Our Trail Onward: Mentoring the Next Generation of Researchers in Forensic Science

2014 Forensic Science R&D Grantees Meeting Agenda

Tuesday, February 18, 2014
Grand Hyatt, Princessa II

Welcome and Opening Remarks
8:00 - 8:15 am

Gerald LaPorte
Acting Director
Office of Investigative and Forensic Sciences

Morning Session I – Off the Beaten Path: Non-Standard Forensic DNA Research
8:15 am - 9:55 am

8:15 am	***Development of DNA-Based Multi-Species Identification and Quantification Assays*** Jillian Ng, Regents of the University of California
8:40 am	***Characterization of X chromosomal short tandem repeat markers for forensic use*** Toni Diegoli, American Registry of Pathology
9:05 am	***An analysis of microRNA variation and stability in forensically relevant body fluids*** Sarah Seashols & Christina Hayes, Virginia Commonwealth University
9:30 am	***Hair Shaft Proteomic Analysis - Improved Evidence Discrimination?*** Pei-Wen Wu, Regents of the University of California

---

Break
9:55 am - 10:10 am

Morning Session II – Sure Footing: Fundamental Forensic Science Research
10:10 am - 11:50 am

10:10 am	***From Basic Research to Routine Use in the Courtroom: Elemental Analysis and Comparisons of Materials with Laser Ablation Inductively Coupled Plasma Mass Spectrometry*** Jose Almirall & Tatiana Trejos, Florida International University
10:35 am	***Review on some Fluid Dynamics Aspects of Bloodstain Pattern Analysis*** Daniel Attinger, Iowa State University
11:00 am	***Quantitative Metrics of Friction Ridge Detail Derived From Information Theory*** Thomas Busey, Indiana University

11:25 am ***Definition of the concept of sufficiency in latent print examination***
Cedric Neumann, South Dakota State University (*Grantee: PSU*)

Lunch Break – On Your Own
11:50 am - 1:00 pm

Afternoon Session I – Trail Blazing: Novel Techniques and Tools for Forensic Analysis
1:00 pm - 2:40 pm

1:00 pm ***Combining strontium and oxygen isotope ratios of hair for human provenancing***
Brett Tipple, IsoForensics, Inc.

1:25 pm ***The Evaluation of Laser Diode Thermal Desorption (LDTD) for High Throughput Analysis of Controlled Substances and Toxicology in Forensic Sciences***
Nichole Bynum, Research Triangle Institute

1:50 pm ***Fluorescent Metal Indicators for the Presumptive Identification of Substances of Abuse through Visual Inspection and Implementation of a Cell Phone Spectrometer Querying a Cloud-based Database***
Richard Blair & David Nash, University of Central Florida

2:15 pm ***Development of Paper Microfluidic Devices for the On-Site Detection of Improvised Explosives***
Kelley Peters, Florida International University

Break
2:40 pm - 2:55 pm

Afternoon Session II – Map It Out: Models in Forensic DNA & Pathology
2:55 pm - 4:35 pm

2:55 pm ***Modeling 3D facial shape from DNA***
Mark Shriver, Pennsylvania State University

3:20 pm ***Pediatric Fracture Printing: Creating a Science of Statistical Fracture Signature Analysis***
Todd Fenton & Jen Vollner, Michigan State University

3:45 pm ***A New Pediatric Head Injury Assessment Tool for possible child abuse cases considering subject-specific child head anatomy***
Jingwen Hu, University of Michigan

4:10 pm ***Investigation of the Impact of Body Temperature and Post-Mortem Interval on Magnetic Resonance Imaging (MRI) of Unfixed Tissue***
Natalie Adolphi & Chandra Gerrard, University of New Mexico

Wrap-up & Adjourn
4:35 pm

2014 Forensic Science R&D Grantees Meeting
Grantee Presenter Abstracts

Development of DNA-Based Multi-Species Identification and Quantification Assays

The Regents of the University of California (2008-DN-BX-K288) ^

Species identification of forensic samples allows analysts to select the appropriate tests for evaluating a wide range of biological evidence. Since most short tandem repeats (STR) analyses are species-specific, it is important to know the species origin of the sample before attempting STR typing. Accurate detection and quantification of target DNA in mixed-species samples are crucial when target templates may be overwhelmed by non-target DNA. Additionally, forensic laboratory accrediting agencies have standards for quantifying extracted DNA templates and optimizing typing tests used in casework to minimize the consumption of limited samples. An accurate taxonomic-identification and quantification assay of forensic DNA samples is important for cost efficient analyses of the evidence and will provide an additional means of forensic inquiry.

