



**The author(s) shown below used Federal funding provided by the U.S. Department of Justice to prepare the following resource:**

**Document Title:** Raman Spectroscopy for Analyzing Body Fluid Traces: Moving towards a Practical Forensic Application

**Author(s):** Igor K. Lednev

**Document Number:** 253070

**Date Received:** July 2019

**Award Number:** 2014-DN-BX-K016

**This resource has not been published by the U.S. Department of Justice. This resource is being made publically available through the Office of Justice Programs' National Criminal Justice Reference Service.**

**Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.**

## Raman spectroscopy for analyzing body fluid traces: Moving towards a practical forensic application

The Research Foundation of SUNY  
University at Albany  
Office for Sponsored Programs, MSC 312  
1400 Washington Avenue  
Albany, NY 12222  
RF # 1122735-1-70484

Igor K. Lednev  
Professor  
Department of Chemistry  
University at Albany, SUNY,  
1400 Washington Ave  
Albany, NY 12222  
Phone: (518) 591-8863  
Fax: (518) 442-3462 (Chemistry)  
E-mail: ilednev@albany.edu  
<https://sites.google.com/site/lednevlab/>

### Final Summary Overview

#### 1 DESIGN AND METHODS

This funded work during the report period was focused on further method development for identification and characterization of body fluid traces using Raman spectroscopy including determining the limit of detection, identifying biological stains on different substrates, investigating potential false positives, and determining the age of the donor. Body fluid samples were purchased from scientific and medical vendors, who provided all relevant donor information (gender, race, age, etc.) if necessary. Human blood for *Objective 1* was procured from volunteers following a protocol approved by the University at Albany Internal Review Board (IRB) and Institutional Biosafety Committee (IBC). All false positive substances used for *Objective 3* were purchased from appropriate sources.

For all objectives samples were prepared for analysis by depositing around 10-50  $\mu\text{L}$ , depending on the project, onto an aluminum foil covered microscope slide and allowed to dry. After drying, samples were analyzed via automatic Raman microspectroscopic mapping to probe multiple points across the sample surface, and collect a dataset that accurately represents the heterogeneous nature of a body fluid stain. The datasets were then subjected to comprehensive data analysis including preprocessing, constructing statistical models, conducting internal and external validation, and developing spectroscopic libraries.

## 2 DATA ANALYSIS

In the beginning of the report period, we completed work towards the objectives of our previous NIJ grant (2011-DN-BX-K551) and published articles on the development of Raman spectroscopic libraries of body fluids to study the spectral changes over time [1, 2], differentiate human and animal blood [3, 4], and determine a donor's sex and race based on their body fluid spectra [5-8]. These studies based only on pristine samples do not answer all of the questions a forensic investigator may have about a body fluid trace found at a crime scene. To address the objectives of the current grant, we studied the limit of detection, common substrates' interference, samples with similar biochemical composition to body fluids to address the problem of potential false positives, and determining the age of a body fluid donor. Specifically, we have developed advanced data analysis methods to eliminate fluorescent interference, reduce dimensionality of the dataset, and exclude interference from cosmic rays and random noise. Algorithms were also developed in order to select variables that vary the most between classes and so are most useful in class differentiation. Pretreated datasets were used to build statistical models to find statistically significant patterns. These models, together with the spectroscopic libraries created earlier, allowed for the development of statistical methods for classifying unknown spectra and determining the necessary error rates.

## 3 STATEMENT OF RESULTS

**3.1 Objective 1:** Determine the limit of detection of the current method to identify dry traces of blood, semen, vaginal fluid, saliva, and sweat.

The limit of detection of Raman spectroscopy was studied in order to find the smallest possible amount of sample used for identification the traces of body fluids. Creating a

technique that is sensitive enough to detect the minuscule sample size often associated with crime scenes is very important.

