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Gabby DiEmma and Erica Fornaro, Editors

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Abstract

The 2022 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.

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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, NIJ provides 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 March 1–2, 2022, NIJ and the FTCoE held the 2022 NIJ Forensic Science Research and Development (R&D) Symposium. 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, Frances Scott, Tracey Johnson, and Danielle McLeod-Henning—have worked to create a phenomenal research agenda. The full 2-day program included 26 presentations and 27 posters from principal investigators and their research partners; these presentations and posters represent accomplishments from NIJ R&D grants awarded during 2015–2020. Most presentations are archived on the FTCoE's website and available to view for free.

Dr. Dutton and Dr. Scott were moderators on Day 1. Dr. Dutton moderated Session I, Impression and Pattern Evidence/Trace Evidence; Dr. Scott moderated Session II, Seized Drugs and Toxicology.

Ms. Johnson and Ms. McLeod-Henning were moderators on Day 2. Ms. Johnson moderated Session III, Forensic Biology/DNA; Ms. McLeod-Henning moderated Session IV, Forensic Anthropology and Forensic Pathology.

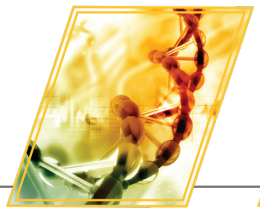
Summary of Oral Presentation and Poster Session Topics

NIJ Forensic Science Research and Development Symposium

March 1–2, 2022 • Virtual Event

Oral Presentation and Poster Session Topics






Sessions I & II

Impression and Pattern Evidence/ Trace Evidence | Seized Drugs and Toxicology

Sessions III & IV

Forensic Biology/DNA | Forensic Anthropology and Forensic Pathology

+ Digital Evidence (Poster Session Only)

701 Unique Attendees

2 Days

85% Desktop
14% Mobile
1% Tablet

Attendees per Session
Sessions I & II: **457**
Sessions III & IV: **378**
Poster Sessions: **321**

70% of attendees found the content engaging

Feedback From Attendees

"**Learning** about new techniques and methods"
"**Connecting** with experts in forensic research"
"**Knowing** new information and discoveries about other forensic science specialties"

26 Oral Presentations

7,483 Live Learning Hours

313 Chat Messages

8 Panel Sessions

27 On-Demand Posters

322 Learning Hours

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SESSION ABSTRACTS

SESSION I

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Moderated by NIJ Program Manager
Gregory Dutton

Validating Conclusion Scales in the Forensic Sciences

NIJ AWARD #: 2018-DU-BX-0212

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John Vanderkolk[2]
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*Presenting author

This project combined two sets of studies to validate the conclusion scales in the fingerprint, footwear, and tool mark disciplines. The first study measured how fingerprint examiners and members of the general public interpreted different articulation statements. The second set of studies measured how fingerprint, footwear, and tool mark examiners would use articulation statements expressed in strength-of-evidence language rather than as source attribution statements. By combining across the two sets of studies, we demonstrate how statements are both used in casework-like comparisons and how the articulation language is interpreted by the consumers of forensic evidence. In the first study, we measured the strength of evidence for six different scales in members of the general public and fingerprint examiners. The statements came from different types of scales, including categorical conclusions, likelihoods, strength of support statements, and random match probabilities. We used an online interface that required participants to first sort the statements correctly in a given conclusion scale and then to place each statement on a single evidence axis that ranged from most support imaginable for same source to most support imaginable for different sources. We found systematic differences between examiners and members of the general public, such that examiners distinguished between *Identification* and *Extremely Strong Support for Common Source*, while members of the general public did not. Statements that included numerical values tended to be placed lower than categorical conclusions, and members of the general public tended to place whatever statement was the highest in its scale at the very top of the evidence axis. The results suggest that laypersons can distinguish between statements meant to represent moderate vs. strong evidence but tend to place categorical conclusions above statements that involve numerical values. The second set of studies compared traditional conclusion statements against statements phrased as strength of evidence for different propositions in the fingerprint, footwear, and tool mark disciplines. Each participant completed 60 comparisons within their discipline that were designed to approximate casework conditions, using either a traditional or a strength of evidence conclusion scale. The scale used on each trial was randomly assigned, and participants knew the scale for that trial as they began the comparison. We found that fingerprint examiners redefined the term *Identification* when the scale was expanded to include *Support for Common Source*, using *Identification* less often than when the traditional scale was assigned. Fingerprint examiners were also much less likely to use *Extremely Strong Support for Common Source* than *Identification*. Footwear examiners treated the traditional and strength of evidence scales similarly, but tool mark examiners were much less likely to use *Extremely Strong Support for Common Source* than *Identification*, similar to fingerprint examiners. The results demonstrate that examiners reserve *Extremely Strong Support for Common Source* for only the comparisons with the most evidence for the common source proposition.

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Assessing Error Rates in Multiple Examiner Groups Using Regression Methods

NIJ AWARD #: 2019-DU-BX-4011

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*Presenting author

This presentation will introduce regression methods to evaluate continuous or ordinal decision scores from large-scale black box studies, especially when error rates of multiple examiner groups are compared. The uncertainty quantification provides inherent sampling variabilities of the black box studies. The true positive rates and true negative rates from our method will vary in different examiner groups and in different characteristics of source subjects. This ensures that the resulting error rates will not be "one size that fits all." Specifically, we applied regression methods to assess error rates of face recognition based on a recent black box study by Phillips et al. (2018). Participants include forensic facial examiners, facial reviewers, super recognizers, fingerprint examiners, and students. They were asked to provide ordinal-scale decision scores for image pairs based on their belief on whether the pairs belong to the same source. Scores are on a 7-point scale, with +3 for the highest confidence of same source to –3 for the highest confidence of different sources. Because human perception may exhibit differences regarding recognition of female faces and male faces, we expect differences between the respective error rates. In addition, it is likely that the characteristics of source subjects may affect the error rates of face recognition. Our regression model includes the ordinal decision score as the response variable. The covariates in this dataset include race, gender, age of source subjects, and examiner groups. To accommodate ordinal decision scores and to incorporate covariates of source subjects from the Phillips et al. (2018) black box study, we employed the ordinal regression instead of the regular linear regression. The resulting covariate-specific receiver operating characteristic curves provide false positive rates and false negative rates conditional on specific values of covariates. More importantly, our analysis provides the association between these error rates and characteristics of source subjects. We also calculated the error rates for each examiner group using the regression method and developed methods for calculating variances of these error rates to quantify the uncertainty for comparing examiner groups. We have also conducted large-scale simulation studies to study the uncertainty of the error rates from simulated data. Our simulation studies show that the error rates tend to have smaller variances as the sample sizes of the black box studies increase.

Reference

Phillips, P. J., Yates, A. N., Hu, Y., Hahn, C. A., Noyes, E., Jackson, K., Cavazos, J. G., Jeckeln, G., Ranjan, R., Sankaranarayanan, S., & Chen, J. C. (2018). Face recognition accuracy of forensic examiners, superrecognizers, and face recognition algorithms. *Proceedings of the National Academy of Sciences, 115*(24), 6171–6176

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Evaluating the Spatial Distribution of Randomly Acquired Characteristics on Outsoles

NIJ AWARD #: 2018-MU-MU-0003

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The foundation of a forensic footwear source association is the agreement between randomly acquired characteristics (RACs) identified on questioned and exemplar test impressions. These wear features are presumed to be randomly acquired and independent. However, independent acquisition does not necessarily mean wear features will be random or uniformly distributed because the factors (such as friction and gait) that dictate their development are not necessarily random or uniformly distributed across an outsole. The aim of this research is to determine if the distribution of RACs in a research dataset can be described by an inhomogeneous Poisson point process based on tread contact and wear. To achieve this goal, RAC spatial frequency from an empirical dataset of shoes was compared against simulated and modeled data assuming a Poisson point process. Deviations in count between the empirical and simulated/modeled predictions were examined using a Poisson rate test and spatial autocorrelation using Moran's I. Results indicate that RAC frequency over 67 percent to 79 percent of an outsole can be reasonably explained as a Poisson point process or a Poisson generalized linear regression model (non-spatial GLM), with tread contact as a predictor. Moreover, if the predictor is extended to include both tread contact and wear, RAC count over 84 percent of the spatial locations on an outsole are well-explained, although autocorrelation over nearly 40 percent of the shoe persists. Overall, results indicate that RACs are not uniformly distributed over an outsole, and if using a Poisson point process, they are underpredicted in the medial ball of the toe and over predicted in the heel and instep.

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Advancing Reporting of Significance From the Analysis and Comparison of Glass Evidence: A Global Collaboration

NIJ AWARD #: 2018-DU-BX-0194

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A standard test method of chemical analysis of glass evidence (ASTM E2927-16a) describes a consensus-based approach to sampling, sample preparation, and multivariate quantitative elemental analysis and also suggests a "match" criterion for the comparison of chemical properties of glass evidence. The result of the application of this "gold standard" method is a binary decision of either finding a difference in the elemental composition (exclusion) or a failure to exclude, based on elemental composition. This presentation aims to improve on this conclusion by demonstrating the utility of likelihood ratio (LR) calculations using different background datasets of glass samples of known manufacturing history or from casework. LRs were calculated using a previously reported multivariate kernel density (Aitken & Lucy, 2004) and calibrated with pool adjacent violators using a method previously reported by the authors (Corzo et al., 2018). Three different test datasets derived from the analysis of glass from known manufacturing origins (>400 samples from four different plants) and using the ASTM analytical method for data collection are interrogated using LR calculations. Five different background databases are used to calculate the LRs. The first background dataset is derived from a collection of elemental data from ~700 different authentic vehicle glass samples collected over 2 decades (collected with National Institute of Justice funding). The second background was provided by the Bundeskriminalamt forensic laboratory in Germany and includes ~430 casework samples from different sources. The third set was donated from a large law enforcement laboratory that has maintained elemental data for glass analysis for more than 3 decades. The last two datasets are also casework from international laboratories. The LRs calculated from comparing glass manufactured at three different plants over relatively short periods (over 2–6 weeks) result in a range of calibrated LR values from very low ($LR \sim 10^{-3}$) when the glass is manufactured at different plants or manufactured weeks or months apart in the same plant to very high ($LR \sim 10^{3}$) when the glass samples either originate from the same source or were manufactured on the same day and in the same plant. Although some of the glass samples being compared may not originate from the same broken window source, they exhibit chemical similarity within these lower and upper bounds, and the LRs presented here facilitate the correlation between chemical relatedness to manufacturing history, specifically the time interval between production. The results from an interlaboratory trial involving 10 laboratories and the analysis of several blind samples and interpretation of the resulting data using the LR calculation are also presented. The overall aim of this research is to improve on the opinion statement provided to the court for the significance of finding matching glass evidence in a particular case.

References

Aitken, C. G. G., & Lucy, D. (2004). Evaluation of trace evidence in the form of multivariate data. *Journal of the Royal Statistical Society Series C (Applied Statistics), 53*, 109–122.

Corzo, R., Hoffman, T., Weis, P., Franco-Pedroso, J., Ramos, D., & Almirall, J. R. (2018). The use of LA-ICP-MS databases to calculate likelihood ratios for the forensic analysis of glass evidence. *Talanta, 186*(15) 655–661. https://doi.org/10.1016/j.talanta.2018.02.027

Attenuated Total Reflection Infrared Microscopic Analysis of Simulated Automotive Paint Smears for Vehicle-Vehicle Collisions

NIJ AWARD #: 2017-IJ-CX-0022

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Kaan Kalkan
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*Presenting author

Paint smears represent a type of automotive paint sample found at crime scenes that is problematic for forensic automotive paint examiners to analyze because there are no reference materials present in forensic automotive paint databases (such as Paint Data Query) to generate hit-lists of potential suspect vehicles. Realistic paint smears are difficult to create in a laboratory and have also proven challenging to analyze because of the mixing of the various automotive paint layers. A procedure based on an impact tester has been developed to create smears (e.g., abraded or deformed clear coats, color coats, and undercoat layers mixed together or top coats and undercoat layers mixed together) to simulate paint transfer between vehicles during a collision. As revealed by attenuated total reflection (ATR) infrared (IR) imaging microscopy and alternating least squares (ALS), there is separation of the automotive paint layers in some but not in other regions of the IR image. In contrast, a paint smear appears intact in all regions using transmission IR imaging microscopy. The unmixing of the layers is only evident with ATR IR imaging microscopy because of its superior spatial resolution. As smeared paint in all likelihood undergoes melting due to the heat generated in a vehicle-to-vehicle collision, it is plausible that a separation phenomenon of the different paint layers may occur during fluid flow due to differences in the viscosities and/or affinities of the layers for the substrate. Data collected from 26 original equipment manufacturer (OEM) paints in simulated collisions using an impact tester with a steel (inert) substrate show that ATR IR imaging microscopy possesses sufficient spatial resolution to isolate the individual OEM layers. The General Motors OEM paint samples used spanned a narrow production year range (2000–2006). For each OEM paint sample, the corresponding smear depended on the conditions used (e.g., the level of the elastic force constant and the damping factor of the springs used to control the sliding of the two metal substrates during a simulated collision). By varying these conditions, the number of distinct layers obtained was tuned for each of the OEM paints investigated. Furthermore, the IR spectrum of each layer extracted from the image using ALS was found to compare favorably to an in-house General Motors OEM paint spectral library (comprising 600 IR spectra) for each layer as the correct match was always found to be in the top five hits. The results of this study indicate that the paint smears developed using the impactor can serve as the basis of realistic proficiency tests for forensic laboratories and could also be used to train forensic scientists.

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

NIJ AWARD #: 2020-MU-CX-0018

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Very small particles (VSP) acquired post-manufacture and trapped in the adhesive along the edges of duct tape rolls have the potential to discriminate among tape segments from different rolls and provide a quantitative association between segments from the same roll. Forensic analysis of duct tape is important in the investigation and prosecution of major crimes where it occurs as blindfolds, bindings, and ligatures. Laboratory methods of examination and comparison are focused on physical and chemical properties of tape backings, adhesives, and reinforcing materials. Correspondence in properties provides very strong evidence that two specimens share a common manufacturing source. However, as for any mass-produced commodity, the associative value is limited to a class association. This concern was specifically identified in the 2009 National Academy of Sciences (NAS) report, together with the suggested remedy that analytical methods be developed to exploit characteristics acquired post-manufacture, during an item's use. Once a roll of tape is used, exposed adhesive along the sides of the roll presents an ideal opportunity for collection, and most importantly retention, of the VSP ubiquitous in our environment. VSP occur with tremendous variety and, when adhering to items of physical evidence, can provide a powerful means of association independent of manufacturing characteristics. The current research focus is development of a practical and effective means to harvest VSP trapped within the adhesive along the edges of duct tape. Procedures are complicated by the presence of adhesive filler and pigment particles that are part of the duct tape adhesive formulations. Sampling methods intended to harvest post manufacture–acquired VSP will inevitably include collection of the filler and pigment particles. These particles must be recognized using substrate controls, and procedures for sampling and analysis need to accommodate the differences in the variety and abundance of these particles. Alternative VSP harvesting approaches include (1) excising the duct tape edges and extraction of particles from the adhesive, (2) swabbing duct tape edges, and (3) using a substrate to "lift" particles from the tape edge. Methods differ in the thoroughness of VSP recovery, amount of effort and skill required, and the yield of VSP particle "signal" above the filler/pigment particle "background." Following method development, the project will harvest VSP from the edges of a population of duct tape specimens and use scanning electron microscopy/energy dispersive X-ray spectroscopy to analyze these VSP and to distinguish them from those particles present as manufactured components of duct tape adhesives. Previously developed statistical and interpretive methods will be used to test the potential of VSP acquired post-manufacture along the edges of duct tape to address the 2009 NAS concerns and support or refute the association of one piece of tape with another.

