National Institute of Justice

Forensic Science Research and Development Symposium

American Academy of Forensic Sciences
76th Annual Scientific Conference

February 20, 2024

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Forensic Technology
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STRENGTHEN SCIENCE. ADVANCE JUSTICE.

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.

Forensic Technology
CENTER OF EXCELLENCE

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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 almost 6,000 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.

RTI INTERNATIONAL

This forum is funded through a cooperative agreement from the NIJ (15PNIJ-21-GK-02192-MUMU), Office of Justice Programs, U.S. Department of Justice.

Greetings,

The National Institute of Justice (NIJ) and its Forensic Technology Center of Excellence (FTCOE) at RTI International welcome you to the 2024 NIJ Forensic Science Research and Development (R&D) Symposium. We are excited to be back as a hybrid event, both in person and virtual.

NIJ is dedicated to improving knowledge and understanding of crime and justice issues through science. As an element of this mission, NIJ sponsors research, development, evaluation, technology transition, and knowledge transfer to strengthen the forensic sciences. We have annually hosted a Forensic Science R&D Symposium at the American Academy of Forensic Sciences (AAFS) Scientific Meeting to highlight recent research by our grantees and promote the transition of this research into practice.

A day-long agenda of oral presentations is the core of the symposium; this year, the event also includes a blended poster session. The symposium is offered at no cost, and it includes an interactive question-and-answer platform as well as break time for networking. Every year, hundreds of attendees join us for a compelling discussion of the latest forensic science research. This year, the NIJ R&D program managers have assembled an interdisciplinary agenda and will serve as your moderators. The program features 16 oral presentations and 34 posters—representing the accomplishments of just a portion of NIJ's diverse R&D portfolio. The sessions include the following:

Session	Program Managers and Moderators
Session I–Physics and Pattern Interpretation/ Trace Evidence	Gregory Dutton, PhD
Session II–Forensic Anthropology and Forensic Pathology	Danielle McLeod-Henning, MFS
Session III–Seized Drugs and Toxicology	Frances Scott, PhD
Session IV–Forensic Biology/DNA	Tracey Johnson, MSFS

After the event, the presentations will be made available to view on demand at no cost. Whether you are experiencing the NIJ Forensic Science R&D Symposium for the first time or are a returning guest, we are pleased to have you—our fellow professionals in the criminal justice and forensic science communities—join us for this shared experience.

Respectfully,

Jeri D. Ropero-Miller, PhD, F-ABFT
Director
Forensic Technology Center of Excellence
Justice Practice Area, RTI International

Lucas Zarwell, MFS, D-ABFT-FT
Director
Office of Investigative and Forensic Sciences
National Institute of Justice

Directors

Jeri D. Ropero-Miller

Dr. Jeri D. Ropero-Miller, F-ABFT, is a Principal Scientist in the Justice Practice Area at RTI International. With expertise in the areas of forensic toxicology and criminal justice research, she has published on postmortem drug studies, emerging drugs, hair drug studies, drug surveillance and intelligence, program evaluation, and technology evaluation and adoption. She supports ongoing projects, including NIJ's FTCOE and its Criminal Justice Technology and Evaluation Consortium, the Bureau of Justice Statistics–funded 2022 Census of Medical Examiners/Coroners' Offices, and the 2023 Census of Publicly Funded Forensic Crime Laboratories. She is a certified Fellow in the American Board of Forensic Toxicology and currently serves on the Forensic Science Standards Board of the National Institute of Standards and Technology, Organization of Scientific Area Committees, and as the Treasurer of the Society of Forensic Toxicologists. She is the 2021–2022 Past President of the American Academy of Forensic Sciences and its 2022 Rolla N. Harger Award recipient for Career Excellence in Forensic Toxicology. 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.

Lucas Zarwell

Lucas Zarwell is the Director of the Office of Investigative and Forensic Sciences at NIJ and leads a team of dedicated scientists who work to facilitate research and implement new technologies nationwide. Prior to this position, Mr. Zarwell served as Chief Toxicologist for the District of Columbia Chief Medical Examiner, DC Pre-Trial Services Forensic Drug Testing Laboratory, and the Armed Forces Institute of Pathology Forensic Toxicology Laboratory. Mr. Zarwell maintains his certification from the American Board of Forensic Toxicology and has a master's in forensic science from The George Washington University. He currently co-chairs the Office of Justice Programs/Department of Health and Human Services Federal Interagency Medicolegal Death Investigation Working Group, which is hosted by NIJ.

NIJ Program Managers

Jillian Conte

Dr. Jillian Conte is a physical scientist in NIJ's Office of Investigative and Forensic Sciences. She has over 15 years of experience in the forensic science field where she has performed casework as a forensic biologist, taught countless undergraduate and graduate students, and held a role in industry. Jillian has earned a doctorate degree in cell and molecular biology (University of the Sciences, Philadelphia, PA), a master's degree in forensic science (Cedar Crest College, Allentown, PA), and a bachelor's degree in biology (Misericordia University, Dallas, PA). Jillian holds a certification from the American Board of Criminalistics in molecular biology and a graduate certificate in Six Sigma. She is a member of the DNA Consensus Body of the Academy Standards Board, International Association of Forensic Geneticists, American Academy of Forensic Sciences, and the Northeastern Association of Forensic Scientists.

Gregory Dutton

Dr. Gregory Dutton is a program manager at NIJ whose portfolios include Trace Evidence—material or chemical traces recovered from crime scenes—and Physics and Pattern Interpretation (e.g., friction ridge, firearms, and footwear). Greg also manages NIJ's Graduate Research Fellowship program, which supports students across all science and engineering fields whose work is relevant to criminal justice. Prior to joining NIJ, Greg was a postdoctoral fellow at the National Institute of Standards and Technology.

Tracey Johnson

Tracey Johnson is a program manager with NIJ's Office of Investigative and Forensic Sciences. She spent the first 12 years of her career at the Armed Forces DNA Identification Laboratory followed by 7 years as a Senior Scientist supporting human DNA identification development. She brings experience with traditional and challenged samples using autosomal STRs, Y-STRs, mitochondrial DNA, and SNP analysis to the position. In her current role, she oversees NIJ's forensic biology research and development portfolio, which seeks to advance technologies across the forensic biology spectrum. Tracey holds a master's degree in forensic science from Marshall University and a bachelor's degree in biology from the University of Arkansas.

Danielle McLeod-Henning

Danielle McLeod-Henning is a physical scientist at NIJ who manages research portfolios in forensic anthropology, forensic pathology, crime scene examination, and related medicolegal death investigation fields. She also facilitates the Forensic Laboratory Needs Technology Working Group, a working group to explore ways to increase casework efficiencies and implement forensic technology innovations within the crime laboratory. Danielle holds a master's degree in forensic science from The George Washington University and a bachelor's degree in anthropology from the Pennsylvania State University.

Frances Scott

Dr. Frances Scott is a program manager scientist at NIJ, where she manages the Seized Drugs and Forensic Toxicology research and development portfolios under the General Forensics portfolio and the Research for Publicly Funded Labs program. Frances received a bachelor of science in chemistry from the University of California at Davis and a doctorate in physical chemistry from The George Washington University.

Steering and Planning Committees

Steering Committee

National Institute of Justice, Washington, DC
Jillian Conte
Gregory Dutton
Danielle McLeod-Henning
Frances Scott
Tracey Johnson
Lucas Zarwell

Project Directors

RTI International, Research Triangle Park, NC
Jeri D. Ropero-Miller, PhD, F-ABFT

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National Institute of Justice, Washington, DC
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NATIONAL INSTITUTE OF JUSTICE
GRADUATE FELLOWSHIP

Apply for NIJ's Graduate Research Fellowship. By supporting outstanding graduate research, NIJ is expanding the future pool of young investigators pursuing research with the potential to provide solutions to issues that affect crime and the fair and impartial administration of criminal and juvenile justice in the United States.

Eligibility

Students must be (1) enrolled full time in a Ph.D. program in a science or engineering field and (2) propose dissertation research relevant to improving criminal or juvenile 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

- $40,500 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.

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

GRF is back in 2024!

There is a single solicitation for NIJ's GRF program in 2024. Eligible applicants in all science and engineering fields are invited to apply.

Disciplines include, but are not limited to:

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

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NATIONAL INSTITUTE OF JUSTICE
PUBLIC LABS RESEARCH SOLICITATION

Introduction

The Research and Evaluation for the Testing and Interpretation of Physical Evidence in Publicly Funded Forensic Laboratories (Public Labs) program's intent is to fund projects that direct the findings of research and evaluation toward the identification of the most efficient, accurate, reliable, and cost-effective methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

With this solicitation, the National Institute of Justice (NIJ) seeks applications for research and evaluation projects that will:

- Identify and inform the forensic community of best practices through the evaluation of existing laboratory protocols.
- Have a direct and immediate impact on laboratory efficiency and assist in making laboratory policy decisions.

Program Overview

The Public Labs program is specifically targeted toward applications from or in partnership with publicly funded laboratories and has the following objectives:

- Assess existing laboratory protocols to improve understanding of the rationales underpinning the processes.
- Evaluate emerging laboratory methods to assess their value.

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Eligibility

NIJ welcomes practitioner-researcher partnerships through this program. Applicants must be, or be partnered with, publicly funded forensic science laboratories that are currently accredited by an independent accrediting or certifying forensic science organization. Publicly funded forensic science laboratories include state, regional, county, municipal, and tribal agencies. For detailed eligibility information, please refer to the solicitation document.

Researcher-Practitioner Collaboration

To facilitate researcher-practitioner collaboration, we are calling on public laboratories to submit contact information to foster collaboration and/or partnerships to assist in preparation of robust research proposals.

Universities and other research organizations are encouraged to use the provided contact information as a way to establish collaboration with the practicing lab as required by the solicitation.

Learn more at NIJ.ojp.gov/topics/forensics/connecting-researchers-forensic-laboratories

Apply Now!

https://nij.ojp.gov/funding/opportunities/nij-fy24-public-labs

Deadline: 2024

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February 2024

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National Institute of Justice

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NATIONAL INSTITUTE OF JUSTICE
FORENSIC SCIENCE R&D GRANTS

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:

- **Foundational/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 policy 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.

NIJ is interested in funding innovative research that aligns with administration priorities and attempts to address the needs of the forensic science community.

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.

Begin the application process early by registering with Grants.gov. Read the solicitation at nij.ojp.gov/funding/opportunities/o-nij-2023-171606.

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NATIONAL INSTITUTE OF JUSTICE

Forensic Science Strategic Research Plan

2022-2026

NIJ developed the Forensic Science Strategic Research Plan to communicate its research agenda and advance its forensic science research mission. The strategic priorities and objectives outlined in this plan closely parallel the opportunities and challenges faced by the forensic science community.

NIJ sponsors research, development, and evaluation to bring innovation to forensic science, better understand the limits of current forensic methods, and inform forensic science policy and practice. NIJ has identified five strategic research priorities for the Forensic Science Strategic Research Plan.

I. Advance Applied Research and Development in Forensic Science

NIJ supports applied research and development that aids the forensic science community through the development of methods, processes, devices, and materials. Applied research and development may result in improved procedures or otherwise resolve current barriers.

II. Support Foundational Research in Forensic Science

NIJ supports research to assess the fundamental scientific basis of forensic analysis. If forensic methods are demonstrated to be valid and the limits of those methods are well understood, then investigators, prosecutors, courts, and juries can make well-informed decisions. This can exclude the innocent from investigation and help prevent wrongful convictions.

To read the entire NIJ Forensic Science Strategic Research Plan, visit
ojp.gov/pdffiles1/nij/304856.pdf

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III. Maximize the Impact of Forensic Science Research and Development

The ultimate goal of NIJ's research and development is to make a positive impact on forensic science practice. For this to happen, the products of research and development must reach the community. These products include peer-reviewed publications, presentations, databases, patents and inventions, software, best practice guides, and more. Implementation of new technology and methods into practice can be aided by NIJ stewardship, in partnership with researchers and practitioners.

IV. Cultivate a Diverse, Highly Skilled Forensic Science Workforce

NIJ supports the development of current and future forensic science researchers and practitioners through laboratory and research experience. Student engagement and the promotion of diversity, equity, and inclusion within the forensic science workforce are critical elements of this effort. NIJ advocates for representation of a diverse range of perspectives in the forensic science community.

V. Coordinate Across the Community of Practice

The forensic science enterprise benefits from collaboration across academic, industry, and government sectors. NIJ serves as a coordination point within the forensic science community to help meet the challenges caused by high demand and limited resources.

AGENDA

At a Glance

February 20: 8:30 a.m.–6:30 p.m. Mountain Standard Time (MST)

MORNING SESSIONS

8:30–8:40	Welcome and Opening Remarks
8:40–10:20	Session I—Physics and Pattern Interpretation/Trace Evidence
10:20–10:35	BREAK
10:35–12:15	Session II—Forensic Anthropology and Forensic Pathology
12:15–1:25	LUNCH BREAK

AFTERNOON SESSIONS

1:25–3:05	Session III—Seized Drugs and Toxicology
3:05–3:20	BREAK
3:20–5:00	Session IV—Forensic Biology/DNA
5:00–6:30	Poster Session

Detailed Agenda

MORNING SESSIONS: 8:40 a.m.–12:15 p.m. MST

8:40–10:20	**SESSION I—Physics and Pattern Interpretation/Trace Evidence** Moderated by NIJ Program Manager Gregory Dutton
8:40–9:05	**Development of Nationwide Reference Population Distributions for Statistically Supported and Objective Testimony in Firearm Evidence Comparisons** Xiaoyu Alan Zheng, National Institute of Standards and Technology
9:05–9:30	**Assessing the Strength of Trace Evidence Fracture Fits Through a Comprehensive, Systematic, and Quantifiable Approach** Tatiana Trejos, West Virginia University
9:30–9:55	**Universal Method for the Detection of Organic and Inorganic Gunshot Residue Based on Fast Fluorescence Mapping and Raman Spectroscopic Identification** Igor K. Lednev, University at Albany, State University of New York
9:55–10:20	**Comprehensive Assessment of Novel Reference Materials and Analytical Methods for the Analysis and Interpretation of Organic and Inorganic Gunshot Residues** Luis Arroyo, West Virginia University
10:20–10:35	**BREAK**

10:35–12:15	**SESSION II—Forensic Anthropology and Forensic Pathology** Moderated by NIJ Program Manager Danielle McLeod-Henning
10:35–11:00	**Pre-grouping of Commingled Human Skeletal Remains by Elemental Analysis** Matthieu Baudelet, University of Central Florida
11:00–11:25	**Potential Postmortem Microbial Biomarkers of Infant Death Investigation** Jennifer Pechal, Michigan State University
11:25–11:50	**Forensic Tool to Identify Fall Characteristics in Infant Skull Fracture** Brittany Coats, University of Utah
11:50–12:15	**Determining Fracture Timing from Microscopic Characteristics of Cortical Bone** Jessica Skinner and Natalie Langley, Mayo Clinic Arizona
12:15–1:25	**LUNCH BREAK**

AFTERNOON SESSIONS: 1:25 p.m.–5:00 p.m. MST

1:25–3:05	**SESSION III—Seized Drugs and Toxicology** Moderated by NIJ Program Manager Frances Scott
1:25–1:50	**Non-contact Detection of Fentanyl and Other Synthetic Opioids: Toward a Generalized Approach to the Detection of Dangerous Drug Classes** Lauryn E. DeGreeff, Florida International University
1:50–2:15	**Expert Algorithm for Substance Identification Applied to the Tandem Mass Spectra of Seized Drugs** Glen Jackson, West Virginia University
2:15–2:40	**Retinal Cannabinoids: Measures of Function and Impairment** Denise Valenti, Impairment Measurement Marijuana and Driving (IMMAD)
2:40–3:05	**Prevalence of Fentanyl and Its Analogues in a Court-Ordered Mandatory Drug Testing Population** Katherine Bollinger, RTI International
3:05–3:20	**BREAK**

3:20–5:00	**SESSION IV—Forensic Biology/DNA** Moderated by NIJ Program Manager Tracey Johnson
3:20–3:45	**Improved Nucleic Acid Recovery from Trace and Degraded Samples Using Affinity Purification** Arati Iyengar, West Virginia University
3:45–4:10	**Forensic STR Sequencing Nomenclature Resource** Katherine B. Gettings, National Institute of Standards and Technology
4:10–4:35	**Comparative Evaluation of Massively Parallel Sequencing STR Kits with the Emphasis on Mixture Deconvolution Utilizing Probabilistic Genotyping** Elisa Wurmbach, New York City Office of the Chief Medical Examiner
4:35–5:00	**Comparative Assessment of Emerging Technologies for Body Fluid Identification** Mirna S. Ghemrawi, Center for Forensic Science Research and Education

5:00–6:30	**Poster Session**

SESSION ABSTRACTS

An asterisk (*) denotes the presenter for presentations that have multiple authors.

MORNING ABSTRACTS

SESSION I—Physics and Pattern Interpretation/Trace Evidence

Moderated by NIJ Program Manager Gregory Dutton

Development of Nationwide Reference Population Distributions for Statistically Supported and Objective Testimony in Firearm Evidence Comparisons

NIJ Award: 2016-DNR-6257-3

Xiaoyu Alan Zheng* and Johannes Soons | National Institute of Standards and Technology

Abstract: Application and validation of the National Institute of Standards and Technology (NIST) statistical framework requires the use of relevant population distributions. A population distribution describes the frequency distributions of a similarity score for same-source and different-source comparisons. Like DNA analysis, these distributions are required to establish a statistical foundation for the estimation of identification confidence limits and false positive error rates. NIST sampled four specific firearm manufacturers (Ruger, Glock, S&W, and Sig Sauer) with 100+ firearms from each to test the systematic error rates associated with the developed protocols. Each firearm was used to test fire a minimum of two test fires using Remington UMC 9 mm ammunition with brass cases and nickel primers. For each population of firearms, all available known matching scores and 10,000 known non-matching scores were calculated. These were used to establish the statistical distributions for further analysis of cumulative false positive and cumulative false negative error rates. These error rates describe the systematic error rate of the NIST analysis protocols. The results show low false positive and false negative error rates using the NIST analysis protocols across all four populations. The research also demonstrates opportunities to generalize the reference population through statistical methods such as the score-based likelihood ratio.

Assessing the Strength of Trace Evidence Fracture Fits Through a Comprehensive, Systematic, and Quantifiable Approach

NIJ Award: 2020-DQ-BX-0012

Tatiana Trejos,* Zachary Andrews, Meghan Prusinowski, and Aldo Romero | West Virginia University
Cedric Neumann | Battelle Memorial Institute

Abstract: Criminal activities, such as sexual assaults, kidnappings, and homicides, often lead to fractured materials. The realignment between fragments left at the scene and items recovered from an individual or object of interest could become crucial evidence during an investigation. These fracture fits are often regarded as the highest degree of association of trace materials because of the common belief that fracture edges produce individualizing patterns; there is a need to demonstrate the scientific validity of this assumption. Currently, the examination of fractured edges involves the subjective judgment of the examiner without consensus-based standard methodologies for the identification of distinctive features, a systematic criterion for informing a fit/non-fit decision, or methods for assessing the weight of the evidence. To help reduce these gaps, the overall goal of this research was to develop an effective and

practical approach that provides an empirically demonstrable basis to assess the significance of trace evidence fracture fits. In particular, the goals were to develop a systematic method for the comparison of fracture fits of common trace materials such as duct tapes, textiles, and automotive plastics; develop a relevant extensive database of nearly 9,000 samples to evaluate performance rates in this field and assess the probative value of a fracture fit using similarity metrics and score likelihood ratios; and evaluate the utility and reliability of the proposed approach and establish consistency base rates through interlaboratory studies. Partnerships among experienced forensic researchers, computational material science physicists, statisticians, and practitioners were crucial to develop strategies to facilitate the future adoption of the developed approaches within crime laboratories. This study identified material-specific relevant features for duct tapes, textiles, and automotive polymers and developed reporting templates to facilitate thorough and systematic documentation of an analyst's decision-making process and minimize risks of bias. It also established criteria for using quantitative metrics, such as the edge similarity score (ESS) that estimates the quality of a fit and the feature prominence score (FPS) that captures the relative features' importance in each comparison. The method yielded relatively high accuracy (85% to 100%). The auto-populated cell options in the reporting template are provided to characterize the influence of the feature on a decision and, together with the ESS and FPS, offer a means to assess the similarity between two given edges and standardized criteria to support their decision. The method demonstrates that most true non-fit pairs receive low ESS (0%–20%) and low FPS (< –5). True fit pairs generally receive high ESS (80%–100%) and high FPS (> +15). This research specifically addressed several research needs in the field (i.e., quantitative assessment of error rates, scientific foundations, standardization, validation, interpretation, casework review, and proficiency assessment). As a result, this study is anticipated to transform current trace evidence practice by providing—for the first time—harmonized examination protocols and decision thresholds, effective mechanisms to ensure transparent and systematic peer-review process and interlaboratory testing, and a quantitative basis that together substantiate the evidential value of fracture fit conclusions.

