RTI Press

Conference Proceedings

April 2021

2021 National Institute of Justice Forensic Science Research and Development Symposium

Nicole S. Jones and Erica Fornaro, Editors

RTI INTERNATIONAL

RTI Press publication CP-0013-2104

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Suggested Citation

Jones, N. S., and Fornaro, E. (Eds.). (2021). *2021 National Institute of Justice Forensic Science Research and Development Symposium*. RTI Press Publication No. CP-0013-2104. https://doi.org/10.3768/rtipress.2021.cp.0013.2104

This publication is part of the RTI Press Conference Proceedings series.

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This forum was funded through a cooperative agreement from the National Institute of Justice (2016-MU-BX-K110), Office of Justice Programs, US Department of Justice. Neither the US Department of Justice nor any of its components operate, control, are responsible for, or necessarily endorse, this forum. The opinions, findings, and conclusions or recommendations expressed in this forum are those of the presenter(s) and do not necessarily reflect those of the Department of Justice. Any products and manufacturers discussed are presented for informational purposes only and do not constitute product approval or endorsement by the US Department of Justice.

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About the Editors

Nicole S. Jones, MS, is the director of the Innovation, Scientific Growth and Training (INSIGHT) Program in the Center for Forensic Sciences (CFS) at RTI International.

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Abstract

The 2021 National Institute of Justice (NIJ) Forensic Science Research and Development (R&D) Symposium is intended to promote collaboration and enhance knowledge transfer of NIJ-funded research. The NIJ Forensic Science R&D Program funds both basic or applied R&D projects that will (1) increase the body of knowledge to guide and inform forensic science policy and practice or (2) result in the production of useful materials, devices, systems, or methods that have the potential for forensic application. The intent of this program is to direct the findings of basic scientific research; research and development in broader scientific fields applicable to forensic science; and ongoing forensic science research toward the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

Introduction

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

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

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

The FTCoE is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of more than 5,000 providing research and technical services to governments and business 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.

On February 16, 2021, NIJ and the FTCoE held the 2021 NIJ Forensic Science Research and Development (R&D) Symposium. This event was held in conjunction with the American Academy of Forensic Sciences' 73rd Annual Scientific Meeting. Hundreds of attendees joined us online for this all-virtual event to learn about NIJ research awards given to several talented researchers spanning the forensic disciplines.

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

This year, members of the NIJ Office of Investigative and Forensic Sciences R&D team—including program managers Gregory Dutton, Danielle McLeod-Henning, and Frances Scott—have worked to create a phenomenal research agenda. The full-day program included 26 presentations and 21 posters from principal investigators and their research partners; these presentations and posters represent accomplishments from NIJ R&D grants awarded during 2015–2019. In Track I, Frances Scott moderated the Seized Drugs and Toxicology session, and Danielle McLeod-Henning moderated the Forensic Anthropology and Forensic Pathology session. In Track II, Gregory Dutton moderated the Impression and Pattern Evidence/Trace Evidence session and the Forensic Biology/DNA session. Most of the presentations are archived on the FTCoE's website and are available to view for free.

SESSION ABSTRACTS

TRACK I, SESSION I

SEIZED DRUGS AND TOXICOLOGY

Moderated by NIJ Program Manager
Frances Scott

The Rapid Forensic Identification of Psychoactive Plant Types by Multivariate Data Analysis of a DART-MS Plant Database, Featuring a User-Friendly Graphical User Interface

NIJ AWARD #: 2015-DN-BX-K057

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The abuse of "legal high" psychoactive plants is a worldwide public health concern that exposes users to dangerous health consequences and even death. A major challenge for law enforcement in regulating widespread abuse of these plants is the paucity of methods by which to identify them. In general, identification of psychoactive plants is limited to only a few species using methods such as color tests and microscopic evaluation, chemical/biochemical methods, and DNA. Although DNA analysis is the gold standard, the genomes for most of the relevant psychoactive plants have not been mapped; therefore, this approach cannot always be used. By and large, the other tests are presumptive and definitive identification is laborious and time-consuming, meaning cases involving these substances are not prosecuted. Developing accurate, fast, efficient, and cost-effective techniques for forensic identification of psychoactive plant materials is crucial. Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) was investigated as an approach for building a species-specific chemical signature database that could be mathematically interrogated to reveal differentiation between and identification of plant species. The rapid acquisition of DART-HRMS mass spectra (i.e., a few seconds per analysis) and the ability to analyze the materials in their native form, without pre-treatment steps, enabled the generation of the vast number of spectral replicates required for database construction. A machine learning–based workflow was implemented in Python for the generation of a discrimination model and identification of plant unknowns. An interactive graphical user interface (GUI) was also developed to simplify the workflow usage for identification of plant materials for end users.

To create the database, 54 psychoactive species—including plants such as *Mitragyna speciosa* (i.e., Kratom) and *Salvia divinorum* (i.e., diviner's sage), representing a range of sample types that included flowers, stems, seeds, leaves, roots, extracts, and brews—were analyzed by DART-HRMS in multiple replicates. A DART-SVP ion source (IonSense, USA) coupled with a JEOL AccuTOF high-resolution time-of-flight mass spectrometer (JEOL USA) operating in positive ion mode was used to collect soft-ionization mass spectra in the m/z 40–800 range. The spectra were corrected for background and mass shifts and aligned along common m/z values for further multivariate analysis. A hierarchical classification tree, divided into three levels (i.e., family, genus, and species), was designed—based on taxonomic relationships between plant species—to reduce the 54 multiclass problem into several simplified multiclass problems. Supervised classifiers such as support vector machine (SVM), linear discriminant analysis (LDA), and k-nearest neighbor (KNN) were used to train the discrimination models in the nodes of a classification tree, and their outputs were fused using the decision fusion approach for sample prediction. Performance analysis using fivefold cross-validation revealed the hierarchical

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

classifier to have 95% prediction accuracy for test samples. Therefore, it enabled plant species identity to be predicted from the raw DART mass spectra of unknowns, despite the complexity of their matrices. The developed screening tool can be readily utilized by crime labs and forensic scientists and does not require sample preparation steps or knowledge of botany.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Increasing Safety, Speed, Sensitivity, and Selectivity of Controlled Substance Analysis

NIJ AWARD #: 2018-DU-BX-0165

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With the influx of synthetic opioids and other novel psychoactive substances into forensic casework over the last several years, laboratories are being required to complete a higher volume of increasingly complex cases containing increasingly toxic chemicals. These issues have translated to growing burdens on laboratories that are facing mounting backlogs. The goal of this project was to re-envision the current analytical caseflow of drug evidence and evaluate if a re-envisioned workflow can lead to increased safety, speed, sensitivity, and selectivity of the examination. The re-envisioned workflow will replace screening samples using colorimetric tests and GC-FID analyses with screening via direct analysis in real-time mass spectrometry (DART-TOF MS) followed by a targeted gas chromatography mass spectrometry analysis. The optimized methods were developed using both fractionated factorial design of experiments and response surface methodology along with other existing statistical processes for establishing reproducibility, repeatability, and sensitivities. Current and re-envisioned workflows will be compared through blind evaluations of 40 samples designed and collected to test common and challenging cases that have been observed in the laboratory. Workflow comparison metrics include time for analysis (analyst time and instrument time), sensitivities, safety, false positive and false negative rates, and overall workflow performance.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Determining the Quality of Mass Spectral Library Searches Using a Quantitative Reliability Metric

NIJ AWARD #: 2018-DU-BX-0184

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Mass spectral library searches are central to identifying unknown compounds in forensic science. Generally, the mass spectrum of an unknown compound is compared with a collection of reference spectra during the library search. The closest matching spectra can then be used to identify the compound. The advantage of a mass spectral library search is that it provides empirical results. However, the misidentification of compounds or the inability to identify a compound resulting from the hit list could present challenges for the analyst. These challenges are often caused by poor mass spectral quality arising from low concentration samples or impurities and background noise. Identification difficulty may also arise from the sparsity of reference standards in the resulting library data. To measure the quality of each library search result independently, a Quantitative Reliability Metric (QRM) was developed. The QRM uses an intra-library comparison of the spectra within the inter-library hit list to provide a quality score of 100% for reliable or ideal matches and lower scores for unreliable results or when reference spectra are missing from the library. The QRM method was applied to library searches for opioids. However, it can be used for any class of drugs or applied to any database to query reference data. The targeted compounds were opiates, both semi-synthetic and synthetic opioids frequently identified through forensic analysis in the Greater Houston Area. In this presentation, we discuss the development and validation of the QRM method. This includes the development of an automated pipeline for chromatographic peak detection and column bleed removal from the mass spectrum prior to the library search.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Characterization of the Vapor Profile of Fentanyl and Related Analogs for Instrumental and Canine Detection

NIJ AWARD #: IAA-2019-20310-DC-DU

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In recent years, there has been an escalation in fentanyl abuse as indicated by a 65% increase in fentanyl reports submitted to the National Forensic Laboratory Information System (NFLIS) from 2016 to 2017, making fentanyl the fifth most frequently identified drug at crime labs by NFLIS in 2017, and drug seizures continued to increase through 2019. The ability to detect bulk product as it crosses our borders and prior to its street distribution is an important part of defeating the problem. However, the high potency of fentanyl is dangerous to users as well as law enforcement officers. Given this current environment, it is imperative to provide a safe and effective method for field detection of fentanyl and related substances for law enforcement officers and first responders. To minimize the risk, noncontact detection methods like vapor detection are preferred. Vapor detection has the benefit of being noncontact and non-intrusive; however, vapor sampling is only feasible when the target vapor is present at concentrations high enough to be detected. Although the low volatility of target analytes like pharmaceuticals can limit instrumental vapor detection, this can be overcome through preconcentration sampling such as using solid phase microextraction (SPME). Biological detectors such as canines are other highly efficient field vapor detectors, and are frequently used in the detection of low volatility analytes, such as explosives and narcotics. Canine detectors overcome low vapor availability by detecting the odors associated with the parent molecule instead of the parent molecule itself. The collection of volatile organic compounds associated with the parent material can be referred to as its vapor signature. This presentation will impact the forensic community by describing the research to determine the vapor signatures of fentanyl and related analogues, and attendees will learn the methods used for the headspace analysis as well as similarities and differences in the vapor signatures of differing fentanyl and fentanyl-related samples. SPME was used to extract volatiles from the headspace of solid fentanyl samples with analysis by gas chromatography/mass spectrometry. Following method optimization, the headspaces of fentanyl samples to include pharmaceutical-grade and street exhibits were measured. Analysis also included a lot-to-lot comparison of the pharmaceutical-grade material, in addition to evaluation to fentanyl analogs. Finally, forced degradation experiments, including thermal, oxidative, and acid degradation, were carried out to determine the origin of the analytes making up fentanyl's vapor signature. A number of analytes were identified in the headspace of solid fentanyl. Analytes making up the vapor signature of fentanyl included benzaldehyde, pyridine, aniline, N-phenylpropanamide, and N-phenyl-4-piperidinone. In future research, the identified vaporous analytes will be used as targets for detection.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Chemical Foundations for a Cannabis Breathalyzer: Vapor Pressure Measurements and a Pilot Breath Collection Study

NIJ AWARD #: NIST IAA DJO-NIJ-19-RO-0008

Rapid decriminalization of cannabis by state governments has led to extensive research and development to invent a cannabis breathalyzer for law enforcement to identify intoxication in a field environment. Several versions of breath collection devices are being marketed for the detection of Δ9-tetrahydrocannabinol (Δ9-THC) in the breath of cannabis users. Alcohol breathalyzers are based on decades of breath-ethanol analysis in thousands of human subjects. At the legal limits of ethanol-breath concentrations, breath can have over an order of magnitude more ethanol than other volatile organic compounds. Alcohol breathalyzers can also determine impairment with as little as one breath. This is due to ethanol's high solubility in blood and very high vapor pressure. These thermophysical and chemical properties are necessary to estimate the partitioning of a compound between blood and breath and the abundance of a compound in breath. It is difficult to measure the thermophysical and chemical properties for terpenes and cannabinoids because of very high boiling points, high molecular weights, or chemical instability. Herein, I present dynamic vapor microextraction, a method specifically designed for measuring the vapor pressures of cannabinoids and terpenes. I will present the first-ever vapor pressure measurements of THC and CBD (cannabidiol) and the vapor pressures of the terpene linalool. The vapor pressure measurements of THC are approximately eight orders of magnitude lower than that of ethanol at 40°C. Finally, I will discuss our partnership with colleagues at the University of Colorado Boulder to obtain breath samples before and after cannabis use. We are examining these samples for cannabinoids, terpenes, and other potential biomarkers that may specifically indicate recent use. Due to the extremely low vapor pressure of THC measured in our laboratory, we have selected impaction filter devices. During this presentation, I will discuss our pilot breath collection study and present the preliminary results.

