National Institute of Justice

Office of Investigative and Forensic Sciences

FY 2011: Forensic Science Discretionary Awards made by the National Institute of Justice’s (NIJ) Forensic Science Research and Development Program

Questions and/or comments should be submitted to:
Forensic.Research@ojp.usdoj.gov

Introduction

This document contains the submitted abstracts of all FY 2011 discretionary awards that were made by the National Institute of Justice’s (NIJ) Forensic Science R&D Program. This is not an official publication of the U.S. Department of Justice. Findings and conclusions reported in this document are those of the authors and do not necessarily reflect the official position or policies of the U.S. Department of Justice. Products, manufacturers, and organizations discussed in these materials are presented for informational purposes only and do not constitute product approval or endorsement by the U.S. Department of Justice.
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FY 2011 Forensic Science R&D Solicitations and Awards

In FY 2011, there were a total of two (2) research and development solicitations released by NIJ on behalf of the Forensic Science R&D Program. These include:

- “Applied Research and Development in Forensic Science for Criminal Justice Purposes”
- “Basic Scientific Research to Support Forensic Science for Criminal Justice Purposes”

Of these two (2) solicitations, a total of thirty-three (33) awards were made by the NIJ to the amount of $14,893,465.

For the purposes of these solicitations, the following definitions apply:

**Forensic** – Of, relating to, or used in legal proceedings or argumentation.¹

**Science** – The observation, identification, description, experimental investigations, and theoretical explanation of natural phenomena.²

**Basic research** – A systematic study directed toward fuller knowledge or understanding of the fundamental aspects of phenomena and of observable facts without specific applications towards processes or products in mind. Basic research may include activities with broad applications in mind.³

**Applied Research** – The systematic study to gain knowledge or understanding necessary to determine the means by which a recognized and specific need may be met.⁴

**Development** – The systematic application of knowledge or understanding, directed toward the production of useful materials, devices, and systems or methods, including design, development, and improvement of prototypes and new processes to meet specific requirements.⁵

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¹ Definition of “forensic” is taken from *Webster’s II New Riverside University Dictionary*.
² Definition of “science” is taken from *Webster’s II New Riverside University Dictionary*.
³ Definition taken from: OMB Circular A—11, Preparation, Submission, and Execution of the Budget, Section 84—Character Classification (Schedule C).
⁴ Ibid.
⁵ Ibid.
#1. Applied Research and Development in Forensic Science for Criminal Justice Purposes

Posting Date: January 5, 2011

Closing Date: April 5, 2011

Awards Made: 22

The intent of the Applied Research and Development in Forensic Science for Criminal Justice Purposes Program is to direct the findings of basic scientific research, research and development in broader scientific fields applicable to forensic science, and ongoing forensic science research toward the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

With this solicitation, NIJ sought proposals for applied research and development projects that will:

- Increase knowledge or understanding necessary to guide forensic science policy and practice, or
- Result in the production of useful materials, devices, systems, or methods that have the potential for forensic application.
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2011-DN-BX-K528

McCrone Research Institute

“Development of a Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals”

Principal Investigator:
Dr. Gary Laughlin
glaughlin@mcri.org

Funding Amount:
$199,712 for 3 years

Abstract: A common microanalytical method for forensic drug analysis is the use of microcrystal tests. Light microscopy and microcrystal tests have been in use for more than 100 years but are sometimes incorrectly regarded as an archaic or useless method. However, they are extremely valuable when automated instrumentation is not available or when one wishes to check for the presence of one or several specific drugs. It might be noted that certain methods of analysis for drug identification, for example those specified by SWGDRUG, require the use of multiple uncorrelated techniques. This indicates that a good use of the light microscope would be to check and confirm the results obtained by other methods. Microcrystal tests, brought up to date with the inclusion of optical properties and compiled in a modern compendium, would be an excellent confirmatory method to give that added degree of confidence in the procedures and in the courtroom. Thus, analysts would not be relying strictly on gross crystal morphology, but would be examining refractive index, birefringence, extinction, and other optical properties.

Microcrystal tests are based on the known morphology and optics of crystals obtained after dissolving the compound and obtaining a precipitate through use of a specific reagent. Because many of these tests were developed in the early part of the 20th century, they do not automatically apply to new drugs which includes new prescription drugs that are abused and diverted from their intended recipients, drugs that come in new delivery mechanisms other than a traditional tablet or powder, e.g., transdermal patches, sprays, etc., new drugs that are commonly abused and the identification of optical isomers of a compound (e.g., identification of either the d- or l- enantiomer or the dl racemate) and especially drugs such as fentanyl, where no known microcrystal tests have been discovered for the purpose of their identification.

This project serves two main purposes and consists of two parts. The first part comprises the compilation of microcrystal tests which have previously been developed for illicit drugs and diverted pharmaceuticals by determining, locating, and compiling analytical data and literature material from the numerous sources (many of which are out of print or difficult to locate) spanning past decades. Such procedures will be vetted and appraised by McCrone Research Institute microscopists, together with
practicing forensic scientists in other collaborative laboratories. The resulting electronic compendium will include recommended protocols and morphologies of crystals (including photomicrographs), infrared spectra of microcrystals, and potential interferences. But most importantly, the compendium will also include optical and crystallographic properties of the resultant microcrystals. This optical data is absent in many references, which is unfortunate because microcrystals of a given substance are unique if optical properties (and not only morphology) are considered. Including this optical data will refine the application of many microcrystal tests and strengthen their use within the criminal justice system. Furthermore, this compendium will be available to all forensic scientists for free access from selected websites.

The second part concerns the development of microcrystal tests for illicit drugs and diverted pharmaceuticals where no current procedures exist. There are a number of such pharmaceuticals which are seeing a marked increase in abuse and misuse nationally, including alprazolam (trade name Xanax) and clonazepam (trade name Klonopin). Microcrystal tests are needed to aid in their identification, particularly among labs with large caseloads or those which are unable to employ GC-MS or other expensive instrumentation. These tests would be vetted and appraised as described above, for incorporation into the electronic Microcrystal Compendium.

**NIJ Point-of-Contact**

**Program Manager:** Frances Scott
2011-DN-BX-K551

Research Foundation of State University of New York

“Raman Spectroscopy for Analyzing Body Fluid Traces, Stain Aging, Differentiation between Races, Genders and Species”

Principal Investigator:

Dr. Igor Lednev

lednev@albany.edu

Funding Amount:

$615,575 for 3 years

Abstract: We are requesting support for the continuation of an ongoing research project ultimately targeting the development of an easy-to-use, portable instrument for the rapid, non-destructive, and confirmatory identification of body fluids in biological stains recovered at a crime scene. The main goals of the proposed study herein are to build a library of Raman signatures, and develop/validate the methodology and software for the automatic analysis of Raman spectroscopic data for (i) determining the age of a biological stain under various environmental conditions, (ii) differentiating human and animal blood, and (iii) determining race and gender based on human body fluid traces including blood, menstrual blood, vaginal fluid, semen, saliva and sweat.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
The analysis of DNA extracted from degraded human source materials is complicated by four major factors: 1) the presence of contaminating human DNA, 2) the presence of non-target DNA whether exogenous or endogenous to the sample, 3) co-extracted polymerase chain reaction (PCR) inhibitors, and 4) the degree to which template molecules have been damaged or chemically modified post-mortem or from the time of deposition of the biological material. These associated problems make the authentication of DNA profiles from low copy number (LCN) and degraded samples particularly problematic. As such, there is continued need to develop and evaluate methods that increase the yield and purity of genetic material extracted from degraded sources. Moreover, there are still poorly understood aspects of how degraded DNA should “behave” during routine laboratory methods, and whether this behavior is useful to differentiate it from contaminating DNA.

The proposed project contains three phases, each focusing on a specific aspect of working with LCN and degraded DNA samples. First, we propose to test commercially available DNA extraction kits, specifically those marketed towards LCN and degraded DNA samples, for the presence of contaminating human DNA. Quantifying the contaminating DNA molecules and determining the strand lengths will establish the level of degradation the DNA fragments have undergone. These observations will allow us to address whether it is possible to discriminate between profiles generated from authentic DNA versus contaminating DNA based on the copy number and intactness of the template molecules.

In the second phase of the project, we propose to test the performance of common protocols and commercially available LCN and degraded DNA sample extraction kits. Using a novel synthesized standard methodology we will directly compare the extraction methods by determining the copy number originally entering the extraction process and the number of copies retained at the end of the protocol. In this way, the performance of various extraction methods can be determined relative to complete recovery of DNA rather than as a comparison to other methods.
For the final phase of the project we propose to test three different “DNA capture” methods for enriching CODIS marker DNA in LCN and degraded samples. Similar to the methods proposed in Phase II, comparison using synthesized standards will determine the efficacies of each method in absolute terms. In addition, evaluation of the three capture methods will assess their respective abilities in removing PCR inhibitors from DNA extractions. Lastly, each method will be applied to capturing CODIS markers from ~500 year old human remains. Our goal is to identify methods that hold promise for more reliably recovering and typing CODIS profiles from LCN and degraded samples.

**NIJ Point-of-Contact**

**Program Manager:** Minh Nguyen
2011-DN-BX-K558

Trustees of Boston University

“Low-template DNA mixture interpretation: Determining the Number of Contributors”

Principal Investigator:

Dr. Jane Kinsel

bumc-era@bu.edu

Funding Amount:

$464,617 for 2 years

Abstract: The purpose of this proposal is to address the need to increase the knowledge and understanding associated with forensic sciences policy and practice and to produce useful systems and methods that have a forensic science application. This proposal is expected to benefit the forensic DNA/biology discipline and meets the objectives of 1) performing applied research to increase the knowledge of the behaviour of DNA evidence and 2) producing novel and useful materials/methods that have the potential to improve complex forensic DNA interpretation.