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SESSION ABSTRACTS

SESSION II

SEIZED DRUGS AND TOXICOLOGY

Moderated by NIJ Program Manager
Frances Scott

Accurate THC Determinations in Seized Cannabis Samples for Forensic Laboratories

NIJ AWARD #: DJO-NIJ-20-RO-0009

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Forensic laboratories have been forced to start differentiating seized cannabis samples as either legal hemp or illegal marijuana with the passage of the 2018 Farm Bill, despite these labs lacking reliable extraction protocols and analytical methods for this purpose. Historically, forensic laboratories have only performed a series of qualitative measurements: macro- and microscopic identification of plant features, colorimetric tests for the presence of tetrahydrocannabinol (THC), and confirmation of Δ^9-tetrahydrocannabinol (Δ^9-THC) by gas chromatography-mass spectrometry (GC-MS). The new legislation declassified hemp as a Schedule 1 controlled substance and defined it as cannabis containing 0.3 percent or less of decarboxylated-Δ^9-THC (total THC). As a result, forensic laboratories are now required to quantify the level of total THC in seized evidence to distinguish it as either hemp or marijuana. The objective of this project was to provide forensic laboratories with the necessary analytical tools to make these measurements confidently through simple, robust, and cost-effective analytical methods. This effort has primarily focused on the development of isotope dilution GC-MS methods, extraction protocols, and a single laboratory validation study. Existing qualitative GC-MS approaches are amenable to quantitative measurements with specific analytical modifications to sample preparation protocols, addition of isotopically labeled internal standards (i.e., Δ^9-THC-d3), and data collection mode from full scan to single ion monitoring mode for quantitation of *m/z* 299. This presentation will summarize the method development and validation results for the determination of total THC in cannabis (hemp and marijuana) plant samples.

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Development of a Validated UHPLC-DAD Method With Optional ESI/TOFMS Detection for Rapid Quantification of Δ^9-THC and Δ^9-THCA Among Sixteen Cannabinoids in Hemp Concentrates

NIJ AWARD #: 2020-DQ-BX-0021

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The Federal Controlled Substances Act of 1970 defined cannabis as a Schedule 1 substance, which made marijuana and hemp—two primary species of cannabis—illegal. Although marijuana is psychoactive, hemp is not. To facilitate the commercial cultivation, processing, and sale of hemp and hemp-derived products, the 2018 Farm Bill excluded hemp from the statutory definition of cannabis if its total concentration of Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ9-tetrahydrocannabinolic acid A (Δ^9-THCA-A) is not more than 0.3 percent. Here, we report an ultra-high performance liquid chromatography–diode array detector (UHPLC-DAD) method with optional electrospray ionization time-of-flight mass spectrometry (ESI/TOFMS) detection for rapid quantification of Δ^9-THC and Δ^9-THCA-A among 16 cannabinoids in hemp concentrates. In the literature, a thoroughly systematic optimization of the liquid chromatography separation of cannabinoids was not reported. In our study, the effects of the type of organic solvent (i.e., methanol and acetonitrile), the content of the organic solvent, the pH of the mobile phase, and the temperature of the column oven were thoroughly investigated. Under the optimized conditions, the achieved minimum resolution of adjacent cannabinoids was 1.58 at 1 µg/mL individual cannabinoid concentration. Method validation was performed according to the International Organization for Standardization (ISO) 17025 guidelines. The linear calibration ranges of all cannabinoids were between 0.02 and 25 µg/mL with R2 ≥ 0.9880. Quality control samples of individual cannabinoids were prepared at 0.04, 1, and 25 µg/mL and analyzed in triplicate on each day, consecutively on 3 separate days, with precision and accuracy within the requirements by ISO 17025. Unlike most published methods that had to analyze the same sample at more than one concentration because of a narrow linear calibration range, samples were analyzed at one concentration with our method (i.e., 20 µg/mL in methanol) because of a wide linear calibration range. Nine samples of hemp concentrates (i.e., cannabidiol [CBD] distillate [90 percent+], cannabigerol [CBG] distillate, cannabichromene [CBC] distillate [99 percent], cannabinol [CBN] terpsolate dabs, Δ^8 hemp distillate, Δ^8 hemp shatter, gelato CBD vape pen cart, clementine CBD vape pen cart, and full spectrum CBD oil 1000 mg) were analyzed in triplicates with relative standard deviation (RSD) ranging from 0.35 to 11.18 percent. Although the recent Δ^8 THC craze concerned chemists, our results clearly showed that Δ^8 hemp distillate and Δ^8 hemp shatter contained 10.28 and 10.52 percent Δ^9-THC, respectively, although other samples contained less than 0.3 percent Δ^9-THC. In the literature, published recovery experiments were limited by the unavailability of cannabinoid-free matrix and the high cost of cannabinoid standards. We solved this problem by spiking abnormal cannabidiol, a cannabinoid not naturally present in cannabis products and commercially available with a reasonable price, into the samples. Our assessment in triplicate showed that the recovery ranged from 94.8 to 103.6 percent with RSD from 1.51 to 8.00 percent.

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Quantitative Analysis of Δ^9-Tetrahydrocannabinol (THC) in the Presence of THC Isomers in Biological Specimens Using Liquid Chromatography Tandem Mass Spectrometry

NIJ AWARD #: 2020-DQ-BX-0017

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The ever-changing climate of cannabis decriminalization or legalization has had a significant impact on forensic testing laboratories. Traditionally, changes within the seized drug community can be utilized to anticipate analyses needed within forensic toxicology laboratories. The conformation and quantitation of Δ^9-tetrahydrocannabinol (Δ^9-THC) and its metabolites in biological matrices is commonplace among testing laboratories. It is essential for forensic toxicology laboratories to adapt to the legal consumption of manufactured cannabis products containing other phytocannabinoids and isomers of Δ^9-THC. 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 (DUI/DUID) and death investigations. In 2020, DFS Toxicology received approximately 10,780 cases. Over the past 5 years, DFS has observed a 25 percent increase in the reporting of quantitative values for Δ^9-THC. In addition to the increased caseload, the median and average whole blood concentration has also increased. Of late, DFS Toxicology has indicated the presence of Δ^8-THC in a variety of case types. Traditional chromatographic methods did not focus on the chromatographic separation of Δ^9-THC isomers because of the lack of prevalence of these compounds. Given the current climate of THC isomers and legislative language surrounding cannabinoids, traditional methods require additional method development and validation to effectively separate, confirm, and quantify not only Δ^9-THC but its commonly observed isomers, including Δ^8-THC, $\Delta^{6a,10a}$-THC, and Δ^{10}-THC. DFS has validated a quantitative method for Δ^9-THC and its associated metabolites (11-hydroxy-Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC) using liquid chromatography tandem mass spectrometry (LC/MS/MS) that provides baseline resolution between Δ^9-THC and its commonly encountered isomers. The method has been validated to meet the requirements set forth by the ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. In addition to the changes in analytes within biological specimens, forensic toxicology laboratories must anticipate an increased caseload with the decriminalization or legalization of cannabis use. It is critical to decrease sample volume and streamline sample preparation to offset the increase in caseload. DFS has been investigating the viability of non-traditional sample preparation techniques and changes in various instrumental parameters to assist in streamlining the protocol for THC analysis in biological matrices while still providing a reproducible and robust analytical method.

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Blood Protein Modification Assay for Retrospective Detection of Abused Drug Exposure

NIJ AWARD #s: 2017-MU-BX-0002 AND 2020-R2-CX-0023

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Hemoglobin (Hb) contains a nucleophilic free thiol moiety at β93Cys that is highly reactive with electrophilic species, including oxidative drug metabolites. The measurement of covalent modifications at Hb β93Cys as retrospective exposure biomarkers has been studied for environmental and occupational toxicants. In contrast, the use of this approach for assessing abused drug exposure in forensic settings has not been explored, despite its potential value as an alternative to hair analysis. In this laboratory, development of a viable assay based on Hb modifications has focused on three critical steps: (1) characterization of covalent adducts formed by selected drugs using an in vitro metabolic assay with a Hb tryptic peptide containing the reactive thiol moiety (i.e., GTFATLSELH93CDK) as a trapping molecule, (2) development of an enrichment method to selectively remove unmodified Hb to allow for greater sensitivity in the detection of covalent Hb thiol adducts, and (3) confirmation of modification of the β93Cys moiety by selected drugs following tryptic digestion and high resolution mass spectrometry (HRMS) peptide analysis of in vitro modified Hb. For the first aim, analysis of adducted peptide formed with acetaminophen (APAP); 3,4-methylenedioxymethamphetamine (MDMA); methamphetamine (METH); and Δ^9-tetrahydrocannabinol (THC) was performed on an Agilent 1290/6530 liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) system. The metabolic trapping assay included human liver microsomes (HLMs), NADPH, G6P, G6PD, target peptide, and drug in pH 7.4 buffer, with incubation for 4 hours at 37°C. Predicted covalent thiol modifications for parent drugs and metabolites were added as target ions to BioConfirm B.08.00 software via Sequence Manager and were used to identify peptide modifications. For the targeted tandem mass spectrometry (MS/MS) studies, the spectra were collected and compared to a theoretical peak list supplied by Protein Prospector, and confirmed peaks were recorded. Adducted Hb peptides were detected and structure confirmed for all four drugs and/or selected metabolites. For the second and third aims, test drugs (i.e., APAP, clozapine, oxycodone, cocaine, and diazepam) were added to the HLM trapping assay along with control human Hb. Following a 6-hour incubation and centrifugation in a 3-kDa spin filter, treated Hb was collected and subjected to an enrichment procedure developed in the Principal Investigator's laboratory using Thiol Sepharose 4B resin for removal of unmodified Hb. After an 18-hour incubation with resin, supernatant containing enriched, drug-modified Hb was collected and subjected to tryptic digestion and peptide analysis on an Agilent 1290/6530 LC-QTOF-MS system. Agilent Qualitative (B.07.00) and BioConfirm (B.08.00) software along with Protein Prospector were used to process tryptic peptide MS and MS/MS spectral data. Full scan data confirmed specific binding at the Hb β93Cys moiety for the drug metabolites N-acetyl-p-benzoquinone imine (NAPQI), clozapine-n-oxide, noroxycodone, hydroxybenzoylnorecgonine, and n-hydroxydiazepam, with peptide mass errors of <5 ppm. Additional MS/MS fragmentation data further confirmed the binding site and adduct structure.

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The ability to detect low levels of drug-induced covalent modifications at the reactive β93Cys moiety of Hb using this enrichment approach and targeted HRMS analysis suggests that the approach could be a useful alternative to hair analysis for long-term biomonitoring of drug exposure. Current work is being conducted to test the applicability for detecting such specific Hb modifications in authentic specimens.

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Development of an Open-Source Direct Analysis in Real Time Mass Spectrometry (DART-MS) Search Software and Library Building Tool for the Analysis of Complex Drug Mixtures

NIJ AWARD #: DJO-NIJ-20-RO-0012

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Facing increasing caseloads and an ever-changing drug landscape, forensic laboratories have been implementing new analytical tools. Direct analysis in real time mass spectrometry (DART-MS) is often one of these tools because it provides a wealth of information from a rapid, simple analysis. The data produced by these systems, although extremely useful, can be difficult to interpret, especially in the case of complex mixtures. Unlike traditional gas chromatography mass spectrometry (GC-MS) systems, software to aid in spectral analysis and interpretation is limited and primitive. The purpose of this proposal was to create software for DART-MS spectral interpretation, library building, and report generation with input from the community that is vendor-agnostic and freely available. Implementing search algorithms developed at the National Institute of Standards and Technology (NIST), the software allows laboratories to gain more insight into their DART-MS data by identifying potential components in a mixture while leveraging multiple in-source collision-induced dissociation (is-CID) spectra to provide quantitative indices for decision-making. This produces results similar to what forensic chemists are accustomed to when they use GC-MS search software. Importantly, the creation of the software, the user experience, and the included features were done in collaboration with five forensic laboratories that represent local, state, and federal levels.

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Fusion of DART-HRMS-Derived Dark Matter and Infrared Spectroscopy for the Identification of New Psychoactive Substances

NIJ AWARD #: 2017-R2-CX-0020

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Novel psychoactive substances (NPS) are recreationally used unscheduled products that elicit a psychoactive response and have a high potential for addiction. Although several are scheduled compounds, there are numerous sites within their scaffolds to which structural modifications can be introduced, resulting in the continued emergence of novel variants that retain their psychoactivity. These "legal highs" enjoy increasing use without the risk of criminal prosecution by law enforcement agencies because of their non-regulated status. Crime laboratories have undertaken structural elucidation studies of suspected NPS using a variety of methods, including colorimetric assays, thin layer chromatography, liquid chromatography, gas chromatography-mass spectrometry, Fourier transform infrared (FTIR), and nuclear magnetic resonance spectroscopies to characterize NPS. However, it is still challenging for crime laboratories to rapidly determine the emerging variants that mostly represent modifications of earlier generations of drugs. Developing accurate, fast, and cost-effective techniques for structure determination of the psychoactive compounds is crucial. In this study, we are investigating the combination of direct analysis in real time high-resolution mass spectrometry (DART-HRMS)–derived neutral loss data and infrared (IR) spectroscopy data to provide a rapid and accurate means by which to distinguish emerging compounds. Data measured using different analytical methods are often complementary, and their fusion enhances knowledge discovery. Although IR spectroscopy can indicate the presence of certain diagnostic structural features that imply the presence of a new psychoactive compound, it does not supply definitive structural information. Complementary DART-HRMS–derived neutral loss data furnishes the structural information. The fusion of these datasets can rapidly yield structural information for the determination of emerging unknowns. In this study, 36 tryptamine standards were subjected to DART-HRMS and IR analysis. DART-HRMS data were collected under collision-induced dissociation conditions at 20, 60, and 90 V in positive ion mode in the mass range of m/z 40–1000. Generation of neutral loss spectra enabled the extraction of the fragment identity information that is essential to interpretation of the spectra. The neutral loss spectra were aligned along common m/z values and merged row-wise for further analysis. For IR data, samples were analyzed using attenuated total reflection (ATR) IR spectroscopy in the wavenumber range of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. The transmittance data were corrected for baseline and multiplicative scattering. The IR and DART-HRMS neutral loss data were fused row-wise and analyzed by hierarchical clustering analysis to reveal the groups of similar structures that can be utilized for identifying the core skeletal features of NPS. The results revealed that the fused IR neutral loss results were able to group similar structures and therefore, fusion resulted in a synergetic effect that enabled accurate elucidation of emerging psychoactive substances. The partial least squares discriminant analysis algorithm was applied on the fused IR neutral loss data to create a screening model for classifying unknown samples. "Leave-one-structure-out" validation, which evaluated the performance of the model for predicting the structure of unknowns, gave 100 percent accuracy, precision, sensitivity, and specificity.

