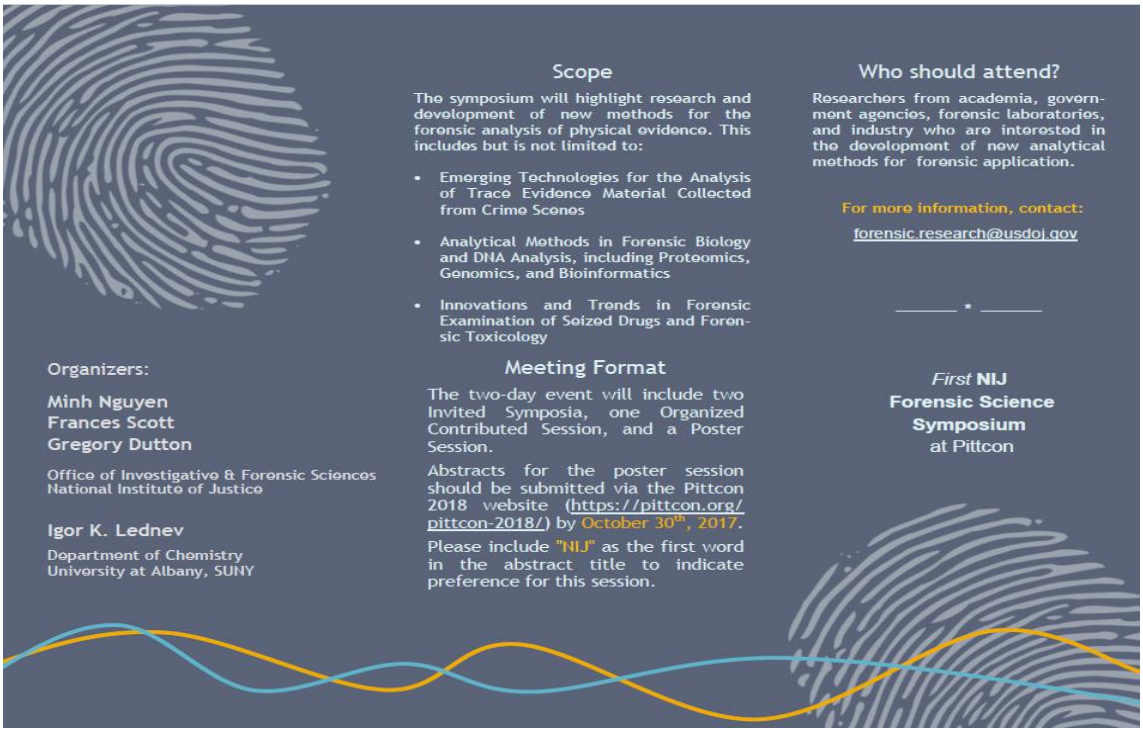
REPORT

First National Institute of Justice

Forensic Science Symposium at Pittcon (NIJ-FSS)

February 26 –March 1, 2018

Orlando, FL



Contents

INTRODUCTION

History of Pittcon

Pittcon is a conference and exposition organized by The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy since 1950. The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy is a non-profit educational organization based in Pennsylvania. Proceeds from Pittcon fund science education and outreach at all levels kindergarten through adult. These include science equipment grants, research grants, scholarships and internships for students, awards to teachers and professors, and grants to public science centers, libraries, and museums. Forensic sessions have been included in the Pittcon

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

technical program since its founding 68 years ago, while Homeland security was added in 2011. Pittcon has an attendance of over 11,000 every year from over 80 countries.

NIJ-FSS

Research and development in forensic science has significantly increased in the United States and the rest of the world during the last decade. The National Institute of Justice Forensic Science Symposium at Pittcon (NIJ-FSS) is a two-day event designed to draw the attention of the wider scientific community to forensic science research. NIJ-FSS is a major annual research and development platform for researchers from academia, government agencies, forensic laboratories, and industry who are interested in the development of new analytical methods for forensic application. This first NIJ-FSS in Orlando was organized by Minh Nguyen, Gregory Dutton, and Frances Scott from the NIJ as well as Igor Lednev from the University at Albany, SUNY.

The NIJ-FSS consisted of two invited symposia, one organized contributed session and a poster session. A total of 18 invited talks and 71 posters were presented during the two-day event. The invited talks given by the leaders in the highlighted subfields of forensic science encompassed a wide range of topics. Presentations were organized into three sessions entitled "Emerging Technologies for the Analysis of Trace Evidence Materials Collected from Crime Scenes", "Analytical Methods in Forensic Biology and DNA Analysis – Proteomics, Genomics, and Bioinformatics" and "Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology." The first two symposia were on Wednesday February 28th from 8:30 to 11:45 and 1:30 to 4:25 respectively with the organized contributed session from 8:30 to 11:45 am on Thursday March 1st. The poster session was from 1:00 to 3:00 in the afternoon on March 1st.

NIJ and Pittcon provided financial support for speakers as well as for students presenting at the poster session. All reasonable expenses of speakers in the invited symposia were covered by Pittcon. Organized contributed session speaker travel was supported by NIJ.

NIJ-FSS was well advertised before and during the conference. Pittcon promoted the technical program using various outlets, including Facebook, Twitter, LinkedIn, Google+, Instagram and Pinterest. A one-hour promotional webinar sponsored by Pittcon was given by one of the NIJ-FSS speakers with 323 people from 53 countries registered. Several speakers from the NIJ-FSS sessions were chosen as Featured Symposia Speakers to be highlighted on the conference website. One speaker was also interviewed by Pittcon TV; the video was widely broadcasted during the conference.

FUTURE MEETINGS

The Second NIJ-FSS is planned to be organized at Pittcon 2019 in Philadelphia, PA, March 17-21. The possibility of further expanding the symposium program for next year has already been discussed.

Schedule

Wednesday Symposia

NIJ - Emerging Technologies for the Analysis of Trace Evidence Materials Collected from Crime Scenes

8:30 AM Introductory Remarks

8:35 AM **Dutton, Gregory** - Forensic Science Research and Development Funding Program at the National Institute of Justice: Opportunities in Analytical Chemistry, Applied Spectroscopy and Bioanalysis

9:10 AM **Dorrestein, Pieter** - Retracing Lifestyles of People from Objects Using Mass Spectrometry

9:45 AM **Gilbert, Jack** - The Burglary Microbiome Project:Detecting Personal Microbiome Signatures at Artificial Crime Scenes

10:20 AM Recess

10:35 AM **Lilien, Ryan** - 3D Surface Topography Analysis and Virtual Microscopy for Firearm Forensics

11:10 AM **Lednev, Igor** - Vibrational Spectroscopyand Advanced Statistics for Detection and Characterization of Gunshot Residue

NIJ - Analytical Methods in Forensic Biology and DNA Analysis – Proteomics, Genomics, and Bioinformatics

1:30 PM Introductory Remarks -

1:35 PM **Siegel, Donald** - NIJ Research – Human Identification by Single Amino Acid Polymorphisms Using Proteomic Mass Spectrometry

2:10 PM **McCord, Bruce** - Forensic Epigenetics, A Novel Method for Body Fluid Identification and Phenotyping

2:45 PM **Shin, Giwon** - Population Haplotype Analysis of 2,543 STRs and their Flanking SNPs Using a Massively Parallel Next-Generation Sequencing Technology

3:20 PM Recess

3:35 PM **Zaaijer, Sophie** - Democratizing DNA Fingerprinting

4:10 PM **Budowle, Bruce** - The Research and Development Progress of Enhancing Mixture Interpretation with Highly Informative STRs

Thursday

NIJ- Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology

8:30 AM **Yu, Jorn Chi Chung** – Application of Headspace Solid Phase Micro Extraction in Chemical Forensics

8:50 AM **Musa, Rabi Ann** – Forensic Identification of Plant-based 'Legal Highs' by Chemometric Processing of Direct Analysis in Real Time Mass Spectrometry (DART-MS)-derived Chemical Fingerprints

9:10 AM **Smith, Ruth** – Statistical and Mass Spectral Tools for the Identification and Characterization of Synthetic Phenethylamines

9:30 AM **Lurie, Ira** – Decreasing the Uncertainty of Peak Assignments for the Chromatographic Separation of Emerging Drugs

9:50 AM Recess

10:05 AM **Peace, Michelle R**. – Evaluation of Drugs Other than Nicotine (DOTNs) in an Aerosol Formed by an Electronic Cigarette

10:25 AM **Gilliland Richard A.** – Analysis of Drug-Protein Modifications in Forensic Toxicology

10:45 AM **Xiao, Yi** – Aptamer-based Assays for On-site Drug Detection

11:05 AM **Manicke, Nicholas** – Post-Mortem Drug Screening Using Paper Spray High Resolution Tandem Mass Spectrometry

NIJ Forensic Science Research & Development Poster Session

ABSTRACTS

Abstracts submitted for the three oral symposia and the poster session are presented here. The presenting author is indicated in bold type. Abstracts are separated into oral and poster presentation, with the oral presentations further divided into the three different sessions.

Oral presentations

Emerging Technologies for the Analysis of Trace Evidence Materials Collected from Crime Scenes Session

Gregory Dutton, Minh Nguyen
National Institute of Justice

Forensic Science Research and Development Funding Program at the National Institute of Justice: Opportunities in Analytical Chemistry, Applied Spectroscopy and Bioanalysis

The National Institute of Justice (NIJ) is the research, development and evaluation agency of the U.S. Department of Justice. NIJ's Office of Investigative and Forensic Sciences maintains a program of external funding for R&D in forensic sciences. This program is a leading federal funder in this mission space, and the portfolio spans a broad range, from fundamental research, to development of prototype devices, to validation of novel instruments and methods. Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of biological and chemical samples. Because of the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded or mixed samples. Balancing that is the need to ensure that analytical methods applied to these challenging samples are objective, rigorously tested, and foundationally valid. These needs drive NIJ's

continuing R&D investments in analytical chemistry and bioanalytical science. Advances in mass spectrometry, electrophoresis, applied spectroscopies, microscopy and microfluidics, among other analytical techniques, have yielded or show promise for successful application to forensics. NIJ anticipates continued interest in advancing these technologies, as well as emerging analytical methods, for forensic application. In this effort, NIJ strives to engage the analytical chemistry and applied spectroscopy research communities to bring novel perspectives to solving forensic problems. An overview of NIJ's research and development portfolio will be presented, highlighting funding opportunities, including the anticipated plans for the FY2018 Research and Development in Forensic Science for Criminal Justice Purposes solicitation and examples of past funded projects in areas relevant to the analysis of trace evidence materials.

Pieter Dorrestein
University of California, San Diego
Retracing Lifestyles of People from Objects Using Mass Spectrometry
You are an investigator that has recovered an expensive artifact to the rightful owner or you are faced with a crime scene and want to retrace what has transpired, how to do this? Basically one collects as many pieces of evidence as possible and then reconstruct the scene or rightful owner of the object based on these clues. Objects or rooms contain many clues about how they are used and the lifestyle characteristics (e.g. food preferences, personal care preferences, medications, etc.) of the people that use them. The vast majority of these clues (I am fairly sure >99.99%) remain hidden and are therefore not used by investigators. In this presentation we will highlight how mass spectrometry, combined with molecular networking, creating reference data sets that reflect lifestyle characteristics and 3D cartography can begin to reveal such lifestyles but also how such lifestyle characteristics can be used to 'reconstruct' the typical use within a building - a step toward crime scene reconstruction from chemical clues. Finally we will discuss why they are not routinely used and what it will take to get these capabilities into real life applications from a community and chemoinformatic and computational standpoint.

Jack Gilbert
University of Chicago
The Burglary Microbiome Project: Detecting Personal Microbiome Signatures at Artificial Crime Scenes
The composition of microbial organisms associated with skin is unique to an individual. This is because the experiences each person has since birth are unique, and it is these physical interactions with the world that allow microbes to colonize and form communities ('microbiomes') on our bodies. Even identical twins, whose microbiota are significantly more similar than other siblings, each have a unique profile. While, an individual's core microbiome is considered stable by the age of 2-3, it can still undergo variation as we change aspects of our lifestyle that cause us to be exposed to different microbial worlds. The skin microbiome is our primary interface with the world and the interface we most readily leave behind when we interact with a space. To date, the evidence to support this has been limited by small studies and anecdotal enquiry. We are performing a systematic analysis of a human population around Miami, FL, to determine categorically whether elements of their lives can be predicted from their microbiome, both on their bodies, and that left behind on surfaces they interact with. In doing so, we will create a list of highly specific microbial biomarkers for particular traits (e.g. young adult female vegetarian, who lives in the suburbs and works in a bakery or bread counter). We will also build a sophisticated artificial neural network and database to enable extrapolation of microbial signature detection to other samples, so that a person's traits can be detected from the microbial community they leave behind. This proof of principle study will form the foundation of a forensic effort in Miami to create a new suite of trace evidence options that can be leveraged by investigators to help shape their interpretation of a crime scene.

Ryan Lilien
Cadre Research
3D Surface Topography Analysis and Virtual Microscopy for Firearm Forensics
The discipline of tool mark analysis is based on the observed phenomenon that through force and contact individual tools can transfer tool-specific marks to a target object. In the case of firearm forensics it is the firearm which leaves unique marks on cartridge cases and bullets. Microscopic examination of these marks allows firearm examiners to assess the likelihood of common origin (e.g. linking a cartridge case found at a crime scene to a test fire from a suspect's firearm). Several 3D scanning technologies, including our recently developed TopMatch scanner, are capable of measuring a high-resolution 3D surface topography in standard units. These topographies represent a one-to-one geometric mapping between the scanned digital surface and the actual physical surface. Virtual Microscopy, or the examination of digital representations of objects rather than the physical objects themselves, offers many novel use cases to the forensic examiner. These include new abilities in remote data sharing/collaboration, visualization, annotations, verification, and proficiency testing. In support of Virtual Microscopy, we developed specialized viewing software, ran a training workshop at a national meeting, and conducted a blind validation study. Each participant utilized the same computer setup loaded with a training tutorial and two virtual CTS-like proficiency tests. Unlike a traditional physical proficiency test, Virtual Microscopy has no inter-operator test set variability. Participants were asked not only to complete a standard results worksheet but also to use the software to indicate regions of tool mark similarity via a 'paintbrush-like' feature in the software. In this presentation, we will share both details of our work in Virtual Microscopy as well as study results. Our work demonstrates the feasibility of the method and represents an important step towards validating this new technology for use in casework and proficiency testing.

Igor Lednev
University at Albany, SUNY
Vibrational Spectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue
Vibrational spectroscopy including Raman microscopy and FTIR has numerous applications in modern forensic chemistry. Vibrational spectroscopic characterization allows for confirmatory class identification of analytes through its high specificity towards molecular structure and composition. The technique is non-destructive, rapid, sensitive and requires little or no sample preparation. Furthermore, portable Raman and FTIR spectrometers are readily available, allowing for crime scene accessibility. Vibrational spectroscopy offers several advantages over the current methodology for GSR analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape. This is contrary to current GSR elemental analysis methods which rely solely on the detection of the heavy metals (lead, barium and antimony). This is problematic since environmental concerns have led to the increased popularity in heavy metal free or 'green' ammunition. The firearm discharge process could be considered analogous to a complex chemical reaction. Therefore, the chemical composition of the products (GSR particles) is directly related to the chemical nature of the reagents (firearm-ammunition combination) and the conditions of the reaction. Preliminary results show that Raman and FTIR data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified according to caliber. This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

Analytical Methods in Forensic Biology and DNA Analysis – Proteomics, Genomics, and Bioinformatics Session

Donald Siegel[1], Erin Butler[1], Emmanuel Chang[2], Adam Essene[1], David Fenyo[3], Samantha Monier[1], Amy Mundorff[4], Tatiana Perez[1], Heyi Yang[1]
New York City Office of Chief Medical Examiner[1], York College CUNY[2], New York University[3], University of Tennessee, Knoxville[4]
NIJ Research – Human Identification by Single Amino Acid Polymorphisms Using Proteomic Mass Spectrometry
The identification of fragmentary and/or commingled human remains following a mass disaster or discovery of a mixed grave is an immediate obligation of the forensic community, not only to decedents' families, but also to aid investigators and document evidence for the criminal justice system. Such incidents as airline crashes, infrastructure failures, industry explosions and natural catastrophes can result in large numbers of fragmentary human remains spread over large areas of land or water. These remains may be burned, contaminated with caustic or other substances (e.g. jet fuel and industrial chemicals), or subject to natural taphonomic processes that destroy tissue or inhibit the DNA polymerases that are used in DNA identity testing. Proteins, which are more abundant and less sensitive to degradation than DNA, offer an alternative bio-identity molecule when DNA is degraded or polymerases inhibited. Recent advances in mass spectrometry instrumentation make it faster, simpler, more accurate and more sensitive than earlier instruments. Similar advances have occurred in the power of bioinformatics to rapidly search and recover annotated protein information from ever increasing databases. These advances have propelled proteomics to the forefront of basic research and pharmaceutical discovery. These same mass spectrometry tools are directly applicable to the forensic identification of individuals. Here we demonstrate the use of proteomic mass spectrometry and bioinformatics for human identification. Using tissue samples form 14 individuals from the University of Tennessee Forensic Anthropology Research Facility (IRB approved) we have identified 13 muscle protein polymorphisms, from among hundreds of proteins, that are present at frequencies greater >5% and can be used for individual identification. All polymorphisms were confirmed by DNA sequencing. Advantages and challenges of human identification through proteomic mass spectrometry will be discussed.

