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## **Raman spectroscopy for analyzing body fluid traces: Stain aging, differentiation between races, genders, and species**

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### **1 DESIGN AND METHODS**

This funded work included three main body fluid studies: sample aging, species determination, and race and gender differentiation. Body fluid samples were purchased from scientific and medical vendors, who provided all relevant donor information (gender, race, age, etc.). Peripheral blood samples used for *Objective 1* were collected in the lab according to an IRB protocol approved by the University at Albany Office of Regulatory Research Compliance. Samples were prepared for analysis by depositing 10-30  $\mu\text{L}$  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 procedures, including preprocessing, constructing statistical models, conducting internal and external validation, determining the error rate and developing spectroscopic libraries.

## 2 DATA ANALYSIS

Previous NIJ funding allowed us to develop multidimensional Raman spectroscopic signatures for body fluids [1-5] that account for the intrinsic heterogeneity of body fluid traces and potential variations within and between donors [6]. However, these signatures alone do not answer all of the questions a forensic investigator may have about a body fluid trace found at a crime scene. We have worked to study the changes in body fluid spectra over time, differentiate human and animal species based on their blood spectra, and determine a donor's race and gender based on their body fluid spectra. In order to do this, we have developed advanced data treatment methods to correct fluorescent baselines, reduce dimensionality, and exclude interference from cosmic rays and random noise. Pretreated data is then used to build statistical models to study datasets and find subtle patterns in the spectral data. These models, together with the spectroscopic libraries created, allowed for the development of statistical methods for classifying unknown spectra and determining the necessary error rates.

## 3 STATEMENT OF RESULTS

### 3.1 Kinetic Studies

**Objective 1:** Determine the age of a biological stain for human blood, menstrual blood, semen, vaginal fluid, saliva, and sweat under various environmental conditions including high and low humidity, photodegradation due to sunlight, etc. Evaluate the effect of stain degradation on the identification/differentiation efficacy of the method.

**Objective 2:** Develop a spectroscopic library and software for the automatic determination of the age of a human body fluid stain and validate its performance.

A kinetics study of body fluids was carried out via *Objectives 1* and *2*. These studies aimed to explore and answer two questions. First- could a Raman spectrum be identified as originating from a specific body fluid after it had aged? Second- could that spectrum also be used to estimate the age of the body fluid trace?

### 3.1.1 Determine the Age of a Peripheral Blood Stain

A total of 6 sets of peripheral blood samples (45 separate microscope slides), from two different donors (one Caucasian male and one Caucasian female), were used for kinetic studies in four different environments (i.e. ambient, increased and decreased temperature, and high humidity (HH)). Two sets of samples of the male donor's blood were stored in an ambient environment, and one set for each of the three other environments. One set of samples of the female donor's blood was stored in an ambient environment only. For blood stored in an ambient environment, spectra have been collected from both donors at 14 time points over 1 year, and at 12 times points over 1 month for the male donor's second set. Spectra have also been collected, for the male donor, at 14 time points over 9 months at increased temperature (32 °C), 17 time points over 9 months at decreased temperature (4 °C), and 13 time points over 1 month at HH (80%). For each time point, one Raman map of 9 spectra was collected for an overall total of 738 spectra.

One partial least squares regression (PLSR) model was built for the male donor's blood stored in each environment, and five PLSR models (three for the male donor and two for the female donor) for the ambient environment, using all spectra up to the latest time point surveyed. Since there were two donors for the ambient environment, four of the five PLSR models were externally validated using the blood spectra from the other donor; for all other environments only internal cross-validation was performed. The relevant statistical metrics (e.g. root mean squared error of cross-validation (RMSECV), coefficient of correlation ( $R^2$ ), root mean squared error of prediction (RMSEP), etc.) are included in Table 1 in the Appendix for all 8 PLSR models built.

All blood samples stored in each environment were compared to previously developed multidimensional Raman spectroscopic signatures in order to confirm the identity as blood. Successful blood identification was achieved for blood spectra collected up to 1 month stored in ambient conditions (both donors), at decreased temperature, and at HH; but only for up to 2 weeks at increased temperature.