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https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Evaluation of Pre-Treatment Parameters in Forensic Hair Testing Using Statistical Design of Experiments (DoE)

NIJ AWARD #: NIJ-2018-75-CX-0037

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There are many differing opinions regarding the optimal methods for hair analysis, especially regarding the decontamination and extraction processes. The statistical technique known as design of experiments (DoE) is useful for assessing pre-treatment methods by evaluating both the individual roles and the combinatorial associations between multiple factors. The most effective decontamination parameters for fentanyl were evaluated. Blank de-identified hair (30 mg) was weighed into an Eppendorf tube and contaminated by adding fentanyl in solution before drying in the vacufuge. The samples underwent decontamination parameters determined using a 24 fractional factorial design. Each sample was randomly assigned specific design points made up of combinations of factors of interest in decontamination protocols. These included aqueous solvent (0.1% SDS or HPLC water), organic solvent (dichloromethane or methanol), and the number of consecutive aqueous or organic washes (three or one). Blocking factors were assigned as sequence of washes (organic first or aqueous first) and wash time (30 minutes or 30 seconds). Wash solutions were analyzed using an Agilent® 1290/6460 LC-QqQ-MS. The optimal extraction parameters for methamphetamine, oxycodone, alprazolam, and nordiazepam were assessed. Samples (20 mg) of Authentic HRM containing drugs of interest were weighed into steel milling jars. The samples underwent extraction parameters determined using a 23 full factorial design. Factors of interest included extraction solvent/sample size ratio (12.5 or 25 µL/mg), particle size (i.e., pulverized into a powder using a ball mill with milling beads for 30 seconds or cut into snippets with scissors), and extraction time (2 or 24 hours). The samples were extracted using a solvent swelling technique, transferred into Eppendorf tubes, and centrifuged for 30 minutes. Post-centrifugation, the eluent was subjected to solid phase extraction using a mixed-mode C18 cartridge prior to LC-QqQ-MS analysis. Analysis of variance (ANOVA) F-tests were performed post-analysis to determine if the parameters were significantly different. The F-test completed in the fentanyl decontamination study indicated that the combinations of factors were more significant than the factors on their own. The F-tests completed in the extraction studies indicated that high-level interactions, such as interactions between two to three factors (methamphetamine and oxycodone), as well as all interactions (alprazolam), were significant. These data suggest that studying variables individually and in combination with each other is important to the evaluation of pre-treatment parameters in forensic hair analysis methods. A consensus statement was made based on the design points with the highest percent recovery to determine which parameters were most effective. The optimal method determined for removing fentanyl from the surface of hair includes one 30-second wash with dichloromethane followed by three 30-second washes with water. The best extraction methods included pulverizing the hair prior to a 2-hour extraction in 12.5 µg/mg extraction solvent (oxycodone and alprazolam), pulverizing the hair prior to a 24-hour

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

extraction in 12.5 µg/mg extraction solvent (nordiazepam), and cutting the hair into snippets prior to a 2-hour extraction in 12.5 µg/mg extraction solvent (methamphetamine). In conclusion, DoE is a valuable approach for determining effective pre-treatment protocols for forensic hair analysis.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Identification of Phase II Opioid Metabolites in Human Hair

NIJ AWARD #: 2019-DU-BX-0021

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RTI International

Although drugs and some metabolites can be measured reliably in hair, it can be difficult—if not impossible—to differentiate drugs deposited in hair because of actual use of the parent drug from drug that is present due to external contamination. External contamination may occur when an individual is in the company of users or from environmental exposure in contaminated environments. Wash procedures are commonly employed as a step to minimize the impact of external contamination; however, there are no standardized wash procedures and washing may incorporate drug into the internal matrix of the hair shaft.

The purpose of this project is to look for unique metabolites in hair that are indicators of consumption in addition to, or in place of, the parent drugs. Phase II conjugated metabolites are ideal markers of use because they are not products of common degradation pathways, as is the case for many Phase I metabolites, and are not commercially available for the purposes of therapeutic use or abuse.

Using hair specimens previously analyzed at a commercial reference testing laboratory, we performed exploratory research using a variety of LC-MS/MS acquisition modes to look for the presence of conjugated metabolites in hair from drug-positive specimens. Our previous targeted research has shown the presence of morphine-3-glucuronide, morphine-6-glucuronide, hydromorphone-3-glucuronide, codeine-6-glucuronide, and oxymorphone-3-glucuronide in user human hair specimens. We have expanded this targeted method to include 6-acetylmorphine-glucuronide. We used a focused metabolomics approach using neutral loss and precursor ion scanning, multiple reaction monitoring, and selected ion monitoring to provide complementary analyses for determination of Phase II glucuronides and other potential Phase II metabolites. Research started with the opioid class of drugs in which hair specimens were selected with concentrations above the proposed National Laboratory Certification Program Guidelines of 200 pg/mg. This presentation will provide an overview of the analytical methods and rationale for choosing these acquisition modes. We will discuss the extraction method used for the opioid drug class and provide insight into identification of unique Phase II metabolites in user hair specimens.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Development and Validation of Two Automated Sample Preparation Techniques for the Comprehensive Screening for Biological Matrices Using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry: A Correlative Analysis of Drug Recognition Expert Evaluations and Forensic Toxicology Results in Suspected Driving Under the Influence of Drugs Cases

NIJ AWARD #: 2018-DU-BX-0168

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Forensic toxicology analyses rely heavily on results obtained from qualitative screening methods. Given the limitations of these methods, an individual case sample tends to require multiple analyses to obtain comprehensive qualitative information. In conjunction with the utilization of multiple analytical methods, sample preparation can be laborious and time-consuming. Automated sample preparation and liquid chromatography–high resolution mass spectrometry (LC-HRMS) can be employed to comprehensively evaluate biological samples for toxicologically significant drugs and metabolites using a single analytical technique. The Toxicology Section of the Virginia Department of Forensic Science (DFS) evaluates biological specimens for the presence of drugs in criminal matters, including driving under the influence of alcohol/driving under the influence of drugs and death investigations. Over a 2-year period, DFS Toxicology observed a 32% increase in the number of qualitative and quantitative drugs reported. Given the number of qualitative and quantitative drugs reported annually and the variety of drugs evaluated, it is essential that a screening technique be comprehensive, efficient, and robust to decrease laboratory turnaround times without limiting the scope of testing. DFS has developed a fully automated solid phase extraction (SPE) sample preparation technique to be used for the comprehensive screening of antemortem and postmortem biological matrices using LC-HRMS. The SPE utilizes 0.5 mL of biological matrix that is extracted and eluted into two fractions for analysis. The in-progress validation will meet ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. Additional method validation experiments will be conducted, including establishing acceptance criteria such as the utilization of mass match, mass accuracy, mass spacing, and mass abundance scoring. These criteria, among others, are critical for establishing false positive and false negative rates. To further evaluate the validated method, over 400 biological matrices will be evaluated using the LC-HRMS method and the results confirmed utilizing other previously validated confirmation and quantitation techniques. Through validation and the analysis of authentic biological specimens, the fully automated SPE extraction is intended to offer a streamlined approached to the comprehensive screening of biological matrices within forensic toxicology laboratories.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

TRACK I, SESSION II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Moderated by NIJ Program Manager
Danielle McLeod-Henning

A DNA Barcoding Strategy for Blow and Flesh Flies Encountered During Medicolegal Casework

NIJ AWARD #: 2019-DU-BX-0022

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Accurate insect identification is critical to estimating time of colonization and postmortem interval during medicolegal death investigations. Insect specimens are currently identified by evaluating morphological characteristics as indications of particular taxonomic groups; however, this process is limited because immature life stages typically lack distinguishing morphologies. Identification may be achieved by rearing live specimens; however, this process is time-consuming, labor-intensive, and not always successful. These deficiencies may be addressed through molecular identification by DNA "barcoding" wherein DNA sequences from unknown samples are matched to references. This technology enables identification of immature specimens, may be performed without specialized forensic entomology training, and requires equipment common to forensic genetics laboratories. DNA barcoding has been demonstrated in numerous entomological surveys of forensically relevant species; however, the technology has not been implemented for medicolegal death investigations. This is partly due to deficiencies in the technology: no single primer set is capable of distinguishing all of the diverse species important to forensic investigations. Instead, multiple primer sets and sequencing reactions are utilized to maximize the species that may be identified. We propose a simplified DNA barcoding strategy for identifying insects commonly encountered in casework at Harris County Institute of Forensic Sciences (HCIFS). The strategy comprises sequencing and phylogenetic analysis of a single barcoding fragment amplified from the mitochondrial COI locus. Using verified reference specimens, we show that the DNA barcoding strategy enables statistically supported identification of species previously encountered in our agency's medicolegal death investigations, in particular, members of blow fly genera *Lucilia, Calliphora*, *Chrysomya, Phormia*, and *Cochliomyia*, the flesh fly genus *Blaesoxipha*, and the scuttle fly genus *Megaselia*. The strategy is advantageous over previous methods in the literature because all target species may be amplified using a single primer set. Identification is demonstrated for larvae and pupae collected during past HCIFS medicolegal death investigations for which species-level identification was undetermined by morphology. Constructing a database of COI sequences from local specimens is an ongoing project. This may be used to provide additional statistical analyses, in particular the interspecific and intraspecific sequence variations, enabling comparisons to local blow, flesh, and scuttle fly populations. This presentation will impact the forensic science community by demonstrating a simplified method for DNA barcoding as a tool for medicolegal death investigations. Future work will include the continued collection of local population data and elucidation of respective interspecific and intraspecific sequence variations. In addition, formal internal validations will be conducted to support casework application.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

The Impact of Drugs on Human Decomposition and the Postmortem Interval: Insect, Scavenger, and Microbial Evidence

NIJ AWARD #: 2018-DU-BX-0180

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Data from the Centers for Disease Control and Prevention show that most Americans take at least one drug (including illicit drugs, prescriptions, and other medications) regularly. This means that deceased individuals in the medicolegal system likely have drugs in their system at the time of death, creating a complex postmortem toxicological profile. Although some studies have examined the effects of certain drugs of abuse on insect development for postmortem interval estimates, no study has addressed the impacts on the decomposers of human remains for a wide range of drugs expected to be used near end-of-life in combination with the diseases they were used to treat. Thus, this study aimed to correlate the presence of drug metabolites within the human body to the diversity and development of necrophagous insects and enteric and soil microbe communities, as well as scavenger preference. Twenty human donors to the Forensic Anthropology Center were placed at the Anthropology Research Facility (ARF) from Spring 2019 to Spring 2020. Peripheral and central blood serum samples were obtained at intake from each donor to screen for drug metabolites in the body at the time of death. Donors were placed on the soil surface at ARF and allowed to decompose naturally. Photos and observations of insect and scavenging activity were taken daily; soil, maggot, and body fluid samples were periodically taken until active decomposition was complete. Game cameras captured scavenging activity, and data loggers tracked temperature and humidity at the site of each donor. Postmortem ultra-high performance liquid chromatography-high resolution mass spectrometry toxicological analysis was performed on donor serum, decomposition fluid, maggots, and soil over the course of decomposition to assess what drug metabolites were present and how they passed from the body to the environment; these results were validated using commercial drug standard stocks. Preliminary results show that it is possible to not only trace parent drugs but also drug metabolites from a decomposing body all the way to its decomposers. As expected, metabolite relative concentrations decrease as they pass through the different matrices. Soil analyses indicate that soil chemistry and microbe communities have different characteristics based on the disease categories of the donors. For instance, individuals with respiratory diseases result in greater soil salt concentrations (as measured by electrical conductivity) during decomposition than non-respiratory challenged individuals, whereas donors with cancer had lower microbial diversity in the soil compared with individuals without cancer. Finally, preliminary analyses indicate that fly colonization and maggot growth may be delayed and species richness decreases under certain drug loading and disease conditions. These analyses indicate that antemortem characteristics of donors have significant impacts on their own decomposition processes as well as the biology and ecology of the organisms that colonize them after death and are ultimately responsible for decomposition rates and trajectories. Such inter-individual variation is not currently taken into account in PMI estimation methods. Our focus on examining intrinsic explanations for this variability is critical for improving estimations of PMI across many forensic disciplines.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Modeling the Fluvial Transport of Human Remains in the Sacramento River, California

NIJ AWARD #: 2016-DN-BX-0159

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The fluvial transport of human remains is a major area of interest within forensic anthropology. Fluvial transport focuses on the potential of a riverine system to physically move human remains downstream. Missing persons cases involving riverine systems pose major challenges to law enforcement because human remains may be moved a long distance from their initial point of entry, especially under high flow rate conditions. To date, very little research has been conducted to model the movement of human bodies in riverine systems, like the Sacramento River, which is California's largest fluvial system. Flow rates are regulated by Shasta Dam at the river's source and are influenced by the numerous tributaries that feed into the river and by the physical characteristics of the river. The highest flow rates occur during winter storm events and the lowest during late fall after irrigation season has ended. Each year, the river claims numerous victims, with most deaths caused by accidental drowning. However, a small number of bodies recovered from the river represent suicides, homicides, or have an undetermined manner of death. Although the Sacramento River spans eight counties, the project area includes five counties from the southern half of the Sacramento River. This research adapts an existing hydraulic model (Hydrologic Engineering Center's River Analysis System; HEC-RAS) to generate a predictive model of fluvial transport rates of victims who entered the river with known dates and locations under low, medium, and high flow rate conditions. The HEC-RAS was originally designed for flood control management but can also simulate advective and dispersive transport of water quality constituents. The HEC-RAS model treats a human body as a "pollutant" and can be used to predict distance from the source given a number of parameters, such as flow rate conditions and river channel properties. The model is calibrated using two sources of information: historical case data on river victims collected from sheriff-coroner's offices and data generated from rescue manikins placed in the river under different flow rate conditions. Historical data were collected on 150 river victim cases from three counties in the project area (spanning 1972–2012), 62 (41.3%) of which had known postmortem interval and transport distance data. This initial stage of data collection showed that most victims are male, and most deaths are accidental. A moderate relationship between postmortem interval and transport distance was found, but postmortem interval only explained a small part of the variation in transport distance. To supplement the historical data, this study uses two types of rescue manikins to simulate the body positions of "floaters" and "sinkers." Data from controlled releases of the floating manikins have been collected under different flow rate conditions from six locations along the Sacramento River. The results demonstrate that river curvature and flow rate play a large role in the route of travel, with repeated tests in the same locations showing similarity in the path the body travels. The project also uses sinker manikins to study transport along the riverbed. Data collected at four locations have shown a significant relationship between type of riverbed substrate and the flow rate and transport distance.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Skeletal Trauma Research in Forensic Anthropology

