National Institute of Justice

Forensic Science Research and Development Symposium

American Academy of Forensic Sciences
72nd Annual Scientific Meeting

Tuesday, February 18, 2020
Anaheim, California
NIJ is the federal government’s lead agency for forensic science research and development as well as the administration of programs that facilitate training, improve laboratory efficiency, and reduce backlogs. The mission of NIJ’s Office of Investigative and Forensic Sciences is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, information exchange, and the development of training resources for the criminal justice community.

Through the research, development, testing, and evaluation process, we provide direct support to crime laboratories and law enforcement agencies to increase their capacity to process high-volume cases and provide needed training in new technologies. With highly qualified personnel and strong ties to the community, NIJ’s Office of Investigative and Forensic Sciences plays a leadership role in directing efforts to address the needs of our nation’s forensic science community.

RTI International and its academic- and community-based consortium of partnerships work to meet all tasks and objectives for the Forensic Technology Center of Excellence (FTCoE), put forward under the National Institute of Justice (NIJ) Cooperative Agreement No. 2016-MU-BX-K110.

The FTCoE is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of more than 5,000 providing research and technical services to governments and businesses in more than 75 countries, RTI brings a global perspective. The FTCoE builds on RTI’s expertise in forensic science, innovation, technology application, economics, DNA analytics, statistics, program evaluation, public health, and information science.

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Greetings,

The National Institute of Justice (NIJ) and its Forensic Technology Center of Excellence (FTCoE) at RTI International want to welcome you to the 2020 NIJ Forensic Science Research and Development (R&D) Symposium. This event is held in conjunction with the American Academy of Forensic Sciences 72nd Annual Scientific Meeting in Anaheim, California. On Tuesday, February 18, hundreds of attendees will join us in person and online to learn about NIJ research awards given to several talented researchers spanning the forensic disciplines.

For more than a decade, NIJ has hosted an annual R&D symposium to showcase scientific innovations and promote research transitioning to practice. The NIJ supports research to advance efficiency, quality, reliability, and capacity in the criminal justice and forensic science communities; this research focuses on developing new technologies, providing proof for evidence-based practices, and evaluating findings for case investigations and legal proceedings.

This year, members of the NIJ R&D team—including program managers Andrea Borchardt, Gregory Dutton, Danielle McLeod-Henning, and Frances Scott—have worked to bring you a phenomenal research agenda. The full-day program includes presentations from principal investigators and their research partners; these presentations highlight 16 NIJ-awarded grants that represent several of the accomplishments from NIJ R&D grants that were awarded during 2015–2018. The two morning sessions comprise Forensic Biology/DNA and Controlled Substances and Toxicology; the two afternoon sessions cover Impression and Pattern Evidence/Trace Evidence as well as Forensic Anthropology and Forensic Pathology.

We are pleased to have you—both national and international stakeholders of the criminal justice system—join us for this event; we are confident that you will learn valuable information to help guide your research endeavors and impending cases. The research shared with you at this event will help to efficiently identify, gather, and process evidence related to crime and death scene investigations in the future. We hope you enjoy this program of research and developments in forensic sciences.

Respectfully,

Jeri D. Ropero-Miller, PhD, F-ABFT  
Director  
Forensic Technology Center of Excellence  
Center for Forensic Sciences, RTI International

Jonathan G. McGrath, PhD, MSFS  
Senior Policy Analyst  
Office of Investigative and Forensic Sciences  
National Institute of Justice
Directors

Jeri D. Ropero-Miller

Dr. Jeri D. Ropero-Miller is the Chief Scientist in the Applied Justice Research Division at RTI International. With expertise in the areas of forensic toxicology and criminal justice research, she has published on topics of postmortem drug studies, emerging drugs, hair drug studies, drug surveillance and intelligence, program evaluation, and technology evaluation and adoption. She supports several ongoing projects, including the National Institute of Justice’s Forensic Technology Center of Excellence and its Criminal Justice Testing and Evaluation Consortium, the Drug Enforcement Administration–funded National Forensic Laboratory Information System, the Bureau of Justice Statistics (BJS)–funded 2018 Census of Medical Examiners/Coroners’ Offices, and the BJS-funded 2019 Census of Publicly Funded Forensic Crime Laboratories. She is certified by the American Board of Forensic Toxicology; is currently the president-elect for the American Academy of Forensic Sciences; and serves on the Toxicology Subcommittee of the National Institute of Standards and Technology, Organization of Scientific Area Committees. She received her doctorate in clinical chemistry and forensic toxicology from the University of Florida College of Medicine. Her work has been extensively published, and she is recognized nationally and internationally for her work in criminal justice research.

Jonathan McGrath

Dr. Jonathan McGrath serves as a senior policy analyst with the U.S. Department of Justice (DOJ) National Institute of Justice (NIJ) in the Office of Investigative and Forensic Sciences located in Washington, D.C. He supports the NIJ Forensic Technology Center of Excellence program, the DOJ Needs Assessment of Forensic Laboratories and Medical Examiner and Coroner Offices, the NIJ Forensic Laboratory Needs Technology Working Group, and the NIJ Drug and Crime Program; he also serves as a vice co-chair for the Federal Medicolegal Death Investigation Working Group. Prior to joining NIJ in 2015, he served as a forensic scientist with the U.S. Customs and Border Protection’s Laboratories and Scientific Services Directorate (CBP LSSD) in Houston, Texas, from 2007 to 2011. He worked at the CBP LSSD headquarters office in Washington, D.C., where he supported CBP’s trade, forensic, and weapons of mass destruction operations programs during 2011–2015. Dr. McGrath holds a doctoral degree in analytical chemistry from Georgia Tech, a master of science in forensic science from the University of Illinois at Chicago, and bachelor of science in chemistry from the University of Dallas.
NIJ Program Managers

Andrea Borchardt
Andrea Borchardt is a senior physical scientist in the Office of Investigative and Forensic Sciences at the National Institute of Justice (NIJ) within the US Department of Justice. She joined NIJ in 2019 after spending 14 years in public and private agencies involved in forensic DNA analysis. She is responsible for the Forensic Biology/DNA component of the R&D program and comanages programs such as Capacity Enhancement for Backlog Reduction, Best Practices for Evidence Management Working Group, and the DNA Efficiency Improvement Working Group. Ms. Borchardt earned both a bachelor of science degree and master of science degree in Molecular and Cellular Biology from The Johns Hopkins University.

Gregory Dutton
Dr. Gregory Dutton is a program manager at the National Institute of Justice (NIJ). His portfolio includes the broad umbrella of trace evidence—microscopic materials, chemicals, or nonhuman biological traces recovered from crime scenes as well as impression and pattern evidence (e.g., latent fingerprints, firearms, and shoeprints). His programs at NIJ seek to bring advances from across the physical sciences into the forensic sciences. Prior to joining NIJ, he was a research fellow at the National Institute of Standards and Technology. Dr. Dutton earned his doctorate in chemistry from the University of Minnesota.

Danielle McLeod-Henning
Danielle McLeod-Henning is a program manager/physical scientist in the Office of Investigative and Forensic Sciences at the National Institute of Justice, US Department of Justice. She is responsible for managing projects in Forensic Science Research and Development, specifically in forensic anthropology, forensic pathology, crime scene examination, and related medicolegal death investigation fields. Ms. McLeod-Henning holds a master's degree in forensic sciences from the George Washington University and a bachelor's degree in anthropology from the Pennsylvania State University.

Frances Scott
Dr. Frances Scott is a physical scientist at the National Institute of Justice, where she manages the Controlled Substances and Forensic Toxicology research and development portfolios under the General Forensics portfolio and comanages the Paul Coverdell Forensic Science Improvement Grants program and the Research for Publicly Funded Labs program. Dr. Scott received a bachelor's degree in chemistry from the University of California at Davis and a PhD in physical chemistry from the George Washington University.
Apply for NIJ’s Graduate Research Fellowship (GRF). By supporting outstanding graduate research, NIJ is expanding the pool of young investigators whose work has the potential to affect issues of crime and the fair and impartial administration of criminal justice in the United States.

Eligibility
Students must (1) be enrolled full time in a Ph.D. program in a science or engineering field and (2) propose dissertation research relevant to improving criminal justice practice or policy in the United States.

Applications are submitted by the university, which must be an accredited academic institution in the United States or its territories.

Benefits
- $35,000 annual student salary
- $12,000 annually for tuition, fees, and administrative costs
- $3,000 annually for research expenses
- Up to three years of funding, usable over a five-year period.

Deadline: April 15, 2020
NEW FOR 2020

There is a single solicitation for NIJ's GRF program in 2020. Eligible applicants in all scientific disciplines, whether in the social and behavioral sciences (SBS) or STEM fields, are invited to apply to the same funding opportunity.

