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About the Editor

Nicole S. Jones, MS, is the associate director of strategic planning and operations in the Center for Forensic Sciences (CFS) at RTI International.

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Abstract

The 2018 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

The National Institute of Justice (NIJ) is the federal government's lead agency for forensic science research and development as well as the administration of programs that facilitates training, improves laboratory efficiency, and reduces backlogs. The mission of NIJ's Office of Investigative and Forensic Sciences (OIFS) 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 OIFS 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 NIJ Cooperative Agreement No. 2016-MU-BX-K110.

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

On February 20, 2018, NIJ and the FTCoE held the 2018 NIJ Forensic Science Research and Development (R&D) Symposium. This event was held in conjunction with the American Academy of Forensic Sciences' 70th Annual Scientific Meeting in Seattle, Washington. 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 Gregory Dutton, Danielle McLeod-Henning, Minh Nyugen, and Frances Scott—worked to create a phenomenal research agenda. The full-day program included 18 presenters and their researcher partners, representing 16 NIJ awards; these awards were received during a 4-year period (2013–2016). The two morning sessions comprised Forensic Anthropology and Controlled Substances and Toxicology; the afternoon sessions covered Trace Microbiome and Forensic Biology/DNA.

FORENSIC ANTHROPOLOGY

The Macromorphoscopic Databank: A New Tool for Forensic Anthropologists

2015-DN-BX-K012

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The purpose of this research is to address a substantial gap in best practice in forensic anthropology. That is, very little reference data are publicly available for the objective analysis of macromorphoscopic traits in assessing ancestry. Consequently, forensic anthropologists rely on their own experience or outdated methods having very little empirical support to estimate a fundamental component of the biological profile. This approach provides no error rates and is not verifiable because it is not replicable, resulting in post hoc trait selection, experience-based justifications, and anecdotal expert judgment with no empirical support.

This presentation addressed this issue by introducing the Macromorphoscopic Databank (MaMD). The purpose of any databank is to serve as a repository of data and to make those data accessible to many end users or practitioners. To that end, the MaMD serves as a repository for macromorphoscopic trait data obtained primarily from recent and well-documented forensic cases or donated skeletal material. To facilitate data sharing and to maximize analytical output, the databank comprises relational databases housing not only macromorphoscopic trait scores, but also demographic data on each decedent. These data include but are not limited to age at death, sex, stature, ancestry, place of birth, occupation, and self-identified ancestry (social race). All data are maintained in these relational databases on a central server, using a database platform to perform the essential managerial and analytical functions necessary for data management.

The MaMD is populated using Macromorphoscopic Traits (v. 1.61), a newly developed data collection program for 17 macromorphoscopic traits. The end user provides provenience information after data are collected, retaining trait scores to a sample-specific database for subsequent submission to the MaMD. The MaMD currently contains macromorphoscopic trait data for 6,670 individuals. Examples of populations for which data are available include samples of modern American black and white, Hispanic, Guatemalan, Colombian, Fijian, Thai, Japanese, Pacific Islander, and Peruvian.

The MaMD and a forthcoming analytical program using classification algorithms appropriate for categorical data will be available for wider use following extensive beta testing, method validation, and inter- and intra-observer error tests. Forensic anthropology laboratories (applied and academic) are encouraged to help validate and beta test this research through a data-sharing model similar in scope and function to the Forensic Anthropology Databank. Further refinement of several classification algorithms and the user interface for the analytical program will be completed within the next year.

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Analysis of Inter- and Intra-Observer Error Associated with the Use of 3D Laser Scan Data of the Pubic Symphysis

2015-DN-BX-K010

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In age-at-death estimation based on visual assessment, objective evaluation and correct diagnosis of age-related skeletal traits are crucial to achieving accurate and reliable age estimates. Nevertheless, the traditional, phase-based methods have been reported to yield inconsistent age estimates both within and between observers.[1,2] The reasons for these discrepancies lie in the fact that accurate macromorphoscopic analysis depends heavily on the interpretation of qualitative trait descriptions, conformity of the bone, and experience of the observer.

Recently, Slice and Algee-Hewitt[3] and Stoyanova et al.[4,5] have introduced three fully computational methods using 3D laser scans of the pubic symphysis that minimize subjectivity in age estimation by reducing both the effects of observer experience in skeletal-trait assessment and methodological bias. However, the reproducibility of these methods has not been fully explored. This is of concern because there is potential for introducing error in the scanning and editing of the raw scans at different times by different observers. In response to this concern, the current study evaluated the repeatability of these novel methods by assessing intra-scan variation, within-/between-observer differences in scan editing, and the impact on age estimation.