Spectral changes in bloodstains stored up to one week in ambient conditions were elucidated in a more robust manner using 2-dimensional correlation spectroscopy. A manuscript is in preparation to include this work, along with PLSR analysis and signature comparisons [P5]. Lastly, all spectra and preprocessing steps used to construct each PLSR model, as well as the models themselves, were saved as part of a reference library for future use.

### 3.1.2 Determine the Age of a Menstrual Blood Stain

A single menstrual blood sample was ordered especially for kinetics studies, which was collected without the usual addition of anticoagulants in order to more accurately mimic blood found at a crime scene. This menstrual blood sample was used to prepare 28 experimental samples, 14 stored in an ambient, room temperature, environment, and 14 stored in a HH (80%) environment. Each sample was mapped to collect 9 spectra per time point. A total of 126 spectra were collected from the ambient environment samples at 14 time points over one year, and 72 spectra were collected from the samples in the HH environment at 8 time points over 2 weeks. These spectra were pretreated to correct their baseline and exclude cosmic ray interference. A PLSR model built using the ambient environment spectra achieved an  $R^2CV$  and  $RMSECV$  of 0.90 and 0.42, respectively. A second PLSR model, based on the HH spectra, resulted in 0.90 and 0.25  $R^2CV$  and  $RMSECV$  rates, respectively.

### 3.1.3 Determine the Age of a Semen Stain

Semen samples were stored in two different environments, warm (34 °C) and cold (4 °C). All samples were analyzed at 15 time points over eight months using automatic Raman spectroscopic mapping. A principal component analysis (PCA) model was built to compare spectra collected after one week and four months in the cold environment, and showed clear separation of the two time points. Semen spectra collected after one week, 2 months, and 8 months in the cold environment were fit to the previously reported multidimensional spectroscopic signature for semen and could be identified as such [1].

#### 3.1.4 Determine the Age of a Vaginal Fluid Stain

Vaginal fluid samples were used for the kinetics study. All spectra were collected with automatic Raman spectroscopic mapping at sixteen time points over 8 months in a cold environment (4 °C), and at seventeen time points in a warm environment (34 °C). Two PLSR models were built using the spectra from the two different environments. A vaginal fluid spectrum collected up to 4 days in the cold environment could be identified as vaginal fluid using our previously developed multidimensional spectroscopic signature [4]. The experimental spectrum fit the signature with a sum of squared errors (SSE) of 3.11, an R<sup>2</sup> of 0.97, and an RMSE of 0.04.

#### 3.1.5 Determine the Age of a Saliva Stain

Saliva samples were used for the kinetics study. All spectra were collected with automatic Raman spectroscopic mapping at 15 time points over 8 months in a warm environment (34 °C). A PLSR model was built using the experimental spectra, which showed a strong relationship between the spectroscopic data and age of the trace. Saliva spectra collected after 8 months in the warm environment could be identified as saliva using our previously reported multidimensional spectroscopic signature [3]. The experimental spectrum from 8 months had an SSE of 4.92, an R<sup>2</sup> of 0.93, and an RMSE of 0.05.

#### 3.1.6 Kinetic Studies Conclusions

The effects of time on the Raman spectra of peripheral blood, menstrual blood, semen, vaginal fluid, and saliva were studied in different environments. Body fluids could be identified using multidimensional spectroscopic signatures up to one month after deposition. In each case, it was observed that the storage environment had significant influence on the spectra, and the identification efficacy of the method depends on both the body fluid of interest and storage conditions.

### 3.2 **Species Differentiation**

**Objective 3:** Differentiate species based on dry traces of blood of human and animal origin.

**Objective 4:** Develop a spectroscopic library and software for the automatic differentiation of human and animal species and validate its performance.

A total of 4,900 peripheral blood spectra have been collected, broken up into 35 spectra for each of 140 donors (10 per species) from the following species: human, cat, dog, rabbit, rat, raccoon, opossum, chicken, frog, fish, cow, horse, pig, and mouse. The animal species were specifically chosen as belonging to one of three categories: (1) bred for domestication (2), consumed by humans, and (3) integrated with human existence. Two partial least squares discriminant analysis (PLSDA) models have been constructed, a binary [7] and a species specific model [8], and a manuscript has been published for each one. Both models demonstrated superb internal classification abilities with zero occurrences of false negative assignments of the human class. The cross-validated sensitivity and specificity for the human class in the binary model were 100% and 98%, respectively, while these values were 100% and 99%, respectively, for the species specific model. Furthermore, both models performed well under two performance measures: a blind test of internal samples and an external validation test; with no misclassifications for the human class. The two models used in tandem can not only reliably discriminate human from animal blood spectra, but also classify unknowns to their specific species class. All spectra and preprocessing steps used to construct both PLSDA models, as well as the models themselves, were saved as part of a reference library for future use.