These objectives are met via the development of a novel approach to interpret complex and/or low template quantities of DNA. This enhanced method to characterize complex DNA mixtures will be accompanied by the development of a complex DNA mixture interpretation tool designed to enhance traditional DNA interpretation by utilizing a likelihood ratio which makes no assumptions regarding the number of contributors or by determining the likelihood that a certain number of individuals contributed to the DNA mixture. Despite a number of commercially available tools, to obtain a likelihood ratio, the number of contributors is still qualitatively assessed by the analyst, where the common approach is to determine the minimum number of contributors based on the number of peaks observed at individual loci. This technique is fraught with problems and it is the intention of this work to utilize an a posteriori probability to determine the probability that a DNA mixture is from n contributors. We propose to overcome these difficulties by using statistical signal processing methods to accurately infer the number of contributors to a DNA stain. Specifically, we will calculate the a posteriori probability (APP) of the number of contributors to a stain based on the genotyping results. The APP is the probability that the stain came from a certain number of contributors given what is observed during genotyping. If it is strongly peaked, i.e. the APP says that there is a particular number of contributors that is highly likely and all others are highly unlikely, then the APP tells us the number of contributors that gave rise to the stain. If not, the APP will nevertheless tell us the range in which the number of contributors is overwhelmingly likely to lie, which can then be used to calculate a range for the LR. The APP formulates the process of assigning a number of contributors, which currently must be performed by subjective judgment, into an accurate, objective process. However, to accurately assess the APP, characterization of drop-out and stutter contributions need to be assessed such that they can be incorporated into the calculation. Determining the probability of drop-out with respect to target and
analytical threshold will be determined – as well as the stability of these empirically derived figures. These new stability studies will test the probability of drop-out over kit lots and time (i.e. instrument calibration) and assess whether the likelihood method approaches currently in the literature - which typically assume the Pr(D) is constant - are appropriate. If the Pr(D) is not stable, protocols to determine Pr(D) given all parameters and variations over time will be taken into consideration. This not only would help crime laboratory analysts in appropriately determining the number of individuals and the uncertainty with respect to the number assumed, but it would ultimately aid in the ability of these laboratories to state the likelihood that it is \( n \) individuals. Furthermore, the LR that is ultimately reported should and can incorporate the uncertainties of the number of individuals into the likelihood calculation.

**NIJ Point-of-Contact**

*Program Manager: Minh Nguyen*
2011-NE-BX-K547

Research Foundation of City University of New York

“Development of an Immuno-Magnetic Procedure for the Separation of Spermatozoa from Vaginal Epithelial Cells”

Principal Investigator:
Dr. Margaret Wallace
MaWallace@jjay.cuny.edu

Funding Amount:
$196,720 for 1 year

Abstract: The main goals of this proposal are to develop a simple, robust, cost and time efficient method for the separation of spermatozoa from vaginal epithelial cells and to disseminate the methods and results to the forensic science community. This proposal outlines the development of an immuno-magnetic technique for the separation of sperm cells from vaginal epithelial cells. A direct immuno-magnetic technique will be used for sperm cell capture. Seven Protein G- magnetic bead (New England Biolabs, Ipswich, MA)- anti-human sperm complexes will be evaluated. Method development will encompass 1) determination of antibody titre, 2) determination of the optimal magnetic bead: antibody ratio, 3) analysis of the effectiveness of sperm cell capture in single source and mixed samples, 3) determination of tissue specificity, 4) evaluation of the quality of the DNA profiles generated from the captured cells and 5) optimization of the sperm-capture procedure. The new method will enhance the efficiency of crime laboratories by reducing the time required for the most time-consuming and labor-intensive step in the analysis of sexual assault swabs. The methods and results will be reported in interim and final reports and made available to the forensic community through publication and presentations.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
2011-DN-BX-K555

Orchid Cellmark Inc

“Forensic Identification of an Individual in Complex Mixtures Utilizing SNP Technology”

Principal Investigator:

Dr. Aaron LeFebvre

alefebvre@orchid.com

Funding Amount:

$224,968 for 1 year

Abstract: Today’s routine forensic work depends in many cases on analyzing DNA evidence with the aim to match against the DNA profile of a suspect/victim. In many instances the DNA evidence found at the scene of crime or in a mega-disaster, such as the 9/11 terrorist attacks, is comprised of DNA from more than a single individual. Commonly used STR genotyping methodologies used today have proven to be inefficient at identification of individuals in complex mixtures composed of more than two individuals mainly because the number of loci do not produce the statistical power required to derive the identity of an individual contributing to a mixed DNA sample. Our proposed research will address this unmet need by developing a method using a SNP assay based on the recent work by Voskoboinik and Darvasi [9] which elucidates a novel strategy for identification of an individual’s DNA in a complex mixture composed of up to 10 individuals. Their strategy employs genotyping a DNA mixture and a “suspect” DNA sample with 1000-3000 SNPs. Their statistical framework shows that this number of markers produces the statistical power required for accurate assessment and inclusion/exclusion of a single contributor in a complex mixture. Our proposal aims to develop and thoroughly test this strategy with real forensic casework-type samples on two different SNP detection technologies currently utilized by the forensic community. We will test a SNP panel that has been pre-selected by Darvasi’s group. In addition, the existing forensic SNP panels developed by the Orchid Cellmark group will be further developed and optimized for this project if so required. Following optimization of a SNP panel, thorough testing of detection limits, sensitivity, and reproducibility studies will be performed prior to testing complex mixtures containing between 3-10 individuals. All testing will be developed and performed on both Illumina’s GoldenGate(R)® genotyping technology and Applied Biosystems’ OpenArray® highthroughput RT-PCR platform as a means to both compare technologies and develop an assay fit for use in the widest range of forensic casework. Future experiments are planned, concomitant with funding for year two, in order to test inhibition, degradation, and other situations common to typical forensic DNA samples.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
Advanced Liquid Logic, Inc

“Automated Multianalyte Screening Tool for Classification of Forensic Samples”

Principal Investigator:

Dr. David Cohen
dcohen@liquid-logic.com

Funding Amount:

$996,237 for 2 years

Abstract: Expanding the science of evidence screening beyond pure serology to include source classification has tremendous potential value in that only the most probative samples are submitted for STR profiling while reducing needless duplication or unnecessary analysis. This will have a substantial positive effect on case processing and adjudication through better utilization of existing STR analysis assets. Source classification is achieved through multianalyte, sample-to-answer screening and is enabled by Advanced Liquid Logic’s Digital Microfluidic technology. The proposed system will accept a sample similar in volume to that required for a confirmatory strip-based test. However, instead of a strip, the proposed system will use Digital Microfluidics to perform, in parallel, purification and analysis of both proteins and DNA. The protein analysis section of the cartridge will test for the presence of 1) hemoglobin and 2) p30/PSA using standard ELISA methods. At the same time, and on the same cartridge, the DNA will be purified, amplified and SNP profiles will be generated. The expert software component of the system will perform exhaustive comparison and analysis of the multianalyte profiles within a case incorporating, where relevant, single-contributor reference samples. The output will be a report with a preliminary classification assigned to each analyzed sample for a given case. The goal of multianalyte sample classification is to quickly provide criminalists and case managers more thorough data to enable more informed decisions.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
2011-DN-BX-K562

Arryx, Inc

“Automated Sperm Detection in forensic DNA analysis: Implications for Rape Kit Analysis”

Principal Investigator:

Dr. Tania Chakrabarty

tchakrabarty@arryx.com

Funding Amount:

$360,748 for 1.5 years

Abstract: In recent years, automation of laboratory practices has been very effectively deployed in sample analysis, allowing much tighter quality control, simpler validation, increased workforce efficiency, lower cost, and higher throughput. In processing forensic samples, automation has played a critical role, focusing on liquid sample handling including DNA extraction, DNA quantitation, and setting up PCR reactions. However, upstream processing steps are still labor-intensive, time consuming, and performed with variability. There are presently few options for automated screening of rape kit elutes for presence of sperm, quantitative cell counting, and precise sperm isolation. Sexual assault evidence samples still require significant manual processing, subject to variability and the negative screening of weakly positive samples. Additionally, commonly used DNA quantitation methods have limited precision, ultimately causing failures in STR profiling.

Arryx has developed a powerful platform for automated microscopy which leverages machine-vision for object recognition and holographic optical trapping (HOT) for cell manipulation within aqueous cell samples on slides and active fluidic disposables. Initially developed for medical diagnostics and human blood typing, using optical traps to probe red blood cells binding to bioconjugated surfaces in an automated machine, this technology holds great promise to advance forensic science.

Ongoing work on upstream forensic processing has focused on the use of HOT to isolate individual sperm from the sexual assault samples on a microscopy platform. Our studies have demonstrated that this method is compatible with downstream PCR-based STR profiling. This automated system for isolation of individual human sperm from elutes will reduce the sample processing time, eliminate DNA carryover from the epithelial fraction to the sperm fraction, and improve the quality and uniformity of sample processing.

We propose to develop an advanced microscopy-based sample characterization system for the evaluation of sexual assault evidence samples. The core of this system will be a set of robust algorithms based on machine vision for scanning cell mixture solutions, characterizing their sperm content, and estimating the sperm DNA available from such samples. This core development will be supplemented with SOP’s (standard operating procedures) for sample handling and operation, along with studies
focused on characterizing its performance. This system will address three critical needs: (1) It can be used for screening sexual assault evidence for the presence of sperm, to determine if further processing should be done. This would improve performance, sensitivity, and uniformity relative to current manual methods which are labor-intensive, slow, and subject to variability. (2) It can be used to quantitate the number of sperm present in a sexual assault sample elute, and thus the amount of sperm DNA present. Current DNA quantitation methods have very high (up to 10-fold) uncertainties, since different quantitation methods such as RT-PCR, End-point PCR, Fluorescence detection for DNA binding dyes, etc, have different detection limits and sensitivities. The presence of various extraction chemicals in the elute also interfere with DNA quantitation. Automated sperm detection and counting will provide better quantitation of sperm DNA and thus lead to higher success rates for STR profiling and human identity matching. (3) It will be integrated into our platform for sperm isolation using HOT, enabling it to perform with a variety of sample input preparations and provide detailed data tracking on the input sample characteristics and the sorted-cell outputs. This work specifically addresses the priority areas of the solicitation: Applied Research and Development in Forensic Science for Criminal Justice Purposes and will advance the area of forensic DNA typing by providing capability to reduce manual labor of processing and to produce more robust and informative DNA analysis of forensic samples.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
2011-NE-BX-K550

Florida International University

“Rapid and Selective Extraction of Male DNA from Rape Kits and other Forensic Evidence using Pressure Cycling”

Principal Investigator:

Dr. Bruce McCord
mccordb@fiu.edu

Funding Amount:

$349,130 for 3 years

Abstract: A major problem in the United States is the huge backlog of rape kits and other forensic samples awaiting analysis in crime labs and evidence lockers. This issue has been the subject of multiple NIJ solicitations and federal hearings. The goal of this proposal is to reduce one bottleneck in the processing of such samples: the long and complex process of separation and differential extraction of sperm cells in the presence of female DNA. We will do this through the development of a novel and highly selective pressure-based DNA extraction procedure that is designed to burst open and extract DNA from male sperm cells while leaving the excess female cells in rape kits or other mixed forensic stains unaffected.