Disciplines include, but are not limited to:

- Anthropology
- Biology
- Chemistry
- Cognitive Science
- Computer Science
- Criminal Justice
- Criminology
- Economics
- Education
- Electrical Engineering
- Geosciences
- GIS
- Information Sciences
- Materials Science
- Mathematics
- Neuroscience
- Pathology
- Physics
- Political Science
- Psychology
- Public Administration
- Public Health
- Public Policy
- Sociology

To learn more and see profiles of past and present GRF fellows, visit NIJ.ojp.gov/GRF.

National Institute of Justice • Strengthen Science • Advance Justice

January 2020

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National Institute of Justice

The research, development and evaluation agency of the U.S. Department of Justice, dedicated to improving knowledge and understanding of crime and justice issues through science.
Pittcon

Pittcon is the world’s leading annual conference and exposition on laboratory science. Pittcon attracts laboratory scientists from industry, academia and government from over 90 countries worldwide.
The National Institute of Justice (NIJ) invites proposals to its Research and Development in Forensic Science for Criminal Justice Purposes program. NIJ funded projects are expected to:

- Increase the body of knowledge to guide and inform forensic science policy and practice; or
- Result in the production of useful materials, devices, systems, or methods that have the potential for forensic application.

Goals
Proposals should address at least one of the following goals:

- **Fundamental/Basic Research Goal**: Improve the understanding of the accuracy, reliability, and measurement validity of forensic science disciplines.

- **Applied Research Goal**: Increase knowledge or understanding necessary to guide criminal justice police and practice related to the forensic sciences.

- **Development Goal**: Produce new materials, devices, systems, or methods that have the potential for forensic application for criminal justice purposes.
New Investigator Opportunities

NIJ is interested in funding scientists new to forensic science research. Proposals for which the principal investigator and any co-investigator(s) qualify under these categories may be given special consideration in award decisions:

- **Junior Faculty**: Non-tenured faculty, typically Assistant Professor or equivalent, who have not received prior NIJ research funding.
- **Senior Faculty**: Established researchers, typically tenured faculty or equivalent, who have not been funded by NIJ in the past 10 years.

Needs of the Community

For more information on what research and technology forensic practitioners would benefit from, see NIJ’s Forensic Science Technology Working Group operational requirements at go.usa.gov/xnvJ3.

Funding Opportunity Anticipated for 2020

NIJ typically posts solicitations for grant proposals once annually. Sign up for email alerts when new NIJ funding opportunities are posted at nij.gov/funding. Begin the application process early by registering with Grants.gov.
AGENDA

Short Agenda

Tuesday, February 18

8:30–8:40  Welcome
8:40–10:20 Morning Session I—Forensic Biology/DNA
10:35–12:15 Morning Session II—Controlled Substances and Toxicology
1:35–3:15 Afternoon Session I—Impression and Pattern Evidence/Trace Evidence
3:30–5:10 Afternoon Session II—Forensic Anthropology and Forensic Pathology
5:10  Adjourn

Full Agenda

Tuesday, February 18: 8:30 a.m.–5:10 p.m.

8:30–8:40  Welcome and Opening Remarks
Office of Investigative and Forensic Sciences, NIJ

Morning Session I: Forensic Biology/DNA
Moderated by NIJ Program Manager Andrea Borchardt

8:40–9:05  Efficient Sequencing and Analysis of Degraded and Trace DNA Samples Using a Novel Targeted Ligation-Free Method
Jannine Forst, Arc Bio, LLC—2017-DN-BX-0140

9:05–9:30  Microhaplotypes: Moving Scientific Research to a Forensic Casework Panel
Kenneth Kidd, Yale University—2018-75-CX-0041

9:30–9:55  DNA Typing Strategies via Real-Time Nanopore Sequencing for Forensic Analyses
Courtney Hall, University of North Texas Health Science Center—2018-DU-BX-0179

9:55–10:20 Development of Entire Mitogenome Reference Data Using an Automated High Throughput Sequencing Workflow
Kimberly Sturk-Andreaggi, Armed Forces DNA Identification Laboratory—DJO-NIJ-17-RO-0219

10:20–10:35—BREAK

Morning Session II: Controlled Substances and Toxicology
Moderated by NIJ Program Manager Frances Scott

10:35–11:00  The Detection and Quantitation of Fentanyl Mixtures by Surface-Enhanced Raman Spectroscopy (SERS) and Chemometrics
Ling Wang, Florida International University—2015-IJ-CX-K006
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<td>Forty-Plus Ways Not to Analyze Beverages for Cannabinoids</td>
<td>Carl Wolf, Virginia Commonwealth University—2017-R2-CX-0029</td>
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<td>12:15–1:35</td>
<td>LUNCH BREAK (on your own)</td>
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<td>1:35–2:00</td>
<td>Afternoon Session I: Impression and Pattern Evidence/Trace Evidence</td>
<td>Moderated by NIJ Program Manager Gregory Dutton</td>
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<td>1:35–2:00</td>
<td>Quantitative Measures for Footwear Impression Comparisons</td>
<td>Steven Lund, National Institute of Standards and Technology—DJO-NIJ-17-RO-0202</td>
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<td>2:00–2:25</td>
<td>Testing the Accuracy and Reliability of Palmar Friction Ridge Comparisons: A Black Box Study</td>
<td>Heidi Eldridge, RTI International—2017-DN-BX-0170</td>
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<td>Rapid Detection of Inorganic and Organic Firearm Discharge Residues by Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemical Sensors</td>
<td>Tatiana Trejos and Luis Arroyo, West Virginia University—2018-DU-BX-0186</td>
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<td>2:50–3:15</td>
<td>Facilitating the Adoption of Glass Evidence Analyses in Forensic Laboratories</td>
<td>Jose Almirall, Florida International University—2015-DN-BX-K049</td>
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<td>BREAK</td>
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<td>3:30–3:55</td>
<td>Afternoon Session II: Forensic Anthropology and Forensic Pathology</td>
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<td>3:55–4:20</td>
<td>Development Responses to Fluctuating Temperatures of a Forensically Important Blow Fly (Cochliomyia macellaria)</td>
<td>Travis Rusch, Texas A&amp;M University AgriLife Research—2016-DN-BX-0204</td>
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<td>4:20–4:45</td>
<td>Understanding the Role of the Thanatobiota in the Decay of “Reproductive Organs” in Human Decomposition</td>
<td>Gulnaz Javan, Alabama State University—2017-MU-MU-0042</td>
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<td>4:45–5:10</td>
<td>Microbial Clocks for Estimating the Postmortem Interval of Human Remains at Three Anthropological Research Facilities</td>
<td>David Carter, Chaminade University of Honolulu; Zachary Burcham, Colorado State University—2015-DN-BX-K016</td>
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Morning Session I: Forensic Biology/DNA

8:40 A.M.–9:05 A.M.

Efficient Sequencing and Analysis of Degraded and Trace DNA Samples Using a Novel Targeted Ligation-Free Method

NIJ Award #: 2017-DN-BX-0140

Presenting author: Jannine Forst, Arc Bio, LLC

Abstract: The field of DNA forensics is limited by the ability of current technology to extract information from trace and degraded DNA samples. Increasingly, researchers are turning to high throughput sequencing (HTS), which has the potential to glean more information from the low quantity of fragmented DNA recovered than other, more traditional target-specific approaches. HTS, however, also has its drawbacks. Most currently available DNA extraction protocols are not optimized for use with HTS in a DNA forensics context. Many preferentially recover larger fragments, which results in the loss of information from the shorter fragments now accessible through the application of HTS. This project has enabled the development of a DNA extraction protocol optimized for the application of HTS to DNA forensics. The untargeted recovery of smaller fragments as well as larger ones will enable more information to be extracted from each sample, an important factor in contexts with low quantities of DNA such as those routinely encountered in DNA forensics. The generation of sequences for HTS is limited by one of its key steps—ligation. The low efficiency of this step creates a bottleneck in the complexity of molecules sequenced and has yet to be overcome by current protocols. Here, we describe our novel ligation-free HTS library generation method developed in pursuit of gathering as much information from forensic DNA as possible. Finally, HTS also requires extensive informatics expertise to fully use the large amount of data generated. To solve this problem, we have developed a bioinformatics pipeline optimized for the analysis of fragmented forensic DNA, with analyses relevant to the field and user-friendly outputs.