Bruce McCord[1]**,** Hussain Alghamin[1], Joana Antunes[1], Kuppareddi Balamurugan[2], George Duncan[3], Deborah Silva[1]
Florida International University[1], The University of Southern Mississippi[2], Nova Southeastern University[3]
Forensic Epigenetics, A Novel Method for Body Fluid Identification and Phenotyping
We have been investigating the potential of epigenetic methylation as a procedure for the identification of body fluids and age at crime scenes. Epigenetic methylation generally appears in the form of methylated cytosines in the genome. These modified bases are typically present in the form of clusters of CpG motifs upstream from gene transcription sites where they are used to control gene expression. Diagnostic epigenetic loci are typically located using whole genome array studies which are then followed by quantitative pyrosequencing. Because methylation information is lost following the PCR, extracted samples are subjected to bisulfite modification which converts unmethlated cytosines into thymines following PCR. Pyrosequencing is next used to provide a quantitative estimate of the level or percent methylation at each CpG site. In addition we have explored the use of real time PCR with high resolution melting capability for bulk analysis of these sites. There are a number of advantages of using epigenetic loci in body fluid identification. These include the fact that samples can be extracted using standard procedures. Because the methylation involves covalent bonding, the method is very stable, permitting analysis of samples over 20 years of age. The procedure is also human specific and resistant to inhibition. Furthermore, epigenetic loci

may also be used reveal phenotypic information such as age and behavior. This occurs due to the fact that certain CPG loci can be influenced by random or environmental processes, resulting in correlations with age or other behavior such as smoking. In this presentation we will discuss our development of epigenetic loci for body fluid and age determination. This will include our work in the analysis of methyl array data, pyrosequencing, real time PCR, and the initial validation of these procedures.

Giwon Shin[1], Susan M. Grimes[2], Hanlee P. Ji[1], Matthew Kubit[1], Billy T. Lau[2], Hojoon Lee[1]
Stanford University School of Medicine[1], Stanford University[2]
Population Haplotype Analysis of 2,543 STRs and their Flanking SNPs Using a Massively Parallel Next-Generation Sequencing Technology

Short tandem repeats (STRs) have a higher mutation rate than single nucleotide polymorphisms (SNPs). STR genotyping is highly informative for genetic applications such as DNA fingerprinting. However, there is little informative genetic analysis of the haplotype structure of STRs and SNPs. This knowledge gap mainly due to the absence of high-throughput technology for these loci. Despite their wide application, the analysis of STRs with next generation sequencing (NGS) methods is limited by several major issues including: i) Only the reads encompassing an entire STR locus are informative; ii) PCR amplification during library preparation can introduce artificial 'stutter' mutations that confound accurate genotyping. Consequently, analysis on STRs requires more sequencing depth with finely controlled target selection, but current methods such as bait-hybridization are of limited utility. Here, we developed a novel targeted sequencing technology (STR-Seq), which can simultaneously genotype thousands of STR loci and phase proximal SNPs with significantly higher accuracy than all other methods. STR-Seq uses single-molecule sequencing in combination with targeted in vitro CRISPR-Cas9 fragmentation. In this study, we evaluated 2,543 STR loci from a population of 1,004 individuals. Our analysis included 436 Marshfield loci, which has been used to characterize the population sample, and an additional 1,915 loci where a proximal SNP was positioned within 100bp of the STR. Overall, we identified approximately 2,000 STR genotypes and 1,000 STR-SNP haplotypes per individual. STR-SNP linkage was extremely low (mean r{2} < 0.1). We discovered a new class of STRs which are highly polymorphic as noted by having 20 or more alleles. Finally, we identified a significant number of STR-SNP haplotypes and describe the geographical and population differences of these previously undescribed, novel genetic markers.

Sophie Zaaijer[1], Yaniv Erlich[2]
New York Genome Center and Cornell Tech[1], New York Genome Center and Columbia University[2]
Democratizing DNA Fingerprinting

DNA re-identification is used for a broad suite of applications, ranging from cell line and tissue authentication to crime scene sample identification. However, current re-identification schemes suffer from high latency. We developed a rapid, and portable strategy to re-identify human DNA, called 'MinION sketching'. Using data from Oxford Nanopore Technologies' sequencer, MinION sketching requires only 3min of sequencing and 60-300 random SNPs to identify a sample, enabling near real-time applications of DNA re-identification. Hands-on preparation of the samples can be reduced to <1 hour. We re-identify individuals using sparse reference files as generated by Direct-to-Consumer companies. Our method potentiates application of MinION sketching for border control, on-site crime scene re-identification of DNA samples and rapid identification of victims after a mass disaster.

Bruce Budowle, Jonathan L. King, Nicole M. Novroski, Maiko Takahashi, Frank R. Wendt, August E. Woerner
University of North Texas Health Science Center

The Research and Development Progress of Enhancing Mixture Interpretation with Highly Informative STRs

The evaluation and interpretation of forensic DNA mixture evidence faces greater interpretational challenges due to increasingly complex mixture evidence. Even with an increased number of CODIS core markers, mixture interpretation is challenging. Massively parallel sequencing (MPS) is a technology that can facilitate mixture interpretation and simplify some complex mixtures. The value of MPS is its ability to provide sequence data in addition to the nominal length of STR alleles. This project focuses on defining current STR marker variation, identifying novel STRs that may be better suited for mixture deconvolution, and development of software tools to facilitate allele calling of STRs typed by MPS. The repeat and flanking regions of 27 autosomal, 7 X-chromosome and 24 Y-chromosome STR markers were characterized in 777 unrelated individuals from four population groups. Additionally novel STRs are being mined from public databases that may facilitate mixture deconvolution. To be able to identify sequence variation within an amplicon software needed to be developed. STRait Razor is a bioinformatics suite used to identify and characterize sequence and length-based polymorphisms in MPS data. STRait Razor consists of two major components: The STRait Razor perl script and the Strait Razor Excel workbook collate, annotate and visualize haplotypes. The latest version provides both a stable code-base that operates on all major operating systems including Microsoft Windows and an indexing strategy tailored to the identification of sequence variants based on anchor sequences. The newest version is 660 times faster than previous versions and maintains all the features as the previous versions.

Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology Session

Jorn Chi Chung Yu, Frank Liu, Austin McDaniel
Sam Houston State University

Application of Headspace Solid Phase Micro Extraction in Chemical Forensics

Chemical forensics has been recently recognized as a new discipline that aims to obtain information from chemical remnants that is relevant to investigative, legal and intelligence questions. In our research work, the application of heated headspace solid phase micro extraction (HHS-SPME) in chemical forensics of marijuana was investigated. Marijuana is currently a Schedule I controlled substance under the federal perspective. Recent changes in some state legislation of marijuana is shifting the forensic task from chemical analysis of marijuana into the determination of marijuana varieties. Legal marijuana may be diverted from its intended use. Regardless the legal status of marijuana in the United States, there is a need to develop an effective and reliable analytical platform and database to determine the variety or source of marijuana evidence for the purpose of law enforcement and forensic intelligence. In this work, headspace chemical signature of standard marijuana samples was extracted by automated HHS-SPME. The extract was injected to a gas chromatography-mass spectrometry (GC-MS) for separation and analysis. The obtained chemical data from different varieties of marijuana were digitized and processed with ensemble learning algorithm in order to build a learning model for the intelligent classification of marijuana varieties. The successful classification of marijuana varieties using headspace chemical analysis combined with machine learning technique will be demonstrated in this presentation. The overall goal in our research group is to develop a forensic analytical system with advanced machine learning technology that is more efficient, robust which will benefit and strengthen the practice of chemical forensics in controlled substance and trace evidence analysis. Future application of HHS-SPME-GC/MS analytical platform in chemical forensics will also be discussed. This research was funded by the National Institute of Justice (Award #2014-R2-CX-K005).

Rabi Ann Musah, Samira Beyramysoltan
University at Albany, SUNY
Forensic Identification of Plant-based "Legal Highs" by Chemometric Processing of Direct Analysis in Real Time Mass Spectrometry (DART-MS)-derived Chemical Fingerprints

The past five years have witnessed a dramatic increase in the abuse of currently unscheduled plant-based psychotropics (PBPs). It continues to be impractical for crime labs to develop standard operating protocols for the myriad of new products that continually appear. We demonstrate here how DART-TOF-MS permits rapid identification of PBP evidence in its native form. Roots, seeds, and aerial plant parts can all be tested directly without the need for the customary sample extraction and other time consuming preparation steps. A single analysis enabled detection of a broad range of both polar and non-polar molecules. In addition, readily identifiable diagnostic chemotaxonomic markers were observed in each case. These included mitragynine and 7-hydroxymitragynine in Kratom, kavain, yangonin, methysticin and dehydrokavain in kava powder, and salvinorins A and B and divinatorin A in Diviner's sage, among several others. The appearance of these compounds in supplements and other products was also useful in identifying the plants from which supplement forms of plant-based legal highs were derived. Multivariate statistical analysis processing of the DART-TOF-MS data by various approaches including principle component analysis (PCA), linear discriminant analysis, and partial least squares discriminant analysis (PLSDA) showed that the various species were readily distinguishable based on their mass spectral fingerprints, and well-defined species-specific clustering was observed. Furthermore, testing of the classification systems with plant material unknowns resulted in correct identifications in all cases. The speed of analysis using this ambient ionization technique dramatically reduces overall analysis time, which makes it practical to quickly generate the large data sets required for the successful application of multivariate statistical analysis to the development of databases of abused substances that can be used for forensic identification and characterization.

Ruth Smith[1], David E. Alonso[2], Alexandria Anstett[1], Fanny Chu[3], Victoria L McGuffin[1]
Michigan State University[1], LECO Corporation[2], Lawrence Livermore National Laboratory[3]
Statistical and Mass Spectral Tools for the Identification and Characterization of Synthetic Phenethylamines

Identification of synthetic designer drugs continues to pose challenges in forensic laboratories due to the high structural similarity among compounds in a given class and the rapid emergence of new analogs. Controlled substance identification is based on comparison of the gas chromatographic retention time and mass spectrum to that of a reference material. However, this visual comparison provides no statistical assessment of the veracity of the identification. Further, identification of new analogs is especially problematic as no reference material is available for comparison. This presentation will describe statistical and mass spectral tools developed to aid in the identification and characterization of designer drugs. The work focused on three structural subclasses of the synthetic phenethylamines (APB-, 2C-, and NBOMe-phenethylamines), a set of which was analyzed by GC-MS. A statistical method based on the unequal variance t-test was developed for the comparison of two mass spectra. Discrimination between compounds within each phenethylamine subclass was achieved at the 99.9% confidence level, except for three NBOMe-phenethylamines which were discriminated at the 99% confidence level. Characterization schemes were also developed to characterize compounds according to phenethylamine subclass based on mass spectral characteristics. The first scheme, based on low-resolution mass spectral data, used characteristic neutral losses to indicate the structural subclass and isotope ratios to determine the substituent. The second scheme, based on high-resolution time-of-flight mass spectral data, additionally incorporated mass defect filters to

increase specificity in subclass characterization. The application of these tools for identification and characterization of synthetic phenethylamines will be demonstrated throughout this presentation.

Ira Lurie
The George Washington University
Decreasing the Uncertainty of Peak Assignments for the Chromatographic Separation of Emerging Drugs
Emerging drugs are synthetically produced alterations of the controlled drugs in order to circumvent laws banning their use as recreational drugs. The identification of existing and new emerging drugs is complicated by the similarity in structure, lack of molecular ions for certain solutes and/ or similarity in mass spectral (MS) spectra for diastereomers and positional isomers, and incomplete chromatographic separations. In order to increase the specificity of analysis emerging technologies such as ultra-high performance supercritical fluid chromatography (UHPSFC) with MS and ultraviolet (UV) detection, gas chromatography (GC) with vacuum ultra violet (VUV) detection, and multi-dimensional ultra-high performance liquid chromatography (UHPLC) will be discussed.UHPSFC which provides complimentary separations to both GC and UHPLC is particularly advantageous for the separation of positional isomers and diastereomers. Peak assignments for the former technique (similar to UHPLC) are aided by the following: MS detection, which generates molecular ions, and UV detection, which can distinguish between classes and subclasses of emerging drugs and differentiate between many positional isomers. For UHPSFC versus UHPLC there was at least a 10 nm blue shift in UV maximum. GC with VUV detection (125-240 nm wavelength intervals) can distinguish most positional isomers. The use of multi-dimensional UHPLC, which provides for significantly increased peak capacity over a one dimensional separation, substantially increases the likelihood of the correct identification of an emerging drug by decreasing the uncertainty of peak assignments using retention time. This project was supported by Awards numbers 2014-R2-CX-K009 and 2016-DN-BX-0169 awarded by the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. In addition the Perkin Elmer Corporation provided financial support for our laboratory through GWU proposal no. 13-04142.

Michelle Peace, Matthew S. Halquist, Haley A. Mulder, Alphonse Poklis, Justin L. Poklis, Joseph Turner
Virginia Commonwealth University
Evaluation of Drugs Other Than Nicotine (DOTNs) in an Aerosol Formed by an Electronic Cigarette
E-cigarettes are an alternate nicotine delivery system. An aerosol is formed when the e-liquid passes over a heated coil, is vaporized, and is then condensed with water in the atmosphere. The size of the droplets formed in the aerosol can vary and the size of the particles contributes to the location where the particles will deposit in the lung. The growing popularity of these products has caused an increase in internet sources promoting the use of drugs other than nicotine (DOTNs) in e-cigarettes. DOTNs include natural and plant-based products, designer drugs, traditional and non-traditional pharmaceuticals. The purpose of this study was to compare the aerosol formed by methamphetamine and methadone to the aerosol formed by nicotine in an e-cigarette. Methadone and methamphetamine were prepared at 60 mg/mL in 50:50 PG:VG e-liquid formulations and were aerosolized for 10 seconds into a 10-stage Micro-Orifice Uniform Deposit ImpactorTM (MOUDI) at a flow rate of 30 L/min. Each stage of the MOUDI represented a different particle size range, from 0.05-18 µm. The aerosol particle sizes for each e-liquid were determined gravimetrically by weighing the stages before and after aerosolization. This experiment was performed at 3.9, 4.3, and 4.7 V on the e-cigarette. The percent of recovered e-liquid was determined for each stage. Stages 8 and 9, representing particle size ranges of 0.31-0.172 µm and 0.172-0.1 µm, consistently collected approximately 90% of the aerosol. Additionally, all 3 voltages produced ultrafine particle sizes, <0.1 µm. Compared to the aerosol of nicotine in an e-cigarette, the e-liquids containing methamphetamine and methadone produced more ultra-fine particles <0.3 µm and had a significantly smaller mean diameter than nicotine. This suggests

that the particles of methadone and methamphetamine produced by an e-cigarette are smaller than the particles of nicotine and are can be efficiently deposited into the lung for absorption into the bloodstream.

Anthony DeCaprio, Richard A. Gilliland
Florida International University
Analysis of Drug-Protein Modifications in Forensic Toxicology
A promising technology for retrospective monitoring of drug exposure in forensic casework involves measurement of covalent modification of free thiol moieties of blood proteins, such as hemoglobin (Hb), by reactive metabolites (RM) of drugs. Since they typically persist for the life of the protein, such protein 'adducts' can provide a much longer window of detection of exposure than is generally possible by direct measurement of parent compound or metabolite. We have previously demonstrated covalent adduction of glutathione and a synthetic thiol peptide in an in vitro human liver microsome (HLM) based assay by 16 drugs with abuse potential, including methamphetamine, morphine, diazepam, Δ {9}-THC, by means of LC-QqQ-MS and LC-QTOF-MS. Current work involves assessment of covalent modification of the reactive {93}Cys thiol of human β Hb by the same drugs as part of the development of a validated assay for retrospective drug exposure. The in vitro assay procedure was modified to facilitate recovery of adducted Hb by utilizing a dialysis membrane to maintain separation of Hb from microsomal components, while allowing for RM to pass through. For the metabolism/adduction assay, each drug was added to a plastic microfuge tube with residual solvent removed via vacuum centrifuge. HLM were added to the tube and combined with NADPH in sodium phosphate buffer (pH 7.4). Hb was then added and tube incubated at 37°C for 18 h and then centrifuged. Analysis of modified protein was performed using positive ESI on an Agilent 6530 QTOF-MS. Data were collected using full MS scan mode to allow for necessary analysis of all protein components. Both whole protein and tryptic peptide analyses to detect modified Hb were conducted. Preliminary results confirm the formation of adducted protein by reactive abused drug metabolites in the in vitro system and provide additional proof of concept for continued development of the assay.

Yi Xiao
Florida International University
Aptamer-based Assays for On-site Drug Detection
On-site detection methods are very important for the screening of drugs of abuse. Chemical spot tests suffer from frequent false-positives and false-negatives, while immunoassays are expensive and have short shelf-lives. These problems can be overcome using aptamers, oligonucleotide-based bio-affinity elements, isolated in vitro via SELEX. Recently, our lab has made several successful attempts to address these problems for aptamer-based on-site drug detection. We first engineered a new cocaine-binding aptamer that exhibited higher target binding affinity than the originally isolated cocaine-binding aptamer. Using this aptamer, we developed a label-free fluorescence assay based on target-dye displacement for sensitive cocaine detection, with a detection limit as low as 200 nM within seconds. We further discovered the specific inhibition of Exo III by the formation of a target-aptamer complex, and employed this feature to develop a label-free fluorescence assay. To achieve ultra-sensitive detection of drugs in biofluids, we developed a general approach to engineer cooperative-binding split aptamers (CBSAs) that exhibit highly responsive target-induced aptamer assembly for signal reporting. We employed the CBSA to achieve specific, sensitive, one-step fluorescence detection of cocaine within 15 min at concentrations as low as 50 nM in 10% saliva. Additionally, we developed an instrument-free colorimetric assay employing enzyme-assisted-target-recycling-mediated aggregation of CBSA-modified gold nanoparticles for visual detection of low micromolar cocaine within 15 minutes. Recently, we have adopted our sensor designs into aptamer isolation and have generated new aptamers that bind to a class of designer drugs. The isolated aptamer can

be directly adopted into our dye-displacement or CBSA-based sensing platforms. We believe by integrating aptamer isolation with sensor design, robust aptamer-based assays can be generated rapidly for on-site detection of any drug of interest.