Universal Method for the Detection of Organic and Inorganic Gunshot Residue Based on Fast Fluorescence Mapping and Raman Spectroscopic Identification

NIJ Award: 15PNIJ-21-GG-04153-RESS

Igor K. Lednev | University at Albany, State University of New York

Abstract: Gunshot residue (GSR) is an important type of forensic trace evidence produced when a firearm is discharged. GSR can be subdivided into two sub-classifications—organic (OGSR) and inorganic (IGSR). Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, also known as SEM-EDS or SEM-EDX, is used to detect and identify GSR particles. The application of this two-step method is limited to IGSR because it relies solely on the detection of heavy metals (lead, barium, and antimony). This is problematic because environmental concerns have led to an increased popularity in heavy metal–free or "green" ammunition. It has been found that in the absence of heavy metals, current elemental analysis techniques are severely hindered when attempting to identify GSR samples accurately. Additionally, the probability of environmental and manufacturing particles being incorrectly assigned as GSR has increased with the onset of green ammunition. OGSR has recently been the focus of many forensic researchers for several reasons. First, the total amount of OGSR generated because of the discharge of a firearm is much larger than the amount of IGSR. Second, OGSR particles are typically much larger than IGSR particles. In addition, the chemical composition of OGSR is quite complex and includes partially burned and unburned smokeless powder, stabilizers, and plasticizers. As a result, it is easier to detect and identify OGSR particles, although new methods are required. This laboratory has developed a new two-step approach

for fast OGSR particle detection using fluorescence spectroscopy followed by a confirmatory identification by Raman microspectroscopy. The method uses a single instrument that combines a confocal scanning Raman and a fluorescence microscope working in reflection mode. In the first proof-of-concept study, the presenter used adhesive tape to collect OGSR particles. Most recently, the presenter significantly expanded this emerging methodology by demonstrating the possibility of detecting and identifying GSR particles on original common substrates (e.g., cotton fabric), eliminating the initial GSR particle transfer stage. The presenter will show the results of these recent studies and then discuss challenges and future steps to develop the proposed two-step method for the detection and confirmatory identification of both OGSR and IGSR particles. In addition, the presenter will discuss the preliminary results of using a portable Raman instrument to detect and identify GSR. The latter approach opens the possibility of bringing the technology to the crime scene.

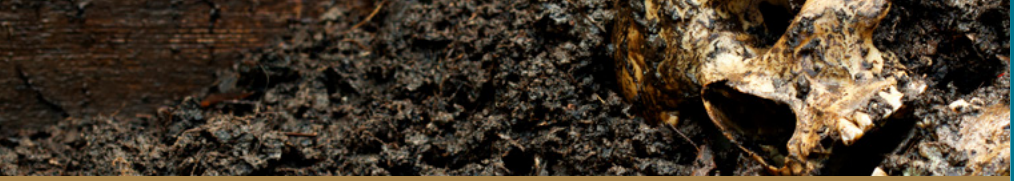
Comprehensive Assessment of Novel Reference Materials and Analytical Methods for the Analysis and Interpretation of Organic and Inorganic Gunshot Residues

NIJ Award: 2020-DQ-BX-0010

Luis E. Arroyo,* Tatiana Trejos, Kourtney Dalzell, Thomas Ledergerber, Leah Thomas, and Madison Lindung | West Virginia University
Matt Staymates | National Institute of Standards and Technology

Abstract: Increased gun violence requires an immediate reaction from the criminal justice system to manage workloads and adapt operations. Thus, technological advances that lead to the accurate reconstruction of events, prompt apprehensions, and meaningful data sharing are critical for timely justice and increased public safety. Identifying traces of gunshot residue (GSR) is one of the forensic services of great interest in these investigations. Nonetheless, essential information to make informed decisions about recovery at the crime scene—while safeguarding the integrity of the evidence and evaluating the evidence under competing propositions—is still needed. The complex nature of GSR transfer and persistence introduces challenges and skepticism in its evidential value. For instance, GSR can be transferred in different ways: direct transfer (primary) or indirect transfer (i.e., secondary, tertiary, or quaternary), opening the question in the courtroom about the presence of GSR on a person of interest because of firing a gun or indirect exposure. This is a question most forensic practitioners cannot answer, yet it is of primary interest to the trier of fact. Thus, this study aims to provide solutions to those needs by enhancing current capacity through technology innovation and increasing knowledge of GSR transfer and persistence. The study addresses a primary demand to include organic constituents in the workflow for increased confidence in the results. The main goal is to establish scientific foundations for best practices for the collective recovery, preservation, storage, analysis, and interpretation of inorganic GSR (IGSR) and organic GSR (OGSR). In the first part of this presentation, the researcher will present findings on the behavior and movement of IGSR and OGSR to assist in evidence interpretation using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The study encompassed over 650 samples, including 247 collections from human skin after firing a gun and 405 synthetic skin and fabric substrates after depositing a characterized IGSR/OGSR standard. Transfer and persistence experiments were evaluated on different substrates (hands, ears, nostrils, forehead, hair, fabrics, and synthetic skin) at different times after firing (0 to 6 hours) and common post-shooting activities (rubbing hands, handshaking, running, washing hands and fabrics). Ground truth knowledge of particle counts and analyte concentrations were used to calculate the recovery for inorganic and organic constituents from clothing and a synthetic skin membrane (StratM®). In the second part of the

presentation, the researcher will discuss the preliminary results of GSR deposition in enclosed environments and evaluate GSR exposure risks on bystanders and passersby. This study uses high-speed and standard videography and laser sheet scattering to investigate visual information about the flow of GSR under various controlled experimental conditions. Also, cost-effective atmospheric samplers and particle counting systems are used to evaluate particle concentrations and distributions in three locations: a shooter, a bystander, and a passerby entering the scene after 10 minutes. The laser-based visualization methods, airborne particle analyzers, and analytical methods offer novel advances to aid in the interpretation of IGSR/OGSR deposition dynamics and exposure risks for non-shooters.



SESSION II—Forensic Anthropology and Forensic Pathology

Moderated by NIJ Program Manager Danielle McLeod-Henning

Pre-grouping of Commingled Human Skeletal Remains by Elemental Analysis

NIJ Award: 15PNIJ-21-GG-04151-SLFO

Matthieu Baudelet* | University of Central Florida
Katie Zejdlik-Passalacqua | Western Carolina University
Jonathan Bethard | University of South Florida

Abstract: Although forensic anthropology has developed quantitative tools to recover, analyze, and reassociate individuals in mass graves, challenges still exist for small bones, fragments, or bones that underwent taphonomic changes. When considering the tediousness of the process, additional approaches for sorting skeletal remains in large, mixed assemblages could be a great resource for the community. Several studies have shown that chemical and physical variation exists between individuals' bones because of several factors, such as diet, health, and living environment. As such, chemical analysis of the osseous remains in a mass grave could help anthropologists tackle some of these challenges. To be helpful, this chemical analysis needs to be affordable, approachable, and available outside of research facilities or field-deployable, if possible. In recent decades, portable spectroscopic instruments have seen an increase in use across the field of forensic analysis, from molecular (e.g., Raman, infrared, nuclear magnetic resonance, mass spectrometry) to elemental analysis (e.g., laser-induced breakdown spectroscopy [LIBS] and X-ray fluorescence spectroscopy). Elemental analysis has shown promising results in anthropology, providing information beyond the bone matrix and giving insights on the trace elements profile for each individual. Although X-ray fluorescence has been a strong focus of research, LIBS is a complementary technique that provides additional elemental information while also being field-deployable, easy to use, and visually non-destructive. This National Institute of Justice–funded study explores how the chemical profile of bones may be incorporated into a method for classifying commingled remains using LIBS. The remains of 45 individuals have been analyzed by LIBS after decomposition at the Forensic Osteology Research Station at Western Carolina University. Using data reduction to determine the elemental profile needed for reassociation, supervised discriminant analysis was used to reassociate elemental profiles to their individuals with accuracies reaching 90% to 100%. This presentation will discuss the current challenges in using elemental profiles for reassociation and new paths for better results in chemical reassociation of remains. Recommendations on how to build collaborative approaches between anthropologists and chemists to improve reassociation of commingled remains will also be discussed.

Potential Postmortem Microbial Biomarkers of Infant Death Investigation

NIJ Award: 2020-75-CX-0012

Jennifer Pechal,* Bethany Mikles, and M. Eric Benbow | Michigan State University
Heather R. Jordan | Mississippi State University
Carl J. Schmidt | University of Michigan

Abstract: The field of forensic science is witnessing a paradigm shift with the emergence of postmortem microbial biomarkers as potential tools in death investigation. This work delves into the practicality of integrating microbial signatures into forensic protocols to enhance the accuracy and reliability of postmortem analyses. This research focuses on elucidating the dynamic interactions between the infant microbiome and decomposition processes, aiming to establish microbial biomarkers as robust indicators of the postmortem interval and other critical factors in death investigation. The researchers conducted a postmortem microbiota survey from a Midwest medical examiner's office to assess the viability of microbial signatures as reliable indicators in a forensic context. To provide robust, variable data, postmortem microbiota were collected from approximately 50 Black and White infants of both sexes and included deaths that were classified as an accident, a homicide, or from natural or unknown causes. Nine individual body sites were targeted for a composite analysis: eyes, ears, nose, mouth, umbilicus, brain, rectum, and cardiac blood. Determining genetic signatures via targeted 16S rRNA sequence analyses and whole genome sequencing will test the utility of the postmortem microbiome to help discern cause and manner of death in infants, especially in cases where no cause of death is apparent. The study findings indicate that the infant postmortem microbiome composition variability was structured by body site, offering valuable insights into the persistence of microbial community structure after death. Microbial community composition appears to correlate with specific circumstances surrounding death. For example, natural and control (e.g., co-sleeping) deaths are highly similar in composition. The practical application of postmortem microbial biomarkers is showcased through case studies and experimental models. By leveraging high-throughput sequencing technologies and advanced bioinformatics tools, the researchers demonstrate the potential for microbial profiling to serve as a supplementary method for forensic practitioners. The non-invasive nature of microbial sampling, coupled with the ability to analyze samples from diverse postmortem environments, underscores the adaptability and practicality of this approach in routine death investigation. Despite promising advances, challenges persist in standardizing protocols, interpreting results, and addressing potential confounding variables. This work highlights the ongoing efforts to establish a robust framework for integrating microbial biomarkers into routine forensic analyses. By aiding in the creation of standardized, best practice recommendations for the analysis of microbiomes in routine case work, the value they add to these cases is highlighted. The presenter discusses the necessity for interdisciplinary collaboration between microbiologists, forensic scientists, and legal professionals to ensure the seamless incorporation of microbial data into the forensic workflow. In conclusion, this research emphasizes the practicality of postmortem microbial biomarkers as valuable tools in death investigation. The integration of microbial signatures has the potential to revolutionize forensic science, providing forensic practitioners with additional reliable information to enhance the accuracy and circumstantial analysis of infant deaths. It is conceivable that an individual microbiome profile will have diagnostic significance once more data are obtained. As this field continues to evolve, collaborative efforts are essential to refine methodologies and establish standardized practices for the routine implementation of microbial biomarkers in death investigation.

Forensic Tool to Identify Fall Characteristics in Infant Skull Fracture

NIJ Award: 2020-75-CX-0014

Brittany Coats,* Yousef Alsanea, Jacob Hirst, Tagrid Ruiz, and Ashley Spear | University of Utah

Abstract: Early identification of child maltreatment is critical to the prevention of adverse outcomes, but child abuse in young infants (<1 year of age) is still highly under-detected. Skull fractures are common in both accidental and abusive head trauma and provide a unique opportunity to assess the validity of caretaker histories of infant trauma based on fracture initiation sites, fracture lengths, and level of complexity. Skull thickness distribution influences skull fracture patterns, but the effect of age and biological sex in early development on skull thickness distribution has not been reported in detail. This study aimed to develop an imaging pipeline for high-resolution, 3D maps of skull thickness to compare distributions between male and female infants 0–12 months of age and identify appropriate age divisions for sex-based anatomical templates for fracture simulations. Institutional Review Board approval was obtained to review computed tomography images of 281 healthy infant skulls (<12 months of age) from Primary Children's Hospital. Serial stacks of axial, coronal, and sagittal images were segmented and aligned in the 3D space. Identification of suture location and thickness extraction at more than 12,000 sites across the infant skull was performed using custom scripts. To compare skull thicknesses at similar relative locations, despite differences in head shape and size, one subject within predetermined age groups (0–4, 5–8, 9–12 months) was selected as a template. The skull distribution in each subject was then fit to their respective template using an iterative closest point algorithm. Thickness was averaged across 132 discrete regions representing approximately 10–50 locations in four cranial bones (right and left parietal, occipital, and frontal bones). Classification optimization was used to identify natural age divisions for each sex. The effect of age and sex was evaluated for each cranial bone using a two-way ANOVA with repeated measures, correlations of thickness with age by sex, and one-way ANOVAs that controlled for location. Classification analysis indicated five age groups (0–2, 2–5.5, 5.5–8, 8–10, 10–12 months) were optimal within early development of an infant skull. Frontal bones were significantly thicker than all other bones for all age groups and sexes ($p<0.0001$). Female infants had thicker skulls than males between 0–2 months of age ($p<0.03$), but male infants had thicker skulls at all subsequent age groups ($p<0.02$), which was more pronounced with increasing age. The rate of skull thickness growth was continuous for parietal bones, but occipital and frontal bones had periods where growth was temporarily stalled. Symmetry between the left and right parietal bones was moderate at young ages (0–5.5 months) and increased with age. The high-resolution imaging pipeline and skull thickness comparison in this study illustrate distinct changes in skull thickness with age that are dependent on biological sex and cranial bone. This suggests unique skull geometry templates are needed to represent male and female infants at five stages of development in the first year of life to predict skull fracture patterns from head impact accurately.

Determining Fracture Timing from Microscopic Characteristics of Cortical Bone

NIJ Award: 2020-75-CX-0015

Jessica L. Skinner,* Natalie R. Langley,* and Samuel Fahrenholtz | Mayo Clinic Arizona
Yuktha Shanavas | State University of New York Upstate Medical University
Brian Waletzki | Mayo Clinic Rochester
Robert Brown and James Herrick | MilliporeSigma
Peter Goguen, Loukham Shyamsunder, and Subramaniam Rajan | Arizona State University

Abstract: This study investigated whether scanning electron microscopy (SEM) is effective for assessing microscopic surface characteristics of experimentally induced fractures in human bone at various postmortem intervals (PMIs). The researchers hypothesized that microscopic fracture characteristics, including delamination, osteon pullout, and microfractures, may vary as bone elasticity decreases, elucidating perimortem and postmortem events more reliably than macroscopic analyses. Thirty-seven unembalmed, defleshed human femoral shafts from male (n=18) and female (n=2) donors aged 33 to 81 years were fractured at experimentally induced PMIs ranging from 1 to 60 warm weather days (250–40,600 accumulated degree hours, or ADH). Temperature and humidity were controlled using a gravity convection oven to simulate PMIs. The bones were fractured with a drop test frame using a three-point bending set-up. Sensors were used to calculate fracture energy, and high-speed photography was used to document fracture events. SEM micrographs were collected from the primary tension zones of each fracture surface. A region of interest was defined within the center of the primary tension zone, and three microscopic fracture characteristics were scored: percentage of delaminated osteons, percentage of osteon pullout, and number of microfractures. The following variables were recorded for each sample: PMI length in ADH, temperature, humidity, collagen percentage, water loss, fracture energy, age, sex, cause of death, and microscopic fracture feature scores. Bone mineral density (BMD) and cortical bone thickness (CBT) were calculated from computed tomography scans of the bones using regions of interest placed at 90° intervals around a cross-section of the shaft. Multiple linear regression showed that osteon pullout, delamination, and microfractures are strong predictors of ADH (adjusted R^2=0.90, F-statistic=95.29 on 3 and 33 DF, $p<0.001$), BMD (adjusted R^2=0.72, F-statistic=32.99 on 3 and 33 DF, $p<0.001$), CBT (adjusted R^2=0.70, F-statistic=28.52 on 3 and 33 DF, $p<0.001$), and water loss (adjusted R^2=0.71, F-statistic=26.23 on 3 and 33 DF, $p<0.001$) but weak predictors of collagen percentage (adjusted R^2=0.10, F-statistic=2.31 on 3 and 33 DF, p=0.09). Although BMD, CBT, and water loss play significant roles in microscopic fracture appearance, collagen percentage does not. This may be because the collagen has not begun to degrade significantly prior to 40,600 ADH. Nonetheless, despite collagen retention, postmortem water loss affects elasticity considerably. The hypothesis that microscopic fracture surface characteristics visible on SEM are more predictive of fracture timing than macromorphological characteristics was accepted. Microscopic fracture surface analysis detects the biomechanical effects of decreased elasticity more reliably and with greater sensitivity than macroscopic analysis. In conclusion, SEM analysis of bone fracture surfaces is a promising technique for distinguishing perimortem and postmortem fracture events.

SESSION III—Seized Drugs and Toxicology

Moderated by NIJ Program Manager Frances Scott

Non-contact Detection of Fentanyl and Other Synthetic Opioids: Toward a Generalized Approach to the Detection of Dangerous Drug Classes

NIJ Award: 15PNIJ-22-GG-04418-RESS

Lauryn DeGreeff* and Thouli Jayawardana | Florida International University
Ashley Fulton, Braden Giordano, and Stephanie Vaughan | U.S. Naval Research Laboratory

Abstract: Field-portable detection of fentanyl has become increasingly imperative in recent years. The opioid epidemic is at its deadliest with the increased fentanyl adulteration of commonly used drugs. Currently, the recommendation for preventative exposure of first responders is to wait for trained personnel to handle suspected scenes. This method is time-consuming and costly. The handheld ion mobility spectrometer (IMS) offers a quick and user-friendly method for the presumptive detection of fentanyl. Through headspace analysis using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS), N-phenylpropanamide (NPPA) was identified as a target analyte in the vapor identification of fentanyl. A method was developed on the handheld IMS using NPPA as the surrogate vapor and successfully identified clandestine samples containing >6% fentanyl. However, the method was unsuccessful at lower or unknown concentrations. To improve sensitivity metrics, a functionalized silicon nanowire (SiNW) array for the pre-concentration of vaporous compounds was developed. Acrylate-based polymers were screened to determine pre-concentration efficiency using a quartz crystal microbalance. The optimal polymer was selected and deposited onto the SiNW array and incorporated into a miniaturized pre-concentrator for mobile devices. This presentation describes the studies leading up to and including the incorporation of the SiNW array pre-concentrator to the handheld IMS. The SiNW-IMS system was able to accurately identify the target analyte, NPPA, at trace levels. Heavily diluted samples with concentrations less than 5% fentanyl were also accurately identified. Limit of detection studies and testing of street-grade samples are currently being conducted.

Expert Algorithm for Substance Identification Applied to the Tandem Mass Spectra of Seized Drugs

NIJ Award: 15PNIJ-21-GG-04179-COAP

Glen P. Jackson,* Alex Adeoye, and Emily Ruiz | West Virginia University
J. Tyler Davidson | Sam Houston State University

Abstract: In previous work, this research group has demonstrated that the expert algorithm for substance identification (EASI) can be used to model and explain more than 90% of the variance in the branching ratios in replicate electron-ionization mass spectra of cocaine, fentanyl analogues, and cathinones. Ongoing work has also included a foundational relationship with unimolecular reaction (fragmentation) rate theory and is most suitable for distinguishing substances from their spectrally similar analogues. EASI provides superior binary classification rates than existing algorithms for electron-ionization mass spectrometry (EI-MS) data. Here,

the algorithm is extended to tandem mass spectra obtained from protonated molecular ions, such as from electrospray ionization (ESI) and direct analysis in real time (DART). In one example, replicate DART-MS/MS spectra were collected for tetrahydrocannabinol (THC) and cannabidiol (CBD) on a triple quadrupole MS at three different collision energies. At each energy, the tandem mass spectra for CBD and THC are visually indistinguishable, so manual classification rates are no better than a coin flip at 50%. After splitting the data into a training set and test set, conventional algorithms that use a consensus spectrum as the exemplar at each collision energy provide classification accuracies of 61%–90%, depending on the collision energy. In contrast, EASI applied to the same dataset provides classification accuracies of 86%–96%. EASI classification rates are also superior to binary classification using the Mahalanobis of each spectrum relative to the training set. Applying EASI to replicate tandem mass spectra from ESI-MS/MS instruments provides superior levels of discrimination, with binary classification rates typically exceeding 99% for structurally related opioids.