By utilizing an undergraduate summer intern, who came from a university in Denmark with her own funding, this objective was also explored via attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy. Spectra were collected from 24 samples of human, cat, and dog blood (8 per species). A PLSDA model was built on the calibration spectra, and externally validated with unknown spectra. Internal and external predictions both resulted in 100% accuracy, and a manuscript has been published on the results [9].

### **3.3 Gender and Race Differentiation**

**Objective 5:** Evaluate the capability of Raman spectroscopy for determining race and gender based on human body fluid traces. Develop statistically confident Raman spectroscopic signatures for various human groups including different races and genders for blood, saliva, and sweat; and races for menstrual blood, semen, vaginal fluid.

**Objective 6:** Develop a spectroscopic library as well as software for the automatic identification of race and gender and validate its performance.

The third study in the funded work explored the potential to differentiate body fluid donor race and gender with Raman spectroscopy. *Objective 5* and *6* sought to collect and analyze spectra from a wide variety of donors, then build statistical models and software to differentiate them according to race and/or gender.

### 3.3.1 Determine Race and Gender Based on Peripheral Blood

For race differentiation, twenty samples of human blood from Caucasian and Black donors (10 per race) were used to collect a map of 9 spectra per donor, for a total of 180 spectra. Genetic algorithm (GA) analysis was used to select the most informative variables in the dataset, and these data points were used to build a support vector machine discriminant analysis (SVMDA) model. The internally cross-validated model achieved a 71% accuracy rate. The calibration model was then validated via the leave-one-out method, and achieved an 81% sensitivity rate and 54% specificity rate for spectral prediction. The sensitivity and specificity rates of the model's donor-wise predictions were 80%. These sensitivity and specificity rates are lower than ideal, and it is possible that expanding the dataset to include more donors, collecting more spectra per donor, or exploring additional statistical methods could improve these rates. This proof of concept study shows that Raman spectroscopy can be used to differentiate between blood from Caucasian and Black donors. A manuscript on this work is to be submitted [P4].

For gender differentiation, sixty human blood samples, 30 female and 30 male, were used. Samples were automatically mapped to collect over 4,500 spectra. Spectra were then averaged to produce one mean spectrum per donor. An SVMDA model was constructed using the mean spectra, and resulted in a 77% sensitivity rate and a 93% specificity rate. This preliminary study yielded a strong calibration classification model, which was saved and can be used for future analysis for external validation, and to predict the gender of unknown donors.

### 3.3.2 Determine Race and Gender Based on Saliva

Sixty saliva samples from 20 Caucasian, 20 Black, and 20 Asian donors (with 30 male and female donors) were used for the race and gender differentiation study. A total of 25 spectra per Raman map were collected from each donor's sample. Two SVMDA models were built using the preprocessed spectra. The race differentiation model had a 65% and an 83% average sensitivity and specificity rate, respectively. The gender differentiation model achieved an 88% sensitivity rate and a 77% specificity rate. This proof of concept study yielded two calibration models, with mixed results.

### 3.3.3 Determine Race and Gender Based on Sweat

Race and gender determination of sweat donors was carried out with data from twenty donors. This included 10 Caucasian, 7 Black, 2 Hispanic, and 1 Asian donor, as well as 13 males and 7 females. Raman mapping was used to collect 105 spectra per sample. An SVMDA model was built on the preprocessed spectral dataset to differentiate between the four races. The internally cross-validated model achieved 99% for both the average sensitivity and specificity rates. A second SVMDA model was built with the same dataset to perform gender determination, and reached an internally cross-validated sensitivity rate of 94% and specificity rate of 99%.