Nicholas Manicke, Josiah McKenna
Indiana University - Purdue University Indianapolis
Post-Mortem Drug Screening Using Paper Spray High Resolution Tandem Mass Spectrometry
There is a need for developing simpler screening methods for drugs in post-mortem samples. Paper spray mass spectrometry is a direct or ambient ionization method in which dried biofluids spots are analyzed directly from paper. Analysis is carried out on dried blood spots directly without sample preparation; total analysis time is about 2 minutes. We have developed a drug screening method for about 140 of the most commonly encountered drugs and drug metabolites in forensic toxicology. In this presentation, we will present the figures of merit, including detection limits, linearity, and selectivity. Whole blood samples were analyzed by first spotting them on chromatography paper contained within a disposable plastic cartridge. A variety of extraction solvents were investigated; the final solvent was 85-10-5-0.01 acetonitrile-acetone-water-acetic acid. Analysis was carried out using an automated paper spray front-end interfaced with a Q-Exactive Focus in MS/MS mode using an inclusion list. Detection of the drugs was based off of the presence of typically two fragment ions (the quantifier and qualifier) within a 5 ppm m/z window. Fragment ion ratios can be used to decrease the potential for interferences; we elected not to use ion ratios to minimize the chance of false negatives. An important contribution of this work is the application of paper spray MS to actual post-mortem samples. We analyzed 30 post-mortem blood samples and compared paper spray MS/MS results against an independent HPLC-MS/MS confirmatory assay. Paper spray showed good sensitivity and selectivity compared with the HPLC-MS/MS assay; the true positive rate of paper spray MS/MS was 92.8% and the true negative rate was over 99%. The results indicate that this approach has good potential as a rapid drug screening method in post-mortem toxicology.

Poster presenters

Younis Abiedalla, Randall C. Clark, Jack DeRuiter
Auburn University
GC-MS, GC-MS/MS and GC-IR Differentiation of Desoxy Cathinone Derivatives: Cyclic Tertiary Amines Related to MDPV
The desoxy phenethylamine analogues in this study represent a combination of alkyl side-chain and cyclic amines (azetidine, pyrrolidine, piperidine and azepane) to yield a set of molecules of identical elemental composition as well as major mass spectral fragment ions (base peaks) of identical elemental composition. These desoxy phenethylamine analogues of the aminoketone designer drug, 3,4-methylenedioxy-pyrrovalerone (MDPV) related to the natural product cathinone were prepared from piperonal (3,4-methylenedioxybenzaldehyde) via the intermediate precursor ketones. The aminoketones and the desoxy phenethylamine regioisomers were each separated in capillary gas chromatography experiments using an Rxi®-17Sil MS stationary phase with the aminoketones showing greater retention than the corresponding desoxyamines. The electron ionization mass spectra for the aminoketones as well as the desoxy phenethylamines yield equivalent m/z 126 regioisomeric iminium cation base peaks. However, the product ion spectra allow for the differentiation of the m/z 126 iminium cations containing the various ring size cyclic tertiary amines and alkyl side-chain. The product ions from the m/z 126 iminium cation produced by the desoxy phenethylamines containing the azetidine, pyrrolidine, piperidine and azepane cyclic amines occur at m/z 70, 84, 98 and 72, respectively. The vapor phase infrared spectra for these desoxy phenethylamines show doublet absorption bands at 1489 cm-1 and 1442 cm-1 characteristic for the 3,4-

methylenedioxy aromatic ring substitution pattern and the unsymmetrical nature of these doublet absorption bands indicates the lack of a carbonyl group at the benzylic position of the alkyl side-chain.

Alex Reinhart, Joel Greenhouse
Carnegie Mellon University
Point Process Modeling with Spatiotemporal Covariates for Predicting Crime
Crime is known to concentrate into 'hotspots': small areas with locally high crime rates. Crime also exhibits 'near-repeat' behavior, where recent crimes tend to locally increase the rate of crime for few days or weeks, perhaps due to retaliation or repeat offenses. Tools to understand the causes and dynamics of these hotspots are limited and don't include near-repeat behavior, while analysis of near-repeats typically ignores hotspots. We propose a spatio-temporal statistical model which accounts for both spatial and temporal variation in crime by modeling hotspots, near repeats, and leading indicator crimes, and demonstrate its performance on a large dataset of crimes in Pittsburgh, Pennsylvania, showing its use for testing criminological theories and predicting crime.

Nikhil Bose[1], Cassandra Calloway[1], Henry Erlich[1], Rachel Gordon[1], George Sensabaugh[2], Shelly Shih[1], Cassandra Taylor[3]
Children's Hospital Oakland Research Institute[1], University of California, Berkeley[2], University of California, Davis[3]
Mitochondrial Genome and Nuclear SNP Probe Capture/Next-Generation Sequencing System for Analyzing Degraded and Mixed DNA Samples
Forensic biological samples that are degraded, limited, or mixed can often fail conventional STR genotyping. When nuclear DNA is degraded, SNPs can serve as an alternative marker since the target variation is a single base pair. However, conventional PCR based SNP systems are unsuitable for highly degraded samples as PCR primer binding sites may not be intact. If nuclear DNA is limited (such as in telogen hair) mitochondrial DNA (mtDNA) can be analyzed since the mitochondrial genome (mtgenome) occurs in high copy number per cell. However, conventional mtDNA sequencing methods, such as Sanger Sequencing, often fails to detect low level DNA mixtures and heteroplasmy. A novel probe capture/Next-Generation Sequencing (NGS) system can overcome limitations of conventional nuclear SNP and mtDNA analysis methods as fragmented target regions can be recovered for high throughput massively parallel clonal sequencing. We have developed probe capture assays targeting the entire mtgenome and 451 nuclear SNPs for analysis of highly degraded and mixed samples. These assays recovered 100% of the mtgenome and >90% (405) SNPs from highly degraded DNA (50-150 bp). With the mtgenome assay, ~100% coverage was obtained from 4000-year-old bones, and 100% coverage from spent cartridge casings. Telogen hairs with limited DNA yielded 100% mtgenome coverage, and >65% SNPs. Furthermore, the clonal nature of NGS aided in analyzing DNA mixtures as low as 10% with 100% recovery of mitochondrial minor variants and >80% of minor contributor SNPs. Also, mitochondrial germline heteroplasmy as low as 5% was characterized. To improve mtDNA mixture analysis, we developed a phylogenetic probability based software for NGS data and successfully determined contributor haplotypes in 50-50 mixtures and in-silico three-contributor mixtures. Therefore, the probe capture/NGS system has the potential to improve mixture resolution and increase recovery of degraded DNA from forensically relevant DNA samples.

David Cunningham[1], Samantha Josselyn[2]
David Cunningham Consulting[1], Eastern Kentucky University[2]
Analysis of Trace Drugs by Direct Analysis in Real Time (DART) Mass Spectrometry
Analysis of trace amounts of drugs is important in a variety of situations, including forensic casework. Here, a method for the facile, rapid collection of traces of drugs from a variety of porous and non-porous surfaces,

including fabrics, is detailed. A small amount of extraction solvent, including an internal standard, is applied to the fabric surface, followed by application of a patterned absorbent disk which resorbs much of the extraction solvent along with dissolved traces of any drug present. Over half of the extraction solvent is recovered in fifteen seconds from many natural and synthetic fabrics, with weights ranging from 64 to 374 mg inch{-2}, by pressing a half-inch diameter patterned glass fiber membrane disk to the wetted area. The patterned disk is then placed in a standard OpenSpot holder of a Direct Analysis in Real Time (DART) mass spectrometer with a data collection time of one to two minutes. Semi-quantitation of low microgram levels of drugs is achieved by comparison of spectra to those from a standard control disk. DART signal generation from the absorbent disks is much longer lived (>2 minutes) than from commercially available OpenSpot cards (~10 seconds) due to the much larger sample capacity of the glass fiber membrane in comparison to the wire mesh of the OpenSpot cards. In an intermediate precision study with four analysts over four days, the average recovery from 190 µg methamphetamine spiked fabric was 8.6 ± 3.2 % indicating that sub-milligram traces of drugs are reliably extracted and measured from fabric. Complete recovery of traces of drug from fabric is not expected since some of the internal standard solution remains in the fabric and the disk may not be placed exactly over the area that was spiked with the drug.

Christopher Maier[1], Marin A. Pilloud[2], Richard G. Scott[2]
Eckerd College[1], University of Nevada, Reno[2]
Combining Cranial and Dental Data to Improve the Estimation of Ancestry in the Forensic Context
Ancestry estimation is central to the construction of a biological profile in forensic anthropology; however, the Daubert standard of evidence requires that methods used in the forensic sciences be testable and have known rates of error. The methods employed in the analysis of cranial morphoscopic and dental morphological traits largely do not meet that standard. Furthermore, no method exists for producing an ancestry estimate from the combination of different datasets. Cranial and dental traits were examined in a sample of 693 individuals from various ancestry groups representative of U.S. populations. Variables were removed from further analyses if they did not differ significantly among ancestry groups or were highly correlated with other variables. The remaining variables were used to produce classificatory models applicable to the estimation of ancestry. Ancestry was estimated correctly in 67%-84% of cases. In general, models that combined cranial and dental data outperformed models based on a single data source; although, the accuracy gained by combining methods was not statistically significant across all groups. The combined data models showed the most marked improvement in estimating the ancestry of Hispanic individuals, suggesting that the cranium and dentition provide different information with regard to ancestry. The methods used to produce ancestry estimates in this research comply with the Daubert standard of evidence, making them applicable to modern forensic casework. Additionally, the results highlight the improvement to ancestry estimation by combining data from different regions of the skeleton, and the utility of the dentition in forensic estimates of ancestry.

Quentin Gauthier[1], Joana Antunes[1], George Duncan[2], Bruce McCord[1]
Florida International University[1], Broward County Sheriff's Office[2]
High-Resolution Melt can be used to Quickly Identify New Loci for Body Fluid Identification Using DNA Methylation Melt Analysis
This project uses High Resolution Melt analysis to quickly screen new potential loci for DNA methylation analysis, with the possibility of creating a confirmatory tool for body fluid identification. DNA methylation is a natural process involving the addition of a methyl group to the 5'carbon of cytosines in a dinucleotide cytosine-guanine (CpG) pair. Whole genome analysis of CpGs using a commercially available array can provide additional loci of interest but it must be followed by a confirmatory PCR-based assay. HRM constitutes a great experimental tool to perform screening for multiple genome locations quickly and has a

great potential to be used as a standard method in forensic laboratories to discriminate body fluids. Blood, buccal swabs, vaginal and semen samples were collected from volunteers. DNA was extracted using the EZ1® DNA Investigator kit (Qiagen, CA) and the BioRobot® EZ1 (Qiagen, CA) and then bisulfite modified using the EpiTect® Fast DNA Bisulfite Kit (Qiagen, CA) in order to convert the unmethylated cytosine to uracil which will cause amplicons with low GC content to melt at a lower temperature than amplicons with high GC content. Primers specific for the CpG of interest were designed using online tools. Bioinformatic analysis was performed using R software to determine relevant CpGs to discriminate blood, saliva and vaginal epithelia. Real-time PCR reactions were performed using either the Epitect® HRM kit (Qiagen, CA) or an optimized master mix on a Rotor Gene 6000 real time instrument (Qiagen, CA). To date this approach allowed us to obtain a 71% success in identifying new CpGs in silico. For example, from 7 CpGs identified as potential blood markers, 5 proved to show a difference in their TM for blood when compared to saliva, semen and vaginal epithelia, using HRM. Due to the high throughput of HRM, we are able to quickly screen loci of interest with an analytical tool appropriate for casework in forensic laboratories.

Haixiang Yu, Obtin Alkhamis, Juan Canoura, Bhargav Guntupalli, Yi Xiao
Florida International University
Rapid and Sensitive Detection of Small-Molecule Targets Based on Cooperative Target Binding and Enzyme-Assisted Target Recycling

Signal amplification via enzyme-assisted target recycling (EATR) offers a powerful means for improving the sensitivity of DNA detection assays, but it has proven challenging to adopt EATR into the aptamer-based assays for small-molecule detection, because insensitive target response of aptamers. Here, we describe a general approach for the development of rapid and sensitive, EATR-amplified small-molecule aptamer sensors using cooperative binding split aptamers (CBSAs). CBSAs contain two target-binding domains, and exhibit enhanced target response compared with single-domain split aptamers. We introduced a duplexed C3 spacer between the two binding domains that offers an ideal substrate for exonuclease III cleavage, enabling EATR signal amplification. As a demonstration, we engineered a CBSA-based EATR fluorescence assay to detect dehydroisoandrosterone-3-sulfate (DIS) in urine samples. This assay achieved a 100-fold enhancement in target sensitivity relative to a non-EATR-based assay, with a detection limit of 1 μM in 50% urine. We further developed an instrument-free colorimetric assay employing EATR-mediated aggregation of CBSA-modified gold nanoparticles for rapid detection of low micromolar concentrations of cocaine. Based on the generalizability of CBSA engineering and the robust performance of EATR in complex samples, we believe that such assays should prove valuable for detecting small-molecule targets in diverse fields.

Shady El Damaty[1], Diana Fishbein[2], John VanMeter[1]
Georgetown University[1], Pennsylvania State University[2]
Prediction of Youth Antisocial Behavior with Neural Biomarkers

The incidence rate of violent crimes in the U.S. follows a similar developmental trajectory as neurobehavioral metrics of impulsivity and aggression; rapidly increasing following puberty and peaking at ages 17-19 before leveling off in young adulthood. Environmental stressors including, but not limited to, factors such as family conflict, exposure to violence and deviant social influences have been suggested to alter the development of healthy brain networks, thereby predisposing adolescents towards adverse outcomes. The dual systems model of adolescent brain development specifies individual differences in outcomes may be explained by the developmental timing of increased risk/reward-related neural activity in the striatum relative to the onset of maturation of inhibitory control circuits in the prefrontal cortex. In this work, we provide evidence for these claims using functional Magnetic Resonance Imaging (fMRI) to

characterize the maturation of striatal and prefrontal cortical networks. 135 youths (ages 11-14) were assessed for deviant social behavior, environmental stressors, drug use and mental health at three time points spaced 18 months apart from 2011-2017. Striatal-cortical network connectivity increased as a function of age, indicating healthy brain development on average in the sampled group ($p<0.001$). Individual differences in network connectivity were explained by behavioral phenotypes and social influences. The strength of striatal-prefrontal cortical connectivity was revealed to be weaker in individuals exhibiting future antisocial behavior ($r=-0.28$; $p < 0.003$), early emotional disturbances ($r=-0.18$; $p < 0.05$) and deviant peer relations ($r=-0.19$; $p < 0.05$). Overall, our results demonstrate the potential utility of brain-based biomarkers for predicting, understanding and potentially preventing the emergence of youth antisocial behavior.

Ikwulono David Unobe[1], John Kalivas[1], Lisa Lau[1], Rene Rodriguez[1], Andrew Sorensen[2]
Idaho State University[1], Utah State University[2]
Recovery of Defaced Serial Numbers Using Lock-In Thermography
Infrared thermal imaging is an evolving nondestructive method useful for identifying local differences in thermal conductivity or regions of plastic strain from subsurface defects in materials. In the case of vehicle and firearm serial numbers, these defects are the underlying deformations that result from the stamping and laser engraving processes. This study utilizes lock-in thermography (LIT) in combination with multivariate data analysis as an alternative to chemical etching; a destructive method currently used to recover defaced serial numbers. The process involves several unique aspects, each of which works to overcome some pertinent challenges associated with the recovery of defaced serial numbers. The thermal differences captured in the infrared images are quite small and not readily visible due to surface irregularities and environmental factors. As such, further enhancement is usually needed to identify and evaluate the subtle variations. Principal component analysis (PCA), a multivariate technique that transforms a dataset of possibly correlated variables into a set of new uncorrelated variables, is employed on the data collected to enhance these variations and aid the recovery of the numbers. Statistical measures are utilized to independently verify and match the recovered numbers to non-defaced equivalents in a pristine library. Prior to computing similarity measures between a library number image and a defaced score image, the images are decomposed to Zernike moment vectors using Zernike polynomials. These vectors contain image features describing shape characteristics of an image. Fusion rules are then applied to the resultant measures to achieve a consensus as to the identification. Results are presented for known defaced numbers on several samples as well as recovery of the defaced serial number on a stolen motorcycle.

William Fatigante, Michael C. Gizzi, Christopher Mulligan, Jamie R. Wieland
Illinois State University
Analytical Validation and Impact Assessment of On-Site Evidence Screening via Ambient Sampling, Portable Mass Spectrometry
Forensic evidentiary backlogs are indicative of the growing need for cost-effective, high-throughput instrumental methods. One such emerging technology that shows high promise in meeting this need, while also allowing on-site investigation, is portable mass spectrometric instrumentation, particularly that which enables the coupling to rapid, ambient ionization methods. In this work, a comprehensive analytical validation of a portable mass spectrometer (MS) featuring a simplified paper spray ionization (PSI) source is undertaken, examining aspects such as spectral accuracy, error rates amongst diverse user classes, reproducibility and method robustness. As such technology is intended for field usage by non-technical operators, an extensive investigation of environmental ruggedness was conducted, observing the effect of ambient temperature, wind speed/direction and relative humidity on collected spectra and associated error rates. While portable MS systems have the potential to serve as a flexible investigative tool during law

enforcement activities, the underlying legal implications of evidentiary data require the discretion of practitioners to ensure both lawful and ethical usage. Demonstrated in this work are usage scenarios relevant to policing, premising the legality of more abstract applications (such as latent fingerprint screening on identifying materials to establish probable cause in traffic stops) through a review of current search and seizure law. A proactive examination of economic impact regarding implementation of portable MS systems in law enforcement is also presented, weighing the potential cost-savings and enhanced investigatory capabilities afforded against traditional, laboratory-based evidence processing. This research serves to help inform and guide criminal justice decision-makers in regards to the potential adoption of portable, forensic instrumentation.