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Expert Algorithm for Substance Identification (EASI) From Mass Spectra

NIJ AWARD #: 2018-75-CX-0033

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Since the introduction of the electron ionization (EI) source by Bleakney in the 1930s, mass spectrometrists have strived to understand the relationships between the structures and spectra of organic molecules. The current state-of-the-art algorithms for database searches provide probabilities on the order of 80 percent that the correct identity of a compound will be ranked in the top three in a list of all possible identities (not accounting for gas chromatography retention time). By developing a superior algorithm for mass spectral comparisons, we hope to increase the confidence and accuracy of identifying substances from their mass spectra and enable inter-instrument or interlaboratory comparisons. Our new algorithm is tied to fundamental concepts of unimolecular fragmentation, which predict that any variation in an instrument's conditions will result in linear relationships between at least some of the ion abundances. The linear behavior derives from instrument effects on the internal energy distribution of the ions and the apparent observation time of fragmentation kinetics, as described by Rice–Ramsperger–Kassel–Marcus or quasi-equilibrium theories. Our algorithm uses a general linear regression model to enable extrapolation from ion behaviors measured on one instrument to make accurate predictions about ion behaviors on other instruments. A binary classifier then uses collective measures of the accuracy of multiple abundance predictions within a questioned spectrum to decide whether or not to identify the questioned sample as a particular drug. Using external validation spectra of hundreds of replicate spectra, the algorithm predicts abundances with a precision that is typically five times better than models that assume a fixed exemplar, as is the normal approach. The algorithm is sufficiently powerful to accurately distinguish between cocaine and its diasteriomers—allococaine, pseudococaine and allopseudococaine—measured on different instruments and to distinguish between fentanyl analogs like valerylfentanyl and isovalrylfentanyl.

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NPS Discovery Toolkits: Real-Time Identification and Dissemination of Information Regarding Novel Psychoactive Substances

NIJ AWARD #: 2020-DQ-BX-0007

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Novel psychoactive substances (NPS) are an increasing danger to public health and safety because there is little knowledge surrounding these emerging and constantly evolving drugs. Many government and forensic laboratories have difficulty staying ahead of these trends because of case backlogs, lack of funding, or lack of appropriate time and instrumentation. Our laboratory has streamlined the process of identifying new drugs by launching our NPS Discovery initiative in 2018. NPS Discovery comprises various open-access reports, giving the most up-to-date information and data regarding NPS in the United States. A primary goal of NPS Discovery is to anticipate drugs becoming available on the illicit drug market and react appropriately to their emergence once identified in authentic forensic samples. Our scientists collaborate with hospitals, police departments, medical examiner and coroner's offices, and other forensic laboratories to help identify and confirm NPS in a wide range of populations. NPS Discovery focuses on monitoring NPS through sample-mining and data mining, characterizing NPS based on their metabolic pathways, and confirming NPS through rapid development and validation of specialized methods. NPS Discovery Toolkits are an integral piece of the dissemination of information as they consolidate all information available for a specific drug. The first toolkit released by NPS Discovery focused on the novel synthetic opioid metonitazene. A drug monograph was released upon the first identification in July 2020, which describes the chemical information of metonitazene and shows experimental data using different instrumentation (e.g., liquid chromatography-quadrupole time of flight-mass spectrometry [LC-QTOF-MS], gas chromatography-electron-ionization mass spectrometry [GC-EI-MS]). The public alert describes demographic information and geographic distribution, a general background on metonitazene, and recommendations for public health and safety personnel. Trend plots show this synthetic opioid first started being seen in forensic casework around late 2020 with seven identifications in Q4 and peak positivity in early 2021 (29 identifications in Q1 2021). Similarly, trend reports show the positivity and combination information for metonitazene. Although primarily found with fentanyl and NPS benzodiazepines (e.g., clonazolam, flualprazolam), metonitazene was seen with other NPS opioids (e.g., flunitazene). The final piece of the NPS Toolkits is the analytical portion, which describes analytical methods for identifying and confirming the presence of the drug. For metonitazene, two liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) methods were evaluated. All instrument parameters are provided. Suggested extraction procedures are described, and assessment of these procedures, including recovery, matrix effects, and process efficiency, is shown. Authentic sample data are provided for any available matrices. For metonitazene, 20 authentic postmortem cases were analyzed, showing it was found with fentanyl, additional NPS, and cutting agents. From this sample set, metonitazene had a mean blood concentration of 6.3 ng/mL (±7.5 ng/mL, range: <0.5–33 ng/mL). In urine, metonitazene had a mean concentration of 14 ng/mL (±13 ng/mL,

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range: 0.6–46 ng/mL). The NPS Discovery Toolkit was uniquely designed to be a "one-stop" resource for laboratories and individuals interested in information regarding a specific NPS. The toolkits provide all known information about a NPS in an open-access format that provides laboratories the ability to identify and quantitate the new drug without delays.

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SESSION ABSTRACTS

SESSION III

FORENSIC BIOLOGY/DNA

Moderated by NIJ Program Manager
Tracey Johnson

Evaluation of Precision ID GlobalFiler NGS STR Panel

NIJ AWARD #: 2018-DU-BX-0166

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The technique of individual identification in modern forensics, DNA typing of short tandem repeats (STRs), has brought a standardized, quantitative method with strong statistical underpinnings to the criminal justice system. Although the fundamental principles behind STR typing have not changed, newly developed instrumentation and informative biological markers have the potential to address limitations of current techniques and to improve throughput at lower costs. The forensic community is beginning to evaluate massively parallel sequencing as a means to overcome these problems. Such methods not only add additional sequencing information but have a nearly unlimited capacity for additional STRs and single nucleotide polymorphism (SNP) markers, thereby enhancing individual identification. These instruments also have the potential for significant improvements in throughput at lower costs. The goal of this study was to evaluate Thermo Fisher's Precision ID GlobalFiler™ NGS STR Panel v2 by using the Ion Chef and the S5 System for forensic casework. To achieve this goal, 24 experimental runs were performed testing GlobalFiler™ NGS STR Panel v2 thoroughly. Although data analysis is still ongoing, the following results will be discussed: concordance, coverage (number of reads), repeatability, number of samples of an experimental run, artifacts, allele coverage ratio, sensitivity, degraded, and mixed DNA samples.

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Verification and Evaluation of a miRNA Panel for Body Fluid Identification Using DNA Extracts

NIJ AWARD #S: 2012-DN-BX-K017, 2016-DN-BX-0163, AND 2019-NE-BX-0005

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Molecular-based approaches for biological source identification are of great interest in the forensic community because of a lack of sensitivity and specificity in current methods. MicroRNAs (miRNAs) have been the subject of many body fluid identification studies because of their robust nature and tissue specificity; however, analysis requires a separate RNA extraction, requiring an additional step in the forensic analysis workflow. The purpose of this project was to build on previous work in our laboratory, wherein we identified a panel of eight miRNAs that can identify blood, semen, menstrual secretions, vaginal secretions, feces, urine, and saliva. As part of two previously National Institute of Justice–funded projects, we conducted a pilot study in which we showed that miRNAs are consistently detectable using several DNA extraction methods commonly utilized in the field for forensic casework. We reported that the miRNA panel for forensic body fluid identification was evaluated using DNA extracts of semen, saliva, blood, and menstrual secretions and was largely concordant with results from samples deriving from RNA extracts. In this project, we evaluated a larger sample set of DNA extracts (50 donors each of blood, semen, vaginal secretions, menstrual secretions, feces, saliva, and urine) and evaluated miRNA expression using the method previously validated for RNA extracts using quantitative reverse transcription PCR (RT-qPCR) analysis of DNA extracts. A quantitative discriminant analysis (QDA) model was developed using these data and demonstrated an accuracy of 91.3 percent in body fluid classification. The model was then tested against other human body fluid sample replicates over time and biological cycles, as well as human organs and tissues, and body fluids from other species to identify potential areas of misclassification. The validated QDA model is now available on a public website for testing by the research community. In conclusion, the panel of miRNA markers was shown to have a high specificity for classifying six biological fluids in DNA extracts using QDA and is a promising alternative to traditional serological tests for forensic casework. This is a simple RT-qPCR assay that uses only a small portion of the DNA extract to classify the body fluid(s) present in the evidence, with no additional sample use or personnel time in producing a separate RNA extract.

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Variation in Laboratory Policies and Procedures Related to Interpretation of DNA Mixtures

NIJ AWARD #: 2020-R2-CX-0049

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The purpose of this presentation is to describe the first phase of the ongoing study, Inter-laboratory Variation in Interpretation of DNA Mixtures. The results of the Policies and Procedures (P&P) Questionnaire and variability in laboratories' standard operating procedures related to DNA mixtures will be presented. The study is being conducted to evaluate the current state of the practice of DNA mixture casework and is not restricted to specific products or statistical approaches. Since 1995, the mixture interpretation process has been continually improved through numerous research and development efforts. Within the past 5 years, the use of probabilistic genotyping has become common in laboratories because it significantly advances the mixture interpretation process. Almost all inter-laboratory studies reported to date were conducted prior to the widespread adoption of probabilistic genotyping software in crime laboratories. This study evaluates the current state of the practice in interpretation of DNA mixtures utilizing either binary or probabilistic genotyping protocols. The scope is limited to variability in interpretation and analysis of electropherograms, thereby eliminating variability due to laboratory processing of physical samples. The project expands on the results and lessons learned from DNA mixture interlaboratory studies conducted to date, most notably the National Institute of Standards and Technology MIX13 study, and addresses concerns regarding complex DNA mixtures raised by the President's Council of Advisors on Science and Technology (PCAST) 2016 Report on Forensic Science in Criminal Courts (PCAST, 2016). The study is composed of four phases conducted to assess the sources of variability in analyzing DNA mixtures: (1) P&P Questionnaire—an online questionnaire to assess laboratory policies and procedures relevant to DNA mixture interpretation (notably systems, types of statistics reported, and parameter settings used); (2) Casework Scenario Questionnaire—an online questionnaire presenting a number of casework-derived scenarios (without DNA data) and asking participants to assess how they would conduct analysis for each scenario; (3) Number of Contributors Subtest—an assessment of suitability and number of contributors, given electropherogram data for 12 mixtures; and (4) Interpretation, Comparison, and Statistical Analysis Subtest—interpretations and statistical analyses given electropherogram data for eight mixtures, each provided with DNA profiles of potential contributors.

Reference

President's Council of Advisors on Science and Technology (PCAST). (2016, September). *Report to the President: Forensic science in criminal courts: Ensuring scientific validity of feature-comparison methods.* https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_final.pdf

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Interpretation of Y Chromosome STRs for Missing Persons Cases

NIJ AWARD #: 2020-DQ-BX-0018

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Y chromosome short tandem repeat (Y-STR) haplotypes have been used in assisting forensic investigations primarily for identification and male lineage determination. The Scientific Working Group on DNA Analysis Methods Lineage Marker Committee published interpretation guidelines for Y-STR typing, which provide helpful guidance. However, these guidelines do not address the issue of kinship analysis with Y-STR haplotypes. Because of the high mutation rate of Y-STRs, there are complex missing persons cases in which inconsistent Y-STR haplotypes between true paternal lineage relatives will arise (e.g., cases with two or more male references in the same lineage that differ in their haplotypes). Therefore, more useful guidelines are needed for interpretation of Y-STR haplotype data. Computational methods and interpretation guidelines have been developed specifically addressing this issue, either using a mismatch-based counting method or a pedigree likelihood ratio method. However, these methods and guidelines have not been adopted by forensic laboratories, likely because of a lack of specific procedures and software to facilitate analyses. The Missing Persons Unit under the Center for Human Identification at the University of North Texas Health Science Center (UNTCHI) specializes in the DNA analysis and identification of missing persons cases and processes >50 percent unidentified human remains of the missing persons cases in the US. It is common within UNTCHI to encounter complex cases that would be better served with enhanced Y-STR interpretation procedures. With the experiences and resources at UNTCHI, more sophisticated interpretation methods and guidelines for Y-STR applications are implemented, which include pairwise comparison with a mismatch-step-based counting method to quickly determine if two profiles are from the same male lineage and a pedigree likelihood ratio–based method to evaluate the evidence weight of Y-STR profiles, particularly for complex missing persons cases. A software program is under development and will be validated to facilitate Y-STR haplotype interpretation, and the software will be made accessible to the forensic community free of charge.