The test data represent triplicate scans of the Suchey-Brooks' (SB) male casts, taken using a 3D desktop laser scanner by a single observer (n=36). Four different observers with various experience levels and training backgrounds independently edited the three sets of the raw scans using the scanner's accompanying software. From the edited scans, shape measures were computed via the subarachnoid hemorrhage (SAH)-Score method,[3] the thin plate splines/bending energy (TPS/BE) method,[4] and the ventral curvature (VC) method.[5] These measures were subjected to various regression models to obtain age estimates for each replicate scan per observer. Finally, a series of the intraclass correlation coefficient was calculated to evaluate observer reliability in scan editing. Extra editing conditions were tested to simulate the situation where the practitioner misidentifies age-related traits because of unfamiliarity with the scan editing protocol. A set of the SB casts was edited with different widths of the margin (2 mm vs. 4 mm vs. 1 cm) left around the symphyseal face and with/without the pubic tubercle, which may affect the VC values as it protrudes ventrally.

Results of this study demonstrate that the raw scans were edited consistently within and between observers and the derived shape measures and age estimates were in excellent agreement among observers. For the test of improper scan editing, simulated with various margin widths, the methods were robust enough to produce consistent and accurate age estimates with an exception of the faces with 1 cm margin. Interestingly, the inclusion of the pubic tubercle for the shape

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analysis did not necessarily yield inaccurate age estimates for the VC method, while it produced significant differences between the documented chronological age and age estimates of the SAH-Score and TPS/BE methods and the two multivariate regression models. These results show high repeatability of the computational methods regardless of the level of observer experience or training background and support using a 3D laser scanner and scanned images to aid in resolving the issue of subjectivity.

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Building a Science of Adult Cranial Fracture

2015-DN-BX-K013

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We presented key findings in new research on adult cranial fracture and potential implications for analyzing forensic cases involving blunt force cranial trauma. The fields of forensic anthropology and pathology currently lack guidelines for making forensically significant assessments about implement type, impact energy, location of impact, and number of impacts based on cranial fracture patterns. In practice, these assessments are often based on practitioners' past case experience and on post hoc applications of biomechanical principles, rather than scientifically informed methods compliant with Daubert standards. In the absence of hypothesis-driven, experimental studies confirming links between injury scenario and fracture pattern, the work of even highly experienced practitioners may be called into question in a court of law.

This research used adult human cadaver heads in a series of biomechanical impact experiments documenting the influence of several forensically relevant impact variables on cranial fracture patterns. Our three primary objectives were to document (1) the influence of implement on the initiation, propagation, sequence, and pattern of cranial fracture; (2) the influence of input energy on these same parameters; and (3) patterns of cranial fracture following sequential blows. The ultimate goal of this project was to provide a body of experimental evidence as a resource for practitioners to make more accurate interpretations of adult cranial fractures.

We performed cranial impact experiments using a pneumatic impact system designed to simulate a blow to the head of an upright individual using three aluminum impactors selected to approximate the shapes of objects commonly implicated in forensic cases: a brick, a baseball bat, and a hammer. The effects of impact energy were investigated in two phases of testing: the first with impacts performed at a base energy level and the second at a high energy level. Each impact experiment was filmed with a high-speed camera that captured fracture initiation and propagation at 10,000 frames per second.

The data set consists of measured and calculated mechanical variables, high-speed video footage of fracture initiation and propagation following impact, photographic and diagrammatic representations of fracture patterns after each impact, and anthropological assessment of ectocranial and endocranial fracture patterns after three sequential impacts.

This research represents a major shift in understanding of cranial fracture initiation and propagation. A key finding of this research is that cranial fractures can initiate in one or more locations peripheral to the point of impact. Additionally, peripherally initiated cranial fractures do not always propagate back to the point of impact, resulting in fractures concentrated remote from the impact site. This finding contradicts recent, widely cited sources in the literature that assert fractures always initiate at the point of impact.

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This presentation highlighted the implications of these results and this research for forensic trauma analysis. Trauma analyses guided by the flawed assumption that fractures occur only at the point of impact result in inaccurate assessments regarding the location of impact and may lead to overestimations of number of impacts.

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Standardizing a Large-Scale, Whole Body CT Image Database

2016-DN-BX-0144

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In 2016, a National Institute of Justice grant was awarded to the Center for Forensic Imaging in the Office of the Medical Investigator (OMI), a statewide, centralized medical examiner's office for New Mexico, to create a free-access Decedent CT Database, which will make 15,250 whole-body computed tomography scans (CTs) available to the research public. Work is currently underway to populate the new database. Populating the database presents numerous challenges in that the majority of fields within the database are free-text fields without any limitations. As a result, even sex can be recorded in multiple ways (e.g., "Male," "male," "M," "m"), limiting the ability of future researchers to query efficiently. To combat this issue, the CT database being developed will use data standards, terminologies, and classification systems.

