identification by this technique, SEM-EDX cannot be used to identify "green" (lead free) ammunition. On the contrary, Raman spectroscopy has been particularly useful in advancing the forensic research available for identification and differentiation of both organic and inorganic GSR, showcased amongst other techniques in two 2017 reviews [58, 59]. Additionally, Raman spectroscopy can identify certain plasticizers and nitrate ester explosive compounds from GSR whereas SEM-EDX cannot, which makes it an overall more comprehensive and selective technique. For a more thorough overview of GSR in general readers are suggested to read a recent review by Blakey et al. [60].

The Lednev research group, with substantial efforts by Justin Bueno, initiated the use of Raman spectroscopy to investigate various research questions surrounding forensic GSR analysis. In a 2012 study, Bueno et al. demonstrated that Raman spectroscopy could differentiate between GSR particles from 9 mm and 0.38 in. caliber ammunition using SVMDA and PLSDA [61]. The authors indicated that the differentiation most likely stems from variations in the chemical composition of the ammunition as well as the discharge combustion conditions, which depend on the firearm type and caliber in particular. As can be seen in Figure 3, the two types of ammunition have slightly different spectra. Also, there is heterogeneity within both types of ammunition as can be seen in the inset and part B of Figure 3; specifically for the peak located at 983 cm<sup>-1</sup>, which was assigned to  $PbSO_4$  (from the primer). More importantly, this figure demonstrates that Raman spectroscopy allows for probing both organic and inorganic components of GSR, regardless of the caliber of the ammunition.



**Figure 3.** (A) The selected Raman spectra collected from 9 mm (red line) and 0.38 (green line) caliber GSR of different caliber GSR particles. Shaded areas indicate the contributions of the inorganic (purple) and organic (green) substituent's from the original ammunition. (B) Illustration of the heterogeneity of GSR at the particle level. The red and blue spectra were collected from different locations on the same GSR particle. Reprinted with permission from J. Bueno, V. Sikirzhytski, I.K. Lednev, Raman Spectroscopic Analysis of Gunshot Residue Offering Great Potential for Caliber Differentiation, Analytical Chemistry, 84 (2012) 4334-4339 [61]. Copyright (2017), American Chemical Society.

Then, in 2013, Bueno and co-workers used attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FT-IR) and PLSDA to differentiate between 9 mm, 0.38 in., and 0.40 in. caliber ammunitions with 100% and 93.3% accuracy for internal cross-validation and external validation GSR samples, respectively [62]. Later that year, Bueno and Lednev expanded upon the caliber differentiation approach by using the combination of FT-IR and Raman spectroscopies, whereby the complementarity of the two techniques demonstrated an increase in both the specificity and sensitivity to 100% as compared to 97% and 94%, respectively, when only FT-IR was used and 98% when only Raman was used [63].

In 2014 Bueno and Lednev used a "tape lifting" technique using double-sided office tape to collect GSR from firings of Winchester "0.38 special" ammunition and 0.40 in. Federal brand "S&W" full metal jacketed ammunition on cloth substrates. By way of ATR imaging, chemical color maps were generated to show specific GSR spectroscopic "fingerprints" of different particles, which were different than those of the cloth substrate and the tape, and a 4.7 µm spatial resolution was established [64]. In their most recent study, Bueno and Lednev used Raman microspectroscopy and PLSDA to analyze and differentiate inorganic GSR (IGSR) and organic GSR (OGSR) on tape lifts from cotton cloths that had been shot with three Winchester brand "0.38 special" (0.38 in. caliber) discharge samples [65].

x from limps of winchester 0.58 special aminuminon and 0.40 in. Pederal healta classed ammuniton and 0.40 in. Pederal healta classed ammuniton on cloth substrates. By way of ATR imaging, che were generated to show specifi López-López and co-workers evaluated the ability to use Raman spectroscopy for analyzing both samples of lead-free and conventional GSR directly from unmodified and bloodstained black, white, and printed cotton targets, as well as samples recovered using the conventional method of swabbing with SEM-EDX carbon stubs [66], as can be seen in Figure 4. Multivariate curve resolution (MCR) analysis was utilized to create chemical color maps for GSR identification, which allowed for detecting chemical components of all four types of ammunition for each of the (bloodstained) substrates surveyed. The Raman spectra on the right in Figure 4 showcase the differences between the GSR particles and the substrate upon which they were deposited, along with the corresponding components used to generate the MCR color maps, where "Component 1" (blue in MCR analysis panels) was for the GSR particle and "Component 2" (red in MCR analysis panels) was for the respective substrate. This group also used SERS to analyze twenty-one different smokeless gunpowders, and then detect macroscopic GSR particles on aluminum stubs collected from cloth targets shot using two different ammunition cartridges [67].