Retinal Cannabinoids: Measures of Function and Impairment

NIJ Award: 15PNIJ-22-GG-04417-RESS

Denise A. Valenti* | Impairment Measurement Marijuana and Driving (IMMAD) LLC
David Calixte, Trevor Kopp, and Rhoda Nankabirwa | University of Massachusetts Boston
Xueling Zou | New England College of Optometry
Holly Kailher | University of Rhode Island

Abstract: There is no direct relationship between measures of cannabinoids in fluids and impairment. There needs to be both a measure of presence of cannabinoids and a separate measure of impairment related to the ability to safely drive a motor vehicle or perform tasks. Measures of impairment need to be objective, free of racial bias, and easily performed on the roadside by law enforcement professionals or in the workplace. Saliva is an efficient means to detect cannabinoids; however, the researchers propose a simple retina test of impairment. This research demonstrates the efficacy of using visual retinal dysfunction as an indicator of impairment with cannabis use. Retinal function was assessed using a PicoNeo3 virtual goggle with eye tracking to measure the functional responses related to contrast and temporal processing. The technology has backlit-striped, 10°-sized squares of variable contrast flashed on a fixed, flat virtual reality (VR) screen. The stripes are of low spatial frequencies that undergo counterphase flickering at a high temporal frequency. The contrasts of the squares are variable. Squares are flashed in 19 locations. The presenter is reporting retinal results from one participant, a chronic cannabis user. The researchers collected multiple variables, including the Fitzpatrick Pigment Scale as an additional variable related to race and genetics. There is a substantial difference in retinal pigmentation across different populations, and it is essential that any technology used by law enforcement is not impacted by such variables. The researchers used a validated cannabis use questionnaire to determine categories of marijuana use: early initiators, chronic users, and casual users. Saliva and blood were taken each visit. This study identified cannabinoids, Δ9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-carboxy-THC in the blood of users, and the amount at both baseline and with acute use was related to frequency of use. The volume of cannabinoids detected after consumption was considerably greater and had a relationship to the post-consumption blood sample. The researchers evaluated the retina before dosing and after dosing. The retinal findings were related to the presence of blood cannabinoids at baseline and after dosing. The use of retinal response as a measure of actual impairment from cannabis use is promising, and further research will provide insight into how the retinal response along with the blood or other biomarkers of THC can be used to further differentiate the impairment and differences of chronic heavy recreational users, medicinal users, and casual recreational cannabis users.

Prevalence of Fentanyl and Its Analogues in a Court-Ordered Mandatory Drug Testing Population

NIJ Award: 2019-R2-CX-0017

Katherine Bollinger,* Megan Grabenauer, Nichole D. Bynum, Jeri D. Ropero-Miller, Nicolas Richardson, and David Heller | RTI International

Abstract: The prevalence of fentanyl and its analogues in the criminal justice system is relatively unknown, although drug testing is frequently conducted in correctional settings. Court-ordered mandatory drug testing (COMDT) using hair is routinely done at large commercial laboratories but does not include testing for fentanyl and its analogues. Limited information is available on prevalence data in the COMDT population to characterize drug use patterns. Phase I of this study analyzed over 400 hair specimens for fentanyl; a selection of fentanyl analogues; and other drugs such as cocaine, methamphetamine, and codeine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). These hair samples were submitted from a COMDT laboratory and previously analyzed at their laboratory from November 2020 through February 2021. Any hair specimens that were positive for opioids on the LC-MS/MS were also analyzed by non-targeted high-resolution mass spectrometry. Hair specimen positivity rates in COMDT were calculated with and without inclusion of fentanyl and fentanyl-related compounds to determine the effect on overall positivity rate when fentanyl targets were included in the hair-testing protocol. Phase II of this study was a retrospective analysis of 5 years of COMDT data from oral fluid and hair collected from 2015 to 2019 in nationally represented COMDT programs. A random, national COMDT sampling of 959,237 oral fluid test results and 65,645 hair test results was analyzed. Specimens in the historical dataset were tested for misused substances by screening with immunoassay and confirmatory testing was performed on a subset of oral fluid and all hair positive specimens. The prevalence of positive drug tests among different demographic groups of the analysis pool and the positivity rates of oral fluid confirmation with and without fentanyl were calculated.

SESSION IV—Forensic Biology/DNA

Moderated by NIJ Program Manager Tracey Johnson

Improved Nucleic Acid Recovery from Trace and Degraded Samples Using Affinity Purification

NIJ Award: 15PNIJ-21-GG-04149-RESS

Arati Iyengar* and Coral Smith | West Virginia University
Michael Rishel, Chrystal Chadwick, John Nelson, and Brian Davis | GE HealthCare Technology & Innovation Center

Abstract: Analysis of challenging samples such as trace or degraded DNA is becoming increasingly common in forensic laboratories. Optimization of DNA recovery from these types of samples is essential to downstream profiling success because only nanogram or sub-nanogram quantities of DNA may be available. There are several commercial kits on the market designed and optimized to extract DNA from such samples; however, these products isolate DNA and disregard all other components within the sample. It is well-documented in relevant literature that other components of a biological sample, such as mRNA and proteins, can hold a wealth of information that can provide investigators with insight into the context of a crime. The sensitivity of short tandem repeat (STR) DNA typing kits has dramatically increased in recent years, to the point where a full DNA profile can now be recovered from trace samples containing only a few cells. Because of this, the source of the DNA profile is being questioned in court, and methods have been developed to link a DNA profile recovered from evidence to a specific body fluid stain by profiling the coding single-nucleotide polymorphisms (SNPs) in mRNA. Additionally, genetically variant peptides are useful when DNA profiles are inadequate. This study aims to develop a novel method to recover the entirety of the nucleic acids in the sample while retaining analytes such as proteins and small molecules for additional analysis using a highly efficient nucleic acid binding vector embedded onto the surface of a paramagnetic bead in one efficient, streamlined workflow. This method has demonstrated highly efficient capture (>99%) and elution (>93%) using spiked DNA. Similar efficiencies were obtained with severely degraded (24–500 base pairs) and low template DNA (1 ng–200 pg), which often present significant challenges when using traditional extraction methods. When compared with a popular commercial magnetic bead–based DNA extraction kit, the new method performs equally well, with the added benefit of multi-analyte retention. The researchers have observed highly efficient capture (>95%) and elution (>89%) of spiked RNA and developed protocols for co-elution and differential elution of DNA and RNA. Protocols are being developed for DNA and RNA recovery from multiple body fluids and case type samples, achieving DNA recoveries >1 ng from trace blood, saliva, semen, and thumb touch samples. Multianalyte recovery of DNA and RNA from semen provided successful STR DNA profiling and amplification of a body fluid–specific mRNA marker. Experimentation is underway for blood, saliva, and touch deposits on glass and polypropylene surfaces and after environmental exposure such as heat and ultraviolet light. With continued optimization, the new method presents great potential for successful recovery of multiple analytes from trace biological samples.

Forensic STR Sequencing Nomenclature Resource

NIJ Award: DJO-NIJ-22-RO-0004

Katherine B. Gettings* and Lisa Borsuk | National Institute of Standards and Technology
Martin Bodner | Medical University of Innsbruck
Jonathan King | University of North Texas Health Science Center

Abstract: This presentation will inform attendees of resources that have been developed via National Institute of Justice funding to support the recently published International Society for Forensic Genetics (ISFG) Short Tandem Repeat (STR) Sequence Nomenclature recommendations. These resources include updates to and expansion of the STRSeq BioProject, tools for STR sequence exploration, and new capabilities for the STRidER allele frequency database. The STRSeq BioProject is a collection of forensic STR sequence GenBank records that was initiated in 2017. Records were added rapidly between 2018 and 2021 based on STR population sample sequencing data generated by the National Institute of Standards and Technology (NIST) Applied Genetics group and partner laboratories. These early records were annotated following the 2016 ISFG DNA Commission Report on STR Sequence Nomenclature Considerations. The recently published 2023 ISFG DNA Commission Report on STR Sequence Nomenclature Recommendations contains new guidance on formatting; thus, all existing STRSeq records (>2,500) are undergoing annotation and metadata updates. In addition, nearly 30 publications of STR sequence population data that have not been considered for STRSeq were identified. From these, an additional >500 GenBank records for new (unique to STRSeq) STR sequences are expected. A suite of tools that are designed to facilitate user access to STR sequences formatted according to the 2023 nomenclature recommendations will also be presented. The Forensic Sequence STRucture Guide, released alongside the 2023 recommendations, is an extensive downloadable Excel file that overlays forensic annotation onto the relevant STR sequences from the GRCh38 Human Genome Reference sequence. Additional annotations include SNPs that users will likely encounter in their STR sequences and overlap between sequence ranges in commercially available STR sequencing kits. Additionally, sequence access and exploration are being improved by updates to the NIST STRBase website, including incorporating STRSeq GenBank records into more easily navigable webpages, developing a string search tool so that users can determine if the sequence(s) of interest are present in STRSeq, and developing an interactive tool for exploring the Forensic Sequence STRucture Guide. Finally, planned updates to the STRidER allele frequency database will be presented, specifically including the capability of serving out sequence-based STR allele frequency data to facilitate the generation of STR sequence-based match statistics and likelihood ratios.

Comparative Evaluation of Massively Parallel Sequencing STR Kits with the Emphasis on Mixture Deconvolution Utilizing Probabilistic Genotyping

NIJ Award: 15PNIJ-22-GG-03560-SLFO

Elisa Wurmbach,* Vishakha Sharma, and Erin Butler | New York City Office of the Chief Medical Examiner

Abstract: The technique of individual identification in modern forensics, DNA typing of short tandem repeats (STRs), has brought a standardized, quantitative method with strong statistical underpinnings to the criminal justice system. Although the fundamental principles behind STR typing have not changed, newly developed instrumentation and informative biological markers have the potential to address the limitations of current techniques and improve throughput at lower costs. The forensic community has begun to evaluate massively parallel sequencing (MPS) to overcome these problems. MPS not only adds additional sequencing information but has a nearly unlimited capacity for additional STRs and single-nucleotide polymorphism markers,

thereby enhancing individual identification. In addition, amplicons can be designed to be the shortest possible length, making them more useful for degraded samples. One of this project's objectives was to evaluate the recently released ForenSeq™ MainstAY kit. This MPS kit tests for Amelogenin, 26 autosomal STRs, and 25 Y-STRs. So far, the researchers have performed several experiments to analyze different conditions, including benchmark (which is defined as following the recommendations of the manufacturer), sensitivity, degraded DNA, throughput, and two-person mixtures. These experiments were executed using both the recommended micro-flow cell and the standard flow cell. The two flow cells differ in their capacity, sequencing time, and cost. Knowing how they work will provide more flexibility in the experimental design. Although data analysis is still ongoing, it seems that the standard flow cell resulted in higher coverage and showed slightly better outcomes than the micro-flow cell.

Comparative Assessment of Emerging Technologies for Body Fluid Identification

NIJ Award: 2020-DQ-BX-0015

Mirna Ghemrawi | Center for Forensic Science Research and Education

Abstract: Biological fluid detection and identification provides important contextual information to a forensic investigation. Although genetic testing can help establish from whom DNA may have come, only serological testing can provide an indication of the body fluid or tissue from which a DNA profile may have originated. The goal of this project was to compare different approaches to body fluid identification, including DNA methylation, mRNA, and proteomic-based methods. This was performed by preparing identical sets of simulated forensic samples and sending them to three different research groups, each specializing in one of these procedures. The researcher also compared these results with contemporary immunochromatographic procedures. The goal of this research was not to determine which procedure was optimum but instead to produce a time stamp showing the current state of the art and revealing the progress to date. Furthermore, the results from this study provide future directions in terms of developing and further optimizing each process. In this study, a comparative assessment of these emerging "omics"-based technologies for body fluid identification (e.g., epigenome, transcriptome, proteome) was performed on four replicates of 58 blind samples. Peripheral blood, menstrual blood, semen, vaginal fluid, saliva, breast milk, urine, non-human samples, and nasal secretions were deposited onto diverse substrates, including denim, leather, and cotton, with volumes as small as 2.5 µL. Some of these samples underwent intentional degradation and inhibition treatments. To assess the performance of each assay, a comprehensive analysis encompassing specificity, sensitivity, and error rates was conducted. The obtained findings also shed light on the capability of contemporary serologic techniques versus emerging technologies. The new omics-based procedures were found to be highly specific with results providing >99.5% specificity and lower error rates than conventional immunochromatographic assays. Additional research and validation studies still remain for these procedures; however, the researcher notes that the technology has been widely heralded and applied in medical diagnostics, and its implementation in forensics is long overdue. The comparative assessment of the strategies discussed in this study provides valuable information to the forensic community, which can aid in the development of new research and facilitate technology transfer.

PRESENTER BIOS

Xiaoyu Alan Zheng

Xiaoyu Alan Zheng is a mechanical engineer in the Sensor Science Division of the National Institute of Standards and Technology (NIST). He has a BS and MS in mechanical engineering from Johns Hopkins University. His primary area of research focuses on advancing the science of forensic firearm and toolmark analysis through documentary standards, measurement methods, state-of-the-art instrumentation, database development, statistical population characterization, and objective similarity metric research and development. He currently leads the development of the Reference Population Database for Firearm Toolmarks to enable the calculation of statistical weight of evidence in firearm toolmark comparisons. He has been the principal investigator on multiple National Institute of Justice grants and is currently on his fourth, where he is investigating the parity between examiner conclusions and score-based likelihood ratios generated by automated systems. He is currently a member of the Subcommittee on Firearms & Toolmarks in the NIST Organization of Scientific Area Committees, the chair of the Technical Advisor committee for the Association of Firearm and Tool Mark Examiners, and the co-chair of the Technical Working Group for 3D Toolmark Technologies. He also has a role in surface texture metrology at NIST and supports U.S. industry needs through American Society of Mechanical Engineers (ASME) B46 documentary standards development and low uncertainty calibrations of roughness, step heights, and other surface measurements. This work also supports international comparability and traceability in surface texture measurements by representing NIST for international key comparison studies.

Tatiana Trejos

Dr. Tatiana Trejos is an associate professor of the Department of Forensic and Investigative Sciences at West Virginia University, where she teaches forensic courses for undergraduate, master's, and doctoral programs. Dr. Trejos's long-term research goal is to develop methods that enhance the reliability and efficiency of trace evidence, providing valuable data to the criminal justice system and streamlining processes. Her research group focuses on building capacity and applying emerging methods to improve data quality and data usage. Dr. Trejos's main research includes developing and implementing field-testing methods, applying statistics to evidence interpretation, and discovering chemical signatures of forensic materials by spectrochemical methods, such as scanning electron microscopy–energy dispersive X-ray spectroscopy, inductively coupled plasma mass spectrometry, laser ablation inductively coupled plasma mass spectrometry, micro X-ray fluorescence spectroscopy, laser-induced breakdown spectroscopy, and mass spectrometry. Dr. Trejos's laboratory investigates trace materials, including glass, tape, paints, polymers, inks, and gunshot residues. She has authored 77 peer-reviewed scientific publications and book chapters in the field of forensic and analytical chemistry. Dr. Trejos has served as a program chair of scientific meetings and guest speaker at over 250 scientific venues. Dr. Trejos received

the prestigious science and technology award "Clodomiro Picado Twight" from the Costa Rican National Academy of Sciences (2015), has been listed on the Forensics Colleges' top 10 forensic chemistry professors list, and received the West Virginia University Eberly College Outstanding Researcher Award (2020). Dr. Trejos has contributed to different scientific working groups. She is a member and technical contact of the ASTM E-30 committee and the National Institute of Standards and Technology (NIST) Chemistry/Instrumental Analysis Scientific Area Committee's Materials (Trace) Subcommittee within the Organization of Scientific Area Committees (OSAC). Within the NIST-OSAC organization, she has served as chair of the Research and Glass Working Groups and a member of the Interpretation and the Physical Fits Groups. Dr. Trejos has served on two NIST Scientific and Technical Review Panels for physical fits and gunshot residue. She also serves as an affiliate member of the Ignitable Liquids, Explosives & Gunshot Residue Subcommittee. Dr. Trejos's contributions include drafting discipline-specific standard guidelines and testing methods; identifying research and development needs in trace evidence; designing and leading interlaboratory studies; and developing plans for training, disseminating, and implementing consensus-based methods. Dr. Trejos serves as director of the American Society of Trace Evidence Examiners.

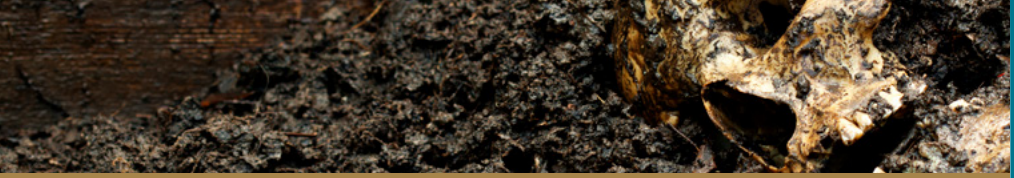
Igor K. Lednev

Dr. Igor K. Lednev is a Williams-Raycheff Professor in chemistry and distinguished professor at the University at Albany, State University of New York. He is an adjunct professor in the Department of Biological Sciences and a faculty member of the RNA Institute. Dr. Lednev is a cofounder and CTO of SupreMEtric LLC (www.supremetric.com), which commercializes a universal method for the identification of body fluid traces for forensic purposes, and Early Diagnostics LLC, which is developing saliva and blood tests for early diagnosis of Alzheimer's disease. Dr. Lednev is a fellow of the Royal Society of Chemistry in the United Kingdom and the Society for Applied Spectroscopy. He has received several prestigious awards, including the 2022 Charles Mann Award for Applied Raman Spectroscopy; Gold Medal Award from the Society for Applied Spectroscopy; Guest Professor Fellowship from the Friedrich-Schiller-University, Jena, Germany; Research Innovation Award from Research Corporation; Chancellor's Award for Excellence in Scholarship and Creative Activities; and the College of Arts and Sciences Dean's Award for Outstanding Achievements in Teaching. Dr. Lednev's research is focused on the development and application of novel laser spectroscopy for forensic purposes, biomedical applications, and fundamental biochemistry. His accomplishments include the development of new approaches for the identification and characterization of biological stains, gunshot residue, hair, and other trace evidence recovered at a crime scene and non-invasive, early diagnoses of neurodegenerative diseases such as Alzheimer's and Parkinson's. Dr. Lednev invented a new method for testing the stability of RNA vaccines and a new approach for drug discovery. The fundamental research is focused on understanding the structure and formation mechanism of amyloid fibrils, which are protein aggregates related to neurodegenerative diseases. U.S. Congressman Paul Tonko acknowledged Dr. Lednev's research accomplishments at the U.S. House of Representatives Hearing on Advancements in Forensic Science in the United States in September 2019. Dr. Lednev was recruited by the United Nations to give a week-long National Training Course on using vibrational techniques to enhance the forensic analysis in Santiago, Chile, in January 2020. Dr. Lednev has served as an advisory member on the White House Subcommittee for Forensic Science. Together with the National Institute of Justice, he organized the first National Institute of Justice Forensic Science Symposium at Pittcon in 2018, which has since become an annual event that includes 34 invited talks and a poster session. Dr. Lednev has co-authored over 270

publications in peer-reviewed journals and has eight patents. His h-index is 74. His work has been covered by media more than 90 times, including 18 TV interviews and publications in the *Wall Street Journal*, *Chemical & Engineering News*, *Forensic Magazine*, and more. Canada Discovery Channel featured his work using Raman spectroscopy for gunshot residue analysis, and 736 people registered for Dr. Lednev's recent keynote podcast on Raman spectroscopy and machine learning for medical diagnostics.

Luis E. Arroyo

Dr. Luis E. Arroyo-Mora holds the position of associate professor in the Department of Forensic and Investigative Sciences at West Virginia University. His extensive research portfolio is dedicated to the comprehensive characterization and analysis of emerging drugs of abuse, pollutants, and various environmental stressors. Employing advanced techniques such as mass spectrometry, Dr. Arroyo explores the identification and characterization of novel psychoactive substances, pesticides, and chemical entities within intricate biological and non-biological matrices. Beyond his primary focus, Dr. Arroyo is engaged in multifaceted research pursuits, including the examination of gunshot residues and drugs using electrochemical and spectroscopic methodologies. Another significant area of his expertise lies in the elemental analysis of printing inks found on counterfeit pharmaceutical packages, a domain where he leverages laser-induced breakdown spectroscopy. Dr. Arroyo's scholarly contributions extend to numerous peer-reviewed scientific publications, showcasing the depth and impact of his research. Furthermore, he has been an active participant in the global scientific community, presenting and coauthoring over 175 presentations and posters at prestigious scientific meetings across North America, Central America, South America, Europe, Asia, and Australia. His work not only contributes to the advancement of forensic and investigative sciences but also demonstrates a commitment to addressing contemporary challenges in substance analysis and environmental forensics.



Matthieu Baudelet

Matthieu Baudelet graduated with a BSc in physics from the University of Lille (France) in 2003, where he began his experience in spectroscopy with Fourier-transform microwave spectroscopy. In 2005, he graduated with an MSc in laser and spectroscopy from the University of Lyon (France) and continued on to complete his PhD in the "Laboratoire de spectrométrie ionique et moléculaire" (LASIM, Lyon) working on laser-induced plasma and spectroscopic analysis, showing the advantages of laser-induced breakdown spectroscopy (LIBS) for biological sensing and food monitoring. He continued his research on laser spectroscopy and sensing as a senior research scientist for the Townes Laser Institute at the University of Central Florida (Orlando, FL) covering the fundamentals of laser-induced plasmas, the application of laser spectroscopies (e.g., LIBS, fluorescence, Raman, Fourier-transform infrared) as fundamental diagnostics and sensing techniques for defense, industrial, environmental, biomedical applications, and the study of propagation of ultrashort laser pulses for sensing purposes at distances up to the kilometer range. From 2012 to 2015, as a research assistant professor of optics in the Laser & Plasma Laboratory at the Townes Laser Institute, he further developed laser spectroscopy for atomic spectroscopy, as well as electronic, vibrational, and rotational molecular spectroscopy, for studying the fundamentals of plasmas, quantitative analysis, and sensing in tabletop and integrated configuration as well as stand-off detection. Dr. Baudelet's applied expertise ranges from forensic science (through a secondary joint appointment at the National Center for Forensic Science) to biomedical diagnostics and manufacturing optimization. Joining the faculty of the chemistry department at the University of Central Florida in 2015 as an assistant professor in the National Center for Forensic Science, he developed his research to focus on the application of laser-based spectroscopic techniques for forensic analysis of tire skid marks, pollen grains, and anthropological remains. He demonstrated the first non-destructive DNA analysis of single pollen grains, opening the path to quantitative and multimodal forensic palynology. The focus on quantitative field forensic anthropology using LIBS has led to the development of chemical standards of hard biomaterials adapted for laser-ablation-based chemical analysis (LIBS and laser ablation inductively coupled plasma mass spectrometry [LA-ICP-MS]). Since becoming an associate professor in 2020, Dr. Baudelet has been furthering the development of multimodal analysis for pollen, tire marks, and forensic anthropology. Another focus of his research is to further the development of better matrix-matched standards of biomaterials for LIBS and LA-ICP-MS. His activities garner interest from the forensic and the biomedical communities as they will contribute to filling the gap in quantitative analysis required to develop new approaches to medical diagnostics and forensic decision-making.