### 3.1.1 Determine the limit of detection of blood

We found that the developed method of Raman microspectroscopy can be used to detect and identify easily any small amount of undiluted blood, which can be deposited by any known method providing the control of the deposited amount. Therefore, we analyzed individual red blood cells (RBCs) as representative components of whole blood. Peripheral blood was collected by pinprick from 10 volunteers following an approved IRB protocol. A total of 50 spectra were collected from all donors, and loaded into a support vector machine discriminant analysis (SVMDA) model previously built to identify body fluids [9]. All of the experimental spectra were correctly identified as peripheral blood. Given that the average RBC concentration in peripheral blood is  $4.0 \times 10^6/\mu\text{L}$ , the limit of detection for the current method has been determined to be 250 fL of blood. Comparatively, 100,000 fL of peripheral blood is needed in order to acquire a full DNA profile. These results show that our current Raman spectroscopic method is more sensitive than DNA profiling by several orders of magnitude. In other words, if the amount of blood is large enough for DNA analysis then our method should definitely detect it.

### 3.2 **Objective 2:** Identify biological stains prepared to simulate real world forensic evidence.

Preparation parameters will include varying substrates, mixing body fluids, and introducing inorganic contamination. Modify the current method to account for these variables and their effects on the spectral properties of blood, semen, vaginal fluid, saliva, and sweat.

#### 3.2.1 Identify biological stains prepared to simulate forensic evidence- Semen

Together with our collaborators from Japan, we were able to train a program called Hypothetical Addition Multivariate Analysis with Numerical Differentiation (HAMAND) to

identify semen on different substrates. Spectra of semen on two different substrates, glass and blue polyester, as well as background spectra of just semen and just the substrates were collected in our laboratory.

These spectra were then sent to our collaborators in Japan for data analysis. The HAMAND program was able to extract signals unique to semen only when semen was present in the combined semen-substrate spectra. No significant extraction was seen when the program was applied to negative controls that contained no semen.

**3.3 Objective 3:** Investigate samples with similar biochemical compositions to body fluids that could potentially result in false positives. Update the developed method to ensure they are not misidentified. Samples will be chosen based on suggestions from consultants.

The purpose of this work is to demonstrate that Raman spectroscopy can be used as a confirmatory method for body fluid identification. A crucial step in this process is to determine that the method does not result in any false positive or false negative assignments like some presumptive tests commonly used for body fluid identification.

#### 3.3.1 Identify and differentiate potential false positive samples from human blood

Raman spectroscopy was used to compare spectra of dried human blood with spectra of different substances. Based on our findings, and collaborative input from the Netherlands Forensic Institute, bleach (6.15% sodium hypochlorite), horseradish, pasta sauce, tomatoes, and parsnips were chosen. Every substance was prepared in triplicate. The average spectrum for each of the substances studied was overlaid with an average Caucasian male blood spectrum previously acquired for the purpose of comparison. Through spectral comparisons, all five aforementioned substances were differentiated from human blood, resulting in zero false positive assignments. Furthermore, multivariate curve resolution analysis was used to compare experimental blood spectra to our previously developed multi-dimensional blood

signature and generate reconstructed spectra. The reconstructed spectra were compared to the experimental spectra of substances using three statistical metrics including root mean squared error (RMSE), sum of squared error (SSE), and the coefficient of determination ( $R^2$ ). For dried blood the RMSE and SSE values were the lowest, while the  $R^2$  value was the highest. In comparison to the other substances, aside from dried horseradish, the values of the three statistical metrics were much worse, particularly for SSE and  $R^2$ . Based on these results, it could be determined, in yet another more confirmative way, that all five substances do not provide a false positive blood identification when Raman spectroscopy is used for the method of analysis. That provided another tool for more robust confirmation that the substances are not falsely identified as blood. Therefore, these results demonstrate that Raman spectroscopy is selective and advantageous over current presumptive tests for correct blood identification.

### 3.3.2 Identify and differentiate potential false positive samples from human saliva

Raman spectroscopy was also used to compare spectra of dry human saliva with spectra of four substances. Based on the Starch-iodine test, the Phadebas® Saliva Test kit, Rapid Stain Identification (RSID)-Saliva kits, and SALIgAE®, which are all presumptive saliva tests, a list of substances that could be mistaken for saliva or that contained  $\alpha$ -Amylase was prepared. Bleach, olive oil, vegetable oil, and laundry detergent have been analyzed so far.

Raman spectra of four substances that could potentially present false positive results were compared to spectra of saliva samples. A principal component analysis (PCA) enabled the differentiation between these substances and dried human saliva. All potential false positive substances clustered separately from saliva sample, thus forming pre-defined groups not overlapping with saliva samples.