**Figure 4**. Microscope images of GSR particles from four ammunition cartridges fired over different cloth samples; their corresponding MCR analysis (blue indicates the component assigned to the GSR particles; red indicates the component assigned to the cloth substrates); and the Raman spectra of the GSR particles, the substrates, and the pure components calculated for the MCR model. (a) Super X GSR particle on white cotton cloth. (b) GECO 9319 Luger SX GSR particle on black cotton cloth. (c) S&B 80 9319 GSR particle on printed (blue and red) cotton cloth. (d) G.F.L. 9 mm Luger GSR particle on white cotton cloth stained with blood. Raman conditions: laser at 455 nm, 6.0 mW, 503magnification objective lens, slit pinhole size 50 lm, 0.01 s 3 30 scans, step size 5 lm (3726, 5829, 7857, and 4248 spectra, respectively).

Reprinted from M. López-López, M.Á.F.d.l. Ossa, C. García-Ruiz, Fast Analysis of Complete Macroscopic Gunshot Residues on Substrates Using Raman Imaging (69:7) pp. 889-893, copyright @ 2015 by Applied Spectroscopy [66]. DOI: 10.1366/14-07816. Copyright 2017, by Permission of SAGE Publications, Ltd.

To date, Raman spectroscopy has demonstrated its keen ability to detect both organic and inorganic GSR, which is a significant improvement over SEM-EDX. Also, distinguishing between different ammunition calibers and between GSR and (bloodstained) cloth substrates has been implemented. However, certain problems still exist that will require further research studies to be investigated. These include differentiating between the same types of bullet from different manufacturers and identifying any potential false positives. Future studies should focus on expanding upon those that have already been established and attempting to solve the existing problems where research is lacking.

## **3. CONCLUSIONS**

Raman spectroscopy has demonstrated its keen ability to detect both organists<br>
SSR, which is a significant improvement over SEM-EDX. Also, distinguisferent ammunition calibers and between GSR and (bloodstained) cloth subst Raman spectroscopy has proven extremely useful for a variety of applications, including those important to solving crimes and maintaining security. This review covers numerous studies utilizing Raman spectroscopy as a possible solution to certain forensic problems, particularly in the areas of forensic serology, and gunshot residue analysis. This selective analytical technique, along with chemometrics and/or the more sensitive Raman spectroscopic method of SERS, has even demonstrated success for sample analysis using portable/hand-held Raman spectrometers; albeit these instruments typically provide lower spectral resolution as compared to benchtop instruments. Through the implementation of these methods for evidence analysis, more information can be supplied to forensic science specialists during a criminal investigation. However, a limited number of these methods have been applied for consistent and reliable use in actual forensic casework.

One potential roadblock for this, and where research should be focused moving forward, is the construction, or supplementation, of spectral libraries for matching unknowns to knowns, as is routinely done in forensic drug analysis, toxicology, and trace evidence analysis. Unfortunately, Raman spectral libraries are either not available, not common, or not widely accessible, which is

Assume Crucial antimation of the antimation crucial contracts and the dield, and the case is closed. What's more is that even though portable/hand-trometers are becoming cheaper and more user-friendly, very few crime labs in stark contrast to mass-spectrometry libraries used in forensic drug analysis. Another reason is the lack of validation of the techniques, which could involve demonstrating their effectiveness on real crime scene samples. In the United States, it is extremely difficult, if not impossible, to obtain forensic evidence for such analyses, even from casework where a verdict has been garnered and filed, and the case is closed. What's more is that even though portable/hand-held Raman spectrometers are becoming cheaper and more user-friendly, very few crime labs possess these instruments and/or have the auxiliary funds to purchase them; not to mention having their staff trained on how to properly use them. Furthermore, in many instances the methodologies have not yet been transferred over from benchtop systems to portable/hand-held systems.

In the future, we may see an influx of Raman spectroscopic methods being implemented into routine case analysis work, but that prospect is still a few years away. However, if the significant progress made over the last decade, including the studies outlined here, is any indication of what's to come, Raman spectroscopy is well on its way to being a highly reliable and advantageous technique for forensic science and security applications.

#### **ACKNOWLEDGEMENTS**

This endeavor was supported by Awards No. 2014-DN-BX-K016 and 2015-R2-CX-0021 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K. Lednev and K.C. Doty). The opinions, findings, and conclusions or recommendations expressed here are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

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MANUSCRIPT

## **HIGHLIGHTS**

- Raman spectroscopy offers great potential for forensic serology
- Raman spectroscopy offers great potential for forensic phenotype profiling
- Raman spectroscopy offers great potential for determining the bloodstain age
- Raman spectroscopy offers great potential for differentiating human and animal blood
- Raman spectroscopy offers great potential for gunshot residue detection and identification

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