Jennifer Pechal

Dr. Jennifer Pechal is an assistant professor at Michigan State University. Her program is focused on using an interdisciplinary research approach to better understand insect–microbe interactions during decomposition. Dr. Pechal's research is specifically centered on those questions to better understand the biological mechanics governing decomposition, which is a vital biological process for all life. The knowledge gained through basic research allows further research avenues to be explored with more directed and applied research questions to solve real-world problems. Expanding the fundamental knowledge of the human postmortem microbial community, their interactions, and the potential impact to use this information during death investigation remains underexplored. To develop robust tools that aid in death determination, researchers must first investigate and describe the microbiomes (structure and function) of infants with deaths resulting from varying conditions. Dr. Pechal has had extensive training in studying microbial community dynamics on decomposing organic matter, such as carrion and human bodies. Her research is focused on decomposition ecology community assemblages and ecosystem dynamics by identifying key microbes present during the decomposition processes. Dr. Pechal has the expertise to (1) collect, process, and quantify the postmortem microbial community structure via nucleotide isolation; (2) use bioinformatics to characterize microbial communities detected using high-throughput sequencing techniques; and (3) model microbial community dynamics in relation to antemortem human health using either Bayesian or frequentist approaches in complex multi-level or hierarchical statistics and performing multivariate statistical analyses of metagenomic data from the characterized microbial communities. Her work strives to maximize the positive impacts insects and microbes can have to improve lives.

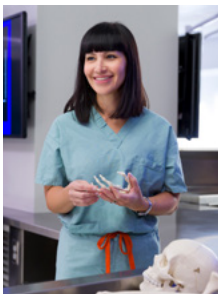
Brittany Coats

Dr. Brittany Coats is a full professor in the Department of Mechanical Engineering at the University of Utah and holds affiliated positions in the Departments of Ophthalmology and Visual Sciences, Pediatrics, and Bioengineering. Her research focuses on neural and ocular injury mechanics, with a specific interest in understanding microstructural features and properties that lead to better injury prediction in infants and young children. Using a combination of imaging, mechanical testing, and computational simulations alongside animal studies, she has investigated the effects of repetitive head rotation on traumatic brain injury, material properties of pediatric brain and eye tissue, age-related changes to vitreoretinal adhesion, and skull fracture mechanics in infants. She works closely with clinicians and judicial representatives to develop data and tools to improve identification of child abuse in children and infants. She graduated with a BS in mechanical engineering from the University of Utah and a PhD in bioengineering from the University of Pennsylvania. Her postdoctoral training was at the University of Pennsylvania and spanned bioengineering, neurosurgery, and ophthalmology. She is an American Society of Mechanical Engineers (ASME) fellow and current chair of the ASME Bioengineering Division's Head and Injury technical subcommittee. She was an associate editor at the ASME *Journal of Biomechanical Engineering* and presents biannually at the U.S. Army Defense Counsel Assistance Program. Her research has been supported by the Department of Defense, the National Institutes of Health, the National Science Foundation, and the National Institute of Justice.

Jessica L. Skinner

Dr. Jessica L. Skinner holds the esteemed position of senior research fellow at Mayo Clinic Arizona, where she serves as the leader of research teams specializing in regenerative rotator cuff repair and forensic science. Her influential role in forensic research, initiated by the impetus of the National Institute of Justice award 2020-75-CX-0015 under the guidance of Dr. Natalie Langley, is at the forefront of innovation in forensic fracture assessment. Armed with a PhD and MS from the University of Wisconsin Milwaukee, Dr. Skinner's academic trajectory laid the groundwork for her career in interdisciplinary team-based research. Her specialization during this academic phase involved identification and preservation efforts for individuals recovered from historic cemeteries, fostering a deep commitment to the fusion of science and humanities for community service. At Mayo Clinic, Dr. Skinner's forensic research delves into bone micromechanics, employing state-of-the-art techniques like scanning electron microscopy to unravel the intricacies of bone elasticity during the postmortem interval. In her current role, she oversees a spectrum of responsibilities, including data collection and analysis, intern mentorship, and the effective dissemination of research findings. Dr. Skinner's research interests traverse microscopic musculoskeletal biomechanics, bone tissue mechanics, and personalized musculoskeletal modeling. With a dedication to making positive contributions to both medicine and forensic science, she aspires to inspire and guide the next generation of forensic scientists.

Natalie R. Langley



Dr. Natalie R. Langley is an anatomist and Board-certified forensic anthropologist who is known internationally for her multidisciplinary approach to research. Her research collaborators include forensic scientists, surgeons and physicians, engineers, biochemists, pharmacologists, software designers, and graphic artists. Dr. Langley is an associate professor of anatomy in the Mayo Clinic Arizona Department of Laboratory Medicine and Pathology. She is also the forensic anthropologist for the Southern Minnesota Regional Medical Examiner's Office at Mayo Clinic Rochester. Dr. Langley is President of the American Board of Forensic Anthropology and a Fellow of the American Academy of Forensic Sciences with 20 years of experience as a researcher, case consultant, and educator. She is also Vice Chair of Curriculum at Mayo Clinic Alix School of Medicine and directs courses in clinical anatomy and histology. Dr. Langley has 15 years of experience with federal grants in various capacities, including completion of six National Institute of Justice grants. Dr. Langley's work on her 2007 National Institute of Justice Graduate Research Fellowship grant earned her the Emerging Forensic Scientist Award from the American Academy of Forensic Sciences. Other grant deliverables include a laboratory manual for osteometric data collection in forensic anthropology (*Data Collection Procedures for Forensic Skeletal Material 2.0*), which is the standard for data collection in the field; software for reconstructing fragmentary skeletal remains (Fragmento); and numerous publications in refereed scientific journals (*Journal of Forensic Sciences, Forensic Science International, Forensic Anthropology, International Journal of Legal Medicine Human Biology, Regional Anesthesia and Pain Medicine, Clinical Anatomy, American Journal of Physical Anthropology, Medical Science Educator,* and *Anatomical Sciences Education*). She is also co-editor of three textbooks designed to teach best practices to burgeoning and experienced forensic anthropologists.

Lauryn DeGreeff

Dr. Lauryn E. DeGreeff is an associate professor of chemistry with the Global Forensic and Justice Center at Florida International University (FIU) in Miami, where she takes a chemistry-based approach to studying olfaction for the purpose of informing field vapor detection and sampling practices. She conducts research in the field of vapor analysis as it relates to canine and instrumental detection, specifically trace vapor sampling, characterization, and generalization. Dr. DeGreeff graduated from FIU with a PhD in forensic chemistry in 2010. Prior to returning to FIU in 2021, she conducted a fellowship at the Federal Bureau of Investigation (FBI) Counterterrorism and Forensic Science Research Unit. Afterward, she spent 10 years as a researcher and principal investigator in the Chemistry Division at the U.S. Naval Research Laboratory in Washington, DC. Dr. DeGreeff regularly lectures on the dynamics of odor for the operational canine detection community and at national and international scientific conferences. She also discusses canine olfactory detection on a number of podcasts aimed at the canine detection community. She has authored many peer-reviewed manuscripts, holds four pending and completed patents, and is the editor of the book titled *Canines: The Original Biosensor*, released in 2022. Her research has earned her a number of awards, including the Federal Laboratory Consortium Award for Excellence in Technology Transfer, the U.S. Navy Civilian Service Award, and Top 30 Influential Woman Role Models by the American Society of Naval Engineers. She participates in two subcommittees of the Organization of Scientific Area Committees (OSAC), Dogs and Sensors and Ignitable Liquids, Explosives and Gunshot Residue, and is on the editorial board for *Forensic Chemistry*.

Glen P. Jackson

Dr. Glen P. Jackson is the Ming Hsieh distinguished professor of forensic and investigative science at West Virginia University, where he also holds a joint appointment in the C. Eugene Bennett Department of Chemistry. Dr. Jackson earned a BS abroad in the United Kingdom and an MS and PhD in the United States, all in analytical chemistry. He is a fellow of the Royal Society of Chemistry and the American Academy of Forensic Sciences. Dr. Jackson's research is broadly defined as forensic and biological applications of mass spectrometry. Recent forensic applications include the mass spectral interpretation and identification of seized drugs, a model to understand the evaporation/weathering of ignitable liquids in fire debris, and the chemical analysis of human hair. His group's research has appeared in more than 90 publications, more than 160 conference and university presentations, and three issued patents. As a principal investigator or co-principal investigator at Ohio University and West Virginia University, he has helped secure more than $5 million in state and federal funding. Since 2016, Dr. Jackson has served as the co-founder and co–editor-in-chief of the Elsevier journal *Forensic Chemistry*. He recently served a 3-year term on the National Institute of Standards and Technology

Organization of Scientific Area Committees (OSAC) Subcommittee on Seized Drugs, and he has taught numerous workshops to practicing forensic professionals. He is an active forensic chemistry consultant and has worked on more than three dozen legal cases. His work has appeared on *Nancy Grace Live*, *Forensic Files II*, Sundance TV, a WRAL *What Remains* podcast, and *Law & Order: Special Victims Unit*.

Denise A. Valenti

Dr. Denise A. Valenti is a certified social equity candidate for research license. The credential is provided by the Massachusetts Cannabis Control Commission. Dr. Valenti has had a long-time interest in and been a long-time advocate for health care equity. She has been a member of the American Public Health Association for over 40 years. She provided direct clinical care with the Indian Health Services and ran a clinic for the indigent in inner city Detroit, MI. Her residency was in Houston, TX, where she provided house calls/homecare in the communities suffering from the greatest health disparity. Dr. Valenti, along with two colleagues, received a grant from the Administration on Aging to develop curricula on aging that addressed the unique needs of a diverse population. The understanding that race, genetics, and culture influence health care outcomes was not part of medical or allied health professional curriculum at the time, and education around such health variables was essential. Today, we take such knowledge for granted. Dr. Valenti is the founder and CEO of IMMAD. IMMAD, or Impairment Measurement Marijuana and Driving, is a company that specializes in education and technology for the responsible use of marijuana. IMMAD focuses on technology for roadside use by law enforcement to determine functional impairment to drive with marijuana use. IMMAD also has a research line to determine cannabis use status with the objective to determine whether the retina could be used as a biomarker of cannabis use disorder. IMMAD has two technologies; IMMAD-VR is a functional test of the eye retinal response for driver impairment detection, whereas IMMAD-CUD has different, more expanded programming relative to cannabis use disorder diagnosis and management. Dr. Valenti has been the principal investigator on federal grants related to cannabis; her current grants from the National Institute of Justice include 2023–2024 Marijuana Intoxication: Roadside Tool for Law Enforcement to Measure Impaired Peripheral Vision, 15PNIJ-22-GG-04417-RESS, and an earlier grant from 2018–2019, Digital Marker of Marijuana Intoxication: Measure of Dysfunctional Retinal Ganglion Cell Response NIH NIDA SBIR Phase I contract-SBIR N43DA-18-1221. Dr. Valenti has had the opportunity to present her research and provide medical education specific to cannabis to a diverse set of groups. For 2023, this included the New England Association of Forensic Scientists in Hartford, Northeastern University Chemistry and Pharmacology of Drug Abuse Conference, Carolina Cannabinoid Collaborative in Raleigh, National Interdisciplinary Cannabis Symposium in Portland, and the Cannabis Science Fair at Harvard. Additional presentation activities included the SXSW 2023 in Austin, International Association of Chiefs of Police Conference in Chicago (2019), International Cannabinoid Research Society Symposium in Bethesda (2019), Association for Ocular Pharmacology and Therapeutics Scientific Meeting in New Orleans (2019), Gerontological Society of America Scientific Meeting in Boston (2019), Northern California High Intensity Drug Trafficking Conference in San Jose (2019), and Association Research Vision and Ophthalmology Annual Meeting in Honolulu (2018).

Katherine Bollinger

Katherine Bollinger is a research forensic scientist in the Center for Forensic Science Advancement and Application at RTI International. She is a Board-certified Forensic Toxicologist with Diplomate status in the American Board of Forensic Toxicology (D-ABFT-FT). She has an MS in forensic science from Virginia Commonwealth University. In her role at RTI, Ms. Bollinger participates in research and business development activities and mentors and provides guidance to interns and early career staff. With over 12 years of experience in laboratory research and development, she has extensive knowledge of analytical instrumentation using targeted analyses, including LC-MS/MS, and non-targeted data acquisition instrumentation, such as liquid chromatography quadrupole time of flight mass spectrometry for forensic toxicology. Ms. Bollinger regularly develops and validates quantitative methods for substances of interest, including drugs of abuse, from multiple matrices such as blood, urine, oral fluid, and hair. She has served as a principal investigator or co-principal investigator on numerous National Institute of Justice Research and Development research projects, and she leads tasks within the National Laboratory Certification Program, the Forensic Technology Center of Excellence, and formerly the National Forensic Laboratory Information System. In addition to research activities, Ms. Bollinger routinely develops evaluation plans and participates in evaluations of emerging technologies of potential interest to the forensic community, often in partnership with public laboratories.

Arati Iyengar

Dr. Arati Iyengar joined the faculty of the Department of Forensic and Investigative Science in West Virginia University in fall 2021. After obtaining a PhD in biotechnology from the University of Southampton in the United Kingdom many years ago, she enjoyed carrying out postdoctoral research on topics ranging from cancer genetics to forensic and conservation genetics in the United Kingdom and Germany for nearly a decade. This experience then led to her first faculty position in a large forensic science department within a modern British university where she taught forensic and conservation genetics to undergraduate and graduate students for another decade. She relocated to the United States in 2018 and after a short stint teaching forensic biology at the University at Albany, State University of New York, she moved to West Virginia University in 2021. She now teaches undergraduate and graduate courses in molecular genetics, population genetics, and forensic biology. Her research interests remain broadly in the fields of forensic genetics and wildlife forensics. She is currently mentoring undergraduate and graduate students working on projects focused on improving DNA recovery and DNA profiling from trace and challenging biological samples and investigating DNA transfer during crime scene examination. She has received funding for her research from West Virginia University and the National Institute of Justice.

Katherine B. Gettings

Dr. Katherine B. Gettings began working in the field of human identity testing in 1998 and obtained her MS in criminal justice, specializing in forensic science, from Virginia Commonwealth University in 2001. She trained and worked in the Forensic Biology and DNA Casework section of the Virginia Department of Forensic Science and later became the technical leader of forensic operations at Bode Technology. Dr. Gettings has testified in numerous criminal cases, taught forensic science courses at the undergraduate and graduate levels, and served as a forensic laboratory auditor/assessor with the National Forensic Science Technology Center and the American Society of Crime Laboratory Directors Laboratory Accreditation Board – International. Dr. Gettings earned her PhD in biological sciences from The George Washington University in 2013, then began her work with the Applied Genetics group at the National Institute of Standards and Technology (NIST). Dr. Gettings's background serves as a useful foundation as she leads the NIST Applied Genetics group's efforts to develop next-generation sequencing infrastructure for the forensic community. These efforts have resulted in multiple publications of sequence-based allele frequency data, the creation and development of the STRSeq catalog of sequences, and initiation of the STRAND (Short Tandem Repeat: Align, Name, Define) Working Group. Most recently, Dr. Gettings chaired an International Society for Forensic Genetics DNA Commission on STR Sequence Nomenclature, the recommendations report of which was published in fall 2023 (Gettings et al., 2023).

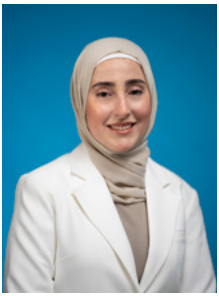
Reference

Gettings, K. B., Bodner, M., Bursuk, L. A., King, J. L., Ballard, D., Parson, W., Benschop, C. C. G., Børsting, C., Budowle, B., Butler, J. M., van der Gaag, K. J., Gill, P., Gusmão, L., Hares, D. R., Hoogenboom, J., Irwin, J., Prieto, L., Schenider, P. M., Vennemann, M., & Phillips C. (2023). Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on short tandem repeat sequence nomenclature. *Forensic Science International: Genetics, 68,* 102946. https://doi.org/10.1016/j.fsigen.2023.102946

Elisa Wurmbach

Dr. Elisa Wurmbach studied biochemistry at Freie University in Berlin, Germany. She is a molecular and developmental biologist by training and received her PhD from the University of Stuttgart Hohenheim (Germany) in *Drosophila* genetics with Professor Anette Preiss. For her first postdoctoral position, she joined Dr. Wilhelm Ansorge's laboratory at the European Molecular Biology Laboratories in Heidelberg, Germany, where she was involved in sequencing the *Arabidopsis* genome and developing microarray methods. In 2000, she moved to the United States for her second postdoc with Dr. Stuart Sealfon at the Mount Sinai School of Medicine, where she generated the first microarray and supervised the qPCR facility. As research assistant professor with Dr. Samuel Waxman, she specialized in genomics and data analysis. In 2007, Dr. Wurmbach joined the Office of Chief Medical Examiner in New York City as city research scientist. She initiated the pigmentation project, for which she was selecting and testing genetic markers to predict eye, hair, and skin coloration. This project was partly funded by the National Institute of Justice (2010-DN-BX-K181) and resulted in four peer-reviewed publications and one book chapter. She also supervised the micromanipulation project (2012-DN-BX-K043) that examined several techniques aiming to deconvolute mixed DNA samples before they become inadvertently pooled in DNA extraction. The outcomes of this study were disseminated in three peer-reviewed publications, one of which was given the PW Allen award for the "Most Meritorious Research Paper" published in *Science & Justice* in 2017. More recently, Dr. Wurmbach became interested in massively parallel sequencing (MPS) techniques. Her laboratory evaluated several MPS-STR kits, including Verogen's ForenSeq™ Signature Prep kit (2015-DN-BX-K005) and ThermoFisher's Precision ID GlobalFiler™ NGS STR Panel v2 (2018-DU-BX-0166), as well as available mitochondrial MPS kits (2016-DN-BX-0172), with the goal of using them for forensic identification in casework. This research led to six peer-reviewed publications and several presentations, including at the Green Mountain DNA Conference, Next Generation Dx Summit, and the Promega Tech Tour. Currently, she is working on evaluating the ForenSeq™ MainstAY kit (15PNIJ-22-GG-03560-SLFO). In addition to her research, Dr. Wurmbach has been teaching molecular biology at Yeshiva University (2008–2010) and molecular biology (2012–2022) and advanced topics in forensic DNA analysis at Pace University (2012–present).

Mirna Ghemrawi



Dr. Mirna Ghemrawi serves as an associate director at the Center for Forensic Science Research and Education, focusing on forensic biology, genetics, and epigenetics. Dr. Ghemrawi holds a faculty appointment and serves as the program director for the Thomas Jefferson University Master of Science in Forensic Biology program. She also serves as a reviewer in scientific journals, including the *International Journal of Legal Medicine* and *Electrophoresis*. Dr. Ghemrawi received her PhD in biochemistry from Florida International University in 2022 where she graduated with the Real Triumphs award. She was invited to contribute to the "Young and Inspiring Scientists Special Issue" in *Electrophoresis*. She was also elected to serve as a co-chair for the 2025 Gordon Research Seminar on Forensic DNA. Prior to moving to the United States for graduate school, Dr. Ghemrawi graduated with a BS in medical laboratory sciences from Haigazian University, Lebanon, on a full merit scholarship from the U.S. Agency for International Development (USAID). Following that award, Dr. Ghemrawi received one of the most prestigious scholarships globally, the J. William Fulbright Foreign Student Scholarship from the U.S. Department of State, Bureau of Educational and Cultural Affairs, where she pursued her master's in forensic science at Purdue University, IN. To date, Dr. Ghemrawi has successfully executed numerous research projects that culminated in peer-reviewed publications and national and international scientific presentations, including the International Society for Forensic Genetics, the American Academy of Forensic Sciences, and the International Symposium on Forensic DNA in Law. Dr. Ghemrawi developed a pyrosequencing-based assay for species identification and an epigenetic-based examination for complex body fluids, and she also worked on investigating the genital microbiome for sexual assault cases. In addition to these awards, Dr. Ghemrawi has a passion for education, science communication, and STEM outreach. She offers pro bono sessions for students interested in applying to graduate schools, communicating science to non-scientists, and connecting students to professionals and experts in the field. She was the first-place winner of the Three-Minute Thesis competition at Florida International University, was the second-place winner of the Massachusetts Institute of Technology Lebanon Challenge hackathon 2020, and received the Best Community Interaction Award for linking high schoolers to universities.