Stephen Raso, Suzanne Bell
West Virginia University
Evaluation of the Production, Detection, and Possible Toxicity of the Pyrolytic Products of Synthetic Cannabinoids

Synthetic cannabinoids have been a vital issue to public health since the mid 2000's as the abuse has steadily induced increasing cases of fatalities. The basis of the fatalities has been due to varying effects on numerous organs, which has made deciphering and counteracting this epidemic challenging. Acute toxic effects include tachycardia, seizures, possible suicidal tendencies and psychotic episodes occur with synthetic cannabinoid abuse not observed in marijuana use. The current project aims to introduce new insight into this toxicity of synthetic cannabinoids versus marijuana. Given that ingestion is typically via inhalation through smoking, an investigation into the pyrolysis of cannabinoids was initiated to evaluate the thermal degradation products as a cause of toxicity. The first objective of the project was to identify the thermal degradation products. Parent cannabinoids were studied using an in-house constructed and optimized apparatus followed by GC-MS analysis, in which over 50 products were observed. Three major trends were seen and allow for predictive breakdowns of compounds not analyzed. The pyrolytics may have an impact on the toxicity, but must be shown to be absorbed by the user, which leads to the second objective of the project. Post mortem blood samples from fatality cases were obtained and are being analyzed for the presence of thermal degradation products. An adaption of a previously reported LC-MS/MS method following liquid-liquid extraction has been optimized for the parent compounds reported in each case as well as their predicted pyrolytic products. Products shown to be present in the blood will be evaluated for acute toxicity as an answer to the current mystery of the cannabinoid epidemic. This new insight into answering the cannabinoid toxicity question could have a major impact in forensic toxicology and clinical pharmacology. This work was supported by the National Institute of Justice [2015-R2-CX-0032].

Brandon Bills, Jeffrey Kinkade, Nicholas Manicke,
Indiana University - Purdue University Indianapolis
Studying Paper Properties to Improve Detection Limits of Synthetic Cannabinoids and Fentanyl Using Paper Spray Mass Spectrometry

Abuse of synthetic cannabinoids and fentanyl has led to an increase of overdoses in recent years. Sample preparation and chromatography are time consuming; simpler mass spectrometry based methods are needed to stream line drug screening. Paper spray is a rapid ambient ionization technique in which a biofluid is spotted and analyzed directly from paper with a macroscopic point without further sample preparation. However, detection limits for these compounds, which are typically in the 1-10 ng/mL range, are too high to detect toxic levels in some cases. Previous work has shown that the spray substrate impacts of ion suppression and recovery, and therefore detection limits, in paper spray. Different types of paper and porous materials have been studied before to evaluate their feasibility, but these studies did not use a systematic approach that clearly identified substrate properties that impacted matrix effects. In this study, an approach

was used in which spray substrates, including filter paper, chromatography paper and manufactured cellulose TLC plates, were selected that were similar with a single property significantly different. Furthermore, these substrates were subjected to modifications to try to reduce the matrix effects. Changes in ion suppression were determined by comparing the signal of the stable isotopic label (SIL) in the solvent while recovery was based on the ratio of the signal of the analyte eluted from the biofluid to the SIL. A general trend was found in that improvements to signal from improved recovery were usually paired with decreases in signal due to ion suppression. Using the properties determined in this study an ideal combination of solvent and paper was proposed for reducing the detection limits in a urine matrix.

Ryan Eller, Noah Herrick, Susan Walsh, Andreas Wollstein
Indiana University - Purdue University Indianapolis
Unearthing New Variants Related to Common Variation in Human Facial Morphology Using Genome Wide Association Study (GWAS) Methods
DNA Phenotyping is an up and coming area within the forensic DNA analyses community that has many possible applications, from casework to anthropological studies. While there are currently several tools available, such as HIrisPlex that aid in the prediction of categorical pigmentation phenotypes, a similar tool to predict facial structure is notably absent. If such a tool were to exist, then this may prove useful to investigators if standard genetic profiling failed to return a match to a DNA database or a 'person of interest.' Determining the variants associated with facial shape and their role in prediction is vital to moving facial morphology prediction in the right direction and reach the prediction levels that are currently seen for other traits. Although research is just beginning to explore common facial variation (i.e. non-disease-related variation), the use of genome-wide association studies (GWAS) to unearth variants has proved beneficial in several studies, such as Paternoster et al., 2012 and Liu et al., 2012, for quantitative variation. Therefore, in this study we concentrated on generating both categorical facial morphology definitions as well as quantitative facial measurements. Building upon previous disease-related and some non-disease related facial morphology studies, we have generated phenotypes using 2D and 3D facial images from an admixed population of over 2500 individuals. Using a 1.7 million SNP array combined with the computationally intensive process of imputation, we were able to exponentially increase our genomic coverage allowing us to increase our statistical power for association of these phenotypic classifications. Using GWAS techniques we have assessed novel and known variants for their association and potential predictive value in common facial feature categories and quantitative measures using a US-based population set. This work was funded by a STEM fellowship from the National Institute of Justice (2015-R2-CX-0023).

Kelly Brinsko Beckert, Christopher S. Palenik
Microtrace, LLC
The Forensic Analysis of 3D Printer Dust Particles
3D printers are becoming increasingly efficient and economical, and thus more widespread and easily accessible to consumers and the general public. Previous research has documented the release of dust particles during the printing process. However, little is known about their morphology and other characteristic features. This study was undertaken as part of a federal research grant (NIJ Grant No. 2015-DN-BX-K033) to characterize these particles so that they may be collected, recognized, and analyzed appropriately. Samples were collected from a variety of 3D printers, representing both consumer- and commercial-grade models. These printers use thermoplastic filaments, typically polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS), though others may be used (nylon, polyvinyl acetate, polyurethane, etc.). Cotton or polyester-flocked swabs were used to collect dust from various surfaces within the printer chamber and surrounding areas up to 10 feet away. Particles produced from ABS filaments are most easily recognized based on color and rounded morphology via light microscopy; FTIR spectra of the particles

confirmed the identification of the ABS polymer. Pigments and the ABS polymer matrix were also identified using Raman microspectroscopy. Dust from PLA printers consistently contained finer, submicron sized particles (relative to background levels) that could be observed by field emission scanning electron microscopy; however, the size of the particles precluded their specific identification as PLA. This presentation will detail the collection procedures employed to find, isolate, identify, and compare 3D printer dust particles, and a discussion of their potential applications and limitations as forensic evidence.

Kelly Brinsko Beckert, Christopher S. Palenik
Microtrace, LLC
Nanoparticles as Trace Evidence: Part I. Recognition and Collection

The use of nanotechnology and engineering of nanomaterials has grown exponentially in the last decade, and has found widespread application across a number of disciplines, including biology, medicine, electronics, energy, optics, and materials manufacturing, among others. These nanoparticles and other subvisible particles are present in nearly all forms of existing trace evidence, yet currently the overwhelming majority of trace examinations focus exclusively upon larger particles. In this era where highly engineered nanoscale materials are being introduced at increasing rates, it is inconceivable that such materials are not being regularly examined as forensic evidence. Practical forensic research is currently being undertaken by the authors in order to systematically develop approaches for the isolation, analysis, and interpretation of particles on the nanoscale, effectively equating the sensitivity of trace evidence to that of DNA analysis. While the smallest particles in this range may require higher resolution instrumentation, the majority of these particles can be characterized effectively by applying the suite of microanalytical methods present in most trace evidence laboratories today (stereomicroscopy, polarized light microscopy, and scanning electron microscopy). Here we present the first part of our research: describing the relevance, classifications, and applications of nanoparticles, then following with information about how these particles can best be recognized and collected in a forensic science laboratory.

Katie M White, Brendan Nytes, Christopher Palenik
Microtrace LLC
Applications of Glass Microspheres as Forensic Trace Evidence

Microspheres are used in an increasing variety of applications, from personal care products to food and industrial applications. Glass microspheres represent a significant subset of the microsphere market and are encountered in cosmetics, paints, plastics, building materials, and other applications. While they are used in a variety of consumer-grade products, their size, transparency, and shape can make them difficult to find or easy to overlook. For example, in solution, an isotropic, glass microspheres may be confused with an immiscible phase. Despite such difficulties, the size range (~5-1,000 µm) and composition (glass), make them accessible and potentially useful indicators of products, activity, or associations. This poster will cover the range of physical, optical, and elemental characteristics of reference microspheres obtained from manufacturers and the ways in which glass microspheres can be located and characterized in industrial and consumer applications, e.g., cosmetics, spackle, and polymers. When present in dust, microspheres may be encountered as free particles, where they may be the sole basis of an association, or they may be encountered in a matrix, e.g., a polymer or ceramic, where they could be used to improve the significance of an association. The results from these analyses illustrate some of the ways in which microspheres can be located, characterized, and interpreted in the context of a forensic investigation.

Katie M White, Christopher Palenik
Microtrace LLC
A Forensic Study of Known Toner Particles

Whether we are aware of them or not, small particles abound in the environments that surround us. Small particles may be engineered for use in manufactured products, be present in dusts generated from man-made industrial processes, or occur naturally in the environment. Some of these particles are just barely visible, while others are so small that they cannot be resolved by the human eye. These subvisible and submicrometer particles (nanoparticles) offer potential as forensic evidence, but they are presently unexploited due to the challenges that their small size present. One example of subvisible particles is the toner powder used in laser printers and copiers. Presently, most existing research on forensic toner analysis focuses on document examination, i.e., analysis of printed toner, rather than on trace evidence. However, toner is widely used, and these small particles are easily transferred and rarely noticed. Identification of trace amounts of toner, e.g., on hands or clothing or in dust, could be used to provide investigative leads or associate them with a scene and/or victim, particularly if the particles are suggestive of a specific toner. This poster will discuss the results from an analytical study of more than 50 different toner samples. This research evaluates microscopic morphologies observed by light microscopy and scanning electron microscopy, and chemical properties determined by Raman spectroscopy, of the known toner samples, providing methods that can be used in the forensic laboratory to identify and classify toner particles. Analytical differences observed within the sample set, the prevalence of background toner particles in different environments, and limitations of this approach will be covered.

Minh Nguyen, Gregory Dutton, Frances Scott
National Institute of Justice
National Institute of Justice - Office of Investigative and Forensic Sciences Research Programs and Funding Opportunities
The National Institute of Justice (NIJ) is the research, development and evaluation agency of the U.S. Department of Justice. NIJ's Office of Investigative and Forensic Sciences maintains a program of external funding for R&D in forensic sciences. This program is a leading federal funder in this mission space, and the portfolio spans a broad range, from fundamental research, to development of prototype devices, to validation of novel instruments and methods. Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of biological and chemical samples. Because of the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded or mixed samples. Balancing that, is the need to ensure that analytical methods applied to these challenging samples are objective, rigorously tested, and foundationally valid. These needs drive NIJ's continuing R&D investments in analytical chemistry and bioanalytical science. Advances in mass spectrometry, electrophoresis, applied spectroscopies, microscopy and microfluidics, among others, have yielded or show promise for forensics. NIJ anticipates continued interest in advancing technologies for forensic application. In this effort, NIJ strives to engage the analytical chemistry and applied spectroscopy research community to bring novel perspectives to solving forensic problems. An overview of NIJ's R&D funding programs will be presented, with the objective of introducing the Pittcon audience to the available options for research support. These range from fellowship opportunities for students, to grant funding programs for basic research with potential for long term impact on forensic science, to applied research and development focused on improving forensic practice immediately.

Timothy Coulther, Penny Beuning, Mary Jo Ondrechen,
Northeastern University
Engineering a Replicative DNA Polymerase for Specific Damage Bypass Capability
Enzyme engineering seeks to create an enzyme, either de novo or by modification of a known protein, with a new desired function. While biocatalysis has advantages over conventional catalytic processes, there most

often are not natural enzymes with the desired properties many commercial applications. For example, many DNA polymerases can replicate DNA accurately at high speeds but are inhibited by bases damaged by environmental mutagens, oxidative stress, or UV light. Polymerases may not be able to insert nucleotides opposite a lesion or may do so with reduced accuracy. Specialized polymerases may be well suited to bypass the damage, but are typically less accurate and efficient, even on undamaged DNA. This accuracy tradeoff allows genomic replication, and life, to continue, but can also contribute to antibiotic resistance or oncogenesis. A hybrid DNA polymerase that retains the accuracy and speed of replicative polymerases, while incorporating specific lesion-bypass abilities, would be a useful biochemical tool. This research focuses on the common oxidative DNA lesion 8-oxoguanine, a small modification yet often mutagenic. The additional hydrogen bond donor allows formation of a Hoogsteen pair with adenosine when 8-oxo-dG resides in the syn conformation. To identify variants for accurate bypass, multiple criteria were used to identify positive mutations. Residue scanning was used to assess mutations' effects on affinity towards both the preferred anti conformation and the mutagenic syn conformation. Our computational method THEMATICS was then used to filter out mutations that affect the electrostatic properties of catalytic residues and therefore are more likely to affect the polymerase activity negatively. These methods are being utilized to identify specific variants for biochemical characterization, including thermal stability, catalytic activity, lesion bypass capability, and fidelity. Supported by NSF-MCB-1517290 and the National Institute of Justice.

Zhengming Ding, Fu Yun
Northeastern University
Deep Multi-Factor Forensic Face Recognition
Forensic face recognition attempts to identify the suspects from a huge amount gallery photos for the current probe image. The key challenges for forensic face recognition are subject to a variety of internal/external impact factors under different views, illuminations, resolutions, modalities, periods when probe images are captured in the surveillance environments without collaborations. Up to now, there is no working face recognition system that has been accepted within the judicial system. To this end, we propose to develop an effective deep structure to better handle the multiple factors within forensic data. Specifically, we build a novel Deep Robust Encoder (DRE) through locality preserving low-rank dictionary to extract robust and discriminative features from corrupted face data, where a low-rank dictionary and a regularized deep auto-encoder are jointly optimized. With the features extracted with our DRE, we adopt standard classifiers, e.g., NNC, SVM, to evaluate the face recognition performance in terms of classification accuracy. We conduct experiments on Matlab 2014 with CPU i7-3770 and 32GB memory size for a personal computer. Experimental results on several face benchmarks verify the effectiveness of our algorithm in better handling multiple factors, compared with the state-of-the-art approaches. This concludes that our proposed model could better address the multiple factors with forensic faces. Finally, this work is supported by the NIJ Graduate Research Fellowship 2016-R2-CX-0013.

Austin Lee Ciesielski[1], David Pretorius[2], Jarrad Wagner[1]
Oklahoma State University[1], Savannah River National Laboratory[2]
Detection of One Pot Methamphetamine Laboratories via Waste Water Effluent Monitoring
The One Pot methamphetamine production method has become the primary method of choice in clandestine drug laboratories across the United States, due to its simplicity and the availability of required materials. This study was undertaken to determine the feasibility of the detection of methamphetamine clandestine laboratories through monitoring waste water effluents. Waste water samples were collected from small and large city municipalities and analyzed via solid phase extraction with liquid chromatography-tandem mass spectrometry for methamphetamine, pseudoephedrine, amphetamine, and an over-reduced product

characteristic of One Pot methamphetamine synthesis, CMP [1-(1',4'-cyclohexadienyl)-2-methyl aminopropane]. A survey of urine samples (N=47, 2% CMP) that were positive for methamphetamine was conducted, and all four target compounds were similarly detected making differentiation of clandestine laboratory effluent from urinary excretion challenging. This work demonstrates the potential for analyzing waste water to detect clandestine One Pot methamphetamine laboratories and methamphetamine abuse within a community.

Kyleen Elwick[1], Bruce Budowle[2], Sheree Hughes-Stamm[1], Jonathan King[2], Xiangpei Zeng[2]
Sam Houston State University[1], University of North Texas Health Science Center[2]

Evaluation of Five Common Extraction Methods for Analysis of Human Remain Samples on Massively Parallel Sequencing Success

Often in missing persons cases bone, teeth, hair, and decomposed tissue are the only samples remaining for identification. Exposure to harsh environmental conditions may also cause DNA degradation, damage, and/or inhibition, making these samples challenging to process. Human skeletal remains are often inhibited by humic acid, melanin, hematin, collagen, and calcium. Inhibitors may be co-extracted with the DNA, can interfere with PCR, and may reduce downstream DNA typing success. Current DNA identification methods include capillary electrophoresis based short tandem repeats (STRs), which are currently the gold standard. Single nucleotide polymorphisms (SNPs) are single base changes in the genome that can also be used for bio-ancestry and phenotypic information. Massively parallel sequencing (MPS) is a newer technology used in the forensic science field. It has the ability to expand our current technologies as more genetic information can be retrieved and simultaneous analysis of different (and more) markers can be incorporated (e.g. iiSNPs, STRs, aiSNPs). An effective DNA extraction method is critical to obtain clean DNA from difficult samples. However, little is known regarding the compatibility of common DNA extraction methods with MPS chemistries. The goal of this study was to evaluate the efficiency of various DNA extraction methods to remove PCR inhibitors from skeletal remains prior to MPS. Samples were extracted using either organic or commercial kits commonly used in forensic laboratories. DNA was extracted from blood, hair, muscle, and bone after being spiked with high amounts of inhibitors. These samples were then sequenced using the Precision ID Library kit and an early access panel for degraded samples on the Ion S5™ System, and the ForenSeq™ DNA Signature Prep Kit on the MiSeq FGx™. Although the two MPS chemistries were differentially tolerant to the inhibitors tested, the results showed that all extraction methods were compatible with both MPS systems.

Rachel Houston, David Gangitano, Sheree Hughes-Stamm
Sam Houston State University

Nuclear, Chloroplast, and Mitochondrial Data of a US Cannabis DNA Database

Since Cannabis sativa is a controlled substance in many parts of the world, the ability to track its biogeographical origin could provide law enforcement with investigative leads regarding its trade and distribution. Using autosomal, chloroplast, and mitochondrial DNA, allows not only for prediction of biogeographical origin of a plant, but also allows for genetic identification. A previously validated 13-autosomal STR multiplex was used to genotype 496 samples. Samples were analyzed from four different sites: 21 seizures at the US-Mexico border, Brazil, hemp seeds, and Chile. In addition, a previously reported multi-locus system was modified and optimized to genotype five chloroplast and two mitochondrial markers. For this purpose, two methods were designed: a homopolymer STR pentaplex and a SNP triplex. For autosomal typing, distinguishable profiles were generated from 381 samples that yielded full STR profiles and 44 duplicate genotypes within seizures were observed. Phylogenetic analysis and case-to-case pairwise comparisons of the 21 border seizures revealed the genetic association of nine seizures that formed a reference population. For mitochondrial and chloroplast typing, subsampling was performed and 141

samples were genotyped. As expected, extensive haplotype sharing was observed; five distinguishable haplotypes were detected. Haplotype sharing was observed between the US border seizures, Brazil, and Chile while the hemp samples generated a distinct haplotype. Results revealed that both autosomal and lineage markers could discern population sub-structure. Phylogenetic analysis of the four populations using neighbor joining were estimated with the GDA software. Parsimony analysis was then performed with the PAUP* software. The STRUCTURE software was employed to investigate the population structure among groups. And finally, the R package, Adegenet, was used to visualize the genetic distance of the populations using Principal Component Analysis (PCA).