NIJ AWARD #: 2019-DU-BX-0040

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Forensic anthropologists contribute to the criminal justice system through expert testimony, the majority of which is skeletal trauma analysis and interpretation. The Organization of Scientific Area Committees Anthropology Subcommittee has identified a major gap in the current knowledge and methods of skeletal trauma research, analysis, and interpretation. Although there has been a major push in forensic anthropology to incorporate biomechanical principles into the understanding of blunt force trauma injury analysis, forensic anthropologists are still lacking the necessary data to conduct comprehensive bone trauma analysis with confidence or error rates. This baseline for interpreting skeletal trauma and providing scientific testimony does not satisfy the Daubert guidelines that require (1) validated studies, (2) peer review, (3) known or potential error rate, and (4) general acceptance. This research—conducted in collaboration with forensic anthropologists and biomedical engineers—provides controlled experimental bone trauma data; the research focuses on fracture mechanics to improve the validity of skeletal trauma analysis and interpretation through precise and repeatable analytical methods in an effort to fill the identified gap.

The goal of this research is to validate the relationship between long bone fracture characteristics and injury mechanisms by quantifying the differential effects of intrinsic and extrinsic variables on fracture characteristics. Two specific goals will be addressed throughout this research project. First, to analyze relationships between skeletal fracture characteristics and intrinsic variables of the individual or the tibia (e.g., age, sex, cross-sectional geometry), as well as evaluate covariation of intrinsic variables. Second, to analyze relationships between skeletal fracture characteristics and extrinsic experimental variables (e.g., loading rate, loading direction).

This innovative project is the first large-scale study (n=100) to conduct dynamic bone fracture experiments to analyze multifactorial predictors of fracture characteristics. The originality of the project is the strategic test matrix that allows for the evaluation of many common assumptions that form the current foundation of simple qualitative trauma analysis. The expected outcomes of this work are (1) quantifiable error rates for trauma analysis based on controlled experimental blunt force trauma testing with a known mechanism and (2) intrinsic and extrinsic explanations for why certain fracture characteristics are observed. These results will have a strong impact by providing forensic anthropologists validation for their expert testimony that satisfies Daubert standards and strengthens their position as forensic experts among the community at large. Furthermore, the creation of a public online forensic anthropology skeletal trauma (FAST) database will perpetuate rigorous training of skeletal trauma analysis for students and professionals at all levels.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

To date, all specimens for the first goal have been procured; pre-test imaging is complete; and development of sample preparation, instrumentation, and testing protocols have been established. At present, we are in the experimental testing and data collection phase of the first goal. Concurrently, the creation of the FAST database is being conducted with a focus on layout and code development. Preliminary data analysis for the first goal will be available for presentation and discussion.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Post-Mortem Iris Recognition

NIJ AWARD #: 2018-DU-BX-0215

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Postmortem biometrics entails utilizing the biometric data of a deceased individual for determining or verifying human identity. Because of fundamental biological changes that occur in a person's biometric traits after death, postmortem data can be significantly different from antemortem data, introducing new challenges for biometric sensors, feature extractors, and matchers. This presentation will address the problem of using iris images acquired after death for automated human recognition and will summarize the National Institute of Justice–funded research efforts aimed at designing a software tool and methodology for enhancing unidentified decedent systems with postmortem automatic iris recognition. The lecture will present results that are unique in several ways. First, it will discuss automatic iris recognition results in a scenario when gallery images are acquired before death (perimortem images) and the probe images are acquired after death from the same subjects. Second, results will be presented from the largest database of perimortem and postmortem iris samples, collected from more than 400 subjects by two independent institutions located in the United States and Poland. Third, the postmortem recognition viability will be assessed using more than 20 iris recognition algorithms, ranging from the classic (e.g., Gabor filtering-based) to the modern (e.g., deep learning–based) approaches, including methods designed by Czajka's research team specifically for the application of iris recognition in forensics. Finally, based on lessons learned in this project, Czajka will provide key directions for future research.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Understanding the Pathology of Homicidal Pediatric Blunt Neurotrauma Through Correlation of Advanced Magnetic Resonance Images with Histopathology

NIJ AWARD #: 2017-DN-BX-0145

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In the United States, approximately one-third of children who are victims of abusive head injury die, and those who survive have worse neurological outcomes compared with children who survive accidental head injuries. Current forensic pathology practice relies on autopsy examination with comprehensive neuropathological examination for the diagnosis of fatal non-penetrating blunt head injury. Although considered the gold standard for forensic pathology, neuropathologic examination may be limited, particularly in the evaluation of traumatic axonal injury. The use of advanced magnetic resonance (MR) imaging may facilitate the forensic examination of brains, allowing the pathologist to target specific regions that may not be macroscopically visible during examination. To evaluate whether MR imaging supplements the neuropathologic evaluation of traumatic brain injury in homicidal pediatric blunt head trauma, this study compares standard routine neuropathologic examination with examination guided by any findings detected on advanced MR. Ex vivo formalin-fixed brains removed at the time of forensic autopsy on children ages birth to 15 years who died as a result of abusive head injury (non-penetrating) underwent 7T MR imaging within a 3D-printed brain holder and included diffusion tensor imaging (DTI), T2 weighted, T1 weighted, and susceptibility weighted imaging (SWI). MR images were visually assessed by a neuroradiologist to evaluate for any focal findings that might help guide pathological evaluation, followed by examination by a forensic neuropathologist, with radiologic/histologic correlation. This study is an ongoing study that has collected data from four control studies (ages 1 month to 1 year) and three blunt head trauma cases (ages 1 year to 5 years). Macroscopic brain examination included histologic sections of the anterior and posterior corpus callosum, internal capsule, midbrain, pons, and cervicomedullary junction with immunohistochemistry for β-amyloid precursor protein (APP), as well as immunohistochemistry for CD68, p62, and glial fibrillary acidic protein (GFAP) on select sections. Thus far, neuropathologic examination has demonstrated traumatic axonal injury patterns with APP, whereas T1W, T2W, and SWI MR has shown no visible evidence of subtle axonal injury or other significant anatomic findings that were not visible by macroscopic examination. Studies are underway using advanced MR imaging, including diffusion tensor imaging, and more cases are being collected for quantitative comparison to controls.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

TRACK II, SESSION I

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Moderated by NIJ Program Manager
Gregory Dutton

Black Box Evaluation of Bloodstain Pattern Analysis Conclusions

NIJ AWARD #: 2018-DU-BX-0214

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This presentation will discuss the results of the Bloodstain Pattern Analysis Black Box Study. This study was conducted to assess the scientific validity and reliability of bloodstain pattern analysis (BPA) by rigorously measuring the accuracy and reproducibility of conclusions reported by practicing BPA analysts. Participation was open to bloodstain pattern analysts who have conducted operational casework within the past 2 years. Each participant completed a background questionnaire regarding general education, BPA-specific training, BPA experience, and other forensic experience. Participants were each asked to analyze 150 bloodstain patterns over a period of 18 weeks. Seventy-five BPA analysts participated, 45 of whom completed all assigned bloodstain patterns. A total of 192 bloodstain patterns (samples) were selected to span a range of complexity to be broadly representative of patterns encountered in casework. These samples included patterns from both controlled collection and operational casework. Most of the bloodstain samples had multiple images presented (1–9 images for each sample, 532 distinct images). Information provided to the participants about the samples and images was limited to the type of surface and whether the surface was horizontal or vertical. The test was conducted using web-based software to present images and collect examiner responses (no physical images or samples were sent to the participants). Because the BPA discipline does not have a preexisting conclusion standard that could be adopted for use in the study, we used three complementary approaches to collect participants' assessments: classifications (limited to mechanisms defined in the OSAC/ASB terminology standard), questions regarding possible mechanism(s) that may have caused the pattern(s), and summary conclusions (short text summarizing observations and conclusions). The study resulted in the following data:

- 815 distinct classification prompts (0–6 classification prompts per sample, mean 4.3), resulting in 27,038 classification responses (mean 33.2 participant responses per classification prompt)
- 223 distinct questions (0–5 questions per sample, mean 1.7), resulting in 5,967 responses to questions (mean 26.8 participant responses per classification prompt)
- 1,760 short text responses (mean 9.2 text responses per sample)

Results and findings to be presented include the extent to which BPA analysts' conclusions are accurate, the extent to which BPA analysts' conclusions are reproduced by other analysts, and associations between BPA analysts' performance and their training and experience.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

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

NIJ AWARD #: 2018-R2-CX-0034

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Fractured metal fragments with rough and irregular surfaces are often found in crime scenes. Current forensic practice visually inspects the complex jagged trajectory of fractured surfaces to recognize a "match" using comparative microscopy and physical pattern analysis. We developed a novel framework, utilizing the basic concepts of fracture mechanics and statistical analysis to provide quantitative match analysis with match probability and the corresponding error rate. The framework is applied to a wide range of metallic fractured articles, including knives, steel bars, and hacksaw blades, and their polymeric replica to assess its applicability and potential to assist forensic scientists in comparative match analysis with quantitative accretions. The computational framework employs the statistics of fracture surfaces to become non–self-affine with unique roughness characteristics at relevant microscopic length scale, dictated by the intrinsic material resistance to fracture and its microstructure. At such a scale, which was found to be greater than two grain-size or other microscopic feature scale, we establish that the material intrinsic properties, microstructures, and exposure history to external forces on an evidence fragment have the premise of uniqueness, which quantitatively describes the microscopic features on the fracture surface for forensic comparisons. The methodology utilizes 3D spectral analysis of the fracture surface topography and classifies specimens with very high accuracy using statistical learning. Overlapping topological images are acquired by confocal microscopy for individual fracture surfaces. Cross correlations of image pairs in two frequency ranges are used to develop statistical models for the distributions among matching and non-matching pairs of images. The model accounts for the use of multiple overlapping images to distinguish the relationship between pairs of fracture surfaces and to develop a decision rule for identifying matches and determining error rates. A set of 60 different fracture surfaces of metallic steel articles were fractured in different ways. In addition, one side of each fracture surface is also replicated with a polymeric replica. The pairs of fracture surfaces or pairs of a fracture surface and replica were correctly classified. 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.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Results of the 2019 3D Virtual Comparison Microscopy Topography Resolution Study (VCMTRS)

NIJ AWARD #: 2018-DU-BX-0216

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Objectives. This presentation will educate the audience about Virtual Comparison Microscopy (VCM) and present the results from the 2019 VCM Topography Resolution Study (VCMTRS). The VCMTR Study was designed to investigate the effect of scan resolution on the accuracy of VCM conclusions and is a follow-up project to the 2018 Virtual Comparison Microscopy Error Rate (VCMER) Study presented at the 2019 Association of Firearm and Tool Mark Examiners (AFTE) meeting.

Proposition. 3D VCM is a powerful tool for microscopic examination, which presents an examiner with a highly detailed visualization of a toolmark surface. As labs begin to incorporate VCM into their standard operations, it is important to understand the effect that scan resolution has on the ability of VCM to be an effective tool. The VCMTR Study (1) investigated the relationship between topographic resolution and inconclusive rates and (2) identified the specific topographic features used by examiners when reaching source conclusions at different scan resolutions.

Synopsis. The 3D VCMER Study was conducted in 2019 and focused exclusively on virtual comparison microscopy of cartridge cases. Seventeen pairs of cartridge cases were assembled and scanned where each pair had the same class characteristics and were either known matches or known non-matches. The selected cartridge cases represent a variety of tool manufacturing/finishing processes and class characteristics. The pairs ranged in complexity to represent the variability experienced in real casework. Four test sets were created from each of the 17 pairs. The first set consisted of high-resolution scans (approximately 2.0 micrometer per pixel). The second, third, and fourth sets contained increasingly down-sampled versions of the originals representing scans down to approximately 12 micrometer per pixel. All scans were collected using Cadre's TopMatch-3D scanning system. Participants analyzed all 17 pairs, but each participant only analyzed each pair at one of the four (randomly selected) resolutions. Each participant therefore saw different scan pairs at different resolutions from high resolution to low resolution. For each test set, participants were asked both to reach a source conclusion (utilizing the 5-point AFTE range of conclusions) and to annotate areas of similarities and differences that were used when reaching their conclusion.