Conventional differential extraction methods used for the separation of DNA from sperm cells and epithelial cells rely on a complicated procedure of selective digestion and separation of epithelial cell fractions followed by sperm cell lysis to generate the male genetic profile. Unfortunately, this process is difficult to automate, has poor recovery, and can be overwhelmed due to the presence of a large number of female cells. Hence, a faster more selective method is required. This proposal described here addresses the issue through the development of a method using pressure cycling technology (PCT) combined with reagents to selectively disrupt sperm or epithelial cells and recover DNA. The extraction procedure is then performed utilizing the Barocycler® NEP 2320, a commercially available instrument from Pressure Biosciences Inc. (South Easton, MA), equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressures with a range of 5-45 kpsi. Samples such as cotton swabs or cuttings of cloth can be directly extracted using this technique by simply placing them in a pressure cell along with an appropriate buffer.

The pressure processing will be coupled with specific reducing agents such as TCEP and DTT which selectively attack sperm cells without damaging female epithelial cells. Using this technique, we can greatly simplify the long and complex processing of rape kits and other mixed forensic samples by selectively extracting the assailant’s sperm cells, while leaving the victim’s DNA behind. Our initial studies using a demo instrument indicate a highly selective extraction of sperm cells over female epithelial cells. Overall these results provide an opportunity to develop a one pot differential extraction
which can selectively generate male or female DNA profiles through the use of variable pressure pulsing and differential chemical lysis.

**NIJ Point-of-Contact**

**Program Manager:** Minh Nguyen
Harris County

“Use of Pressure Cycling Technology to Enhance DNA Yield and Profile Success in Touch Samples”

**Principal Investigator:**

Dr. Roger Kahn

rkahn@hctx.net

**Funding Amount:**

$76,778 for 1.5 years

**Abstract:** Touch samples are potentially relevant evidence in almost every type of criminal case. Unfortunately, in a majority of cases they simply do not provide sufficient DNA yield to generate interpretable DNA results. One method utilized to overcome low level DNA yield is to increase the number of PCR cycles for these sample types. Doing so does result in increased success with respect to DNA results; however, this methodology is also highly controversial and fraught with problems such as allelic drop out, contamination and elevated stutter products. Ideally the way to successfully generate profiles from touch DNA samples is to increase the yield from extraction. Pressure cycling technology (PCT) can be used to address the issue of low level DNA yield during the pre-extraction stage. PCT (Pressure BioSciences, South Easton, MA) uses cycles of alternating high hydrostatic and ambient pressures to assist in the recovery of DNA from a variety of sample types, including but not limited to swabs, hairs, tissues, and liquid samples. PCT alters conformations and interactions of biomolecules, destabilizes secondary structures and does not denature or inhibit enzymes. The severe changes in pressure allow for molecular interactions to be controlled and because of baroporation, DNA is released into solution while maintaining the sample’s morphological integrity. The instrument, which is capable of processing up to three samples simultaneously, is either manually or computer controlled, can cycle pressure between ambient and 40,000 PSI, and offers a working temperature range of 4°- 37°C. This project will validate the use of PCT on evidence samples from a variety of sample types.

Based on the validation results on sample yields and sample types that demonstrate the best way to use this methodology, a decision will be made whether to implement the technology on DNA case samples from touch evidence and other low DNA yield samples such as hairs. Comparison of results from historical data from HCIFS touch samples or low DNA yield samples such as hairs will provide the forensic community with a thorough evaluation of the best practices for this technology.

**NIJ Point-of-Contact**

**Program Manager:** Minh Nguyen
2011-DN-BX-K559

Florida International University

“Comprehensive Forensic Toxicological Analysis of Designer Drugs”

Principal Investigator:

Dr. Anthony DeCaprio

adecapr@fiu.edu

Funding Amount:

$143,225 for 1 year

Abstract: In recent decades, clandestine drug lab operators have attempted to bypass controlled substance laws and legal regulations with “designer” compounds similar to current drugs of abuse, including methamphetamine, ecstasy, and khat. Presently, “bath salts” have erupted onto the drug scene containing analogs of cathinone that have produced severe side-effects in users across the globe. These “legal highs” have been sparking concern with law enforcement and emergency bans have been placed on the sales of such items. Designer drugs often carry unknown safety profiles, a high potential for abuse, unknown potency, and serious health consequences, especially when ingested unknowingly. Easy access via the internet has made such compounds more available to the general public. While seizures of these compounds only account for about 3% of all drug seizure cases in the world, severe intoxications and fatalities are not uncommon. These drugs are difficult to identify from a forensic standpoint due to the large numbers of compounds classified as “designer drugs”, the constant introduction of new structures, inadequate accessibility to standards, and the relatively limited frequency of occurrences.

With the high number of designer drugs currently on the market, few comprehensive screening techniques are available for the detection of these compounds in biological specimens. Inadequate information is available with which to assess the detectability of these drugs in currently available immunoassays designed to target amphetamine, methamphetamine, MDMA, or benzylpiperazine derivatives. For this reason, systematic research is needed in order to thoroughly understand the activity of these compounds in preexisting immunoassay platforms and to assess the need for novel assays directed towards designer drugs as a class.

In addition, comprehensive confirmatory techniques are required for the detection and quantitation of multiple classes of designer drugs and their major metabolites in human specimens. This project will develop and compare novel liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) methods for the analysis of the designer drug compounds. These methods will be applicable to both known drugs and previously uncharacterized novel modifications of known drugs, including phenethylamines, tryptamines, piperazines, and cathinone derivatives. Specific aims of the project include the following:
1. Determine the cross-reactivity, false-positive rate, and false-negative rate for a broad range of designer drugs in commercially available screening immunoassays. Studies will focus on ante-mortem blood specimens using ELISA-based assays and urine specimens using EMIT-based assays.

2. Develop improved comprehensive confirmatory analytical methods to detect a broad range of designer drugs and their major metabolites, including previously unknown structures. Applicability of LC-triple quadrupole-MS vs. GC-triple quadrupole-MS based approaches will be assessed and compared. Parameters to be optimized include sample extraction and pretreatment, derivatization for GC analysis, and selection of internal standards, in addition to evaluation of standard method validation criteria.

**NIJ Point-of-Contact**

**Program Manager:** Frances Scott
“Smartphone Technology for Capturing Untreated Latent Fingerprints”

**Principal Investigator:**

Mr. James Moulton

jmoulton@eoir.com

**Funding Amount:**

$208,085 for 1 year

**Abstract:** The capture of latent fingerprints in the field can sometimes be a daunting task. Although various systems exist to collect fingerprints, they can be bulky and require the use of chemicals that are difficult and messy to deal with in the field. Furthermore, when the latent fingerprint is detected, the ability to determine if a residue existed on the finger is an even tougher task. EOIR Technologies proposes to utilize its expertise in electro-optics (EO), spectral measurements, and systems integration to: 1) determine the feasibility of reliably collecting latent fingerprints with current high-definition photography, 2) determine the feasibility of spectrally detecting the presence of nefarious fingerprint residues, and 3) develop a prototype latent fingerprint EO collection system. The technology being exploited is the EO fluorescence of latent fingerprints in the UV/Blue wavelengths and the spectral detection capability of materials in the Visual-Near-Infrared (VNIR) and Short-Wave-Infrared (SWIR) regions of the electromagnetic spectrum. EOIR will utilize its remote sensing laboratory (RSL) with existing cameras and VNIR/SWIR Spectro-Vista Corporate (SVC) spectrometer. A prototype system will be developed using Smartphone technology that will be ruggedized and portable for field use. The National Forensic Science Technology Center (NFSTC) will act as a collaborative partner to provide forensics expertise as well as independent testing and evaluation of the developed prototype.

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
University of Tennessee, Knoxville

“Computerized Reconstruction of Fragmentary Skeletal Remains for Purposes of Extracting Osteometric Measurements and Estimating MNI”

Principal Investigator:
Dr. Mohamed Mahfouz
utkegrants@utk.edu

Funding Amount:
$514,495 for 2 years

Abstract: The purpose of this research project is to improve forensic anthropology practice and policy by facilitating more complete and accurate analyses of fragmentary human skeletal remains. Project personnel will develop and launch free user-friendly software that will enable forensic anthropologists to quantify and reconstruct fragmentary human skeletal remains (crania, pelves, humeri, and femora are the focus of the initial platform; additional elements will be added with subsequent releases of the software) from three-dimensional surface files generated by computed-tomography or laser scans. First, the system will serve as an osteological case or scene management tool. All scanned skeletal remains from each scene will be reviewable within the application. Initially the software will provide a minimum number of elements (MNE) estimate of scanned material (Grayson 1984). Following osteological protocols developed in forensic anthropology and bioarchaeology (Adams and Byrd 2008; Herrmann and Devlin, 2008), MNE estimates will allow for the determination of the Minimum Number of Individuals (MNI) represented at the scene. Once the MNI is determined, the software will provide a fully reconstructed bone along with automated measurements for the user to apply to regression equations, discriminant functions, or to use with software such as Fordisc 3.0 (Jantz and Ousley 2005). As a secondary option, the software will provide sex and ancestry classification options using nonlinear classifiers. This software will have applications in individual forensic casework as well as in situations with commingled remains, such as mass graves or mass disaster scenarios.

The development of this new technology is possible due to recent advances in statistical atlas bone modeling (Mahfouz et al. 2007a; Mahfouz et al. 2007b). A statistical bone atlas is an average mold (or template mesh) that captures the primary shape variation of a bone and allows for the comparison of global shape differences between groups or populations, as well as for the rapid generation of automated computer measurements. This research team has used the powerful exploratory capabilities of statistical atlases previously to investigate and improve upon forensic techniques (Jantz and Mahfouz 2009; Mahfouz et al. 2007a; Mahfouz et al. 2007b; Shirley 2009; Shirley et al. in press). The proposed project will expand the usefulness of the atlas into the analysis of fragmentary and commingled remains. In addition, forensic anthropologists will be provided with a means to quantify and reconstruct remains that are damaged or fragmentary, thereby enhancing analyses in challenging cases. The data
management aspect of the application will allow forensic anthropologists to digitally inventory complex commingled scenes; if geospatial data is integrated with each fragment then the refitting process can proceed geographically. Therefore, the developed application will significantly impact forensic anthropologists’ and crime scene investigators’ ability to reconstruct mass disasters, commingled mass graves, and highly fragmentary individual burials or surface scatters.