Carrie Mayes[1], Sharee Hughes-Stamm[1], Sarah Seashols-Williams[2]
Sam Houston State University[1], Virginia Commonwealth University[2]

Identifying Forensically Relevant Body Fluids Using a Capillary Electrophoresis Based miRNA Multiplex

In many cases, probative information may be gained from evidence through the identification of the source body fluid in addition to identification of a person. While serological tests are commonly used by crime laboratories, they are generally presumptive in nature due to differing levels of specificity and sensitivity. However, nucleic-acid based methods have been proposed as a means to provide a more confirmatory method of body fluid identification (BFID). These methods allow for the identification of a greater number of body fluids as well as the ability to co-analyze with DNA and consume less evidentiary sample. Recently, miRNAs have been suggested as a biomarker for BFID due to their small size (19-22 nucleotides), making them ideal for analyzing highly degraded samples. In this study, we generated both DNA and miRNA profiles from single co-extracted samples using capillary electrophoresis-based methods. For the miRNA analysis, we expanded on a previously reported linear primer system in order to include additional markers. In this panel, an 8-marker system was designed to differentiate venous blood (miR-451 and miR-142-3), menstrual blood (miR-141-3 and miR-412), semen (miR-891 and miR-10), and saliva (miR-205). In addition, an endogenous reference gene (let-7g) was included to confirm successful reverse transcription and amplification. Each primer set was evaluated in singleplex to assess cross-reactivity between body fluids and genomic DNA as well as to determine optimal amplification conditions. All samples tested yielded full STR profiles from the DNA fraction and let-7g amplification from the RNA fraction. Although some cross-reactivity was observed, a presence/absence scheme was developed to distinguish between venous blood, menstrual blood, semen, and saliva.

Douglas Armstrong, Madeline Ausdemore, Cedric Neumann, Christopher P. Saunders
South Dakota State University

Development and Properties of Kernel-Based Methods for the Interpretation and Presentation of Forensic Evidence

The chemical analysis of forensic traces often results in complex, high-dimensional data. The statistical inference of the source of these traces requires assigning probability distributions to the data. Chemometric techniques often involve data reduction and machine learning such as principal component analysis, neural networks, or support vector machines. However, while these are useful for discrimination and classification, they are difficult to use in the probabilistic framework necessary to properly quantify the weight of forensic evidence. One approach proposes to circumvent this problem by reducing the data dimension by assigning pairwise similarity scores between i.i.d. objects that compose the evidence (in an analogous manner to support vector machines) and to build probabilistic models based on the univariate distribution of scores, disregarding the dependency between multiple scores calculated pairs of objects which reuses a common object. In this presentation we will summarize recent advances made on kernel-based models to propose a probabilistic and multiclass version of SVM. Our model captures the dependencies between pairwise scores

from a hierarchical sample and models them in the kernel space using a linear model. Our model is flexible to accommodate any kernel satisfying basic conditions and as a result is applicable to any type of complex high-dimensional data. An important result of this work is the asymptotic multivariate normality of the scores as the data dimension increases. As a result, we can: 1) model very high-dimensional data when other methods fail; 2) determine the source of multiple samples from a single trace in one calculation. We will provide examples of real-life problems using data from very small particles and dust analyzed by SEM/EDX, and colors of cotton fibers quantified by microspectrophotometry.

Fatih Inci[1], Duygu Cimen[1], Utkan Demirci[1], George Duncan[2], Gozde N. Durmus[1], Leonard Klevan[2], Morteza Miansari[1], Mehmet O. Ozen[1], Yeseren Saylan[1]
Stanford University[1], Broward County Sheriff's Office[2], DxNow Inc.[3]

Automation of Differential Extraction with Sperm Quantitation Using Microfluidic Platform for Forensic Applications

Rapid and efficient processing of sexual assault evidence is an urgent need in forensic DNA analysis to accelerate forensic investigation and reduce casework backlogs. One of the major challenges in the processing of sexual assault cases is to differentially extract evidence samples, separation of the victim's cells (epithelial) from the perpetrators cells (sperm). Current methodologies and techniques consist of multiple time consuming steps, including selective cell lysis, centrifugation and differential DNA separation. However, there is no integrated rapid sperm isolation and differential extraction platform which includes cell isolation and quantitation, in preparation for downstream genomic analyses. In this abstract, we present a microfluidic platform technology to specifically capture sperm cells and differentially extract male DNA in complex forensic samples. To capture sperm, the microfluidic chips are modified with a unique oligosaccharide molecule, which is located on the extracellular matrix (i.e., zona pellucida (ZP)) of the oocyte to denote a ligand for human sperm-oocyte binding. Our platform isolates sperm with >90% of capture efficiency, and removes up to ~93% of epithelial cells from heterogeneous cell populations. Captured sperm are then lysed on-chip for potential downstream genomic analyses. In collaboration with the Broward County Sheriff's Office, we have also presented that these microchips can selectively and efficiently capture sperm from mock sexual assault samples in a cost-effective manner. This next generation differential extraction process considerably reduced assay-time to 80 minutes, providing an inexpensive alternative to multi-step, labor-intensive differential extraction, thus potentially accelerating identification of suspects; advancing public safety.

David A. Stoney, Paul L. Stoney
Stoney Forensic, Inc.

Application of Particle Characterization Methods (Such as SEM/EDS) in Support of Particle Combination Analysis

Particle combination analysis using very small particles (VSP) is a new approach, highly significant for its potential to expand the number of cases to which trace evidence can meaningfully contribute and for its ability to include a quantitative statistical approach to data interpretation. The laboratory analyses are highly efficient, utilizing existing crime laboratory personnel and equipment. Prior research, employed reasonable choices of analytical and statistical parameters which were sufficient to demonstrate feasibility and potential. Systematic development and validation of these methods requires that the analytical and statistical parameters be more critically examined, and that the key factors influencing the performance of the methods be identified. Determination of the key factors and the magnitude of their effects will result in a significantly improved capability and provide necessary input to experimental designs that will permit systematic improvement and optimization. This will enable transition of particle combination analysis to practice and contribute to the fundamental advancement of a new quantitative and broadly applicable approach to trace

evidence. Well-documented factors and effects for one VSP analysis protocol will allow parallel, collaborative assessments of alternative options for high efficiency analysis of VSP (such as micro Raman methods, microXRF, genetic analysis, or alternative SEM/EDS protocols). This project was supported in part by Award Nos. 2012-DN-BX-K041 and 2015-DN-BX-K046 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

Yasmine Ahmed[1], Valentina D'Elia[2], Vladimir V. Ermolenkov[1], Carmen García Ruiz[2], Igor K. Lednev[1], Gemma Montalvo[2]
University at Albany, SUNY[1], University of Alcalá[2]
Ultraviolet Resonance Raman Spectroscopy for the Detection of Cocaine in Oral Fluid
Detecting and quantifying cocaine in oral fluid is of significant importance for practical forensics. Up to date, mainly destructive methods or biochemical tests have been used, while spectroscopic methods were only applied to pretreated samples. In this work, the possibility of using resonance Raman spectroscopy to detect cocaine in oral fluid without pretreating samples was tested. It was found that ultraviolet resonance Raman spectroscopy with 239-nm excitation allows for the detection cocaine in oral fluid at 10 [micro]g/mL level. Further method development will be needed for reaching the practically useful levels of cocaine detection.

Marisia Fikiet[1], Masahiro Ando[2], Hiro-o Hamaguchi[2], Igor K. Lednev[1], Gregory Mclaughlin[1]
University at Albany, SUNY[1], Spectroscopic Science Laboratory[2]
Overcoming Substrate Interference in Raman Spectroscopy for Forensic Purposes
Raman spectroscopic mapping has become more and more popular as an analytical method. One drawback of Raman microspectroscopic analysis is interference from substrates. Our lab has evaluated several approaches to overcome this problem targeting the identification of body fluid traces for forensic purposes. First, we varied the excitation wavelength, but no one wavelength worked well for all the common substrates. Using different excitation wavelength for different substrates was impractical. Then background subtraction was attempted and works very well for homogeneous substrates, but not for heterogeneous ones. Here we report on a new universal approach based on Raman hyperspectroscopy and MCR data analysis. The program was used for substrates that are fluorescent and heterogeneous and was able to extract the body fluid signal in all cases. This project was supported by Award No. 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

Meghan G. Fogerty, Rabi Ann Musah
University at Albany, SUNY
Detection and Identification of Plant-Based Legal Highs Based on Headspace Chemical Signatures Determined by SPME-Facilitated Direct Analysis in Real Time Mass Spectrometric Analysis
The United Nations Office on Drugs and Crime has identified 20 plant species that are increasingly being used as 'legal highs' (i.e. psychoactive products that remain unscheduled). Their legal status shields users and traffickers of these products from prosecution. Although the bulk of these materials are not cultivated in Europe or the United States, they are imported from various regions of the world including Asia, Africa and South America, and are thus readily available. A major challenge to the legislation of the use and sale of these substances is that it is extremely difficult to distinguish them from innocuous plant-based products such as herbs, spices, and foods. The ability to do so would be especially useful for Border Protection

Agents, so that commercial cargo can be screened for their presence. We sought to address this issue by developing a method that would enable detection of diagnostic small-molecule chemical signatures indicative of the presence of plant-based legal highs. In this approach, the headspace volatiles of 'legal high' plant materials are concentrated on polydimethylsiloxane (PDMS) SPME fibers, which are subsequently analyzed by direct analysis in real time-high resolution mass spectrometry (DART-HRMS). The observed chemical signatures are then subjected to multivariate statistical analysis approaches to enable classification and identification of the plant material. Kernel discriminant analysis (KDA) of the DART-MS data showed that the headspace signature could be used to accurately identify the bulk material. External validation was also performed to assess the reliability of the technique and was 100% accurate in all tests. These results demonstrate proof-of-concept for the creation of a database against which cargo-container derived headspace can be screened for the detection and identification of plant-based legal highs. This work was supported in part by the United States National Institute of Justice (grant 2015-DN-BX-K057).

Kristen Leigh Fowble, Ashton D. Lesiak, Rabi Ann Musah
University at Albany, SUNY
A Validated and Rapid Method for the Quantification of Psychoactive Materials in Complex Plant Matrices Using Direct Analysis in Real Time-High Resolution Mass Spectrometry
The quantification of natural products in complex matrices is a common practice in a number of fields, including medicine and forensics, among others. When the analyte of interest is contained within a complex plant matrix, the steps towards its quantification are far from straightforward. Our objective was to establish a procedure for the quantification of psychoactive materials found in plants being abused as 'legal highs', using an ambient ionization mass spectrometry technique that would greatly reduce the sample preparation typically required using traditional methods. Specifically, we describe an FDA validated method for the quantification of psychoactive atropine in Datura spp. seeds using direct analysis in real time-mass spectrometry. Calibration curves for atropine in a 1:1 v/v ethanol/water solvent using atropine-D3 as an internal standard were obtained over a linear range of 0.49 to 500 ppm. The limits of detection and quantification were determined to be 0.49 ppm and 0.98 ppm, respectively. D. stramonium, D. ferox and D. inoxia seeds were extracted and analyzed. Average concentrations of 15.32 +/- 0.15 ppm of atropine in the D. stramonium extract and 1.07 mg/g per seed were determined. The results compared well with reported levels determined using traditional approaches. Atropine in seed extracts of D. inoxia and D. ferox fell below the lower limit of quantification, but the extrapolated concentrations of atropine in their extracts were 0.83 +/- 0.14 ppm and 0.76 +/- 0.05 ppm, respectively. The results indicate that DART-MS can be used as a rapid means to quantify atropine in plant samples while avoiding significant sample preparation steps. Furthermore, the method can be applied to the quantification of other biomarkers in plant materials, despite the complexity of the plant matrix. This work was supported by the National Institute of Justice (grant 2015-DN-BX-K057).

Jan Halamek, Erica K. Brunelle, Lenka Halamkova, Crystal Huynh
University at Albany, SUNY
Novel Concept for Fingerprint Analysis
For years, fingerprint samples have been believed to be useful only for matching purposes using the unique ridges, shapes, and sizes. The traditional pictorial comparison used for the past century has been useful for the identification of some individuals, but many fingerprint samples have been labeled 'unusable' due to smudging, smearing, or any one of a myriad of reasons that could cause inconclusive matches. These samples can, however, still be of use. The chemical composition contains sebum, sweat, and a variety of lipids found to be secreted from the fingertips. The methods our group has developed use the amino acid content in fingerprints to differentiate between male and female fingerprints -one of the many possible

characteristics that can be identified. The research described here further explores the concept of determining attributes of an originator via the fingerprint contents mentioned above. Currently, research has focused on utilizing amino acids with known cascades specifically to see if decreasing the number of amino acid targets would interfere with the ability to determine if the sample is from a male originator or a female originator. This previously studied physical trait was chosen both to prove the viability of a single analyte assay for fingerprint analysis and to provide corroboration for the data obtained by the multi-analyte assay from our previous work. Additionally, we have explored the development of a paper-based strip for the colorimetric detection of metabolites. The systems presented here are designed to be versatile and adjustable enzyme cascades that will produce easily interpretable results. The successful development of this concept would lead to a new treatment of fingerprints as a source of evidence. This project could revolutionize on-site forensic analysis, as a result, accelerating the rate of criminal investigations.

Crystal Huynh, Erica K. Brunelle, Jan Halamek, Lenka Halamkova
University at Albany, SUNY
New Concept for Fingerprint Analysis: Bioaffinity-Based Systems Utilizing Amino Acids
Fingerprint analysis refers to the process of comparing fingerprint patterns by an expert and/or an automated fingerprint identification system. This method has become a universally accepted method for identification. This method of pictorial comparison is also one of the few forensic areas that have yet to see improvement in the past years, even with the recent increase of interest in forensics. Currently, the analysis ends with this matching methodology causing the field to be dependent on the presence of a stored matching print or a matching print from an individual that is physically present. Due to this limitation, many analyses result in inconclusive outcomes. What is often overlooked is that those latent prints are created by sweat and sebum emulsions excreted by the fingertips. Those emulsions have their own unique chemical compositions for each individual making them possible biological samples for analysis. Our lab has developed a bioaffinity-based cascade for the determination of biological sexes from the chemical composition of the sweat/sebum left as the latent prints. The research presented here addresses the current limitations in fingerprint analysis using a bioassay system that focuses on the components of fingerprints. Bioaffinity-based assays have been developed for the determination of biological sexes from those components. In one assay, L-amino acid oxidase was used to target the amino acids present in the sebum and sweat left on latent fingerprints. Further research has led to the testing of authentic fingerprint samples collected from various surfaces as well as the development of other bioaffinity-based assays capable of differentiating between biological sexes via less complex systems. Other bioaffinity-based assays will also be developed in the future for the determination of other physical attributes such as age group and ethnicity.

Cameron M. Longo, Rabi Ann Musah
University at Albany, SUNY
Establishing Exposure to Plant-Based Psychoactive Materials by Mapping Diagnostic Biomarkers in Fingerprints—A MALDI Mass Spectrometry Imaging Study
One approach to evading prosecution for illicit drug use is to utilize currently unscheduled substances such as psychoactive plants. Barriers to legislating the use of such substances include the absence of standard protocols for their identification and the non-existence of methods that establish a connection between the abuser and the substance abused. To address this, our objective was to develop a technique by which evidence of contact with these mind-altering substances could be linked to an individual, as this would provide valuable information to law enforcement and healthcare practitioners alike. We demonstrate here that prior handling of plant-based psychoactive materials can be established through detection of diagnostic biomarkers by SpiralTOF matrix-assisted laser desorption ionization high-resolution mass spectrometry imaging. Furthermore, the compounds remain detectable in the fingerprint even after the print has aged.

Plant products representing several relevant species were handled by rubbing the material between the fingers, whereafter fingerprints were deposited and prepared for analysis by SpiralTOF MALDI MS. Ion images of selected m/z values showed the spatial distributions of diagnostic small molecules indicative of exposure to the psychoactive plant materials. These included dimethyltryptamine from Mimosa hostilis and Psychotria viridis, and harmala alkaloids derived from Peganum harmala, as well as others. Importantly, the observed images were identical to those generated using endogenous lipids such as oleic acid. Plant biomarkers in fingerprints remained detectable for at least one week after being deposited. The findings illustrate that handling of psychoactive plant material by an individual can be definitively established solely through visualization of fingerprint images based on plant biomarkers. This work was supported by the United States National Institute of Justice (grant 2015-DN-BX-K057).