Results. A total of 102 participants completed the study, including 77 examiners qualified to perform independent casework in the United States or Canada. In this presentation, we will describe overall study results, conclusions, and inconclusive rates. Results will be broken down by test set, toolmark class, and scan resolution. Presented results will include our summary annotation maps, which are visual topographic overlays indicating the percentage of participants that utilized each portion of the 3D surface when reaching their conclusion.

The results of this study add to the growing support for the use of 3D VCM as a viable alternative to traditional light comparison microscopy within the

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

discipline of firearm examination. The results also provide evidence that decreases in resolution corresponds with a decrease in overall accuracy of examiners to reach definitive conclusions.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Determining Fingerprint Age with Mass Spectrometry Imaging of Triacylglycerols

NIJ AWARD #: 2019-DU-BX-0134

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Fingerprint analysis is a common way to identify a suspect(s) in forensics. However, it has been notoriously difficult to determine the time since deposition, or fingerprint age, which is crucial to correlate the fingerprint with a criminal event, especially when the suspect can make a legitimate claim to have visited the place before the crime. Many different approaches have been taken to address this issue, including monitoring physical degradation, chemical profiling with GC-MS, and measuring chemical diffusion, but none has been successful. In this work, we apply matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) for the chemical profiling of fingerprints and demonstrate that it can be used to determine fingerprint age utilizing ambient ozonolysis of triacylglycerols (TGs). For all fingerprint samples, the donor touched their forehead a single time prior to depositing the fingerprint on a glass slide precleaned with methanol. Samples for aging were stored under ambient conditions for various time points in a room with no windows and received fluorescent room light for approximately 8 hours per day. Intact TGs can be readily and reliably detected by MALDI-MS, especially using a combination of sodium and gold as a matrix. After MALDI-MS analysis, fingerprints can be kept for court evidence because there is minimal damage to the analyzed surface. TGs have a broad distribution spanning the mass range of 740–890, due to variations in fatty acyl chain length and varying levels of unsaturation. For the first few days of fingerprint aging, the gradual decrease of unsaturated TGs is clearly observed whereas there is no apparent change for the saturated TGs. The decay is even faster for multiple unsaturated TGs. Along with this decay, an appearance of two other distributions with slightly lower masses in the range of 640–800 is observed. According to the elemental composition analysis, they have shorter carbon chain lengths than original TGs but have one or two more oxygens (O7 or O8). After a series of experiments using aged TG 50:1 standard in comparison to aged fingerprint TGs, the O7 and O8 compounds could be explained as ozonolysis products as previously proposed by Blanksby and coworkers. In aging experiments with fingerprints from three volunteers, a reproducible decay curve was obtained; however, individual differences were also noted. In subsequent experiments, the particular individual with slow decay also contained higher amounts of fatty acids, wax esters, and diacylglycerols in the fingerprints, suggesting the slow decay of unsaturated TGs is due to competing reactions. No further aging occurred when the fingerprints were kept in a sealed container, suggesting fingerprints kept by law enforcements would not degrade before the analysis. Reproducible results were also obtained when the experiment was performed with carbon forensic powder, which is commonly used by forensic practitioners.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Application of Morphologically Directed Raman Spectroscopy (MDRS) for the Forensic Examination of Soils

NIJ AWARD #: 2019-DU-BX-0017

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The potential of soil analysis is not being realized in modern forensic laboratories. Soil can associate an unknown specimen from a shoe, tire tread, or shovel with a known and can also provide investigative leads. Although numerous cases have demonstrated this capability, criticisms of forensic soil analysis (e.g., subjective, labor-intensive, time-consuming) have resulted in a considerable decline in its use in forensic investigations. The failure to collect and analyze soil evidence has created countless missed opportunities, and the criminal justice community is missing out on a valuable and powerful type of physical evidence that has been proven to help in the investigation and adjudication of cases. Consequently, there is a need for a statistically supported, automated, and objective analytical method for soil analysis. Particle-correlated Raman spectroscopy (PCRS), also known as particle-driven or morphologically directed Raman spectroscopy (MDRS), is a novel yet reliable analytical technique that can add significant value to the forensic examination of soil evidence. PCRS is capable of delivering particle size distribution and microscopic morphological characteristics for the particles present within a soil sample, and at the same time provides secure mineral identification. The research presented here focuses on the method optimization for soil mineral analysis with PCRS. The parameters for the chemical identification of minerals via Raman spectroscopy (e.g., laser wavelength, laser power, exposure time, magnification, grating), the imaging of particles (e.g., contrast/illumination method, magnification, targeted morphological analysis), and dispersion of the particle mixtures were optimized using response surface modeling of a multi-level experimental design. This research presents the first steps in achieving the overall aim of developing a robust, automated, and objective analytical method for the analysis and comparison of soil minerals using PCRS.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Raman Microspectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue

NIJ AWARD #: 2016-DN-BX-0166

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Raman spectroscopy is a technique that can provide confirmatory class identification of analytes through low-intensity laser light scattering. The technique is nondestructive, rapid, sensitive, and requires little or no sample preparation. Furthermore, portable Raman spectrometers are readily available, allowing for crime scene accessibility. Raman spectroscopy offers several advantages over the current methodology for gunshot residue (GSR) analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape. This is contrary to current GSR elemental analysis methods, which rely solely on the detection of the heavy metals (lead, barium, and antimony). This is problematic because environmental concerns have led to the 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 accurately identifying GSR samples. Additionally, the probability of environmental and manufacturing particles assigned (incorrectly) as being GSR has increased with the onset of "green" ammunition. Until recently, the application of Raman spectroscopy for GSR analysis was largely unexplored, although this approach is not dependent upon detecting metals and is more capable of differentiating environmental contaminants and GSR. Therefore, a Raman spectroscopic method displays numerous advantages in specificity when compared with current techniques. The firearm discharge process is analogous to a complex combustion reaction. Therefore, the chemical composition of the products (GSR) is directly related to the chemical nature of the reagents (ammunition). In this study, we have investigated the effect of the weapon used on the Raman spectroscopic signature of GSR. The same ammunition was fired from three different handguns and the resulting GSR was examined using Raman spectroscopy and advanced statistics. It was demonstrated with statistical confidence that Raman spectroscopic characteristics of organic GSR particles are independent of the firearm used and determined by the chemical composition of the ammunition. This discovery is important because it significantly simplifies the analysis of GSR using Raman spectroscopy linking it to the ammunition. Further investigation is required and is in progress in our laboratory to determine the selectivity of the methods with respect to the ammunition manufacturer and a specific type of ammunition produced by the same manufacturer. This study demonstrates the capability of Raman microspectroscopy for on-scene, nondestructive, identification and analysis of GSR. This method has the potential to greatly impact the forensic science community by increasing the accuracy (and discriminatory power) of GSR detection. The most direct application for this research is a method to exclude a specific ammunition as producing an evidentiary GSR sample. The comparison of a laboratory-generated GSR sample discharge and an evidentiary GSR sample can be made without extensive preliminary studies. This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

TRACK II, SESSION II

FORENSIC BIOLOGY/DNA

Moderated by NIJ Program Manager
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Population Distribution and Factors Affecting Individual DNA Shedding Propensity

NIJ AWARD #: 2018-DU-BX-0203

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The distinction between active and passive DNA transfer is critical for determining the probative value of trace DNA. Instances of detectable passive transfer have been linked to individual shedding propensity (Lowe et al., 2002; Szkuta et al., 2017). The ability to test a person of interest for shedding propensity would benefit evidence interpretation in specific cases. Cell count data seem promising (Kanokwongnuwut et al., 2018), but reproducibility over time remains an issue, and an alternative sampling location independent of handwashing would have benefits. This study explores shedding propensity results using skin surface tape lifts collected from eight sample types (left and right washed and unwashed fingers, big toe, upper arm, nape, and below ear) collected over a 3-week period. Each donor (n=30) also provided thumbprints on glass before and after handwashing. Thumbprints were stained with Diamond Dye (Promega Corp.). All tape disks were extracted with QIAamp DNA investigator kit (Qiagen), quantitated with Quantifiler Trio, and typed using GlobalFiler (both Thermo Fisher Scientific). Short tandem repeat (STR) results were examined for profile quality and mixture status. The presence of foreign alleles was used to correct the quantitation values for the presence of non-self DNA. Cell count data were evaluated for reproducibility over the 3 weeks. Variance testing (using a classical one-way random effects model) showed that for unwashed hands, donor-to-donor variability is low when compared with the daily variation for the individual donors. For washed hands, donors show more differences to each other but still a large variance in the day-to-day collections. Pearson r values showed moderate correlations between weeks for washed but not unwashed hands. Average DNA concentrations over all three collections were highest for ear samples, followed by nape and unwashed fingers. Washed fingers always had less DNA than unwashed fingers. Arm samples had low DNA concentrations but the most consistent values over the three collections. Quantitation values showed strong Pearson correlations between left- and right-hand washed and unwashed fingers and washed and unwashed states from the same hand. Sebaceous to palmar skin comparisons resulted in moderate correlations for some of the fingers to arm or nape. Cell count and quantitation data for fingers showed a continuous distribution with no obvious gaps between low and high shedders. There was no correlation between cell count data and DNA concentrations. STR profile quality was generally consistent with the amount of DNA amplified. STR results for unwashed hands showed a higher percentage of DNA mixtures. Using only self-DNA allele counts for washed hands and criteria similar to the Lowe et al. (2002) study, out of 28 volunteers, 3 individuals were observed to be consistently low shedders (10.7%). The majority of volunteers (20, 71.4%) were intermediate shedders, and 5 (17.9%) individuals were always high shedders. Variance over three separate collections and the use of either arm or nape results for shedder status determination is still being examined.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

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Persistence of Touch DNA for Forensic Analysis

NIJ AWARD #: 2018-DU-BX-0192

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Touch DNA evidence (i.e., the low quantities of DNA that can be left behind when someone touches an object) is increasingly important in criminal investigations. However, many important questions exist regarding basic properties of touch DNA, including the expected stability of touch DNA samples following exposure to different environmental conditions. This effort seeks to provide comprehensive, well-controlled studies that provide practical data on the environmental persistence of touch DNA, culminating in a predictive regression model incorporating relevant variables to enable prediction of DNA stability based on estimated environmental conditions. Pilot studies were conducted to evaluate the impact of temperature, humidity, and exposure time on the quantity and quality of both touch DNA and purified human DNA. These pilot studies established methodologies for precise control of environmental conditions. Four environmental conditions were achieved and stably maintained (CV < 0.04) over the course of two weeks: high temperature/high humidity (36°C, 83% relative humidity), low temperature/low humidity (21°C, 27% relative humidity), and the remaining pairwise combinations. Purified human DNA samples were obtained from the Coriell Institute (n=5), and touch DNA samples were obtained from two donors on stainless steel bolts (n=10). DNA samples were collected from the substrates at several time points after controlled environmental exposure for 0 to 14 days. DNA was purified and quantified using the Quantifiler HP kit, which provides a DNA quantity for each sample as well as a degradation index (i.e., a measure of DNA integrity based on the relative ability to amplify a long compared with a short DNA segment). Initial statistical analysis was performed using ANOVA with post-hoc Tukey test and regression analysis. Decreased DNA quantity and increased degradation index were observed for the high temperature/high humidity condition, suggesting that the combination of these two environmental factors may have a bigger impact on DNA stability than either condition alone. Significant differences in DNA quantity were not observed across the two time points included in the experiment (7 and 14 days). Not unexpectedly, given the known variability in touch DNA quantities across different individuals, donor identity was the major source of variability in the initial experiments. Ongoing studies aim to increase donor number, institute a formal randomization scheme to reduce this source of variability, and leverage non-parametric statistical methods. Pilot studies are also underway to investigate the effects of UVB light on touch DNA samples. It is well established that UV light damages DNA, but exposure experiments often use irradiance levels and wavelengths not applicable to real-world forensic scenarios. UVB bulbs commonly used in reptile terrariums are being used to mimic relevant irradiance levels and wavelengths of UV light representative of natural sunlight. Collectively, these studies will provide comprehensive, well-controlled data and predictive models regarding the stability of touch DNA in real-world scenarios.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Towards Developing Forensically Relevant Single-Cell Pipelines by Incorporating Unsupervised Clustering

NIJ AWARD #: 2018-DU-BX-0185

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The power of probabilistic software tools that draw inferences from forensic DNA stains is limited by the input: the multi-cellular bulk-processed DNA signal. Consequently, the evaluation of complex samples can, therefore, render little information. Single-cell methods have been proposed as a solution and methodologies that enable single-cell electropherogram (EPG) creation have been developed, but the inferential framework remains undeveloped. We present one step in the solution to the computational conundrum of realizing the promise of single-cell technologies: the introduction of a computational pre-processing step, prior to likelihood ratio (LR) computation, which clusters EPGs into groups by unknown genotype. When successful, each group consists of EPGs from a single genetic source for which the LR can be evaluated by adaptation of current probabilistic methods.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