### 3.3.3 Identify and differentiate potential false positives from semen

Raman spectroscopy was used to compare various common substances and household items that either give the appearance of semen or share a similar (bio)chemical composition with semen. Twenty-three substances that could potentially be misidentified as semen were analyzed. The list of substances was made based on the presumptive tests giving false-positive results (i.e. Acid-Phosphatase test, Prostate-Specific Antigen test, the spermine test, and various enzyme tests), samples that fluoresce similar to body fluids under Wood's Lamp and Polilight, and samples that give the visual appearance of semen. This list includes semen-free vaginal fluid, blood, various milks and cheeses, contraceptive foam, plant tissue, onions, apples, fruit juices, KY Jelly lubricant, and various teas.

A partial least squares discriminant analysis (PLSDA) model indicated that none of these substances would be mistaken for semen using the Raman method. The components responsible for providing false positive results in some presumptive tests did not interfere with the model's ability to differentiate the Raman spectra. The preprocessed averaged dried semen spectrum can be distinguished visually from the averaged spectrum of each false positive substance analyzed thus far. Furthermore, the use of statistical comparisons between measured spectra provided another tool for confirmation that the substances are not falsely identified as semen.

### 3.3.4 Identification of potential false positive substances conclusions

Raman spectroscopy was found useful in nondestructive discrimination of body fluid (i.e. blood, saliva, semen) and some substances resulting in false positive assignment to these body fluids. That gives a potential for real crime scene investigation to exclude non-relevant samples. For the majority of substances the Raman signature is different than that of the body fluid due to differences in (bio)chemical composition of these samples. However, to avoid

human error and bias in evaluating results, the statistical approaches were made. They could determine, in yet another, more confirmative way, that all substances do not provide a false positive body fluid identification when Raman spectroscopy is used. This provided another tool for more robust confirmation that the substances are not falsely identified as body fluid.

**3.4 Objective 4:** Collaborate with an accredited forensic lab to analyze case-realistic samples to validate the developed method, and test the practical accuracy and robustness.

Our collaborators (practitioners) advised us that they would be interested in validating our method using realistic samples on common substrates. Therefore, we needed to complete our work on the method development, which would allow us to overcome substrate interference. As described in *section 3.2. Objective 2*, we have developed such method due to the collaboration with Japanese Software Company very recently. We had no time to implement this newly developed method for case-realistic samples.

**3.5 Objective 5:** Evaluate the capability of Raman spectroscopy to determine the age of a donor based on dry body fluid traces. Develop statistically significant Raman spectroscopic signatures for specific age groups for blood, semen, vaginal fluid, saliva, and sweat traces.

The determination of a body fluid donor's age is an important piece of information to know in any forensic investigation. We have begun to target attaining this information using Raman spectroscopy and advanced statistical analysis. We completed the experiment, spectra treatment and building a statistical model. We are at the last stage of completing the model validation. We plan to complete this work before the end of the grant period.

**3.5.1 Determine the age of a peripheral blood donor**

Raman spectra were collected from 3 blood donors in the “young” age range (11-13 years old) and 20 donors in the “old” range (43-68 years old). All blood donors were



Caucasian males so that race and gender variations were eliminated. A PLSDA model was built to differentiate Raman spectra of bloodstains from donors split into two different age groups (i.e. “young” and “old”). The internal cross-validated (CV) prediction results were promising, with sensitivity and specificity values of 0.98 and 0.91, respectively, for the “old” class of blood donors. However, the model has yet to be externally validated, which would represent a more stringent form of model performance testing.

### 3.5.2 Determine the age of a semen donor

Raman spectra were collected from 37 semen donors, ranging in age from 22 to 57. The population included 13 Caucasian, 12 Black, and 12 Hispanic donors. Multivariate data analysis was applied to the spectra to search for a quantitative relationship between the spectral data and the donors’ ages. Regression and classification algorithms were applied to the calibration dataset, using three different labeling schemes for donor age. All models were CV by leave one out (LOO), such that all of the spectra from one donor were removed from the dataset at a time. The most promising results were obtained when the donors were split into two groups: less than 30 years old and 40 years and older. Donors between these two age groups were removed from the dataset. A CV PLSDA model separated these two age groups with 48% accuracy that is not satisfactory. We will continue working on the data analysis to understand what else can be done to improve the differentiation power of our method.