The goal of this project was to develop a multispecies identification assay that utilizes the strengths of DNA-based taxon identification and simultaneously quantifies the target species' DNA with the accuracy of nuclear markers in a simple and quick multiplex TaqMan® real-time quantitative PCR assay. Our primers and probes were designed to identify sequences within conserved nuclear genes of target species containing sufficient polymorphisms to differentiate one target species from another. We developed two triplexes targeting human-dog-cat and poultry species (duck, chicken, and turkey) and validated each with simulated forensic conditions for species-specificity and limit of detection (determined through dilution, degradation, inhibition, and population studies). Two quadriplexes, the human-dog-cat triplex with the addition of an internal positive control and a livestock (cattle, goat, horse, sheep, and pig with a single target for goat and sheep), were also designed and validated. Using the same approach for animal DNA testing and quantification (i.e., targeting regions of the genomes for species-specific primers and probes), we developed an assay for marijuana species testing and DNA quantification.

The human-dog-cat assays employ primers and probes that interrogate the human THO1 locus and the MC1R locus in dogs and cats in a species-specific manner. The assay can detect and quantify as little as 0.4 pg of human and cat nuclear DNA, respectively, and 4.0 pg of dog nuclear DNA in mixed samples. Our poultry and livestock assays target species-specific regions of nuclear genes (including CD4, MC1R, and ACTB) and have limits of detection and quantification ranging from 5 to 50 pg of DNA. Our marijuana (*C. sativa*) assay interrogates the trnL and trnL 3' exon -trnF intragenic spacer region and can identify and quantify the *C. sativa* species down to 4 pg of DNA. Attempts were made to pool all animal targets into one multiplex assay using high-resolution melt (HRM) technology with promising initial results.

Most assays developed under this grant were thesis projects of master students in the UC Davis Forensic Science Graduate Program. The students were the lead researchers for each assay and were involved in every step of design, optimization, and validation of the assays.

Characterization of X chromosomal short tandem repeat markers for forensic use

American Registry of Pathology (2011-DN-BX-K401) ^

The use of X chromosomal short tandem repeat (STR) markers has been greatly increasing in the forensic setting. The marker system offers the potential to provide information in addition to that obtained from autosomal STR systems currently used at crime laboratories and the courtroom, and in certain scenarios, markers on the X chromosome may be the only means of obtaining this information. In-depth characterization of the marker system is the first step in maximizing the power of this additional tool in the forensic arsenal.

In previous work performed at the Armed Forces DNA Identification Laboratory (AFDIL), two mini-X chromosomal STR multiplexes capable of amplifying 15 total markers were developed. These assays use techniques and instrumentation that is already in use in most laboratories for autosomal STR analysis. Developmental validation of the system was completed and allele frequencies have been recorded in a number of population groups. Therefore, a tool with which to further characterize this marker system already exists.

The 1991 report of the International Society for Forensic Genetics (ISFG) relating to the use of DNA polymorphisms in paternity testing has been used as a guideline for the evaluation of other marker systems such as autosomal and Y STRs. Several requirements of this report remain unresolved for X chromosomal STRs. First, mutation rates must be known in order to adequately handle possible mismatches attributable to mutational events. Second, questions of independent assortment and linkage disequilibrium must be addressed. Both criteria have been thoroughly investigated here through the study of relevant family groups and populations. Additionally, population data generated as a result of these studies is also reported, contributing to the continuing characterization and potential utility of these markers.

Lastly, mixture interpretation is an important part of the forensic scientist's role in evaluating evidence from a crime scene, where mixed stains are common. The potential use of gonosomal STR markers to aid in the interpretation of such mixtures was investigated, and a multiplex was developed and characterized for this purpose. While it is clear from this initial development and characterization study that the MIXplex cannot solve all of the questions surrounding the interpretation of a mixed profile, there are benefits to its use in certain situations that justify continued study.

The opinions or assertions presented hereafter are the private views of the speaker and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the US Army Medical Research and Materiel Command or the Armed Forces Medical Examiner System.

An analysis of microRNA variation and stability in forensically relevant body fluids

Virginia Commonwealth University (2012-DN-BX-K017) ^

Current serological techniques are often subjective, and new molecular based serological techniques could provide a confidence level that is currently lacking in traditional methods. microRNAs (miRNAs) are small non-coding RNAs that regulate cellular processes through modulation of proteins at the translational level. miRNAs are very stable, both because of their small size and also due to the fact that, when they are secreted, they are typically either encased in a protective lipid vesicle or encapsulated in a protein or cholesterol matrix. These properties make miRNAs more resistant to degradation than naked nucleic acids. Thus, it is likely that miRNAs, when fully evaluated, will make excellent candidates for body fluid identification.

Recently, miRNA analysis of body fluids has been the subject of some interest in the forensic community. While blood, semen and vaginal fluid are easily extractable using conventional kits, stains that contain urine, sweat, and feces proved a challenge for the isolation of high quality and quantity of miRNAs.