A Universal Method for Biological Stain Identification and Analysis Using Raman Spectroscopy

NIJ AWARD #: 2017-DN-BX-0135

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Traces of body fluids discovered at a crime scene are a potential source of DNA, which is a major individual evidence in the modern forensic investigation. We have reported on the application of Raman spectroscopy for nondestructive, confirmatory identification of biological stains at a crime scene, including dry traces of sweat, vaginal fluid, semen, saliva, and blood. The method allowed for differentiating animal and human blood as well as menstrual and peripheral blood. In addition, the method was further developed for determining the time since deposition for bloodstains for up to 2 years. The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, nondestructive laser light. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms. A typical Raman spectrum provides a unique vibrational signature of the material. Nonresonance Raman spectroscopy is not destructive for the sample. A portable Raman spectrometer is a reality now that should allow identification at the crime scene. It would be of great help for criminal investigation to develop a phenotype profiling immediately at a crime scene based on a rapid analysis of biological stains. With this goal in mind, the possibility of race, sex, and age differentiation based on Raman spectroscopy of body fluid traces have been investigated. Specifically, advanced statistical analysis of spectroscopic data was used to discriminate between Caucasian and African American donors based on dry peripheral blood traces. In addition, the differentiation of a donor's sex based on bloodstains and saliva traces as well as race differentiation based on traces of semen have been demonstrated. Raman spectroscopy and chemometrics have been used to analyze blood from human donors and differentiate between them based on their chronological age. In this presentation, three new project developments will be discussed. First, the expansion of the body fluid identification method to include urine: the high-specificity and confirmatory nature of the method has been demonstrated. Second, the potential of the method to result in false positives was investigated. Specifically, it was demonstrated that no environmental contaminants, which were tested as potential interferences for blood and semen, resulted in false positives. A new universal model based on random forest statistical approach was developed and tested; 100% identification of all body fluids with no false positives was demonstrated. Third, a proof-of-concept study was reported showing great potential for Raman spectroscopy to differentiate smokers and nonsmokers based on dry traces of saliva. This result expands further the capability of the method for phenotype profiling based on dry traces of body fluids immediately at the crime scene. This project was supported by Award No. 2017-DN-BX-0135 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

The Effect of Storage Conditions on Estimates of the Age of Dried Bloodstains

NIJ AWARD #: 2018-DU-BX-0206

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The relationship between RNA degradation and the age of a bloodstain has been suggested by the work of several investigators. A prior study from this laboratory described a qPCR assay that was effective at estimating the age of bloodstains stored in an environmentally controlled laboratory for periods of up to 1 year. In this study, the effect of the environmental conditions on the rate of RNA degradation during storage was analyzed. Bloodstains were prepared on stain cards and stored in one of nine different environments for periods of up to 24 weeks. At selected times during the storage term, RNA was extracted, reverse transcribed, and the integrity of select transcripts analyzed. Three temperatures (37°C, 20°C, and 4°C) and three relative humidities (rH) (75%, 35%, and 10%) were combined pairwise. The rate of RNA degradation was found to increase five- to tenfold in stains stored at 37°C versus those stored at 20°C. The rate of RNA degradation was faster for stains stored at 20°C compared with those at 4°C but differed only two- to fourfold. Multivariate regression analysis suggests elevations in temperature or rH will accelerate RNA degradation and will do so to a similar extent. It is clear from the data that the integrity of the transcriptome in dried bloodstains is better preserved in a cold and dry environment. Investigations are ongoing to develop an approach for the estimation of sample age that incorporates the environmental conditions of a crime scene into the age estimate.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

An Epigenetic Multiplex Capable of Discriminating Body Fluid Type, Age, and Phenotype

NIJ AWARD #: 2017-NX-BX-0001

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In certain situations, such as sexual assault and child abuse, where the assailant has access to the victim, the presence of DNA is not as crucial as determining the body fluid type. Unfortunately, many of the common techniques for body fluid identification are presumptive or have poor sensitivity when compared with polymerase chain reaction (PCR). In our laboratory, we have developed a series of epigenetic markers based on DNA methylation for body fluid, as well as for age and phenotyping. The goal of the current National Institute of Justice (NIJ)–funded project has been to develop a multiplexed PCR approach to the determination of body fluid origin and biological age. The project conserves samples by utilizing a single DNA cocktail to detect multiple loci simultaneously. Standard DNA extracts are used, making the process easy to integrate into any DNA laboratory workflow. Initial work on the project involved the development of a compatible set of loci for the trace determination of blood, semen, saliva, and vaginal epithelia. A variety of epigenetic loci were screened using DNA array technology, real-time PCR, and pyrosequencing. Next, a body fluid multiplex was created for pyrosequencing by adjusting PCR conditions and primers. Four loci were selected, including BCAS4, CG06379435, VE 8, and ZC3H12D. Once successful amplification was demonstrated, expert systems were created using agglomerative hierarchical cluster analysis and latent profile analysis to permit rapid determination of body fluid type. Following this project, additional work was performed to identify age-specific markers in a variety of body fluids. This information was used to create a larger multiplex for application to moving particle semi-implicit methodology, which is currently being optimized. The result of this project demonstrates the successful application of forensic epigenetics as a useful adjunct to short tandem repeat typing.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

POSTER ABSTRACTS

POSTER ABSTRACTS I

SEIZED DRUGS AND TOXICOLOGY

Coupling Raman Spectroscopy with Ambient Sampling, Portable Mass Spectrometry for On-Site, High-Throughput Evidence Confirmation on a Single Instrumental Platform

NIJ AWARD #: 2017-R2-CX-0022

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Forensic evidentiary backlogs are indicative of the growing need for cost-effective, high-throughput chemical identification methods, and two emerging technologies that show high promise in meeting this need are surface-enhanced Raman spectroscopy (SERS) and paper spray ionization–mass spectrometry (PSI-MS). Both Raman and MS techniques are regarded for their discriminating power, denoted by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) as "Category A" methodologies. Although these two techniques have been demonstrated for rapid evidence screening, neither individually fulfills the two-tiered identification guidelines recommended by SWGDRUG for generating prosecutorial data, requiring a continued role for off-site, laboratory processing. Correspondingly, efforts have been taken to integrate these techniques (coined SERS-PSI-MS) into a portable prototype that could enable field-based, yet court-admissible, evidence identification. Here, aspects of this instrument development project will be addressed, including design phases and proof-of-principle testing, characterization and validation of later stage prototypes, and investigation of authentic evidence samples to show feasibility and practicality of the proposed two-tiered methodology. Specific detail will be given on efforts to incorporate 3D printing technologies to develop cartridge-based PSI sources capable of usage as (1) physical transfer swabs for collection of trace drug residues from surfaces, (2) disposable PSI ionization sources, and (3) dual-use, SERS-active materials. Method robustness to operating variables and positioning will also be discussed because it was examined to optimize the technique for general usage by experienced and novice users alike. Of interest to this study was demonstrating the discriminatory power of the combined SERS-PSI-MS method toward evidence present as complex mixtures (e.g., cutting agents, adulterants) or exhibiting structural similarities (e.g., novel synthetic analogues). Using protocols to examine design of experiments processes, a large sample, blinded error rate study was undertaken to determine the overall reliability of drug identification when employing SERS-PSI-MS. Built into this study were pseudo-random events to test the efficacy of training efforts, particularly deducing carryover and potential hygiene events. Other validation aspects of the technique that will be discussed include spectral accuracy, detection limits, sample throughput, and inter- and intra–day-to-day variability. The results presented herein demonstrate the potential of coupling of SERS and PSI-MS to significantly reduce error rates for chemical identification and generate prosecutorial evidence on-site.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

POSTER ABSTRACTS II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

NMDID: A New Resource for Forensic Anthropology

NIJ AWARD #: 2016-DN-BX-0144

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The Office of the Medical Investigator is a statewide, centralized medical examiner's office for New Mexico. A National Institute of Justice (NIJ) grant was awarded in 2016 to create the New Mexico Decedent Image Database (NMDID). It is a free-access, searchable dataset of full-body computed tomography (CT) scans and associated demographic, lifestyle, and health data. The development of this database allows multitudes of investigators to conduct research, especially within the forensic community. It is available at https://nmdid.unm.edu. The sample includes 15,243 decedents who died between 2010 and 2017, which accounts for approximately 11% of deaths in New Mexico. Over two-thirds of the scans have no discernable decomposition. Additionally, 10,000 are male, 30% are Hispanic, and 13% Native American. Natural causes of death and accidents account for 73% of the sample, with the remainder of deaths due to suicide, homicide, and unknown causes. Each individual has demographic, lifestyle, and health data from both the medical examiner's database and interviews with next of kin. The available information can differ greatly between individuals but can include up to 69 variables—such as education; occupations; habitual activities; number of children; country of origin for decedent, parents, and grandparents; health history; medications; socioeconomic status; and medical diagnoses. The presentation will focus on how to perform queries of the data, download metadata and further narrow down the information, and download CT images. To date, there are 277 users of the database from 30 countries, with 70 requesting access to the CT images. Reasons for accessing the database have included research, education, and art. Projects have included COVID-19 research, age estimation, cancer treatment, automobile safety, biomechanics, and morphometric analyses. Funded by NIJ grant number 2016-DN-BX-0144; statements made are solely the responsibility of the authors.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Auto-Curation of a Large Human Decomposition Image Collection

NIJ AWARD #S: 2016-DN-BX-0179 & 2018-DU-BX-0181

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Human decomposition is a complex process that may be influenced by a plethora of factors. As a result, the field is lacking scientifically grounded methods to answer key forensic issues, such as how, when, and where specific environmental conditions affect the decomposition pattern and result in mummification, the effects of scavenging on human remains, or the effect of factors such as body weight on the decomposition process. We aim to enable more research on the human decomposition process by producing a large, curated dataset of photos depicting human donors undergoing decomposition in various weather conditions and seasons. Specifically, we focus on the challenge of extracting the decomposition-relevant knowledge from the content of the photos, because manual processing of the complete collection is time- and cost-prohibitive. We propose to use unsupervised and semi-supervised machine learning methods with specially designed user interfaces that facilitate manual inspection and tagging of a large collection of photos to automate and accelerate the image data enrichment process to ensure that it provides means to conduct forensic research on human decomposition on a massive scale. We apply our approach on an image dataset of over 1 million photos depicting human decomposition collected at the Anthropology Research Facility, University of Tennessee. Our approach is an iterative method that first clusters photos into groups of similar images. Only relatively few clusters then need to be inspected to tag these clusters with meaningful labels and, potentially, to merge clusters representing similar phenomena. This step is supported via a specialized interface that makes both tagging and merging extremely efficient, so that thousands of images can be processed with minimal manual effort. Our major algorithmic innovation involves combining the information obtained from the manual tagging and cluster adjustments by experts with the implicit constraints imposed by the protocol used to obtain the photos and by the nature of the decomposition process. Specifically, the protocol assures that every body part is depicted in the set of images taken of the same subject on any single day. Subjects are placed in the fresh stage and are photographed until they reach a skeletonized state of decomposition. This results in an iterative semi-supervised approach that combines clustering with classification and uses a suitably designed penalty for violating the constraints. Our approach has led to dramatic improvement in the accuracy of the automatic classification on generated 33,000 tags indicating the location of decay characteristics in 3,555 photos and on body part labeling of 54,361 photos. We expect to extend the approach to the entire dataset of over 1 million images to make it into a valuable resource for research on human decomposition. Other large digital forensic collections may also benefit from the ability to apply our method for image content extraction at scale.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

A Conceptual Framework for Constructing and Integrating Insect Thermal Profiles into Death Investigations

NIJ AWARD #: 2016-DN-BX-0204

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Blow flies have an exceptional ability to locate and colonize dead and decomposing remains. Thus, blow flies are regularly used in death investigations to estimate forensically important timelines, such as the time of colonization as related to the postmortem interval. However, several factors may prevent adult blow flies from immediately colonizing said remains—ranging from blocked access (e.g., sealed oil drum) to environmental temperature (e.g., heat waves or cold snaps). Currently, we lack a comprehensive conceptual framework for understanding the thermal constraints of adult blow fly survival, activity, and oviposition—three critical elements required for colonization. Through a series of experiments with two blow fly species (*Cochliomyia macellaria* and *Chrysomya rufifacies*), we quantified both the upper and lower thermal limits for survival, activity, and oviposition. We also quantified oviposition performance, measured as the number of eggs laid, and egg viability (i.e., percentage of eggs hatched) over a range of temperatures (10–45°C) at which adult blow flies were observed to be active. Not surprisingly, each thermal parameter resulted in a different range of temperatures. Adult blow flies survived over the widest range of temperatures, were active over a narrower range of temperatures, and oviposited over an even narrower range of temperatures—plus, egg viability was observed over the narrowest range of temperatures. We integrated these data into a single unified framework that has general applicability across forensically important insects. We will explain the basic experimental criteria required for each thermal parameter measured and how the thermal parameters are collectively used to develop blow fly thermal profiles. We will also discuss both the biological meaning and forensic significance of these thermal profiles. For instance, the range of temperatures over which a species can survive provides information about where and when a given species can live. This provides forensically relevant information related to where a body was colonized. Alternatively, the temperature range over which blow flies oviposit provides information about their physiological constraints. This provides evidence for a potential delay in colonization, which affects the accuracy of forensically important timelines—such as the postmortem interval (i.e., time since death). We will also provide examples from forensic entomology casework in which the implementation of this framework could address fundamental questions related to death investigations observing insect colonization and the rare cases lacking insect colonization, even though the body had no physical barriers inhibiting insect access. We propose that using this conceptual framework to construct blow fly thermal profiles will improve the use of insects in death investigations and consequently improve testimony by forensic entomologists.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

How Skin Color Affects Bruise Assessments by Alternate Light: Results of a Randomized Controlled Trial

NIJ AWARD #: 2016-DN-BX-0147

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Problem Statement. According to the National Crime Victimization Survey, less than half of all violent victimizations are reported to the police. Research suggests victims of violence may be more likely to engage in the criminal justice process if their physical injuries are identified and documented. Unfortunately, individuals with dark skin tones are disadvantaged by current practices assessing soft tissue injuries by the naked eye. To overcome this challenge, a national protocol recommends the use of alternate light to improve the visibility of subtle injuries on adults and adolescents. However, there is limited research to show how skin color affects alternate light wavelengths in the detection of cutaneous light absorption originating from trauma.