POSTER ABSTRACTS

An asterisk (*) denotes the presenter for presentations that have multiple authors; when available, the presenters' headshots are included in the abstracts.

SESSION I: Physics and Pattern Interpretation/Trace Evidence

Physics and Mathematical Models for Error Quantifications in Comparative 3D Microscopy for Physical Match Analysis

NIJ Award: 15PNIJ-21-GG-04141-RESS

Ashraf F. Bastawros,* HyeokJae Lee, Joshua Berlinski, Ranjan Maitra, and William Meeker | Iowa State University
Lauren Claytor | Virginia Department of Forensic Science
John Vanderkolk | Indiana State Police Laboratory (Retired)

Ashraf F. Bastawros

Abstract: 3D microscopy provides a means to analyze distinctive microscopic patterns inherent in fractured surfaces, aiding forensic match analysts in mitigating the subjectivity associated with comparative microscopy in forensic evidence examination. Despite its utility, the repeatability and reproducibility of features generated by 3D microscopy have been insufficiently investigated, with limited understanding of the impacts of the microscope operator or sample alignment on the measurement system. The topography imaging process introduces various sources of variation, encompassing those from the 3D microscope, equipment operator, and physical characteristics of the measured fracture surface. To assess the measurement system's quality, steel rods broken under tensile or bending loading conditions are generated. Five inexperienced microscope operators are trained, and they repetitively image pairs of matching surfaces akin to comparative microscopy. After the third imaging iteration, each operator utilizes a fixture to align the fractures during imaging. The topologies of matching images yield a multivariate similarity measure for the two surfaces, incorporating the aforementioned sources of variation. A gauge repeatability and reproducibility (R&R) model is employed to scrutinize these diverse sources. The model formulation details are presented within the context of the available data for each fracture method. The resultant sources of variation are discussed, highlighting differences for each dataset in the context of physical distinctions between fracture types. Notably, although all matches and non-matches are correctly classified irrespective of the imaging fixture, gauge R&R reveals that utilizing the fixture enhances the measurement system by minimizing within-operator variability. These findings offer insights into the quality of the dataset acquired by 3D microscopy measurement systems, establishing a valuable framework for training automated fracture matching algorithms. Moreover, they provide guidance on enhancing imaging processes and procedures for various fractured surfaces encountered in crime scenes.

Physics and Statistical Models for Physical Match Analysis Utilizing 3D Microscopy of Fracture Surfaces

NIJ Award: 2018-R2-CX-0034

Ashraf F. Bastawros,* HyeokJae Lee, Joshua Berlinski, Ranjan Maitra, and William Meeker | Iowa State University
Lauren Claytor | Virginia Department of Forensic Science
John Vanderkolk | Indiana State Police Laboratory (Retired)

Ashraf F. Bastawros

Abstract: Forensic fracture matching relies on the principle that no two objects break identically because of the variables involved in the fracture process. The development of objective comparisons starts with imaging the topological features of the fracture pairs and performing mathematical and statistical comparisons. The scales of the fracture topologies are rooted in the principles of fracture mechanics. For hardened metals, commonly examined in the forensic laboratory, plastic flow is suppressed in favor of brittle fracture. The fracture or failure commences when the fracture strength is exceeded over a characteristic distance from the crack tip. This fracture characteristic scale is about two grain diameters in size for materials with grain-like structures. Within this scale of observation, the fracture surface roughness exhibits self-affine scaling properties. The fracture surface character becomes more complex and non–self-affine at larger length scales, exhibiting unique roughness characteristics dictated by the material intrinsic properties, microstructure, and exposure history to external forces. The researchers exploited this deviation scale to ascertain the individuality of a pair of fracture fragments. The researchers used 3D microscopy to map the fracture surface's topological details at a scale about 10 times the fracture process scale and employed surface spectral analysis, using the mathematical framework of fast Fourier transforms to identify these critical scales. The researchers applied the statistical correlation analysis and statistical learning tools to develop a classification rule for matching and non-matching. The study found that this scale is about two grain–sized or micro feature–sized, rendering the required imaging scale to be about 20 times of such scale. Multivariate statistics were employed to develop quantitative topological descriptions, evaluated from 3D spectral analysis of overlapping topographical images, to provide premise of uniqueness for forensic comparisons. The statistical learning tool performance is tested on a robust training dataset and validated on a set of 38 different broken pairs of either knives broken in bending, or stainless-steel rods with similar grain sizes, broken in tension or bending. The generality of the framework under different modes of loading is examined by application to a set of 10 twisted knives to failure. All broken pairs were classified with very high accuracy. The framework lays the foundations for forensic applications with quantitative statistical comparison across a broad range of fractured materials with diverse textures and mechanical properties.

Evaluation of Mobile Technology for Detection of Inorganic and Organic Gunshot Residues in Firearms-Related Investigations

NIJ Award: 2020-DQ-BX-0010

Kourtney Dalzell,* Leah Thomas, Madison Lindung, Tatiana Trejos, and Luis Arroyo | West Virginia University

Kourtney Dalzell

Abstract: Firearms-related incidents represent one of the major outcomes of gun violence and a pervasive and ongoing societal issue bringing death and injuries to a significant portion of the population. The investigation of such events is initiated at the scene of the incident. Once specimens are collected from suspects or materials,

they are usually sent to laboratories for processing, an inquiry that may take several weeks to be finalized. Therefore, forensic services can greatly benefit from effective real-time screening methods that can be implemented at the crime scene. In recent years, research efforts have boosted mobile devices as practical alternatives, offering rapid screening capabilities to improve workflow in forensic laboratories. Indeed, laboratories rely upon their interpretation using the gold standard in gunshot residue (GSR) detection, scanning electron microscopy–energy dispersive X-ray spectroscopy. However, the surface interrogation of the unknown material in pursuit of particles and later spectroscopic analysis can take 2–8 hours per sample, and no organic information is obtained. This research group proposed using electrochemistry and laser-induced breakdown spectroscopy (LIBS) as complementary methods to bridge these gaps and provide screening methods that may aid in more timely analysis, improved decision-making, and triage from scene to laboratory. In the last decade, this research group has developed LIBS methods for GSR applications as a rapid, reliable technology that can streamline laboratory and crime scene processes. This poster compares a laboratory LIBS unit to a mobile instrument using authentic hand samples from shooters (100 samples) and non-shooters (200 background samples). A significant novelty of the mobile instrument is its enhanced imaging, which allows quick searching and visualization of GSR particles for single micron–sized particle examination. Both instruments obtained accuracy better than 98.8%, demonstrating their suitability for trace inorganic GSR detection from skin specimens. This study evaluates LIBS capabilities for GSR detection in other substrates commonly encountered at a crime scene and is tested in mock crime scene situations. Also, due to their rapid, cost-efficient, and compact platform, electrochemical methods using disposable screen-printed carbon electrodes are proposed for GSR screening at the laboratory and point of care. GSR residues were extracted from typical aluminum/carbon adhesive collection stubs and analyzed via square-wave anodic stripping voltammetry. Both benchtop and mobile electrochemical instruments were compared for the assessment and classification of authentic shooter samples by monitoring a panel of inorganic and organic GSR elements and compounds, including lead, antimony, copper, 2,4-dinitrotoluene, diphenylamine, nitroglycerin, and ethyl centralite. Performance rates obtained by assessing authentic hand samples collected from over 100 known shooter and 200 non-shooter samples compared to their benchtop counterparts will be presented. Results demonstrated the accuracy of the mobile electrochemical and LIBS instruments at 96.5% and 98.9%, respectively, for correctly classifying a sample as positive for GSR. The capabilities and limitations of these devices were further evaluated with mock case scenarios that simulated common firearm-involved situations and assessed the workflow for using the two methods in succession for the screening of GSR. These results highlight the potential for mobile devices as a viable option for rapid and reliable GSR detection.

Assessing the Reliability of Modern µXRF Technology for Expanded Impact on the Forensic Examination and Interpretation of Trace Evidence: Glass Evidence

NIJ Award: 15PNIJ-22-GG-03571-SLFO

Troy Ernst* | Michigan State Police, Grand Rapids Forensic Laboratory
Zachary Andrews and Tatiana Trejos | West Virginia University
Cedric Neumann | Battelle Memorial Institute
Ruthmara Corzo | National Institute of Standards and Technology

Troy Ernst

Abstract: Glass fragments are among the trace materials most often submitted to forensic laboratories, as their physical and chemical examination can provide valuable information in forensic investigations. These disciplines use state-of-the-art methodologies

that hold substantial scientific grounds and consensus-based protocols. However, some challenges come in hand with the advancements of modern technology and the changing manufacture of mass-produced materials. For example, micro-X-ray fluorescence (μXRF) is an elemental analysis technique widely used in forensic laboratories that recently experienced a significant shift in their detection systems (e.g., silicon drift detectors [SDDs] vs. silicon lithium detectors). Among the advantages of SDDs are improved sensitivity and precision, which can lead to superior informing and discriminating power. However, the lagging of research in this area has not caught up with the rapid adoption of the new technology by public laboratories, exposing them to potential increased error risks. Moreover, the manufacture and global marketing of glass has evolved in past years, leaving a void in current literature and datasets based on decades-old collections and instrumentation. Another challenge with μXRF examinations is the subjectivity associated with spectral data interpretation. Thus, the trace community and organizations such as the National Institute of Standards and Technology Organization of Scientific Area Committees and the Department of Justice Forensic Science Technology Working Group have identified these research needs as a high priority. This project's overarching goal is to address these immediate operational needs by developing and validating improved protocols for collecting, examining, and interpreting contemporary glass evidence. To achieve that, this multidisciplinary team (forensic practitioners in a publicly funded laboratory, forensic researchers, and statisticians) proposes to (1) identify the significant sources of variability when using modern μXRF SDDs and provide specific sampling and interpretation recommendations for soda-lime glass casework comparisons; (2) assess the accuracy, discrimination, and informing power of elemental analysis of μXRF SDDs for contemporary broken glass from portable electronic devices; and (3) validate objective and quantitative metrics for μXRF spectral comparison and probabilistic interpretations of glass evidence. The creation of an extensive dataset of over 4,000 μXRF glass spectra is used to test error rates, providing a one-of-a-kind repository that can strengthen the current foundations and modernize standard methods. Findings on datasets of vehicle glass windshields and architectural glass originating from the same source (same windowpane) and different sources are reported in this poster for small full-thickness glasses (~1–2 mm thick) and smaller irregular glasses (~30–50 μm thick) to simulate worst-case scenarios where the sample size is limited. The use of quantitative similarity metrics is also evaluated as a more objective means to compare spectral data. This study is anticipated to lead to best practices for more efficient and objective decision-making processes and increased reliability in the analysis and interpretation of physical evidence and has already provided relevant input to an updated version of the ASTM E2926 standard method. The project deliverables are designed to have maximum impact and become rapidly adopted by forensic laboratories.

Elucidation of the Effect of Heat and Sun Exposures on Hair Colored by Permanent and Semi-Permanent Colorants Using Surface-Enhanced Raman Spectroscopy (SERS)

NIJ Award: 15PNIJ-21-GG-04169-RESS

Dmitry Kurouski,* Aidan P. Holman, and Mackenzi Peterson | Texas A&M University

Dmitry Kurouski

Abstract: Confirmatory identification of hair colorants can be used to establish a connection between a suspect and the crime scene or demonstrate the absence of such connections. A growing body of evidence shows that surface-enhanced Raman spectroscopy (SERS) could be a confirmatory, minimally destructive, and fully non-invasive analysis of hair colorants. In SERS, a signal provides information about the chemical structure of both permanent and semi-permanent

dyes present on hair and is enhanced a million-fold using noble metal nanostructures. However, it is unclear whether the information of hair colorants can be revealed if hair was contaminated or exposed to harsh environments such as sunlight and heat. This poster will discuss the effect of short- and long-term heat exposure on SERS-based analysis of hair colored with blue and red permanent and semi-permanent dyes. The poster will also discuss the extent to which water and ultraviolet radiation can alter SERS-based accuracy in identification of colorants on hair. The results show that heat, ultraviolet radiation, and water exposure of colored hair causes chemical changes in the dyes, which results in significant changes in the spectroscopic signature of these colorants. Therefore, the effect of environmental factors should be strongly considered upon their SERS-based examination to avoid both false-positive and -negative identification of chemical dyes.

Assessing the Reliability of Modern µXRF Technology for Expanded Impact on the Forensic Examination and Interpretation of Trace Evidence: Tape Evidence

NIJ Award: 15PNIJ-22-GG-03571-SLFO

Lacey Leatherland,* Charlotte Vogler, Zachary Andrews, and Tatiana Trejos | West Virginia University
Ruthmara Corzo | National Institute of Standards and Technology
Cedric Neumann | Battelle Memorial Institute
Troy Ernst | Michigan State Police, Grand Rapids Forensic Laboratory

Lacey Leatherland

Abstract: Recent advances in micro-X-ray fluorescence spectroscopy (µXRF) technology provide increased sensitivity and precision compared with other methods of analysis, such as scanning electron microscopy – energy-dispersive spectroscopy (SEM-EDS). At the same time, this new XRF technology is cost-effective and more widely adopted by public laboratories for other trace material analyses. However, µXRF systems equipped with modern silicon drift detectors are underused for tape evidence. Moreover, current analytical standards have not progressed as swiftly as the technology. Thus, organizations such as the National Institute of Standards and Technology Organization of Scientific Area Committees and the American Society of Trace Evidence Examiners have identified the need to develop and normalize objective means of interpreting and comparing spectral data to provide a basis for the development of consensus-based criteria standard methods. This study evaluates the accuracy, discrimination, and informing power of the µXRF elemental analysis of electrical tapes. This is accomplished by modern µXRF silicon drift detector systems to assess the variability within and between rolls of electrical tapes and through an interlaboratory study to evaluate inter-examiner and instrumental variability. The study also reports performance rates of various quantitative spectral comparison methods and further validates them through the examination of a contemporary dataset of 50 tapes originating from different sources, including a variety of manufacturers (3M™, DiversiTech®, Shurtape®, NSI Industries™, Utilitech™, Intertape Polymer Group™, Amazon®), countries of origin (United States, China, Taiwan, Poland), quality grades (high, medium, low), and label brands (Scotch®, Temflex™, Morris Products™, Duck Brand®, Shurtape®, Easy-Wrap™, WarriorWrap®, Utilitech™, Intertape Polymer Group™, 3M™, AmazonCommercial®). In addition, five rolls from this set were selected to analyze intra-roll homogeneity and three rolls to assess variability within a package of multiple rolls. A subset of 10 tapes was distributed for an interlaboratory study within eight participating laboratories. This dataset serves as a repository to provide a more robust foundation for current XRF examinations and provides examiners with a more objective means of spectral interpretation. Spectral data are evaluated using spectral overlay, comparison intervals of integrated peak ratios, and a spectral contrast angle ratio

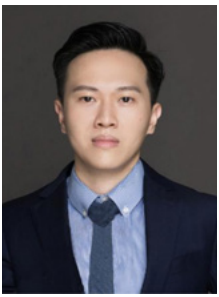
(SCAR). The SCAR method is advantageous because it provides a quantitative metric of the level of similarity considering the within- and between-source variations. SCAR values below 1.5 are typically observed for same-source samples, whereas larger ratios are observed as the spectral differences increase (>1.5 to 60). Recommended comparison criteria are provided based on the lowest combination of false exclusion and false inclusion error rates. Good discrimination power (91%–93%) and accuracy (92%–94%) are observed, depending on the comparison criteria. In this poster, the researchers report and compare various sources of variation in the data (i.e., within roll, between packages, between rolls, inter-examiner/instrumental variation). Finally, the effects of common fingerprint development chemicals on the elemental profiles of electrical tape are presented along with recommendations for proper workflow for tape evidence examinations across multiple disciplines (i.e., trace, DNA, fingerprints).

Enhancing Fire Pattern Analysis with Experiments on Architectural Finishes Impact and Developing Data-Driven Tools

NIJ Award: 15PNIJ-22-GG-04442-RESS

Shuna Ni* and Stanislav I. Stoliarov | University of Maryland, College Park
Pengkun Liu* and Pingbo Tang | Carnegie Mellon University

Shuna Ni



Pengkun Liu

Abstract: This project studies the effects of architectural finishes on fire patterns and uses the resulting test data in combination with other data to develop data-driven tools for quantitative fire pattern analysis. Interpreting fire patterns on walls and other surfaces is integral to investigating fire incidents. Architectural finishes may potentially influence these patterns. Unfortunately, prior studies of fire patterns did not account for the impact of such finishes on the results of fire pattern analysis or conclusions drawn from this analysis, despite fires frequently occurring in enclosures with finished surfaces. This project seeks to fill this knowledge gap by conducting extensive fire experiments in burn cells with drywalls featuring a range of common architectural finishes, including paints varying in sheen level, color, and chemical composition, and various wallpapers. This project focuses on plume-generated fire patterns. Some tests will also incorporate the effect of sprinkler water. A comparative analysis of all the test results is ongoing, focusing on (1) damage features of a burn cell's fire-exposed surfaces, (2) geometry features of a fire pattern, (3) spatial distribution of fire patterns over the surfaces of a burn cell, and (4) drywall calcination contour maps. Those comparisons have allowed us to (1) identify whether architectural finishes magnify or diminish the degree of fire damage, taking into account both surface damage features and drywall calcination; (2) determine if architectural finishes have a significant impact on the overall geometrical shapes of a fire pattern; and (3) determine if the impact of architectural finishes is reflected in the size of a fire pattern, even if its overall geometrical shape remains consistent. The underlying mechanisms of how architectural finishes influence fire patterns can be accurately described by examining the variances in fire test data (e.g., burning process, temperature, heat flux, and mass loss) and by correlating those data with the properties of architectural finishes such as flammability and thermal inertia. In addition to experiments, the researchers are developing data-driven tools for fire pattern analysis to enhance the traditionally qualitative process, using both experimental data from the previously mentioned tests and other sources and synthesized data. The data-driven tools developed in

this project encompass three primary functions: (1) fire damage evaluation models to assess surface fire damage, applicable to both finished and unfinished drywall surfaces; (2) fire pattern classification models to categorize a fire pattern's shape (e.g., triangular, columnar, conical); and (3) spatial feature extraction and reasoning models to extract the spatial relationship among multiple fire patterns in a room. The first and the second functions use 2D images but map results onto a 3D reconstructed fire scene, while the third function extracts spatial information from 3D images. The extraction of a fire pattern from a fire scene is conducted using a human–computer interaction approach with reasoning segmentation via a large language model. The information provided by these data-driven tools offers crucial insights into the development of a fire within a compartment and helps fire investigators determine the origin of a fire.

Analysis of Small Particles Adhering to the Edges of Duct Tape as a Means to Make Associations in a Way That Is Independent of Manufactured Characteristics

NIJ Award: 2020-MU-CX-0018

David A. Stoney* and Paul L. Stoney | Stoney Forensic, Inc.

David A. Stoney

Abstract: Forensic analysis of duct tape is important for the investigation and prosecution of major crimes, where it is used as blindfolds, bindings, and ligatures. Methods are needed that go beyond comparison of manufactured characteristics of duct tape rolls. Once tape rolls are put into use, exposed adhesive along tape edges presents an ideal opportunity for collection of the very small particles (VSPs) that are ubiquitous in the environment. The researchers have previously reported the development of effective methods for recovering VSPs from the edges of duct tape and the differentiation of environmentally acquired VSPs from the particles present as part of the manufactured adhesive composition. Thirty rolls of partially used silver-colored duct tape were collected from residences within Fairfax County, VA. VSPs were harvested from the tape edges using the previously reported methods. VSPs attributable to the adhesive itself were differentiated after which the acquired VSPs were used, along with previously reported methods of particle combination analysis, to test the ability to (1) discriminate among the 30 tape rolls and (2) link tape segments to rolls. Discrimination among tape rolls was tested by separating the acquired tape edge VSPs from each roll into training and test datasets. Using classification criteria developed from the training datasets, test datasets were correctly classified for 96.7% of the rolls (29 of 30). Linking tape segments to rolls was tested using the same classification criteria. Separate comparisons were made for left edge, right edge, and combined VSP datasets. There were 100% correct associations (30/30) when all three comparisons indicated the same source and 90.9% correct associations (40/44) when two or three comparisons indicated the same source. The correct source was identified by one or more comparisons 53 of 60 times (83.3%). This project has established that once rolls of duct tape have been put into use, the exposed adhesive on the edge of the duct tape roll collects and retains VSPs. These particles, acquired post-manufacture, are a source of individuality for the specific roll of tape, providing a means to distinguish it from the many other rolls of the same make and manufacture. Particle combination analysis of acquired tape edge VSPs allows effective discrimination among duct tape rolls and provides quantitative associations linking tape segments to source rolls. These findings address the requirement that associations be based (in part) on post-manufacture acquired characteristics. The impact is significant due to the frequent occurrence of duct tape evidence in major crimes. This project has also resulted in new methods for (1) the efficient and effective recovery of acquired tape edge particles from duct tape segments and (2) characterization of duct tape adhesives based on small particles included as fillers in their adhesive formulations.