The project described here strives to overcome the three obstacles detailed previously so that HTS can be more accessible, widespread, and effectively used for DNA forensics, allowing for the full potential of this technology to be realized within the field. The project goal is to develop a complete sample pipeline optimized specifically for DNA forensics, from DNA extraction through to bioinformatic analysis. This is demonstrated using a set of forensic samples, analyzed using a curated set of 179 forensically relevant SNPs that provide information on physical characteristics, ancestry, and kinship. With only 30 million sequences per sample, we are able to distinguish between individuals and determine relatedness between different samples, along with providing information on other potential investigative leads.
**Microhaplotypes: Moving Scientific Research to a Forensic Casework Panel**

NIJ Award #: 2018-75-CX-0041

Presenting authors: Kenneth Kidd*, Curt Sharfe, Andrew J. Pakstis, Neeru Gandotra, and William C. Speed; Department of Genetics, Yale University School of Medicine

**Abstract:** Lack of a commercial product for implementing microhaplotypes into forensic casework is a “chicken-egg” problem: until there is an agreed-upon panel, no commercial entity will market a panel and conversely no laboratory will invest in the admittedly powerful technology until there is a commercial kit. Most proposed microhap panels have been identified as having good statistical characteristics by screening large public databases. However, to date, only three panels have been tested by actual sequencing of multiple individuals: (1) 87 microhaps (Turchi, Melchionda, Pesaresi, & Tagliabracci, 2019); (2) 38 microhaps (Bennett et al., 2019); and (3) 90 microhaps we are validating at Yale (unpublished data). A Venn diagram shows 24 microhap loci common to these three panels; all 24 have extensive population data from the Kidd et al. (2017) study. Some loci have been analyzed in more than one multiplex, making these markers highly specific and likely robust to the technical vagaries of PCR and sequencing. Yet, these are unlikely to comprise a final panel because several labs are identifying more informative markers. Our new panel of 90 microhaps includes 44 new loci with high Ae, making many of them significantly more informative than any of the 24 in the intersection of the three studies. These 90 have now been sequenced on 155 individuals. The actual proof obtained from sequencing many individuals for large numbers of highly informative microhaps may finally motivate companies to develop commercial kits.

**References:**


**DNA Typing Strategies via Real-Time Nanopore Sequencing for Forensic Analyses**

NIJ Award #: 2018-DU-BX-0179

Presenting author: Courtney Hall, University of North Texas Health Science Center

**Abstract:** Forensic DNA analysis exploits the high variability of short tandem repeat (STR) markers to differentiate individuals. Typical STR typing workflow consists of polymerase chain reaction amplification followed by size-based separation and detection via capillary electrophoresis. Despite the power and reliability of current techniques, sequence-level variations are masked in the profiles generated. Detection of hidden nucleotide variations
within and around common forensic STR markers significantly increases the resolving power and aids in interpretation of more challenging samples. Adoption of deep-sequencing platforms in forensic laboratories would preclude complete dependence on size-based profiles, providing the most comprehensive representation of the genetic variability at STR loci. Although massively parallel sequencing (MPS) platforms have attracted significant interest from the forensic research community, the high startup fees, complex computational infrastructure, and extensive training requirements hinder widespread implementation in routine casework. The majority of forensic laboratories cannot allocate the funding needed to simultaneously maintain current STR typing workflows and implement MPS. Oxford Nanopore Technologies offers the ability to obtain deep-sequencing data for STR loci on the pocket-sized MinION device. Moreover, this nanopore-based sequencing method is scalable, portable, and capable of simultaneously interrogating the entire panel of forensic markers, making it an efficient and cost-effective alternative to mainstay MPS technologies.

DNA samples were evaluated at 22 autosomal STRs, 23 Y-STRs, and Amelogenin. Primer sets targeting 800 base pair amplicons were designed to interrogate the repeat and flanking regions. Loci were amplified in multiplex PCR for the long amplicons and the shorter Promega PowerSeq 46GY System with varying input DNA amounts and cycle numbers. Amplicons were barcoded and sequenced on the ONT MinION device to produce 1D read data. The unique current disruptions in the raw read data were then decoded with the Guppy base caller. High-quality sequencing data were obtained for the STR loci interrogated for both the long and short amplicons. A customized data analysis pipeline is being developed to align resultant reads, predict size-based allele designations, and identify single nucleotide polymorphisms within the flanking regions. Following compilation of consensus sequences and variant reports for each sample, these size-based allelic designations will be compared to those generated via capillary electrophoresis. Evaluation of concordance between the STR typing approaches will provide valuable insight into the reliability of nanopore sequencing data, ultimately setting the foundation for future development of STRs for biomedically relevant regions and potential forensic applications.

9:55 A.M.–10:20 A.M.

Development of Entire Mitogenome Reference Data Using an Automated High Throughput Sequencing Workflow

NIJ Award #: DJO-NIJ-17-RO-0219

Presenting authors: Kimberly Sturk-Andreaggi*, Joseph D. Ring, Cassandra R. Taylor, and Charla Marshall, Armed Forces DNA Identification Laboratory and SNA International

Abstract: Next generation sequencing (NGS) of the mitochondrial genome (mitogenome) will become more commonly employed in forensic laboratories due to its efficiency in both data production and analysis. For example, at the Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL), NGS protocols for low- and high-quality mitochondrial DNA (mtDNA) sample processing have been validated and routinely used since 2016. Although mitogenome sequence data are now produced more commonly, reference databases of sufficient size are required for population frequency estimates to support forensic casework. With funding from the National Institute of Justice, the AFMES-AFDIL is generating a total of 4,000 US and 1,000 global mitogenomes with NGS.

The methodological approach employs a long-range target enrichment, followed by polymerase chain reaction–free library preparation, and sequencing of 96 samples on an
Illumina MiSeq run. This protocol has been shown to produce negligible artifacts, such as nuclear mitochondrial DNA (NUMT) pseudogenes, to ensure accurate mitogenome sequence reporting. For efficiency, the workflow was automated to reduce hands-on processing time by seven hours per plate. Automation furthermore increased the consistency in library quality. A robust bioinformatic pipeline using the CLC Genomics Workbench was optimized to enable rapid analysis, with analyst review taking less than 5 minutes per sample. The entire process, from PCR to European DNA Profiling (EDNAP) Group’s mitochondrial DNA population database (EMPOP)–ready profile, can be completed in two weeks for each 96-sample set, while maintaining several sets simultaneously in progress.

As of August 2019, nearly 2,000 US and 250 global mitogenome sequences have been generated and reviewed using this workflow. Of these samples, which were processed by only two analysts in a single year, a first pass success rate of 85% was observed. Within each US population (i.e., Caucasian, African American, Hispanic, Asian, and Native American) the proportion of unique haplotypes averaged 91%. Point heteroplasmy (PHP) was observed in 498 of the nearly 1800 completed samples. Of those samples with PHP, a majority (79%) exhibited only a single heteroplasmic site, and the maximum was four PHPs in a single sample. Of the 621 observed PHPs, 61% were found in the coding region. Consistent with previous studies, the following control region positions were found to be PHP hotspots in the dataset of 1,800 samples: 146 (n = 12), 152 (n = 6), 204 (n = 14), 16093 (n = 25), 16129 (n = 10), and 16311 (n = 6). In contrast, only 15 coding region positions showed PHP in two samples and 15924R was observed in three different samples. Therefore, coding region PHPs are more individually discriminating than those in the control region. When considering all PHPs, the proportion of unique haplotypes per population was 97%.

In addition to expanding the mitogenome database for population frequency estimates, the results obtained will help create guidelines for mitogenome analysis, provide information on single nucleotide polymorphism mutation rates across the mitogenome, and further refine phylogenetic knowledge.

Disclaimer: The opinions and assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the United States government.

Morning Session II: Controlled Substances and Toxicology

10:35 A.M.–11:00 A.M.

The Detection and Quantitation of Fentanyl Mixtures by Surface-Enhanced Raman Spectroscopy (SERS) and Chemometrics
NIJ Award #: 2015-IJ-CX-K006

Presenting author: Ling Wang, Florida International University

Abstract: The abuse of opioids has become a critical issue in United States over the past 5 years. Newly developed synthetic fentanyl analogs continue to appear in street drugs, resulting in increased threats to the public health. Since the appearance of these new fentanyl analogs, prior screening methods, such as immunoassays, have had difficulty detecting and analyzing the multiplicity of opioid analogs in the market. We have been working on an alternative screening method using Surface-Enhanced Raman Spectroscopy (SERS) coupled with metal nanoparticles and aggregating agents. SERS is a rapid screening method that provides molecular fingerprint signals at toxicological concentrations. The procedure is simple and fast, and it is convenient for use in both point-of-care analysis and laboratories.
The new method can distinguish fentanyl analogs with a benchtop Raman instrument; the method can also detect fentanyl, cocaine, and heroin at low to sub ng/mL concentrations, as well as distinguish fentanyl in mixtures with cocaine or heroin, even at levels of 0.5% or lower with a portable Raman instrument.

The SERS method we have developed uses gold/silver nanostars in colloidal form, which are mixed with magnesium chloride and aggregated. Next, drug samples are added to those aggregated silver/gold nanostars and allowed to incubate for 5 minutes. On the surface of the aggregated nanostars, the creation of hot spots produces localized surface plasmon field effects resulting in an improvement in SERS enhancement. The SERS spectrum provides molecular vibration information that can identify individual compounds. Chemometrics, such as linear discriminant analysis and principal components analysis, are then used to create a model to cluster classes of drug samples and to distinguish single drugs and their mixtures. The resultant data assist in calculating the percentage of fentanyl in the mixture based on the composite spectra.

The SERS method permits a rapid, easily operated presumptive test for opioids. It is orthogonal to mass spectrometry and is sufficiently sensitive to detect compounds at toxicological levels. As a result, SERS should be particularly useful for screening trace levels of seized drugs including fentanyl analogs, mixtures with heroin and/or cocaine, and other novel psychoactive substances.