A step-by-step process is under way to determine the best standards to implement. First, we are searching the Unified Medical Language System to identify all of the standards for a particular concept (e.g., race) that exist. Second, each standard is then identified and compared for usefulness in this particular database. Standards can be implemented as is, modified, or rejected. If all current standards are rejected for use in the database (or none are found), a new standard will be proposed and implemented.

The free-access Decedent CT Database is slated to be available by the end of 2018.

Reference

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CONTROLLED SUBSTANCES AND TOXICOLOGY

Liver "Doesn't DIE" or at Least its Enzymes, and Other Useful Information Discovered While Evaluating the Effect of Sample Preparation Techniques on Matrix Effects and Absolute Recovery of Opiates in Liver Tissue Using UPLC-MS/MS

2016-DN-BX-0148

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With the "opioid crisis" currently occurring in the United States, the ability to analyze biological specimens for opiates (i.e., heroin and oxycodone) and opioids is very important. Blood is the most common sample analyzed for drug concentration. In forensic toxicology, blood is collected from a living person for human performance testing cases and analyzed as whole blood. However, in postmortem cases, blood is no longer a true whole blood because bodily processes have stopped. Blood in the thoracic and abdominal cavity can become contaminated as a result of postmortem redistribution. This contamination can affect the determination of manner and cause of death, which can have dire criminal and/or civil consequences. Analysis of the liver in conjunction with blood is a routine way to circumvent this issue. Disadvantages of analyzing the liver are the interferences by protein and fatty matrices and potential putrification. These disadvantages necessitate effective cleanup or sample preparation before analysis of the liver.

Newer sample preparation techniques are primarily designed for blood or urine analysis, and the use of difficult matrices such as the liver has occurred without the complete understanding of the effects of the liver on the analysis of the drug(s) of interest. The newer sample preparation techniques are predominantly based on traditional techniques with manufacturers' improvements. These improvements include solid-phase extraction, liquid-liquid extraction, and filtration. However, these techniques have limited published data regarding tissue matrices such as the liver. For these techniques to be effectively used for liver analysis, matrix effects and absolute recovery must be evaluated. We evaluated these techniques using opiates (codeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone, and 6-acetylmorphine [6AM], a heroin metabolite) as the model class of drugs. The sample preparation techniques evaluated were performed following manufacturers' guidelines whenever possible or using a laboratory validated liquid-liquid extraction technique. Prepared liver samples were analyzed using a previously validated ultra-performance liquid chromatography-mass spectrometry (MS)/MS method.

We discovered that liver homogenates fortified with 6AM contained no detectable concentrations of 6AM after a couple of hours. After several attempts and much research, it was determined that while the liver hepatocyte may die in a couple of days, the liver enzymes within the hepatocyte are still active for at least 4 months when stored frozen. While the majority of the sample preparation techniques had a published or recommended protocol for use with tissue samples, the results varied greatly among the methods evaluated. The observed

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matrix effects varied between −35% and +50%, and recoveries varied between 30% and 122%. These variations were even observed between different opiates analyzed using the same sample preparation technique.

The liver is a difficult matrix to analyze. Sample preparation is not as simple as for blood or urine, and even chemically similar drugs do not always extract similarly. We observed that not all sample preparation techniques are effective or reliable for extracting opiates from liver tissue.

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Evaluating Trends in Novel Psychoactive Substances Using a Sentinel Population of Electronic Dance Music Festival Attendees

2015-IJ-CX-K012

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Novel psychoactive substances (NPSs), ingested for their euphoric and stimulating effects, have become widely circulated at electronic dance music (EDM) festivals and have had several adverse events associated with their use at festivals in the United States. Many of these products have been found to contain unregulated phenethylamines, cathinones, and synthetic cannabinoids. The range of NPSs currently available on the market has continued to grow because of their widespread availability over the Internet and constant variation in product composition to circumvent changes in legislation.

We conducted a longitudinal study of drug use by attendees at three major EDM festivals in the United States between 2014 and 2017. Each year, our peer recruiters contacted participants outside the venue and solicited survey participation and provision of a biological sample to compare their admitted drug use with actual drug use and to identify novel emerging substances and metabolites of these substances in these fluids.