In order to determine the optimal method for each body fluid, several commercial miRNA isolation kits as well as other described methods were tested on blood, semen, menstrual blood, vaginal fluid, urine, sweat, and saliva samples. The methods were first compared on efficiency of miRNA extraction by total RNA yield through UV spectroscopy, and secondly through an RT-qPCR method for quality assessment. In order to determine the relative abundance level of known endogenous miRNAs and small RNAs amongst individuals, a comparison of several individuals was made for each body fluid. This data showed that of the two miRNAs (miR-16 and miR-21) assayed, relative miR levels in each body fluid varied little (<3 CTs, or 8-fold) regardless of contributor, but as expected, a large variation between body fluids was observed (4-7 CTs, or up to a 128-fold difference). Finally, stability of miRNAs in forensically relevant biological fluids was evaluated through heat treatment, ultraviolet radiation, basic and acidic pH exposure, and detergent treatment. The dried body fluids showed some susceptibility to harsh treatment, but in most cases miRNAs were still detectable. Thus, miRNAs could represent a highly stable species for body fluid identification methods in forensic science.

Hair Shaft Proteomic Analysis - Improved Evidence Discrimination?

The Regents of the University of California (2011-DN-BX-K543) ^

This project explores whether probative information from human hair shaft samples is obtainable by analyzing their profiles of constituent proteins. Such information could increase the value of hair as evidence. While a thorough microscopic examination of hair provides valuable information, the search for more objective criteria by which to judge hair matches continues. Hair evidence is ordinarily supplemented by DNA evidence whenever possible, but in the great majority of cases only mitochondrial DNA from the shaft is available. Information from proteomic characterization is complementary to that from microscopic examination and DNA analysis.

Our hair studies exploit recent advances in protein identification by mass spectrometry coupled with database searching. Initial efforts focused on sample preparation, since the hair shaft is recalcitrant in its native state to protease digestion and contains a substantial fraction (≈15%) of its total protein that is cross-linked by isopeptide bonds through transglutaminase activity. Our results demonstrated that tryptic digestion is effective in generating identifiable peptides from the hair shaft, including the cross-linked material. Thus, in addition to the well-studied keratins and keratin-associated proteins, various other cytoplasmic as well as histone, junctional and membrane proteins are detectable. Subsequently, application of this approach to inbred mouse strains has shown they can be distinguished by their pelage hair proteomes. Mouse strains provide homogenous populations to test the discrimination of analytical methods before addressing the generally highly heterogeneous human population; in this sense, an inbred mouse strain is equivalent to a single individual (with both sexes) from the human population.

In this project, conducted by students in our Forensic Science Graduate Group, the hypothesis has now been tested that the proteome of human hair shaft can distinguish among humans. This hypothesis is based on observations that over 80% of our genes are differentially expressed among individuals. The hair shaft provides a discrete subset of proteins that we compared among subjects of Caucasian, Korean, African and African-American ancestry. Within each ethnic group, differences in protein profiles were substantial, similar to those among inbred mouse strains, and permitted distinguishing among individuals. As a consequence of the high variance among individuals, however, differences between ethnic groups, as judged by pairwise comparisons, were much less marked. In a related study among Caucasian subjects, hair shafts from scalp, axillary, beard and pubic regions were distinguishable in protein profiles.

Present results provide a basis for investigating further the utility of hair protein profiling in distinguishing among individuals. Additional study of population variations may permit further development of databases to distinguish individuals by hair profiles.

From Basic Research to Routine Use in the Courtroom: Elemental Analysis and Comparisons of Materials with Laser Ablation Inductively Coupled Plasma Mass Spectrometry

Florida International University (2009-DN-BX-K252) ^

The chemical analysis and comparison of a number of solid matrices of interest to forensic scientists is made possible by the progression from fundamental research and method development, to validation in several forensic laboratories, the publication of international standards and, finally, to courtroom use and acceptance. The story of high-sensitivity elemental analysis of materials including glass, paint, soils, precious metals, diamonds, paper and ink on paper begins with the reporting of analytical techniques in the scientific literature and continues with the adaptation and optimization of the analytical methods by forensic scientists to suit these specific matrices.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been called the "gold" standard for solid-sampling and high-sensitivity elemental characterization of materials providing sub-ppm detection limits of elemental analytes encompassing almost 70% of the periodic table. In addition, LA-ICP-MS provides true quantitative analysis data that can be used in numerical/statistical hypothesis testing to determine "match" and also to populate databases that are useful to determine the probability of a match for a given elemental profile. The story develops further when several forensic laboratories collaborate on method development and optimization as reported by the European Union funded NITECRIME effort (2000-2005) [1] and continued by the NIJ-funded Elemental Analysis Working Group (EAWG) effort (2008-2012) [2]. The final report includes interlaboratory trials that report high-quality performance of these methods for the analysis of glass in forensic laboratories [3] and the publication of international (ASTM) analytical consensus standards for the examination these materials [4].

More than 30 forensic laboratories around the world now routinely employ the use of LA-ICP-MS for materials characterization on every continent (except Antartica) and the history of elemental analysis provides a good model for how forensic method development should progress from basic research to routine use and acceptance in the courtroom.

1. C Latkoczy, M Dücking, S Becker, D Günther, J Hoogewerff , JR Almirall, JA Buscaglia, A Dobney, R Koons, S Montero, G van der Peyl, W Stoecklein, T Trejos, J Watling, V Zdanowicz, Evaluation of a standard method for the quantitative elemental analysis of float glass samples by LA-ICP-MS, J. of Forensic Sciences, 2005, 50 (6), 1327-1341.