### 3.5.3 Determine the age of a saliva donor

A classification model was built to determine the age of human saliva donor using Raman spectroscopy. Raman spectra were collected from 58 saliva donors, ranging in age from 19 to 73 years. Mapping was used to collect 25 spectra per sample. Radial basis function kernel support vector machine discriminant analysis (RBF-SVM-DA) was applied to the training dataset to identify relationships between the spectral data and the donors’ ages. To

determine which model to use the model was trained several times during cross-validation, leaving out 20% of training dataset in turn. To measure the model by how well it predicts, the left out data has been assessed on average. Once the model was trained, it was applied to test set to make sure that model is generalizable. A CV RBF-SVM-DA model separated these two age groups with 85% accuracy and a subsequent external validation has shown prediction results with accuracy of 86%. Our results demonstrate that Raman spectroscopy can be used as an analytical tool to reveal information about the age of human saliva donors. To generate summary performance estimates of the test set, we used the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The AUC was estimated at 0.87 (95% CI, 0.82-0.92) from the ROC curve which means that our classifier has an 87% probability to correctly classify the age group of each spectrum.

#### 3.5.4 Determine the age of a sweat donor

A total of twenty human sweat samples were analyzed. Sweat donors were chosen with variations in sex and race. Dataset with a total of the 1,928 spectra was divided into sweat donors' age between 14 and 33 (14 samples) and between 42 and 57 (6 samples). The first group (donors' age 14-33) consisted of 1,304 spectra, and second group (donors' age 42-57) of 624 spectra.

The internal CV prediction showed sensitivity of 0.67 and 0.55 for the younger and older donors, respectively. The method was validated with a ROC curve, which can also determine the best threshold for donor level classifications. The best results were obtained with a threshold of 31% giving 5 misclassifications of 20 subjects on donors' level.

### 3.5.5 Donor's age identification conclusions

The preliminary studies discussed above show that Raman spectroscopy has the potential to narrow down the age determination of body fluid donor. That approach was applied for blood, semen, saliva, and sweat traces so far. The changes in (bio)chemical composition of different body fluids with age of a donor can provide distinctive characteristics in their Raman signature. Due to only minor quantitative changes of specific components, the advanced data analysis was applied to enable discrimination process. More studies and validations are yet to be done. However, preliminary findings show promise for application Raman spectroscopy in nondestructive donor's age identification from body fluids.

### 3.6 **Additional work**

Additionally, Raman spectroscopy capabilities of the CBex handheld Raman instrument were studied by one, self-founded undergraduate student. The CBex handheld Raman instrument performance was compared to the performance of the Renishaw inVia Raman microscope. The CBex handheld Raman instrument was determined to be 8.67 times less sensitive than the Renishaw inVia Raman microscope.

## 4 **PROJECT FINDINGS**

Raman spectroscopy was found sensitive to detect a single red blood cell (RBC) in the study on limit of detection of blood. Based on its Raman spectrum, a single RBC was correctly identify as peripheral blood using previously built model for identification of body fluids using Raman spectroscopy [9]. Given that the average RBC concentration in peripheral blood is  $4.0 \times 10^6/\mu\text{L}$ , the limit of detection for the current method has been determined to be 250 fL of blood. These results show that our current Raman spectroscopic method is more sensitive than DNA profiling by several orders of magnitude.

In conjunction with our collaborators in Japan we were able to train HAMAND to identify semen on different substrates. The method was able to identify semen stain on glass and blue polyester. These two substrates were chosen as been the most problematic for Raman spectroscopic measurements because of strong fluorescence interference and heterogeneous composition, respectively. The HAMAND program was able to extract signals unique to semen in both these cases demonstrating outstanding performance of the method.

A wide selection of substances was chosen as a source of potential false positives including chemicals, which give false-positive results based on currently used presumptive tests, and compounds, which look alike dry blood, saliva, and semen. Raman spectra of these compounds were compared to the spectra of dry body fluids. For all analyzed fluids, results indicated that our method is selective, shows no false positives and, consequently, advantageous over current presumptive tests.