Shelby R. Khandasammy[1], Mathew S. Boll[1], Igor K. Lednev[1], Alex Rzhevskii[2]
University at Albany, SUNY[1], Thermo Fisher Scientific[2]
The Detection and Identification of Organic Gunshot Residue via Fluorescence Mapping and Raman Spectroscopy for Forensic Purposes
In this proof-of-concept study, a novel method for the detection and identification of organic gunshot residue (OGSR) was developed. This method consisted of a two-step process: the first step utilized highly sensitive fluorescence hyperspectroscopy to image a selected area and detect particles with specific size and emission properties. The particles of interest were then characterized with Raman spectroscopy to confirm their OGSR origin. This process was undertaken using 9 mm caliber gunshot residue particles on adhesive tape substrates. Two samples were investigated: one having a known number of OGSR particles and the other possessing an unknown number of particles. Investigation of these two samples demonstrated that the developed method is effective for the detection and identification of OGSR particles. Meanwhile, debris and other artifacts were successfully identified as non-GSR particles. This method presents a potential means for forensic analysts to screen for the presence of OGSR particles. This 'double-screening' of the samples-by first using fluorescence and subsequently utilizing Raman spectroscopy in order to investigate the samples-provides a twofold method that allows for the accurate and effective detection and identification of OGSR particles. This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

Ewelina Mistek, Igor K. Lednev
University at Albany, SUNY
Identification of Species' Blood by Attenuated Total Reflection (ATR) Fourier Transform Infrared (FT-IR) Spectroscopy
Blood is one of the most common and informative types of biological evidence found at a crime scene. In forensic investigations it is crucial to identify the origin of a blood stain. However, current standard methods employed for the analysis of blood samples are destructive and time-consuming. In this study, attenuated total reflection (ATR) Fourier transform-infrared (FT-IR) spectroscopy was used as a confirmatory, nondestructive, and rapid method for identifying species based on blood. Bearing in mind forensic purposes, differentiation of human and nonhuman blood samples was targeted, and partial least squares discriminant analysis (PLSDA) model demonstrated complete separation between human and animal donors. The models also distinguished between three separate species, namely human, cat, and dog. The method was validated in two external manners: using unknown samples outside of the dataset from human, cat, and dog blood, as well as samples which were more different than any in the training dataset (of different species, breeds, and genders). Classification predictions of unknown blood donors performed by the model resulted

in 100% accuracy. This study demonstrates ATR FT-IR spectroscopy's great potential for blood stain analysis and species discrimination. Furthermore, the commercial availability of portable ATR FT-IR instruments affirms the potential for the implementation of such blood stain analyses both at a crime scene as well as in the lab. This project was supported by Awards No. 2011-DN-BX-K551 and 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, USA, Department of Justice (I.K.L.).

Ewelina Mistek, Kyle C. Doty, Lenka Halamkova, Igor K. Lednev, Claire K. Muro
University at Albany, SUNY
Race Differentiation by Raman Spectroscopy of a Bloodstain for Forensic Purposes
Bearing in mind forensic purposes, a nondestructive and rapid method was developed for race differentiation of peripheral blood donors. Blood is an extremely valuable form of evidence in forensic investigations so proper analysis is critical. Because potentially miniscule amounts of blood traces can be found at a crime scene, the ideal method is nondestructive while providing substantial information about the sample. In this study Raman spectroscopy was applied with advanced statistical analysis to discriminate between Caucasian and African American donors based on dried peripheral blood traces. Spectra were collected from 20 donors varying in sex and age. Support vector machines discriminant analysis (SVMDA) was used for differentiation between the two races. An outer loop subject-wise cross-validation (CV) method served to evaluate the performance of the SVM classifier for each individual donor from the training data set. The performance of SVMDA, evaluated by the area under the curve (AUC) metric, showed 83% probability of correct classification for both races, and a specificity and sensitivity of 80%. This preliminary study shows promise for distinguishing between different race donors of human blood. The method provides rapid and reliable results without any preparation, destruction, or consumption of the sample—thus making it an ideal method for real life crime scenes. This project was supported by Awards No. 2011-DN-BX-K551 and 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.).

Jalissa Thomas, Marisia Fikiet, Igor K. Lednev
University at Albany, SUNY
Designing a Methodology for Body Fluid Identification with a Portable Raman Spectrometer
Identification and preservation of body fluid traces is a crucial step in forensic science. These traces are potential sources of DNA evidence, which can be instrumental in making convictions in criminal cases. This step is also very difficult, due to the specificity, imprecision, and destructive nature of current investigative tests. Our laboratory has recently developed a non-destructive, universal method of identifying body fluid traces using a desktop Raman spectrometer. However, it is important that trace evidence be analyzed and identified in situ, and immobile desktop instruments are unable meet this need. This has created a need for portable instruments for use in the field. We have worked on translating this methodology for larger spectrometers to a portable instrument. We have designed a specialized tool for sampling with this instrument, thus making the methodology more specific. We also focused on assessing the instrument's ability to differentiate between body fluids. Spectra of several body fluid were acquired and run against verified models of identification for larger Raman spectrometers.

Ahmed Eltawil, Ahmed Khorshid
University of California, Irvine
Wearable Interbody Communication Sensors for Body Area Networks
Wearable devices are rapidly being adopted as means of improving health care services. However, most wearable platforms are limited to a single point of contact location with the human body due to area and

power consumption restrictions, dictated by the wireless interface. An emerging technology that holds the potential for solving such issues is Intra-body Communication (IBC), where the signal is harmlessly confined to the human skin rather than propagated in the air. Using this approach, multiple sensors, distributed on the human body, can intercommunicate without the need for an air interface, leading to ultra-compact, precise, low-power sensors. A single wireless hub, can connect to distributed IBC sensors to serve as the gateway to the external world using traditional air interfaces (e.g. Bluetooth etc.). Distributing such small, low-power, smart sensors in a well studied pattern over the human body will allow users to monitor vital signals, such as body temperature and electrocardiogram with unprecedented accuracy. Such data can then be analyzed for training or operational safety purposes where stress profiles and reactions can be accurately identified. One of the initial research goals of the project is to understand and compare the main potential data carriers for IBC; namely using electro-magnetic waves, ultrasonic waves and magnetic coupling. Pros and cons of each approach are studied and the frequency response of different body tissues to these modalities are quantitively documented. Based on these studies, a model for intra-body communications, using electromagnetic galvanic coupling has been developed and adopted as the appropriate data carrier for this emerging technology. In the proposed model, biological parameters of the human body are accurately modeled, as well as assumed to be variable, taking into consideration the impact of important factors; such as age and weight, on these parameters and thus on the overall system profile.

Ramya Mohan[1], Sarah McElligott[1], Ruben Salgado[1], Rachna Dhir[1,2], Jesus Rivera[1], Steven Herrera[1], Seth Menser[3], Prof. Joanna McKittrick[4], Prof. Alexander Balandin[1], Prof. Charles Wyman[1,2], Prof. David Kisailus[1].
University of California, Riverside[1]; Center for Environment Research and Technology, UCR[2]; San Diego Zoo Global, San Diego[3]; University of California, San Diego[4]

Heat Dissipating Strategies in Pyrophytic Plant Follicles

Existing structural materials are thick, rigid, heavy, and cumbersome for use as protective clothing, necessitating the development of lightweight, thin and flexible materials that shield from high-velocity projectiles and overheating. Nature, over millions of years of evolution, has designed hierarchically structured bio-composites with superlative damage tolerance capabilities. Oftentimes, these materials feature multi-functional capabilities, such as thermal robustness coupled with mechanical resistance. One such multifunctional bio-composite is found in pyrophytic plants, that have evolved fire-resistant follicle valves to protect their encapsulated seeds. In addition, these valves also protect seeds from pecking and abrading predators. The follicle valves are made of organic polymeric components and yet exhibit remarkable thermal and mechanical tolerance, due to their microstructure and chemical components. The follicle valves consist of three distinct layers - a dense lignified exocuticle called the exocarp, a thick-walled cellulosic fibrous mesocarp, and an endocarp consisting of thin-walled cellulose fibers. Through thermal analyses, we determined that the exocarp shows significantly higher circumferential thermal conductivity when compared to the through-thickness direction, indicating that it possibly functions as a heat-dissipating shield. In addition, heating the follicle valves in air to ~200C revealed an expansion of the exocarp, called intumescence. This creates localized insulating pockets which could further inhibit flame penetration. Upon heating the follicle valves to ~600C, the plant organics carbonize to form a dense graphitic phase - Carbon-8, a known insulator, resulting in yet another flame retarding mechanism. Design strategies from these investigations will be used as guidelines to develop next-generation multifunctional materials for NIJ applications with thermally resistant synthetic polymers like polyimides.

Zhenjiang Xu[1], David O. Carter[2], Jack Gilbert[3], Greg Humphrey[1], Rob Knight[1], Jessica L. Metcalf[4], Se Jin Song[1]

University of California San Diego[1], Chaminade University of Honolulu[2], University of Chicago[3], Colorado State University[4]

Evaluating the Skin Microbiome as Trace Evidence on Common Surface Types

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 and whether they persist over timescales relevant to forensic investigations. Here we investigate 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 also determined that skin microbial signatures persisted on ceramic and plastic surfaces for at least one day, and became less accurate over time. Overall, we find 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 even when the reference microbiome was collected a year ago. We conclude that skin microbes are uniquely positioned to augment friction ridge comparison when sufficient ridge detail is not available to make a positive identification. Thus, the potential for microorganisms to reveal whether a particular person has touched an object is substantial.

Yasmine Moustafa, Candice Bridge
University of Central Florida/National Center for Forensic Science

The Evaluation of Sexual Assault Evidence Using Direct Analysis In Real Time Time-of-Flight-Mass-Spectrometry (DART-TOFMS) and Gas Chromatography (GC-MS)

Suspects in sexual assault cases are commonly identified by analyzing seminal fluid that remains at the crime scene for the DNA profile. However, the use of condoms in such crimes has been on the rise, prevention the deposition of biological fluid. As a result, sexual assault evidence should also include the analysis of condom lubricants to support the current analysis that is being conducted. However, to ensure that this type of analysis is beneficial for sexual assault investigations, an important consideration is the risk of false positives from the inherent residues of personal hygiene products (PHPs) found on the skin of the victim or at the crime scene. Therefore, the aim of this study is to present a classification scheme to mitigate the predicament of misidentifying unknown samples. In this study, 32 samples including 12 personal lubricants, 10 condoms, and 10 PHPs, were analyzed using DART-TOFMS and GC-MS. The results were statistically treated using hierarchical cluster analysis, principal component analysis, and linear discriminant analysis. The statistical classifications from both DART-TOFMS and GC-MS were compared to evaluate the advantages and disadvantages of each analytical method to differentiation sexual lubricants from PHPs in sexual assault evidence. The presentation will disclose a classification scheme to differentiate sexual lubricants from PHPs using two instrumental methods. Additionally, the use of statistics in providing accurate classification and discrimination will be discussed. The attendees will learn how unknown samples can be classified into sample groups and how instrumental methods for sexual assault evidence can be evaluated and compared.

Danielle Green, Melanie J. Beazley, Candice Bridge
University of Central Florida/National Center for Forensic Science

Identification and Quantification of Sexual Lubricant Degradation Pathways from Exposure to the Vaginal Bacterial Environment

Due to the use of DNA analysis for identification, an increasing number of offenders are using condoms to mask their identity from law enforcement. During a sexual assault, lubricant from a condom can be transferred to the victim. In forensic lubricant analysis, the major components of condom lubricants, such as polyethylene glycol (PEG) and 1-octylamine, are used as indicators of the presence of sexual lubricants. Bacteria natural to the vaginal cavity, including members of the Pseudomonas genus, help maintain a healthy environment. Metabolic processes of these bacterial strains can use residual lubricant that remains in or near the vagina as a possible energy source thus leading to sample degradation of the lubricant. The degradation caused by microbial exposure makes it necessary to understand how the microbes change the condom lubricant components and the overall chemical profile of the lubricant. The degradation of two common condom lubricant components, PEG and 1-octylamine, was studied using a common vaginal microbe, Pseudomonas putida (P. putida). Toxicity tests were conducted to determine the viability of P.putida in the presence of PEG and 1-octlyamine. Growth was positive at concentrations ranging from 25 ppm to 100 ppm, in the presence of both components. Subsequently, P. putida was inoculated with PEG and 8 samples were collected in triplicate over 36 hours. Lubricant degradation studies were conducted and demonstrated that P.putida might be using the PEG as a possible carbon source. Chemical degradation was measured using direct analysis in real time-time-of-flight mass spectrometry and gas chromatography-mass spectrometry. Instrumental data was evaluated and analyzed using chemometric methods. Further understanding the interactions of bacteria found in the vaginal cavity and condom lubricants can provide forensic science and sexual assault investigation communities with a new analytical timeline for vaginal samples collected after a sexual assault.

Brooke R. Baumgarten, Candice Bridge, Mark Maric, Caterina R. Vadell-Orsini
University of Central Florida/National Center for Forensic Science

Preliminary Characterization of Sexual Assault Lubricants: Comparison Between DART-TOFMS, GC-MS, and FT-IR

Unfortunately, sexual assaults are a reality in today's society. Increasing use of condoms reduces potential of recovering DNA evidence, and a novel approach for the analysis of other trace evidence is required. The characterization and classification of lubricants is a relatively new approach for analyzing unknown trace evidence that could be collected from the crime scene or the victim. In this study, 20 samples from different sexual lubricant manufacturing types were tested: water-based, silicone-based, oil-based, and organic/edible lubricants, and personal hygiene products which could also be used in sexual assaults. Instrumental methods were developed for direct analysis in real time-time of flight mass spectrometry (DART-TOFMS), gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR). Analytical protocols were designed to increase the identification of unique components in these lubricants to develop a classification scheme for unknown samples. Neat lubricants, as well as solvent extracts, were analyzed in both positive and negative ionization modes using DART-TOFMS in replicates of five. Neat lubricants and extracts were also analyzed via FTIR in triplicate, and extracts were analyzed by GC-MS in triplicate. Multivariate statistical techniques were used to identify unique markers that describe each class within the larger dataset. Classification schemes were developed for each instrument individually. The outcomes of the classification schemes are expected to separate the different manufacturing types into groups, and sub-classes within each manufacturing type. The classification schemes developed from this preliminary study will affect the forensic trace evidence community by aiding in future exploitation of evidence found at a crime scene based on the data, thus providing investigative leads and innovative techniques in the analysis of trace evidence.

Leondra Lawson-Johnson, Mark Dadmun
University of Tennessee, Knoxville
Coupling the Effects of Temperature and Humidity to Enhance the Cyanoacrylate Fuming Method for Retrieval of Latent Prints

The cyanoacrylate fuming method (CFM) is a widespread chemical process used in forensics to reveal latent prints on surfaces via the anionic polymerization of ethyl cyanoacrylate (ECA). The ECA monomer reacts with biological components, such as the amino acids found in sweat, which serve as initiators. Empirical studies have shown the polymerization of ECA at room temperature produces the most polymer when the relative humidity is around 80 percent. Previous studies in the Dadmun group have also shown that the anionic polymerization of ECA at low temperatures produces a larger quantity of high molecular weight polymer. Although the exclusive effects of relative humidity and temperature on the growth of poly(ethyl cyanoacrylate) (PECA) from deposited prints during fuming have been thoroughly investigated, our research exceeds the scope of current knowledge by evaluating the collaborative influence these parameters have on the method as well as how these parameters affect the characteristics of the resultant PECA polymer. To test the combined effects of humidity and temperature on the efficiency of the CFM, latent fingerprints on glass slides were placed in a fuming tank and fumed at different humidity levels and surface temperature settings. The amount of polymer accumulation and quality of the prints were compared. Our data findings suggest although there is more PECA observed at higher relative humidity, lower relative humidity provides more high molecular weight polymer, which is more ideal for the quality of the polymer and visual detail of the prints. This holistic study provides forensic scientists with more optimum procedures to obtain fingerprints from surfaces without deteriorating the evidence in the process. This research is funded by the National Institute of Justice Grant: 2015-IJ-CX-K015.

Christy J. Mancuso, James Ehleringer
University of Utah
Evidence Recorded in Fingernails: Oxygen and Strontium Isotopes Reveal Travel Histories

Stable isotope (SI) analysis of keratin tissues have been used to reconstruct geographic location across multiple disciplines. Drinking water contributes to the oxygen isotope (δ18O) signal that reflects geographic locations, while strontium isotope ratios (Sr87/Sr86) come from environmental sources and relate to geologic formations. These SI combinations form unique signatures that can be used to understand an individual's travel history. This study focuses on the SI patterns of fingernail clippings from residents of the Salt Lake City, Utah region (SLC) that traveled to locations outside of the USA before returning home (NIJ STEM Graduate Fellowship, 2014-DN-BX-0003). We hypothesized that the δ18O and Sr87/Sr86 would change, as precipitation SI values and geologic formations differed between their region of travel and SLC. The δ18O values were consistent with reported travel histories. SI values were similar to SLC residents when the volunteers resided in the area and as they moved to their new locations they diverged. In contrast, Sr87/Sr86 of the fingernail clippings displayed different patterns. We found that the Sr87/Sr86 reflected the location of where the volunteer clipped their nails, resulting in an unexpected pattern that was attributed to how the isotopes are incorporated into keratin protein. δ18O are incorporated during the protein formation and once formed the isotope signature does not change. Our findings suggest that Sr87/Sr86 are incorporated into the fingernail keratin through environmental or bathing waters and reflect an individual's most recent location. As one of the first multi-isotope studies on human fingernails, we report on new travel-related isotope signals that can be obtained from δ18O and Sr87/Sr86 measurements. The study has also allowed us to look into greater detail at isotope incorporation into fingernails and how these values can vary among individuals.

Shannon T. Krauss[1], Ryan M. Aubrey[1], James Landers[1], Aeren Q. Nauman[2], Brain Root[1]

University of Virginia[1], TeGrex Technologies[2]

A Centrifugal Microfluidic Device with On-Board Reagents and Smartphone Colorimetric Detection for Narcotics and Explosives Identification

Rapid screening of narcotics and explosives is critical for ensuring public safety and controlling crime, especially at the state and local levels of law enforcement. Colorimetric reactions have been extensively used for on-site explosives and narcotics analysis as a rapid, user-friendly, and inexpensive detection platform. Although current colorimetric field methods enable on-site testing, such methods rely solely on subjective interpretation of color with a variety of operational issues including poor training, differences in color interpretation, varied chemical response due to improper mass of sample, all with no multiplexing capability. For an enhanced system to be a major improvement, it must be fully automated to reduce subjectivity, inexpensive, handheld, capable of rapid screening and parallel processing, and include an on-board optical detector. Here, we describe a centrifugal microfluidic system that accepts single-use, disposable microchips that, with embedded reagents, costs <$1 and are compatible with a modified Sony Discman® to drive fluid flow and chemical reaction. An integrated Android cellphone functions as the colorimetric detector with a custom-built 'app' for interpreting the average pixel color density and associating it with a specific narcotic or explosive. This prototype system (microdevice, instrument and smartphone) was used for multiplexed testing for the presence of various explosive and narcotics material from a single input, such as cocaine, methamphetamine, TNT, and ammonium nitrate. Color analysis was used to determine quantitative hue values to associate with positive results for each analyte of interest. These threshold values were then applied to the custom-built cellphone app for user-friendly analysis.