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
Michigan State University

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

**Principal Investigator:**

Dr. Todd Fenton
fentont@msu.edu

**Funding Amount:**

$681,147 for 2 years

**Abstract:** In medicolegal death investigations, current techniques for interpreting pediatric cranial trauma are of questionable reliability due to a lack of baseline data that matches pediatric cranial fracture patterns with known impact scenarios. This research will address this significant gap in best practice through a multidisciplinary effort that: (1) continues the development of experimental data from an experimental animal model, to help correlate input forces and cranial fracture patterns; (2) develops a pattern recognition method for ‘fracture-printing’ to be used in the identification of injury causation, initially based on this “ground truth” data from an animal model; and (3) collects data on human pediatric deaths involving blunt force cranial fracture and known impact scenarios from current forensic case files at medical examiner offices across the country to establish a database (The Pediatric Cranial Fracture Registry).

This research will develop automated pattern recognition methods to classify cranial fracture patterns based on contact interface, impact energy, and head constraint condition based on subject age. The predictive analysis will use classification models that are generated using experimentally produced data (e.g. digital images of cranial fractures) and are accompanied with the “ground truth” data (i.e. contact interface, impact energy, and head constraint condition). The ultimate aim of this research will be that for a given cranial fracture pattern in a subject of a given age, we will be able to compute a statistical probability that a particular impact condition was the cause. Future studies will develop a computer program that will automatically generate a fracture feature set based on pediatric human fracture pattern inputs that can be compared to a known database, to help predict the most likely cause of a particular fracture print in a forensic case.

This proposal brings together a team of established researchers in forensic pathology, forensic anthropology, orthopaedic biomechanics, pattern recognition and machine learning, and database
development to work on this significant gap in best practice. This research builds on studies that have been performed during a recently funded NIJ research project titled “A Forensic Pathology Tool to Predict Pediatric Skull Fracture Patterns” (Award No. 2007-DN-BX-K196).

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
Florida International University

“Graphical User Interface for a Multi-Factorial Age-At-Death Estimation Method Using Fuzzy Integrals”

**Principal Investigator:**
Dr. Daniel Wescott
dwescott@fiu.edu

**Funding Amount:**
$417,595 for 3 years

**Abstract:** A standardized method for combining multiple indicators of age from a single skeleton into a single, accurate, and repeatable age-at-death estimation is needed in forensic anthropology. To date there are no “best practice” guidelines in forensic anthropology for combining multiple indicators of age. Most currently published multi-factorial methods are not appropriate for forensic anthropology because they cannot be applied to a single skeleton, do not perform better than univariate methods, do not provide a confidence in the point estimate or prediction interval, or are restricted to a certain types of age indicators.

Recently, we presented a multi-factorial approach that uses the Sugeno fuzzy integral to produce a confidence in skeletal age-at-death. This method is novel and has multiple advantages over other multi-factorial methods. Our procedure allows investigators to use nearly any well established and tested age-at-death indicator methods and fuse the information about the accuracy of the methods with other types of quantifiable information that cause uncertainty in the age-at-death estimation. No other method allows for the fusion of information about the quality of the bone, the appropriateness of the method for the target age group, or inter-observer error in the methods used. Other advantages of the fuzzy integral method are that it can be easily used for a single skeleton, it can be used for both adult and immature skeletons, it can be customized to meet the investigator’s needs on specific cases, and it provides informative graphs and a standardized reproducible way to generate linguistic descriptions of age-at-death estimations.

We propose to develop an easy-to-use graphical user interface (GUI) that will allow forensic anthropologists to submit age-at-death indicator and bone quality information and obtain an age-at-death estimation, a measure of the confidence in the estimation, and additional results (numeric, graphical, and linguistic) regarding the type of graph and degree of specificity of an age-at-death estimation based on multiple indicators of age. In order to reach the largest audience possible and avoid requiring pricy or trendy software and libraries that have to be installed on individual personal computers and different operating systems, we propose that the GUI be web-based.
Development and testing of the GUI will be conducted over a two year period in six phases using a strategic team of experts that have the scientific, theoretical, and technological experience and expertise in forensic anthropology, computer engineering, and fuzzy set theory and fuzzy logic to successfully complete the project. The first year will primarily be devoted to designing the GUI and building the core libraries and algorithms. The second year will focus on development and testing of the GUI and preparing it for distribution to the forensic community.

The benefit of this project to the forensic science community is an open source library and GUI that provides forensic anthropologist with an easy-to-use and standardized method for combining multiple indicators of age into a single, accurate age-at-death estimation. The method is also important to the forensic science community in that it makes the qualitative fuzzy set analysis procedure explicit so that forensic anthropologists, law enforcement agents, lawyers, and other members of the medicolegal community can understand how to interpret the results. Currently there is no standardized method or best practices in forensic anthropology for combining multiple indicators of age.

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
2011-DN-BX-K539

University of Central Florida

“Degraded Ignitable Liquids Database: An Applied Study of Weathering and Bacterial Degradation on the Chromatographic Patterns of ASTM E 1618 Ignitable Liquid Classes”

Principal Investigator:

Dr. Michael Sigman

Michael.Sigman@ucf.edu

Funding Amount:

$470,545 for 2.75 years

Abstract: The identification and classification of ignitable liquid residue in fire debris can be complicated by weathering (evaporation) and biological degradation of the residue. The ASTM E 1618-10 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry emphasizes the need for laboratories to consult libraries (databases) of GC-MS data for ignitable liquid references, including a set of weathered and biologically degraded samples. The National Center for Forensic Science (NCFS) at the University of Central Florida and the Technical Working Group for Fire and Explosions (TWGFEX) Ignitable Liquids Database Committee have collaboratively produced the Ignitable Liquids Reference Collection and Database (ILRC) and a Substrates Pyrolysis Database, both of which are freely available online and consulted daily by fire debris analysts throughout the U.S. and other countries from around the world. Although the ILRC contains in excess of 500 datasets for ignitable liquids from the ASTM E 1618 classes, the collection contains relatively few weathered samples and no biologically degraded samples. This proposal would remedy this situation by creating a new database of weathered and biologically degraded ignitable liquids and would provide another valuable tool for the fire debris analysis community. The proposal involves six distinct activities: (1) Development of an online database to hold the degraded ignitable liquids data and link to the existing ILRC; (2) select a set of 50 “fresh” ignitable liquids for weathering from the existing ILRC; (3) weather the set of 50 ignitable liquids to six specified levels of evaporation, analyze and enter the data into the database; (4) biologically degrade the same set of 50 ignitable liquids for four specified time periods, analyze and enter the data into the database; (5) assess the possibility of misclassification due to weathering and biological degradation and (6) formulate a set of best practice guidelines to assist the fire debris community in evaluating the influence of weathering and biological degradation on the interpretation of casework samples. The data will be placed in a new Degraded Ignitable Liquids Database and linked to the existing ILRC in order to preserve the integrity of the current database, and weathered samples in the current database will be transferred to the new database. Weathering will be conducted by Dr. Sigman, Mary Williams and students at the National Center for Forensic Science (NCFS). Biological degradation will be conducted by Dr. John Goodpaster and his students at Indiana University Purdue University Indianapolis (IUPUI). Samples of ignitable liquids will be taken from the
ILRC or purchased by NCFS and samples shipped to IUPUI. Non-degraded samples of the newly purchased ignitable liquids will also be analyzed at NCFS and entered into the existing ILRC following classification by the TWGFEX Ignitable Liquids Database Committee. The degraded samples generated at NCFS and IUPUI will be analyzed at those institutions and samples from IUPUI will be archived on activated carbon and shipped to NCFS for analysis under a strictly controlled ILRC protocol. The datasets generated at NCFS and IUPUI will serve two purposes: (1) the results will be published by those respective researchers to further forensic science and (2) the datasets will be used to evaluate the influence of liquid degradation on correct positive ASTM class association rates. The final activity, best practices guidelines, will be formulated by the TWGFEX Ignitable Liquids Database Committee and publicized on the database and through presentations at AAFS. This proposal also serves to provide partial support for TWGFEX, which is otherwise without support, and will allow a highly productive portion of this TWG to continue providing valuable resources to the community.

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
2011-DN-BX-K553

University of Central Florida

“Comparison of Microspectrophotometry and Room-Temperature Fluorescence Excitation-Emission Matrix Spectroscopy for Non-Destructive Forensic Fiber Examination”

Principal Investigator:
Dr. Andres Campiglia
Andres.Campiglia@ucf.edu

Funding Amount:
$241,257 for 2 years

Abstract: The purpose of this project is to advance the state-of-the-art of nondestructive methodology for forensic fiber examination. Non-destructive techniques that can either discriminate between similar fibers or match a known to a questioned fiber - and still preserve the physical integrity of the fibers for further court examination - are highly valuable in forensic science. When microspectrophotometry (MSP) is used in the study of fiber evidence, variations within a fiber source lead to the recommendation that multiple spectra be collected from each fiber to properly characterize the sample. A positive association is determined when “the questioned spectrum is consistent in all absorbance values to at least one of the known spectra” and exclusion is determined when “either the suspect spectrum is totally different to that of any known fiber, or it falls outside the range produced by the known spectra”. Although this methodology is sufficient for comparison of profiles with obvious differences, it is possible that a statistic based assessment may be better suited to exclude different samples with very similar profiles. When fibers cannot be discriminated by non-destructive tests, the next reasonable step is to extract the questioned and known fibers for dye analysis with a more selective technique. Because dye extraction destroys the evidence, the possibility for fiber court examination will no longer exist.

We will introduce a highly discriminating approach based on fluorescence microscopy. Our proposition focuses on the total fluorescence emission of fibers. In addition to the contribution of the textile dye (or dyes) to the fluorescence spectrum of the fiber, we will examine the contribution of intrinsic fluorescence impurities – i.e. impurities imbedded into the fibers during fabrication of garments - as a reproducible source of fiber comparison. Fiber comparison will be made via data formats known as room-temperature fluorescence excitation emission matrices (RTF-EEM). We will compare the discrimination power of this approach to MSP. We will provide a rigorous statistical basis for the comparison of MSP and RTF-EEM data from the analysis of fibers of questioned and known origin. We will test the statistical tool on data datasets representative of forensically relevant samples. We will provide detailed methods for the use of commercial or public sector software for the analysis of MSP and RTF-EEM data. We will investigate spectral changes that might occur in textile fibers as a result of exposure to environmental conditions such as laundering, exposure to cigarette smoke and weathering. With the examination of these effects through comparison of fibers, we will gain a better understanding
of textile physical, chemical and spectral changes that might affect fiber comparison via MSP and/or fluorescence microscopy.