### 3.3.4 Determine Race Based on Semen

Samples from 23 semen donors, 10 Caucasian and 13 Black, were analyzed by Raman spectroscopy for race differentiation. An Artificial Neural Network (ANN) model was built with three layers (1478-

20-2) and cross-validated by the bootstrap method. A total of 40 bootstrapping iterations were performed, with the data split in half for calibration/validation. The cross-validated sensitivity and specificity of the ANN model was 95%. The model has been saved in the software system so that it can be edited, validated, and used again. This preliminary study shows that while it is possible to differentiate semen samples based on Raman spectra, our models can only separate Caucasian and Black donors.

### 3.3.5 Determine Race Based on Menstrual Blood

Menstrual blood from 5 Caucasian and 10 Black donors was used to differentiate between the two races by collecting 15 spectra from each donor. Several PLSDA and SVMMDA models were built on the preprocessed spectra, using GA selected peaks, to differentiate between the two races. The best PLSDA model resulted in 55% sensitivity and 99% sensitivity. The best SVMMDA model resulted had a 91% sensitivity rate, and a 59% specificity rate. Despite rigorous data treatment and analysis, no statistical model could be constructed that could reliably classify menstrual blood spectra according to donor race. The results do not suggest that Raman spectroscopy can be used to differentiate between menstrual blood donors' races.

### 3.3.6 Gender and Race Differentiation Conclusions

The preliminary studies discussed above show that Raman spectroscopy has the potential to obtain a “genetic profile” of a body fluid donor. Gender and race differentiation was achieved with over 90% sensitivity and specificity with sweat and semen spectra. The final models built for gender differentiation of peripheral blood and saliva donors and those built for race differentiation of peripheral blood and menstrual blood resulted in sensitivity and/or specificity rates below 85%. It is possible that these results could be improved through the expansion of donor populations or spectral datasets, or by further developing statistical pretreatment methods and models. More work is required to explore these additional options and validate more advanced statistical models.

**3.4 Objective 7:** Develop combined software and evaluate major performance characteristics and limitations of the developed method as both a presumptive and confirmatory test.

We have developed several spectral libraries and statistical models for the funded work. These were built through the use of MATLAB (MathWorks, Inc.) and the PLS Toolbox (Eigenvector Research, Inc.). All of the spectral libraries and models developed have been saved in their native file format so that they can be used, edited, and/or updated in the future. Each individual project required specific approaches to both data preprocessing and modeling procedures. These various approaches were necessary because each problem was unique, and a universal method is not possible at this time. As a result, it was premature to combine all models into one singular software package.

#### **4 PROJECT FINDINGS**

The effects of time on the Raman spectra of peripheral blood, menstrual blood, semen, vaginal fluid, and saliva were studied in several different environments. Semen and saliva could be identified with their multidimensional spectroscopic signatures up to 8 months after deposition in a cold (semen) and warm (saliva) environment. Peripheral blood could be identified using its multidimensional spectroscopic signature up to one month after deposition in the ambient, high humidity, and decreased temperature environments, and up to 2 weeks after deposition in the cold environment. Vaginal fluid could be identified up to 4 days after deposition. As expected, the storage environment had a substantial impact on the identification efficacy of the method.

Peripheral blood from humans and 10 other animal species were successfully differentiated using Raman spectroscopy. A binary model was built to separate human from non-human animal blood, as well as a more advanced model that could specifically discriminate between different species.

Finally, genetic profiling via Raman spectroscopy was carried out with peripheral blood, menstrual blood, saliva, sweat, and semen to differentiate donors on the basis of race and/or gender. These

preliminary studies showed that such a technique is possible, and further work is necessary to finalize the method.

## **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) opening the opportunity for immediate genetic profiling based on body fluid traces, (ii) determining the approximate time at which a crime may have occurred, and (iii) limiting the amount of evidence collected and documented for further analysis to only that which is the most relevant to the crime.

## APPENDIX

### Tables

Table 1. Statistical metrics for all PLSR models constructed from human peripheral blood stored in different environments up to varying time points.