2. T Trejos, R Koons, S Becker, T Berman, J Buscaglia, M Duecking, T Eckert-Lumsdon, T Ernst, C Hanlon, A Heydon, K Mooney, R Nelson, K Olsson, C Palenik, E Pollock, D Rudell, S Ryland, A Tarifa, M Valadez, P Weis and JR Almirall, Cross-validation and evaluation of the performance of methods for the elemental analysis of forensic glass by μ-XRF, ICP-MS and LA-ICP-MS, Anal. Bioanal. Chem., 2013, DOI 10.1007/s00216-013-6978-y.

3. T Trejos, R Koons, P Weis, S Becker, T Berman, C Dalpe, M Duecking, J Buscaglia, T Eckert-Lumsdon, T Ernst, C Hanlon, A Heydon, K Mooney, R Nelson, K Olsson, E Schenk, C Palenik, E Pollock, D Rudell, S Ryland, A Tarifa, M Valadez, A van Es, V Zdanowicz and JR Almirall, Forensic analysis of glass by μ-XRF, SN-ICP-MS, LA-ICP-MS and LA-ICP-OES: evaluation of the performance of different criteria for comparing elemental composition, J. Anal. At. Spectrom., 2013, 28, 1270-1282. DOI: 10.1039/C3JA50128K.

4. (2013) Standard Test Method for the Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons. ASTM (in press).

Review on some Fluid Dynamics Aspects of Bloodstain Pattern Analysis

Iowa State University of Science and Technology (2010-DN-BX-K403) ^

This review highlights some key relationships between the disciplines of bloodstain pattern analysis (BPA) in forensics and that of fluid dynamics (FD) in the physical sciences. The purpose of this talk is to build bridges between BPA and FD, as well as to describe collaborative research opportunities.

While the BPA and FD disciplines each have a rich history, interactions between these communities have never been strong. From its infancy in 19th century Germany, BPA has aimed for practical answers to specific questions: “How did a bloodletting incident happen?” FD was born in the 17th and 18th century, in England, Switzerland and France. In contrast to BPA, FD aims to quantitatively describe the motion of fluids (gases or liquids), and the causes of the motion, with general equations. BPA typically solves the indirect problem of inspecting stains in a crime scene to infer the most probable bloodletting incident that produced these patterns. FD typically defines the initial and boundary conditions of a fluid system, and from there describes how the system evolves in time and space, most often in a deterministic manner. In both the BPA and FD communities, scientists study the motion and phase change of a liquid in contact with air, or with other liquids or solids. We will show that each community could benefit from a deeper understanding of the other. Specifically, BPA can obtain new quantitative tools and methods, while FD may be presented with new flow problems.

This review focuses on three aspects of BPA: first we first describe the physical forces driving the motion of blood as a fluid. We then describe two key phenomena relevant to BPA: the generation of drops and their trajectories. For each of these topics, we review relevant literature from the BPA community, and then from the FD community. We then show the connections between both disciplines, describing how well the problems are understood and what opportunities exist for new research and the development of novel tools and methods.

Quantitative Metrics of Friction Ridge Detail Derived From Information Theory

Indiana University (2009-DN-BX-K226) ^

Standard quantification metrics of friction ridge information rely on hand-coded features such as the location and orientation of minutiae. However, currently human examiners outperform computer-based methods, in part because they may use information such as ridge width, curvature and pore structure. In this project we derive a set of feature detectors using eye gaze data collected while latent print examiners completed comparison tasks. These feature detectors characterize the available information in latent prints, given the detectors and the statistics of the entire database.

The feature detectors themselves provide a level of activation for each location in the print. Although the pattern of activation is relatively non-diagnostic, the inputs can be used to derive a measure of feature diagnosticity using a self-information metric. The self-information metric is an extension of information theory (Shannon, 1948). The basic idea behind the approach is that rare signals are the most informative, and that the feature detectors provide a representation of friction ridge impressions that can be quantified using information theory.

In order define the probability of an event or a feature in a print, one must construct an intermediate representation, akin to an alphabet. We have chosen to use Independent Component Analysis (ICA) as our means to discover the underlying basis set for friction ridge features. These approaches have been widely used in face recognition research. ICA components also produce sparse representations and features that are as independent as possible.

The basis functions can be applied at each location in an image to produce a set of activations for this location. The activation of each basis function can be compared against an overall frequency histogram for this basis function to compute the likelihood of observing an activation this strong. This has been quantified into a self-information metric by Bruce and Tsotsos (2009).

To test this model, we take a new set of fixations from experts on a new set of images and try to predict where the experts will look based on the self-information metric. We can accurately classify the expert fixations (compared with random locations) with more than 80% accuracy given a logistic regression classifier. The eyetracker accuracy places limits on how high performance can rise, and given this it is unlikely we would see accuracy above 90%. Thus the metric is quite good at identifying those regions in a novel print that an expert would find diagnostic.