The estimation of the donor's age using Raman spectroscopy of dry traces of peripheral blood, semen, saliva, and sweat was investigated. Raman spectroscopy combined with advanced statistical analysis showed a definite promise for differentiating groups of donors based on their age although further work is necessary to complete this study.

## **5 IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE**

The potential advantages of applying Raman spectroscopy to forensic body fluid identification and analysis are vast, including significantly increasing the amount of information obtained, while reducing the cost and time of analysis, as well as preserving evidence integrity through a non-destructive, confirmatory test. The development of portable instrumentation should significantly improve the efficiency of crime scene investigations by (i) identifying a body fluid from very small sample amount, (ii) identifying body fluids deposited on various substrates, (iii) limiting the amount of evidence collected and documented for further analysis to only stains, which are relevant to the crime, and (iv) narrowing down the investigation by determining the age of a body fluid donor.

## Scholarly Products from Funded Project

### I. Publications

1. Doty, K. C., Muro, C. K., Bueno, J., Halámková, L. & Lednev, I. K. What can Raman spectroscopy do for criminalistics? *J. Raman Spectroscopy* **2016**, 47, 39-50. doi: 10.1002/jrs.4826.
2. C.K. Muro, K.C. Doty, J. Bueno, L. Halámková, 渡邊大助 and I.K. Lednev. 振動分光光学□法科学に革命をもたらす新展開. *J. Spectroscopical Research Japan* **2016**, 65, 3–32. (Review, in Japanese).
3. Penido, C.A.F.d.O., Pacheco, M.T.T., Lednev, I.K. & Silveira Jr., L. Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse. *J. Raman Spectroscopy* **2016**, 47, 28-38. doi: 10.1002/jrs.4864.
4. Muro, C.K., Doty, K.C., Bueno, J., Halámková L., and Lednev, I.K. Vibrational spectroscopy for forensic applications. Chapter 2 in *Forensic Science: A multidisciplinary Approach*. Ed. E. Katz and J. Halamek. Wiley-VCH **2016**, pp 5-54.
5. Manheim, J., Doty, K.C., McLaughlin, G., and Lednev, I.K. Differentiation of human, cat, dog, and synthetic hair by attenuated total reflection (ATR) Fourier transform-infrared (FT-IR) spectroscopy for forensic purposes. *Appl. Spectrosc.* **2016**, 70, 1109–1117. doi: 10.1177/0003702816652321.
6. Muro, C.K., Fernandes, L.d.S., and Lednev, I.K. Sex determination based on Raman spectroscopy of saliva traces for forensic purposes. *Anal. Chem.* **2016**, 88, 12489–12493, doi: 10.1021/acs.analchem.6b03988.
7. Mistek, E., Halamkova, L., Doty, K.C., Muro, C.K., and Lednev, I.K. Race differentiation by Raman spectroscopy of a bloodstain for forensic purposes. *Anal. Chem.* **2016**, 88, 7453–7456 (**Made a journal cover**). doi: 10.1021/acs.analchem.6b01173 (Letter).
8. Muro, C.K., Doty, K.C., Fernandes, L.d.S., and Lednev, I.K. Forensic body fluid identification and differentiation by Raman spectroscopy. *Forensic Chem.* **2016**, 1, 31-38 (**Made a journal cover; the most downloaded and second most cited article from the journal**). doi:10.1016/j.forc.2016.06.003.
9. Doty, K.C., McLaughlin, G., and Lednev, I.K. A Raman "spectroscopic clock" for bloodstain age determination: the first week after deposition. *Anal. Bioanal. Chem.* **2016**, 408, 3993-4001. doi: 10.1007/s00216-016-9486-z.
10. Muro, C.K. and Lednev, I.K. Identification of individual red blood cells by Raman microspectroscopy for forensic purposes. *Anal. Bioanal. Chem.* **2017**, 409, 287-293, (**Made a journal cover**). doi: 10.1007/s00216-016-0002-2.
11. Sikirzhitskaya, A., Sikirzhitski, V., and Lednev, I.K. Determining gender by Raman spectroscopy of a bloodstain. *Anal. Chem.* **2017**, 89, 1486–1492. doi: 10.1021/acs.analchem.6b02986.