The evolution of oral fluid as a suitable biological matrix for drug detection coupled with the ease of sample collection provided the ability to collect a larger sample set. Between three sample collections, 1,067 oral fluid samples were collected. Our survey responses suggest as many as 79% of attendees at the events we studied admitted to recent drug or alcohol use. The most common response was alcohol (49%), and the second most common answer was marijuana (31%), followed by "molly" (10%), "MDMA" (methylenedioxymethamphetamine) (3%), and "ecstasy" (2%), the latter three accounting for a total of 15% of responses.

Across all years, the diversity of NPSs confirmed in biological specimens has continued to evolve. NPSs confirmed in 2014—such as alpha-PVP, methylone, and 4-fluoroamphetamine—have decreased in positivity, with compounds such as ethylone, dibutylone, butylone, and n-ethyl pentylone positivity increasing, along with the resurgence in positivity for MDMA. Positivity rates also generally increase over the days of the festival, with the last day having the highest positivity for NPSs. Related to the survey results, there is a large discrepancy between the users believing they are ingesting the NPSs and NPSs being confirmed in their biological samples.

These results stress the importance of updating laboratory-based methods for detecting emerging drugs, the ability to distinguish between isomeric NPSs, and the utility of this target population for monitoring trends.

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Assessing the Impact of Portable Mass Spectrometers for On-Site Drug Evidence Processing

2015-IJ-CX-K011

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Forensic evidentiary backlogs are indicative of the growing need for cost-effective, high-throughput instrumental methods. One such emerging technology that shows promise in meeting this need, while also allowing on-site investigation, is portable mass spectrometric (MS) instrumentation, particularly instrumentation that enables the coupling of rapid, ambient ionization methods. Such technology has the potential to assess the probative value of chemical evidence at the crime scene, requiring only pertinent samples to be sent to off-site laboratories for confirmation, which eases the burden of casework and therefore reduces the magnitude of backlogged evidence. Screening of physical evidence at the crime scene also has the capability to rapidly determine whether a criminal investigation is needed and to provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial.

Through National Institute of Justice funding, a cross-disciplinary team of researchers developed direct-evidence screening methods on a commercially available, portable MS system, culminating in a fieldable instrument that is simplistic in operation, yet robust to the needs of today's forensic and law enforcement practitioners. A rigorous analytical validation using common and emerging illicit chemicals was performed to ensure that reliable and reproducible use by nontechnical operators is feasible and to facilitate future court admissibility of field-collected forensic data. A significant aspect of this project was designed to not only anticipate but also predetermine the legal and economic impacts of adopting this technology for field use to inform and guide forensic science policy and practice. In an effort to ascertain the legal implications of adopting this technology for field use, we examined the current state of US search and seizure law to recommend legal investigation strategies by law enforcement, including the potential legality of using this technology to prompt a "probable cause" search. To assess the financial viability of instrument-based analysis of forensic evidence in the field, we created fiscal impact models to compare this proposed methodology to the current system of off-site evidence processing at publicly funded laboratories in terms of both cost and processing time.

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Rapid Peptide Analysis Utilizing Matrix-Assisted Inlet Ionization and Paper Spray Ionization Mass Spectrometry

2013-DN-BX-K020

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This presentation demonstrated a simple method for analyzing peptides and large biomolecules that can be easily implemented in drug identification laboratories, often using preexisting equipment with little to no sample preparation.

The ease with which consumers can purchase performance-enhancing peptides and cosmetic peptides online is astounding. Hidden behind the anonymity of the Internet, online vendors sell peptide products at minimal cost with no questions asked. To avoid legal action, such products are marketed for research purposes only. Unfortunately, consumers purchase these products to inject into the body to promote muscle growth or cosmetic augmentation. Although the sale of peptides may be legal, it is a major concern. Counterfeit peptide sales are known to be fairly common, especially those reportedly containing recombinant human growth hormone. These trends lead to questions regarding the authenticity of the peptides being sold and, more important, concerns regarding health and safety.

Many crime laboratories do not have protocols in place for successfully identifying peptides and large biomolecules. The workhorse of forensic drug analysis, gas chromatography–mass spectrometry, is limited to the analysis of relatively small molecules that are readily vaporized at the inlet. High-resolution mass spectrometers capable of ambient ionization, however, are well suited for analyzing peptides. Moreover, such instrumentation is becoming more prevalent in forensic laboratories as ambient ionization techniques, such as direct analysis in real time (DART), continue to gain popularity.

Through this project, we developed a protocol to analyze peptide samples using a time-of-flight mass spectrometer that is typically used for DART-MS analysis. In many cases, this technique required no external ion source or additional equipment aside from the mass spectrometer.

