



### E82 Phenotype Profiling Based on Raman Spectroscopy of Biological Stains: A Blood Test for the Donor Age

Igor K. Lednev, PhD\*, State University of New York at Albany, Albany, NY 12222; Kyle C. Doty, PhD, Silver Spring, MD 20904

**Learning Overview:** After attending this presentation, attendees will better understand the potential forensic application of Raman spectroscopy. The implementation of advanced statistics for the analysis of spectroscopic data and the evaluation of the accuracy and reliability of the conclusions made will be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the accuracy and effectiveness of biological stain analysis for forensic purposes.

This presentation will report on the development of a universal, non-destructive, and confirmatory method for characterizing biological stains. The all-in-one method has a capability to identify the body fluid, determine human or animal origin, time since deposition and phenotype profile, race, sex, and chronological age, specifically.

Traces of body fluids discovered at a crime scene are a potential source of DNA, which is a major individual evidence in the modern forensic investigation. The application of Raman spectroscopy for non-destructive, confirmatory identification of biological stains at a crime scene, including dry traces of sweat, vaginal fluid, semen, saliva, and blood, has recently been reported.<sup>1</sup> The method allowed for differentiating animal and human blood as well menstrual and peripheral blood.<sup>2,3</sup> In addition, the method was further developed for determining the time since deposition for bloodstains for up to two years.<sup>3</sup>

It would be of great help for criminal investigation to develop a phenotype profile immediately at a crime scene based on a rapid analysis of biological stains. With this goal in mind, the possibility of race and sex differentiation based on Raman spectroscopy of body fluid traces has been investigated.<sup>3</sup> Specifically, advanced statistical analysis of spectroscopic data was used to discriminate between Caucasian and African American donors based on dry peripheral blood traces. In addition, the differentiation of the donor's sex based on bloodstains and saliva traces, as well as race differentiation based on traces of semen, has been demonstrated.

The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, non-destructive laser light by a solid, liquid, or gas sample. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, non-resonance Raman spectroscopy is not destructive for the sample. A portable Raman spectrometer is a reality now that should allow the identification at the crime scene.

In this proof-of-concept study, Raman spectroscopy and chemometrics have been used to analyze blood from human donors and differentiate between samples based on Chronological Age (CA).<sup>4</sup> Three groups of donors were selected including newborns (CA of <1 year), adolescents (CA of 11–13 years), and adults (CA of 43–68 years). A Support Vector Machines Discriminant Analysis (SVM) model was constructed, which demonstrated high accuracy in correctly predicting blood donors' age groups by showing the cross-validated sensitivity and specificity over 0.96. Overall, this preliminary study demonstrates the high selectivity of Raman spectroscopy for differentiating between blood donors based on the CA. The demonstrated capability completes this suite of phenotype profiling methodologies, including the determination of sex and race. CA determination has particular importance since this characteristic cannot be determined through DNA profiling, unlike sex and race. When completed, the developed methodology should allow for phenotype profiling based on dry traces of body fluids immediately at the scene of crime. The availability of this information within the first few hours since the crime discovery could be invaluable for the investigation.

*This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.*

#### Reference(s):

1. Khandasammy, S.R., Lednev, I.K, et al.. Bloodstains, Paintings, and Drugs: Raman Spectroscopy Applications in Forensic Science. *Forensic Chemistry* 8, 111–133 (2018).
2. Doty, K.C. & Lednev, I.K. Differentiation Of Human Blood From Animal Blood Using Raman Spectroscopy: A Survey of Forensically Relevant Species. *Forensic Science International* 282, 204-210 (2018).
3. Doty, K.C. & Lednev, I.K. Raman Spectroscopy for Forensic Purposes: Recent Applications for Serology and Gunshot Residue Analysis. *Trends Anal Chem* 103, 215-222 (2018).
4. Doty, K.C. & Lednev, I.K. Differentiating Donor Age Groups Based on Raman Spectroscopy of Bloodstains for Forensic Purposes. *ACS Central Science* 4, 862-867 (2018).

#### Serology, Bloodstain, Phenotype Profile