		Latest time point	#LVs	#VB splits	RMSEC	RMSECV	R <sup>2</sup> (cal.)	R <sup>2</sup> (CV)	RMSEP	R <sup>2</sup> (pred.)
Ambient (male)	Set 1	168 hrs (1 week)	4	9	0.02	0.13	1.00	0.97	0.34	0.97
		8760 hrs (1 year)	3	10	0.12	0.17	0.99	0.98	0.32	0.97
	Set 2	720 hrs (1 month)	3	10	0.05	0.12	1.00	0.98	N/A	N/A
Ambient (female)		168 hrs (1 week)	3	9	0.09	0.14	0.98	0.96	0.42	0.91
		8760 hrs (1 year)	4	10	0.06	0.15	1.00	0.98	0.31	0.95
High temperature (32 °C)		6480 hrs (9 months)	4	10	0.05	0.13	1.00	0.99	N/A	N/A
Low temperature (4 °C)		6552 hrs (9 months)	4	10	0.29	0.33	0.92	0.90	N/A	N/A
High humidity (80%)		720 hrs (1 month)	4	10	0.17	0.32	0.96	0.85	N/A	N/A

### Scholarly Products from Funded Project

#### I. Publications

- P1. Doty, K. C.; Muro, C. K.; Bueno, J.; Halamkova, L.; Lednev, I. K. *What can Raman spectroscopy do for criminalistics?* Journal of Raman spectroscopy. DOI: 10.1002/jrs.4826.
- P2. Muro, C. K.; Doty, K. C.; Bueno, J.; Halamkova, L.; Lednev, I. K. *Forensic Applications of Vibrational Spectroscopy*. Forensic Science – Chemistry, Physics, Biology and Engineering for Justice. Wiley. In Press.
- P3. Mistek, E.; Lednev, I. K. Identification of species' blood by attenuated total reflection (ATR) Fourier transform infrared (FT-IR) spectroscopy. *Analytical & Bioanalytical Chemistry*, 2015. **407**(24): p. 7435-7442.
- P4. Mistek, E.; Halámková, L.; Doty, K. C.; Muro, C. K.; Lednev, I. K. A forensic investigation of the distinction between Caucasian and African American blood donors by Raman spectroscopy. In preparation.
- P5. Doty, K. C.; McLaughlin, G.; Lednev, I. K. A Raman 'Spectroscopic Clock' for bloodstain age determination: The first week after deposition. In preparation.
- P6. Muro, C. K.; Doty, K. C.; Bueno, J.; Halamkova, L.; Lednev, I. K. *Vibrational Spectroscopy: Recent Developments to Revolutionize Forensic Science*. *Analytical Chemistry*, 2015. **87**(1): p. 306-327. **(A critical review invited by journal Editorial Board, highlighted on the journal cover, Lednev was interviewed for Audio/Podcast, translated into Japanese by the Spectroscopic Society of Japan)**
- P7. McLaughlin, G.; Doty, K.C.; Lednev, I.K. *Discrimination of human and animal blood traces via Raman spectroscopy*. *Forensic Science International*, 2014. **238**: p. 91-95.
- P8. McLaughlin, G.; Doty, K.C.; Lednev, I.K. *Raman Spectroscopy of Blood for Species Identification*. *Analytical Chemistry*, 2014. **86**(23): p.11628-11633.
- P9. Sikirzhyskaya, A.; Sikirzhyski, V. and Lednev, I.K. *Raman spectroscopy coupled with advanced statistics for differentiating menstrual and peripheral blood*. *Journal of Biophotonics*, 2014. **7**(1-2): p. 59-67. **(Highlighted on the journal cover)**