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Assessment of Sexual Assault Kit (SAK) Evidence Selection Leading to Development of SAK Evidence Machine-Learning Model (SAK-ML Model)

NIJ AWARD #: 2019-NE-BX-0001

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The purpose of this presentation is to share our research steps and progress in developing a sexual assault kit evidence machine-learning model (SAK-ML Model). Our first steps have consisted of analyzing patient and assault factors in sexual assault cases that are associated with development of short tandem repeat (STR) Combined DNA Index System (CODIS)-eligible profiles for a publicly funded laboratory in the Mountain West region of the United States. The findings from this step have been submitted for an American Academy of Forensic Sciences (AAFS) podium presentation titled, "Sexual Assault Victim and Assault Characteristics and Development of CODIS-eligible STR DNA Profiles." Our next steps include completing this analysis for female and male victims, because we have found substantial differences in development of STR DNA CODIS-eligible profiles based on sex. We have then completed the analysis with the outcome variable of partial or full STR DNA profiles of a foreign contributor (enough loci to qualify for National DNA Index System or State DNA Index System CODIS entry). Additionally, we are analyzing the differences between a test-all-swabs approach and a selected swab approach in development of probative STR DNA profiles. We have data from two additional states, which we will be analyzing for replicability of our results. The next steps are to implement the machine learning model in three publicly funded laboratories for different regions. In addition to our findings in development of a machine learning model, our research has yielded new information on the following topics related to sexual assault kit DNA analysis findings (examples):

- The effect of victim bathing/showering on development of CODIS-eligible profiles of foreign contributors
- The impact of victims' age on development of CODIS-eligible profiles of foreign contributors
- The differences between DNA tests on development of CODIS-eligible profiles
- Sexual assault cases in which the suspect was excluded following DNA analysis of sexual assault kits (~85 cases)

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Interpretation of Y-STR Evidence

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The introduction of Y chromosome short tandem repeat (Y-STR) evidence in the United States remains under challenge, especially for cases involving small populations. We believe that assessment should consider the genetic nature of Y-STR profiles and should not be overly affected by the size of a database. The strength of the evidence should increase with the number of loci in matching Y-STR profiles. A review by Andersen and Balding (2021) in *Genes* made similar points by referring to the importance of relatedness, mutation, and database in assessing the strength of evidence of matching Y-STR profiles. We have conducted simulations of Y-STR profiles affected by genetic drift and stepwise mutation, and we have assembled a database of 50,000 profiles from published data. These two actions have guided our empirical version of the Andersen and Balding review. The simple counting method for Y-STR profiles provides a numerical value that depends on the size of the database. A profile seen X times in a database of n profiles has a population proportion estimated as X/n, with an upper 95 percent confidence limit of 3/n when X=0. These values are the same whether the profile has 10 or 20 typed loci, and it leads to awkward interpretation with databases where not every profile has the same number of loci. Moreover, the population proportion does not address the question of how likely it is to see a profile that has already been seen once: this conditional probability allows for incorporating relatedness or population structure. The kappa method is less dependent on the database size but does provide conditional probabilities. Empirical work confirms the expectation that the strength of matching Y-STR profiles increases with the number of loci they contain. The rate of increase diminishes with the number of matching loci. The first result is captured by the use of theta: classical theory predicts theta decreases with more loci but does not predict the diminishing rate of decrease. We note an apparent inconsistency of combining the theta method (with its explicit dependence on the number of loci) with an upper confidence limit on haplotype frequency (with its explicit ignoring of the number of loci). The population genetic theory that underpins the theta method is also the basis of the discrete Laplace method, and those values will be presented for our new database. A quite different approach, also using population genetic theory, uses computer simulations to predict the actual number of men with a particular profile.

Reference

Andersen, M. M., & Balding, D. J. (2021). Assessing the forensic value of DNA evidence from Y chromosomes and mitogenomes. *Genes, 12*, 1209. https://doi.org/10.3390/genes12081209

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SESSION ABSTRACTS

SESSION IV

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Moderated by NIJ Program Manager
Danielle McLeod-Henning

Discovering Clandestine Human Remains Using Unmanned Aerial System Remote Sensing

NIJ AWARD #: 2019-DU-BX-0027

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The use of unmanned aerial systems (UAS) or drones has become more ubiquitous as their acceptance in the public sector has become more prevalent. Public safety and private search organizations are turning more to drones as a force multiplier and highly economical tool to enhance investigative techniques, including the detection of clandestine human remains and documentation of outdoor scenes. Currently, research is being conducted at the Forensic Anthropology Research Facility using a variety of UAS and sensors to detect graves and disturbed earth. The goal is to develop flexible best practices and an open-source graphical user interface for visualization of algorithm outputs that can be used by law enforcement and civilian search and recovery teams. This presentation introduces preliminary findings that will aid medicolegal investigators in the discovery of clandestine human remains using UAS remote sensing. The research at Texas State University has focused on human remains both deposited on the surface and subterranean. As a result, the study has concentrated on aspects that are associated with deposition, sensor type, and signal/image processing and machine learning. Deposition factors include body position, burial depth, soil type, and soil moisture. Several processing strategies will be discussed, from low-level signal/image processing-based anomaly detection via size contrast filters to higher-level object detection and localization using bounding box-based and semantic segmentation based deep neural networks with a variety of sensors, including visible, near infrared, long wavelength infrared, and multispectral. The research has also explored how season, ambient temperature, and time of day affect the technique of detection and documentation. For example, long wavelength infrared appears very useful for detecting buried remains if used in the mornings when the grave soil and surrounding soil differ in temperature. Surface remains, however, can be detected in full sunlight when cloud cover is low. In general, the results have demonstrated that the detection of clandestine human remains using UAS aided remote sensing has significant potential but requires an understanding of the technological and operational capabilities and constraints of the systems and the strategies for collecting, processing, and interpreting sensor data.

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Microbial Clock of Human Decomposition Accurately Estimate Postmortem Interval

NIJ AWARD #S: 2015-DN-BX-K016 AND 2016-DN-BX-4194

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Nearly 42 percent of homicide and non-negligent manslaughter cases went unsolved in the United States from 2010 to 2019. Accurately estimating the postmortem interval (PMI), or time since death, can be critical to solving death investigations because it can aid in identifying the deceased, accepting/rejecting alibis, and corroborating witness statements, but this can be challenging. In fact, only a small fraction of death investigations have a reliable PMI. One emerging area of forensic science aims to use decomposition processes and byproducts as physical evidence in their own right. One form of physical evidence that is always associated with decomposing remains is the postmortem microbiome, and it has proven useful for estimating PMI. This study provides the first analysis in using multiple microbiome 'omic techniques for the accurate estimation of postmortem interval, leveraging three anthropological facilities to place 36 human bodies to decompose outdoors over multiple seasons. This study used amplicon DNA, metagenomic DNA, and metabolite datasets to build random forest machine learning models with the goal of using 'omic data feature abundance and geographic location as predictors of PMI. These results show that model accuracy was dependent on taxonomic resolution and the site where the sample was derived. The models generated from 16S rRNA genes isolated from the corpse skin and classified at the species level provided the most accurate predictive power over the first 21 days of decomposition. Interestingly, taxonomic abundances in the gravesoil predicted PMI more accurately than functional features (e.g., gene and metabolite abundances) suggesting either community structure correlates best with decomposition time or higher resolution is required for other 'omic data types. Furthermore, microbial abundance was more important for predicting PMI than geographic location suggesting shared trends between the three anthropological facilities exists, which allow for the use of cross-facility models. This study conveys that accurate, microbially based models for aiding in the estimation of PMI for death investigations can be obtained from multi-omic data, including cost-effective, amplicon sequencing data.

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Progress Towards the Development of a Database of Chemical Fingerprint Signatures for Species Identification of Necrophagous Insects

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In death investigations, carrion insects collected on or near a body can be used for estimating the postmortem interval. However, to exploit entomological evidence in this fashion, accurate species identification is critical. Traditional methods of insect species identification often rely on rearing larvae to adulthood so that the readily visualized gross morphological features can be used to make the final identification. Newer techniques aimed at identifying species in various life stages, such as DNA typing, are expensive, time-consuming, and limited by a lack of a full, comprehensive database of insect profiles for comparisons. Moreover, there may be instances in which the eggs or juvenile insects cannot be reared because they are nonviable, like when they have been exposed to drugs, toxins, or extreme conditions such as freezing, high temperatures, submergence in fluids, or anoxic conditions. With no quick and reliable method to use for species attribution, entomological evidence often remains an underutilized forensic investigative tool. Therefore, a technique to identify species of blowflies in all life stages, and whether viable or not, is critically needed to enable rapid species identification of *Calliphoridae* spp. insects. We demonstrate here that chemometric processing of direct analysis in real time high-resolution mass spectrometric (DART-HRMS) data acquired from analysis of insects can be used to rapidly accomplish species identification for all insect life stages. Starting with the least easily identifiable life stage, eggs, six species of blowflies (*C. vicina*, *L. coeruleiviridis*, *L. sericata*, *P. regina*, *Phoridae* spp., and *Sarcophagidae* spp.) were allowed to lay fresh eggs on pork liver for collection. The eggs were suspended in 70 percent aqueous ethanol for preservation, and this solution was analyzed via DART-HRMS. Statistical analysis of the spectra obtained from this analysis displayed intraspecies similarities and interspecies differences as a consequence primarily of the variations in amino acid profiles of the ethanol suspensions. Subsequent analysis of larvae and pupae of *C. rufifacies*, *C. vicina,* *L. coeruleiviridis*, *L. sericata*, *P. regina*, and *Phoridae* spp. ethanol suspensions yielded similar results for establishing the species identity of juvenile life stages. In addition, seven species of adults (*C. rufifacies*, *C. vicina*, *L. coeruleiviridis*, *L. sericata*, *P. regina*, *Phoridae* spp., and *Sarcophagidae* spp.) were also differentiated based on chemometric processing of DART-HRMS spectra of their ethanol suspensions. Expansion of this work will be used to develop a DART-HRMS spectral database capable of matching data acquired from sample unknowns for the identification of necrophagous insect evidence collected from remains. Additional species under study include *C. cadaverina*, *C. macellaria*, *P. terranovae*, and *Muscid* spp., for a total of 11 species investigated to date using statistical analysis techniques such as Linear Discriminant Analysis, Kernel Discriminant Analysis, and Kohonen Artificial Neural Networks.

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Exploring Phenotypic Variation Throughout Ontogeny and Its Impact on Forensic Anthropology

NIJ AWARD #S: 2015-DN-BX-K409 AND 2019-DU-BX-0039

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Cranial and dental metrics and morphological traits are frequently used to estimate population affinity as part of the adult biological profile. However, the ontogenetic patterns and the variation of these indicators in subadult individuals have seldomly been explored, primarily because of the lack of available modern subadult reference samples. This study is the first to analyze and compare ontogenetic patterns and variation of four craniofacial and dental indicators and determine the onset at which these indicators stabilize to match patterns observed in adults. A sample of 1,081 contemporary US subadults between birth and 20 years were queried from the Subadult Virtual Anthropology Database (SVAD). The data—33 craniofacial interlandmark distances, 13 cranial macromorphoscopic traits, 21 permanent and 12 deciduous dental morphology traits, and four dental measurements of all permanent and deciduous teeth—were collected from computed tomography (CT) scans, following standardized protocols adapted from dry bone standards for the purpose of this research. All protocols with the associated intra- and inter-observer error and agreement rates are freely available through the SVAD Zenodo Community. There were two approaches taken for each technique: a variable level approach and module/unit approach. For example, the growth trajectory of each interlandmark distance on the cranium was explored as well as the multivariate relationships of the cranium through ontogeny. Additionally, each type of variable required a unique set of analyses—metric data were explored with multivariate adaptive regression splines and canonical variate analysis while the morphological data were explored with frequency data, chi-square statistics, and random forest analysis. When possible, data from contemporary adults were incorporated into the analyses to confirm stabilization of growth. The results offer a novel approach to understanding human growth and development and human variation, informing practitioners on the transition from subadult to adult, and importantly, informing how these patterns impact the techniques used to estimate parameters of the biological profile.

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Human Identification from Computed Tomography Derived 3D Models Using Part-to-Part Comparison Analysis

NIJ AWARD #: 2019-DU-BX-0031

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The presentation will examine the real-world application of using computed tomography (CT) derived three-dimensional (3D) models of the L1–L5 vertebra of antemortem CT (AMCT) scans of known individuals and compare them against true postmortem CT (PMCT) scans to establish the validity of using this technology for personal identification. This presentation will provide the results of the initial findings of an ongoing study utilizing part-to-part comparison of the lumbar vertebra for personal identification. The confirmation of identification for an unknown individual is a critical part of forensic practice, especially in disaster victim identification (DVI). The comparison of antemortem imaging for the purposes of personal identification is a common tool in pathology, odontology, and anthropology. A simulated version of this study was conducted successfully and served as a proof of concept of using 3D-rendered lumbar vertebra comparisons as a means for personal identification (Decker & Ford, 2019). This study applies those findings to test true AMCT scans against true PMCT scans for the purposes of personal identification. The University of Leicester, East Midlands Forensic Pathology Unit utilizes PMCT extensively in their daily practice and DVI situations. For this project, Leicester acquired 23 matching AM scans for individuals who passed through their facility for PMCT scanning. The University of Leicester anonymized the scans so researchers at the University of South Florida Health Department of Radiology were blinded to the identities of the AM and PM scans. Each scan was imported into the Mimics Innovation Suite v. 24 (Materialise). The L1–L5 vertebra were then isolated and modeled via segmentation and thresholding. Each series of 23 AM vertebra was registered with a target unknown PMCT-derived vertebra. A part-to-part comparison was conducted for each vertebra, and a percent match was measured. A threshold of ±1 mm was set for the part comparison. Every unknown PMCT L1–L5 was correctly matched to the corresponding AMCT L1–L5, signifying complete accuracy for this sample. A receiver operating characteristic curve was calculated to determine 100 percent sensitivity and specificity with a cutoff point of a 0.735 match ratio. True identifications had an average match ratio of 0.945 ± 0.048. Negative identifications had an average match ratio of 0.367 ± 0.01. With the increased use of PMCT in the forensic sciences, there is an equal increase in the availability and opportunity to utilize 3D tools. This study has demonstrated the utility of 3D part-to-part comparison for successful personal identification.

Reference

Decker, S. J., & Ford, J. M. (2019). Forensic personal identification utilizing part-to-part comparison of CT-derived 3D lumbar models. *Forensic Science International, 1*(294), 21–26.

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Solving Cases of Sudden Unexpected Natural Death in the Young Through Comprehensive Postmortem Genetic Testing

NIJ AWARD #: 2018-DU-BX-0204

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Sudden Unexpected Natural Death (SUND) in the young (≤50 years old) presents vexing challenges for forensic pathologists when comprehensive forensic investigation (e.g., scene, autopsy, toxicology, microbiology testing, and metabolic screening) offers no clues toward a cause of death. Cardiac arrhythmia diseases and sudden unexpected death in epilepsy have been reported as possible mechanisms of death in cases with negative autopsy findings. As clinical evaluation of the decedent cannot be performed in the postmortem setting, testing disease-associated genes becomes important for establishing a diagnosis of the cause of SUND. It is imperative to identify the underlying etiology and any genetic causes behind positive findings (e.g., massive pulmonary embolus, hypertrophied or dilated heart, dissected and ruptured thoracic aorta). Furthermore, the testing results can translate to potentially lifesaving intervention and clinical care for high-risk surviving family members. This project has two aims: at the case level, we aim to identify the underlying cause of death for a large cohort of SUND cases in the young (>1,000) and provide answers to the families of the deceased; at the cohort level, we aim to ascertain the diagnostic yield of molecular testing in SUND and identify strategies for improvement. To achieve these aims, the Molecular Genetics Laboratory in the New York City Office of Chief Medical Examiner (NYC OCME) has validated several molecular testing panels for SUND (a cardiac-focused 132-gene panel, an epilepsy-focused 159-gene panel, a thoracic aortic dissection and rupture–focused 20-gene panel, and a thrombophilia-focused 5-gene panel) using massive parallel sequencing technology. Sequence variants were interpreted using the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines. Among the cohort of over 1,000 tested SUND cases, there were ongoing open cases and previously unsolved cold cases. All testing was performed by our in-house laboratory, which is accredited by the College of American Pathologists. We will present the diagnostic value of each testing panel in SUND and illustrate the power of molecular testing in resolving cause of death through case examples. This project is one of four Forensic Science Research and Development grants awarded to our laboratory by the National Institute of Justice (NIJ) over a period of 10 years. Those grants supported the implementation of molecular diagnostics in NYC OCME with cutting-edge testing technologies, genetic counseling, advanced interpretation of genetic variants through collaboration with various clinical programs in familial and functional studies and testing of previously unresolved cases. NIJ's continuous grant funding support enables NYC OCME to fulfill our commitment to forensic science, criminal justice, and the surviving families.