11:00 A.M.–11:25 A.M.

**Portable SERS-PSI-MS Dual Analysis Platform Using Gold Nanoparticle-Embedded Paper for Trace Detection of Illegal Drugs**

NIJ Award #: 2017-R2-CX-0022

Presenting author: Jeremy D. Driskell, Illinois State University

**Abstract:** Forensic laboratory backlogs are replete with seized drug samples. Shifting analysis toward the point of seizure would save significant time and public funds. Recent advances in portable analytical instruments offer simplistic on-site operation with requisite analytical performance to revolutionize forensics science and law enforcement. To date, studies have demonstrated that both portable mass spectrometers equipped with an ambient ionization source and handheld Raman spectrometers accurately identify chemicals and are ideal candidates for on-site evidence screening. However, independently these portable techniques do not fulfill the two-tiered identification guidelines recommended by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) for generating prosecutorial data. Development of a two-tiered identification strategy for controlled substance testing that relies on two independent methods could entirely circumvent the need for forensic laboratory testing and provide the greatest positive impact on forensics labs and the criminal justice system.

To this end, we present the development of a dual-method analytical tool using Raman spectroscopy and paper spray ionization mass spectrometry (PSI-MS). Both methods are capable of ambient analysis with fieldable instruments, yet Raman is often limited to bulk analysis. Critical to this work is the development of a gold-nanoparticle (AuNP) embedded paper swab to extend the capability of Raman spectroscopy to trace evidence via surface-enhanced Raman scattering (SERS). The developed plasmonic paper was characterized according to its SERS enhancement and compatibility with PSI analysis. Proof-of-principle is established with the analysis of five representative drugs, and detection limits on the scale of 10–100 ng are achieved for both PSI-MS and SERS. A 3-D printed cartridge was
designed and fabricated to facilitate seamless transition between the two techniques, affording efficient testing at 3 minutes per sample. After developing a standard operating procedure, SERS-PSI-MS was piloted in an error rate study (N = 500), which yielded an excellent success rate of 99.2%. In addition, several isomeric compounds were also studied, showing facile discrimination based on SERS spectra even when MS and MS2 spectra were indistinguishable.

The results presented herein demonstrate the potential of coupling of SERS and PSI-MS to significantly reduce error rates on chemical identification and generate prosecutorial evidence on site.

11:25 A.M.–11:50 A.M.

**Forty-Plus Ways Not to Analyze Beverages for Cannabinoids**

NIJ Award #: 2017-R2-CX-0029

Presenting authors: Carl Wolf*, Heidi L. Brightman, Justin L. Poklis, and William J. Korzun, Virginia Commonwealth University

**Learning Overview:** After attending this presentation, attendees will understand the factors involved in selecting a method for the analysis of cannabinoids in beverages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting approaches to determining acceptable methods for analyzing marijuana-infused beverages.

**Background/Introduction:** In the last decade, use of cannabinoids in the United States has increased tremendously. This increase has been primarily in marijuana-infused products, initially as food products, and now as beverages. These products contain the psychoactive drugs delta-9-tetrahydrocannabinol (THC) and/or cannabidiol (CBD), as well as other cannabinoids, and are sold because of their reputed medical and recreational properties. Regulation of these beverages is determined by the states in which they are legal. Federally, the US Drug Enforcement Administration (DEA) has classified marijuana as a Schedule I substance. The only US Food and Drug Administration (FDA)–approved formulation of THC is Marinol®, and the only FDA-approved formulation of CBD is Epidiolex®. In 2018, the Agriculture Improvement Act (Farm Bill) legalized hemp. The DEA, FDA, and Farm Bill do not address the formulation of these beverages, nor do they address the standardization of methods for potency analysis of these beverages. There is an increased need for accurate methods to determine THC and CBD content in these beverages. The three most common beverage matrices are fermented (beer/ale), brewed (tea/coffee), and high sugar (soft drinks). Each of these matrices creates its own concerns, which need to be addressed when trying to analyze beverages as a single class. Simple dilution methods with an aqueous or organic solvent are not plausible for all beverages due to the potential for varying complex matrices (e.g., plant material, pulp, sugar), and the low solubility of cannabinoids in aqueous solvents.

**Objective:** To develop a method for the forensic analysis of cannabinoid-infused beverages.

**Methods:** More than 40 different methods were evaluated for process efficiency (%PE). These included simple dilutions, rapid solid phase extraction (SPE), and quick easy cheap effective rugged safe (QuEChERS) methods, in which the aqueous and organic solvents were substituted or buffered. To account for the low solubility of cannabinoids in aqueous solvents, the beverage was prepared by solubilizing the cannabinoids in an emulsion (surfactant [2% fruit pectin], carrier oil [12.6% canola oil]) then adding the beverage (85.40%). %PE was determined at 14 mcg/mL (n=3) in two different sets of samples for
each extraction method. The before set was fortified before the extraction method, and
the non-extracted external standards (NEET) were prepared in methanol. The %PE was
determined using the post-extraction addition method. The %PE was calculated by using
the average peak areas of the before fortified samples and dividing by the average peak
area of the NEET and multiplying by 100. An extraction method had an ideal %PE if the
determined %PE were within 75%–125% for all three matrices evaluated. A method had an
acceptable %PE if determined %PE variations in all three matrices were <± 15%. Samples
analysis was performed using a previously presented and published high performance
liquid chromatography tandem mass spectrometry cannabinoid method.

**Results:** In most extraction methods, %PE was similar between the fermented and brewed
matrices, but the high sugar matrix was more than 20%–50% higher. The %PE for the
United Chemical Technologies Clean Screen FAS/THC 200mg/3mL (UCT THC) column
%PE levels for fermented, brewed, and sugar matrices were at 40, 45, and 54%, respectively.
Sample preparation involved 25 mcL of beverage, 225 mcL water, and 250 mcL acetonitrile,
which was added to the UCT THC column and eluted with 80 psi of air, and the eluate was
collected and analyzed.

**Conclusion:** No extraction method was determined to be ideal, but the UCT THC column
was acceptable.

This project was supported by the National Institute of Justice (NIJ) Research and
Development in Forensic Science for Criminal Justice Purposes Grant 2017-R2-CX-0029.

11:50 A.M.–12:15 P.M.

**Investigating the Rise and Fall of Opioids Using Data Acquired by Liquid
Chromatography Time of Flight Mass Spectrometry (LC-TOF-MS)**

NIJ Award #: 2017-DN-BX-0169

Presenting author: Judith Rodriguez Salas, Fredric Rieders Family Foundation: CFSRE

**Abstract:** The prevalence and illicit use of both prescription opioids and novel opioids
has steadily increased over the last decade. Overdoses resulting from opioid consumption
have contributed to a national epidemic. There are several limitations preventing the
identification of new compounds, including absence of reference material, similarity of
chemical structures, and lack of specialized instrumentation. Additionally, interlaboratory
variability in what drugs are tested for and the regional nature of cases processed
further limit the data. The net result is that opioid-involved deaths are overlooked and
underreported.

The objective of this research was to mine raw analytical data acquired using an Agilent
1290 liquid chromatograph coupled to an Agilent JetStream 6230 time-of-flight mass
spectrometer (LC-TOF-MS) with a continually updated database to identify emerging
opioids as well as monitor traditional opioids to create time-course trend reports.

All analytical data was collected from postmortem and driving under the influence (DUID)
cases at our collaborating laboratory (NMS Labs, Horsham PA). Agilent MassHunter
Qualitative Analysis software was used for compound identification. Data files collected
between January 2018 and December 2019 were reprocessed against a frequently updated
database containing more than 170 opioids. Data analysis was divided into three categories:
(1) routine opioids, (2) novel opioids, and (3) emerging opioids. All previously acquired
analytical data was retrospectively mined to determine the date of first identification for
analytes not included in the original scope of testing.
Between January and June 2019, there were 40,093 cases that tested positive for a routine opioid: 9,414 positive for fentanyl, 3,339 for heroin, and 3,613 for morphine. During that same time frame, 531 tests were found positive for novel opioids, which included para-fluoroisobutyrylfentanyl (p-FIBF) (n = 129), valerylfentanyl (n = 125), and carfentanil (n = 61) being the most prevalent. The only emerging opioid identified during the 6 months was o/m/p-fluorofuranylfentanyl, first identified in January 2019. Three positive cases were identified in 2019, and two were retrospectively identified in December 2018. From the retroactive data mining, 12 emerging opioids were identified that were not detected at the initial time of testing. Benzylfentanyl was identified a total of 9 times during this period, 3 of which were before it was included into the scope of testing in June 2018. Isopropyl U-47700 was identified in 10 cases, with the first identification occurring in May 2018. Data mining between March and April 2018 identified isopropyl U-47700 in five cases; however, it has not been detected since October 2018.