## II. Conference Presentations

- C1. I.K. Lednev, invited talk at RamanFest, 3rd International Conference on Advanced Applied Raman Spectroscopy, Xiamen, China. May 9, 2015.
- C2. I.K. Lednev, invited seminar at State Key Laboratory, University of Xiamen, Xiamen, China. May 4, 2015.
- C3. I.K. Lednev, invited talk at 13th Annual Forensic Science Symposium. Cedar Crest College. Allentown, PA. March 28, 2015.
- C4. I.K. Lednev, invited talk at PITTCON 2015, March 8-12, 2015, New Orleans, Louisiana.
- C5. J. Manheim, K.C. Doty, G. McLaughlin, I.K. Lednev, “Differentiation of Human, Animal, and Synthetic Hair by ATR-FTIR Spectroscopy.” Poster presentation at the 67th annual American Academy of Forensic Sciences (AAFS) meeting in Orlando, FL. February 19, 2015.
- C6. K.C. Doty, G. McLaughlin, I.K. Lednev, “Discrimination of Human and Animal Blood Traces Via Raman Spectroscopy.” Poster presentation at the 67th annual American Academy of Forensic Sciences (AAFS) meeting in Orlando, FL. February 19, 2015.
- C7. I.K. Lednev, “Raman Microspectroscopy of Body Fluid Traces: Intrinsic Method Selectivity.” Poster presentation at the 67th annual American Academy of Forensic Sciences (AAFS) meeting in Orlando, FL. February 19, 2015.
- C8. K.C. Doty presented “Differentiation of animals based on Raman spectroscopy of blood traces” at the 6<sup>th</sup> Annual Life Sciences Research Symposium in Albany, NY. December 12, 2014.
- C9. I.K. Lednev gave an invited talk “Raman Microspectroscopy and Advanced Statistics for the Analysis of Biological Stains and Gunshot Residue” at Eastern Analytical Symposium and Exhibition. Somerset, NJ. November 17-19, 2014. The trip was sponsored by the Coblenz Society.
- C10. J. Manheim presented “Differentiation of Human, Animal, and Synthetic Hair by ATR-FTIR Spectroscopy” at the Eastern Analytical Symposium in Somerset, NJ. November 17, 2014.
- C11. K.C. Doty presented “Discrimination of human and animal blood traces via Raman spectroscopy” at the annual Northeastern Association of Forensic Science meeting in Hershey, PA. November 4, 2014.
- C12. I.K. Lednev gave an invited talk “Raman Microspectroscopy for Identification of Body Fluid Traces” at SciX 2014 Conference of the Federation of the Analytical Chemistry and Spectroscopic Societies. Reno, NV. October 2014.
- C13. J. Manheim presented “Differentiating Human and Animal Hair by ATR-FTIR Spectroscopy” at the STEM Undergraduate Research Conference at the University at Albany in Albany, NY. August 20, 2014.
- C14. K.C. Doty presented “Discrimination of human and animal blood traces via Raman spectroscopy” at the International Conference on Raman Spectroscopy (ICORS) in Jena, Germany. August 15, 2014.

- C15. I.K. Lednev was invited by the Taiwan Raman Spectroscopic Association to give a plenary talk at the Second Taiwan International Symposium on Raman Spectroscopy, June 23-24, 2014, in National Dong Hwa University, Hualien.
- C16. I.K. Lednev, Invited lecture on Forensic applications of vibrational spectroscopy at a student's Raman Summer Camp held in Hualien County, Taiwan, June 24-25, 2014.
- C17. I.K. Lednev, Kickoff Forensic Research Symposium at the University at Albany, Albany, NY. May 29, 2014.
- C18. I.K. Lednev, Grace Van DerVoort Memorial Lecture at the Sage College, Troy, NY. March 26, 2014.
- C19. I.K. Lednev, Invited talk at PITTCON 2014. Chicago, IL. March 2-6, 2014.
- C20. K.C. Doty. "Raman Spectroscopy of Blood for Species Identification." Life Sciences Research Symposium V at the University at Albany. October 18, 2013.
- C21. G. McLaughlin. "Forensic Method Development and the Raman Spectroscopic Analysis of Bone Tissue." Forensic Research Symposium at the NYSP FIC. Albany, NY. September 13, 2013.
- C22. A. Sikirzhyskaya. "Raman Spectroscopy Coupled with Advanced Chemometrics for the Identification of Body Fluid Traces: Mixtures and Contamination." Forensic Research Symposium at the NYSP FIC. Albany, NY. September 13, 2013.
- C23. C. Muro. "Race Prediction by Raman Spectroscopy of Semen Traces." Forensic Research Symposium at the NYSP FIC. Albany, NY. September 13, 2013.
- C24. K.C. Doty. "Blood Species Differentiation Using Raman Spectroscopy with Applied Chemometrics." Forensic Research Symposium at the NYSP FIC. September 13, 2013.
- C25. I.K. Lednev gave an invited seminar at Boston University, Boston, MA. May 29, 2013.
- C26. I.K. Lednev was a guest speaker at PerkinElmer Inspiring Innovation Workshop and spoke on the application of vibrational spectroscopy for forensic purposes. CNSE, Albany, May 16, 2013.
- C27. I.K. Lednev gave an invited seminar at FBI Laboratories, Quantico, VA, April 2013.
- C28. I.K. Lednev gave an invited seminar at College of Pharmacy and Engineering, University of Iowa, Iowa City, IA, April 11, 2013.
- C29. A. Sikirzhyskaya. Raman Spectroscopy for the Identification of Body Fluid Traces: Semen and Blood Mixtures. PITTCON Philadelphia, PA, March 17-21, 2013
- C30. V. Sikirzhyski. Raman Spectroscopy and Advanced Statistics for Forensic Studies. Invited talk at PITTCON Philadelphia, PA, March 17-21, 2013
- C31. G. McLaughlin. Substrate Interference and the Spectroscopic Identification of Body Fluids. AAFS Washington D.C., February 20, 2013
- C32. I.K. Lednev gave two talks at the 65<sup>th</sup> Anniversary Meeting of the American Academy of Forensic Sciences (AAFS). Washington, DC. February 18-23, 2013.
- C33. I.K. Lednev gave an invited seminar at the University of Puerto Rico, Mayaguez, January 2013.