Using matrix-assisted inlet ionization mass spectrometry, as well as paper spray ionization, we successfully analyzed various peptide standards and case samples. An array of peptides was also purchased from an online vendor to determine the authenticity of the products using this technique. Direct inlet ionization was accomplished by placing the samples in solution with the matrix compound 3-nitrobenzonitrile and introducing approximately 5 µL directly into the mass spectrometer inlet with a micropipette. In a matter of seconds, electrospray ionization–like spectra were obtained. Molecular masses were calculated using a mass spectral interpretation software package.

To further characterize the peptides, we used a simple enzymatic protein digestion procedure. Matrix-assisted inlet ionization was employed to analyze the resulting peptide fragments. We assigned possible identifications for each peptide by comparing each digested spectrum against an online peptide

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database. Combined with the molecular mass attained from analyzing the intact peptides, we identified each peptide at a reasonable level of certainty.

Finally, we explored peptide analysis using paper spray ionization. This ionization method was favored for extremely large peptides for which inlet ionization was not feasible. By using both inlet ionization and paper spray ionization as complementary techniques, the vast majority of peptide samples that would be encountered in a crime laboratory could be readily characterized.

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TRACE MICROBIOME

Developing Reliable Methods for Microbial Fingerprinting of Soil Evidence: Collection, Contamination, Storage, and Analysis

2015-DN-BX-K031

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Soil can represent valuable trace evidence, helping link a victim or perpetrator of a crime to a scene. Research has shown that the microbial makeup of soils is variable enough that a soil sample can be linked to a specific location with a high degree of accuracy. However, most studies have examined freshly collected or frozen samples, neither of which mimic a forensic scenario, wherein the evidentiary soil will have aged ex situ for some period of time, potentially allowing temporal or environmental change in its bacterial makeup.

In the current set of studies, we examined the influence that evidence stored at room temperature has on ex situ soil. Soils from different habitats (agricultural field, coniferous forest, dirt road, yard) were placed on evidence items (t-shirts and trowels) and allowed to age. Soil samples were collected weekly and then monthly. Known soil samples were also collected and stored at −80°C, −20°C, 4°C, and room temperature and sampled over time. In a second study, we examined the influence of the human microbiome on soil evidence by having volunteers wear t-shirts for 24 hours, after which we applied soils from different habitats. Samples were again collected over time. Finally, we examined the influence of blood (a good growth medium for some bacteria) on soil evidence by mixing soil with fresh swine blood at 10:1, 1:1, and 1:10 ratios and applying it to t-shirts and trowels. Evidence was either dried or stored wet and sampled over time.

We isolated bacterial DNAs using a MoBio PowerSoil kit, the V3/V4 region of the bacterial 16S rRNA gene was amplified using barcoded universal primers, and sequences were obtained on an Illumina Mi-Seq. We processed sequence data and analyzed the data using bacterial abundance charts (generally at the taxonomical class level), nonmetric multidimensional scaling, and Random Forests.

Bacterial profiles from all evidence changed temporally, showing increases in the bacterial classes Actinobacteria and bacilli and decreases in Sphingobacteria and Acidobacteria. At later time points, the evidence soils were more likely to misclassify with the knowns, unless the knowns were also aged, in which case the evidence samples classified well. The human microbiome had no perceptible influence on soil profiles, with evidence soils always classifying with the correct habitat of origin. Likewise, blood on evidence had no temporal classification effect when the evidence was dried or when the stain was primarily soil (10:1). In contrast, greater ratios of blood (1:1) stored wet misclassified after 1 week, while 1:10 misclassified even on day 0, showing extremely high levels of bacilli and reduced levels of actinobacteria.

Overall, the results demonstrate that soil bacterial profiling is highly effective for identifying its place of origin, although time and storage conditions must carefully be considered.

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Evaluating the Skin Microbiome as Trace Evidence on Common Surface Types

2014-R2-CX-K411

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Each of our bodies is covered in billions of microbial cells, which we shed on objects that we touch. Recent work on the built environment (human-made structures) has highlighted the ubiquity of human microbiome signatures in these human-dominated ecosystems. Previous work has demonstrated that the transfer of skin microbes to surfaces can associate objects with individual people and that the microbial signatures are generally stable within a person, raising the potential that these microbial fingerprints could provide important physical evidence. However, a knowledge gap exists about whether skin microbes transfer to different material types, whether they persist over timescales relevant to forensic investigations, and whether they are stable on a decedent's skin after death.

