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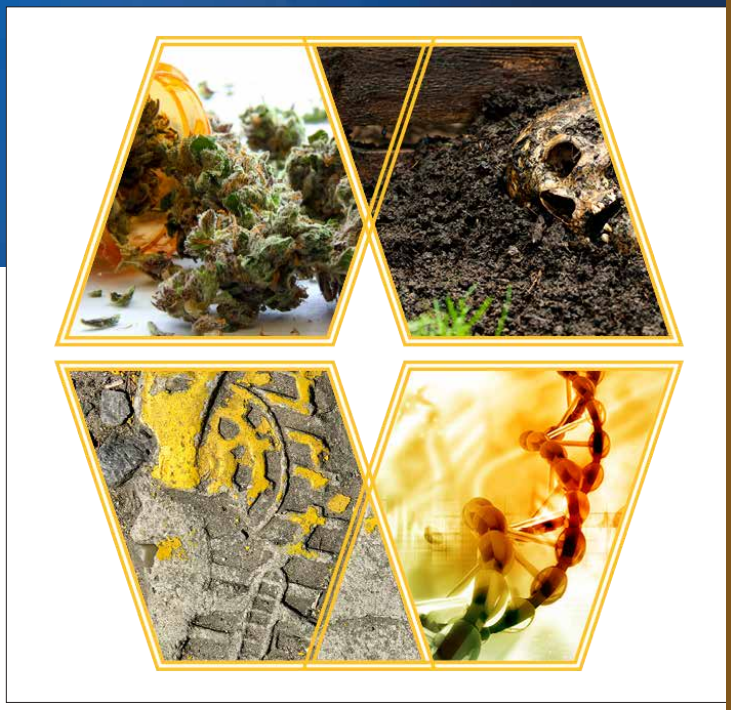

2020 National Institute of Justice Forensic Science Research and Development Symposium

Nicole S. Jones and Erica Fornaro, Editors

RTI INTERNATIONAL

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About the Editors

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

Erica Fornaro, AA, is a senior project management specialist in CFS at RTI International.

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Abstract

The 2019 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, we provide direct support to crime laboratories and law enforcement agencies to increase their capacity to process high-volume cases and provide needed training in new technologies. With highly qualified personnel and strong ties to the community, NIJ's Office of Investigative and Forensic Sciences plays a leadership role in directing efforts to address the needs of our nation's forensic science community.

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

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

On February 18, 2020, NIJ and the FTCoE held the 2020 NIJ Forensic Science Research and Development (R&D) Symposium. This event was held in conjunction with the American Academy of Forensic Sciences' 72nd Annual Scientific Meeting in Anaheim, California. Hundreds of attendees joined us in person and online to learn about NIJ research awards given to several talented researchers spanning the forensic disciplines.

For more than a decade, NIJ has hosted an annual R&D symposium to showcase 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 R&D team—including program managers Andrea Borchardt, Gregory Dutton, Danielle McLeod-Henning, and Frances Scott—have worked to create a phenomenal research agenda. The full-day program included presentations from principal investigators and their research partners; these presentations highlighted 16 NIJ-awarded grants representing several of the accomplishments from NIJ R&D grants that were awarded during 2015–2018. The two morning sessions comprised Forensic Biology/DNA and Controlled Substances and Toxicology; the two afternoon sessions covered Impression and Pattern Evidence/Trace Evidence as well as Forensic Anthropology and Forensic Pathology.

MORNING SESSION I

FORENSIC BIOLOGY/DNA

Moderated by NIJ Program Manager
Andrea Borchardt

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

2017-DN-BX-0140

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

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

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Microhaplotypes: Moving Scientific Research to a Forensic Casework Panel

2018-75-CX-0041

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

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

2018-DU-BX-0179

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

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

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

DJO-NIJ-17-RO-0219

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

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

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

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

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

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MORNING SESSION II

CONTROLLED SUBSTANCES AND TOXICOLOGY

Moderated by NIJ Program Manager
Frances Scott

The Detection and Quantitation of Fentanyl Mixtures by Surface-Enhanced Raman Spectroscopy (SERS) and Chemometrics