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POSTER ABSTRACTS

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Physical Match Analysis Utilizing 3D Microscopy of Fractured Surface

NIJ AWARD #S: 2015-DN-BX-K056 AND 2018-R2-CX-0034

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Fractured surfaces carry unique features and details that can provide an accurate quantitative comparison to support comparative forensic analysis of those fractured surfaces. Silicone casts are widely used by practitioners in the comparative analysis of forensic items to perform such comparison. In this work, we assessed the reliability of silicon cast replica of the topological surface details of a fractured article to reproduce its original features and to determine the size (or wavelength) of features that can be faithfully reproduced. A statistical analysis comparison protocol was applied to a set of 3D topological images of fractured surface pairs and their replicas to provide confidence in the quantitative statistical comparison between fractured items and their replicas. In this study, a set of 10 stainless-steel samples were fractured from the same metal rod under controlled conditions and were cast using a standard forensic casting technique. Six 3D topological maps with 50 percent overlap were acquired for each fractured pair. Spectral analyses were utilized to identify the correlation between topological surface features at different length scales of the surface topology. We selected two frequency bands over the critical wavelength (which is greater than two-grain diameters) for statistical comparison. Our statistical model utilized a matrix-variate-t distribution that accounts for the image overlap to model the match and non-match population densities. A decision rule was developed to identify the probability of matched and unmatched pairs of surfaces. The decision rule employs a uniformed prior of 0.5. The proposed methodology correctly classified the fractured steel surfaces and their replicas with a posterior probability of match exceeding 99.96 percent. Moreover, the replication technique shows the potential to accurately replicate fracture surface topological details with a wavelength greater than 20 μm, which far exceeds the range for comparison of most metallic alloys of 50–200 μm. The developed framework establishes the basis of forensic comparison of fractured articles and their replicas while providing a reliable quantitative statistical forensic comparison, utilizing fracture mechanics–based analysis of the fracture surface topology.

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Identification of Low Explosives and Their Post-blast Residues via Gas Chromatography (GC) Coupled With Vacuum Ultraviolet (VUV) Spectroscopy

NIJ AWARD #: 2018-R2-CX-0015

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Analysis of explosives (intact and post-blast) is of interest to the forensic science community to qualitatively identify the explosive(s) in an improvised explosive device (IED). This requires high sensitivity, selectivity, and specificity. Forensic science laboratories typically utilize visual/microscopic exams, spectroscopic analysis (e.g., Fourier Transform Infrared Spectroscopy) and gas chromatography/mass spectrometry (GC/MS) for explosive analysis/identification. However, GC/MS has limitations for explosive analysis because of difficulty differentiating between structural isomers (e.g., 2,4-dinitrotoluene, 2,5-dinitrotoluene, and 2,6-dinitrotoluene) and thermally labile compounds (e.g., ethylene glycol dinitrate, nitroglycerine, and pentaerythritol tetranitrate) because of mass spectra with very similar fragmentation patterns. The development of a benchtop vacuum ultraviolet spectrometer coupled to a gas chromatography (GC/VUV) was developed in 2014 with a wavelength region of 120 nm to 430 nm. GC/VUV can overcome limitations in differentiating explosive compounds that produces similar mass spectra. This work encompasses analysis of explosive compounds via GC/VUV to establish the sensitivity, selectivity, and specificity for the potential application for forensic explosive analysis. Nitrate ester and nitramine explosive compounds thermally decompose in the VUV flow cell resulting in higher specificity caused by fine structure in the VUV spectra. These fine structures originate as vibronic and Rydberg transitions in the small decomposition compounds (e.g., nitric oxide, carbon monoxide, formaldehyde, water, and oxygen) and were analyzed computationally. The thermal decomposition process was further investigated for the determination of decomposition temperatures for the nitrate ester and nitramine compounds which range between 244°C and 277°C. Nitrated compounds were extensively investigated to understand the absorption characteristics of the nitro functional group in the VUV region. The nitro absorption maximum appeared over a wide range (170–270 nm) with the wavelength and intensity being highly dependent upon the structure of the rest of the molecule. Finally, the GC/VUV system was optimized for post-blast debris analysis. Parameters optimized include the final temperature of a ramped multimode inlet program (200°C), GC carrier gas flow rate (1.9 mL/min), and VUV make-up gas pressure (0.00 psi). The transfer line/flow cell temperature was determined not to be statistically significant.

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Fire Pattern Indicators, How Reliable Are They?

NIJ AWARD #: 2020-R2-CX-0047

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As demonstrated by the devastating events over the last few years, wildland fires dramatically impact the environment, human life, and property and cause significant economic losses. Furthermore, because of the rapid expansion of the wildland–urban interphase and the effects of climate change, the frequency and intensity of these events have increased dramatically. To address this issue, it is necessary to characterize and understand the phenomena related to the ignition of wildland fires. To achieve this goal, the accurate determination of the area of origin and cause is critical. In the United States, the National Fire Protection Association's Guide for Fire and Explosion Investigations (NFPA 921) and the National Wildfire Coordinating Group's Guide to Wildland Fire Origin and Cause Determination are the primary documents that provide guidance on conducting wildland fire investigations. Both of them present systematic approaches based on fire pattern indicators. A fire pattern indicator is a physical object or artifact that displays changes when affected by a fire. An overall fire pattern is derived from the accurate analysis of individual fire pattern indicators and shows the general fire progression. Fire pattern indicators are commonly categorized based on their scale, either as macro- or micro-scale indicators. Macro-scale indicators appear on vegetation, landscape features, and the environment where the fire is spreading. Micro-scale indicators correspond to the localized effect of the fire, such as the partial burning on a single face of an object. Because of this, micro-scale indicators are only observable by getting up close to an object. The reliability of the fire pattern indicators is an ongoing discussion. To the best of our knowledge, there are no systematic scientific studies that support any assessment of the indicators. Through a collaborative effort between Worcester Polytechnic Institute and the US Department of Agriculture Forest Service, and with the support of the New Jersey Forest Fire Service (NJFFS), this research project proposes the development of a first dataset based on experimentation at field and laboratory scales that assesses the reliability of fire pattern indicators related to fire behavior and local fire conditions used in wildland fire investigation. The field-scale experiments will be conducted within the framework of the prescribed burns conducted regularly by the NJFFS, allowing for the study of the creation of fire pattern indicators under controlled and realistic conditions. At this scale, the generation of macro-scale indicators will be studied, and the thermal exposure conditions that lead to the generation of micro-scale indicators will be quantified. At the laboratory scale, a wind tunnel will be used to study the effects of wind, slope, and vegetation on creating micro-scale and scaled-down macro-scale indicators. Combining the two types of experiments will allow establishing robust and statistically representative datasets about the generation of fire pattern indicators within the range of tested conditions. Finally, each fire pattern indicator will be analyzed for its reliability in providing information related to the spread of the fire. The reliability criteria will be developed in agreement with the NJFFS fire investigators and historical investigation data.

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Physical Characteristics of Spatter Stains on Textiles: Influence of Impact Surface Texture, Blood Drop Volume and Blood Drop Velocity

NIJ AWARD #: 2018-R2-CX-0033

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Bloodstain pattern analysis (BPA) is a forensic technique for crime scene reconstruction through analyzing bloodstains (e.g., size, shape) and their patterns (e.g., distribution, location) to recreate the blood shedding event. BPA practice on textiles has been hindered because of the highly distorted bloodstains resulted from complex surface properties and multi-physical processes involved. Here, we report the latest findings regarding interpreting bloodstains on textiles for deriving the volume, impact speed, and angle of impact of corresponding airborne porcine blood drops. "Dynamic" stains were created by gravity-driven perpendicular impact of airborne blood drops (14 μL) on three types of cotton fabrics (i.e., plain-woven, twill, jersey knit), with seven different impact speeds between 0.3 and 6.2 m/s. On the other hand, seven different volumes of blood drops (0.1–16 μL) were dispensed by using a micropipette to create "static" stains on those three distinct fabrics. For "dynamic" stains, the complete stain formation processes were also recorded by utilizing a customized multiscale imaging system, which enables a transient analysis of the stain characteristics and underlying physics. In total, a database of 75 dynamic bloodstains, 63 static bloodstains, and affiliated video footage of stain formation process has been constructed for investigating the yet missing link between the airborne blood drops and resultant bloodstains on textile surfaces at the scene. The acquired dataset provides the forensic community benchmark data for validating BPA on specific fabrics and new perspectives on the role of drop volume and impact speed in bloodstain formation on textiles. Analysis validates that the bloodstain area is independent of the airborne blood drop impact speed when no splashing occurs upon impact for the three types of fabrics. This suggests the utilization of a new quantitative parameter (i.e., irregularity factor) to reconstruct the drop impact speed when practicing BPA on absorbent textiles. Analysis of the static stain data evaluates the appropriateness of extending a nondestructive approach, which was proposed to determine the original drop volume for large drops (drop volume > 30 μL) only on knit fabrics to typical spatter drops (drop volume < 16 μL) on a broader range of fabrics.

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Post-Blast Explosives Attribution

NIJ AWARD #: 2018-DU-BX-0193

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Forensic science practitioners are often called upon to 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. 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, there is currently no capability to link the explosive charge to its source via post-blast trace residues. 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, a field test was conducted to recover post-blast explosive samples from controlled detonations of multiple explosive materials, including Royal Demolition eXplosive (RDX), trinitrotoluene (TNT), and ammonium nitrate-aluminum (AN-AL). Samples were collected and analyzed via multiple analytical techniques, including high-performance liquid chromatography mass spectrometry (HPLC-MS) for quantitation of organic explosives and profiling of small molecules, inductively coupled plasma mass spectrometry (ICP-MS) for aluminum quantitation of AN-AL samples and profiling of trace elements, and isotope ratio mass spectrometry (IRMS) for isotope ratios of carbon and nitrogen in RDX and TNT or nitrogen and oxygen in AN-AL. Signature results from each of the techniques show some consistency between pre- and post-blast explosive materials and thus could be relevant for source attribution. The IRMS results for AN-AL are some of the most promising, yielding some overlap between the pre- and post-blast spreads of the data, with oxygen showing slightly better overlap than nitrogen. The median post-blast isotope ratio for oxygen is 20.7‰, and the median pre-blast isotope ratio is 20.2‰. Although these are not perfectly overlaid, the reference standard deviation of 0.4‰ for oxygen brings the medians closer together with the overall spreads of the data overlapping considerably. This trend is similar for nitrogen, with the post- and pre-blast median isotope ratios at –0.82 percent and –1.04‰ respectively, and the reference standard deviation at 0.2‰. In addition, trace element profiling results for AN-AL are quite promising with 33 elements detected in both pre- and post-blast samples, with several of those elements having consistent abundance pre- to post-blast. Finally, small molecule profiling by HPLC-MS of TNT samples yielded four compounds detected in both pre- and post-blast, with all four having consistent abundance. These compounds were not detected in any blanks or controls and were detected only in samples where the explosive compound itself was detected, therefore enhancing the confidence that these signatures are specific to the explosive. With these results, this proof-of-concept study shows promise that pre-blast explosive signatures may be preserved post-blast, allowing the forensic community to potentially benefit from a novel approach to attribute explosives after detonation.

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DVME (Dynamic Vapor Microextraction) and Its Potential for Fire Debris Analysis

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Vapor or headspace sampling methods provide many advantages for the chemical analysis of forensic artifacts. They provide a clean sample for instrumental analysis, do not require direct contact with potentially dangerous artifacts, are non-invasive, and can be non-destructive. The Fluid Characterization Group at the National Institute of Standards and Technology is working to advance forensic science by developing new instruments to sample vapors in the laboratory and the field. Dynamic vapor microextraction (DVME) is a small-volume purge-and-trap sampling method that concentrates vapor-phase analytes onto a section of a porous layer open tubular capillary column. The sample container is heated within an oven, but the majority of the capillary is coiled outside the oven in a chilled enclosure; sub-ambient temperatures enhance adsorption efficiency and stabilize reactive species. Headspace analytes are swept by inert gas into the inlet of the capillary, and the flow rate and total collection volume are measured at the outlet. Breakthrough is also measured. DVME has been applied to the analysis of pure compounds under tightly controlled laboratory conditions to determine vapor pressures with low uncertainty. These measurements focus on low volatility and reactive compounds such as cannabinoids. DVME has also been applied to the extraction of volatile compounds from a simulated shipping container in a preliminary investigation of uncontrolled field conditions. Debris from structural fires is typically evaluated for ignitable liquid (IL) residue by passive headspace concentration onto activated carbon strips (ACSs), followed by solvent elution and analysis. The high affinity of ACSs for hydrocarbons requires the use of carbon disulfide, a dangerous neurotoxic solvent, to recover the IL compounds. By contrast, DVME can recover characteristic IL compounds from laboratory-generated fire debris with a relatively benign solvent: acetone. However, preliminary experiments were conducted with a small quantity of debris in crimp-capped vials, and several instrumental factors would not be reasonable for the analysis of authentic fire debris. This presentation will discuss whether the DVME method could be a practical alternative to existing headspace concentration methods for IL extraction and improvements to the laboratory DVME instrument for this purpose. Instrumental factors include capillary dimensions, adsorbent phase, oven temperature, capillary temperature, gas flowrate, and collection volume. This presentation will discuss investigations with pure compounds, fuel surrogates, weathered gasoline, diesel fuel, and simulated fire debris, including water-soaked debris. Outcomes include target compound recovery, repeatability, carbon number and carbon class distribution, and the stability of adsorbed vapors. This presentation will highlight significant first-order effects identified by sensitivity analysis with simulated fire debris in casework containers. Instrumental factors that significantly influence IL extraction direct future research on optimization, whereas non-significant factors identify elements that might be modified to facilitate laboratory adoption. The results also provide a better understanding of the robustness of the DVME method to realistic differences in the composition of authentic fire debris.