Accounting for Covariates in Forensic Error Rate Assessment and Evidence Interpretation

NIJ Award: 2019-DU-BX-4011

Larry Tang* and Ngoc Ty Nguyen | University of Central Florida
Martin Slawski and Emanuela Marasco | George Mason University
Chris Saunders and Semhar Michael | South Dakota State University
Dannica Ommen | Iowa State University
P. Jonathon Phillips | National Institute of Standards and Technology

Larry Tang

Abstract: These error rates reported by recent forensic black box studies are mainly obtained by pooling all the decisions from examiners or computer algorithms with same-source or different-source pairs. These measures report the average error rates across a population of examiners for evidence sources. It would be ideal to account for covariates such as (1) source subjects' covariate information, including their demographics or source images' attributes and quality and (2) examiners' covariate information, such as their training background and demographics. Appropriately accounting for covariates in error rate assessment and evidence interpretation requires sophisticated statistical analyses with modern statistical concepts and methods. In this poster, the researchers will present this National Institute of Justice–funded work on the receiver operating characteristic (ROC) regression framework for error rate quantification by allowing covariates specific to source subjects and examiners. The researchers will discuss statistical techniques by fitting ROC regression in order to relate covariates to error rates quantified by the ROC curve. The resulting covariate-specific ROC curves in face recognition, handwriting, and latent print databases will model the relationship between covariates and decision scores, given the error rates for specific values of covariates. The researchers will also present an R-Shiny app to facilitate the implementation of the developed methods for black box studies.

Utilizing eDNA from Four Biological Taxa Associated with Geologic Evidence for Sample-to-Sample Comparisons and Study Site Separation

NIJ Award: 2020-R2-CX-0035

Teresa M. Tiedge* and Kelly A. Meiklejohn | North Carolina State University

Teresa M. Tiedge

Abstract: Geologic materials, such as soil and dust, are valuable types of trace evidence submitted to crime laboratories. Forensic geologists aim to analyze the inorganic components (e.g., mineral content) and determine their physical properties (e.g., color and pH) for sample-to-sample comparisons or to identify an evidentiary sample's origin. However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information. DNA metabarcoding is a commonly used approach to identify the biological taxa that are present in environmental samples by amplifying and sequencing short, informative regions of the genome and is not restricted by sample amount. The goal of this research was to determine the utility and stability of environmental DNA from four biological taxa associated with soil and dust for sample-to-sample comparisons and sample origin determination. To accomplish this, four taxa—bacteria (16S), fungi (ITS1), arthropods (COI), and plants (ITS2, trnL)—recovered from each sample were characterized (n=1,026)

via DNA metabarcoding. An initial soil isolation study was performed to determine the most suitable approach (picking/scraping, swabbing, and sonication) to remove soil from mock evidence for environmental DNA analysis. Following soil removal, DNA was isolated using the PowerSoil® Pro Kit. DNA extracts were amplified using PCR primers specific to 16S, ITS1, ITS2, COI, and trnL. Libraries were then prepared and sequenced on an Illumina® MiniSeq™. Raw sequencing reads were then processed through a bioinformatic pipeline that identifies amplicon sequence variants via DADA2 and searches the amplicon sequence variants against GenBank for taxonomic identification. Picking and scraping of soil produced the highest amount of DNA compared with swabbing ($p=0.0025$) and sonication ($p=0.0068$). Although all three methods recovered similar taxonomic assignments, picked and scraped samples tended to cluster together more consistently with the soil reference in multidimensional space and thus was the method chosen for soil isolation in subsequent experiments. Following the soil isolation study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a 1-year period. Mock items included (a) soil scraped from t-shirts, boot soles, and trowels; (b) exposed dust collected from brick pavers using polyurethane swabs; and (c) dry dust from air filters (~1" × 1" area used). DNA was isolated from mock geologic evidence using the PowerSoil® Pro Kit, and libraries were prepared using custom indexed primers and subsequently sequenced using the Illumina® MiSeq™. After sequencing, the bioinformatic pipeline was used to process sequencing reads to characterize the bacterial, fungal, plant, and arthropod communities. Important findings from this research include the following: (1) despite the low DNA concentrations of dust samples, it was still possible to characterize the biological communities in dust; (2) the wet lab workflow successfully recovered taxa associated with mock forensic evidence; and (3) it is apparent that there were changes in the biological communities over time and between locations. This poster will also include a preliminary assessment of temporal and spatial variables on the recovery of bacteria, fungi, arthropods, and plants from mock geologic evidence.

Assessing Methods to Enhance and Preserve Proteinaceous Impressions from the Skin of Decedents During the Early Stages of Decomposition While Examining Environmental Variations Across Seasons

NIJ Award: 2019-R2-CX-0070

Jessica Zarate* | Madonna University
Jane Wankmiller | Northern Michigan University

Jessica Zarate

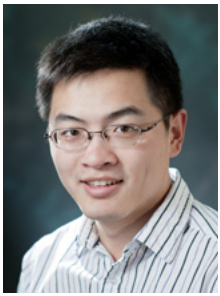
Abstract: Homicides and violent crimes often result in bloodshed; the constant substrate involved in physical altercations in the commission of violent crimes is human skin. Thus, it is likely blood impressions are left on the skin of living victims or decedents during these violent interactions. However, skin is one of the least studied substrates in the impression discipline. In some cases, impressions are clearly visible, but it is much more likely that they are latent and not readily visible. There have been cases where the enhancement of blood impression evidence on human skin was possible, but it is not standard practice, especially when blood impressions are latent. Although visible blood impressions are best enhanced in situ at the scene of the crime, most often these impressions on decedents are not enhanced until the body is moved to the medical facility for autopsy, increasing the possibility of damage to the impression evidence from handling or moving the body. Because it is semi-porous, skin is a difficult substrate to enhance through chemical enhancement methods, which may cause background staining that results in suboptimal visualization of the impressions. In addition to the staining of skin, visualization

of impression details may be obstructed by competing background patterns, such as dermal scales, hairs, wrinkles, and variations in skin tones. Two commonly used dye stains, Amido Black and Hungarian Red, have been used to enhance blood impressions on human skin, and a newer method, Zar-Pro™ Fluorescent Blood Lifters, has also been used in preliminary studies to lift and enhance blood impressions from decedent skin effectively. A comparative analysis between methods conducted in collaboration with the Forensic Research Outdoor Station at Northern Michigan University assessed the effectiveness of enhancing semen smears and blood impressions on decedent skin during the early stages of decomposition. All three enhancement methods demonstrated effectiveness in recovering proteinaceous materials with Amido Black and Hungarian Red primarily effective as in situ dye stains. The dye-stained impressions were not reliably lifted using BVDA Gellifters®, thus not removing the substrate variables that can impede visualization of impressions on skin. The Zar-Pro™ Fluorescent Lifters were able to effectively lift and fluorogenically enhance proteinaceous materials in the form of blood impressions and semen smears from decedent skin through 10 days of active decomposition. A statistical assessment of the enhancement methods was conducted among examiners to verify the efficacy of results. During the early stages of decomposition, donor skin will deteriorate; thus recoverable impressions will also be degraded or damaged, yet this degradation is not perilous for the recovery of proteinaceous materials as long as the epidermal skin is still intact. Even during active decomposition, skin—arguably one of the most difficult substrates for impression recovery—can produce viable impressions, and the recoverability of this vital evidence can now be re-evaluated by practitioners in the field.

Recovery and Analysis of Both Volatile and Less-Volatile Compounds from Ignitable Liquid Residues on Substrates/Debris by SPME-DART-MS

NIJ Award: 2020-DQ-BX-0003

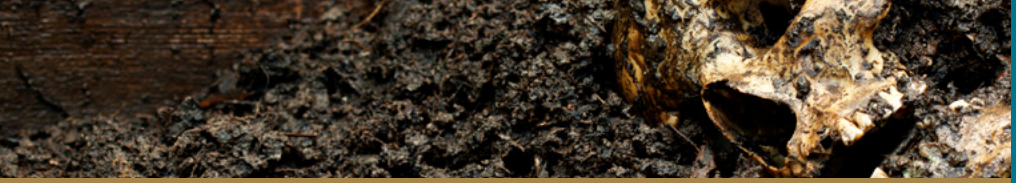
Mengliang Zhang,* Shruthi Perna, Victoria Bascou, and Ngee Sing Chong | Middle Tennessee State University



Mengliang Zhang

Abstract: The detection of ignitable liquid residue (ILR) is critical to the arson investigation process, which may potentially identify the cause of a fire. The most commonly used method to analyze ignitable liquids (ILs) is gas chromatography–mass spectrometry (GC-MS), which is used primarily in the detection of the volatile components in ILR. Direct analysis in real-time mass spectrometry (DART-MS) has shown success in profiling the non-volatile or less-volatile components in IL, which are likely to be contained in the fire debris and could therefore yield corroborating evidence on the use of specific ILs in the investigation. However, the substrates and fire debris tend to cause interference in analysis. In this study, solid-phase microextraction (SPME) was coupled with DART-MS to investigate the matrix effect and optimize the extraction parameters to reduce interference. Gasoline and paint thinner were used as model ILs to evaluate the SPME-DART-MS method. A previous study has shown that fuel additives and polyethylene glycol could be the marker compounds for gasoline and paint thinner, respectively. Two parameters (i.e., temperature and time) associated with SPME were evaluated by using a two-factor central composite design. The full second-order polynomial model that fits the data was constructed, and the optimum condition was reported based on the modeled response surface. The ILRs on wood, paper, sand, fabric, and fire debris were studied to examine their impact on extraction efficiency and analytical interference. The ILs were also mixed with water to simulate wet fire debris that has been exposed to water during fire suppression. The influence of water on the detection of ILR will be discussed. The SPME-DART-MS results indicate that both volatile

and less-volatile marker compounds for gasoline and paint thinner were recovered from the substrates and fire debris, and their ion patterns matched well with the gasoline and paint thinner liquid samples analyzed directly by DART-MS. As expected, the effective extraction of those marker compounds required a relatively high temperature (i.e., 150°C and 120°C for gasoline and paint thinner, respectively). In the presence of a matrix, a higher extraction temperature and longer extraction time could benefit the extraction efficiency. The desorption of ILR on the SPME fiber was achieved by inserting the fiber into the DART-MS helium gas stream under 300°C for 1 minute, and no carry-over residues were observed. In conclusion, the SPME-DART-MS has shown promise in ILR detection as an important complementary tool. The chemical information yielded by this method is typically not observed in the current GC-MS-based practice. Since SPME is one of the standard strategies for ILR extraction and DART-MS is becoming more available in forensic laboratories, the implementation of SPME-DART-MS for ILR detection could be achieved without much need for capital expenditure.



GIS Application for Building a Nationally Representative Forensic Taphonomy Database

NIJ Award: 2020-DQ-BX-0025

Madeline M. Atwell,* Katherine Weisensee, Carl Ehrett, D. Hudson Smith, and Patricia Carbajales-Dale | Clemson University

Madeline M. Atwell

Abstract: Estimating the time since death, or the postmortem interval (PMI), poses a significant challenge to medicolegal death investigations when human remains are discovered because of insufficient methodologies. Despite decades of research, existing studies involving PMI estimation are significantly hindered because of reliance on small sample sizes, environmental homogeneity, and inconsistent definitions of the stages of decomposition. These inconsistencies impede the successful identification of unidentified human remains and the reconstruction of events surrounding their death. To transcend enduring methodological issues involving PMI estimation, the researchers developed an ongoing collaborative forensic taphonomy reference database called geoFOR. The geoFOR application is a case entry platform and data repository. The app pairs this comprehensive dataset with ArcGIS and machine learning models to deliver improved PMI estimations. Specifically, the geoFOR app offers forensic practitioners a platform to enter case information, including observations regarding body size; the presence of trauma; and uniform descriptions concerning characteristics of decomposition, insect, and scavenger activity. The app automates weather data collection from the location of discovery using the Global Historical Climatology Network through the National Oceanic and Atmospheric Administration. After case submission, the app delivers a PMI prediction directly to practitioners using a statistically robust regression model. The advanced cross-validated machine learning PMI predictive model results in an R^2 value of 0.8, and users receive a predicted PMI with an 80% confidence interval. The geoFOR database currently contains over 2,600 cases derived from medicolegal death investigations and human decomposition research facilities across the United States and internationally. Data collection is ongoing as new and existing collaborators enter case information. The size and comprehensive nature of the geoFOR dataset allows for the application of machine learning methods for estimating PMI. Estimating PMI using machine learning is a novel concept in the field that can address major limiting gaps in PMI estimation. The impact of the geoFOR application in the advancement of forensic anthropology is two-fold: foremost, it provides the most comprehensive reference database to date, comprising thousands of individuals across varied environments. Many forensic studies are confined to small sample sizes, similar geographic regions, non-human proxies (e.g., pig carcasses), and donors of forensic decomposition research facilities, which are not necessarily reflective of the realities of medicolegal forensic cases. Second, the automation of weather data collection and deliverable PMI estimations using machine learning models offer unprecedented data-driven results that can help successfully narrow the search parameters for unknown decedents, which can expedite identification and more accurately inform investigators about the circumstances surrounding their deaths. GeoFOR also follows an Open Science Framework through data sharing that promotes methodological integrity and reproducibility. These principles encourage fairness, equity, and inclusion within the research community.

Skeletal Blast Trauma: Determining the Effect of Known and Experimental Blast Events on Trauma Patterns, Fracture Behavior, and Blast Scene Recovery Approaches

NIJ Award: 2020-R2-CX-0041

Petra Banks* and Nicholas P. Herrmann | Texas State University

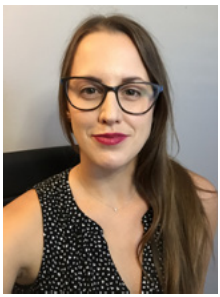
Petra Banks

Abstract: Skeletal blast trauma is still a relatively unexplored field. Although there are detailed data on the forces and appearance of blast injuries in soft tissue, such as blast lung or inner ear damage, much of the data on skeletal blast trauma is either derived from warfare case studies or from compiled generalized trauma data from military research. Minimal data have been collected regarding civilian skeletal blast trauma. In particular, few studies have compiled these and other skeletal trauma data into comparable datasets. To examine comparable trauma, this study explored the physical distribution of skeletal trauma for blast events, falls from height, aircraft crash trauma, and motor vehicle/pedestrian (MVP) collisions. Analyses were conducted using Chi-squared analysis, comparing trauma presence and absence at the zone, element, region, and axial versus appendicular levels. These data were collected from deidentified decedent case files from medical examiner and coroner offices from across the United States. In addition, blast trauma and aircraft crash trauma data from the Defense POW/MIA Accounting Agency (DPAA) were included to provide a comparison from non-civilian contexts. Hard tissue trauma events were recorded by bone element, limb, and specific bone location using osteological zones described in CORA, a database designed and built by DPAA. Case-specific data were recorded, including a description of the cause of the event, basic demographic information, and a general inventory of the remains to indicate whether the absence of trauma is due to a lack of preservation or a lack of injury. The collected data include 227 individuals from blast events, 70 individuals from aircraft crash events, 42 falls from height, and 50 MVP collisions. Demographically, male individuals predominate the sample of traumatic events ($n_M = 672$, $n_F = 48$). By age, both blasts and aircraft crashes skewed younger, with most cases occurring between age 20 and 25. However, although the rest of aircraft crash cases outside of the 20–25 year cohort were distributed equally, a large portion of blast events outside of this range occurred between the ages of 15 and 20. Both of these younger values are likely from the inclusion of the DPAA data, reflecting the age of military personnel in combat. The ages of individuals in falls and MVPs were statistically normally distributed. At all levels, there were significant differences in trauma frequencies. Axial versus appendicular comparisons resulted in significant results with a p-value of 0.01 between every combination of events. By region, all event types were statistically significant when examined together. When individually compared, there were more regions of dependence (non-significance) between blasts, falls, and MVPs, while aircraft crashes and MVPs were significant across all regions. Aircraft crashes and blasts had dependence at hands and thoracic vertebrae, and aircraft crashes and falls had dependence at cervical vertebrae. These trauma distribution data are being used to generate a predictive model of trauma event type using random forest modeling, which will provide investigators with important trauma analysis comparisons to help assess trauma causes.

Improving Identification of Unknown American Indians and Hispanic/Latinx Americans

NIJ Award: 15PNIJ-21-GG-04139-SLFO

Kelly R. Kamnikar,* Esteban Rangel, and Amaya Abeyta-Brown | The University of New Mexico
Nicollette S. Appel and Heather J.H. Edgar | The University of New Mexico and New Mexico Office of the Medical Investigator
Natalie Adolphi | New Mexico Office of the Medical Investigator
Stephen D. Ousley | The University of Tennessee



Kelly R. Kamnikar

Abstract: Stature estimation is a core component of the biological profile in forensic anthropology research and casework. Stature estimates using a mathematical framework are derived from equations that use single or multiple long bone lengths in combination with prediction intervals to create a stature range. The researchers provide mathematical equations for estimating stature for modern American Indians in New Mexico. This research draws on postmortem computed tomography scans from forensic casework (n=222) available from the New Mexico Decedent Image Database. The researchers regressed four long bone length measurements from the humerus, femur, and tibia on measured cadaver length to create 14 combinations of equations. These equations were calculated for the entire sample, by sex, by broad American Indian language group, and age and sex in combination. The most appropriate equations for each group were determined through various methods of model accuracy and efficiency testing. An independent test sample comprising forensic casework from the New Mexico Office of the Medical Investigator demonstrates that the equations created here are accurate and precise, with most overestimating stature by approximately 1 cm. The researchers provide recommendations for the use of these equations in a forensic setting and introduce a downloadable macro sheet for estimating stature available for use by practitioners.

Expanding and Validating the Microbiome Database for Estimating the Postmortem Interval

NIJ Awards: 2015-DN-BX-K016, 2016-DN-BX-0194, 2019-DU-BX-0025, 15PNIJ-22-GG-04402-MUMU

Jessica L. Metcalf* and Victoria Nieciecki | Colorado State University
Zachary Burcham | University of Tennessee Knoxville
Sibyl Bucheli and Aaron Lynne | Sam Houston State University
David O. Carter | Chaminade University Honolulu
Rob Knight | University of California San Diego

Jessica L. Metcalf

Abstract: Estimating the postmortem interval (PMI) in death investigations is important because it helps with reconstructing death scenes, identifying the deceased, issuing death certificates, and distributing assets defined in wills. However, PMI can be challenging to estimate, especially if no last communications or visual sightings are available. Previously, the researchers used 36 human cadavers at three anthropological facilities over four seasons (three cadavers per facility per season) and sampled skin and cadaver-associated soil daily for 21 days to characterize the decomposer microbial community. The researchers discovered a universal set of key microbial decomposers that assembled despite location, season, or climate. These universal key decomposers underlie an accurate random forest regression model for predicting PMI (calculated as accumulated degree days) from microbiome normalized abundance patterns with

species-level taxonomy data (16S rRNA gene amplicon data) of the skin predicting PMI within approximately ±3 calendar days (awards 2015-DN-BX-K016 and 2016-DN-BX-0194). However, several knowledge gaps still exist. First, the model only used forensic facilities in two climates present in the United States (Köppen-Geiger classification: "temperate without a dry season and hot summer" and "arid steppe cold"; Beck et al., 2018). Therefore, a gap in knowledge and data exists for building a model that predicts PMI from cadavers in other climates. Second, the model only includes data from outdoor donor decomposition. Therefore, a knowledge gap exists about whether the model is useful for indoor decomposition scenarios. These knowledge gaps are being addressed through samples collected from donors placed across forensic anthropology facilities in a third major climate type (Köppen-Geiger classification: "cold without a dry season warm summer") across North America (award 15PNIJ-22-GG-04402-MUMU) and in built structures approximating an indoor death scene (award 2019-DU-BX-0025). These data will clarify whether a universal model for predicting PMI is possible, or if predictions are more accurate with climate/environmental specific models. The researchers will validate and estimate error for PMI prediction by collecting an independent test set of samples collected from donor bodies placed at multiple forensics anthropological facilities (including facilities not represented in the training data set), as well as other forensically relevant opportunities. The researchers synthesize across multiple National Institute of Justice–funded projects to determine next steps for developing a tool useful to forensic science practitioners.

Reference

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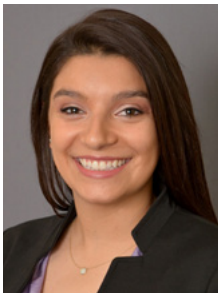
Finding the Missing and Unidentified: The Application of Predictive Modeling, Ground-Penetrating Radar, and Small Unmanned Aircraft-Mounted Infrared Imagery for the Detection of Unmarked Graves

NIJ Award: 2020-R2-CX-0043

Mariah E. Moe | Texas State University



Mariah E. Moe

Abstract: The migration crisis along the U.S.–Mexico border has claimed the lives of over 8,000 individuals in the last two decades, and Texas marks the state with the highest number of deceased undocumented migrants found. In many cases, partly because of overwhelming numbers, these individuals are buried in cemeteries throughout the South Texas region without a thorough examination and often without documentation of their burial location. This creates a situation in which the remains of individuals may become lost within cemeteries without the possibility of identification. Finding their graves is the first step toward returning their identity and repatriating them to loved ones. Prior efforts to locate these unmarked graves have been performed by Operation Identification and the Forensic Border Coalition and includes interviews, pedestrian surveys, and geophysical prospecting with moderate success. However, a thorough examination of current and alternative geophysical methods followed by establishing the ground truth through excavation has not been conducted. This poster will discuss the results of the geophysical survey and subsequent excavation of a cemetery in South Texas known to bury unidentified migrants who perished in their attempt to cross the U.S.–Mexico border. Two areas in the cemetery were identified by county employees to contain unidentified migrants, one active area in which burials are continuing to occur and the other

in which the last burial occurred several years prior. Both areas were surveyed using ground-penetrating radar and drone-based infrared imaging, although only the more recent section was excavated due to budgetary and time constraints. This study revealed that ground-penetrating radar is not an ideal method in the search for unmarked graves given the complex soil composition in South Texas; however, it does have the potential to identify subsurface anomalies associated with older burials where other methods fail. It should not be used as the sole method for determining the presence of burials but can provide an initial picture of the subsurface environment. Infrared imaging, as obtained using a DJI Mavic 2 Enterprise Advanced system, is more adept at detecting faint topsoil and vegetation changes associated with more recent burials but fails to identify older graves with less topographical variation. This poster will discuss the benefits and limitations of the equipment used and the environmental factors to consider when conducting grave searches, particularly in highly active cemeteries. In complex environments, including those in which the burials of interest are suspected to be several years old, more sensitive technology may be required to achieve a satisfactory output.