The opioid crisis presents a large public health and safety concern in the United States. Fentanyl continues to be implicated in approximately 1,450 cases each month, and heroin is implicated to a lesser extent. By comparison, the novel opioids have rapidly declined in positivity and are seen with much less frequency. However, emerging opioids not previously reported are still being identified. The data show that retroactive data mining can be a valuable tool to determine the prevalence and date of first appearance of novel compounds, giving a comprehensive perspective of the rise and fall of synthetic opioids over time.

Afternoon Session I: Impression and Pattern Evidence/Trace Evidence

1:35 P.M.–2:00 P.M.

Quantitative Measures for Footwear Impression Comparisons
NIJ Award #: DJO-NIJ-17-RO-0202

Presenting authors: Steven Lund*, Martin Herman, and Hari Iyer, National Institute of Standards and Technology

Abstract: We present National Institute of Standards and Technology (NIST) research on the quantitative evaluation of footwear evidence. This talk describes the current capabilities of the NIST footwear impression image comparison tools by illustrating each component of the comparison workflow: image annotation, image alignment, image comparison, and score interpretation.

During annotation, an expert examiner manually marks up a questioned impression by identifying regions of perceived outsole contact and noncontact and provides a clarity mask that indicates how clearly the expert can tell the difference between the two across different regions of the impression. The expert is also asked to roughly outline perceived RACs in test impressions, and conveys the size scale (i.e., pixel resolution) for each image. The original images and their respective annotations are provided as inputs to the automated workflow. The automated workflow begins by performing a nonrigid alignment between the test and questioned impressions. When test impressions from other shoes of the same design and size are available, a discrimination heat map is evaluated to quantify how informative each region of the test impression might be regarding which specific shoe left an impression. Taking the discrimination heat map and the clarity mask into account, the automated workflow then conducts a multi-stage comparison with dedicated stages for wear patterns and for each RAC identified in the test impression.

The comparison scores obtained from each phase of comparison are interpreted in the context of scores obtained from ground-truth-known reference comparisons, giving
reference comparisons most similar to the current case the most weight. When available, we compare multiple test impressions from different shoes of the same make, model, and size to the questioned impression as additional context. We will illustrate how these comparison results provide an empirical basis for assessing how strongly a given questioned impression singles out a shoe of interest from other shoes with the same outsole design.

2:00 P.M.–2:25 P.M.

Testing the Accuracy and Reliability of Palmar Friction Ridge Comparisons: A Black Box Study

NIJ Award #: 2017-DN-BX-0170

Presenting authors: Heidi Eldridge*, RTI International; Marco De Donno and Christophe Champod, University of Lausanne

Abstract: After attending this presentation, attendees will be aware of the results of a recent large-scale black box study that measured the performance of expert friction ridge examiners to establish a discipline error rate estimate for the comparison of palmar impressions. This is the first study to specifically measure performance on the palmar comparison task.

This presentation will impact the forensic community by providing an error rate estimate that can be used by examiners in court when testifying to the results of palm comparisons. These results provide the first step in establishing the foundational validity of palmar comparisons, as defined by the recent President’s Council of Advisors on Science and Technology (PCAST, 2016) report.

In 2011, a team of researchers from the Federal Bureau of Investigation (FBI) and Noblis published the first large-scale black box study measuring the accuracy of fingerprint examiners (Ulery, Hicklin, Buscaglia, & Roberts, 2011). They reported a low rate of false positives (0.1%) and a rather high rate of false negatives (about 7.5%). The FBI/Noblis study dealt only with marks and prints originating from the distal phalanges of fingers (fingerprints). However, anecdotally it is estimated that approximately 30% of comparison cases involve palm impressions. It has been unknown up to now whether examiners are equally accurate at both tasks. This presentation provides the results of a recent large-scale black box study that measured examiners’ accuracy when conducting exclusively palm comparisons.

This presentation reports on the results recorded both during the analysis phase and the comparison phase by a total of 226 fingerprint examiners who carried out a total of 12,279 determinations in analysis and 9,460 decisions following comparison. The pool of cases comprised 526 cases (questioned and known palm impressions) of known ground truth (i.e., the source of the unknown impressions was known to the researchers before conducting the study). Both known mated pairs and known nonmated pairs were presented. Participants first performed a suitability analysis on unknown marks; thus, not all unknown marks proceeded to comparison (those deemed by the examiner to be unsuitable were not presented with a known exemplar to compare). Unknown marks and known exemplars varied in quantity and quality of features to reflect the complexity of casework.

Two online Shiny applications are also presented for exploring the results of the study and the data’s associated confidence and credibility intervals. The implications of these results on the reporting of “error rates” associated with palm print examinations will be discussed along with the implications and incidence of “questionable” conclusions that may not be supported by a consensus panel.
Rapid Detection of Inorganic and Organic Firearm Discharge Residues by Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemical Sensors

NIJ Award #: 2018-DU-BX-0186

Presenting authors: Tatiana Trejos* and Luis Arroyo, West Virginia University

Abstract: Rapid and accurate detection of firearm discharge residues (FDR) is highly desirable in circumstances that require a fast response to protect the welfare of citizens and provide reliable information to make informed decisions. Quick screening methods for FDRs are particularly useful, but currently difficult, in cases involving shootings. The scientific validity of this field relies on extensive research and standardization of the existing methods. There are still some remaining challenges in this area in terms of speed of analysis, preservation of the evidence, accuracy, and interpretation of results. Consequently, there is a critical need to develop methods to improve the speed and reliability of these determinations. The long-term goal of this study is to develop a comprehensive approach to overcome these major concerns in FDR detection and to improve current capabilities in the criminal justice system. This research study aims to develop and validate fast tests—laser-induced breakdown spectroscopy (LIBS) and electrochemical sensors—for FDR detection and statistical models for the interpretation of the evidence.

The proposed methodologies were validated through a set of over 700 samples and in-house gunshot residue (GSR) control standards. The first collection consists of 600 samples representing hand traces from background populations and from the hands of the shooters who fired standard ammunition and nontoxic ammunition. The rapid scanning of the laser beam allowed the identification of multiple emission lines per target element in less than one minute, with repeatability better than 11% relative standard deviation (RSD) and limits of detection for the target species in the range of 0.2–200 ng. Electrochemical analysis via square-wave voltammetry and disposable carbon electrodes allowed the simultaneous detection of inorganic and organic GSR markers in less than 5 minutes per sample (repeatability better than 8% RSD, detection limits 0.007–5 mg/mL, linearity > 0.991). Four different approaches—critical threshold, logistic regression, naïve Bayes, and neural networks—were applied to examine the performance of each method alone and collectively. The combination of LIBS and electrochemical methods provided overall accuracy between 87% and 100%, depending on the type of prediction model applied.

A second collection set includes 150 specimens for estimation of firing distance and identification of residues on fabrics, wood, drywall, glass, and specimens with blood. LIBS created 3D chemical maps for shooting distance estimations and identification of bullet holes. Statistical methods, like principal component analysis and multivariate discriminant analysis, were performed to estimate shooting distances and identify the presence of...
FDR. Color tests led to misclassification of 26%–70% of the unknown shooting distances, while the LIBS method correctly classified 100% of the blind test samples. Additionally, LIBS was able to correctly identify elemental profiles from the modern ammunition, expanding current capabilities in the reconstruction of events at crime scenes. Scanning electron microscopy–energy dispersive X-ray spectroscopy, liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry were used to cross-validate the results.

The comprehensive approach presented in this study demonstrates the versatility and reliability of LIBS and electrochemistry methods for detection of FDR. The results of this research increase the existing knowledge in firearm discharge residues and demonstrate the potential of ultrafast and portable screening methods to transform current practice.

2:50 P.M.–3:15 P.M.

Facilitating the Adoption of Glass Evidence Analyses in Forensic Laboratories
NIJ Award#: 2015-DN-BX-K049

Presenting author: José R. Almirall, Florida International University

Abstract: Forensic laboratories that invest in staffing and equipping a state-of-the-art trace evidence facility report multiple benefits to the justice system derived from these investments. This presentation describes a strategy to facilitate the adoption of standard test methods resulting from NIJ-funded research and working group coordination. This presentation also describes how an “implementation package” including instrument specification, procedures, and validation assistance can be transferred to any forensic laboratory willing to take on the challenge of setting up a state-of-the-art facility while substantially reducing the learning curve normally associated with that effort.

The example of a glass evidence examination and comparison “implementation package” will be presented to include personnel training, access to reference materials, completed validation studies (including peer-reviewed reports), standardized test methods of analysis that have undergone Standards Development Organization and Organization of Scientific Area Committee review and approval, and focus on rational evidence interpretation guidelines using likelihood ratio reporting based on NIJ-funded research (Akmeemana et al., 2019; Corzo et al., 2018; Hoffman et al., 2018). This implementation package can be used to effectively reduce the barriers to quickly and inexpensively providing this important forensic service to your community. An added benefit is the creation of a network of labs—using the same set of procedures, validation, interpretation, and reporting—that can share databases and experience, enabling the tracking of trends and improvements that are easily transportable among network members.