The success of this approach demonstrates that experts are likely fixating on regions that are most diagnostic, and the visualizations produced by the metric can help novices learn to fixate on regions that experts consider most useful for the task. The metric can also suggest regions that experts should rely on but perhaps may not be, as well as regions that are more common than experts might believe. This is especially important in an age of large databases, where it is becoming more common to find AFIS results that contain highly similar but non-matching prints.

Definition of the concept of sufficiency in latent print examination

The Pennsylvania State University (2010-DN-BX-K267) ^

The examination of friction ridge skin impressions relies on the observation and comparison of various friction ridge features, such as the general pattern of the ridge flow, minutiae, number and location of ridge pores, and shape of ridge edges. The observation and comparison of such friction ridge features between skin impressions recovered on crime scenes and control prints from individuals enables latent print examiners to form opinion on the identity of their source.

Examiners generally claim to rely on the quality and quantity of features present on the latent print, and in agreement/disagreement with the control print to form opinions on the commonality of the source of both prints. Pairs of impressions displaying sufficient features in agreement are deemed to be from the same source; pairs of impressions displaying insufficient information in agreement, but no significant discordances are deemed to be inconclusive; finally, pairs of impressions displaying disaccording features are deemed to be from different sources.

A research project was designed to investigate the level of sufficiency required by more than 150 examiners when comparing 15 pairs of latent/control prints. The results of our study show that examinations' conclusions are strongly correlated with the quality and quantity of minutiae present on the prints, and that there is a general trend between the quantity and quality of information observed during the examination and the resulting conclusion. However, our results also show that the appreciation of sufficiency is extremely variable between multiple examiners considering a given pair of latent/control print; and between different pairs of latent/control prints. In other words, our data show that the decision to identify a person as the source of a latent print, not only depends on the quality/quantity of information present on the latent print, but also on the examiner conducting the comparison.

Unfortunately, it is not possible to firmly correlate the resulting decisions with given levels of quality/quantity, nor with demographic variables (such as training and experience) related to the examiners. And therefore, it is not possible to support the claim that the current implementation of the concept of sufficiency is reliable and reproducible.

This presentation will provide an overview of the results obtained during this study, and will also provide examples of poor practices from some of the participants that have resulted in the misinterpretation and misapplication of the concept of sufficiency when comparing latent and control prints. From these results, it is clear that the solution to better practices in latent print examinations is rooted in a better definition, standardization and documentation of the Analysis stage of the examination process, possibly with the use of tools to quantify the quality and quantity of information present on a latent print. Such SOPs and tools will greatly reduce the observed variability and poor practices, and therefore increase the confidence in fingerprint identifications.

Combining strontium and oxygen isotope ratios of hair for human provenancing

IsoForensics, Inc. (2011-DN-BX-K544) ^

Understanding an individual's travel-history can be an important piece of information in a forensic investigation. The analysis of stable oxygen (O) isotopes of human hair has assisted in the reconstruction of the geographic-movement histories of individuals in criminal investigations. The oxygen isotope value ($\delta^{18}O$) of human hair records the $\delta^{18}O$ value of drinking water at the time a hair segment is formed. Thus, it is possible to get a linear record of an individual's travel. However, depending on the environmental gradients in water $\delta^{18}O$ values, the estimated geographical regions can be spatially narrow or broad. Strontium (Sr) isotope ratios ($^{87}Sr/^{86}Sr$) of human hair can provide a complementary isotopic approach to measurement of ^{18}O in hair for human provenancing, as Sr from local waters is incorporated into hair through washing and showering. Over time, the deposited Sr migrates to the interior of a strand of hair. $^{87}Sr/^{86}Sr$ ratios record independent geographic information and thus further constrain the estimated regions to a subset of locations predicted by $\delta^{18}O$ values.

To understand the linkages between the Sr in keratinous tissues and an individual's environment, human hair and tap waters were collected from fifty-five cities throughout the contiguous United States, and the $^{87}Sr/^{86}Sr$ ratios were measured on paired hair-water samples. Paired tap water and hair $^{87}Sr/^{86}Sr$ ratios were strongly correlated and displayed a 1:1 relationship, indicating little to no fractionation between Sr isotope ratios in water and hair. While hair and leachate $^{87}Sr/^{86}Sr$ ratios were generally significantly correlated, in several hair-leachate pairs, the values were different, suggesting differences between the externally- and internally-bonded Sr in the hair. These differences between external and internal Sr content may be the result of recent travel/movements reflecting variations in the $^{87}Sr/^{86}Sr$ ratio of exogenous Sr water source. Thus, by comparing the $^{87}Sr/^{86}Sr$ ratios of hair and leachate, recent movements may also be detected. Our findings indicate community-specific Sr isotope signals are reflected in the isotope ratios of the hair of individuals, and in turn, the $^{87}Sr/^{86}Sr$ ratios of human hair provides unique geographic information relating to the locality where an individual resides. As the O and Sr isotope systems are both geographically controlled but reflect different physical and chemical environmental processes, we suggest that the paired analyses of both O and Sr isotope ratios in hair allow for greater refinement in reconstructing the travel-movement history of an individual.