Towards an "Eggs-perimental" Approach for Species Determination of Blow Fly Eggs to Facilitate Estimations of Postmortem Interval

NIJ Award: 2020-MU-MU-0016

Rabi Ann Musah* and Alexa Figueroa | University at Albany, State University of New York
Jennifer Y. Rosati | John Jay College of Criminal Justice


Rabi Ann Musah

Abstract: Medicolegal forensic entomology is the utilization of "carrion insects" that colonize human and animal remains to estimate the time since death, commonly referred to as the postmortem interval (PMI). Blow flies (*Calliphoridae*) usually arrive at the body within a few minutes of death, with the remains serving as a feeding, breeding, and oviposition (egg laying) medium and refuge from extreme weather, predation, and resource competition. Because of the well-established correlation between the species of insects that feed on remains and the different stages of corpse or carrion decomposition, along with the well-known species-specific insect life cycle timelines, it is possible to "back-calculate" the approximate time of death by assessing when the eggs from which the retrieved entomological evidence hatched were laid. Accordingly, because it is presumed that the eggs were laid on the remains shortly after death occurred, the determination of when the eggs were laid serves to approximate when death occurred. Accurate species identification is critical for PMI determination, but conventional methods are often time-consuming and resource-intensive and require specialized expertise and laboratory resources. Typically, the juvenile life stages (eggs, larvae, and pupae) are the specimens collected at the scene, but visually determining species identity is challenging because of their similar appearance across species, particularly for the eggs. For this reason, if the retrieved specimens are viable, it is customary for an experienced entomologist to rear them to adulthood for species identification to be based on their more visually apparent distinguishing morphological features. In the absence of the necessary resources to accomplish this task, entomological evidence often remains underused. Therefore, an alternative rapid method for accurate determination of the species identity of retrieved entomological evidence is greatly needed to facilitate PMI estimation using underused entomological evidence such as eggs. Reported in this poster is the development of a chemometric method that enables determination of the species identity of necrophagous species eggs from within the *Calliphoridae* family through their direct analysis in real-time high-resolution mass spectrometry–derived chemical signatures. Chemometric processing of the

70% aqueous ethanol suspensions of eggs, disaggregated by species identity, exploits intraspecies similarities and interspecies differences to enable accurate species identification of *Calliphora vicina*, *Calliphora vomitoria*, *Cynomya cadaverina*, *Lucilia illustris*, *Lucilia sericata*, and *Phormia regina*. Accordingly, the application of Kernel discriminant analysis to the data enabled species identification with an accuracy of 87.35%. As an extension of this work, solid-phase microextraction–facilitated gas chromatography–mass spectrometry analysis of *Lucilia sericata* eggs over time revealed that they emit a range of volatiles as a function of their development progression. The compounds emitted can potentially provide valuable insights on the age of the evidence, thereby providing more refined and accurate PMI-relevant information. Future investigations aim to establish a comprehensive database containing species-specific chemical signatures for identifying entomological evidence, thus enhancing the evidentiary value of immature life stages such as eggs.

An Examination of Musculoskeletal Markers in Modern Populations for Forensic Analysis and Identification Purposes

NIJ Award: 2020-R2-CX-0042

Emilie L. Wiedenmeyer | Texas State University

Emilie L. Wiedenmeyer

Abstract: Forensic anthropologists provide biological profile estimations (sex, age, and stature) of unknown decedents to narrow down the list of missing persons and accelerate positive identifications. The addition of estimated activity level or occupational type holds promise to enhance the biological profile by further individualizing a decedent's remains. Entheseal changes (i.e., osseous changes occurring in musculoskeletal junctions within the body) have long been used to reconstruct lifeways and demographic indicators of archaeological populations. However, few attempts have been made to apply this knowledge of lifestyle and demographic indicators in a modern forensic medicolegal context to help with identification efforts. This study examined 691 individuals within modern donated human skeletal collections across the United States. Entheseal changes in the shoulder and elbow of each individual were scored following the Coimbra method published by Henderson and colleagues (2016). Various statistical comparisons and multivariate models were used to assess whether entheseal changes of the upper limb reflected biosocial information of the skeletal donors included in the study sample. Mixed factor analysis was used to incorporate all data into a single model to explore general relationships between entheseal scores and known donor data. Additional statistical tests explored more specific questions, such as Pearson's Chi-squared tests to find direct correlations between entheseal scores and reported demographics and random forest models to test the predictive strength of entheseal changes in predicting categories of labor and occupational type. Results indicated that age at death is the most consistent explanatory variable to affect developmental rates of formative and resorptive entheseal changes in the upper limb. However, variables such as biological sex, body mass index, and socioeconomic status were found to have varying effects, often accentuated with increasing age. Formative entheseal changes, such as bony spurs and textural changes, were found in higher frequencies in individuals with increasing body mass indices and those who reported working more manual labor jobs. Additionally, resorptive entheseal changes, such as erosive lesions and increased porosity, were seen in higher numbers within individuals of lower body mass, especially in older female individuals and older individuals working nonmanual labor jobs. Random forest models revealed that entheseal changes are more likely to accurately predict general manual versus nonmanual labor categories as opposed to more informed categories of occupation, rising as high as 74% accuracy when

using only entheseal scores and biological sex estimates. However, these results are likely skewed by male dominant manual laborers, as only 5% of women represented in the study reported working in manual labor jobs. Results from this study show promising avenues for entheseal changes in forensic work but also display their possible multifactorial origin, likely affected by many aspects of lived experience. Furthermore, the results of this study were limited because of the lack of Black, Indigenous, and People of Color (BIPOC) representation within the study sample, a common bias of modern donated skeletal collections. Additional research is needed to further explore how entheseal changes are distributed among individuals with diverse backgrounds and life histories to better assess potential forensic applications.

Reference

Henderson, C. Y., Mariotti, V., Pany-Kucera, D., Villotte, S., & Wilczak, C. (2016). The new 'Coimbra Method': A biologically appropriate method for recording specific features of fibrocartilaginous entheseal changes. *International Journal of Osteoarchaeology, 26*(5), 925–932. https://doi.org/10.1002/oa.2477

Development of a Colorimetric Breath Analyzer for THC

NIJ Award: 15PNIJ-22-GG-04437-RESS

Emanuele Alves,* Wagner Pacheco, Savanah Allinson, and Kyrah Golden | Virginia Commonwealth University

Emanuele Alves

Abstract: This presentation will demonstrate the development of a Δ9-tetrahydrocannabinol (THC) breathalyzer for the detection of recent Δ9-THC use in the field. After visiting this poster, attendees will better understand the mechanism of the colorimetric reaction applied to detect Δ9-THC and the application of this reaction to a support base that will work as a prototype for a colorimetric-based Δ9-THC breathalyzer. This presentation will impact the forensic science community by showing the development of the basic chemical foundations needed for the development of a portable colorimetric device for the on-site detection of Δ9-THC in air exhaled for the early detection of driving under the influence of marijuana. Today, the recreational use of marijuana is legal in 31 U.S. states and the District of Columbia. Several studies show that after legalization, a 6.0% increase in injury crash rates and a 4.0% increase in fatal crash rates were observed. Several factors must be considered to reduce driving under the influence of marijuana, such as preventive, educational, and punitive activities. On-site detection of recent marijuana use is one of the possible measures to be adopted. Alcohol breathalyzers are currently used to help law enforcement perform site evaluations quickly and easily. Thus, a Δ9-THC breathalyzer could bring similar advantages. Unfortunately, this is not yet a reality for Δ9-THC. The current devices are collection devices where the exhaled air is stored and must be further analyzed in a laboratory to allow Δ9-THC identification. In this project, the development of the Δ9-THC breathalyzer is based on the application of an additive manufacturing solid device made by 3D printing. To create this support, a commercial Anycubic® 3D polymerizable resin was mixed with different Fast Blue dyes and evaluated in the presence of several cannabinoids. The Fast Blue dye family is known to produce a colorimetric response in the presence of THC. Several dyes from the Fast Blue family were studied (B, BB, and RR), in concentrations varying from 1% to 6% w/v. The results showed that the Fast Blue B in a concentration of 4% w/v in the solid resin is capable of reacting with 0.01 µg with a linear response ranging from 0.01–0.50 µg of Δ9-THC. Achieving lower concentrations is extremely important because the concentration of Δ9-THC in exhaled air is described to be in the range of 1 ng/30 L of exhaled air. The results obtained in this work are the initial fundamental chemical foundation needed for the construction of a reliable semi-quantitative breathalyzer device to be applied in U.S. monitoring of driving under the influence of drugs.

Illuminating the Dark: Molecular Networking as a Novel Psychoactive Substance Identification Strategy

NIJ Award: 15PNIJ-21-GG-04171-COAP

Maia Bates* and Abby Helm | University of Wisconsin–Madison
Heather Barkholtz | Wisconsin State Laboratory of Hygiene and University of Wisconsin–Madison

Maia Bates

Abstract: Due to the ever-changing drug landscape, forensic laboratories are increasingly using untargeted techniques for presumptive substance identification. One of the most promising untargeted techniques is liquid chromatography–high-resolution mass spectrometry (LC-HRMS). Untargeted LC-HRMS technology allows for sensitive and selective detection of a vast number of diverse drugs, toxins, and metabolites extracted from complex biological matrices. Although untargeted LC-HRMS methods have many advantages, no one method that works efficiently for all analytes has been described. This work sought to assess the performance of an untargeted extraction and LC-HRMS method for the National Safety Council's Tier I drugs of abuse in whole blood. An untargeted analyte extraction and data acquisition method was developed and validated for Tier I drugs of abuse and metabolites. Target analytes were extracted from whole blood using 96-well Agilent Captiva EMR-lipid cleanup plates. A Waters Xevo G2-XS quadrupole time-of-flight mass spectrometer with electrospray ionization was used in positive and negative ionization modes for data acquisition. A data-independent acquisition mode, MS^E, was used to collect precursor and product ion data within one run. Peak detection was performed using the Waters UNIFI 3D peak algorithm and *m/z* retention time pairs to match data against an in-house spectral library. Performance of the outlined methods were measured using the following criteria: analyte recovery, limit of detection (LOD), ionization, interferents, carry-over, and precision. A total of 32 analytes were considered and method performance data were consolidated according to drug class and chemical structure. LODs were below recommended screening cut-off concentrations for the majority of analytes; several had sub–nanogram per milliliter LODs. Some exceptions were amphetamine, lorazepam, and oxymorphone, which were not detected at the recommended screening cut-off concentration. Extraction recoveries varied depending on the physical and chemical properties of the analytes and ranged from 9% to greater than 100%. Ionization also varied significantly with an average of 94% and a standard deviation of 43.8%. Endogenous and exogenous interferents were assessed, and none were determined. In addition, carry-over was not observed at the concentrations considered. Cannabinoid compounds posed the greatest challenge in this work; Δ9-tetrahydrocannabinol and the 11-hydroxy metabolite were not ionized sufficiently for detection at the recommended cut-off concentrations. However, the 11-nor-9-carboxy-Δ9-tetrahydrocannabinol analyte was ionized and included in the performance assessment data. The method described in this work is efficient and robust with minimal limitations. However, establishing untargeted LC-HRMS screening assays is a difficult process, and it is imperative that laboratories consider the strengths and limitations of these methods across diverse drug classes and chemical properties. Results from this work will be used to inform future assessment of the National Safety Council's Tier II drugs of abuse.

Advanced Microfluidic Technology for Automated, Rapid, and Objective Laboratory Screening of Seized Drugs

NIJ Award: 15PNIJ-21-GG-04176-COAP

Shannon Krauss | RTI International

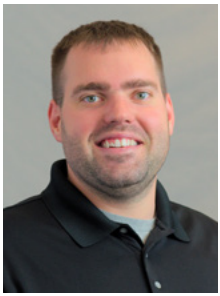
Shannon Krauss

Abstract: When samples are seized for subsequent forensic laboratory analysis, a workflow involving multiple laboratory techniques is used to achieve a sufficient level of selectivity for a scientifically supported conclusion. Color testing continues to be reported as the predominant seized drug screening method because of the advantages of low cost, rapid results, and simplicity in which color changes are observed visually. However, limitations can include the subjectivity of color interpretation, the fully manual procedure, the presence of interferents, incorrect results reported, and multiple color tests for classification, which all provide the potential for user error or unreliable results. Because these results ultimately contribute to criminal justice outcomes, it is important to explore methods aimed at increasing confidence in these tests and providing improvements to incorporate unbiased and quantifiable metrics. This presentation will discuss a method for improved automation and objective analysis through digitization to address seized drug color testing challenges. This method uses custom software that was integrated into a small-scale technology platform for sample processing and analysis. This platform enabled the translation of routine color tests (e.g., cobalt thiocyanate, Ehrlich) to capture and record the resultant color changes for downstream objective analysis in an adaptable format. This format allows for the future implementation of additional or alternative tests to address the evolving drug landscapes and regulations.

Confirming the Presence of Novel Psychoactive Substances in Forensic Samples from Medicolegal Death Investigations

NIJ Award: 15PNIJ-22-GG-04434-MUMU

Alex J. Krotulski,* Sara E. Walton, Melissa F. Fogarty, and Joshua DeBord | Center for Forensic Science Research and Education
Donna M. Papsun | NMS Labs
Barry K. Logan | Center for Forensic Science Research and Education and NMS Labs



Alex J. Krotulski

Abstract: As the recreational drug supply becomes more volatile and dynamic in the United States, it is now critical to conduct comprehensive postmortem toxicology testing in cases where drug overdose is suspected. Ideally, practitioners should seek testing using novel and innovative methods with up-to-date scopes of analysis that include traditional drugs, novel psychoactive substances (NPS), and adulterants. New synthetic drugs continue to appear in medicolegal death investigations, and it is increasingly common to encounter NPS used in combination with traditional drugs (e.g., fentanyl) to elicit more customized pharmacological effects, a phenomenon that has been confirmed though drug material testing and forensic toxicology analysis. The Center for Forensic Science Research and Education (CFSRE)—a nonprofit, federal grant–funded, state-of-the-art forensic laboratory—conducts drug testing and drug market surveillance using liquid chromatography quadrupole time-of-flight mass spectrometry and liquid chromatography tandem quadrupole mass spectrometry. In 2018, the CFSRE launched NPS Discovery—an open access drug early warning system—to develop and provide testing

resources focused on new and emerging drugs to forensic practitioners. The primary goal is to disseminate actionable new drug data to various stakeholders in public health and safety. The CFSRE maintains a battery of novel testing workflows to confirm the presence of specific drug substances emerging in forensic casework, including the latest novel benzodiazepines, opioids, stimulants, hallucinogens, and cannabinoids. In 2022, the CFSRE analyzed nearly 2,000 forensic toxicology samples. Fentanyl (63%) was the most commonly detected drug, found in combination with NPS benzodiazepines (etizolam, 24%; flualprazolam, 19%; and bromazolam, 8%). Fluorofentanyl (19%) was the most frequently detected NPS opioid but when excluded, nitazene analogues (e.g., metonitazene, isotonitazene) comprised the most detections. Dimethylpentylone (5%) was the most encountered NPS stimulant. Synthetic cannabinoid positivity was low compared with previous years, and MDMB-4en-PINACA and ADB-BINACA were the two most detected. Xylazine (11%) was commonly detected alongside fentanyl. The year-over-year drug landscape has differed greatly since 2018. Today, apart from fluorofentanyl, fentanyl analogues have been largely eradicated from the recreational opioid supply, replaced by novel nitazene analogues often accompanied by NPS benzodiazepines. The combination of opioids (traditional or novel) with NPS benzodiazepines has increased significantly as "benzo-dope" use is now common but still less than "tranq-dope" (xylazine-fentanyl) use in most jurisdictions. Synthetic cannabinoid–related fatalities decreased in the dataset because of control measures in China; however, deaths involving these drugs continue. Specific and detailed case examples will be included in this poster.

Developing an Approach to Standardize the Naming of Novel Psychoactive Substances

NIJ Award: 2020-DQ-BX-0007

Alex J. Krotulski,* Sara E. Walton, Max Denn, Brianna Stang, and Barry K. Logan | Center for Forensic Science Research and Education

Alex J. Krotulski

Abstract: Novel psychoactive substances (NPS) continue to appear in forensic casework with increasing regularity as they are mixed with or substituted for traditional drugs or purchased online as legal or alternative "highs." When NPS are detected by forensic laboratories, their name (and associated identity) is reported on the final forensic report. This information is used downstream by various local, state, and federal agencies, including medical examiner and coroner offices when certifying deaths and the Centers for Disease Control and Prevention when consolidating information on drug morbidity and mortality. Accurately reporting and tracking NPS is contingent on the proper use of nomenclature and consistency between laboratories. Mismatches in NPS naming (e.g., N,N-dimethylpentylone vs. dipentylone) can cause unnecessary confusion and mistakes in communication, interpretation, and reporting. A central authority on NPS naming is needed; however, the framework for naming must first be established. NPS nomenclature is complex, and not all substances under the NPS classification are necessarily new. Some are derived from previous pharmaceutical drug discovery patents but repurposed for illicit use, while others are "old" drugs that have resurfaced or are being used in a new or different way. Some drugs are named based on initials of the inventor and numbers based on the series in which they are discovered (e.g., JWH-018 and John W. Huffman). Some drugs are named based on abbreviations of their structure features with numbers (e.g., AP-237 and aryl piperazine). Some drugs are given fabricated names that become common language (e.g., fentanyl, etonitazene, alprazolam). The Center for Forensic Science Research and Education, through its NPS Discovery program and in collaboration with Cayman Chemical, has launched an initiative to help standardize the

manner in which NPS are named. The goal is to develop tools and techniques with enhanced workflows to name new and old drugs more accurately and comprehensively. This will allow storage and consolidation of information in a database that is easily accessible and searchable and rapid dissemination of information about the existence of drugs, literature, trends, effects, and more to the forensic science community. Currently, the Center for Forensic Science Research and Education and Cayman Chemical are developing naming resource documents for synthetic cannabinoids and NPS opioids, specifically the nitazene analogues. Synthetic cannabinoid naming is the most structured under the NPS umbrella, using a semi-systematic alpha-numeric scheme that correlates back to the structure. However, with the constant emergence of new synthetic cannabinoids, this process needs to be documented yet flexible to include evolving chemistries. The recent emergence of the synthetic cannabinoids BZO-HEXOXIZID (formerly MDA-19) and CHO-4'Me-5'Br-FUBOXPYRA (formerly CH-FUBBMPDORA) are examples of these naming efforts and ways the naming scheme has helped standardize the language across the forensic science community. For the nitazene analogues, all names are based on the prototypical drug in the series, etonitazene. Modifications to etonitazene are reflected within the name or as prefixes. N-pyrrolidino etonitazene is an example of the naming efforts within this group. The primary outcome of this initiative will allow the forensic science community to further standardize drug naming and avoid unnecessary communication issues between forensic laboratories, reporting entities, and other stakeholders.