Research has shown that effective countermeasures to hit-and-run accidents include driver education campaigns similar to those used to reduce drunk driving, reducing the incentive to flee (Lueders, Hainmueller, & Lawrence, 2017) and raising public awareness of the high likelihood of getting caught. Providing trace evidence analyses in your jurisdictions and broadcasting the successes in solving hit-and-run accidents will increase submission of essential trace evidence by crime scene investigators and may, in fact, motivate drivers not to run after a crash, thereby providing the opportunity to render aid to an injured victim and potentially save lives.


Afternoon Session II: Forensic Anthropology and Forensic Pathology

3:30 P.M.–3:55 P.M.

OSTEOID, a New Forensic Tool: Developing a Practical Online Resource for Species Identification of Skeletal Remains

NIJ Award #: 2018-DU-BX-0229

Presenting author: Heather Garvin, Des Moines University

Abstract: Approximately 30%–40% of cases received by forensic anthropologists end up being nonhuman (i.e., animal bones) and not of forensic significance. Although the forensic anthropologist can quickly determine whether the remains are human based on their expert knowledge in human osteology, agencies typically also want to know to which species they belong. In a way, providing the faunal (animal) species identification bolsters the confidence in the nonhuman determination. Forensic anthropologists without extensive zooarchaeological collections are limited to a few (expensive) books containing comparative photographs when making identifications.

The aim of this project is to create a free, practical, and easy-to-use online tool (OSTEOID), where an individual can use simple measurements and morphological information to determine: (1) whether a bone is human, and (2) if it is nonhuman, to which species it belongs. High-quality photographs will guide the user through choosing the correct element (e.g., humerus). At which point, the user can then input any available basic measurements (e.g., maximum length), and the program will return high-quality color photographs of the potential species it could be (based on size) for visual comparison and identification. Links to three-dimensional (3D) surface models will also be made available. Practitioners may even build their own comparative collections from the 3D prints.

Beyond being a source for practicing forensic anthropologists, OSTEOID will also be available to death investigators, crime scene personnel, coroners, medical examiners, and law enforcement. These agencies can use OSTEOID to rule out the possibility that discovered remains are human, reducing time and costs associated with subjecting easily identifiable animal remains to medical examiners’ offices and forensic anthropological analyses. Finally, the photographs and 3D models can be used to train future practitioners in comparative osteology.

At present, we are in the data collection phase of this grant. OSTEOID has defined 53 measurements that could be consistently taken across 28 different species (including mammals and birds), which are simple enough that individuals without osteological
training can easily take them without specialized equipment. These measurements are being collected from a minimum of 30 specimens of each species, so that the size range of elements by species can be incorporated into the online database, from which searches will be performed to narrow potential species. Statistical analyses (e.g., discriminant function analyses) will be carried out to evaluate which measurements are the most diagnostic, and those measures will be retained for the online program.

3:55 P.M.–4:20 P.M.

Development Responses to Fluctuating Temperatures of a Forensically Important Blow Fly (Cochliomyia macellaria)
NIJ Award #: 2016-DN-BX-0204

Presenting author: Travis Rusch, Texas A&M University AgriLife Research

Abstract: Forensic scientists investigate crimes by piecing evidence together in order to reconstruct past events. Forensic entomologists, in particular, use insect evidence to estimate forensically important timelines in death investigations, such as the time of insect colonization (TOC), which can be inferred as the time of death given certain assumptions. To do this, forensic entomologists take advantage of the temperature sensitive development rates of necrophagous insects, such as blow flies, and use them as biological clocks in death investigations. However, changes in temperature do not affect all organisms equally, nor do changes in temperature affect the same organism equally at all life stages.

These phenomena raise serious concerns for forensic entomologists, yet little is known about the developmental responses of immature blow flies to fluctuating temperatures. Most temperature-development data sets consist of exposing larvae to a series of constant temperatures to determine how fast larvae develop at a given temperature. However, larvae experience a variety of temperatures on a corpse due to microclimates on decomposing body created by daily weather cycles. Therefore, exposure to constant temperatures may result in under- or over-estimation of larval development rates, which reduces the accuracy of estimating forensically important timelines (e.g., TOC or PMI).

To begin addressing this concern, we exposed forensically important immature blow flies (Cochliomyia macellaria) to both constant and fluctuating temperature regimens and compared their development rates (1/development time). Each treatment consisted of the same mean temperature (25°C) but differed in magnitude of fluctuation (± 0°C, 5°C, or 10°C). Furthermore, because blow fly activity is greatest during the morning and evening in warm environments, we altered the initial ramping directions (i.e., hot or cold) for the treatments of 25±5 and ±10°C to simulate either (1) a morning oviposition event followed by warming afternoon temperatures or (2) an evening oviposition event followed by cooling overnight temperatures. We recorded development times of each treatment for each development stage (egg, larvae, and pupae) and the percentage surviving to adult (i.e., emergence).

We found that not only does a greater magnitude of temperature fluctuation affect development time, but the initial direction of the fluctuation causes differences in development time across stages, with a difference in total development time (egg to emergence) of up to 44 hours. We hope these findings shed further light on the issue of temperature dependent blow fly development in fluctuating environments and how this may affect estimates of forensically important timelines.
**Understanding the Role of the Thanatomicrobiota in the Decay of “Reproductive Organs” in Human Decomposition**

NIJ Award #: 2017-MU-MU-0042

Presenting author: Gulnaz Javan, Alabama State University

**Abstract:** After attending this presentation, attendees will understand how to use 16S rRNA amplicon sequencing analyses to characterize the thanatomicrobiota of reproductive organs from actual cadavers in criminal cases (e.g., homicide, suicide, and overdose). Specifically, attendees will learn methods to assess the microbial diversity after death using cases with postmortem intervals (PMIs) between 3.5 and 240 hours.

This presentation will impact the forensic science community by revealing the specific bacterial signatures associated with the uterus and prostate of cadavers with different manners of death. These signatures could help to improve trace evidence regarding characteristics of manner of death for criminal cases.

Human organs decompose at different rates and in different ways. For example, human prostate glands and uteri are the last internal organs to deteriorate during putrefaction. However, the reason for this phenomenon has not been elucidated. To determine whether the bacteria associated with these organs differ from other organs and whether the taxonomic signature is associated with the PMI, we applied 16S rRNA amplicon sequencing to tissues associated with 21 prostate glands and 13 uteri collected at autopsy from criminal casework cadavers. The 16S rRNA V4 region was amplified and sequenced from each sample, and nonparametric statistics were used to determine the resulting microbiota profile and its association with cadaver characteristics.

Both the uterus and prostate had a significantly greater alpha diversity compared to other organs, as well as maintaining a significantly different microbial composition (beta diversity) as determined by unweighted UniFrac. The prostate was significantly enriched for two 16S rRNA absolute sequence variants (ASVs) associated with the Bacteroidia, one in the family Comamonadaceae (genus *Limnohabitans*) and another in the family Oxalobacteraceae. Uterine tissues were enriched for only two ASVs, including a single ASV in the class Bacilli (family Lactobacillaceae, genus *Lactobacillus*) and a single ASV in the class Gammaproteobacteria (family Enterobacteriaceae, unknown genus). Prostate tissues had a significant underrepresentation of 4C0d-2 ASV (order MLE1-12) and a single Clostridia ASV (family Lachnospiraceae, unknown genus). It is possible that these organisms may associate with differential decay rates. Natural deaths were enriched for class 4C0d-2 (order MLE1-12) and ASVs in the classes Bacilli (family Lactobacillaceae, *Lactobacillus zeae*), Gammaproteobacteria (family Enterobacteriaceae, unknown genus), and Saprospirae (family Chitinophagaceae, genus *Sediminibacterium*). Among victims of accidental death, a single Bacilli ASV (order Lactobacillales, unknown family) and Gammaproteobacteria (family Enterobacteriaceae, unknown genus) were enriched. Homicide victims did not exhibit enrichment of any bacterial taxa. Currently none of these signals was a significant predictor of manner of death.
Microbial Clocks for Estimating the Postmortem Interval of Human Remains at Three Anthropological Research Facilities
NIJ Award #: 2015-DN-BX-K016

Presenting authors: David Carter*, Laboratory of Forensic Taphonomy, Forensic Sciences Unit, Division of Natural Sciences and Mathematics, Chaminade University of Honolulu; Zachary Burcham* and Jessica Metcalf, Department of Animal Sciences, Colorado State University