12. Doty, K.C., Muro, C.M., and Lednev, I.K. Predicting the time of the crime: bloodstain aging estimation for up to two years. *Forensic Chem.* **2017**, 5, 1-7. doi: 10.1016/j.forc.2017.05.002.
13. Muro, C.M. and Lednev, I.K. Race differentiation based on Raman spectroscopy of semen traces for forensic purposes. *Anal. Chem.* **2017**, 89, 4344–4348. doi: 10.1021/acs.analchem.7b00106 (Letter).

## II. Conference Presentations

1. Kyle Doty gave an oral presentation entitled “*A Raman ‘Spectroscopic Clock’ for Bloodstain Age Determination: The First Week After Deposition*” at the University at Albany Life Sciences Research Symposium VII; September 25, 2015, Albany, NY.
2. Dr. Lednev gave a plenary session talk entitled “*Raman Spectroscopy as a Versatile Tool for Fundamental Research and Practical Applications*” at SciX-2015, the annual conference of the FACSS; Sept. 27 - Oct. 2, 2015, Providence, RI.
3. Dr. Lenka Halámková gave an invited oral presentation entitled “*Advanced Statistics of Raman Spectroscopic Data for Disease Diagnostics and Forensic Purposes*” at SciX-2015, the annual conference of the FACSS; Sept. 27 - Oct. 2, 2015, Providence, RI.
4. Kyle Doty presented a poster “*A Raman ‘Spectroscopic Clock’ for Bloodstain Age Determination: The First Week After Deposition*” at SciX-2015, the annual conference of the FACSS; Sept. 27 - Oct. 2, 2015, Providence, RI.
5. Kyle Doty gave an invited oral presentation entitled “*Discrimination of Animal and Human Blood Using Raman Spectroscopy and Chemometrics*” at SciX-2015, the annual conference of the FACSS; Sept. 27 - Oct. 2, 2015, Providence, RI.
6. Kyle Doty gave an oral presentation entitled “*A Raman ‘Spectroscopic Clock’ for Bloodstain Age Determination: The First Week After Deposition*” at the 41<sup>st</sup> annual meeting of the Northeastern Association of Forensic Scientists; Oct. 13 – 17, 2015, Hyannis, MA.
7. Dr. Lednev gave an oral presentation entitled “*Determining Donor Gender Based of Blood Stains Using Raman Spectroscopy*” as the annual American Academy of Forensic Sciences conference; February 22-27, 2016, Las Vegas, NV.
8. Kyle Doty gave an oral presentation entitled “*A Raman ‘Spectroscopic Clock’ for Bloodstain Age Determination: The First Week After Deposition*” at the annual American Academy of Forensic Science meeting; February 22-27, 2016, Las Vegas, NV.
9. Niraj Shah presented a poster “*Raman spectroscopy for determining gender based on sweat traces*” at the Greater Capital Region Science and Engineering Fair – RPI, March 19, 2016.
10. Dr. Lednev gave a Keynote lecture “*Raman Microspectroscopy and Advanced Statistics for Forensic Applications and Medical Diagnostics*” at the 32<sup>nd</sup> International Symposium on Microscale Separations and Bioanalysis; April 3-7, 2016, Niagra-on-the-Lake, ON, Canada.