Purpose. The purpose of our study was to determine which wavelengths within the narrow-band visible (NBV) and ultraviolet spectrums improved detection of light absorption on areas of trauma over time.

Methods. A randomized controlled trial was designed to prospectively bruise 157 healthy adults using controlled application of a paintball pellet to a randomly selected upper arm. Participant diversity was assured through quota sampling of six skin color categories determined using colorimetric skin color data collected from the lateral right deltoid. Using a cross-over design, the bruised area was examined 21 times over a 4-week period using an alternate light source (ALS) and white light in random order. The presence of light absorption was assessed using wavelength peaks within UV (365 nm) and NBV (415 nm, 450 nm, 475 nm, 495 nm, 515 nm, 535 nm) spectrums and filters (yellow, orange, red). Multilevel models were used to account for the correlated repeated measures data collected in this study. The probability of detecting light absorption for individual wavelength/filter combination was calculated and estimates were made for each skin color category (area around the bruise).

Results. Across each skin color category, the expected probability of detecting areas of absorption under alternate light wavelengths using 415 nm and 450 nm with a yellow filter was higher compared with white light or any other tested wavelength (i.e., dark skin 415 nm: 0.90, 95% CI: 0.87–0.93; white light: 0.81, 95% CI: 0.77–0.85). Ultraviolet was limited in its effectiveness to individuals with light skin (i.e., very light skin: 0.93, 95% CI: 0.90–0.95; dark skin: 0.20, 95% CI: 0.17–0.24).

Conclusion. ALS wavelengths of 415 nm and 450 nm provide a greater probability of detecting light absorption in areas of trauma across skin tones. With further development and evaluation of evidence-based practice guidelines, ALS is an ideal adjunctive tool to complement the physical assessment of injuries on diverse populations.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

POSTER ABSTRACTS III

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Security Crystals: NIR-to-NIR Upconverting Nanoparticles for Fingerprint Identification and DNA Extraction

NIJ AWARD #: 2017-IJ-CX-0026

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Over the past 3 years, our research group has been focusing on using upconverting nanoparticles (UCNPs, β-NaYF4:Yb/Tm) to develop fingerprints. This effort was successful with oleic acid-capped near-infrared (NIR)–emitting UCNPs (Baride et al., 2019). More recently, we have been focused on developing a system for reading fingerprints with UCNPs and for altering the UCNP surface such that DNA within the fingerprint can be captured and examined. Latent fingerprints and DNA are among the most personally diagnostic of all forensic evidence but have been difficult to obtain from the same fingerprint. Our approach has been to replace the oleic acid capping the UCNPs with acetic acid. We hypothesize that the phosphate-ligand in DNA should interact with the lanthanide ions in the UCNPs due to the reduction in steric hindrance. Initial results from zeta potential, polymerase chain reaction (PCR), and infrared spectroscopy have shown that DNA binds to the UCNPs at pH 4 and releases back into solution at pH 8 for solutions with ~300 ng of DNA. Because fingerprints typically contain on the order of 10 ng of DNA, experiments are underway to examine lower levels of DNA. With respect to our NIR-emitting UCNP fingerprint reader systems, we have developed an NIR-based laboratory system, utilizing a 980 nm laser, and are developing a portable NIR light-emitting diode system that can utilize an NIR-enabled cell phone. In addition, a senior design group in Computer Science has developed an Android app for detecting fingerprints, identifying minutiae, and determining the identity of the latent fingerprint producer from a database for use with a cell phone. This app consists of a server that runs the fingerprint recognition software, a database management system that manages the datasets and metadata, and an Android app that connects to the server and uploads images to be processed. The user interfaces with the software from a mobile app, where they can either take pictures of fingerprints using the device camera or import images from a separate device. The app allows the user to select images to analyze, after which the image gets uploaded to a server that runs automatic fingerprint recognition software. It can perform image enhancement on the fingerprint, and then it attempts to extract the minutiae from the enhanced image. The server will then use the extracted features to match the uploaded fingerprint against a database of known fingerprints. The server will query the database for a list of fingerprints to match the uploaded fingerprint. Once the server has completed the matching step, it will return the results back to the android app, which will display the results to the screen.

Reference

Baride, A., Sigdel, G., Cross, W. M., Kellar, J. J., & May, P. S. (2019). Near infrared-to-near infrared upconversion nanocrystals for latent fingerprint development. *ACS Applied Nano Materials, 2*, 4518–4527. https://doi.org/10.1021/acsanm.9b00890

Statistical Error Estimation for an Objective Measure of Similarity to a Latent Image

NIJ AWARD #: 2017-IJ-CX-0029

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The goal of the grant project is to exploit state-of-the-art computational resources and modern methods of statistical analysis to make statistically well-founded assessments of the rarity of individualizing information relative to a latent image. The forensic science problem at issue is that the assessment of latent prints from crime scenes is based largely on human interpretation and claims that these assessments have zero error rates are not scientifically plausible. The grant research relates to the following scenario for latent fingerprint examination: a region of interest (ROI) is specified in the latent image, and the latent ROI is consistent with the quality areas of a particular exemplar image of interest. Therefore, the scenario for modeling is that it supports a Latent Print Examiner's report concerning a latent image and a specific exemplar. The grant (1) defined an objective measure of similarity of any fingerprint to a latent image; (2) used a very large, randomly selected set of known non-mate images to the latent to create a model predicting random similarity to the latent image; and (3) demonstrated the prediction of random similarity to a latent image using images from the NIST SD 27 Special Data Base and additional images. The grant exploited the technology underlying the LatentSleuth Latent Fingerprint Examination Workstation to compare fingerprint images based their Level 2 similarity with a latent image. The LatentSleuth technology creates a powerful, comprehensive quantification of Level 2 characteristics that provides the ability to find the best orientation and location for overlaying the Level 2 characteristics of the latent image onto any particular fingerprint image. The LatentSleuth technology that warps the Level 2 characteristics of the latent image to any fingerprint image is the basis for the grant's objective measure of similarity to the latent image for a fingerprint image. The grant research has shown that it is computationally feasible to warp the latent image to a large set of known non-mate fingerprints for the latent image. Hence, it has been computationally feasible for the grant to measure similarity to the latent image for a large set of known non-mate fingerprints for the latent image. The measured similarity from a large set of known non-mate fingerprints that are randomly selected from a fingerprint database statistically represents the measured similarity for all fingerprint images in the database. The grant research used an objective measure of similarity applied to a large set of known non-mate fingerprint images, randomly selected from a database, to statistically estimate a database random match probability for a specific latent image. The statistical model is a null model in that it predicts rarity for the objective measure of similarity to the latent image when it is computed for any non-mate fingerprint image. A very small rarity prediction for the fingerprint of interest contradicts the null model and suggests that the fingerprint image is, in fact, a true mate. The predicted rarity is conservative in that it is derived from an analysis of Level 2 characteristics only.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Post-Blast Explosives Attribution

NIJ AWARD #: 2018-DU-BX-0193

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Forensic science practitioners in both domestic and Department of Defense organizations are often called upon to build cases and attribute crimes using trace evidence remaining at a crime scene. The ultimate goal of these investigations is to associate a crime with a suspect or suspects to prevent further attacks through exclusion, exoneration, arrest, and criminal prosecution of potential perpetrators. However, fundamental questions remain regarding evaluation and interpretation of trace evidence for post-blast explosives attribution. The explosive charge is an attractive component for attribution in crimes involving explosives because it is key to the functioning of the device, and there are limited pathways for acquisition. However, unlike pre-blast attribution where signatures from explosive manufacturer reference samples can be compared with recovered samples, there is currently no capability to link the explosive charge to its source via post-blast trace residues. Attributing the explosive post-blast is a challenge because very little explosive material remains after detonation, which limits the analytical techniques that can be used. The purpose of this study is to determine whether pre-blast attribution signatures are preserved after detonation and whether they can be recovered from a blast site and measured at a detectable level. In this study (NIJ award #2018-DU-BX-0193), a preliminary field test was conducted to recover post-blast explosive samples from controlled detonations of multiple explosive materials, including RDX, TNT, and ammonium nitrate-aluminum (AN-AL). Samples were processed according to adapted methods 1–2 to extract both the explosive compound of interest and other chemical components that could potentially serve as signatures for attribution. Samples were subsequently analyzed via multiple analytical techniques, including high-performance liquid chromatography mass spectrometry for polar and non-polar small molecules, inductively coupled plasma mass spectrometry for trace elements, and gas chromatography combustion isotope ratio mass spectrometry or elemental analyzer isotope ratio mass spectrometry for isotope ratios of carbon, nitrogen, and oxygen. Preliminary results have shown promise that carbon, nitrogen, and oxygen isotope ratios remain consistent pre- and post-detonation and thus could be relevant for source attribution. For RDX, the average difference in isotope ratios between pre- and post-detonation is 0.33‰ (max difference = 0.58‰) for carbon and 0.2‰ (max difference = 1.20‰) for nitrogen. For TNT, the average difference is 0.2‰ (max difference = 1.36‰) for carbon and 0.5‰ (max difference = 1.77‰) for nitrogen. Finally, for AN-AL, the average difference is 0.13‰ (max difference = 0.54‰) for oxygen and 0.10‰ (max difference = 0.37‰) for nitrogen. The analytical measurement uncertainties (standard deviation of repeated analyses of isotopic reference standards) were less than or equal to 0.51‰ across all isotopes and material types. These results indicate that isotope ratio signatures of explosive compounds look to have been preserved after detonation in this small sample set. With this proof-of-concept study, the forensic community will benefit from a novel approach to attribute explosives after detonation.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

The Use of Computational Models for Fire Investigation

NIJ AWARD #: 2017-DN-BX-0163

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Investigations provide a means to identify the cause of a fire and collect data that may provide insight about the development and spread of the fire. By determining the cause of a fire and identifying products and phenomena that contributed to fire spread, investigators may be able to prove guilt or innocence in criminal proceedings, assign blame in civil proceedings, or contribute to the knowledge base that informs the fire protection community in future designs. Data such as area of fire origin, time until target products ignite, time to flashover of a compartment, and influence of ventilation on the dynamics of the developing fire are critical to understanding the cause of the fire and to reducing the number and severity of fires. Computational models are increasingly relied upon in fire investigations to improve the understanding of fire dynamics and fire-induced fluid flows. Models that are currently available range in complexity from simple algebraic heuristics derived from fundamental physical concepts and empirical data to generalized, physics-based computational fluid dynamics codes that require a wide range of property values as inputs and may require significant computational resources. The models used in this study are maintained with on-going verification and validation conducted by the National Institute of Standards and Technology with the support of the Nuclear Regulatory Commission. The understanding of the accuracy of the models and the inherent uncertainties in each model are currently based on fire measurements generated with well-characterized and, in many cases, steady-state heat sources like natural gas-fuel burners or liquid hydrocarbon pool fires. Realistic fuels encountered in fire investigations are often fueled by natural and synthetic solid materials. These fuels are three-dimensional (as opposed to a two-dimensional burner surface), and the foam plastics used in furnishings tend to melt, drip, and flow during burning. Fires with these fuels are characterized by non-steady burning where rapid transitions in energy and fuel output are possible. This research focused on evaluating the ability of three types of models commonly used in fire investigations to predict characteristics of the fire environment generated from gas burners and from burning a single upholstered furniture item. A quantitative analysis of the accuracy of predicting plume and compartment temperatures, flow velocities, flame heights, heat fluxes, oxygen concentrations, and additional measurands was provided for each model. The accuracy of the predictions from all the models was found to be better in the gas burner experiments than in the furniture experiments. All models were sensitive to the definition of the heat release rate and the geometry of the burning item. More research is necessary to develop recommendations for fire investigators on how to model burning furniture. This research constitutes a starting point to understanding the limitations of the application of fire models with "real world" fuels to ensure a given model is appropriate and physical phenomena are accurately represented.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Forensic Interpretation of Multiple Concurrent Audio Recordings