To investigate properties of skin microbiome transfer to surfaces, we investigated the antemortem skin microbiome in a set of experiments at University of California, San Diego, and the postmortem microbiome in collaboration with the City and County of Honolulu Medical Examiner. At University of California, San Diego, we investigated the effect of surface type (wood, plastic, metal, glass, and ceramic tiles) on the ability of skin microbes to transfer to an object. By applying machine learning methods using a Random Forests classifier, we discovered that plastic and ceramic surfaces were most accurate for classifying the correct participant, followed by glass and metal. We determined that skin microbial signatures persisted on ceramic and plastic surfaces for at least 1 day and became less accurate over time. We found that microbiome trace evidence samples can be tracked back to individuals with high accuracy and can be used to narrow pools of suspects even when multiple people have touched a surface and when the reference microbiome was collected 1 year previously.

Additionally, we investigated the skin microbiomes of recently deceased individuals at 15 death scenes in Honolulu, Hawaii. We discovered that skin microbiomes were fairly stable after death and during morgue storage prior to autopsy. Further, we found that household and personal objects could still be linked with a person after death. We conclude that skin microbes are uniquely positioned to augment friction ridge impressions when sufficient ridge detail is not available to make an identification. Thus, the potential for microorganisms to reveal whether a particular person has touched an object is substantial.

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Forensic Geosourcing Potential of the Human Microbiome

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Next-generation sequencing technology has advanced our understanding of the human microbiome, enabling researchers to predict various physiological states based on microbial community composition and function across diverse body sites. In addition to overall physiology, the composition of the human microbiome also correlates with geographical location, subsistence, and culture. Here, we evaluated the use of microbiome data from different body sites to predict geographical provenance of subjects, using 16S rRNA data and combining classic multivariate and machine learning techniques. The results indicate that the microbiome of diverse body sites can be used to predict geographical origin of a sample or subject at narrow geographical scales, with high accuracy, sensitivity, and specificity, and linking specific geolocators to particular human groups and body sites. The data also indicate that diverse body sites have varying geolocation accuracy, with specific skin and oral samples showing higher performance than stool. As an outgrowth of the skin, the microbial communities of human hair shafts show significant body site and geographical compositional differences indicating that hair shafts have the potential to be used to predict the source location of the hair. As such, the potential of the human microbiome as a reliable and specific geolocation tool in forensics should be considered, along with efforts to formally implement geosourcing from microbiome data in forensic investigations. The Forensic Microbiome Database (FMD) (http://www.fmd.jcvi.org) is a publicly available analysis resource that correlates 16S rRNA data obtained from multiple human body sites to metadata as it relates to forensics. The goal of the FMD is to be used as a valuable tool to predict the geographical location of subjects using human microbiome data. The FMD is an actively updated database and will be expanded through the inclusion of paired oral and stool microbiome samples from 100 healthy adult women residing in Hong Kong, Barbados, Chile, and two cities in South Africa. The addition of these large-size datasets will strengthen the FMD's geolocation prediction model and incorporate multiple currently underrepresented geographical locations worldwide.

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Candidates of Skin Microbiomes for Human Identification

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Human microflora on the skin may be a high-copy source of DNA for identifying individuals. This study used supervised learning methods and Propionibacterium acnes pangenome presence/absence features and nucleotide diversities of stable clade-specific markers to determine classification accuracies of the microbial source. The hidSkinPlex was developed and evaluated on bacterial control samples and then on eight individuals from three body sites. All samples (n =72) regardless of body site origin (foot, manubrium, and hand) were correctly classified with up to 94% accuracy, and body site origin could be predicted with up to 86% accuracy.

Forensic human DNA typing relies primarily on characterization of short tandem repeat (STR) markers (residing on autosomal and Y chromosomes). The types of evidence presented to the laboratory today often contain mixtures of human DNA from multiple sources and/or contain low amounts, making interpretation of mixed or partial profiles difficult or inconclusive. In these cases, alternative markers, such as sequencing the high-copy mitochondrial genome, or methods to enhance sensitivity of detection sometimes are considered. The human microbiome possibly may be considered another high-copy number genetic marker because microbial cells may be at a ratio of 1:1 to 10:1 to human cells. Indeed, microbes on human skin may be more readily deposited than human cells. Thus, human microbiome flora may be a potential target to complement partial or inconclusive STR profiles to increase resolution for human source attribution. Previous studies have demonstrated the potential to use microbiome profiling for forensic applications; however, a method has yet to identify stable features of skin microbiomes that produce high classification accuracies for samples collected over reasonably long time intervals. A novel approach has been developed to classify skin microbiomes and associate them with their donors.