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Experimental and Numerical Investigations for the Prediction of Depth of Calcination of Gypsum Plasterboards Under Fire Exposure

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Quantitative analysis of gypsum calcination caused by fire is of great use in fire investigations. The rate and the depth of calcination through the gypsum board are dictated by the heat and mass transfer through it. The present study compares the experimental results of gypsum calcination to the results predicted by a one-dimensional unsteady computational model. Controlled laboratory-scale experiments are conducted with gypsum wallboard exposed to a uniform heat flux. During this exposure, the internal temperature profile is recorded using an array of 12 thermocouples, placed at different depths inside the gypsum board, and the depth of calcination is measured every minute of the experiment when exposed to a uniform heat flux. This process is repeated for several different heat fluxes ranging from 10 kW/m^2 to 100 kW/m^2 using either a radiant burner or a premixed burner placed at different distances from the gypsum board. To understand the repeatability of the depth measurements, the mean and standard deviations of the depth of calcination from multiple trials for the same heat flux are analyzed. The effects of heat flux and the duration of exposure on the depth of calcination are quantified. The same constant heat flux is used as a boundary condition to predict the depth of calcination of the gypsum board using a validated in-house one-dimensional unsteady computational model that solves the mass, species, momentum, and energy conservation equations assuming local thermodynamic equilibrium. The dehydration of the gypsum board, coupled with the heat and mass transport through it, has been modeled by considering the gypsum board as a homogeneous porous material. Because the transport of species inside the porous gypsum board is extremely difficult to measure experimentally, the transport of water vapor has been analyzed numerically by considering the diffusion because of the concentration gradients, convection because of the pressure gradient, the water vapor generation during calcination, and its removal because of re-condensation. The internal temperature profile from the experiments is compared to the temperature profile predicted by the model. The percentage of dehydration predicted by the model is compared to the depth of calcination measured in the experiments. The rate of calcination is higher for the greater heat fluxes. The propagation of the dehydration front is found to be highly non-linear both in the experimental measurements and numerical predictions. The nonlinearity in the propagation of the dehydration front during gypsum calcination has been explored.

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A Novel Approach for Detection and Identification of GSR Based on Laser Spectroscopy

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Gunshot residue (GSR) is an important type of trace evidence often associated with a violent crime. Traditionally, scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, also known as SEM-EDS or SEM-EDX, is used for detection and identification of GSR particles. The application of this two-step method is limited to inorganic GSR (IGSR) because it relies solely on the detection of the heavy metals (e.g., 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 GSR has increased with the onset of "green" ammunition. Organic GSR (OGSR) has been the focus of many forensic researchers recently for several reasons. First, the total amount of OGSR generated because of the discharge of a firearm is much larger than the amount of IGSR. Second, OGSR particles are typically much larger in size than IGSR particles. In addition, the chemical composition of OGSR is quite complex and includes partially burned and unburned smokeless powder, stabilizers, and plasticizers. As a result, it is easier to detect and identify OGSR particles, although new methods are required. Our laboratory has developed a new two-step approach for fast detection of OGSR particles using fluorescence spectroscopy followed by a confirmatory identification by Raman microspectroscopy. The method utilizes a single instrument combining a confocal scanning Raman and fluorescence microscope working in reflection mode. In our first proof-of-concept study, we used adhesive tape as a method of collecting OGSR particles. Most recently, we have significantly expanded this emerging methodology by also demonstrating the possibility of detecting and identifying IGSR particles. In addition, we explored the capability of the method for detecting GSR particles on an original common substrate (cotton fabric), eliminating the initial GSR particle transfer stage. In this presentation, we will show and discuss the results of these recent studies and discuss challenges and future steps for the proposed two-step method development for the detection and confirmatory identification of both OGSR and IGSR particles. 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.

When Bloodstain Ellipticity Depends on More Than the Impact Incidence Angle

NIJ AWARD #: 2020-DQ-BX-0006

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In Blood Pattern Analysis (BPA), detectives rely on the ellipticity, or aspect ratio, of a blood stain to determine the incidence angle and, ultimately, the area of convergence. More generally, the shape and size are assumed to depend solely on the dynamics at impact through an incidence angle, Reynolds number, and Weber number. However, recent experiments on horizontal surfaces have shown that microscopic surface residues, including the oil from fingerprints, will affect the size of the circular bloodstain, and it is unclear how these findings might extend beyond perpendicular impact. Here, we demonstrate that when drops of blood impact a surface obliquely, the presence of microscopic residues can alter both the size and the ellipticity of the final stain. Through systematic experiments with human blood, we map the shape and size of stains over a range of impact incidence angles, Reynolds numbers, and Weber numbers for varying surface conditions, noting that contact angle hysteresis is more relevant than surface contact angle alone. Our results highlight that practitioners should exercise caution when calculating the area of convergence if they suspect that microscopic residues might be present because these residues can affect stain ellipticity and, by extension, introduce systematic errors in area-of-convergence calculations.

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Expanding the Scope and Efficiency of 3D Surface Topography Analysis in Firearm Forensics

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Three-dimensional (3D) virtual comparison microscopy (VCM) is a powerful tool for microscopic examination that presents an examiner with a highly detailed visualization of a toolmark surface. VCM can be used for establishing common source, triaging evidence, documenting conclusions, recording electronic notes/images, conducting blind verification, sharing data among multiple sites, and enabling remote access. VCM offers several advantages over light comparison microscopy (LCM) in the areas of access, speed, documentation, and quality. For example, after evidence and test fires are scanned at a 3D microscope, the topography data may be viewed down the hall or off-site on a second computer where examiners can annotate the surfaces, link items with common source, capture screenshots, record text notes, and export structured case notes for inclusion in laboratory information management systems (LIMS). Several recent studies, such as our VCM Error Rate Study and our VCM Topography Resolution Study, each involving over 100 participants, provide strong support for the quality of VCM examination. These studies establish 3D VCM as a viable alternative to traditional light comparison microscopy within the discipline of firearm examination. In this presentation, we will provide a high-level overview of 3D VCM within the crime laboratory and review the state of validation of this new technology.

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Reaction Kinetics of Fingerprint Aging Studied by Laser Desorption/Ionization Mass Spectrometry

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This presentation will inform attendees of the major factors attributing to fingerprint aging and a proposed kinetics formula. The research described proposes a method to determine the age of a fingerprint without destroying the physical print. This would allow criminal justice officials to determine the validity of a suspect's alibi by establishing the relevance of a fingerprint to the crime timeline. Additionally, after analysis using laser desorption/ionization mass spectrometry (MALDI-MS), the physical print would still be available for conventional forensic analysis. Fingerprints are invaluable physical evidence because of their ability to link individuals to crime scenes. Our previous work also demonstrates that ozonolysis of unsaturated triacylglycerols (TGs) in fingerprints can potentially determine fingerprint age (Hinners et al., 2020). This current study expands our understanding of TG degradation with three experiments aimed at determining key factors impacting the aging process and proposing a preliminary kinetics formula. First, the impact of ozone concentration on TG degradation in fingerprints was assessed. Groomed fingerprints from the same donor were collected onto clean glass slides. All samples were then aged in a climate chamber (Memmert), in which the environmental factors were controlled. To understand the impact of ozone concentration, fingerprints were aged for 2 hours at increasing ozone concentrations (0 ppb, 100 ppb, 200 ppb, 300 ppb). Second, to understand which factors would significantly impact the rate of ozonolysis, key environmental factors were investigated, including temperature, humidity, and light while maintaining constant ozone concentration (100 ppb). Fingerprints were aged for 2 hours while modulating the environmental factors. The following variable ranges were examined: temperature (10°C, 30°C, 50°C, 60°C), humidity ($3.44gm^{-3}$, $6.88gm^{-3}$, $10.3gm^{-3}$), and light (fluorescent, ultraviolet). Third, a kinetics formula was obtained by aging TG standards with varying degrees of unsaturation. The standards were spotted on glass slides. Samples were aged in the climate chamber for an extended time (0–14 hours) at 100 ppb of ozone at different temperatures (20°C, 30°C, 40°C). All aged fingerprints and standards were sprayed with 10 mM sodium acetate and sputtered with gold. The samples were then analyzed in positive mode using a Q-Exactive HF (Thermo Scientific) equipped with a MALDI source (Spectroglyph). The data were then analyzed using Xcalibur's Qual browser and a home-written Python code. The ozone concentration experiment results support the hypothesis that unsaturated TG degradation increases as ozone concentrations increase and that the climate chamber aging is a reasonable approximation of ambient aging. When examining other environmental factors, the second experiment suggests that as temperature increases, unsaturated TG degradation increases significantly. However, both humidity and light did not result in a significant variation in unsaturated TG degradation. Therefore, it was determined that only ozone concentration and temperature have a significant effect on TG ozonolysis. These

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factors formed the basis for the third experiment on kinetics. Data from the kinetics experiment produced a preliminary kinetics equation that resembles pseudo-first-order behavior. Future studies will verify the validity of this equation with fingerprint samples.

Reference

Hinners, P., Thomas, M., & Lee, Y. J. (2020). Determining fingerprint age with mass spectrometry imaging via ozonolysis of triacylglycerols. *Analytical Chemistry, 92*(4), 3125–3132. https://doi.org/10.1021/acs.analchem.9b04765.

Kendrick Mass Defect Plots: Guided Selection of Compounds for Fingerprint Aging Methods

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Kendrick mass defect (KMD) plots will be described in the context of forensic evidence to demonstrate the potential value that they bring to fingerprint chemical analysis. This work demonstrates the use of a high-resolution mass spectrometry (HRMS) data analysis technique, borrowed from petroleomics, to easily visualize degradation compounds within sebaceous fingerprints. The data visualization technique has been used in many other areas, such as rapid assignment of polymer series, but it is the first time it has been applied to forensics. Determining fingerprint age is a challenging but important task to suggest whether the evidence is relevant to the crime timeline. So far, there has been no definitive way to determine fingerprint age. We have recently demonstrated that ambient ozonolysis of unsaturated triacylglycerols has a potential to determine the time since deposition of latent fingerprints (Hinners et al., 2020). Additionally, the laser desorption/ionization mass spectrometry (LDI-MS) technique we use is ideal for fingerprint chemical analysis because there is almost no destruction to fingerprint details during the analysis, unlike gas chromatography–mass spectrometry or liquid chromatography–mass spectrometry. As we are developing a model to determine the fingerprint age, however, the complexity of the aging process has become a serious bottleneck to understand the chemical reactions involved, especially because it results in the complex convoluted mass spectra. This work focuses on selecting spectral features that can be used to reliably model the time since deposition of a fingerprint. By employing KMD plots, one can readily assign compound classes, assess degradation pathways, and have guidance in the selection of compounds to monitor during fingerprint aging. Groomed sebaceous fingerprints were acquired from a single individual onto precleaned glass slides. Fingerprints were aged in the ambient laboratory environment for 0, 3, and 7 days. After aging for the desired time, fingerprints were stored in an opaque slide holder within a desiccator to mitigate further aging. Gold was sputtered as a matrix, and samples were analyzed with matrix-assisted LDI-MS instrumentation. The results corroborate that triacylglycerols, diacylglycerols, and wax esters degrade primarily by similar ozonolysis processes and species with more than one unsaturation can undergo multiple ozonolysis processes. Though these trends are intuitive based on previous work, the KMD plot analysis suggests that there should be some thought put into selecting which compounds to monitor during degradation. Challenges to resolve compounds that arise from degradation are readily observed by overlapping series in the KMD plots. The overlapped compounds can then be avoided during analysis while using lower resolution mass analyzers, which are likely to be adopted in the forensic field. From the results, we can select multiple classes of compounds to monitor during the ozonolysis degradation process and avoid others when there are interferences.

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With these considerations in mind, better fingerprint aging models can be developed in the future. We plan to use this information to develop these models and investigate other analytical approaches to further parse apart challenging spectral regions.

Reference

Hinners, P., Thomas, M., & Lee, Y. J. (2020). Determining fingerprint age with mass spectrometry imaging via ozonolysis of triacylglycerols. *Analytical Chemistry, 92*(4), 3125–3132. https://doi.org/10.1021/acs.analchem.9b04765.

Comparison of Weathering Profiles of Ignitable Liquids by GC/MS and DART-MS

NIJ AWARD #: 2020-DQ-BX-0003

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In many arson cases, fire is initiated by employing ignitable liquids (ILs). Therefore, in the arson investigation process, the identification of IL and IL residues at the scene plays a key role in identifying the cause of the fire and distinguishing the ILs used to initiate the fire. The most commonly used technique to analyze the fire debris is gas chromatography mass spectrometry (GC-MS); however, it has a major limitation of analyzing only the volatile components in ILs. The non-volatile or less volatile components in IL are likely to be contained in the fire debris and hence could yield corroborating evidence on the use of specific ILs in the investigation. Direct analysis in real time mass spectrometry (DART-MS) is a sensitive tool with excellent analytical sensitivity to analyze non-volatile components in ILs. In this study, the IL weathered chemical profiles are compared by GC-MS and DART-MS methods. In the present study, the four ILs selected were gasoline, diesel, kerosene, and Japan Drier. Aliquots of 1–10 mL of each IL were weathered at three different temperatures, 30°C, 90°C, and 210°C, to different degrees of weathering at 30–99 percent mass reduction of IL before being analyzed. To analyze the IL samples on DART-MS, an automated sample introduction apparatus with Linear Rail Enclosure that holds consumable Quickstrip™ sample cards was used. A 5-µL sample volume was spotted on the Quickstrip card after diluting the sample in chloroform. For the GC-MS analysis, 20 µL of the sample was added to 1 mL of chloroform followed by an injection of 1 µL with a split ratio of 1:50. The DART-MS data were analyzed by averaging the mass spectra of ~60 scans after the spectral background subtraction. The mass spectral profiles of gasoline and Japan Drier are relatively consistent irrespective of weathering percentage. In the case of diesel and kerosene, as the weathering percentage increased, the mass spectral patterns shifted toward the higher mass range. The total ion chromatogram (TIC) of GC-MS data of all ILs have shown a distinctive pattern corresponding to the extent of weathering. Collectively, for the highly weathered IL samples, the relative peak intensities would increase for the less volatile compounds. To evaluate the experimental factors in the sample preparation, such as weathering percentages and weathering temperatures, Analysis of Variance-Principal Component Analysis (ANOVA-PCA) was used. The data indicate that the weathering percentage is the major factor that is responsible for the variance of the TIC data. The GC-MS data suggest that it is challenging to correlate the weathered IL to the non-weathered IL because the weathering process significantly alters the relative quantities of the IL. The DART-MS data of ILs provided less-variable profiles and therefore may not be used to predict the weathering percentage of IL. However, the capability of DART-MS in detecting the less volatile IL constituents at high *m/z* values imparts its unique ability to discriminate among different types of ILs. Examples of these non-volatile additives include polyisobutylene succinimides and polyether amines in gasoline and carboxylate salts of zirconium, manganese, and cobalt in Japan Drier.