NIJ AWARD #: 2019-DU-BX-0019

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The widespread use of handheld smartphones and other devices capable of recording audio and video has resulted in the likelihood that user-generated recordings (UGRs) may be presented as evidence in a criminal investigation. Combined with other recordings from body cameras worn by law enforcement, dashboard camera systems, residential and commercial surveillance systems, and more, the availability of UGRs may offer important audio forensic insights. User-generated audiovisual recordings of a public event will involve multiple recording devices at different spatial locations. The recordings may start and stop at different times, have differing technical format specifications, and will seldom have sufficiently reliable time stamp information for exact synchronization. When two or more audio devices are operating concurrently from different spatial locations while recording the same sound sources, the audio recordings will not be identical, but there will be some degree of correlation among the recordings. However, the sound received at each microphone will differ due to (1) the directionality of the source and microphones, (2) the differing distance between the source and each microphone, (3) the presence of acoustic noise and reverberation, and (4) the likelihood that one or more of the recording devices may be moving during the recording. Thus, there is a need to determine how best to combine the available audio information within the asynchronous framework. This presentation will include several examples of forensic interpretation for incidents involving multiple UGRs and will explain the reliability and authenticity concerns associated with user generated material.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Key Factors in Particle Combination Analysis

NIJ AWARD #: 2017-IJ-CX-0030

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Prior research, using reasonable choices of analytical and statistical parameters, has shown the high potential for very small particles (VSPs) to associate items of physical evidence. Systematic improvement of these methods, leading to optimization and transition to practice, requires the identification of key factors that affect method performance. A set of key factors has been determined for the particle combination analysis of VSPs using an automated scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS) procedure and associated data analysis. A Thermo Scientific™ Explorer™ 4 Analyzer was used for SEM/EDS analysis of VSPs collected from common items of physical evidence (handguns, cell phones, drug packaging and face masks) (Stoney et al., 2018). Experiments were conducted with a screening objective for factor identification focused on five variable classes (1) particle detection parameters, (2) SEM/EDS analysis parameters, (3) the number and choice of elements being analyzed, (4) particle size, and (5) data filtration parameters. Dependent variables were the analysis time and VSP selectivity. Particle size limits and magnification have been identified as key particle detection factors affecting the selectivity of VSP populations. A narrower size range (2.5–40 µm vs. 0.3–80 µm) and a higher magnification (2400× vs. 1200×) result in statistically significant greater selectivity. The minimum EDS counts parameter has been identified as a key SEM/EDS analysis factor affecting the selectivity of VSP populations. A higher minimum count (1,000 vs. 500) results in statistically significant greater selectivity. Each of four SEM/EDS analysis independent variables has been identified as a factor affecting the analysis time. Statistically significant shorter analytical times arose with greater maximum EDS duration (9 seconds vs. 6 seconds), lower minimum EDS counts (500 vs. 1,000), lower target EDS counts (2,500 vs. 5,000), and a higher element threshold (3% vs. 1%). The number and choice of elements has been identified as a factor affecting both selectivity and analytical time. Greater selectivity was shown by a broader set of 27 elements (vs. a 9-element subset and two alternative 18-element subsets). Results indicate that that there are minor differences in selectivity from particle size (within the range of 2.5–40 µm, given equal numbers of particles), and no differences from data filtration parameters (given the higher element thresholds of 3%). The next step is the optimization of the methodology based on the identified set of key factors.

Reference

Stoney, D. A., Neumann, C., & Stoney, P. L. (2018). Discrimination and classification among common items of evidence using particle combination profiles. *Forensic Science International, 289*, 92–107. https://doi.org/10.1016/j.forsciint.2018.05.024

Occurrence and Utility of Non-Identifiable Fingermarks

NIJ AWARD #: 2016-R2-CX-0060

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This presentation results from a 3-year investigation into the occurrence and utility of latent print correspondences that are insufficient for identification. Latent prints that have no value for identification (NVID), or insufficient characteristics for identification, often have discernible characteristics that could form the basis for lesser degrees of correspondence or for probability of occurrence within a population. Latent fingerprints were collected from nine different state and local jurisdictions. Fingermarks included in this study were those (1) collected in the course of investigations using existing jurisdictional procedures, (2) originally assessed by the laboratory as NVID, (3) confirmed by expert review as NVID but showing at least three clear and reliable minutiae in relationship to one another, and (4) determined to show at least three auto-encoded minutiae. An expected associative value (ESLR) of each of these marks was measured, without reference to a putative source, based on modeling within-variability and between-variability of automated fingerprint identification systems (AFIS) scores. This method incorporated (1) latest generation feature extraction, (2) a minutiae-only matcher, (3) validated distortion functions, and (4) NIST SD27 database calibration. Observed associative value distributions were determined for violent crimes, property crimes, and for prints meeting existing objective measurements of latent print quality. Non-identifiable fingermarks (NIFM) were found to occur commonly. They occur much more frequently than identifiable prints and in locations where identifiable prints do not commonly occur. They can occur in clusters, showing activity and involvement, and in locations of material impact within case contexts. These NIFM have significant expected associative value. Most have a very high value, and all have some value. The average for this study was 1/380,000. These results were presented and reviewed together with experts within various constituencies, including forensic laboratory scientists, crime scene investigators, police investigators, prosecutors, academic forensic scientists, defense attorneys and judges. Consensus findings were that the overall potential utility of NIFM evidence is very high. There are a range of limitations and constraints facing different applications and different constituencies. Some areas have a high, immediate potential impact at the current level of development. Other areas have significant potential but require improvements and developments either in the method or within the broader forensic community in order to realize the potential. Areas with immediate potential impact include cold case investigations, post-conviction cases and more general investigative applications. Performance within these areas would be significantly affected by turn-around times and the effectiveness of the application of AFIS searching methods. Mainstream use as evidence would require standardized and vetted methods, with consensus scientific support and clear definition of limitations. Important considerations (as with all new methodologies) would be how the method is implemented in the laboratory, requirements for reporting and practices for testimony. NIFM evidence also shares a number of currently

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

recognized issues and concerns in common with other forms of physical evidence. Notably, these include how quantitative scientific assessments should be expressed and the potential misuse of non-definitive associations. The results also indicate that latent print collection and retention practices should be adapted to anticipate the use of NIFM evidence.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Validating the Sexual Lubricant Database Using True Known and Unknown Samples for Forensic Analysis

NIJ AWARD #: 2018-MU-BX-0002

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Sexual assaults are an unfortunate reality and include situations in which the assailant is either known or unknown. Classifying a sample of an unknown sexual lubricant without directly comparing it with a known sample would provide a capability that has not been operationalized in the United States. Lubricant analysis for identifying and comparing trace amounts in sexual assault cases is relatively new in the field of forensic science compared with other trace evidence disciplines. Most research focuses on the identification of trace levels of polymers from the main lubricant component, but it is necessary to identify the critical parameters to conduct daily analysis of unknown lubricants, that is, post-coitus residue collection, storage, extraction, and identification. The development of the Sexual Assault Lubricant (SAL) database provides the foundation of a more objective manner of forensic lubricant analysis. This database provides the community a way to classify a true unknown sample collected in sexual assault cases; however, to advance this tool forward it is necessary to evaluate the use of this database for real-world samples and analysis. A characterization scheme for unknown lubricants has been developed as a result of National Institute of Justice (NIJ) grant 2016-NE-RD-0001, but the next step is to define a way to operationalize its use for sexual assault cases. This can be achieved by determining the accuracy of analyzing true unknown and known samples considering that many of the minor and unique components can be absorbed into the human skin or wear away during the act of sex or any appropriate use. This project aims to develop the necessary guidelines for forensic laboratories to analyze unknown lubricant samples, including collection, storage methods, screening methods, lubricant extraction protocols, analysis, and classification. Because it is necessary to provide direct comparison between degraded unknown samples, it is necessary to develop a protocol for degrading known lubricant samples in the laboratory for analytical comparison. This will allow for the determination of false positive and false negative error rates throughout the forensic analytical process. At the end of the project, optimal collection, storage, and extraction protocols will be developed. Additionally, an evaluation of classifying true unknown lubricants that were purchased for this project specifically and those residues collected after sexual intercourse will be conducted to determine error rates. Newly acquired lubricant samples will be added to the SAL database at the end of the project.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Characterization and Analysis of Lithium-Ion Battery Fire Signatures and Debris

NIJ AWARD #: 2018-MU-BX-0004

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According to the Fire Protection Research Foundation, in the United States it is assumed that normal lithium-ion cells from reputable manufacturers will exhibit failure rates of less than approximately 1 in 1 million. Although overwhelmingly safe, given the large numbers of cells of varying quality in devices and appliances, notable fires involving lithium-ion batteries have occurred in the past and challenged fire forensic investigators to determine in what scenarios lithium-ion batteries can initiate a fire and what post-fire signatures exist to determine if the battery was more likely the cause of the fire or a victim of the fire. Characterization of the ignition characteristics of 18,650 cells and the post-failure properties of the cells are crucial first steps for determining whether batteries were the cause of fire. States of charge/health, oxygen concentration of the environment, and proximity to adjacent secondary fuel packets of varying properties are all critical factors that change battery thermal failure behavior (e.g., ignition and propagation) and post-failure conditions (e.g., residual mass) and complicate the investigation process. This study involves studying thermal failure of cylindrical format 18,650 cells under varying states-of-charge and in controlled environments with either inert or normal oxygen environments to quantify the temperature of vented gases and heat flux from these gases to surrounding objects. Tests provide visual demonstrations (e.g., flame height) of thermal runaway in different configurations and ignition propensity and flame spread over standard furnishing type materials. Finally, the internal propagation of 18,650 cells inside laptop power banks and the subsequent jet flame issued from the laptops were studied to investigate the effect of a common appliance package on the propagation of individual cell thermal runaway. Overall, in this study, experimental work on 18,650 cells in multiple configurations seeks to provide some basic data for fire investigators to use in assessing the likelihood of a battery to cause a fire as either a single cell or when used in a power bank system.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

POSTER ABSTRACTS IV

FORENSIC BIOLOGY/DNA

Preservation of Forensically Relevant Biological Materials with Commercial Off-the-Shelf Antioxidants and Chelators

NIJ AWARD #: 2015-R2-CX-K037

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After attending this presentation, attendees will have a better understanding of DNA degradation and preservation in blood, semen, saliva, and vaginal cell samples on cotton swabs. Attendees will also gain knowledge on the use of accelerated aging to simulate aging of mock biological evidence samples when real-time aging is not feasible. In forensic science, proper evidence collection and storage techniques are important to prevent or minimize DNA degradation in biological samples. It may not always be possible to achieve ideal storage conditions for evidence, and many current forensic evidence collection substrates (e.g., swabs, cloth) do not include methods for DNA preservation, leaving the DNA vulnerable to degradation by hydrolysis and oxidation. To preserve the integrity of the DNA and reduce the risk of DNA degradation, biological evidence should be preserved from environmental damage as soon as it is collected. The ability to apply a DNA preservative directly to the swab would minimize the risk of DNA degradation and could allow for the generation of higher quality DNA profiles. Currently, there are few options available to the forensic community for long-term preservation of biological evidence. The goal of this research was to identify the optimal method for the long-term preservation of forensic DNA evidence using commercial-off-the-shelf (COTS) preservatives that can be applied directly to evidence collection substrates. To accomplish this goal, the preservative effects of three chelators and four antioxidants were examined on forensically relevant biological materials deposited on cotton swabs. Chelating agents and antioxidants interfere with DNA degradation caused by hydrolysis and oxidation. The chelating agents examined in this study were desferrioxamine (DFOA), diethylenetriamine pentaacetic acid (DTPA), and ethylenediaminetetraacetic acid (EDTA); the antioxidants were α-tocopherol, astaxanthin, hydroxytyrosol, and zinc. Blood, semen, saliva, eluted vaginal cells, and eluted buccal cells were applied to cotton swabs. All swabs were dried at room temperature for approximately 1 hour, and then each preservative solution was applied directly to the tips of the swabs. The swabs were dried at room temperature overnight, placed in cardboard swab boxes, and stored at room temperature, 37°C, and 50°C with ambient relative humidity conditions or 75%–85% humidity. At 10 time points, samples were extracted on the Qiagen® QIAsymphony® DNA Investigator® Kit, quantified with the Quantifiler® Trio DNA Quantification Kit and amplified with the GlobalFiler™ PCR Amplification Kit. Capillary electrophoresis was performed on the 3500xL Genetic Analyzer for Human Identification, and data were analyzed with GeneMapper ID-X® v1.5 using an analytical threshold of 125 RFU and a stochastic threshold of 600 RFU. The DNA yields, degradation index values, and STR profile qualities were evaluated and compared for each biological material, preservative, storage condition, and time point. The results from this study will explore the efficacy of the examined preservatives, the stability of the biological materials under both ambient and increased humidity conditions, and the effectiveness of the accelerated aging technique.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

A Rotational Platform-Driven Microdevice for Differential Separation, Purification, and Amplification of Sexual Assault Forensic Samples