This study compared two features types: *Propionibacterium acnes* pangenome presence/absence features and nucleotide diversities of stable clade-specific markers. Supervised learning was used to attribute skin microbiomes from 14 skin body sites from 12 healthy individuals sampled at three time points over a more than 2.5-year period with various accuracies for multiple skin body sites. With feature selection, we identified a subset of markers (187 markers from 12 clades) from each body site that are highly individualizing. We compared the classification accuracies in a formal model testing framework, and the results indicate that nucleotide diversity performed better than presence/absence encodings. These selected features provided a preliminary marker panel for developing a robust and reproducible method for skin microbiome profiling for forensic human identification. Likely for forensic applications, informative targets will need to be enriched, because they are for current human identification methods. Targeted enrichment and sequencing using a panel of the most informative markers are being sought for microbiome profiling for forensic identification to obtain high coverage at stable informative sites. A multiplex panel has been designed and is being tested empirically

for classification accuracy and sensitivity at various sites on the human skin, including the currently low-informative foot region. Performance assessment is under way, and preliminary data indicate that the candidate panel can characterize human-based selected microbes even at initially low-abundant body sites.

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FORENSIC BIOLOGY/DNA

Multi-locus Match Probability Dependencies

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With the recent increase in the number of Combined DNA Index System (CODIS) core loci from 13 to 20, would more loci provide even higher discriminatory potential? Or are we approaching the point of diminishing return?

Donnelly discussed the question: "after the observation of matches at some loci, it is relatively much more likely that the individuals involved are related (precisely because matches between unrelated individuals are unusual) in which case matches observed at subsequent loci will be less surprising. That is, knowledge of matches at some loci will increase the chances of matches at subsequent loci, in contrast to the independence assumption."[1] Laurie and Weir provided a theoretical prediction of dependencies in match probabilities, focusing on the effects of mutation,[2] following an earlier treatment of the effects of population size by Cockerham and Weir.[3] Weir provided an empirical demonstration[4]: among 15,000 forensic short tandem repeat profiles the ratios of multi-locus match proportions to products of single-locus proportions were 1.000, 1.000, 1.008, 1.034, and 1.041 for two, three, four, five, and six loci, respectively.

The hope has been that using the single-locus "theta-corrections" at each locus would compensate for the between-locus dependencies. Some caveats for this approach will be presented, based on theoretical, simulation, and empirical results for autosomal and Y chromosomes and autosomal single nucleotide polymorphism. This work suggests consideration of a multi-locus population structure parameter theta as a predictor of match probability for multi-locus profiles.

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Record Linkage of CODIS Profiles with SNP Genotypes

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Forensic-genetic work in the United States relies largely on the Combined DNA Index System (CODIS) markers, a set of 20 (until recently, 13) microsatellite loci in heavy use since the 1990s. One premise that has influenced forensic practice—figuring in discussions of both backward compatibility of single-nucleotide polymorphism (SNP)–based systems with the CODIS database and of genetic privacy—is that the information provided by the CODIS loci is completely distinct from the information provided by larger sets of SNPs. Although the associations between CODIS markers and specific genetic variants known to influence phenotypes are weak, there may still be a connection between CODIS records and SNP information if pairs of CODIS and SNP genotypes can be identified as coming from the same person—that is, if CODIS and SNP records can be linked. We present a method for assessing whether a particular set of genotypes from the CODIS markers is likely drawn from the same person (or an identical twin) as a set of genome-wide SNP genotypes, extracting a signal arising from linkage disequilibrium between CODIS and SNP markers. In subsets of a dataset of 872 people, 90%–98% of records of the original 13 CODIS markers can be linked to corresponding SNP records and vice versa. As more short tandem repeat (STR) markers are used, accuracy improves, reaching 99%–100% when ~30 STRs are used. Our results suggest possibilities and limits for backward compatibility of SNP-based databases with existing forensic-genetic databases, and it also raises privacy concerns associated with maintaining forensic databases containing even small numbers of genetic markers.

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Microhaplotypes Analyzed by Massively Parallel Sequencing Are Valuable Forensic Tools

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With the advent of massively parallel sequencing (MPS), microhaplotypes (microhaps) have become a valuable new type of DNA marker for use in forensics. Microhap loci are small (<300 base pairs [bp], generally <200 bp) regions of DNA with two or more single nucleotide polymorphism (SNPs) defining three or more common haplotypes (alleles) in multiple populations. Microhaps have the potential to be very informative in (1) identifying and deconvoluting mixtures of DNA, (2) identifying biological relationships, (3) identifying biogeographic ancestry, and (4) providing very small random match probabilities. Publications and presentations to date have demonstrated proof of principle, using typing of the individual SNPs and statistical phasing into haplotypes with lengths ranging from 11 bp to about 250 bp. MPS typing by collaborators has confirmed the broadly accepted accuracy of statistical phasing of data for population allele frequencies.