2015-IJ-CX-K006

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

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

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

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

2017-R2-CX-0022

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

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

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

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Forty-Plus Ways Not to Analyze Beverages for Cannabinoids

2017-R2-CX-0029

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

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

Background/Introduction: In the last decade, use of cannabinoids in the United States has increased tremendously. This increase has been primarily in marijuana-infused products, initially as food products, and now as beverages. These products contain the psychoactive drugs delta-9-tetrahydrocannabinol (THC) and/or cannabidiol (CBD), as well as other cannabinoids, and are sold because of their reputed medical and recreational properties. Regulation of these beverages is determined by the states in which they are legal. Federally, the US Drug Enforcement Administration (DEA) has classified marijuana as a Schedule 1 substance. The only US Food and Drug Administration (FDA)–approved formulation of THC is Marinol®, and the only FDA-approved formulation of CBD is Epidiolex®. In 2018, the Agriculture Improvement Act (Farm Bill) legalized hemp. The DEA, FDA, and Farm Bill do not address the formulation of these beverages, nor do they address the standardization of methods for potency analysis of these beverages. There is an increased need for accurate methods to determine THC and CBD content in these beverages. The three most common beverage matrices are fermented (beer/ale), brewed (tea/coffee), and high sugar (soft drinks). Each of these matrices creates its own concerns, which need to be addressed when trying to analyze beverages as a single class. Simple dilution methods with an aqueous or organic solvent are not plausible for all beverages due to the potential for varying complex matrices (e.g., plant material, pulp, sugar), and the low solubility of cannabinoids in aqueous solvents.

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

Methods: More than 40 different methods were evaluated for process efficiency (%PE). These included simple dilutions, rapid solid phase extraction (SPE), and quick easy cheap effective rugged safe (QuEChERS) methods, in which the aqueous and organic solvents were substituted or buffered. To account for the low solubility of cannabinoids in aqueous solvents, the beverage was prepared by solubilizing the cannabinoids in an emulsion (surfactant [2% fruit pectin], carrier oil [12.6% canola oil]) then adding the beverage (85.40%). %PE was determined at 14 mcg/mL (n=3) in two different sets of samples for each extraction method. The before set was fortified before the extraction method, and the non-extracted external standards (NEET) were prepared in methanol. The %PE was determined using the post-extraction addition method. The %PE was calculated by using the average peak areas of the before fortified samples

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and dividing by the average peak area of the NEET and multiplying by 100. An extraction method had an ideal %PE if the determined %PE were within 75%–125% for all three matrices evaluated. A method had an acceptable %PE if determined %PE variations in all three matrices were <± 15%. Samples analysis was performed using a previously presented and published high performance liquid chromatography tandem mass spectrometry cannabinoid method.

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

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

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

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

2015-R2-CX-K038

Judith Rodriguez Salas

Fredric Rieders Family Foundation: CFSRE

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

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

All analytical data was collected from postmortem and driving under the influence (DUID) cases at our collaborating laboratory (NMS Labs, Horsham PA). Agilent MassHunter Qualitative Analysis software was used for compound identification. Data files collected between January 2018 and December 2019 were reprocessed against a frequently updated database containing more than 170 opioids. Data analysis was divided into three categories: (1) routine opioids, (2) novel opioids, and (3) emerging opioids. All previously acquired analytical data was retrospectively mined to determine the date of first identification for analytes not included in the original scope of testing.

Between January and June 2019, there were 40,093 cases that tested positive for a routine opioid: 9,414 positive for fentanyl, 3,339 for heroin, and 3,613 for morphine. During that same time frame, 531 tests were found positive for novel opioids, which included para-fluoroisobutyrylfentanyl (p-FIBF) (n = 129), valerylfentanyl (n = 125), and carfentanil (n = 61) being the most prevalent. The only emerging opioid identified during the 6 months was o/m/p-fluorofuranylfentanyl, first identified in January 2019. Three positive cases were identified in 2019, and two were retrospectively identified in December 2018. From the retrospective data mining, 12 emerging opioids were identified that were not detected at the initial time of testing. Benzylfentanyl was identified a total of 9 times during this period, 3 of which were before it was included into the scope of testing in June 2018. Isopropyl U-47700 was identified in 10 cases, with the first identification occurring in May 2018. Data mining between March and April 2018 identified isopropyl U-47700 in five cases; however, it has not been detected since October 2018.