NIJ AWARD #: 2016-NE-BX-0002

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The ever-growing sexual assault kit backlog currently facing forensic laboratories is made worse by the prevalence of mixture samples that contain DNA from both the victim and the suspect. The most commonly used method of separating and analyzing these samples involves a decades-old technique that is time-consuming and often still requires back-end mixture interpretation, which can lead to a high rate of inconclusive reporting. This work has developed a novel bead-mediated antibody binding assay for separating sexual assault mixtures and a microdevice platform for automation of the separation, DNA preparation, and short tandem repeat (STR) amplification steps. The binding assay utilizes streptavidin-coated beads bound to cell-specific antibodies to capture relevant in-tact cells. After binding, the size of the beads prevents bound cells from flowing into the unbound cell chamber. A simple enzymatic DNA liberation assay and downstream custom multiplex STR amplification are completed in just under 50 minutes. The sexual assault microdevice is fabricated from five layers of inexpensive PeT transparency sheets, bonded with toner and heat-sensitive adhesive. The microdevice hardware consists of a spin motor, Peltier clamp, laser, and related circuitry. Fluidic movement within the microdevice is exerted via centrifugal force only, without bulky pumps or actuators; laser-opened tap valves allow for fluidic movement between chambers. Microdevices can be further rotated with the mounted servos, which add flexibility to the fluidic architecture and provide improved fluid mixing. Upon commercialization, the entire platform could be condensed into a footprint roughly the size of a large shoebox. After an extensive literature search, selected antibodies were initially screened using flow cytometry. The best performing antibodies were then used to test the bead binding assay. When tested in-tube, sperm-specific SPAG-8 and CRISP2 antibodies performed best, capturing 70.09% and 52.61% of sperm DNA, respectively. Mucosal epithelial-specific antibody CK4 performed similarly, showing 68.98% of semen sample DNA in the unbound fraction. However, when semen-vaginal mixtures were tested in-tube, SPAM-1/PH-20, AKAP3, and CK4 outperformed SPAG-8 & CRISP2, generating STR profiles that had 10-fold more male DNA contribution than female contribution in the bound fraction (SPAM1/PH-20, AKAP3) or unbound fraction (CK4), essentially producing single-source male profiles. Semen and vaginal swab samples separated on the microdevice platform using SPAG-8 antibody-coated beads showed the expected single-source STR profiles in the bound and unbound fractions respectively, as desired. However, due to fluidic movement and valve limitations, the hardware and the microdevice itself were redesigned. Although semen-vaginal mixtures tested on the updated system produced a 142% enrichment of the male profile in the bound fraction, both bound and unbound fractions displayed clear mixtures at most loci. This work represents significant progress toward a quick, inexpensive, small-footprint alternative to current differential extraction methods, which could be easily and quickly implemented into forensic labs. In order to bring this platform to market, further optimization

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

of the binding assay is needed to minimize non-specific binding of non-target cells and non-specific desorption of the target cells. Modifications to the binding mechanism or antibody delivery methods could further improve binding and should be explored.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Paper Microfluidic Single-Walled Carbon Nanotubes Chemiresistive Biosensor Arrays for Body Fluids Identification

NIJ AWARD #: 2019-NE-BX-0006

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Identifying the body fluid left at a crime scene is an important component in forensic science because identifying the body fluid is often the key in a criminal investigation and is considered in court. Although tests for detecting body fluids are available, they have low sensitivity and selectivity, consume a significant quantity of valuable sample, and are labor-intensive. Furthermore, current identification methods are limited to one body fluid at a time and hence are inherently more time-consuming, require large sample volume, and are expensive and thus can negatively influence the outcome of a court case. The objective of the proposed research is to develop a point-of-use/on-site multiplexed nanobiosensor array that is digital/electronic for identification of multiple body fluids—blood, saliva, semen, urine, and sweat—through highly sensitive, quantitative, selective, facile, rapid, and cost-effective detection of 10 body fluid protein biomarkers. To detect these protein biomarkers simultaneously in a small sample volume, we will develop an array consisting of 10 nanobiosensors, each consisting of ultrahigh sensitivity single-walled carbon nanotubes chemiresistor transducer functionalized with an antibody of high specificity/selectivity against a specific target antigen. The ability of multiplexed and ultrahigh sensitive quantitative analysis provided by the array nanobiosensor would increase analysis specificity and thereby make body fluid identification infallible, reduce sample requirement, reduce time, and lower forensic analysis cost and make identification more infallible. In order to make these sensors amenable to on-site, multiplexed analysis from a small single sample as well as lightweight, semi/fully automated, easy to use, low cost, disposable, and with no/low environmental burden, single-walled carbon nanotubes chemiresistor nanobiosensors will be integrated with paper-based microfluidics. Furthermore, to facilitate performing forensic analysis at remote crime scenes, that is on-site, with various environmental conditions the sensor platform will be integrated to mobile/smartphone platform for analysis, data processing and communication. We expect that the proposed sensor system is potentially transformative because the expected outcomes will significantly improve forensic analysis of body fluids and thereby investigative lead and case investigation.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Evaluation of MPS Technology on Tissues Collected from the Body Farm for Forensic Identification

NIJ AWARD #: 2016-DN-BX-0172

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The National Missing and Unidentified Persons System (NamUs) estimates that over 600,000 individuals go missing in the United States every year, with several thousand becoming cold cases over time. New York ranks as the second highest state in the nation for number of reported unidentified human remains (2000–2004) and accounts for nearly 25% of all unidentified cases nationwide. The DNA Missing Persons Group at the New York City Office of Chief Medical Examiner processes hundreds of missing persons cases each year. Often, only body parts (e.g., limbs) or degraded tissues are available and consequently offer limited physical characteristics to make a match in missing persons databases. Routinely, DNA is first used in an attempt to amplify genomic markers (STRs) to generate an identity and sex profile that can be compared with DNA databases. If database comparisons are unsuccessful, or if the genomic DNA is too degraded to yield a profile, mitochondrial DNA is analyzed by sequencing the regulatory region, in particular the hyper-variable (HV) regions HV1 and HV2. The ability to utilize mtDNA to make forensic identifications on degraded tissues was evaluated using massively parallel sequencing (MPS) technologies. Tissue samples were obtained from the University of Tennessee Anthropological Research Facility Body Donation Program, where bodies are placed outdoors to decompose naturally. Tissues from one individual were collected over a defined period (0 to 40 days) in the fall of 2015 and then examined. Tissues at early collection dates showed little degradation, whereas with longer exposure the tissues became darker and softer. The Quantifiler™ Trio DNA Quantification Kit was used to determine degradation index and nuclear DNA concentration. Only the tissues collected at day 0 and day 10 (low Total Body Scores) showed sufficient DNA for STR profiling. Mitochondrial specific PCR revealed that the HV regions of the mtDNA could be amplified. MPS sequence analysis was performed utilizing the Promega's PowerSeq™ CRM Nested System and MiSeq reagent kit V3 on Ilumina's MiSeq FGx® instrument with the use of GeneMarker HTS (SoftGenetics) for data analysis. Profiles were uploaded to the EMPOP database.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Improving Results from Touch DNA Evidence with Optimized Direct PCR Methods

NIJ AWARD #: 2019-DU-BX-0009

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After attending this presentation, attendees will have a better understanding of the effectiveness of multiple direct polymerase chain reaction (PCR) amplification-compatible touch DNA sample collection methods and the advantages of direct PCR for touch DNA evidence compared with standard DNA processing procedures. Improved methods to generate high-quality DNA profiles from touch DNA samples are of considerable interest to forensic DNA laboratories. Touch DNA evidence samples are collected from items on which skin cells have been deposited after being worn, handled, or touched. Although the amount of DNA transferred to an object can vary from sample to sample, it is generally understood that touch DNA samples contain low amounts of DNA. Direct PCR, a sample processing method in which an evidence swab or substrate punch is added directly to an amplification reaction without prior extraction or quantification, has been identified as a method that may improve the generation of genotyping data from such samples. Direct PCR allows for the maximum amount of DNA template to be present in a reaction by eliminating DNA loss that occurs during DNA extraction, quantification, and concentration. The main objective of this study was to determine effective sample collection method(s) for use on touch DNA evidence items prior to direct PCR processing. Nine direct PCR-compatible collection methods were used to collect touch DNA from eight porous and nonporous substrates. Collection was performed with cotton swabs, nylon flocked swabs, and FTA paper that were moistened with sterile water, moistened with 0.1% Triton X, or left dry. Additionally, three types of fabric substrates were sampled via cutting. For each collection method, processing method, and substrate type, eight replicates were prepared from three donors. Samples were processed with two methods: (1) standard processing with DNA extraction and quantification and (2) direct PCR. The samples that underwent standard processing were extracted on the Hamilton Vantage liquid handling system with the Qiagen Investigator® STAR™ Lyse&Prep Kit and concentrated with Microcon® DNA FastFlow concentration devices. Quantification was performed on the concentrated samples with the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher). For direct PCR, the collected samples were placed directly in a 96-well plate for amplification. Amplification for both the extracted and direct PCR samples was performed with Thermo Fisher's GlobalFiler™ PCR Amplification Kit. Capillary electrophoresis was performed on the 3500xL Genetic Analyzer for Human Identification, and data were analyzed with GeneMapper ID-X® using an analytical threshold of 125 RFU and a stochastic threshold of 600 RFU. The results from this study will identify effective methods for collecting touch DNA for use with direct PCR. Furthermore, these results will support the use of direct PCR with evidentiary samples. By identifying effective sample collection methods and circumventing the extraction, quantification, and concentration processes, maximum quantities of DNA can be targeted, laboratory personnel error and exogenous DNA contamination may be minimized, and overall sample processing time and cost could be reduced.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Decontamination of Crime Scene Equipment: An Evaluation of Current Methods and Best Practices

NIJ AWARD #: 2018-DU-BX-0226

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A 2016 needs assessment conducted by the National Institute of Standards and Technology's Organization of Scientific Area Committees for Forensic Science (NIST/OSAC) Crime Scene Investigation (CSI) Subcommittee identified the decontamination of crime scene equipment as a gap that must be addressed. Currently, there are no widely accepted standard operating procedures (SOPs) or best practices for crime scene investigators to follow regarding equipment decontamination. Furthermore, there has been no peer-reviewed published research to date that assesses the effectiveness of the different decontamination methods that are currently employed by crime laboratories and law enforcement agencies involved in CSIs. Ineffective decontamination of crime scene equipment can lead to cross-contamination between scenes as well as secondary transfer to evidence after contact with equipment. With increased sensitivity of current DNA analysis protocols, the ineffective decontamination of CSI equipment has implication for wrongful convictions due to potential secondary DNA transfer. To address this gap, and in response to the NIST/OSAC needs assessment, RTI International performed a comprehensive evaluation of several decontamination methods on commonly used CSI equipment to provide the community with evidence-based recommendations of effective decontamination protocols. RTI identified reusable CSI equipment that is most likely to be contaminated with biological material after use at a crime scene based on literature searches, crime laboratory SOP reviews, and discussions with crime scene practitioners. Seven types of crime scene–related equipment were used to determine the extent of the effectiveness of nine frequently used decontamination methods. The total amount of DNA remaining on the equipment after a controlled decontamination was quantified using Quantifiler™ Trio DNA Quantification Kit and processed for STRs with GlobalFiler™. Quantifiler™ Trio includes a degradation index that estimates the quality of DNA in potentially degraded samples. The outcomes of this research will provide the CSI and forensic DNA communities the following: (1) an understanding of the possibility of introducing biological contaminants to crime scene equipment during scene processing and the threat of cross-contamination between scenes; (2) a determination of whether the threat of biological contamination varies between equipment; (3) an assessment of the effectiveness of current methods in reducing true contamination threats; and (4) recommendations for the formulation of best practices regarding biological decontamination methods.

https://doi.org/10.3768/rtipress.2021.cp.0013.2104

Evaluating and Optimizing DNA Extraction and Amplification Protocols for Microbiome-Based Forensic Applications

NIJ AWARD #: 2016-DN-BX-0196

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Human microbiota displays both specificity and inter-individual variability, which enables it to be a suitable candidate for forensic applications. As a person's microbes can be transmitted to human-touched objects, it is hypothesized that microbial DNA from touched objects could be isolated and used to identify a particular individual. However, the methods of microbial DNA extraction for forensic applications must first be optimized due to typically low DNA yields. To investigate and optimize extraction of microbial DNA, human-touched objects were identified in an office environment and sampled using four different commercial swabs: cotton, rayon, HydraFlock, and polyester. DNA from the swab samples were extracted using the bead-beating method and quantified using both NanoDrop ND-1000 spectrophotometer and Qubit 3.0 fluorometer. DNA were then amplified by using 24 different primer combinations after the optimization using a gradient polymerase chain reaction (PCR). The samples collected using cotton and rayon swabs yielded higher amounts of DNA than other swabs. We identified that keyboards and computer mice provided the highest DNA yields among human-touched objects. Among 24 primer sets, the 515F(Parada)–1064R and 515F–1064R primer combinations showed the best results with an annealing temperature of 62°C. This study evaluated different swabs and various primer combinations for small subunit ribosomal RNA genes, which provides a practical insight into microbiome-based forensic applications.

 https://doi.org/10.3768/rtipress.2021.cp.0013.2104

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RTI Press publication CP-0013-2104