Abstract: The co-evolution of microbial decomposers and vertebrate carcasses has resulted in conserved cross-kingdom ecological interactions and metabolic decomposition pathways. Therefore, microbial succession during decomposition of vertebrates may be repeatable and generalizable enough to develop predictive tools to estimate the postmortem interval (PMI) by utilizing microbiome data. Over the past several years, our research lab has been developing a microbial clock to estimate how long human remains have been decomposing. In a large-scale, collaborative research project, we placed 36 donated human cadavers at three anthropological research facilities, located in distinct geographic regions in the United States (Colorado Mesa University, Sam Houston State University, University of Tennessee Knoxville). At each facility, three bodies were placed outdoors each season for four seasons. Skin and soil swabs were collected daily from each body for 21 days of decomposition. From these swabs, we characterized the decomposer microbial community by amplicon sequencing (16S and 18S rRNA gene) to reveal composition and diversity, shotgun metagenomics to reveal potential gene function, and metabolomics to assess small molecules generated by the microbes. Data were used to train machine-learning models to predict PMI. 16S rRNA gene amplicon data revealed PMI prediction errors of 2–4 days within each facility over 21 days of decomposition in the spring season and approximately 3.5 days when all facilities were used in model construction. Models included a temperature-based accumulated degree day. Additional modeling will include data from summer, fall, and winter seasons, as well as other -omic data and variables (e.g., humidity) to generate the most robust predictive model possible for these data. Overall, we demonstrate that microbiome tools provide a potentially powerful new tool for the forensic science community.
José R. Almirall

José R. Almirall is a Professor in the Department of Chemistry and Biochemistry and Director of the National Science Foundation–funded Center for Advanced Research in Forensic Science (CARFS). Professor Almirall has authored one book and ~145 peer-reviewed scientific publications in the fields of analytical and forensic chemistry (h-index ~ 40). Prof. Almirall and his research group have authored and co-authored five ASTM standards within the fields of forensic chemistry. His group has been awarded five patents related to volatile organic chemical (VOC) sampling and analysis using capillary microextraction. The primary applications developed in the Almirall laboratory for CMV have been the sampling, preconcentration and analysis of explosives and of VOCs associated with explosives and drugs. He has also served as a consultant to the United Nations Office on Drugs and Crime, the government of Spain, and the US government, most recently serving as a judge of a Department of Homeland Security Challenge to detect opioids in parcels at mail facilities. Dr. Almirall is also interested in the standardization of analytical methods used by forensic scientists and currently chairs the Chemistry Scientific Area Committee of the National Institute of Standards and Technology–funded Organization of Scientific Area Committees. He currently serves as consultant to the International Atomic Energy Agency on the forensic analysis of materials and has served as chair of the Fire Scene Investigation working group of the American Academy for the Advancement of Science. Dr. Almirall is also interested in commercializing technology and has started Air Chemistry, Inc., to commercialize capillary microextraction of volatiles. Prof. Almirall is also the Editor-in-Chief of Forensic Chemistry, an Elsevier journal.

Luis Arroyo

Luis Arroyo is an Assistant Professor at the Department of Forensic and Investigative Science at West Virginia University. Dr. Arroyo teaches graduate and undergraduate courses in forensic chemistry and forensic toxicology. Dr. Arroyo’s primary research focuses on the characterization and analysis of emerging drugs of abuse, pollutants, environmental stressors, and gunshot residues. Dr. Arroyo has ample expertise in analytical instrumentation, including the application of electrochemistry, mass spectrometry (gas chromatography with tandem mass spectrometry), liquid chromatography with tandem mass spectrometry, direct analysis in real time mass spectrometry, laser ablation inductively coupled plasma mass spectrometry), and spectroscopic methods (Raman, Fourier-transform infrared spectroscopy, laser-induced breakdown spectroscopy) in a diversity of matrices of forensic interest. Dr. Arroyo has authored several peer-reviewed scientific publications and has presented over 90 papers and posters at scientific meetings in North America, Central America, South America, Europe, and Australia. The National Institute of Justice has recently funded Dr. Arroyo’s research in novel psychoactive substances and gunshot residues.
Zachary Burcham

Zachary Burcham is a postdoctoral researcher in Jessica Metcalf’s laboratory in the Department of Animal Sciences at Colorado State University where he has been a faculty member since 2018. Dr. Burcham completed his PhD at Mississippi State University and his undergraduate studies at the University of Tennessee. His research interests lie in the use of multi-omics approaches to study host and environmental microbiomes, with a focus on the postmortem microbiome function and structure.

David Carter

David O. Carter is Professor of Forensic Sciences at Chaminade University of Honolulu, Hawaii, USA. He has been interested in medicolegal death investigation since 1999 and has been attending death scenes since 2000. Dr. Carter has consulted with several investigative agencies around the world and regularly collaborates with his local medicolegal death investigation agency, the City and County of Honolulu Department of the Medical Examiner. Dr. Carter’s research in the Laboratory of Forensic Taphonomy focuses on corpse decomposition and the estimation of postmortem interval. Dr. Carter is particularly interested in the relationships between decomposition and the structure and function of postmortem microbial communities.

Jeremy Driskell

Jeremy Driskell is an Associate Professor of Analytical Chemistry at Illinois State University. Prof. Driskell has nearly 20 years of experience in developing point-of-need analytical devices with specific expertise in the area of surface-enhanced Raman spectroscopy (SERS). Prof. Driskell’s research program aims to demonstrate the utility and potential impact of SERS-based detection applied to point-of-care diagnostic testing and on-scene forensic analysis by investigating the mechanism of SERS enhancement to design optimized SERS substrates. Prof. Driskell’s research in these areas is funded by Department of Defense—Defense Threat Reduction Agency (DOD-DTRA), the National Science Foundation, and the National Institute for Justice. As an independent investigator, Prof. Driskell was selected for a 2013 DOD-DTRA Young Investigator Award and featured as a 2015 “Emerging Investigator in Analytical Sciences” by Analytical Methods and a 2016 “Emerging Investigator in Analytical Sciences” by Analyst.

Heidi Eldridge

Heidi Eldridge has been a latent print examiner for over 12 years. Ms. Eldridge is a Certified Latent Print Examiner and is a member of the Board of Directors for the International Association for Identification, sits on the Journal of Forensic Identification editorial board, and was a member of Scientific Working Group on Friction Ridge Analysis, Study and Technology. She is now a member of the Friction Ridge Subcommittee of the Organization of Scientific Area Committees.
and the Academy Standards Board Friction Ridge Consensus Body. Ms. Eldridge has been teaching latent print testimony for over 10 years and is a doctoral candidate in forensic science at the University of Lausanne. After 11 years as a practitioner, she is now a Research Forensic Scientist with RTI International, working on multiple research projects in latent fingerprints.

Jannine Forst

Ever the explorer, Jannine Forst has spent most of her career chasing after elusive DNA. Starting in the field of ancient DNA, she worked on detecting ancient tuberculosis DNA in a variety of archaeological bone samples during her doctoral work at the University of Manchester in the UK. As a postdoctoral scholar, Dr. Forst delved into the next-generation sequencing of archaeological charred grain to investigate the origins of agriculture. From there, she moved to her next postdoctoral position at the University of California, Santa Cruz, and studied the human population genetics of Machu Picchu to better understand the role and impact of this important archaeological site on Incan society.

Most recently, Dr. Forst has used her expertise in capturing low quantities of fragmented DNA towards next generation sequencing method development at a biotech start-up called Arc Bio. Here, as a research scientist, she leads a project developing new library preparation methods and bioinformatic pipelines to better detect trace DNA both with and without a high background of non-target DNA.

Heather Garvin

Heather Garvin is currently an Associate Professor of Anatomy at Des Moines University, where she teaches medical students, continues her human skeletal research, and conducts forensic anthropology cases for the State of Iowa. Dr. Garvin began her journey in forensic anthropology as an undergraduate volunteer at the C. A. Pound Human Identification Laboratory at the University of Florida. Graduating with bachelor’s degrees in anthropology and zoology, she then earned a master’s in forensic and biological anthropology from Mercyhurst College and completed a doctorate in functional anatomy and evolution from Johns Hopkins University School of Medicine. From 2012 to 2017, she taught undergraduate and graduate students in forensic anthropology at Mercyhurst University and was heavily involved in casework and research. Dr. Garvin became a Diplomate of the American Board of Forensic Anthropology in 2017 and has served on the editorial board for the Journal of Forensic Sciences since 2015. Her research interests include forensic anthropological methods, human skeletal variation, functional morphology, 3D scanning, and geometric morphometrics. She has more than 30 publications and 50 national presentations related to this research and is a Fellow in the American Academy of Forensic Sciences and a Member of the American Association of Physical Anthropology.
Courtney Hall

Courtney Hall is a doctoral student in the Department of Microbiology, Immunology, and Molecular Genetics at the University of North Texas Health Science Center (UNTHSC). She holds a bachelor's degree in forensic science from Saint Edward's University and a master's in forensic genetics from UNTHSC. In addition to assessing the potential applicability of nanopore-based sequencing in forensic DNA analysis, her current research under Dr. John V. Planz focuses on the development and implementation of targeted enrichment strategies for methylation detection using this platform. She received a pre-doctoral fellowship in the Neurobiology of Aging and Alzheimer’s Disease Training Program to study the role of epigenetic modifications in the progression and pathology of age-related disorders.