Jennifer Szekely, Raquel Green, Cara Lewis, Sarah Seashols-Williams, Michael Valle
Virginia Commonwealth University

Developmental Validation of a miRNA Panel for Forensic Body Fluid Identification

MicroRNAs (miRNAs) are small non-coding RNAs 18-25 nucleotides in length that have been evaluated as potential markers for the identification of forensically relevant body fluids. Due to their short length and high resistance to degradation, they provide potential for robust detection in degraded samples. High-throughput sequencing (HTS) of eight forensically relevant biological fluids was used to identify miRNAs with tissue-specific expression. Candidate miRNAs were developed and the expression patterns were assessed, identifying a panel of miRNAs plus two endogenous reference miRNAs that allow for normalization of expression without evaluation of the RNA or known input quantity. This panel uses expression detection through reverse-transcription quantitative PCR (RT-qPCR) to differentiate feces, urine, blood, menstrual secretions, and saliva. Candidates for vaginal secretions and perspiration did not readily prove to be reliable, so additional screening for distinguishing miRNAs was conducted. Biological fluid identification was found to be reliable across population samples of mixed ages, ethnicities, and gender, and detectable at picogram-level RNA quantities. Performance of miRNAs in DNA extractions for body fluid identification was assessed and compared to paired RNA extracts. Detection in compromised samples, limit of detection, and species specificity was evaluated according to developmental validation guidelines. Future work will include expansion of population samples and further validation of the markers. Upon completion, this microRNA panel has the potential for rapid and inclusive discrimination of the body fluids encountered in forensic evidence, and capability for rapid implementation. This project was supported by Award No. 2016-DN-BX-0163, awarded by the NIJ, Office of Justice Programs, U.S. DOJ to SJSW. The opinions, findings, and conclusions or recommendations expressed are those of the authors and do not necessarily reflect those of the DOJ.

Hergen Eilers, Benjamin R. Anderson, Natalie Gese, Ray Gunawidjaja, Steven Livers
Washington State University

Temperature Sensors for Forensic Fire/Arson Investigations

We are developing particle-based luminescent temperature sensors that can be embedded in building materials such as paint, and be used in case of fire and arson investigations to help determine the temperature and heating duration that occurred during the fire. In addition, these sensors can also be used during testing in fire laboratories for a better understanding of surface temperatures. The motivation behind our development is twofold. First, as part of a fire/arson analysis, investigators are often trying to determine temperature and burn-time, as this information can provide clues to how and where the fire started and how it evolved. Investigators use indicators such as color and deformation patterns of various materials, including discolored or melted metal, crazed glass, depth of char, spalling, etc. to extract temperature and burn-time information. However, in many cases these indicators are ambiguous at best and false at worst. Second, fire tests under controlled conditions typically use thermocouples to measure and record temperatures. While they work well to measure gas temperature, they have some inherent disadvantages for measuring surface temperatures. Our sensors consist of lanthanide-doped metal oxide precursors. As these materials are heated, they undergo irreversible transitions such as decomposition, nucleation, grain growth, and phase transitions, changing the crystal field around the lanthanide dopants. Upon excitation with an appropriate wavelength, the sensors emit light that is characteristic of the dopant. However, the specific wavelengths, line widths, intensities, intensity ratios, etc. depend strongly on the condition of the host material. As the sensor material undergoes temperature-induced changes, the emission properties change. Using laboratory-based calibrations, temperature and heating duration can now be determined.

Justin Wade Firestone, Myra Cohen, Massimiliano Pierobon
University of Nebraska Lincoln

Building Assurance Cases for Synthetic Biology to Validate Functionality and Detect Illegal Use

Recent research advances in modifying and controlling cells' DNA have created a booming field of biological engineering called synthetic biology. In synthetic biology, engineers manipulate and modify living organisms to alter their functionality, sometimes creating entirely novel behaviors. Early successes have shown promise in developing new fuel sources, pollution mitigation, and intelligent drug delivery systems. In synthetic biology, designs are first built using biological modeling and then implemented in a laboratory. These synthetic organisms can be considered living programs that can sense, respond, and interact with humans while they persist in the natural environment. We argue that we should view these as safety-critical devices, which should be regulated and certified in order to assure their safety and restrict the potential for illegal use. Because synthetically engineered organisms follow a cycle of reproduction and replication, they can mutate, and adapt to environmental changes and evolve new behaviors over time. In this poster, we propose using an assurance case, an argument structure often used in other safety-critical systems to reason about safety, and we introduce an orthogonal dimension we call the 'Assurance Timeline.' The Assurance Timeline can be used to reason about the dynamic, evolving aspects of these systems. We present a case study based on a real application to illustrate our ideas.

Glen P. Jackson, Mayara P. Matos
West Virginia University

Using Stable Isotopes to Determine Class Characteristics of Human Hair Donors and the Carrion Source of Blow Flies

We describe several chemical methods of hair analysis that can be used to provide characteristic traits about human donors. In one approach, the abundance of amino acids in hair is used to predict sex, age and geographic origin of a hair donor, or to distinguished diabetic patients from a control group. In an alternative method, stable carbon isotope ratios of amino acids in hair is used to classify donors based on body mass index and age group, among other traits. In both approaches used here, human hair from known donors was

first washed and hydrolysed using acid hydrolysis. In one approach, the absolute abundance of the amino acids in human hair was determined by GC-MS of derivatized free amino acids from the hair hydrolysates. In the second approach, bulk and amino-acid-specific carbon isotope ratio analysis was used as input variables for classification. Statistical techniques such as canonical discriminant analysis (CDA) were used to overlook the covariance of amino acid values between individuals caused by dietary factors and instead highlight the selective differences caused by grouping factor(s) such as age, body mass index and sex. Using leave-one-out cross-validation, CDA is able to predict the body mass index of donor's hair sample with about 80% success rate. Using leave-one-out cross-validation, age group and sex can be predicted with better than 80% success rate. The compound-specific isotope ration approach has also been applied to the analysis of amino acids in blowflies. We show that blow fly larvae, pupae and adult flies can all be linked to specific meat sources (carrion) because the extent of fractionation for each amino acid is either negligible or reproducible. This latter work demonstrates that blowflies can be used as a proxy for human flesh because the carbon in the flies originates in a predictable way from their human host.

Carolina Frasson, Thiago Paixao, William Reis
University of São Paulo
Electrochemical Detection of Picric Acid Using a Laser Scribed Paper-Based Device
Picric acid (2,4,6-trinitrophenol) is a military explosive used for the manufacture of weapons and fireworks. The detection of this compound is highly relevant for forensic investigations, national security and environmental health application.{[1,2]} In this present work, the explosive detection was performed using a new paper-based carbon device fabricated using a CO[2] laser scribing process, without the need of chemical reagents or controlled environment conditions. The disposable sensor shown good electric conductivity and a design comprising all the three electrodes pattern (counter, working and reference). For the electrochemical characterization, Differential Pulse Voltammetry (DPV) method was optimized in order to achieve the best analytical figures of merit to quantify the picric acid. The best DPV parameters (Step (ΔE[s]) and Amplitude Potential (ΔE[a])) were ΔE[s] = 9.0 mV, ΔE[a] = 70.0 mV. The interference of some well-known interfering species such nitrate, sulfate and iron were evaluated and non-interference was observed for picric acid detection. The analytical curve under the optimized conditions ranged from 0.48 to 6.9 mmol L{-1} and limit of detection was estimated as 0.16 mmol L{-1}. Financial support: FAPESP, CAPES and CNPq.

Benjamin Figueroa
University of Washington
Label-Free Chemical Imaging of Latent Fingerprints with Stimulated Raman Scattering Microscopy
Analyzing sensitive materials such as latent fingerprints (LFPs) has always been of paramount importance to forensic scientists both for the morphological visualization of the ridge patterns left behind by an impression and for the extraction of chemical information on foreign material. Because fingerprints have long been the gold standard for personal identification in forensic investigations, methods for cultivating and enhancing the visualization of LFPs are continuously evolving. One important challenge is to identify suspicious chemicals present in fingerprint residues, which requires chemical imaging capability. Recently, vibrational spectroscopy has shown that LFP analysis through tape-lift, Raman mapping, and multivariate data analysis presents a useful tool for forensic investigation. However, there are still major difficulties in terms of acquisition speed, poor spatial resolution, and lack of sensitivity. In this presentation, I will demonstrate the feasibility of using non-destructive, label-free stimulated Raman scattering (SRS) microscopy to quickly and easily extract LFP patterns from different substrates. Contrary to what has been reported, no obvious fingerprint degradation or lipid diffusion is observed with either glass or stainless steel substrate. Importantly, we demonstrate that trace exogenous chemicals can be detected in fingerprints.

Furthermore, we present a novel approach of chemically imaging an LFP directly off transparent adhesive tape, bypassing the need for dusting and staining to further simplify visualization and eliminate cross contamination.

Katlynn Agosta[1], Eric B. Monroe[2], Thomas Spudich[1], Kelsey Vancil[1]
Maryville University[1], Library of Congress[2]
Determination of Trace Metal Content in Paper Using Inductively Coupled Plasma- Optical Emission Spectroscopy for Analysis of Historic Papers
Library and museums preserve a wide range of paper materials from their collections. Knowing the composition of the paper, including elemental composition, greatly helps to improve their ability to preserve paper-based objects from the collections. Paper from a collection of books, for instance, may be tested using small (0.1-0.2 g) samples. The results of trace metal analyses can shed light into the production methods used to produce the paper as well as indicate potential problems as some elements are known to induce degradation. A method developed for forensic applications was utilized to examine samples from the William James Barrow book collection, which contains books from the years 1507 to 1899 that have been studied both by Barrow's lab in the 1960's as well as the Library of Congress as part of an ongoing research program. Pages were tested with both text and without text to account for any metals within the ink that was used. Samples from 12 different books from different years were digested and analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy and then the data was compared for any differences across the examined materials.

Ilaria Ferrante, Abdenour Achour, Massimo Santoro, Nicola Watson, David Wevill,
Markes International
Rapid Analysis of Fire Debris for Arson Investigation Using Micro-Chamber/Thermal Extractor Sampling with TD–GC–MS
The fire debris analysis offers vital evidence to a forensic investigation. It helps to validate any suspicion of the intentional use of ignitable liquids to initiate a fire or accelerate it. Analysis of materials from crime scenes requires reliable sample preparation in order to efficiently determine the presence of ignitable liquid residues (ILRs). Several well-established methodologies have been approved by the American Society for Testing and Materials (ASTM). Although recent various improvements, some of these methods still have associated drawbacks. In this study, we present results of the application of Micro-Chamber/Thermal Extractor (µCTE) to the analysis of ignitable liquids (diesel and gasoline) in fire debris. We tested ignitable liquids on two substrates: Wooden dowel and Tea towel cloth. We determined that the µCTE not only has the advantage of rapid sampling but allows to have a great level of control over the sampling conditions for more consistent profiles. Moreover, it offers high sensitivity advantages associated with pre-concentration thermal desorption (TD) and gives the analyst the ability to easily modify the method and develop ideal sampling conditions depending on the suspected accelerant or the material being analyzed. Ensuring legally defensible evidence in arson investigation, sample re-collection option allows to achieve multiple re-run of the same sample. Furthermore, it conserves both the fire debris sample and the TD tube. We also assessed the carryover effect for the diesel and gasoline samples prior to the next sample analysis and the results were less than 0.050%, which could be considered difficult for other systems.

Heather Jordan[1], Mark E. Benbow[1], Zachary M. Burcham[1], Jennifer L. Pechal[2], Carl J. Schmidt[3]
Mississippi State University[1], Michigan State University[2], University of Michigan[3]
Postmortem Microbiomes: Transformative Progression in Forensic Science
This presentation will overview progressive data from NIJ-funded research using culture-based methods, high-throughput molecular analyses, and innovative collaborations between scientists and forensic

practitioners investigating changes in human post-mortem microbiome (HPMM) structure and function, to correlate decomposition of a once living host, and estimated PMI. Part one of this presentation describes HPMM community, transmigration, and gene expression of commensal bacterial species in a controlled setting using quantitative PCR, and next generation sequencing. Results suggest bacterial transmigration and expression, and specific microbiome profiles may be sufficiently predictable for estimating a PMI range. Part two focuses on describing data and demographics of a HPMM Database. In a first-of-its-kind effort, we have collected swab samples from over 1,000 bodies received during routine death investigations from partnership with a major, metropolitan city medical examiner's office in Michigan. Metadata and demographics to be discussed for the database are as follows: sex, ethnicity, age, location at the time of discovery, date and time pronounced dead, the autopsy date, stature of a body, the manner and cause of death, and the estimated PMI range. Also, microbiomes of a subset of 120 cases show important associations of specific microbial communities and individual taxa with PMI estimates and manner of death. Results suggest that the HPMM is highly variable when evaluated within the context of routine case investigations of a large city population, but that HPMM profiles emerge that are potentially useful for forensic science. Altogether, this project greatly expands on early HPMM studies, and demonstrates potential real-world utility of microbes in forensic science. Samples from this largest known human postmortem microbiome dataset to-date will serve as the foundation for further studies and research into the microbiome after death.

Tracy-Lynn Elise Lockwood, Marya Lieberman
University of Notre Dame

Development of a Paper Analytical Device for the Detection of Illicit Drugs

The presumptive drug tests currently used by law enforcement officers in the field are often critiqued by the public due to their high false positive rates and subjective interpretation. Each test pouch uses a colorimetric chemical reaction to identify the illicit drug, however, many other substances such as cutting agents can interfere with the reagents. To combat this, a paper-based analytical device (PAD) has been developed and combines 12 colorimetric tests to create a 'color- barcode' for each illicit substance. The PAD uses less solid than current presumptive tests, costs less than the test pouches, and can be used in fewer than 5 minutes. A portable light box ensures consistent lighting on the PAD so the colors can be read accurately regardless of the environment. A limit of detection study for cocaine, crack cocaine, heroin, and methamphetamine and a blinded sample study will be discussed." David Nash, University of Central Florida, Low-Cost Handheld and Cloud-Based Data Analysis for Improved Identification of Substances of Abuse, “For decades, law enforcement officers have been using color tests to presumptively identify suspected drugs. In recent years, color tests have received negative media coverage for false positives resulting from testing donut glaze, dry wall, sugar, tea leaves, soap, and other non-illicit substances. Handheld Raman devices have shown to be a superior testing method, but the price for one device is not affordable for most law enforcement agencies. This work stems from prior NIJ-funded research and seeks to further develop a drug-indicating test strip, handheld fluorescence spectrometer to analyze the test strip, and a mobile app with access to a reference database of drug standards. The drug-indicating test strip is made using copper (I) iodide, a compound known to form fluorescent products with amines (specifically alkaloids). The copper (I) iodide testing method yields unique photoemission data for each substance based on its chemical structure, allowing for the discrimination between similarly-structured controlled substances and designer drugs. With the help of data matching software, this method will hopefully provide law enforcement officers with a better method for presumptive field drug testing and crime lab analysts a method for high-throughput drug screening.

Nagapratima Kunapareddy

US Naval Research Lab

Controlled Substance Detection with Multi-Wavelength Resonance-Raman Spectroscopy

The Swept-Wavelength Optical resonance-Raman Device (SWOrRD) is an NRL patented spectroscopy platform that combines the advantage of resonance-Raman spectroscopy with multi-wavelength operation. This instrument rapidly illuminates samples at multiple laser wavelengths from deep-UV to the near-infrared and is used to generate two-dimensional Raman signature maps of materials. We have obtained Raman signature maps of controlled substances and cutting agents using deep-UV illumination wavelengths. Illuminating at multiple wavelengths not only provides information about the resonance behavior of target substances but also can potentially provide better identification of these substances in mixtures. We will discuss sample characterization, sample degradation considerations and limits of detection. The goal of this research is to use SWOrRD as an analytical technique for the rapid identification of controlled substances in mixtures.

Ayari Takamura[1], Tomoko Akutsu[1], Takeaki Ozawa[2], Ken Watanabe[1]

National Research Institute of Police Science (Japan)[1], The University of Tokyo[2]

Non-invasive Identification of Body Fluid Samples Using Infrared Spectroscopy and Innovative Chemometric Strategies for Forensic Applicability

Vibrational spectroscopy has shown great potential to identify body fluid (BF) samples non-destructively, based on the characteristic spectral patterns. In order to improve the applicability to more practical forensic demands, we explored multivariate spectral analysis for objectively discriminating BFs, involving soft response against unexpected samples and robust response against sample-aging. ATR FT-IR spectra from five types of BFs, blood, saliva, semen, urine and sweat, showed the characteristic spectral patterns with donor- and spatial-variations. A multivariate discriminant modeling method, Partial Least Squares-Discriminant Analysis (PLSDA), enabled to classify the spectral patterns of five body fluid types correctly. In addition, the combination with Q-statistic technique after PLSDA spectral regression allowed the soft response to exclude unexpected sample's spectra as outliers. Aging for several months caused distortion of the spectral patterns of BFs. The standard multi-class discriminant model constructed with fresh BFs' data was not effective to discriminate the aged BFs due to lack of learning of aged BFs' spectra and low efficiency of modeling. Then, we proposed a novel model structure using dichotomous classification tree, where a two-class discriminant model was equipped at each node. The proposed model built with fresh BFs' data drastically improved the discrimination accuracy for aged BFs. Moreover, the novel model was beneficial to reduce the experimental cost for model construction against sample-aging. The combination of vibrational spectroscopy and chemometric strategies aiming for forensic requirements provides an innovative approach to identify BFs non-invasively with advantages of objectivity and soft and robust applicability. This work was supported by the Japan Society for the Promotion of Science (Grants-in-Aid for Young Scientist (B) 17K18380 to A. T. and Grants-in-Aid for Scientific Research (S) 26220805 to T.O.).