A full analysis of 130 loci evaluated on 83 population samples (>5,000 individuals) was recently published. Analyses of the data demonstrate theoretically the near certainty of detecting a mixture (unambiguous presence of three or more alleles in a sample) using just the 28 loci with the highest global average effective number of alleles. Mixture analyses using MPS have also been performed, demonstrating an enhanced mixture detection capability by detecting the minor contributor at a 40:1 ratio when the mixture is sequenced in a 36-locus multiplex starting with only 250 pg. Both greater sensitivity and absence of stutter interference make microhaps much better than the forensic short tandem repeat polymorphisms (STRPs) in the interpretation of mixtures. Data on an additional 38 loci are now available for those 83 population samples, and 65 of the loci have been extended to 96 population samples.

Anthropologic analyses of the microhap data on these 96 populations show that up to 10 biogeographic clusters can be defined. Because they are multiallelic and many loci can be multiplexed, microhaps are also excellent at quantifying biologic relationships without worrying about high mutation rates. These MPS analyses allow the unequivocal conclusion that these loci are of great value for forensic questions that cannot be answered using just the CODIS STRP loci. The very large number of microhap loci characterized on at least 83 populations from around the world provides a resource for sets of markers targeted to specific questions and circumstances, such as highly degraded DNA. Definitions and allelic data of the published loci are accessible in ALFRED (https://alfred.med.yale.edu; supported by NIJ 2016-DN-BX-0162) under the keyword *microhap*; the newly characterized data are being added to the database.

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Production of High-Fidelity Electropherograms Results in Improved and Consistent Match-Statistics: Standardizing Forensic Validation by Coupling Laboratory Data with an In Silico DNA Pipeline

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Samples containing low-copy or complex DNA mixtures are routinely encountered in operations. The signals acquired from these sample types are difficult to interpret because they do not always contain all of the genotypic information from each contributor, and the loss of genetic information is associated with sampling and detection effects. This study developed a validation scheme to help mitigate the effects of the loss of genetic information. We developed a process by which high-fidelity electropherograms (EPGs) are produced consistently, improving interpretation by all probabilistic genotyping systems.

We developed a systematic approach to forensic DNA signal optimization and validation. Specifically, we developed ReSOLVIt (Resolving Signal for Objective Laboratory Validation), a simulation-based tool that allows for quick evaluation of multifarious laboratory scenarios, illustrated how the careful evaluation of a single-copy DNA signal from multiple scenarios provides a mechanism by which to choose laboratory conditions that consistently lead to a high-fidelity signal, and demonstrated that optimized conditions lead to improved and consistent likelihood ratios for true contributors and noncontributors.

We devised ReSOLVIt, which generates synthetic EPGs in a laboratory-specific manner, using a large number of single-source profiles of known genotype provided by the laboratory. From these data, we modeled the distribution of the peak heights at noise positions as a function of the starting template amount using a log-normal distribution. We also acquired the electrophoresis sensitivity, which is used to generate the DNA height distribution, from the single-source experimental data procured from the laboratory. Other pertinent laboratory conditions—such as the number of polymerase chain reaction cycles, injection time, and starting template mass, for instance—are input parameters and are easily modified by the user.

Because ReSOLVIt uses a simulation approach based on experimental data acquired from the laboratory, each laboratory can explore multifarious scenarios cost-effectively. Metrics such as signal copy-to-noise resolution and false-positive and false-negative signal detection rates are used to select tenable laboratory conditions that result in a high-fidelity signal in the single-copy regime. We demonstrate that the metrics acquired from simulation are consistent with experimental data obtained from two capillary electrophoresis platforms and various injection parameters. Once good resolution is obtained, analytical thresholds can be determined using detection error trade-off analysis, if necessary.

Decreasing the limit of detection of the forensic process to one copy of DNA is a powerful mechanism by which to increase the information content on alleles from minor components of a mixture, which is particularly important for

probabilistic system inference. By using another fully continuous probabilistic system, CEESIt (Computational Evaluation of Evidentiary Signal), we demonstrate that if the forensic pipeline is engineered to produce a high-fidelity EPG signal, then the likelihood ratio (LR) of a true contributor increases and the probability that the LR of a randomly chosen person is greater than one decreases. CEESIt has been developed to not only compute the LR but also the probability that the LR is greater than one for millions of randomly chosen contributors, making it a powerful validation tool. This systematic, in silico, laboratory-specific, computational-based approach to improve allele information content is potentially the first step toward standardizing the bio-analytical pipeline and DNA validation process across operational laboratories.

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