Gulnaz Javan

Gulnaz Javan is an Associate Professor and the Director of the Forensic Biology Program at Alabama State University. Since 2013, the Javan Thanatos Lab at Alabama State University has accessed national and international cadaver samples obtained at autopsy for forensic microbiology and genetic studies. Dr. Javan's interests include investigating the microbial and genetic factors associated with the decomposition of human corpses’ internal organs from the time of death throughout the process of decay. The tissues from dead bodies are typically collected from criminal casework cadavers from Montgomery, Alabama, and Pensacola, Florida, medical examiners’ offices (nationally); and Pavia University in Italy and Tampere University in Finland (internationally). Dr. Javan has an extensive publication record in forensic science and microbiological literature and has coined novel terms in the forensic field such as “thanatomicrobiome” (microbiome of death) and “Postmortem Clostridium Effect” to describe the proliferation of microbes for the determination of time and cause of death. She has also received the Lucas Grant through the Forensic Science Foundation in 2016 and a grant with Dr. Jack Gilbert from the National Institute of Justice in 2017 focused on the study of the thanatomicrobiome and metabolome compositions for the determination of the cause of death in medicolegal investigations.

Kenneth Kidd

Kenneth K. Kidd, Professor Emeritus of Genetics and Senior Research Scientist at Yale University, is a human population geneticist. Dr. Kidd has published over 550 scientific papers on a variety of subjects before and during his 46-year career at Yale. His research has included medical genetics, gene mapping, database design, pharmacogenetics, and a variety of molecular methodologies. His long-standing interest in human population genetics has been combined with his laboratory’s expertise in molecular technology to examine human genome diversity at the DNA level. In the late 1980s and early 1990s, Dr. Kidd’s expertise in both population and molecular genetics provided helpful expert testimony in getting DNA accepted in the courts. After serving on the advisory panels for DNA identification of victims of the World Trade Center Attack and of Hurricane Katrina, he began research in
his lab on panels of single nucleotide polymorphisms (SNPs) for various uses in forensics as an extension of his active research on human genetic diversity. His group designed ALFRED, the large ALlele FREquency Database, and FROGkb, the Forensic Reference/Resource on Genetics knowledge base. Since 2013, Dr. Kidd has also been recognized for his development of microhaplotypes as a new type of forensic marker suited for the coming transition from capillary electrophoresis to massively parallel sequencing as a common method in forensic practice. His lab continues to be very active in identifying single nucleotide polymorphisms and microhaplotypes useful in forensics.

Steven Lund

Steve Lund is a Statistician at the National Institute of Standards and Technology. He received his doctorate in statistics from Iowa State University in 2012. He is an affiliate and past member of the Organization of Scientific Area Committees Footwear and Tire subcommittee.

Judith Rodriguez Salas

Judith Rodriguez graduated from the University of Maine at Augusta with a bachelor’s degree in biology. She later obtained a master’s degree in forensic science from Arcadia University, focusing on forensic biology through her research in a “Method Development of Direct Quantification for Sexual Assault Samples to Be Incorporated in the Direct PCR Workflow.” During her internship, Ms. Rodriguez shifted her focus to forensic toxicology, and after the completion of her degree, she worked as the toxicology mentor for the Forensic Science Mentoring Institute class of 2018.

Currently, Ms. Rodriguez serves as a research technician and toxicologist at the Center for Forensic Science Research and Education (CFSRE). She is involved in several projects, including the International Toxic Adulterant Database project that detects, analyzes and identifies toxic adulterants in seized drugs and National Institute of Justice grant-funded research for the retrospective datamining of new psychoactive substances including fentanyl analogs and novel opioids. Judith also provides laboratory support during courses and internships for graduate level toxicology students, as well as ongoing support for various other projects currently being conducted within the CFSRE lab.

Travis Rusch

Travis Rusch obtained his bachelor’s degree from the University of Wisconsin—Stevens Point and his doctorate from Arizona State University. He is currently a postdoctoral research associate at Texas A&M University. Dr. Rusch is a broadly trained thermal biologist investigating how animals function across landscapes. While he has examined the behavioral and physiological responses of lizards to altered thermal environments, his focus has shifted to the thermal biology of necrophagous insects. This system provides a comprehensive understanding of ecological systems while offering exciting applications in forensic
entomology and disease ecology. Dr. Rusch is currently working on a project that examines how fluctuating temperatures alter the development of forensically important insects, such as blow flies. This research will improve estimates of forensically important timelines, such as time of insect colonization on dead bodies, which will help improve estimates of the postmortem interval in death investigations.

Kimberly Sturk-Andreaggi

Kimberly Sturk-Andreaggi is a Research Scientist in the Emerging Technologies (formerly Research) Section at the Armed Forces Medical Examiner System’s Armed Forces DNA Identification Laboratory (AFMES-AFDIL). She received her undergraduate degree in Bioengineering from the University of Pittsburgh in 2003, and her Master of Forensic Sciences from the George Washington University in 2005. She is currently working on her doctoral degree in medical genetics at the University of Uppsala in Sweden. Over her 15+ year tenure at the AFDIL, Ms. Sturk-Andreaggi has contributed to and managed various projects including mitochondrial DNA coding region single nucleotide polymorphism panels, species identification, low template techniques, automated processing, and the development of population databases. For the last several years, her primary focus has been on the use of next-generation, or massively parallel, sequencing for human identification.

Tatiana Trejos

Tatiana Trejos is an Assistant Professor of the Department of Forensic and Investigative Sciences at West Virginia University. Dr. Trejos teaches forensic chemistry and research design courses at the undergraduate and graduate levels. Dr. Trejos’ primary research interest includes the application of chemometrics to evidence interpretation and the discovery of chemical signatures of forensic materials by spectroscopic methods, such as scanning electron microscopy–energy dispersive X-ray spectroscopy, inductively coupled plasma mass spectrometry, laser ablation inductively coupled plasma mass spectrometry, X-ray microfluorescence, and laser-induced breakdown spectroscopy. Dr. Trejos’ recent research focuses on the analysis of trace evidence materials, inks, and gunshot residues.

Dr. Trejos has authored over 35 peer-reviewed scientific publications and book chapters in the field of forensic chemistry and has presented over 140 oral presentations and posters at scientific meetings worldwide. Dr. Trejos is the recipient of the prestigious science and technology award “Clodomiro Picado Twight” from the Costa Rican National Academy of Sciences (2015). She has contributed to different scientific working groups, including the EU-funded NITECRIME group, the National Institute of Justice (NIJ)–funded Elemental Analysis Working Group, and the NIJ-funded Glass Interpretation Working Group. One of the most relevant achievements of these professional groups is the development of technically sound and consensus-based standards to improve forensic practice (e.g., American Society of Testing Materials standard methods). Tatiana was appointed by NIST to serve as a member of the Materials (Trace) Subcommittee within the Organization of Scientific Area Committees, where she currently serves as chair of the Glass Task Group, and member of the Interpretation, Research, and Physical Fit Groups.
Ling Wang

Ling Wang is a postdoctoral associate in Dr. Bruce McCord’s lab at Florida International University (FIU) in Miami, Florida, USA. Dr. Wang obtained both her master’s and doctorate chemistry, forensic science track, at FIU. Since her doctoral program, she has worked on seized drug analysis with chemosensors, biosensors, colorimetric reagents, surface-enhanced Raman spectroscopy (SERS), and electrochemistry. Presently, she is working on the development of new electrochemical sensors and platforms for opioid detection as well as a new project involving the use of microwave-based extraction methods for rapid DNA analysis. Dr. Wang has published four peer-reviewed papers and has presented her work in a variety of national and international venues including Pittcon, American Academy of Forensic Sciences, Florida American Chemical Society, and nanoFlorida.

Carl Wolf

Carl Wolf received his bachelor’s in chemistry from Gannon University, Erie, Pennsylvania in 1986, where he received the CRC Press’ Outstanding Freshman Chemist Award. Dr. Wolf received his master’s in criminal justice, with a forensic science option from Virginia Commonwealth University (VCU) in 1994, and received his doctorate in pathology, with a focus on forensic toxicology from the Medical College of Virginia (MCV) Campus of VCU in 2005.

Dr. Wolf has been employed at MCV Hospitals since 1987 in various roles in the Clinical and Forensic Toxicology Laboratories. Dr. Wolf regularly consults and/or lectures on toxicology and drug testing issues and has given expert testimony in the Commonwealth of Virginia and the State of North Carolina. Dr. Wolf has contributed to over 100 presentations and peer-reviewed publications. Dr. Wolf is a full member of the Society of Forensic Toxicologists. He is a Fellow of the American Board of Forensic Toxicologists (ABFT), and has been certified by ABFT since 2001. In 2007, Dr. Wolf was a member of the group that received an Educational Innovation Award from the School of Medicine at the MCV campus of VCU for developing and maintaining an online continuing education program for chronic nonmalignant pain management. In 2017, Dr. Wolf received a grant from the National Institute of Justice (NIJ) to study the matrix effects that liver tissue has on the analysis of opiates, and in 2018, he received a NIJ grant to study the stability of cannabinoids in marijuana-infused edibles.