11. Mathew Boll (Igor K. Lednev) presented a poster at the 8<sup>th</sup> Annual EAS ACS Undergraduate Research Symposium at Sienna College; April 13, 2016, Loudonville, NY.
12. Niraj Shah presented a poster "*Raman spectroscopy for determining gender based on sweat traces*" at Shaker High School Science Symposium; April 21, 2016, Latham, NY.
13. Claire Muro presented a poster "*Forensic Body Fluid Differentiation*" at the Eigenvector University; April 26, 2016, Seattle, WA. Awarded first prize.
14. Mathew Boll (Igor K. Lednev) presented a poster at the 2016 Undergraduate Research Conference at the University at Albany; April 29, 2016, Albany, NY.
15. Dr. Lednev spoke on "*Raman Microspectroscopy for Forensic Applications and Medical Diagnostics*" at the 99<sup>th</sup> Canadian Chemistry Conference and Exhibition; June 5-9, 2016, Halifax, NS, Canada.
16. Dr. Lednev gave a plenary lecture at the 25<sup>th</sup> International Conference on Raman Spectroscopy (ICORS); August 14-19, 2016, Fortaleza, Brazil.
17. Dr. Lednev gave an invited talk entitled "*Multivariate Analysis of Raman Spectral Data for the Identification of Body Fluid Traces*" at SciX 2016; September 18-23, 2016, Minneapolis, MN.
18. Mathew Boll presented a poster "*Identifying Hair Dyes Using Infrared Spectroscopy*" at the 2<sup>nd</sup> annual Undergraduate Chemistry Research Symposium at the University at Albany; October 6, 2016, Albany, NY.
19. Marisia Fikiet gave an oral presentation entitled "*Universal Detection of Body Fluid Traces In Situ with Raman Hyperspectroscopy for Forensic Purposes*" at the 8<sup>th</sup> Annual Life Science Research Symposium at the University at Albany; November 4, 2016, Albany, NY.
20. Ewelina Mistek gave an oral presentation entitled "*Identification of species' blood by attenuated total reflection (ATR) Fourier transform-infrared (FT-IR) spectroscopy*" at the 8<sup>th</sup> Annual Life Science Research Symposium at the University at Albany; November 4, 2016, Albany, NY.
21. Dr. Lednev gave invited seminar at Gakushuin University; December 13, 2016, Tokyo, Japan.
22. Dr. Lednev was invited seminar speaker at the National Research Institute of Police Science; December 14, 2016, Kashiwa City, Japan.
23. Dr. Lednev gave invited seminar entitled "*Raman Hyperspectroscopy for Forensic Purposes and Medical Diagnostics*" at the University of Puerto Rico; January 19, 2017, Mayaguez, Puerto Rico.
24. Dr. Lednev gave invited talk entitled "*Forensic Analysis: From the Lab to the Crime Scene*" at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON); March 5-9, 2017, Chicago, IL.
25. Marisia Fikiet presented a poster "*Universal Detection of Body Fluid Traces In Situ with Raman Hyperspectroscopy for Forensic Purposes*" at the Pittsburgh Conference on

Analytical Chemistry and Applied Spectroscopy (PITTCON); March 5-9, 2017, Chicago, IL.

26. Robert Rosenblatt gave an oral presentation entitled “*Raman Spectroscopy of Blood False Positives*” at the SUNY Undergraduate Research Conference (SURC)- East; Suffolk County Community College; April 21, 2017, Brentwood, NY.
27. Marisia Fikiet presented a poster “*Universal Detection of Body Fluid Traces In Situ with Raman Hyperspectroscopy for Forensic Purposes*” at the one-day SAS symposium (Society for Applied Spectroscopy, NY-NJ Chapter) at the University at Albany; April 26, 2017, Albany, NY.
28. Mathew Boll gave an oral presentation entitled “*Differentiation of Hair Using ATR FT-IR Spectroscopy: A Statistical Classification of Dyed and Non-dyed Hairs, an Honors Thesis*” at the 14<sup>th</sup> Annual Undergraduate Research Conference at the University at Albany; April 28, 2017, Albany, NY.
29. Dr. Lednev gave invited talk entitled “*The University at Albany - NY State Police Crime Laboratory collaboration on forensic research and development*” at the annual meeting of the American Society of Crime Lab Directors (ASCD); April 30, 2017, Dallas, TX
30. Dr. Lednev gave invited talk at the 5<sup>th</sup> Conference on Advanced Applied Raman Spectroscopy (RamanFest) at the Purdue University; June 1-2, 2017, West Lafayette, IN.
31. Samantha Ingenito gave an oral presentation entitled “*Raman Spectroscopy Capabilities of the CBex Handheld Raman Instrument*” as the keynote speaker of the 7<sup>th</sup> Annual Bethpage Science Research Symposium; June 6, 2017, Bethpage, NY.
32. Dr. Lednev gave invited talk entitled “*Raman Microspectroscopy for Forensic Purposes*” at the International Conference on Advanced Vibrational Spectroscopy (ICAVS); June 11-16, 2017, Victoria, BC, Canada
33. Valentina D’Elia, Gemma Montalvo, Carmen García Ruiz, Vladimir Ermolenkov, Igor Lednev- poster presentation entitled “*Detection of Cocaine in Oral Fluid by Ultraviolet Resonance Raman Spectroscopy*” at the International Conference on Advanced Vibrational Spectroscopy (ICAVS); June 11-16, 2017, Victoria, BC, Canada.
34. Ewelina Mistek presented a poster “*Forensic Applications of Vibrational Spectroscopy*” at the 2017 Green Mountain DNA Conference; July 24-26, 2017, Burlington, VT