The personnel associated with this project have a strong commitment to the forensic science education and mentoring. All students under the tutelage of PI Tipple are measuring the $^{87}Sr/^{86}Sr$ ratios of biologic and geologic materials using techniques developed with this project to address wildlife forensics, anthropologic, and geoforensics questions. To date, several students have been co-authors on peer-reviewed analytical chemistry and forensic science publications and presentations. In addition to direct mentorship, PI Tipple and Professor Ehleringer (Senior Research Scientist at IsoForensics, Inc.) use data collected from this project in multiple general education and stable isotope forensic science lectures.

The Evaluation of Laser Diode Thermal Desorption (LDTD) for High Throughput Analysis of Controlled Substances and Toxicology in Forensic Sciences

Research Triangle Institute (2012-R2-CX-K004) ^

The laser diode thermal desorption (LDTD) source is a high throughput source that allows for ultra-fast sample introduction in mass spectrometry (MS) and can be interfaced to multiple MS platforms from a wide range of instrument manufacturers. As this is a supplemental instrument to expand the capability of an existing MS, it is less expensive for the laboratory to implement than an entire new MS platform. While this technique is finding successful application in many fields, its further use in forensic laboratories requires a careful consideration and demonstration of the validity of this technique.

The purpose of this study was to evaluate the applicability of LDTD in areas of forensic interest. Specifically, we evaluated the instrument's performance as a high throughput source coupled with MS for the detection of controlled substances, including newly emerging designer drugs, drugs used in facilitated drug crimes, and those relevant to postmortem toxicology. The LDTD does not use chromatographic separation therefore it is important to determine whether this technology provides sufficient sensitivity and selectivity to allow for screening and quantification of analytes.

Approximately forty drugs, selected from common drug classes such as antidepressants, amphetamines, and benzodiazepines were extracted from urine and blood by solid phase extraction (SPE) or liquid-liquid extraction (LLE). The samples were analyzed by a Phytronix LDTD source coupled to an AB SCIEX API-4000 MS operated in multiple reaction monitoring mode (MRM). The number of ion transitions monitored for each drug and internal standard were two and one, respectively. Linearity was established by analyzing six non-zero concentrations over five separate runs (n=5). Accuracy and precision were determined by analyzing QC samples at three concentrations (n=15).

The majority of drugs analyzed in this study produced LPA results that were comparable to those accepted in forensic toxicology laboratories. For example, validation results of benzoylecgonine in urine gave a between-run (n=15) and within-run (n=3) %CV of less than 16% and 20% respectively, and overall accuracy (n=45) of 95%. Results of diazepam in urine gave a between-run (n=15) and within-run (n=3) %CV of less than 16% and 19%, respectively and overall accuracy (n=45) of 104%. The use of MRM allows for selectivity of most analytes; however for some drugs further method development (e.g. derivitization) is needed in order to differentiate them from possible interferences.

The LDTD source is a promising new technology to provide forensic laboratories with an option for high throughput sample screening and quantification in the analysis of controlled substances and forensic toxicology. For the majority of drugs analyzed in this study, LDTD produces linearity ranges that are comparable to those obtained by LC-MS/MS. The lack of chromatographic separation can potentially cause problems with selectivity so careful method development and validation are critical to successful implementation of this technology.

Fluorescent Metal Indicators for the Presumptive Identification of Substances of Abuse through Visual Inspection and Implementation of a Cell Phone Spectrometer Querying a Cloud-based Database

University of Central Florida (2012-R2-CX-K005) ^

Conventional methods of presumptive drug testing can yield unreliable results, creating a need for time-consuming confirmatory methods to identify an unknown. We have developed a presumptive test based on luminescent d10 metal cluster compounds, specifically d10 iodides. Chemical compounds that contain amine functional groups, such as alkaloids, form complexes that emit in the visible when irradiated with UV light. Substances of abuse in which conventional methods yield indistinguishable results (i.e. cocaine, phencylcidine, and diphenhydramine) or no results at all (i.e. benzylpiperazine) can be identified using this test. This method can be used to screen unknown sample and streamline the application of confirmatory tests. We have also developed a 3d-printed spectrometer that can be used with the camera on a smart phone to obtain the emission spectra. An Open Source app is used to analyze and compare the emission spectrum to those recorded in a cloud-based database that contains the spectral information for commonly encountered substances, the identification of the unknown sample can be rapidly determined.

Development of Paper Microfluidic Devices for the On-Site Detection of Improvised Explosives

The Florida International University (2012-DN-BX-K048) ^

In recent years there had been a dramatic increase in the use of improvised devices due to better controls placed on commercial and military explosives. In addition, a large number of procedures describing methods to prepare improvised explosives have been placed on the internet making it easier for terrorist and amateur bomb makers to use them. Therefore, police and forensic evidence collection teams need a rapid, inexpensive, simple method in order to identify these types of materials in the field. Commonly used materials for this type of explosive preparation include fertilizers and industrial chemicals containing oxidizers such as chlorates, perchlorates, and nitrates as well as other less stable compounds, such as peroxides. Due to these materials having a wide range of volatility, polarity, and composition, multiple analyses must be run in a lab in order to identify the explosive material. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) can be used to identify organic compounds while ion chromatography (IC) and capillary electrophoresis (CE) can be used to identify inorganic ions. Metals can be detected using scanning electron microscopy/elemental diffraction spectroscopy (SEM/EDS) or x-ray diffraction (XRD). These devices are large, expensive pieces of instrumentation that are not portable, requiring the sample to be sent to a laboratory for testing. This process increases the amount of time before any analytical information on the identity of the explosive can be provided to on-site personnel.