- C34. I.K. Lednev gave an invited seminar at Eastern New York section of the American Chemical Society. Albany, NY. January 2013.
- C35. G. McLaughlin. “Raman spectroscopic analysis of trace evidence.” 38<sup>th</sup> Annual Meeting of the Northeastern Association of Forensic Scientists. Saratoga Springs, NY, November 13-16, 2012.
- C36. I.K. Lednev. “Raman Spectroscopy for Forensic applications.” Invited seminar at the University of South Carolina, November 2012.
- C37. I.K. Lednev. “Raman Spectroscopy for Forensic applications.” Invited seminar at the University of Central Florida, October 2012.
- C38. I.K. Lednev. Lead lecture on “Raman Spectroscopy for Forensic Applications.” 23<sup>rd</sup> International Conference on Raman Spectroscopy (ICORS). August 12-17, 2012. Bangalore, India.

### **Participants**

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

Dr. Vitali Sikirzhytski, Postdoctoral Research Associate – experimental design, advanced statistical analysis, software developments, preparation of reports and manuscripts.

Dr. Lenka Halamkova, Research Scientist - Advanced statistical analysis of experimental data.

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.

Aliaksandra Sikirzhytskaya, Ph.D. student - experimental design, experimental work, advanced statistical analysis, preparation of reports and manuscripts.

Gregory McLaughlin, Ph.D. student – experimental design, experimental work, statistical analysis, preparation of reports and manuscripts.

Aliea Afnan, M.S. student (not supported from this grant) – experimental work, preparation of reports. Successfully graduated with M.S. Degree in Chemistry with emphasis in Forensic Chemistry.

Ewelina Mistek, Undergraduate Student from Business Academy Aarhus (Denmark) as an intern to get research experience in the field of forensic chemistry – Experimental work, preparation of reports, preparation of spectroscopic data for further analysis.

Jeremy Manheim, Undergraduate Student supported by STEM-2014 Undergraduate Fellowship – Experimental work, preparation of reports, preparation of spectroscopic data for further analysis.

Luciana de Souza Fernandes, Undergraduate student visiting from the Federal University of Viçosa in Viçosa, Brazil, not supported by this grant - Experimental work, preparation of reports, preparation of spectroscopic data for further analysis.

Lais Nascimento Viana, Undergraduate Student from the State University of Rio de Janeiro, Brazil as an international exchange student through the Brazil Science without Borders

program – Experimental work, preparation of reports, preparation of spectroscopic data for further analysis.

## References

1. Virkler, K. and I.K. Lednev, *Raman spectroscopic signature of semen and its potential application to forensic body fluid identification*. Forensic science international, 2009. **193**(1): p. 56-62.
2. Virkler, K. and I.K. Lednev, Raman spectroscopic signature of blood and its potential application to forensic body fluid identification. Analytical and bioanalytical chemistry, 2010. **396**(1): p. 525-534.
3. Virkler, K. and I.K. Lednev, Forensic body fluid identification: the Raman spectroscopic signature of saliva. Analyst, 2010. **135**(3): p. 512-517.
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