**NIJ Point-of-Contact**

**Program Manager:** Gerald Laporte
North Carolina State University

“Microfluidic System for Automated Dye Molecule Extraction and Detection for Forensic Fiber Identification”

Principal Investigator:

Dr. Thomas Dow

thomas_dow@ncsu.edu

Funding Amount:

$537,098 for 2 years

Abstract: The objective of the proposed project is to improve the front end of a forensic analysis process; specifically, to develop a micro-fluidic device that can automatically extract dye molecules from a small fabric sample and route the extracted molecules through a series of chemical separation processes for identification. Due to the quantity of textile materials in the environment, there is a high probability of fiber transfer during the commission of a crime. In a typical case, a sample of known origin (fiber from suspect’s shirt) is compared to an evidence sample (sample found at a crime scene). The objective is to test the hypothesis that both samples have the same source. A wide variety of analytical techniques are available and each provides different type of information. To maximize productivity and efficiency while preserving the evidence, the fiber examiner begins with non-destructive microscope techniques that can discriminate samples based on morphology (e.g., fiber shape, color, etc.). If additional specificity is needed to prove the hypothesis, more sensitive techniques are employed but typically at the cost of destroying part of the evidence. These processes include high performance liquid chromatography to identify based on hydrophobicity, UV-visible spectrometry to identify based on color and capillary electrophoresis to identify based on migration time through an electric field and time-of-flight mass spectrometry to identify exact mass of ions. This combination of analytical techniques can be done serially and will provide specific dye identification. Prior to the use of these analytic techniques, dye molecules must first be separated from the fiber sample.

Although useful and pertinent research has been conducted to optimize extraction techniques, little effort has been made to provide technology that allows the Forensic Scientist to streamline the extraction process and integrate it with their current analytical techniques. This project will deliver a microfluidic system that automatically extracts the dye from a minute fiber sample (< 1 mm length) and prepares it mass spectrometer analysis with minimal handling. Currently no mass spectrometer manufacturer provides such an automated extraction system. Rather the extraction is done in a series of independent and discontinuous steps which may introduce contaminants. To identify textile dyes, dye molecules must be separated from the fiber using an extraction solvent. The solvent is then evaporated and the dye molecules dissolved in a buffer solution appropriate for mass spectrometry. These methods require large solvent volume (~100 μl) and therefore a large fiber sample to obtain detectable dye
concentration. In the proposed system the trace evidence examiner will place the micro-fiber sample in a well, close and seal the lid and start the process. Streamlined dye extraction will allow identification from smaller samples, minimize the risk of contamination, and it will improve fiber analysis repeatability by offering a standard methodology with minimal operator input. This is particularly important for natural fibers since mass spectrometry may be the only method with sufficient discriminatory resolution to produce a match.

One of the main themes in the 2009 NAS Report on forensic sciences was that improved scientific support for forensic analysis is needed. One of the main points is the need for objective analytical methods as opposed to subjective examinations as a means of reducing the potential for errors. Increasing repeatability and traceability in virtually all fields of forensic evidence processing - save DNA analysis - is necessary. Streamlining processes such as fiber analysis and making them more repeatable and reliable using detailed chemical analysis has the potential to pay dividends in the search for a more equitable and thorough justice system. Fewer false convictions and more successful prosecutions are the goals.

**NIJ Point-of-Contact**

**Program Manager:** Gerald Laporte
Microtrace LLC

“Raman Spectroscopy of Automotive and Architectural Pigments: in situ Identification and Evidentiary Significance”

Principal Investigator:
Dr. Christopher Palenik
cpalenik@microtracescientific.com

Funding Amount:
$242,727 for 1 year

Abstract: Pigments are encountered in many kinds of trace evidence, including automotive, architectural, paints, inks, fibers, and plastics. Traditionally, pigments have been studied by polarized light microscopy, microchemistry, infrared spectroscopy, py-GC/MS, SEM/EDS and X-ray diffraction. Limitations inherent to each of these techniques have constrained the practical use of pigment identification in the analysis of trace evidence. Raman spectroscopy, which is becoming more widely available in forensic science laboratories, is the first analytical technique to provide the spatial resolution, sensitivity and specificity necessary to identify pigments in situ.

This application proposes to continue our current grant with NIJ “Fundamentals of forensic pigment identification by Raman microspectroscopy.” The initial grant focused on developing the fundamental research needed to address pigment identification in samples of forensic interest. This research has included:

- Validation and reproducibility studies to illustrate the reliability of pigment reference samples and Raman microspectroscopy as a pigment identification method.
- The collection of a database of Raman spectra, which represents the great majority of commercially available organic pigments in existence.
- The development of a systematic method of pigment characterization from Raman spectra.
- The production of a “Practical Manual of Pigment Identification” intended for use at the laboratory bench by trace evidence examiners.

This research has resulted in the most systematic examination of organic pigments ever available to the forensic community.

With this wealth of reference pigment information in place, the next natural stage of research is to study pigments in actual paint samples. From an investigative (intelligence) perspective, it was shown in the Green River Murders that the identification of paint pigments can help to identify the manufacturer of a
paint. For comparative investigations, the extent to which pigment identification can provide further discrimination is presently unknown. While pilot studies conducted by Microtrace have shown that as many as four pigments can be identified in a single paint sample, no systematic study of pigments in paints have been conducted. To address these and other topics, we propose to conduct a pigment analysis of three hundred paint samples collected from automotive and architectural paints. The results of these will provide insight into several unexplored areas:

- **Bulk in situ identification.** Analysis of paint samples with no sample preparation to determine the range of pigments that can be routinely identified in paint evidence.

- **Identification of pigments present at trace levels.** Development of methods utilizing thin sections and the spatial resolution of confocal Raman microspectroscopy in select samples to identify pigments present at low levels.

- **Evidentiary significance of pigment evidence.** Through the analysis of numerous paint samples, how many pigments can be readily identified and how common is each pigment?

All of these topics need to be addressed prior to implementing pigment identification as a laboratory tool; however none of the above questions have been previously addressed in the forensic community. The fundamental data collected in the initial grant provides, for the first time, the reference data needed to approach these questions. Preliminary research suggests that these discrete questions can be systematically addressed. The results would be compiled as an addendum to the “Practical Manual of Pigment Identification” being compiled under the initial grant and would included expanded sections on sample preparation, analysis, interpretation and evidentiary significance. This information would be directly applicable to casework in any forensic laboratory with a macro-, micro-, or even handheld Raman spectrometer. While directed specifically at paint evidence, this research would be of utility to other areas forensic sub-disciplines including fiber examination (e.g., pigmented fibers), ink characterization, and the analysis of other colored polymers.

**NIJ Point-of-Contact**

**Program Manager:** Gerald Laporte
2011-DN-BX-K552

Illinois State University

“Accessing the Probative Value of Physical Evidence at Crimes Scenes with Ambient Mass Spectrometry and Portable Instrumentation”

Principal Investigator:

Dr. Christopher Mulligan
researchoffice@ilstu.edu

Funding Amount:

$396,780 for 3 years

Abstract: The amount and variety of evidence collected a typical crime scene is extensive. While many significant analytical methods have been established over the years, forensic laboratories cannot keep up with the demand, and in many cases, significant backlogs of evidence have amassed. While this points to a need for more rapid, streamlined technologies for forensic analysis, a significant reduction in collected evidence, leading to a subsequent reduction in backlogged evidence, would come from the ability to access the probative value of chemical evidence at the crime scene itself, allowing only pertinent samples to be sent to off-site laboratories for confirmation. Screening of physical evidence at the crime scene also has the capability to rapidly determine whether a criminal investigation is needed and provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial. To assist in the reduction of collected samples while increasing the overall quality of said evidence, it would beneficial for forensic science practitioners to have technology at their disposal that is not only portable, allowing the screening of potential evidence before collection, but also flexible in terms of chemical species and sample substrates that can be analyzed. This flexibility, in particular, would allow this technology to be robust towards the ingenuity of criminals and emerging threats.

In an effort to fulfill the current technological needs of forensic science practitioners and associated laboratories, we seek to create a broadly-applicable, portable chemical detector based on a state-of-the-art mass spectrometer capable of “ambient” detection, i.e. detection of target compounds or “analytes” in their native environment and state without prior preparation. The proposed technology will allow sensitive analysis of gas, liquid, and solid-phase chemicals, as well as chemical traces on everyday surfaces, at low concentrations with high chemical specificity. While an array of forensic applications will be investigated, special consideration will be given to trace analysis of common illicit drugs and abused pharmaceuticals from substrates commonly found at crime scene investigations. Novel sampling methods will be coupled with this technology to allow the flexibility to analyze large surface areas, as well.

The principal scientific questions that will be addressed in order to gauge performance of the proposed technology include: (i) can a portable mass spectrometer be adapted to allow direct analysis of solid,
liquid, and gas-phase chemical species? (ii) can evidence be effectively screened in a non-destructive nature? (iii) is physical transfer of chemical residues more effective that direct surface analysis? (iv) is the developed technology on par with current methods in terms of reliability, reproducibility, selectivity, and sensitivity? (v) is this technology robust in terms of the current and changing needs of forensic science and law enforcement personnel?

Project investigators will use the findings of this research, as well as interactions with local forensic science practitioners, to develop and deliver an optimized portable MS instrument prototype to NIJ for evaluation and testing, along with appropriate operational documentation and a spectral library of samples of interest. Quarterly financial reports, semi-annual progress reports, and a final technical report including a detailed description of the project findings and a thorough discussion of the implications of the project on current criminal justice practice and policy will be completed at appropriate intervals.