The use of paper microfluidics allows for the development of very inexpensive analytical devices based on designs printed in wax-based ink on chromatography paper. These wax designed channels can direct liquid samples toward individual sections of the paper containing colorimetric test reagents. Based on the design, a simple device can perform five or more simultaneous analyses while costing very little, since the basic design components (paper, wax, and small quantities of reagents) are all very inexpensive. These devices can also be easily stored for long-term performance and due to their low cost could revolutionize on-site forensic testing.

The paper microfluidic devices are capable of detecting nitrate, nitrites, peroxides, urea nitrate, ammonium, chlorate, perchlorate, chloride, sugar, cyclonite, trinitrotoluene, zinc, aluminum, magnesium, and sulfate. This allows for the design of 3 different chips for the detection of multiple components in an improvised explosive, such as flash powders and other low explosives, along with post-blast identification capabilities. The development of this paper based sensor will allow law enforcement and forensic evidence collection teams a simple, reliable device for the presumptive testing of unknown evidence in the field. These paper devices can be easily-stored and take up very little space allowing for them to be easily brought into the field with the team.

Modeling 3D facial shape from DNA

Pennsylvania State University (2008-DN-BX-K125) ^

The primary goals (specific aims) of this research are:
1. Identify genes underlying variability in facial features within and among European and West Africans populations. This aim includes population sample collection, phenotyping of 3D photos, whole-genome marker genotyping, and gene mapping.

2. Assay independent population samples to test for replication of significant mapping results. Replication of whole genome mapping results is critically important especially for complex and multifaceted traits like human facial features.

3. Test for the ability of human observers to recognize the effects of individual genes on facial features and to match facial photographs with corresponding computer-generated facial reconstructions based on functional locus genotype.

We have accomplished these aims, which have been documented in a Ph.D. dissertation successfully defended by Denise Liberton, “An Investigation into Genes Underlying Normal Variation in Facial Morphology in Admixed Populations.”

Pediatric Fracture Printing: Creating a Science of Statistical Fracture Signature Analysis

Michigan State University (2011-DN-BX-K540) ^

The goal of this presentation is to inform attendees about the development of a pattern recognition method for 'fracture printing' used in the differentiation of impact energy level for a porcine model while keeping the contact interface, contact location and head restraint condition constant for various age groups. Algorithms developed for this method will be demonstrated in a proto-type interface deliverable that classifies cranial fracture patterns with a high degree of accuracy using a porcine dataset.
Pediatric deaths involving cranial fracture are challenging cases for both forensic pathologists and anthropologists as little foundational research exists on the interpretation of fracture. Toward that end, this study utilizes a porcine dataset (n = 63) for which heads were dropped in a controlled laboratory setting onto a rigid aluminum surface with the site of impact at the center of the right parietal bone. The cranial injuries were charted and pattern recognition software was used to differentiate the cranial fracture patterns into low or high impact energy. The analyses were run with three different groups of data: young porcine specimens (1-9 days old); older porcine specimens (10-18 days old); and both age groups combined. Decision trees with the leave-one-out method were utilized to determine which characteristics were useful in differentiating energy levels for each group. The hypothesis of the study was that fracture patterns for drops of differing energy levels can be statistically determined with a high level of accuracy.

The algorithms used 31 variables in a step-wise fashion by selecting the best performing suite of variables for classification of impact energy level. Both the young (n=34) and old (n=29) groups separately selected the total fracture length of the right parietal to classify fracture patterns at 82.4% and 100% correct classification, respectively. The combined age porcine group (n=63) was classified with a 95.2% accuracy based on the total fracture length of the right parietal, total fracture length of the occipital, and number of fractures on the right parietal.

These algorithms have been integrated into an interface where a fracture pattern can be uploaded into a system which will automatically extract features including: fracture length per bone, fractures per bone, number of initiation points, intersecting fractures and fractures that cross sutures. The interface requires the user to select the impact surface, impact scenario (gravity-dropped versus entrapped head restraint) and input the age of the subject. The interface will then classify the uploaded image as a high or low energy impact with an appropriate classification rate, as well as display similar fracture patterns from the dataset.

The refinement of these energy classification algorithms, and the development of the interface classification algorithms, will be the next steps in the project. The ultimate goal of this research will be that for a given cranial fracture pattern in a human subject of a given age, we will be able to compute a statistical probability that a particular impact condition was the cause.