Breath Measurements of Acute Cannabis Use (BACE): Towards Reliable Determination of Recent Use

NIJ Award: DJO-NIJ-22-RO-0003

Tara M. Lovestead,* Jason A. Widegren, and Kavita M. Jeerage | National Institute of Standards and Technology

Tara M. Lovestead

Abstract: The modern alcohol breathalyzer is a reliable indicator of alcohol intoxication based on 100 years of development. The first device, the Drunkometer, used a balloon to capture breath samples and was tested on extremely intoxicated individuals. Two decades later, the "Breathalyzer" quantified ethanol concentration in breath. Today, the National Institute of Standards and Technology (NIST) and other National Metrology Institutes provide aqueous and gaseous reference materials to deliver gases with known ethanol concentrations to evaluate hundreds of devices from dozens of manufacturers approved by the National Highway Traffic Safety Administration for evidentiary purposes. Evolution from benchtop proof of concept to reliable field use was achieved with ethanol, a small water-soluble and volatile compound with well-characterized properties. Ethanol is consumed in large quantities; a standard drink contains 14 grams. Systemic ethanol can be accurately quantified from one exhalation with current sensor technology when device temperature is controlled and protocols to eliminate mouth ethanol contamination are followed. Δ9-tetrahydrocannabinol (THC), the main psychoactive compound found in cannabis, is lipophilic, has low volatility, and is consumed in small quantities, creating challenges for quantification in breath. THC quantification from multiple exhalations has employed sampling devices designed to trap less-volatile compounds, primarily based on interception or impaction. Device processing and high-resolution mass spectrometry are typically performed in an analytical laboratory. Indirect, non-quantitative approaches that rely on machine learning algorithms have also been investigated. No matter the strategy, infrastructure akin to alcohol breathalyzers for evaluation, calibration, and quality control is not yet available. The researchers are focusing on developing reference materials and delivery

systems to deliver breath surrogates with known THC quantities to any sampling device or sensor technology to establish ground truth for the device's performance. The three-pronged approach uses (1) vapor pressure measurements of cannabinoids and cannabis-associated compounds, (2) numerical simulation to identify important parameters to control when delivering breath surrogates containing vapor and aerosol reference materials, and (3) limited human subjects studies with different devices to understand the mode of collection for THC and other compounds in breath with both targeted and untargeted analyses. Although the researchers published the first-ever vapor pressure measurements for THC and cannabidiol (CBD) in 2017, the measurement uncertainty was high, and the temperature range was limited. The researchers have since developed dynamic vapor microextraction to rapidly collect vapor samples while controlling the sample temperature, pressure, and composition. Dynamic vapor microextraction performance has been validated with vapor pressure measurements on the reference compound n-eicosane ($C_{20}H_{42}$). The relative standard uncertainty in the resulting vapor pressure data was about 2%, which is state of the art for measurements in the pressure range studied. Vapor pressure data for the cannabis-associated terpene linalool and for the cannabinoids THC, CBD, and cannabinol will be presented as well as numerical simulations of the effect of aerosol diameter and velocity on capture in an impaction filter device. These data are essential to prototyping initial reference materials and delivery systems for establishing ground truth for the performance of any device intended to determine recent cannabis use.

Application of Insights from High-Level Density Functional Theory for the Differentiation of Marijuana and Hemp

NIJ Award: 15PNIJ-22-GG-04423-SLFO

Rabi Ann Musah,* Parandaman Arathala, Megan I. Chambers, and Benedetta Garosi | University at Albany, State University of New York

Rabi Ann Musah

Abstract: Marijuana and hemp are two varieties of the same species, *Cannabis sativa*, that differ in terms of the levels of tetrahydrocannabinol (THC) present: marijuana is a Schedule I substance that contains >0.3% THC by dry weight whereas hemp, designated as an agricultural product, has ≤0.3% THC. The criminal justice implications of these designations require that accurate methods for the differentiation of hemp and marijuana be available. Although such approaches exist, there remains a need for the development of rapid alternative methods for hemp and marijuana differentiation that can be used to triage the large influx of samples encountered by forensic laboratories, so the strained resources required for THC quantification can be prioritized for further analysis of only those samples in which marijuana levels of THC are believed to be present. In this regard, analysis of the plant material by direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) has been shown to rapidly furnish a chemical fingerprint profile that readily reveals a characteristic peak at nominal *m/z* 315, which is diagnostic for protonated THC. However, THC has a number of other isomers, most notably cannabidiol or CBD (the cannabinoid most prevalent in hemp); therefore, the observation of *m/z* 315 is not itself indicative of whether the product is marijuana or hemp. Notably, in addition to the peak at *m/z* 315, a peak at nominal *m/z* 629 consistent with the presence of the protonated dimer [2M + H^+] of THC or CBD is also observed. The structures of the possible dimer complex combinations such as CBD••CBD, THC••THC, and CBD••THC were investigated by performing high-level density functional theory calculations. The results show that even though the computations revealed the THC••THC homodimer complex to be more stable than the others, studies on the effect of temperature on the population of the dimer complexes showed that the

populations of the THC••THC and CBD••THC dimers decrease as temperature increases and become negligible at ≥200 K, whereas the population of the CBD••CBD dimer increases with temperature. It was thus observed that at the temperature at which DART-HRMS analysis reveals the presence of the protonated dimer (350°C), the peak at *m/z* 629 corresponds to the most stable CBD••CBD protonated homodimer when CBD levels are high (i.e., in hemp). On the other hand, when marijuana samples with high THC content and negligible CBD content were investigated, *m/z* 629 was not observed. Subsequent analysis of marijuana and hemp samples by DART-HRMS, designated by the ≤0.3% THC (hemp) and >0.3% THC (marijuana) thresholds, revealed the predictions observed by density functional theory calculations to be true. The results show that detection of a peak at *m/z* 629 when analyzing *C. sativa* plant material by DART-HRMS could serve as a means to differentiate hemp and marijuana.

Accurate THC Determinations in Seized Cannabis-Derived Finished Products for Forensic Laboratories

NIJ Award: DJO-NIJ-22-RO-0002

Walter Brent Wilson | National Institute of Standards and Technology

Walter Brent Wilson

Abstract: Hemp was removed from the U.S. Drug Enforcement Administration controlled substances list after the 2018 Farm Bill, and hemp has been defined as cannabis containing 0.3% or less of decarboxylated Δ9-tetrahydrocannabinol (Δ9-THC). Forensic laboratories are now required to have access to reliable analytical methods for differentiating between hemp and marijuana in seized cannabis samples. In response, the National Institute of Standards and Technology (NIST) developed a Cannabis Research Program to help provide forensic laboratories the necessary tools to quantitatively measure Δ9-THC in cannabis plant and cannabis-derived finished products. This poster focuses on the optimization of a sample preparation procedure previously published at NIST for the determination of Δ9-THC in commercial hemp oil samples that required 70 minutes. The goal of the new research was to minimize the sample preparation time to make it more desirable to forensic laboratories (<15 minutes) and expand to include cannabis vape cartridges. The new sample procedure was developed and evaluated at NIST for approximately 60 commercial and seized cannabis vape cartridges. Product labels for the seized cannabis samples often included a total THC mass fraction of 80% to 90%; however, in many cases, the actual mass fractions of Δ9-THC were lower.

Moving Forward with Direct PCR: Touch DNA Samples and CODIS Eligibility

NIJ Award: 2019-DU-BX-0009

Abigail Bathrick,* Anna Salmonsen, and Jon M. Davoren | Bode Technology

Abstract: After attending this poster presentation, attendees will have a better understanding of how direct polymerase chain reaction (PCR) affects Combined DNA Index System (CODIS) eligibility for touch DNA samples and how direct PCR success rates compare with standard processing. Direct PCR is a DNA processing method in which a sample is added directly to an amplification reaction without prior extraction or quantification. This study examines the CODIS eligibility of GlobalFiler™ (GF) and PowerPlex® Fusion 6C (PPF6C) profiles obtained from touch DNA samples collected from plastic microscope slides, metal tools, handgun grips, vinyl shutters, brass cartridge casings, foam cups, concrete bricks, unfinished wooden tool handles, denim, wool, and polyester with various methods. For GF processing, collection from the non-fabric substrates was performed with Puritan® cotton swabs, Copan microFLOQ® direct swabs, and Whatman non-indicating FTA™ paper, which were either moistened with sterile water or 0.1% Triton™ X-100 or left dry. The GF work was used to identify optimal direct PCR-compatible touch DNA collection methods for each substrate for further testing with PPF6C. Puritan® cotton swabs and Copan microFLOQ® swabs moistened with sterile water or 0.1% Triton-X or left dry were used to collect DNA from the non-fabric substrates for amplification with PPF6C. Fabrics were sampled via cutting for both amplification systems. For each collection method, processing method, non-fabric substrate type, and amplification system, eight replicates were prepared from three donors. One donor was used for each type of fabric. Samples were processed with two methods: (1) standard processing with DNA extraction and quantification and (2) direct PCR. The extracted and direct PCR samples were amplified with GF and PPF6C. No changes were made to the thermal cycling parameters, reaction mixtures, or reaction volumes validated for regular casework processing (25 µL, 29 cycles). Profiles with alleles at a minimum of eight of the original CODIS core loci and match rarities of at least 1 in 10 million were considered CODIS-eligible. CODIS-eligible profiles were tallied across all collection methods for each substrate, processing method, and amplification system, and success rates were determined by calculating the percentage of profiles that were CODIS-eligible for each processing method. For GF, direct PCR produced higher CODIS eligibility success rates than standard processing for touch DNA samples collected from plastic slides, polyester, metal tools, handgun grips, foam cups, and wood tool handles. For PPF6C, direct PCR produced equivalent or higher CODIS eligibility success rates than standard processing for touch DNA samples collected from plastic slides, denim, wool, polyester, metal tools, vinyl shutters, handgun grips, foam cups, and wood tool handles. These results support previous findings that CODIS eligibility for direct PCR profiles is highly dependent on the substrate from which samples are collected and may be affected by the system used for amplification. There are advantages to using PPF6C when certain inhibitors are present and when touch DNA is collected with cotton swabs, whereas GF is advantageous when microFLOQ® swabs are used. Direct PCR results may be further improved through PCR reaction optimization and additional post-PCR cleanup steps.

Quantifying the Accuracy of Two Innovative Forensic Genetic Identification Techniques

NIJ Award: 2019-DU-BX-0028

Maria Flores | The University of California, Los Angeles

Maria Flores

Abstract: Forensic investigation of DNA samples from multiple contributors has become commonplace. These complex analyses use statistical frameworks accounting for multiple levels of uncertainty in allelic contributions from different individuals, particularly for samples containing few molecules of DNA. These methods have been thoroughly tested along some axes of variation, but less attention has been paid to accuracy across human genetic variation. Here, the researcher quantified the accuracy of DNA mixture analysis over 83 human groups. This research used Forensim, a free open-source R package, to simulate forensic genetic profiles of contributors, generate mixtures of those contributors, and calculate likelihood ratios. This likelihood ratio calculation was performed under the assumption that the genotypes of all non–person of interest contributors in the simulated mixture are known under both the defense and prosecution hypotheses. This research found higher false inclusion rates for mixtures with more contributors and for groups with lower genetic diversity. Even for two-contributor mixtures where one contributor is known and the reference group is correctly specified, false inclusion rates are 10^{-5} or higher for 56 out of 83 groups. This means that some false inclusions may be expected when multiple tests are performed. These false positives could be lessened with more selective and conservative use of DNA mixture analysis.

py_ped_sim - A Flexible Forward Genetic Simulator for Complex Family Pedigree Analysis

NIJ Award: 2019-DU-BX-0028

Miguel Guardado* and Ryan Hernandez | University of California, San Francisco
Shalom Gutierrez, Joaquín Magaña, Sthen Campana, Kaela Syas, Emily Samperio, Cynthia Perez, Bernice Chavez, and Selena Hernandez | San Francisco State University
Elena I. Zavala | University of California, Berkeley
Rori Rohlfs | University of Oregon

Abstract: Large-scale family pedigrees are heavily used across various disciplines, including human genetics and evolution. These family trees are invaluable tools for understanding genetic factors and tracing genetic ancestry over generations. However, there is currently a lack of software available to simulate different pedigree structures with genomes accurately, which limits our understanding of how genetic inheritance functions within various family contexts. To address this gap, the researchers have developed a Python command line–based tool called py_ped_sim that facilitates the simulation of pedigree structure and genomes for pedigrees. This framework represents pedigrees as directed graph data structures, enabling easy conversion between standard pedigree formats and their seamless integration with the forward population genetic simulator, SLiM. Notably, this software allows for the simulation of variable offspring count within a specific set of parents and half-siblings. The researchers validated the accuracy of this software by simulating genomes onto diverse family pedigree structures and observed that the estimated kinship coefficients closely approximated expected values. py_ped_sim is a user-friendly and open-source solution for simulating pedigree structures and conducting pedigree

genome simulations. It empowers medical, forensic, and evolutionary genetics researchers to gain deeper insights into the behavior of genetic pedigree analysis. By using py_ped_sim, scientists can comprehensively explore and understand the dynamics of genetic inheritance and relatedness within families.

DNA Typing Strategies for Identification of Human Remains via Real-Time Nanopore Sequencing

NIJ Award: 15PNIJ-22-GG-04414-MUMU

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Rupesh Kesharwani and Fritz Sedlazeck | Baylor College of Medicine
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Abstract: Short tandem repeat (STR) markers evaluated via capillary electrophoresis continue to be the gold standard for human remains identification in forensic investigations because of their high variability and robust database of comparative samples. However, capillary electrophoresis excludes valuable sequence-level information both within and around STRs and is not suitable for mitochondrial DNA (mtDNA) or single-nucleotide polymorphism (SNP) analysis, both of which are valuable in cases where STR analysis fails, such as cases of damaged and degraded remains. Human remains are frequently encountered in forensic laboratories, coming from crime scenes, mass graves, historical samples, mass disasters, and military conflicts. The problem faced by forensic laboratories when analyzing such samples is they must choose between depleting sample volumes by repeating individualizing STR analysis or performing costly, time-consuming, and less discriminatory mtDNA analysis. New DNA sequencing methodologies combined with novel enrichment techniques may provide a more effective platform for human remains identification that overcomes the most common challenges associated with the processing of bone fragments, aged tissue, and hair samples. Using the custom bioinformatic pipeline, STRspy, the researchers designed a streamlined method capable of producing reliable length- and sequence-based STR profiles from data generated on the newest and most affordable next-generation sequencing platform, Oxford Nanopore Technologies' (ONT's) single-molecule sequencer. The researchers combined this process with targeted sample enrichment via RNA probe capture for a robust analysis of forensic markers, including STRs, SNPs, and mtDNA from a single sample. The presenter will discuss the results of the project to date, detail the pros and cons of a bait capture + ONT–centered approach to human remains identification, and provide insight into the adjustments to the platform that are necessary to harness the true potential of ONT sequencing for the identification of human remains.

Optimizing the Analysis of DNA from Burned Bone Using Ancient DNA Techniques

NIJ Award: 2019-DU-BX-0044

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Adam Ben Rohrlach | University of Adelaide and the Max Planck Institute for Evolutionary Anthropology
Laura Fulginiti | Maricopa County Office of the Medical Examiner
Giovanna Vidoli and Joanne Devlin | University of Tennessee

Cody E. Parker

Abstract: Identifying human remains using DNA analyses is a vital component of forensic investigation. These highly accurate analyses generally rely on the recovery of high-quality endogenous DNA that may not be available, especially when extracting from highly degraded source material. The decomposition of DNA can alter the amount of DNA retained in source tissue and its base composition and quality, making downstream analysis problematic. As such, the field of ancient DNA analysis has invested heavily in the development of optimized protocols for sampling, extracting, and analyzing DNA recovered from archaeological remains. In a forensic context, the use of these same techniques in modern degraded skeletal samples may increase the likelihood of successful DNA identification. The exposure of tissue to extreme temperatures affects DNA recovery and quality in a similar fashion to that observed in archaeological remains. Additionally, although soft tissue may still be present, many current guidelines recommend removing and discarding this charred tissue because it is hypothesized that the extreme levels of morphological degradation render this substrate unusable for DNA identification. Here, the researchers present a systematic investigation comparing forensic and ancient DNA laboratory protocols: the Dabney 2019 extraction protocol (Dabney & Meyer, 2019) and the Lorielle 2007 protocol (Loreille et al., 2007). This study examines DNA yields across a range of levels of thermal alteration on different skeletal locations and an assessment of DNA preservation in severely charred soft tissues using the QIAGEN DNeasy® blood and tissue extraction kit. Ten donor cadavers were systematically exposed to extreme temperatures (i.e., burned) at the University of Tennessee Anthropology Research Center. From each donor, approximately 10 samples representing all regions of the body (i.e., thorax, long bones) were collected and sent to Arizona State University for processing. Each sample was then visually examined and assigned a burn score on a 1–5 scale, with 1 being the least thermally altered and 5 being the highest based on observed morphological condition. Using both extraction protocols, DNA was isolated from each skeletal sample and from corresponding tissue samples. The resulting DNA extracts were then assessed for total DNA recovery (Qubit™ HS DNA assay and Agilent TapeStation D5000 HS), endogenous DNA content (Quantifilier™ Trio), and short tandem repeat (STR) profile recovery (Promega Powerplex Fusion 6C). Preliminary results indicate that the standard DNA quantification techniques (Qubit™ fluorometry, TapeStation, and Quantifiler™ Trio) are not reliable predictors of actual DNA recovery. However, the detection of any DNA using these metrics does directly correlate to successful STR profile recovery. Additionally, this study found that charred tissue samples consistently returned higher concentrations of both raw and endogenous human DNA and more robust STR profile recovery. This indicates that a re-evaluation of previously established sampling guidelines for severely thermally altered remains recommending the removal of this substrate may be necessary moving forward. In terms of the skeletal samples, the complete

demineralization protocol developed by Lorielle et al. (2007) generally performed well at lower to medium levels of thermal alteration whereas the ancient DNA Dabney protocol (Dabney & Meyer, 2019) was more suited for STR profile recovery at higher estimated levels of thermal alteration.

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Species Identification in Forensic Casework Using Proteomics

NIJ Award: 15PNIJ-22-GG-03566-SLFO

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Nicole Slattengren and Ashley Spicer | California Department of Fish and Wildlife
Barry Baker | United States Fish and Wildlife Service

Glendon Parker

Abstract: A significant portion of wildlife crime is focused on the international trafficking of furs from endangered and protected species. Protection for these species includes the international Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) treaty, enforced through the Federal Endangered Species Act, and state-level regulations such as the California Endangered Species Act and the California Fur Ban (FGC §2023). These furs are not amenable to DNA typing because of the harsh chemicals used in fur processing that degrade DNA, rendering the fur unusable for species identification. To address this gap in law enforcement, this project is developing proteomic workflows to identify protein markers for species identification of fur from a single hair shaft. The Proteomic Fur Project is engaged in processing 45 species, 29 of which are relevant to the California Fur Ban, and 16 *Felidae* species that are a focus of the United States Fish and Wildlife Office of Law Enforcement. Two approaches are being used: (1) the measurement of relative reference proteome efficiency in aligning peptide sequences to data and (2) the identification, discovery, and characterization of species-specific peptide biomarkers. One example is muskrat (*Ondatra zibethicus*), a major economic driver of the wild-caught fur trade. Triplicate hair shafts from three individuals were processed (n=9) and submitted to mass spectrometry. Resulting spectra were aligned with peptide sequences from a custom muskrat reference proteome. A total of 17 peptides from 11 proteins were detected and aligned to the muskrat proteome with 100% specificity and sensitivity. The average intensity of the top nine peptides was greater than 109 ions, four orders of magnitude above the detection limit. These candidate peptide biomarkers will help establish sensitive, specific, and robust targeted assays for species identification of degraded samples and therefore represent a powerful new tool for wildlife forensic casework.

Testing New Methods for Degraded DNA Recovery and Next-Generation Sequencing

NIJ Award: 2018-DU-BX-0218

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Laura Fulginiti | Maricopa County Office of the Medical Examiner
Adam Ben Rohrlach | University of Adelaide and the Max Planck Institute for Evolutionary Anthropology

Erin M. Rawls

Abstract: Attendees will learn how technical advances used to isolate and sequence genomic DNA from ancient remains opens new doors for the analysis of modern degraded tissue. The presenter will discuss the efficiency of isolating genomic DNA using ancient DNA and modern forensic extraction methods and their success rates for subsequent short tandem repeat (STR) and mitochondrial genome analyses. Attendees will also learn about DNA damage patterns as assessed using bioinformatic analyses of genomic data revealing how hot desert environments impact DNA preservation in skeletal remains in the American Southwest. According to the National Missing and Unidentified Persons System (NamUs), there are over 600,000 unidentified human remains in the United States as of February 2023. On average, 4,400 are added each year, of which roughly 1,000 remain unidentified. Degradation of these remains presents technical challenges for their identification by researchers and government agencies alike. Techniques used to isolate ancient DNA from archaeological samples could be efficient in cases of highly degraded forensic remains. For example, a method for DNA extraction (using guanidine hydrochloride) was developed by Dabney et al. (2019) to recover DNA fragments as small as 30–50 bp in size. This method has been used to successfully recover analyzable mitochondrial DNA (mtDNA) data from paleoanthropological samples as old as ~400,000 years. For this study, the Maricopa County Office of the Medical Examiner provided 75 skeletal samples representing 42 individuals who have remained unidentified by standard forensic procedures, such as STRs. DNA from bone and teeth samples was extracted using the Dabney protocol and a forensic protocol developed by Loreille and colleagues (Loreille et al., 2007) for degraded samples. The DNA extracted was used to create double- and single-stranded libraries. These libraries were then used for targeted enrichment of the mtDNA genome performed using biotinylated mitochondrial RNA baits synthesized from the H. sapiens Representative Global Diversity Panel (197 mtDNA sequences) (Daicel Arbor Biosciences, Ann Arbor, MI). Single-nucleotide polymorphism (SNP) capture was completed using a custom SNP panel targeting ~4,200 SNPs (Daicel Arbor Biosciences, Ann Arbor, MI). These enriched libraries were then subjected to Illumina® sequencing. The researchers found that the Dabney extraction method resulted in an average 4.4-fold improvement in DNA yield compared with the Loreille extraction method. From the double-stranded DNA libraries, the researchers generated mitochondrial genomes ranging from 0.3–246.8× depth of coverage with average fragment sizes of 89 bp from 62 samples. Sequencing reads were not recovered from 13 samples, likely because of a lack of sufficient DNA. Analyses of the mtDNA sequence data from the single-stranded libraries and the sequencing of the genome-wide SNP enriched libraries are currently underway. Using these data and additional analyses of DNA damage patterns and preservation across skeletal elements and environmental contexts, the researchers aim to identify the optimal means of DNA recovery from degraded skeletal tissues.

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