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POSTER ABSTRACTS

SEIZED DRUGS AND TOXICOLOGY

Site Noninvasive Sensing of Illicit Compounds

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As society advances, the advancement of analytical detection methods for illegal substances and their metabolites must advance in a parallel fashion. As such, there is a need for accurate, rapid testing methods for drugs and alcohol for medical purposes and for law enforcement personnel in the field. The current technology used for testing bodily fluids for drugs and alcohol requires invasive sampling of blood or urine and a laboratory with trained staff to analyze the samples. The need for laboratory testing creates a time-related bottleneck for the return of results to the requesting parties and introduce smore room for error as the submitted sample ages between collection and analysis. This process can potentially lead to delaying any legal proceedings that depend on the accurate analysis of the sample. Particularly, the detection methods for blood alcohol content (BAC) are outdated, as alcohol has been legal to consume for decades across the country and current on-site BAC testing methods can still only be used as preliminary evidence for suspected operation of machinery or driving while intoxicated charges. Additionally, the recent legalization of marijuana for medical and recreational use in many states has led to a push for on-site testing capabilities to determine if a person is under the influence of the psychoactive component of marijuana, tetrahydrocannabinol (THC), at the time of questioning under what is likely a zero-tolerance use policy. The aim of this research is to develop a non-invasive sensing concept for targeting and quantifying certain substances of interest in sweat, because it is easily collected from most individuals in a non-invasive manner. Two concepts for non-invasive sensing in sweat have been developed: one for ethanol quantification relating to BAC and one for THC metabolite detection. These methods utilize enzymatic and immunoassay components, are non-invasive, and provide colorimetric feedback. This method of result reporting provides an opportunity for advancing these systems to on-site detection via handheld ultraviolet-visible spectrophotometry devices and even smartphones with specialized software for colorimetric analysis via the built-in camera. This technology could pave the way for a simple, on-site visual test for multiple kinds of drugs and alcohol that law enforcement and medical staff could operate in the moment of need. This would prevent the need for the individual involved to travel to a facility to have blood samples drawn for laboratory testing, thereby losing valuable time as the body continues to metabolize these compounds.

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Building the Chemical Foundation Necessary for a Reliable Cannabis Breathalyzer

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Chemical analysis of real-world forensic artifacts is challenging. The important compounds that provide evidence of guilt, fraud, adulteration, or innocence can be trace or reactive and are typically in complex mixtures (e.g., identifying accelerant in fire debris to investigate suspected arson). Vapor sampling is an attractive alternative to analyzing the forensic artifact itself. Vapor collection and sampling is standoff, non-invasive, and provides a cleaner sample for analysis. Vapor sampling is the basis for the roadside alcohol intoxication breathalyzer test. Alcohol breathalyzers are common in law enforcement, and their development is based on decades of research in 10,000s of individuals to understand the partitioning behavior of alcohol between blood and breath. Proper use of breathalyzers requires training and routine device calibration and standardization. Several versions of breath collection devices are being marketed for the detection of Δ^9-tetrahydrocannabinol (THC) in the breath of cannabis users. THC and other cannabinoids are non-volatile and chemically unstable, creating currently unsolved reliability challenges that did not exist for the alcohol breathalyzer. Additionally, the partitioning behavior of cannabinoids between blood and breath is not well known, and a clear link for blood cannabinoid concentration to intoxication does not exist. The Fluid Characterization Group at National Institute of Standards and Technology (NIST) is working to advance forensic science for law enforcement by leveraging our expertise in chemical characterizations and separations toward cannabis breathalyzer development. We have recently completed a pilot study detecting cannabinoids in breath aerosols of cannabis users with legal market products. This work was done through an Institutional Review Board–approved Interagency Agreement with collaborators at the University of Colorado Boulder (CU). CU researchers obtained breath samples at baseline intake sessions and before and after cannabis use, which were subsequently transported to NIST for analysis. We received 35 samples from 14 recruits and determined that the quantity of THC in post-use samples was comparable to the quantity detected in baseline and pre-use samples. Looking beyond THC, we developed an apparatus that generates and extracts cannabis smoke to investigate the compounds that may be inhaled, and potentially exhaled, by cannabis users. The use of XCMS, a platform used to generate statistical values for differences in liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry chromatograms, has led to the characterization of a previously unidentified biomolecule that is not present in the plant extract but present in the smoke extract of four low-THC cannabis strains. This finding indicates that smoke is an underexplored matrix for the detection of biomolecules that may be important for consideration in the design of a cannabis breathalyzer. Last, our group develops novel instrumentation to measure fundamental thermophysical properties of compounds critical to cannabis breathalyzer development. We recently designed a novel instrument technique, Dynamic Vapor Microextraction, and have made extremely low uncertainty vapor pressure measurements of the terpenoid linalool common to cannabis strains. These measurements are difficult to perform well because linalool is reactive and decomposes over the course of a typical experiment. Furthermore, it is often difficult to source and maintain high-purity samples, which directly impacts measurement accuracy and repeatability.

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Optimization of Forensic Hair Analysis Methods Using Statistical Design of Experiments (DoE)

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There are many differing opinions regarding the optimal methods for forensic hair analysis. The Society of Hair Testing gives general guidelines suggesting that hair samples should be washed with at least one organic solvent and aqueous solution and should be homogenized in some way prior to extraction. In addition, the literature reports a variety of different extraction methods, including enzymatic, acid/base, and solvent techniques. This lack of consensus regarding best practice methods for forensic hair analysis contributes to bias in hair testing across multiple laboratories. In the present work, the statistical technique known as Design of Experiments was used to determine the most effective decontamination and extraction parameters for diazepam, nordiazepam, methamphetamine, cocaine and metabolites, oxycodone, morphine and metabolites, and fentanyl. For decontamination studies, 30-mg aliquots of blank de-identified hair were weighed into an Eppendorf tube and externally contaminated by adding drug in solution and then drying in the vacufuge. The samples underwent decontamination with parameters determined using a 24 fractional factorial block design. Decontamination parameters included aqueous solvent (0.1 percent SDS or HPLC water), organic solvent (dichloromethane or methanol), and 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 Liquid Chromatography Triple Quadropole Mass Spectrometer (LC-QqQ-MS). For extraction studies, 20 mg aliquots of authentic Hair Reference Material (HRM) were weighed into steel milling jars. The samples underwent extraction with parameters determined using a 23 full factorial design. Factors of interest included extraction solvent volume/sample weight ratio (12.5 or 25 µL/mg), particle size (i.e., pulverized into a powder using a ball mill with milling beads for 30-s or cut into snippets with scissors), and extraction time (i.e., 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 C8/SCX cartridge prior to LC-QqQ-MS analysis. Finally, to compare extraction techniques, 20-mg aliquots of authentic HRM were pulverized into a powder prior to a 2-hour extraction in 12.5 µL/mg extraction solvent/sample weight ratio, transferred into Eppendorf tubes, and centrifuged for 30 minutes. The samples underwent SPE prior to treatment by three extraction techniques: enzymatic degradation (incubation in dithiothreitol and proteinase K), solvent swelling (incubation in a mixture of methanol, acetonitrile, and 2 mM ammonium formate 25:25:50, v/v/v), and base extraction (incubation in 1 M NaOH). Although it was determined that there was not one best practice method that applied to all drugs or metabolites of interest, a consensus statement was determined using the summary of optimized parameters. The optimal method for forensic hair analysis was found to include decontamination by one 30-minute wash with water followed by three 30-minute washes with dichloromethane, pulverizing the hair into a powder prior to a 2-hour extraction in 12.5 µL/mg extraction solvent/hair weight ratio and extraction using a solvent swelling technique.

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POSTER ABSTRACTS

FORENSIC BIOLOGY/DNA

Exploration of RNA Degradation in Dried Body Fluid Stains as a Means of Estimating the Age of the Samples

NIJ AWARD #: 2018-DU-BX-0206

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In previous presentations to this group, a correlation between the state of RNA degradation in dried blood stains and the age of the stain has been demonstrated. Using a novel qPCR assay that quantifies the abundance of small RNA fragments mapping to the 5' and 3' ends of several transcripts, it is possible to calculate a ΔCq value reflective of the state of degradation of the transcript. A rising ΔCq value reflects increased RNA degradation. In past work, we have shown that the environmental conditions of temperature and relative humidity (rH) affect the rate of RNA degradation. Moreover, the temperature and the rH of the environment in which dried blood stains are stored accelerate the degradation rate about equally over storage periods of up to 24 weeks. Sunlight exposure represents another possible variable in the environment an evidentiary stain might be found in. To assess the effect of sunlight exposure, dried blood stains were prepared and stored in a sealed plastic container located on the roof top of a crime scene facility located on campus. Stains were situated in the container such that they could be exposed to available sunlight all day. A second cohort of stains was wrapped in foil to prevent sunlight exposure, and those stains were stored in the same container as the exposed stains. The plastic container was filled with a drying agent and also contained a device that recorded temperature, rH, ultraviolet (UV) exposure, and total light exposure every hour for the entire storage term (i.e., up to 8 weeks). Periodically during the storage term, a sample of exposed and unexposed stains were removed from storage and subjected to RNA extraction and transcripts were reverse transcribed into cDNA. The state of degradation for several RNA transcripts was determined. Results showed that sunlight exposure accelerates the rate of RNA degradation in dried blood stains. However, the potency of sunlight to accelerate degradation is only 2- to 3-fold in comparison to the 5- to 10-fold increase in the degradation rate imposed by raising temperature or rH. A detailed understanding of the effects of the environmental conditions on RNA degradation will be required to develop a mathematic approach that incorporates a crime scene environment into a useful estimate of sample age.

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Quantifying and Qualifying the Influence of Standard Laboratory Procedures on Aged, Degraded, and/or Low Copy Number DNA

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In this study, we tested the influence of increased polymerase chain reaction (PCR) extension time, volume, and primer concentration on the success of amplifying aged, degraded, or low copy number DNA compromised by co-extracted PCR inhibitors. Extension time during PCR is a variable typically manipulated with regard to targeted amplicon size, with longer amplicons taking more time to make and copy than shorter ones. However, we hypothesized that increased extension times may also compensate for PCR inhibition. Comparing with our standard 15-second extension time, we evaluated the effects of extensions lasting 1, 5, and 10 minutes during each round of PCR. We further hypothesized that increased reaction volumes, which result in increased physical distance between inhibitors, template DNA, and the other reagents, would suppress the efficacy of PCR inhibitors. First, standard 20 µL quantitative PCRs (qPCRs) were scaled up to 40, 60, 80, 100, and 125 µL while maintaining standard template and reagent concentrations. Second, qPCRs maintaining standard input volumes for a 20 µL reaction were filled up to 40 µL and 125 µL with water. Our last hypothesis experimented with increased primer concentration and its ability to improve amplification of inhibited DNA eluates. Using 1 µM and 2 µM of each primer (4.17× and 8.33× concentration over our standard 0.24 µM, respectively), we compared the resulting successes in amplification. We recovered DNA from vertebrae morphologically assessed to be of rockfish (genus *Sebastes*) from the archaeological record to test these ideas. Our qPCR tests covered three short (<185 bp) subsections of the rockfish mitochondrial cytochrome (cyt) b gene. DNA eluates recovered from these samples are ideal to test our ideas because they are variably compromised by PCR inhibitors. Our expectations for each element of this study were that a successful approach to subduing the influence of PCR inhibitors (1) will lead to decreased Cq values, indicating more DNA is available for amplification than with standard treatment, and (2) produces a statistically significant increase in successful PCR amplification. We hypothesized that increased extension times may overcome some PCR inhibition. We found support for this in increased amplification of rockfish DNA in the cyt b1 and cyt b3 qPCRs utilizing 1-minute extension compared with the standard 15 seconds. We refer to this approach as "extended extension PCR." However, there was no appreciable reduction in Cq values as predicted. Cyt b2 reactions behaved differently, where 1-minute extension returned fewer amplicons than standard conditions (and actually was associated with higher, not lower, average Cq). We hypothesized that restraining inhibitors from influencing PCR might also arise from increasing reaction volumes, which lessens frequency of contact between inhibitors and the template DNA and other reagents. This prediction was unmet. At the extremes, the 125 µL reactions of standard concentration and the 40 µL and 125 µL dilute reactions performed poorly. Thus, we do not recommend volume increases as an effective means of subduing inhibitory influences. Lastly, we hypothesized that increased primer concentration might improve amplification of inhibited DNA eluates. We found no support for this.

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A Universal Method for the Identification of Body Fluid Traces Using Raman Spectroscopy: Moving Towards a Practical Forensic Application

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DNA profiling is one of two major individual evidence in modern forensic investigation practices. Body fluid traces are the main source of DNA evidence and can be used for reconstructing the event. Most current tests for the identification of body fluid traces are based on biochemical reactions and suffer from cross reactivity. In-field tests for body fluids are presumptive, and confirmatory identification requires laboratory environment. Most importantly, a separate test is required for each body fluid, which complicates the identification protocol. We have reported on the development of a universal method for the identification of all main body fluids—including blood, semen, vaginal fluid, saliva, sweat, and urine—using Raman spectroscopy. The test has a potential for being confirmatory and applicable at the crime scene because portable Raman spectrographs, including handheld instruments, are commercially available and widely used in various fields. A compact Raman instrument is currently on Mars sending spectral data as we speak. It was chosen from many other state-of-the-art analytical approaches because it provides a wealth of (bio)chemical information. If it can be used on Mars, the instrument can be easily adopted and delivered to crime laboratories and other law enforcement units. Our laboratory pioneered the application of Raman spectroscopy for the identification and analysis of body fluid traces more than a decade ago—the 2009 article by Virkler and Lednev in *Forensic Science Internationa*l has been cited 650 times. The development of the method in the University laboratory has been supported by the National Institute of Justice since then. As a result, the method is ready to be implemented to forensic practice after a proper validation. A university spin-off, SupreMEtric LLC (www.supremetric.com), has recently been created for the commercialization of the patented novel technology. The company secured a Small Business Technology Transfer grant from the National Science Foundation for building a working prototype. The developed novel approach for the identification of body fluid traces is based on an intrinsic specificity of Raman spectroscopy to the total biochemical composition of the sample. In addition, Raman spectroscopy is nondestructive to the sample, and the analysis could be done without even "touching" the biological stains discovered on common substrates. Most recently, we made significant progress in the development of novel chemometric approaches for eliminating the substrate interference during such analyses. In addition, we have investigated potential environmental interferences. In this presentation, we will discuss the latest development of the technology with specific focus on its demonstrated capability to become a universal and confirmatory test for the identification of body fluid traces. 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.

The Development of Epigenetic Markers for Body Fluid Analysis and Phenotyping

NIJ AWARD #: 2017-NE-BX-0001

Determining the type and origin of body fluids in a forensic investigation can provide important assistance to criminal investigations. We have developed a set of epigenetic markers that produce unique and specific patterns of DNA methylation that can be used to identify blood, semen, buccal cells, and vaginal epithelial cells. This new method easily fits within the workflow of a standard forensic DNA laboratory. Our procedure involves testing DNA extracted from human samples, which is then amplified using bisulfite modified polymerase chain reaction. Specific primers amplify the region of interest, and the quantitative methylation profile of each locus is determined by pyrosequencing. The versatility of these new markers is presented by showing the results of validation studies on sensitivity, human specificity, stability, and mixture resolution. We also will demonstrate recent results on the specificity of saliva markers. Saliva, composed of different cell types, exhibits differences in methylation percent depending on the collection method. Thus, a buccal/lip swab is different than spit, potentially allowing the differentiation between deposition methods. Lastly, we present additional data on the utilization of pyrosequencing for age and other phenotypic information such as smoking status. The results of these methods can provide important information in criminal casework.