The opioid crisis presents a large public health and safety concern in the United States. Fentanyl continues to be implicated in approximately 1,450 cases

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each month, and heroin is implicated to a lesser extent. By comparison, the novel opioids have rapidly declined in positivity and are seen with much less frequency. However, emerging opioids not previously reported are still being identified. The data show that retrospective data mining can be a valuable tool to determine the prevalence and date of first appearance of novel compounds, giving a comprehensive perspective of the rise and fall of synthetic opioids over time.

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AFTERNOON SESSION I

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Moderated by NIJ Program Manager
Gregory Dutton

Quantitative Measures for Footwear Impression Comparisons

DJO-NIJ-17-RO-0202

Steven Lund*
Martin Herman
Hari Iyer
National Institute of Standards and Technology
* Presenting author

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

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

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

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

2017-DN-BX-0170

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

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

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

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

Two online Shiny applications are also presented for exploring the results of the study and the data's associated confidence and credibility intervals. The implications of these results on the reporting of "error rates" associated with palm print examinations will be discussed along with the implications and incidence of "questionable" conclusions that may not be supported by a consensus panel.

Keywords: error rate, black box, fingerprints

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Rapid Detection of Inorganic and Organic Firearm Discharge Residues by Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemical Sensors

2018-DU-BX-0186

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Rapid and accurate detection of firearm discharge residues (FDR) is highly desirable in circumstances that require a fast response to protect the welfare of citizens and provide reliable information to make informed decisions. Quick screening methods for FDRs are particularly useful, but currently difficult, in cases involving shootings. The scientific validity of this field relies on extensive research and standardization of the existing methods. There are still some remaining challenges in this area in terms of speed of analysis, preservation of the evidence, accuracy, and interpretation of results.

Consequently, there is a critical need to develop methods to improve the speed and reliability of these determinations. The long-term goal of this study is to develop a comprehensive approach to overcome these major concerns in FDR detection and to improve current capabilities in the criminal justice system. This research study aims to develop and validate fast tests—laser-induced breakdown spectroscopy (LIBS) and electrochemical sensors—for FDR detection and statistical models for the interpretation of the evidence.

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

A second collection set includes 150 specimens for estimation of firing distance and identification of residues on fabrics, wood, drywall, glass, and specimens with blood. LIBS created 3D chemical maps for shooting distance estimations and identification of bullet holes. Statistical methods, like principal component analysis and multivariate discriminant analysis, were performed to estimate shooting distances and identify the presence of FDR. Color tests led to misclassification of 26%–70% of the unknown shooting distances, while the LIBS method correctly classified 100% of the blind test samples.

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Additionally, LIBS was able to correctly identify elemental profiles from the modern ammunition, expanding current capabilities in the reconstruction of events at crime scenes. Scanning electron microscopy–energy dispersive X-ray spectroscopy, liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry were used to cross-validate the results.

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

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Facilitating the Adoption of Glass Evidence Analyses in Forensic Laboratories

2015-DN-BX-K049

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

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

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

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AFTERNOON SESSION II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Moderated by NIJ Program Manager
Danielle McLeod-Henning

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

2018-DU-BX-0229

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

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

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

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

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Development Responses to Fluctuating Temperatures of a Forensically Important Blow Fly (*Cochliomyia macellaria*)

2016-DN-BX-0204

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

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

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

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

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Understanding the Role of the Thanatomicrobiota in the Decay of "Reproductive Organs" in Human Decomposition

2017-MU-MU-0042

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

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

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

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

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Microbial Clocks for Estimating the Postmortem Interval of Human Remains at Three Anthropological Research Facilities

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

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