**NIJ Point-of-Contact**

**Program Manager:** Gerald Laporte
“Examining the Effects of Environmental Degradation on the Optical Properties of Manufactured Fibers of Natural Origin”

Principal Investigator:
Dr. Gary Laughlin
glaughlin@mcri.org

Funding Amount:
$370,539 for 3 years

Abstract: With the production of manufactured fibers of natural origin increasing in recent years, products such as azlon and polylactic acid fibers are likely to become more common in regular case work in the forensic science laboratory. However, little is known about the changes occurring in their optical and physical properties as an effect of moisture, sunlight exposure, and exposure to various temperatures. This study investigates the effects of such degradation on the optical properties of selected fibers (polylactic acid, azlon, and rayon). These fibers, which are often proclaimed by manufacturers as being biodegradable (because they are made from naturally occurring proteins, sugars, or cellulose) are expected to show the most change compared to synthetic fibers such as polyester or nylon. Environmental conditions such as exposure to water (saltwater and freshwater), UV light, and hot and cold temperatures will be explored while documenting any change in optical properties. Polarized light microscopy observations including morphology, pleochroism, refractive index, dispersion, birefringence, extinction characteristics, sign of elongation, solubility, and thermal behavior would be monitored throughout two years of exposure to these conditions. Infrared spectra will also be collected at different time intervals to complement light microscopy data. Noticeable changes in optical properties of these types of fibers could prove to be important in a forensic setting, notably in fiber comparison and identification.

NIJ Point-of-Contact

Program Manager: Gerald Laporte
Michigan State University

“Developing Guidelines for the Application of Multivariate Statistical Analysis to Forensic Evidence”

**Principal Investigator:**
Dr. Ruth Smith
rwsmith@msu.edu

**Funding Amount:**
$272,220 for 1.5 years

**Abstract:** The recent National Academy of Sciences’ National Research Council (NRC) report entitled “Strengthening Forensic Science in the United States: A Path Forward,” drew attention to several limitations in the current state of forensic science. Among these, the need to quantify “measures of uncertainty” in the comparison of forensic evidence was highlighted. With the exception of DNA analysis, statistical assessments of questioned and known samples are not widely implemented in other forensic disciplines.

Currently, comparison of questioned and known samples mainly involves a visual examination of the data generated. Such visual comparisons have the potential to introduce subjectivity and do not ascribe any statistical confidence to the association, or ‘match’, between samples. Since complex data are generated from the instrumental techniques more commonly used for analysis, multivariate statistical procedures are needed for such statistical comparisons. The goal of the research proposed here is to evaluate numerous statistical procedures for the association and classification of different types of forensic evidence, in keeping with recommendations outlined in the NRC report.

This study will initially use three very different data sets to investigate the statistical procedures: ignitable liquids analyzed using gas chromatography-mass spectrometry, controlled substances analyzed using infrared spectroscopy, and bacterial populations in soil analyzed using real-time polymerase chain reaction. Pretreatment procedures will be investigated initially, to remove artificial sources of variance from the data that are commonly introduced when using instrumental techniques. Multivariate statistical procedures will then be applied to evaluate association, discrimination, and classification of questioned samples with respect to reference standards within each data set.

A manual generated from this research will outline advantages and disadvantages of each of the statistical procedures evaluated, along with special considerations according to evidence type. The manual will demonstrate applications using the data collected as part of the proposed research and will
be made available for dissemination among forensic practitioners. The results of this research will be one of the first steps necessary in facilitating the routine adoption of multivariate statistical procedures in forensic casework.

**NIJ Point-of-Contact**

**Program Manager:** Gerald Laporte
#2. Basic Scientific Research to Support Forensic Science for Criminal Justice Purposes

Posting Date: January 12, 2011

Closing Date: April 12, 2011

Awards Made: 11

With this solicitation, NIJ sought applications for funding basic scientific research in the physical, life, and cognitive sciences that is designed to increase the knowledge underlying forensic science disciplines intended for use in the criminal justice system. Applicable physical, life and cognitive sciences may include:

- Life Sciences (e.g., biology, genetics).
- Physics.
- Medicine/Dentistry (e.g., neurology, pathology, odontology).
- Mathematical Science.
- Computer Science.
- Chemistry and Pharmacology.
- Psychology.

Basic scientific research proposals to this solicitation should describe the anticipated impact of the basic scientific research on one or more forensic science disciplines and should be designed to lead to:

- Subsequent applied research and advanced technology developments in forensic science-related technologies intended for use in the criminal justice system, and/or
- New and improved crime laboratory functional capabilities that result in faster, more robust, more informative, less costly, or less labor-intensive identification, collection, preservation, and/or analysis of evidence.

Proposals whose principal investigators are defined as “new investigators” may, in appropriate circumstances, be given special consideration in award decisions. To be considered a “new investigator” for purposes of this solicitation, one of the two sets of criteria below must be satisfied for all principal investigators:

- The principal investigator must have, no earlier than April 1, 2007, received an initial appointment in the United States to a full-time junior faculty position at a university or to an equivalent full-time staff scientist position in a research institution; must have at the time of application submission hold such a full-time appointment, and must never have received NIJ funding for a research project, other than a Graduate Research Fellowship program grant.
- The principal investigator must be an established researcher who receives research funding originating from a federal science agency, but has not successfully competed for NIJ funding as a principal investigator or collaborative researcher in the last 10 years. The investigator must hold a full-time appointment in the United States to a faculty position at a university or an equivalent position as a scientist on the staff of a research institution at the time of application submission.
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2011-DN-BX-K534

Baylor College of Medicine

“Extending the Microbial Forensic Toolkit through Whole Genome Sequencing and Statistical Phylogenomics”

Principal Investigator:

Dr. Michael Metzker

mmetzker@bcm.edu

Funding Amount:

$581,213 for 1.25 years

Abstract: Microbial forensics is an emerging field that presents enormous challenges for both the scientific and legal communities. Unlike human forensic analysis, microbial pathogens of humans represent a highly diverse set of organisms known to cause disease. Microbes have also developed a number of elaborate mechanisms for generating natural genetic diversity, including high mutation and recombination rates as well as the horizontal transfer of gene(s). One major goal of microbial forensics is to use this genetic diversity to identify the source of a pathogen used to commit a crime. While phylogenetic analysis of nucleotide variation within a small number of genes has been used in past forensic studies to assess relationships among pathogens, a large fraction of those genomes remain uncharacterized, ignoring useful information contained in the presence or absence of different genes and other structural variation. Additionally, complex evolutionary processes that generate variation in phylogenetic signal across genomes, such as lateral gene transfer, incomplete lineage sorting, recombination, and convergent selection, have not been accounted for in current forensic studies. Recent advances in next-generation sequencing (NGS) technologies and phylogenetic analysis of complete genomes (phylogenomics) have the potential to significantly alter the technological approaches used in characterizing case samples. This proposal seeks to expand our existing scientific work on HIV forensic studies by developing a robust ‘pathogen toolkit’ for source identification across a range of biological agents. We will do this by (i) gathering whole genome sequences from multiple isolates of forensically relevant pathogens, (ii) characterizing the overall genomic diversity of these isolates, and (iii) testing for the signatures of evolutionary processes usually ignored in phylogenetic forensics. Initially, we will fully sequence the genomes of HIV isolates already collected in the course of previous forensic work. Expanding beyond HIV, methods are also proposed to enrich for desired microbial isolates prior to sequence analysis as pathogens typically exist in complex mixtures. Comprehensive surveys to characterize the diversity of pathogens found naturally are proposed to better understand (i) the extent of natural genomic diversity determines the limits of pathogen source identification and (ii) the ‘microbial background’ within local geographic regions. This is important in providing unrelated control groups to assess the relatedness of case samples. Appropriate controls safeguard against misinterpretation of the scientific evidence, which might lead to wrongful
incrimination. Model microbial systems that will be characterized are *Salmonella* sp., *Vibrio cholerae*, and *Francisella tularensis*. Proposed studies will test for more complete evolutionary processes and identify situations in which simplistic analyses may be misleading. Our results will greatly extend the available data and statistical rigor of microbial forensic work with direct applications to the field of criminal justice.

**NJJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
Abstract: Sudden unexplained deaths are one of the most vexing challenges facing medical examiners today. Even after a thorough autopsy, including toxicology, microbiology, review of clinical history and scene investigation, the cause of death often remains unknown. Such deaths, when unobserved, can leave medical examiners and law enforcement with difficult decisions. This is particularly true in the case of young children who are often found only in the presence of a caregiver, or when there are there are multiple child deaths in a family.

In recent years, advances in molecular genetics have begun to push back this shroud of uncertainty and shed light on the genetic contribution to sudden deaths. But even here, the number of known genes remains relatively small, accounting for only 10 to 15% of cases.

There are two major roadblocks impeding the discovery of new genes that may contribute to sudden unexplained deaths. The first is a lack of the large number of cases necessary to give statistical power to a genetic study. The second is the requirement for a thorough medicolegal investigation of every case before it can be included in a study. This latter point is crucial, since failure to exclude cases where the cause of death can clearly be attributed to other factors confounds results by mistakenly including a "normal" genome with the genomes of the affected population. These formidable obstacles can most readily be addressed in a metropolitan area where the population is large (and by necessity will have a significant number of cases), and where medical examiners are in training - learning and practicing the latest methods for evaluating pediatric deaths.

We believe that the New York City Office of Chief Medical Examiner is one such municipality. The NYC OCME is a nationally recognized training center for medical examiners, and is perhaps unique in having frozen tissue samples from several hundred well-defined cases of sudden deaths. In addition, the Molecular Genetics Laboratory of NYC OCME now sequences selected exon from six genes known to be risk factors for sudden death as a routine part of forensic death investigations, and is, therefore, experienced in sequencing technology and interpretation.

In this application, we propose to sequence the entire coding regions of 52 candidate genes believed to be involved in sudden unexplained deaths in 200 samples, as well as sequence all identified variants in
an additional 50 cases and 1,000 gender and ethnically matched controls. Data will be evaluated for new variants as well as combinations of variants that alone may not be disease causing, but in concert may predispose victims to sudden death. To achieve these goals, we propose to use the massive parallel sequencing technology available in next generation sequencing platforms.

We believe this application not only addresses this NIJ Solicitation for “basic scientific research designed to increase the knowledge underlying forensic science disciplines intended for use in the criminal justice system,” but also directly addresses the recent recommendations made by the National Research Council’s report *Strengthening Forensic Science in the United States: A Path Forward*, which specifically stated – “Investigations of unexplained sudden deaths, especially in young people and infants, would benefit from greater access to molecular diagnostics.”