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Comparison of GlobalFiler™ and PowerPlex® Fusion 6C for Direct PCR Amplification of Touch DNA Samples

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After attending this presentation, attendees will have a better understanding of using direct polymerase chain reaction (PCR) to amplify touch DNA samples with the GlobalFiler™ (GF) and PowerPlex® Fusion 6C (PPF6C) amplification systems. Direct PCR, a DNA processing method in which a sample is added directly to an amplification reaction without prior extraction or quantification, has been identified as a method that may improve DNA profiles from low-yield touch DNA evidence samples. Direct PCR eliminates the DNA loss, labor, and costs associated with DNA extraction, quantification, and concentration. This study compares GF and PPF6C amplification of touch DNA samples collected from plastic microscope slides, metal tools, handgun grips, vinyl shutters, brass cartridge casings, foam cups, concrete bricks, unfinished wooden tool handles, denim, wool, and polyester with various methods. Previous work identified optimal direct PCR-compatible touch DNA collection methods for these substrates. Puritan® cotton swabs and Copan microFLOQ® swabs moistened with sterile water or 0.1 percent Triton-X or swabs that were left dry were used to collect DNA from the non-fabric substrates. The fabrics 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 extracted and direct PCR samples were amplified with GF and PPF6C. No changes were made to the thermal cycling parameters, reaction mixtures, or reaction volumes validated for regular casework (25 µl, 29 cycles). For most substrates, the direct PCR and standard processing methods produced profiles of comparable quality with both amplification systems. Direct PCR with PPF6C may have a greater ability to amplify DNA when certain inhibitors are present. Denim and wool samples subjected to direct PCR with GF produced respective medians of 0 and 13.5 alleles, whereas PPF6C produced respective medians of 20 and 29.5 alleles. Direct PCR with both GF and PPF6C was unsuccessful for samples collected from concrete bricks and cartridge casings. Additionally, in some instances, a collection method produced higher quality results with one amplification system compared with the other. For example, when DNA was collected from wood handles, PPF6C produced significantly higher quality profiles ($p<0.05$) when dry cotton swabs were used, whereas GF produced significantly higher quality profiles ($p<0.05$) when dry microFLOQ swabs were used. These results support previous findings that the success of direct PCR is highly dependent on the substrate from which samples are collected. Additionally, the system used for amplification may affect direct PCR success. There are advantages to using PPF6C when certain inhibitors are present and when touch DNA is collected with cotton swabs, whereas GF is advantageous when microFLOQ swabs are used. The examined GF and PPF6C amplification methods were found to be effective for direct PCR of touch DNA samples collected from certain substrates. Direct PCR results may be further improved through PCR reaction optimization and additional post-PCR clean-up steps.

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Developing Multiplex Microhaplotype Sequencing for Mixture Detection and Deconvolution

NIJ AWARD #: 2018-75-CX-0041

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There are two aspects of mixture analysis: determining whether a specific profile could be part of a mixture (i.e., inclusion or exclusion of a subject) and fully (or partially) deconvoluting the mixture to determine the contributing genotypes at the loci. The combinatorics of independent microhaplotype (MH) loci makes full deconvolution difficult. Exclusion of a subject is probabilistic and does not require that all MH loci targeted in the multiplex sequencing assay are informative so long as some reach sufficient read coverage based on the assays established performance parameters for sensitivity and reproducibility. Not seeing the alleles of the subject at any of the loci with sufficient minor contributor reads amounts to exclusion to this level of certainty. In contrast, inclusion involves seeing the alleles of the subject at the informative loci based on read depth of the minor contributor. We have undertaken dilution series for both single-source and mixture samples to determine how much DNA needs to be present to be detected. Using our 24-plex MH panel and empirically established read thresholds, 100 percent of known homozygous variant alleles (n=48) from a single-source dilution sample were accurately detected from 0.156 ng DNA input, and 92 percent were genotyped from a 0.039 ng input sample. For two-sample mixtures analyzed in serial dilutions at 10:1, 20:1, 30:1, 40:1 ratios, the read depth for specific single nucleotide polymorphisms (SNPs) in four MHs was inspected. A linear dilution-dependent change in the percentage of reads for each SNP was observed that correlated to the expected mixture read ratios at these loci. For a two-sample mixture A/B, the 10:1 dilution showed that 92.6 percent of total 5,590 reads correctly called the alleles in Sample A whereas 7.2 percent of reads were for Sample B. Even better, in the 40:1 mixture the allele in Sample A received 97.4 percent of 7,842 reads whereas 2.46 percent of the reads were for Sample B. This analysis of low template samples and mixtures with high major to minor contributor ratios demonstrate our method's ability to recover probative information from such challenging sample types. The large number of alleles ($A_e > 4$) at each of these 24 loci yields a high probability that two random individuals will have different alleles at several loci, thereby assuring the detection of a mixture. We have shown that most of these MH have sufficient read depth in our multiplex that several different alleles at a locus can be identified reliably. We are developing web-based tools to aid in MH sequence data analysis (https://mmhseq.shinyapps.io/mMHseq). Many forensic labs are establishing sequencing capabilities to increase information that can be extracted from Combined DNA Index System (CODIS) short tandem repeat (STR) loci. Microhaplotypes are highly polymorphic and independent loci that could be multiplex sequenced with CODIS STRs to maximize the power of forensic DNA analysis, especially for mixtures.

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POSTER ABSTRACTS

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Software Tool and Methodology for Enhancement of Unidentified Decedent Systems with Postmortem Automatic Iris Recognition

NIJ AWARD #: 2018-DU-BX-0215

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The purpose of this National Institute of Justice (NIJ)-funded project was to design and deliver a complete methodology and the software tool that enhances unidentified decedent systems with a capability for comparisons of perimortem and postmortem iris images. In this presentation, I will present the final products and accomplishments of the project. These include (1) a methodology of automatic postmortem iris recognition with the use of three different artificial intelligence methods, including modern neural network–based image processing and classification, and (2) a methodology of human-machine pairing to increase the chances of correct matching between perimortem and postmortem iris samples. The designed artificial intelligence methods, in addition to a judgment whether the analyzed samples originate from the same subject, offer the "explainability" feature that presents salient image regions to assist human examiners in their work. The demo of the created software tool will be presented, and a short introduction to iris recognition (to provide a context) and medical commentary related to postmortem iris recognition will be given. I will also present the database of near-infrared and visible-light postmortem iris images, collected from more than 250 cadavers, with single cases acquired even 68 days after death. This dataset was deposited at the National Archive of Criminal Justice Data and is available to other researchers. This presentation should also demonstrate a very successful cooperation among computer scientists (University of Notre Dame and Michigan State University) developing new computer vision algorithms to solve a difficult problem of automatic postmortem human iris identification and the law enforcement institution (Dutchess County Medical Examiner's Office, NY) that collected operational data and offered a set of observations and requirements from their perspective. This NIJ-funded project has de facto pioneered the applications of artificial intelligence in forensic iris recognition. A discussion of future research directions in this area will be provided as part of this lecture.

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Body Mass Estimation Using Bone Micro- and Macro-structure: A Practical Approach Using CT Imaging and Computer Analysis

NIJ AWARD #: 2020-R2-CX-0048

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Body mass (BM) is generally not included in the biological forensic profile because of the lack of methods to estimate it accurately from skeletal remains, especially for obese individuals. Accurate BM estimates would provide additional information for matching unknown and missing person profiles and improve age-at-death estimation and facial approximation. Current understanding of bone functional adaptation argues that BM should be reflected in aspects of the weight-bearing skeleton. Therefore, we propose to develop a forensic analysis framework to estimate BM from multiple hierarchical levels of bone structure measured using x-ray computed tomography (CT)—a commonly accessible imaging technology. Here, we report on the initial investigation of gross morphological changes to the external shape of the femur as a possible quantitative BM marker. Femora from 40 male individuals (Texas State University Donated Skeletal Collection) were scanned using a Canon Aquilion Precision™ ultra-high resolution CT scanner. The sample was composed of 20 individuals who had been living with a body mass index (BMI) between 20–25 and 20 individuals with a BMI > 30. Precision CT provides a unique capability to acquire imaging data at two spatial resolutions: (1) standard, with 0.5-mm slice thickness, representative of commonly available clinical CT and (2) ultra-high, with 0.25-mm slice thickness, representative of new CT generation currently entering service. This will enable investigation of image texture markers of trabecular microstructure as BM predictors at different detail levels. Surface models of the femora (2,500 vertices and 5,000 faces) were generated from DICOM CT image stacks of the standard resolution scans (512×512 matrix, 0.625×0.625×0.5 mm voxels) using Matlab 2021b followed by isotropic remeshing and normalization in MeshLab 2021.07. To capture bone shape variations associated with BM, a statistical shape model (SSM) of the femora was then developed from the meshes following the framework of dense point correspondences. Vertex homology between the femora was established using Coherent Point Drift. Following registration to remove location, orientation, and size, the corresponding vertex coordinates were subjected to dimension reduction analysis to yield principal modes of shape variability. To verify that the SSM can accurately represent individual bone shape, SSMs were built from leave-one-out subsets of the femora, and an optimal combination of principal SSM modes that best match the excluded femur was obtained with a least-squares fit. The average distance between the vertices of the SSM fit and the target femur ranged 2.5–3 mm across the leave-one-out experiments (mean of ~2.8 mm), demonstrating the desired accurate representation capability of the SSM. Current work involves least absolute shrinkage and selection operator (LASSO) regression and variable selection to identify SSM components that predict BM. In the next stage of the analysis, the SSM will be applied to identify homologous trabecular bone regions across the skeletal samples. Textural biomarkers of cancellous microarchitecture will be obtained in these corresponding sub-volumes and used to augment the BM estimation model. The resulting predictive models will be implemented in a stand-alone software application to aid forensic examination of skeletal remains.

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GIS Application for Building a Nationally Representative Forensic Taphonomy Database

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Postmortem interval (PMI) determination is a critical piece of information to determine when human remains are discovered. An accurate determination of PMI can facilitate the identification of an unknown individual and help reconstruct the events around the time of death. A major weakness with the current state of the research is the lack of a reference dataset with a large number of cases from which research questions regarding factors impacting the rate of decomposition can be addressed. This presentation will be used to demonstrate an application that can be used by practitioners using crowdsourced data to collect information from scenes where human remains are found. This research utilizes a spatially coded, geographic information system (GIS) application that is accessible from mobile devices and tablets, among other devices. Forensic investigators working on a case use the app to record basic scoring information on the state of decomposition and to upload photos of the state of decomposition, and the GIS software records the location of the discovery. The application will be available for use by investigators from across the country and internationally to develop a large reference sample that will be used to create improved models for determining PMI. To encourage practitioners to submit data from casework, the application has a built-in calculator that provides practitioners with immediate benefits—it calculates the accumulated degree days based on the location the remains are recovered. Accumulated degree days is a commonly used variable for many of the available methods for estimating PMI. However, it is a time-consuming process for forensic investigators to recover information from nearby weather stations to calculate accumulated degree days. Using the geoFOR application, the investigator inputs the information about the case and the application automatically compiles information from the National Oceanic and Atmospheric Administration, with weather stations available worldwide, to determine the accumulated degree days quickly. The decomposition process is influenced by a wide range of factors both intrinsic to the individual and extrinsic environmental variables. The only way to develop accurate models of this complex system is to have sufficiently large sample sizes. In the absence of a large dataset, scientific investigation of PMI is severely limited. The small sample sizes and dependence on case studies has limited research in PMI. Previous research has demonstrated that there are multiple factors that impact the rate of decomposition and to develop a robust and multifaceted model a representative and large dataset is needed. For the field to move forward and to develop models that are reliable, useful, and with known error rates, a large dataset is necessary and currently missing from the field. The forensic community can work together to construct a reference set to build models of decomposition and improving methods for determining PMI when remains are discovered.

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POSTER ABSTRACTS

DIGITAL EVIDENCE

Forensic Interpretation of User Generated Audio Recordings

NIJ AWARD #S: 2019-DU-BX-0019 AND 2017-DN-BX-0179

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The widespread use of handheld smartphones and other devices capable of recording audio and video means that user-generated recordings (UGRs) are increasingly presented as evidence in criminal investigations. We research and implement the means for scientific and reliable comparison and synchronization of audio recordings of the same incident captured concurrently by multiple unsynchronized recording devices at the scene. When two or more audio devices are operating concurrently from different spatial locations while recording the same sound source, we would not expect the recordings to be identical, but we would expect a good correspondence, or correlation, among the recordings. This project increases the audio forensic knowledge base by developing new and innovative techniques to synchronize and process multiple concurrent ad hoc audio recordings from a crime scene obtained from body cameras, cell phone videos, surveillance cameras, dashboard cameras, and other recording devices. The availability of UGRs may offer important audio forensic insights. The proposed examination methodology includes time synchronization, noise reduction, and spatial position estimation. The proposed methods of forensic handling of UGRs also entails establishing best practices for assessing authenticity and integrity of the recorded information. Our goal is to understand the limitations of forensic interpretation of evidence obtained from UGRs, especially in terms of audio bandwidth, recording quality, and questions of authenticity.

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FileTSAR+: An Elastic Network Forensic Toolkit for Law Enforcement

NIJ AWARD #: 2020-DQ-BX-0008

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Nearly every type of criminal investigation (both cyber and traditional) involves some form of digital evidence and usually more than one type of digital device. As a result, law enforcement relies on specialized digital forensic investigative tools to acquire, evaluate, process, and present the probative data in a forensically sound manner to the criminal justice system. Although there are several well-known and reputable commercial products for capturing and analyzing evidence that originates from computers, there are too few complete tools of the same caliber for network forensics. In addition, the rapid rise in technology has consequently led to data overflow and the Big Data problem. In response to this problem, the National Institute of Justice (NIJ) requested proposals through the "Developing Improved Means to Collect Digital Evidence" program (NIJ-2016-8976) to develop innovative tools to "process large-scale computer networks for digital evidence in a forensically sound manner that preserves the probative value of the evidence that the computer network may contain." This research team was awarded a grant to develop the Toolkit for Selective Analysis & Reconstruction of Files (FileTSAR) for large-scale computer networks (>5,000 computers). The success of FileTSAR attracted law enforcement agencies of different sizes from around the world. Many are eager to deploy the toolkit in their own information technology infrastructure. However, the original goal of FileTSAR was to capture network traffic and restore digital evidence, in its original file format, in large enterprise network settings. As such, FileTSAR requires high-performance storage units and assumes high-performance servers or workstations are available on premise within the law enforcement agency it is deployed. To serve all-size law enforcement agencies, we proposed the creation of FileTSAR+, which was funded by the NIJ's Research and Development in Forensic Science for Criminal Justice Purposes FY2020 program. FileTSAR+ is the dockerized version of FileTSAR. It can be downloaded and installed on a single machine to process captured network traffic on a smaller scale than FileTSAR.

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