A New Pediatric Head Injury Assessment Tool for possible child abuse cases considering subject-specific child head anatomy

The Regents of the University of Michigan (2012-DN-BX-K045) ^

Establishing whether a head injury to a child was the result of a short fall or abuse is a fundamental problem in forensic investigation. Current injury assessments on possible child abuse cases are mainly based on clinical evidence and experts' professional experiences. However, expert opinions regarding how a head injury occurred to a child are often not based on scientific evidence and sometimes in direct conflict, which can result in wrongful legal action.

The goal of this 3-year research project funded by the National Institute of Justice is to establish a new paradigm for developing and using subject-specific pediatric head finite element (FE) models to provide objective and accurate assessments for judging the consistency of head injuries and the stated causes in infants or young children. The modeling paradigm will provide useful, accurate and objective information to both clinicians and forensic investigators which will help determine the true causes of a pediatric head injury.

In the first year of this project, we've been able to collect head CT scans from 56 child subjects, and developed a method to rapidly generate a head FE model for fall simulation accounting for the subject-specific head geometry. Moreover, in-depth investigations of 15 pediatric fall cases were also conducted for computational reconstruction to ensure the accuracy of the model. With this model, a fall case described by the parents or caregivers can be quickly reconstructed in high accuracy, which will provide additional evidences to determine the true causes of a pediatric head injury.

In this study, a multidisciplinary research team was formed, including engineering undergraduate and graduate students, forensic investigators, scientists, clinicians at the emergency department, doctors from the Child Protection Team at the University of Michigan, and other medical professionals. This project has created many educational outreach opportunities for undergraduate and graduate students. For example, 2 summer internships are provided to the undergraduate students each year through this project, and a master student is taking part of this project as a direct study course. In this study, students has the opportunities to work on analyzing medical images, building models, as well as going to the scene for fall investigation. The exposure of the state-of-the-art technologies and cutting-edge research in the forensic science has provided learning experiences that is beyond regular class learning. In addition, due to the multidisciplinary nature of this project, engineering students and professionals have chances to interact with medical doctors and staff members to learn medical aspects of child fall and abuse cases. The Child Protection Team also educates and trains all the team members in this project on child abuse and neglect.

Investigation of the Impact of Body Temperature and Post-Mortem Interval on Magnetic Resonance Imaging (MRI) of Unfixed Tissue

University of New Mexico Health Sciences Center (2012-DN-BX-K019) ^

Due to the excellent sensitivity of Magnetic Resonance (MR) imaging to subtle differences in soft tissues, MR enables non-invasive anatomical imaging with superior soft tissue contrast relative to x-ray computed tomography (CT). However, in the post-mortem imaging setting, the sensitivity of MR to subtle changes in tissue properties presents both problems and new opportunities. Normal temperature-dependent and post-mortem interval (PMI)-dependent changes in MR image contrast, not encountered in clinical MR of live subjects, have the potential to confound the identification of pathology or injury in the forensic setting. Thus we hypothesize that post-mortem Magnetic Resonance (PMMR) will eventually find broader application if the dependence of image contrast on temperature and normal post-mortem changes is better-understood.

We are currently investigating temperature-dependent changes in a systematic way, by measuring the tissue-specific MR parameters T1, T2, and the apparent diffusion coefficient (ADC) as a function of temperature for a variety of fresh (unfixed) ex vivo mammalian tissues. From these data, we are identifying those tissue parameters that show a strong, consistent, linear dependence over the temperature range of interest (0 to 40 oC); non-invasive measurement of those parameters may then be used to prospectively determine subject temperature. In the second phase of the project, PMMR imaging protocols will be optimized with respect to subject temperature, utilizing the tissue data obtained in the first phase. The optimized protocols will then be implemented and tested using intact animal subjects. In the third phase of the project, the MR tissue parameters (T1, T2, and ADC) will be measured as a function of PMI and decomposition temperature for a variety of tissue types to determine the impact of decomposition on MR image contrast.

Measurements of the tissue relaxation time T1 demonstrate that overall, most body tissues (e.g., muscle, kidney cortex, spleen) exhibit a monotonic increase in T1 with temperature. Further, the tissue relaxation time T2 depends very weakly on temperature for nearly all tissues with the exception of fat, which shows a significant decrease in T2 as the temperature is reduced from 20 to 10 oC. Together, these findings account for previously observed decreases in tissue at low temperatures. Preliminary data demonstrate an increase in the ADC of brain tissue as a function of PMI over the 2 – 14 day range, which may be useful for estimating PMI.

Currently, this project is providing a training opportunity for a promising young forensic scientist, Chandra Gerrard, a certified MR technologist who is currently completing her Bachelor's of Science degree at the University of New Mexico. The successful completion of the proposed basic studies will provide a framework for improving PMMR imaging of unfixed tissues, applicable to a variety of fields, including forensic investigation. We anticipate that this work will provide further training opportunities in future applied research studies aimed at developing optimized MR imaging methods for specific applications in the forensic setting, such as the evaluation of specific injuries or pathologies to determine cause of death, or the estimation of time of death.