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
2011-DN-BX-K533

The Regents of the University of Colorado

“Characterization of Bacterial and Fungal Communities Associated with Corpse Decomposition Using Next Generation Sequencing”

Principal Investigator:

Dr. Rob Knight

rob.knight@colorado.edu

Funding Amount:

$894,629 for 2 years

Abstract: Elucidating the time since death and locating clandestine graves are crucial in many forensic cases, but can often be a challenge. Biotic signatures of corpse decomposition, such as chemicals or the succession of insects, are commonly used to determine the post-mortem interval and to detect gravesoil, but no method is successful under every scenario (Carter et al. 2008b). Therefore, the development of new forensic tools is important. Microbes are ubiquitous in the environment and they play a key role in regulating the speed of decomposition, but microbial communities are not currently utilized to their full potential as a forensic tool. Testing whether changes in microbial communities are predictable over the timeline of decomposition is crucial for assessing whether microbes can be used as a ‘biological clock’ to assess time since death. Large-scale surveys of microbial diversity have become possible only recently. Due to advances in culture-independent DNA methods and sequencing technologies, recent research has revealed that microbial communities are quantifiable and predictable across habitats such as the human mouth and skin (Costello et al. 2009) and soil (Lauber et al. 2009). Taking advantage of 16S (bacteria) and 18S (fungal) ribosomal gene sequencing and computational pipelines developed by PI Knight and Co-PI Fierer, we aim to characterize the succession of bacterial and fungal communities during the decomposition of corpses, and to test whether corpse decomposition leads to distinct microbial signatures in grave-associated soils. The proposed experiments address two basic questions: Is the succession of microbial communities associated with corpse decomposition predictable and potentially useful for estimating the postmortem interval? And, do characteristic decomposer communities of bacteria and fungi measurably change the endogenous soil community, enabling detection of clandestine gravesites? We propose a three-phase research project coordinated by an interdisciplinary research team, which will bring together experts in high-throughput sequencing, microbiology, ecology, and forensics research. For phase 1, we propose an experiment to assess the succession of corpse and gravesoil communities on sterile and untreated soils. For phase 2, we propose an experiment across multiple soil types to assess the specificity of decomposer communities to the endogenous soil community. Finally, to determine the specificity of decomposer communities associated with mammalian taxa, we will survey pig and human-corpse associated gravesoils, which were sampled as part of previous studies by Co-PI’s Carter and Vass. This basic research will assess the
usefulness of tracking compositional changes in bacterial and fungal communities as a tool for forensic taphonomy.

**NIJ Point-of-Contact**

**Program Manager:** Danielle McLeod-Henning
2011-DN-BX-K543

University of California - Davis

“Human Hair Proteomics – Improved Evidence Discrimination”

Principal Investigator:

Dr. Robert Rice
rhrice@ucdavis.edu

Funding Amount:

$151,150 for 2 years

Abstract: This project investigates a method to obtain potentially probative information from human hair samples by analyzing their profiles of constituent proteins. Such information would 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. This project exploits recent advances in protein identification by mass spectrometry coupled with database searching. Previous and ongoing work has shown that mouse strains can be distinguished by their pelage hair proteomes using a small number of sentinel proteins. 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. The hypothesis will be tested that the proteome of human hair shaft can distinguish among humans by comparing the proteomes of hair samples provided by subjects (20 total) from Caucasian, Korean and African ancestry. The hair will be trypsinized and the protein profiles will be determined by a shotgun approach. From the data, a small subset of sentinel proteins (5-10) will be chosen that are distinctive among the samples analyzed. In the second phase of the work, the samples will be reanalyzed by a targeted approach. For this purpose isotopically labeled sentinel proteins or proteotypic peptides from them will be used as internal standards for relative quantitation. The normalized profiles of proteotypic peptides from sentinel proteins will be compared to find whether individual donor profiles can be reliably distinguished. If the comparison of protein profiles gives promising results, future work can concentrate on increased sample sizes for determining the limits of discrimination in the population, increasing the method sensitivity, developing a processing protocol compatible with mitochondrial DNA extraction, optimizing the panel of sentinel proteins and extending the approach to hair from other anatomic sites.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
"DNA Forensics using Single Molecule Technology: From DNA Recovery and Extraction to Genotyping Degraded and Trace Evidence without PCR"

Principal Investigator:
Dr. Matthew Antonik
antonik-m@ku.edu

Funding Amount:
$587,597 for 3 years

Abstract: Methods of characterizing low copy number and degraded DNA samples with little or no amplification using single molecule biophysical techniques will be studied. Fluorescence and atomic force microscopy techniques for imaging single molecules are now widespread. Quantitative characterization of the number, length, size, and mobility are routinely applied to biological samples, and these same techniques can be applied to characterize forensic DNA samples. In particular, DNA samples containing only trace amounts of DNA or DNA degraded by age, radiation, or chemical erosion will be studied. Such samples are challenging to analyze due to the inherent limitations of PCR amplification. By adapting the sensitivity and specificity of single molecule techniques to forensic analysis, low copy number and degraded samples can be imaged with a minimum of manipulation and little or no amplification. Such technology will make a large impact by providing means of interrogating samples which are currently beyond the reach of existing technology. To apply technologies originally developed for relatively pristine laboratory samples to forensics, specialized specialized protocols will be developed. Issues of sample purity will be addressed by characterizing the effectiveness of current purification protocols and developing specialized protocols for the additional purification that may be necessary for single molecule investigations. Suitable methods of quantifying DNA markers will be determined. Possibilities include using florescence correlation spectroscopy, step-wise photodestruction of fluorophores, AFM imaging of lengths, and fluorescence localization experiments.

In addition, single molecule techniques may be used to complement existing protocols. For example, single molecule techniques excel at detecting heterogeneities and may be useful in characterizing mixtures. The ability to detect and measure small quantities can provide specific information about sample composition that investigators need to properly interpret the data. Finally, the basic science associated with this project can clarify how different contaminants inhibit PCR and will therefore suggest new protocols for sample treatment to make current molecular biology approaches more effective.

NIJ Point-of-Contact

Program Manager: Minh Nguyen
Abstract: A group of experienced investigators at the University of Washington, the University of Auckland and ESR, the New Zealand government forensic agency, propose to continue their collaborative efforts to address population genetic issues in the interpretation of forensic DNA profiles. Although DNA typing has had a major beneficial effect on the criminal justice system in the United States, there are still issues where doubts are being raised about how best to quantify the evidential strength of matching profiles and to present that strength appropriately in court. The investigators have published many scientific papers and three textbooks and are well-positioned to consider the following topics:

Relatedness and Inbreeding Remains identification and familial searching are two of the activities that exploit the genetic nature of DNA profiles. Related individuals have similar profiles, although the current panels of forensic STR markers do not allow distinguishing among different classes or relatedness. The implications of adding lineage markers, more STR markers, or SNP markers will be explored. Tests of relatedness, as opposed to calculating likelihood ratios or specified degrees of relatedness will be developed.

Population Structure The interpretation of matching DNA profiles was improved by the “theta-correction” that allows for population structure, and the use of population-wide allele frequencies as surrogates for frequencies in a relevant sub-population. It is proposed to clarify the meaning of “theta” and to develop appropriate estimates to replace current ad-hoc assumed values. Use will be made of an extensive collection of published allele frequencies from around the world.

Lineage Markers Mitochondrial sequence and Y-chromosome STR data have the potential of improving relatedness inference, familial searching and the recovery of forensic profiles from de-graded samples. It is proposed to work further to remove current uncertainty on how to quantify the evidential strength of these lineage markers when the profiles of interest have not been seen in a database.
Mixtures As DNA typing technology becomes more sensitive, it is more likely that evidentiary samples contain DNA from multiple contributors. The investigators on this proposal were part of a Commission of the International Forensic Science Genetics group that recommended likelihood ratios, as opposed to “Random Man Not Excluded” calculations be used for mixtures. They now propose to conduct further theoretical and empirical studies to amplify that recommendation, especially for low template DNA typing.

**NIJ Point-of-Contact**

**Program Manager:** Minh Nguyen
2011-DN-BX-K530

Auburn University

“Analytical and Synthetic Studies on Designer Drugs of the Piperazine Class”

Principal Investigator:

Dr. C. Randall Clark
clarkcr@auburn.edu

Funding Amount:

$484,819 for 2 years

Abstract: This project will address issues of resolution and discriminatory capabilities in controlled substance analysis providing additional reliability and selectivity for forensic evidence and analytical data on new analytes of the piperazine class. A number of piperazine-containing compounds have appeared on the illicit drug market in recent years including N-benzylpiperazine (BzP), 1-(3-trifluoromethylphenyl)piperazine (3-TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), 1-(3,4-methylenedioxybenzyl)piperazine (3,4-MDBP) and 1-(4-bromo-2,5-dimethoxybenzyl)piperazine (BrDMBP). While some of these piperazines are commercially available others are designer analogues that have been synthesized in clandestine labs.

Exploration and designer development in the piperazine drugs using models based on substituted amphetamines and related phenethylamines is likely to continue for many years. Current clandestine recipes/procedures used for amphetamine-type molecules can be applied directly for piperazine synthesis. Thus, clandestine labs will not need to learn any new synthetic techniques. Restricting the availability of piperazine would require placing dozens of substances from commercial sources around the globe under federal control. Therefore, legal control of the key precursor substance, piperazine, will not prevent the further clandestine/designer exploration of this group of compounds. It could be argued that isomer differentiation is not necessary in forensic drug science because of the Controlled Substance Analog Act. However, the courts expect forensic drug chemistry to be able to identify a substance as an individual compound, not report it as an unknown member of a large group of isomeric substances. Furthermore, the forensic chemist must identify the compound in order to know if it falls under the Controlled Substance Analog Act. These circumstances all point to the strong need for a thorough and systematic investigation of the forensic chemistry of these substituted piperazines.

The broad objective of this research is to improve the specificity, selectivity and reliability of the analytical methods used to identify ring substituted benzylpiperazines, phenylpiperazines, benzyloypiperazines, phenethylpiperazines and related compounds. This improvement will come from methods which allow the forensic analyst to identify specific regioisomeric forms of substituted piperazines among many isomers of mass spectral equivalence. Mass spectrometry is the most common method of confirmation in forensic analysis. This project will provide methodology and analytical data to
discriminate between those regioisomeric and isobaric molecules having the same molecular weight and major fragments of equivalent mass (i.e. identical mass spectra). Furthermore, this work will anticipate the future appearance of some designer piperazines and develop analytical reference data and analytical reference standards for these compounds.

The initial phase of this work is the organic synthesis of the regioisomeric piperazines and in this phase of the work more than 90 substituted piperazines of potential forensic interest will be evaluated. Complete chemical characterization, using tools common to forensic science labs such as MS and IR will be carried out on each of the compounds. The chromatographic retention properties for each series of isomers will be evaluated by gas and liquid chromatographic techniques on a variety of stationary phases. These studies will establish a structure-retention relationship for the regioisomers and isobaric piperazines on a number of chromatographic stationary phases.