## Participants

Dr. Igor K. Lednev, Professor – Principal Investigator.

Ewelina Mistek, Ph.D. Student – Experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts, and conference presentations.

Marisia Fikiet, Ph.D. Student – Experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts, and conference presentations.

Claire Muro, Ph.D. Student – Experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts.



Kyle Doty, Ph.D. Student – Experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts, and conference presentations.

Tatiana Quinones-Ruiz, Ph.D. Student – Experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts.

Dr. Lenka Halamkova, Research Scientist – Advanced statistical analysis of spectroscopic data, preparation of reports and manuscripts, and conference presentations.

Kelsey Auman, Undergraduate student – Experimental work, preparation of reports.

Mathew Boll, Undergraduate student – Experimental work, preparation of reports. Not supported from the grant. Works on related forensic project.

Selina Casadei, Undergraduate Student – Experimental work, preparation of reports. Not supported from the grant. Works on related forensic project.

Taylor Casey, Undergraduate Student – Experimental work, preparation of reports. Not supported from the grant. Works on related forensic project.

Samantha Ingenito, Undergraduate Student – Experimental work, preparation of reports. Not supported from the grant. Works on related forensic project.

Robert Rosenblatt, Undergraduate Student – Experimental work, preparation of reports. Not supported from the grant. Works on related forensic project.

Sera Nakisli, undergraduate students from Stony Brook University, conducts research as a summer intern/volunteer.

Emanuel Apolinario C Oliveira, Undergraduate Student visiting from the Federal University of Pernambuco in Recife, Brazil, not supported from this grant – Experimental work, preparation of reports, preparation of spectroscopic data for further analysis.

Niraj Shah, Research volunteer from the Shaker High School, Latham, NY.

## References

1. Doty, K.C., G. McLaughlin, and I.K. Lednev, *A Raman “spectroscopic clock” for bloodstain age determination: the first week after deposition*. Analytical and Bioanalytical Chemistry, 2016. **408**(15): p. 3993-4001.
2. Doty, K.C., C.K. Muro, and I.K. Lednev, *Predicting the time of the crime: bloodstain aging estimation for up to two years*. Forensic Chemistry, 2017.
3. McLaughlin, G., K.C. Doty, and I.K. Lednev, *Discrimination of human and animal blood traces via Raman spectroscopy*. Forensic Science International, 2014. **238**(0): p. 91-95.
4. McLaughlin, G., K.C. Doty, and I.K. Lednev, *Raman spectroscopy of blood for species identification*. Analytical Chemistry, 2014. **86**(23): p. 11628-11633.
5. Muro, C.K., L. de Souza Fernandes, and I.K. Lednev, *Sex determination based on Raman Spectroscopy of saliva traces for forensic purposes*. Analytical chemistry, 2016. **88**(24): p. 12489-12493.
6. Sikirzhyskaya, A., V. Sikirzhyski, and I.K. Lednev, *Determining Gender by Raman Spectroscopy of a Bloodstain*. Analytical chemistry, 2017. **89**(3): p. 1486-1492.
7. Mistek, E., et al., *Race differentiation by Raman spectroscopy of a bloodstain for forensic purposes*. Analytical chemistry, 2016. **88**(15): p. 7453-7456.
8. Muro, C.K. and I.K. Lednev, *Race differentiation based on Raman spectroscopy of semen traces for forensic purposes*. Analytical chemistry, 2017. **89**(8): p. 4344-4348.

9. Muro, C.K., et al., *Forensic body fluid identification and differentiation by Raman spectroscopy*. *Forensic Chemistry*, 2016. **1**: p. 31-38.