Katherine Gettings, Lisa A. Borsuk, Peter M. Vallone

National Institute of Standards and Technology

STRSeq: A Resource for Sequence-Based STR Analysis

The STR Sequencing Project (STRSeq) was initiated to facilitate the description of sequence-based alleles at the Short Tandem Repeat (STR) loci targeted in human identification assays. STRSeq data are maintained as GenBank records at the U.S. National Center for Biotechnology Information (NCBI)). Each GenBank record contains: observed sequence of an STR region, annotation of the repeat region ('bracketing' consistent with the guidance of the International Society for Forensic Genetics) and flanking region polymorphisms, information regarding the sequencing assay and data quality, and backward compatible

length-based allelic designation. STRSeq GenBank records are organized within a BioProject at NCBI (www.ncbi.nlm.nih.gov/bioproject/380127), which is sub-divided by Commonly used autosomal STR Loci, Alternate autosomal STR Loci, Y-chromosomal STR loci, and X-chromosomal STR loci. Each of these categories is further divided into locus-specific projects. The BioProject will initially contain aggregate alleles across 4,612 samples submitted by four laboratories: National Institute of Standards and Technology (NIST, the project organizer), University of North Texas Health Sciences Center, Kings College London, and University of Santiago de Compostela. In addition to providing a framework for communication among laboratories, the ability to search the BioProject can be leveraged as QC for rare sequences encountered in forensic casework. Future plans for this NIJ-funded effort include a pathway for researchers to submit additional alleles and customized interface tools.

Amy Sorensen Holmes, David Gangitano, Sheree Hughes-Stamm, Madeline Roman
Sam Houston State University
Alternate Methods for Collection, Preservation & Processing of DNA from Decomposing Human Remains
Natural and man-made mass disasters often result in large numbers of casualties. One of the most important considerations following a mass fatality event is victim identification. However, identification efforts may be complicated by harsh environmental conditions, limited facilities, loss of electricity and refrigeration. If remains cannot be stored or identified quickly, the body decomposes and the DNA in those tissues degrades making DNA typing more difficult. This project investigated the effectiveness of various in-field methods for collecting DNA from decomposing human remains. In addition, several alternate DNA preservation, purification, and amplification strategies were also tested in order to facilitate faster, more direct DNA identification. Skin and tissue samples were collected from three decomposing human cadavers over a two-week period at the Southeast Texas Applied Forensic Science Facility in Huntsville, Texas. Three protocols were used to collect DNA from decomposing cadavers in the field: 1) swabbing skin with 4N6FLOQSwabs, cotton, and foam swabs, 2) inserting a swab into a small incision in the thigh, and 3) skin/muscle biopsy. Biopsy punches were compressed onto FTA Elute cards prior to storage or stored in a liquid tissue preservative that facilitates leaching of DNA into solution for quicker and more direct amplification. All samples were stored at room temperature for one month. Samples were processed in two ways: 1) standard forensics DNA workflow including DNA extraction, quantification, amplification, and capillary electrophoresis and 2) rapid purification and/or direct amplification. DNA quantification and STR typing data will be presented in order to compare the success of each sampling, storage, and processing strategy. The novel combination of in-field sample collection methods, tissue preservation and more rapid, direct sample processing has great potential for forensic application and ultimately criminal justice investigations.

Brian Musselman, Joseph Tice, Frederick Li
IonSense
Rapid Screening of Controlled Substances Utilizing an Integrated Direct Analysis in Real Time (DART) Mass Detection System in a Mobile Configuration
Routine screening techniques are employed by crime labs for identifying controlled substances in seized drug samples and determining the samples that need to be further analyzed using confirmatory techniques. These screening techniques commonly include quick colorimetric tests and UV or fluorescence spectrometry, all of which falls under Category C in SWGDRUG's categorization scheme. Although these techniques are rapid, they have the lowest potential discriminating power. In order to increase laboratory efficiency and turnaround time, screening techniques with higher potential discriminating power is desirable. Use of a Direct Analysis in Real Time - Mass Spectrometry (DART-MS) system for more accurate screening for controlled substances with speeds comparable to techniques such as color tests and FTIR is presented. In order to utilize less accurate mass data for these measurements we have developed a

DART-MS database that is up-to-date with the most current drugs. All data were acquired using a more cost-effective platform for use in forensic laboratories. We demonstrate here a program that enables the creation and search of the new low resolution DART-MS databases. A continual effort is being made to develop the database to include a wide array of controlled substances and drugs of abuse using certified reference materials for approximately 250 substances, which includes primarily opioids, cannabinoids, stimulants, benzodiazepines, novel psychoactive compounds. Preliminary tests thus far have shown no false negatives and all false positives were attributed to isomeric compounds. Potential for use of this system in the mobile laboratory setting using a thermal desorber and real time analysis software will be discussed.

Joseph Aldstadt, Ryland J. Bullis, Elliot S. DiMilo, Kelsey A. Holbert
Univeristy of Wisconsin-Milwaukee
Development of a Novel Benchtop SIA-Proton NMR Method for the Determination of 'Bath Salt' Amphetamines

We describe the development, optimization, and application of a novel hyphenated technique, Sequential Injection Analysis-Nuclear Magnetic Resonance Spectroscopy (SIA-NMR). SIA-NMR combines the automated sample pretreatment capabilities of SIA with the qualitative power of proton NMR to create a high throughput, small footprint and low cost instrument with applications in a variety of areas, including forensic science. Over the past decade, the emergence of 'New Psychoactive Substances' (NPS) such as synthetic cathinones have created a confounding problem in forensic analysis. Small structural modifications to traditional psychoactive substances circumvent laws and foil presumptive testing techniques, such as color tests, thin layer chromatography, and infrared spectroscopy. Historically, NMR has been underused in the forensic laboratory primarily owing to its poor sensitivity and selectivity as well as its high cost and large footprint. Recently, low-field benchtop proton NMR spectrometers have emerged; however, their small footprint is also accompanied by relatively poor sensitivity and selectivity. In addition to interfacing SIA to proton NMR, we are studying the use of solid-phase extraction (SPE) and liquid-liquid extraction (LLE) in the SIA manifold to pre-concentrate samples and remove potential matrix interferences. We found strong cation-exchangers to be optimal SPE sorbents for amphetamines such as the cathinones, and we will describe the optimization of the SIA-SPE method in terms of loading and elution conditions. We will also describe our results for on-line LLE, particularly in terms of phase selection, extraction conditions, and system timing. NMR spectra of NPS simulants were also obtained to determine the analytically useful peaks in terms of intensity, selectivity, and linearity. This work was sponsored by the Research Growth Initiative at UW-Milwaukee.

Haley A Mulder[1], Blair Curless[2], Michelle R. Peace[1], Justin L. Poklis[1]
Virginia Commonwealth University[1], Mercer University[2]
The Unexpected Identification of 5-Fluoro MDMB-PINACA Commercial Cannabidiol (CBD) 'Therapeutic' E-liquids

Electronic cigarettes were developed as a method for nicotine delivery. When the e-cigarette is activated, the e-liquid is aerosolized and inhaled. Cannabidiol is a significant active ingredient of C. sativa and C. indica and has been purported to have anti-convulsant, anti-nociceptive, and anti-psychotic properties. CBD has not been approved by the FDA for medical purposes. 5-fluoro MDMB-PINACA (5F-ADB) is reported to have high cannabimimetic activity. In 2014, the Drug Enforcement Agency (DEA) reported 2,311 incidents involving medical intervention or death from 5F-ADB, and, in January 2017, placed it on Schedule I. Two commercial e-liquids were submitted to our laboratory for analysis. The same two products and an additional product were then purchased directly from the manufacture. These e-liquids were analyzed using a Direct Analysis in Real Time Mass Spectrometry (DART-MS) and a Gas Chromatography Mass Spectrometry (GC/MS). Active ingredients were identified using commercial standards based upon

retention time and mass spectral comparisons. All the e-liquids were determined to contain CBD. Three of the four were determined to contain 5-fluoro MDMB-PINACA and the other was determined to contain dextromethorphan, a cough suppressant. These e-liquids were advertised to contain only CBD. The website where they were purchased from stated that these produces do not use synthetics or other psychoactive compounds in their products. 5F-ADB can lead to dangerous consequences, particularly where the users are unaware and are using for therapeutic reasons. This project was supported by NIJ Award No. 2016-DN-BX-0150, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice and the National Institutes of Health Award No P30DA033934. The opinions, findings, and conclusions or recommendations expressed in this abstract are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Kevin Dunk[1], Adel Elsohly[2], Daniel Fletcher[2], Matthew Francis[2], Joseph Heanue[1], Hayuta Jain[1], Kevin Limtao[1], Peter Lloyd[1], Kara Lynch[3], Michael Lynn[4], Ayodele Ogunkoya[2], Roger Tang[1], Jacob Wolf[1]
Triple Ring Technologies[1], University of California, Berkeley[2], University of California, San Francisco[3], Hound Labs[4]

Development of a Portable Marijuana Breathalyzer

As the recreational use of marijuana becomes more common in response to legalization, there is a need to address the safety issues associated with cannabinoid impairment behind the wheel and at the worksite. Law enforcement officers and others must have tools to quickly and conveniently assess recent marijuana use and link it to impairment, much like alcohol breathalyzers that allow rapid indication of alcohol impairment. Breath capture of tetrahydrocannabinol (THC) is a new method for testing during the presumed impairment window that offers significant advantages over oral fluid or blood sampling in roadside and workplace environments. We have developed a novel portable instrument that uses fluorescence detection to quantify the amount of THC in breath. We have tested the performance of this system in human studies under institutional review board approval. We will describe the design and operation of the automated instrument and the associated chemistry assay. The system captures breath samples on a substrate, elutes the substrate with a solvent, binds THC with a fluorescent label, and isolates the bound THC for quantifiable optical detection. The device is suitable for use in roadside tests and in ongoing lab studies. Our human studies have allowed us to elucidate factors that affect the amount of THC available for capture from breath. Kinetic data will be presented showing the range of THC levels under various conditions. We demonstrate that the level of detection in breath needed to determine marijuana use throughout the window of impairment is below 50 picograms per liter of breath. Next, we will show results that validate our fluorescence chemistry against gold-standard mass-spectrometry measurements. We have begun testing to establish the correlation between breath levels of THC and impairment. We will discuss the field use of the device in multiple high-speed track tests where we show for the first time breath levels of THC in impaired drivers.

Bert Ungethuem[1], Alexander Bohnhorst[2], Andreas Walte[1], Stefan Zimmerman[2]
Airsense Analytics, Germany[1], Leibniz University Hanover[2]

FAT-IMS - First Results With an Integrated Combination of FAIMS and TOF-IMS

Ion mobility spectrometers (IMS) are systems which can detect many different toxic industrial chemicals in very low concentrations. Their ambient pressure operation makes them suitable for small, portable hand held systems. Other important parameters are selectivity and cost when talking about application out of the lab. In this talk we will address the selectivity of small IMS devices. Two different approaches based on ion mobility are known for separation of ions in an electric field. The classical time of flight measurement at low electric field strength (TOF-IMS) and filtering of ions at asymmetric low and high field strength (FAIMS or DMS). Both methods use different properties of the ions for separation. We investigated the

possibility to combine the two methods in one drift tube. We will present theoretical considerations and numerical simulations of the approach. As well we will present the first measurements with an IMS that utilizes the separation using the TOF approach superimposed by the FAIMS separation. We will demonstrate the ability of the new approach to separate ions, which are not distinguishable in a classical TOF-IMS because of the same low field mobility (i.e. same K0). This approach enables one to combine orthogonal ion separation mechanisms in one drift tube. In the second part of the talk we will address the costs of building an IMS tube. We will introduce a low cost printed circuit board (PCB) IMS. Using this state of the art production technology makes it possible to achieve very low material costs, while maintaining analytical purity and good performance. Based on a low cost IMS we will introduce a new hand held detection system - GDA-P. To extend the detection capabilities of the system the GDA-P includes selectable other orthogonal sensor such as a dedicated electrochemical cell (EC) or alternatively a photo ionization detector (PID).

Shuang Wang, Keyong Hou, Haiyang Li, Jinxu Li, Qingyun Li, Weimin Wang
Dalian Institute of Chemical Physics, China
Portable Acetone-Assisted Photoionization and Chemical Ionization Ion Trap Mass Spectrometer for On-Site Rapid Detection of Illegal Drugs

Novel Aspect: A portable acetone-assisted photoionization and chemical ionization ion trap mass spectrometer was designed and characterized for on-situ analysis of illegal drugs. Introduction: The portability, sensitivity and accuracy are tough issues for mass spectrometry to achieve on-situ analysis. Ion trap coupled with discontinuous atmospheric pressure interface realized the portability. A novel ionization source based on acetone-assisted photoionization and chemical ionization was designed and implemented to realize the high sensitivity and accuracy for on-site rapid detection of illegal drugs. Methods: A commercial 10.6 eV VUV krypton (Kr) discharge lamp with acetone dopant gas was used for soft positive ionization. Liquid and solid sample was collected on a thin film of PTFE, and dry air carry the gaseous samples generated from thermal desorption sampler into the ionization chamber. Acetone is added into the ion source and assists the ionization of the analytes. Results: Twenty-seven different illegal drugs were texted, the ionization source is soft and their characteristic ions are [M+H]+. The analysis time is less than 2 min, and the LODs of the drugs are at pg level for standard samples. The addition of acetone improve the signal intensity as high as 18-fold for methamphetamine. The accuracy of the drug identification is improved by using tandem mass spectrometry analysis, and the pattern recognition of drugs was carried out using precursor ion and characteristic fragments. The qualitative accuracy of illegal drug components in plant-based drug samples (such as opium ointment) was improved. Illicit drugs samples in drink were also detected. Acknowledgements: The National Natural Science Foundation of China (No. 21375129, 21675155), the National Key Research and Development Program of China (No. 2016YFC0800902), the National Science & Technology Pillar Program of China (No. 2013BAK14B04) and partially supported by DICP (Grant: DICP ZZBS201701).

Jose Almirall
Florida International University
Center for Advanced Research in Forensic Science (CARFS),A New NSF/NIJ-Funded Industry University Cooperative Research Center (IUCRC)

The Center for Advanced Research in Forensic Science (CARFS) is an NSF/NIJ-funded multi-university Industry University Cooperative Research Center (IUCRC) program that enables industry-relevant, pre-competitive research via a multi-member and sustained partnerships among industry, academe, and government. FIU's CARFS Center includes faculty and students from five different academic institutions (FIU, Northeastern, George Washington, Texas A&M, and University of South Alabama) with more than

100 faculty and students participating. The Center also includes 21 active industry board members representing large, medium and small companies as well as State/Federal/Local government and non-profits. The CARFS mission is to support and perform cutting-edge, pre-competitive fundamental research in science, engineering, and technology areas of interest to industry that will drive innovation and the U.S. economy. The IUCRCs offer a platform for significant leveraging of financial investment by members to accelerate the forensic science knowledge base in emerging technologies and manufacturing sectors and develop an industrially savvy workforce to benefit the forensic science enterprise and the US economy. The CARFS have funded more than 25 faculty with ~ $ 500.k of seed funding to initiate 19 research projects of interest to the industry advisory board. To read more about CARFS, please go to: www.forensic-research.org A summary of the CARFS, IUCRC and a list of the funded research projects will be described.

Georgiana Gibson-Daw, Bruce McCord
Florida International University
Rapid Direct PCR: A Method for Complete Sample Processing Obtaining STR Based Genotypes in 15 Minutes or Less

It is often extremely important to rapidly screen suspect samples at border controls and police stations where the individuals in question can only be detained for short periods of time. Current DNA typing methods provide the best biometric information yielding identity, kinship and geographical origin, but they are not sufficiently fast to permit identity of a suspect DNA in real time. Current rapid DNA systems take about 90 minutes and involve complex extraction and analysis procedures. This time for sample processing can be greatly diminished with the use of rapid and direct PCR methods which make use of new mutant polymerases, designed for increased processivity as well as resistance to inhibitors, and new thermal cycler designs can make the analysis even faster. With the combination of these enzymes and instruments we have created a method for multiplexed PCR amplification in under 10 minutes. When coupled with microfluidic electrophoresis, we can produce complete genotypes in 15 minutes. To do this we have been testing specially engineered enzymes Z-taq and Omnitaq along with direct buffer systems to rapidly amplify a 7 loci STR multiplex with no extraction step needed. The designed multiplex includes D5S181, D13S317, D7S820, CSF1PO, D16S539, Penta D and Amel which have sizes between 106 and 454 bp in size. By using off the shelf components and commercially available enzymes it is possible to create a procedure that acts as a quick, highly informative sample screening process that also retains sufficient DNA for later manual processing using standard STR. The results of this study demonstrates the application of ultrahigh speed direct PCR for the successful amplification a 7 loci multiplex. With such a procedure in place any crime lab can produce a nearly instantaneous genotype from buccal samples. Additionally instruments are easily portable and so genotypes could also be obtained from onsite locations such as airports of sites of mass disasters.

Nelyan López Pérez, John A. Howarter
Purdue University
Non-Destructive Technique for Body Armor Lifetime Predictions

Ensuring the performance and safety of body armors during service is crucial for institutions such as the United States National Institute of Justice (NIJ). The NIJ's commitment to safety prompts the research of the degradation of high performance fibers like Kevlar, Twaron and Dyneema. Degradation, the reduction of the mechanical properties such as strength and toughness, occurs when a material is exposed to various common environmental factors like sweat, humidity, temperature and sunlight. In order to understand how one of these factors affects fibers, it is isolated from other. Currently, the effects of water and pH are studied. The amount of water and its effects on the fibers mechanical properties are tested through tensile testing and dynamic mechanical analysis (DMA). Single fibers and yarns are tested through these destructive

methods, however, a non-invasive method that uses anti-particles can be used to detect changes in the structure of fibers as they deteriorate. Positron annihilation lifetime spectroscopy (PALS), emits positrons (the antiparticle of an electron) which reside in low electron density areas such as the free volume of polymers and voids. Once a positron interacts with an electron, they bound and form a new particle called the positronium. The time between the emission of a positron and the detection of positronium particle is related to the size of the low-density area. As the fibers degrade, areas such as the free volume also change in dimensions. A distribution of the size and amount of free volume is offered through PALS, which can then be used as a complementary technique to the previously mention mechanical tests. Comparing the changes in free volume to those in the mechanical properties gives a broader understanding of the degradation process and rate for high performance fibers, and can later be implemented for the lifetime prediction of new and used body armors.