The results of this project will significantly increase the forensic drug chemistry knowledge base for piperazine-type designer drugs. When compounds exist which produce the same mass spectrum (same MW and fragments of equivalent mass) as the drug of interest, the identification by GC-MS must be based entirely upon the ability of the chromatographic system to resolve these substances. This project involves the synthesis and generation of complete analytical profiles as well as methods of differentiation for those regioisomeric and isobaric substances related to the aromatic ring substituted benzyl, phenyl, benzoyl, and phenethyl piperazines. The following application is a request for support to carry out this investigation.

**NIJ Point-of-Contact**

**Program Manager:** Frances Scott
2011-DN-BX-K531

Florida International University

“Separation and Identification of Drugs of Abuse Using ESI-IMS-MS”

Principal Investigator:

Dr. Jose Almirall
almirall@fiu.edu

Funding Amount:

$241,447 for 2 years

Abstract: The recent development of the concept of chiral ion mobility spectrometry (CIMS) allows rapid separation and identification of enantiomers and other stereoisomers within seconds. Ion mobility spectrometry (IMS) is a widely accepted analytical method used in a variety of detection scenarios including trace detection of controlled substances. IMS is listed by SWGDRUG as a category B technique, in the same class of specificity as gas and liquid chromatography. However, IMS application in the forensic science laboratory has been limited because of its poor resolution compared to chromatographic and mass spectrometry techniques. We propose capitalizing on the recently completed development of a commercial high resolution IMS that will enable a CIMS to have separation performance comparable to that obtained by chromatographic methods. The high resolution CIMS will also include a unique sample introduction system that allows liquid samples to be directly analyzed. A combined Electrospray/Secondary Electrospray Ionization (ESI/SESI) source will be used, not only to eliminate the traditional radioactive ion source normally employed in IMS, but also to allow introduction and detection of non-semivolatile controlled substances by ESICIMS.

Most importantly, the CIMS system has the ability to separate stereoisomers of controlled substances, and then detect them in the form that is of interest. A total of 16 chiral drugs will be investigated representing a large number of illicit drugs and pharmaceutical preparations that are of current interest to forensic scientists. The Almirall research group already maintains an Excellims ESI high resolution chiral IMS system that is coupled to a quadrupole mass spectrometer and we propose to develop methods for high resolution separation of drugs by ESI-IMS with the already available instrumentation in the Almirall laboratory. This project will involve close collaboration between the FIU team of researchers, scientists at Excellims Inc., (the developer of the only commercial ESI-IMS and CIMS instrument) and the scientific staff of the drug analysis section of the Miami-Dade Police Department Forensic Services Bureau in Miami, FL. The aims of the project are 1) a fundamental investigation of the use of ESI-IMS-MS for the purpose of separation of controlled substances commonly encountered in the forensic laboratory and 2) research to improve the understanding of chiral separations in the gas phase using CIMS of compounds that are currently difficult or impossible to analyze by other methods. Preliminary results by the developer of the instrumentation and also in the Almirall laboratory suggest that chiral separation of drugs of abuse is attainable but the exact mechanism of action is still not well
understood. The ESI-IMS-MS already installed in the Almirall laboratory will be used for the research requiring some fundamental experiments to optimize the ion chemistry in the IMS and to study the selection of the best chiral modifiers to be used in the gas-phase separations. The coupling of a SWGDRUG category B technique (IMS) with a category A3_ technique (MS) would provide an additional tool for forensic scientists for the fast (on the order of a few seconds) analysis of drugs using a high resolution separation and unambiguous identification of organic compounds. This tool will be useful for the identification of chiral drugs that require the enantiomer to be identified but also for drug analysis in general as ESI/SES sample introduction would offer an alternative for the analysis of other drugs (such as GHB) that are thermally labile and do not survive the temperatures of a GC injector but would be amenable to ESI-IMS-MS analysis. Commercial ESI-CIMS-MS instruments are currently available in the ~ $ 165.k price range and are becoming less expensive.

**NIJ Point-of-Contact**

**Program Manager:** Frances Scott
University of Utah

“Prediction of Drug Interactions with Methadone, Buprenorphine and Oxycodone from in vitro Inhibition of Metabolism”

Principal Investigator:

Dr. David Moody
david.moody@utah.edu

Funding Amount:

$1,058,604 for 1 year

Abstract: A near epidemic of opioid-related deaths has surged over the past decade. While not the primary cause, a mitigating factor is drug interactions that increase the concentration of active opioid to higher concentrations than intended. This factor is not only involved in causation, but also must be considered in the forensic toxicology interpretation during investigation of cases. Three highly used, and all too often abused, opioids are methadone, buprenorphine and oxycodone. While some knowledge exists on certain drugs that cause drug interactions with these opioids, this is limited, and a major focus for methadone and buprenorphine has been with the antiretrovirals.

We have recently developed and validated sensitive liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assays to study the in vitro metabolism of methadone, buprenorphine and oxycodone. To assist in increasing knowledge about opioid drug interactions we propose to test the hypothesis that in vitro inhibition of opioid metabolism can predict potential drug interactions. To test this hypothesis, we will use our LC-MS/MS assays to:

1. Each drug will first be tested at three different concentrations in human liver microsomes. This will be done with and without a 15-minute pre-incubation with the drug to test for metabolism-based inhibition.

And

2. Drugs showing ≥ 25% inhibition will be further tested using cDNA-expressed cytochrome P450s, or UDP glucuronosyltransferases relevant to the specific opioid.

   a. Those with no pre-incubation effect will be tested at eight concentrations to determine a 50% inhibitory concentration (IC50).

   b. Those with a pre-incubation effect will be tested at selected times and 4 concentrations using a primary and secondary incubation system to determine a concentration of inactivator required for half-maximal rate of inactivation at saturation (KI) and maximal rate of inactivation at saturation (kinact).
3. These data will then be compiled along with literature values for interactions to provide a relative ranking of interaction potential.

The results of these findings will be disseminated through presentations at the annual meeting of the Society of Forensic Toxicologists and through publication in peer-reviewed journals. In this manner we propose to add to the knowledge base concerning the basic science of drug interaction potentials for three highly used opioids. The PI, an NIJ new investigator, and his colleagues are aptly suited to carry out these studies.

**NIJ Point-of-Contact**

**Program Manager:** Frances Scott
IAA

Ames Laboratory

“3D Characterization and Comparison of Fracture Surfaces”

Principal Investigator:

Dr. Ashraf Bastawros

bastaw@iastate.edu

Funding Amount:

$355,000 for 2 years

Abstract: As New Investigators we propose to investigate the underlying scientific basis for forensic analysis of fractured and torn surfaces, by employing the fundamentals from the field of fracture mechanics and the nature of the material behavior. This quantitative approach has the potential to enhance the ability of forensic scientists to capture, visualize and analyze fracture patterns, and possibly provide new methodologies for trace evidence. The project will employ spectral analysis of 3D fracture surface topography-measurements to associate or to differentiate fracture surfaces in the performance of physical comparisons. We will utilize an understanding of material failure mechanisms (developed in the field of fracture mechanics), with digital image analysis, to construct protocols for the association (or exclusion) of pairs of surfaces.

A material’s fracture surface consists of 3-D features, with associated spatial frequency signatures, that are dictated by the material’s intrinsic microstructure and external loading history. The topography of a fracture surface is dependent on the ratio of the local material resistance to fracture vs. the local stress state (i.e. load severity), and this relationship can be used to forensically compare fracture surfaces. The quantitative expression of complex microstructural details, combined with the quantitative characteristics of applied load, have the potential to provide quantitative signatures for fracture surfaces, expressed as distributions of the spatial sizes and orientations of the features of a fracture surface. These, then, can be used to support the discriminant analysis of fracture match, yielding a statistical expression of fracture match.

A 3D spectral analysis of fracture surface, based on the use of white light non-contact surface profilometers, will be evaluated to provide fracture surface measurements. The proposed analysis will be self-calibrated for fracture-feature-characteristics identification. This self-calibration should strengthen the methodology, and should expand its potential application across a broad range of fractured materials, with diverse textures and mechanical properties. Moreover, it would provide ease of use for forensic examiners, especially when a user-friendly interface with the analysis tools is developed.
The analytical protocol will be examined to access fracture-match threshold(s), reliability and uncertainty(ies) of measurement. Successful preliminary work supported by the USDOE Ames Laboratory-Midwest Forensic Resource Center suggests a two-year development. First phase, Year-1, a detailed validation study will be conducted on controlled laboratory samples from prey tool steel, with focus on analysis tool assessment and improvements. Efforts will focus on morphological measurement practices, development of a mathematical framework for describing non-continuous fracture events, and establishment of measurement uncertainties. Second phase, Year-2, a broader range of materials class such as metals, glass and plastic fragments will serve as the subjects for further protocol applicability and evaluation. We will explore the role of environmental degradation effects (moisture, heat/cold and corrosives) on the topology of fracture surfaces, to ascertain the applicability of the technique to weathered specimens. Our forensic collaborator will examine the testing protocol to identify its shortfalls and possible improvements.

Assuming developmental success, the proposed technique may be utilized in; (i) Evaluating the 3D surface characterization for representative metal, glass and plastic fragments, (ii) analysis of fracture fragments or torn sections where a visual jig-saw match between fragments cannot be established, (iii) understanding the role of environmental deterioration of fracture surfaces, and (iv) possible expansion to address fibrous materials and torn tabs. A detailed report with the scientific findings and implications will be generated, for forensic analysts’ use of spectral analysis of 3D fracture surface topography-measurements to associate, or differentiate, metal, ceramic and plastic fracture surfaces. This research will be conducted in response to the NIJ’s expressed need for knowledge underlying forensic science disciplines, and in collaboration with forensic scientists working in a forensic laboratory.

NIJ Point-of-Contact

Program Manager: Danielle McLeod-Henning
 IsoForensics, Inc.

“Isotope Analyses of Hair as a Trace Evidence Tool to Reconstruct Human Movements: Combining Strontium Isotope with Hydrogen/Oxygen Isotope Data”

Principal Investigator:
Dr. Brett Tipple
brett@isoforensics.com

Funding Amount:
$342,606 for 2 years

Abstract: Recent critical advances in high resolution multi-collector inductively coupled plasma mass spectrometry (HR-MC-ICP-MS) technologies allow for increased application of strontium isotope (87Sr/86Sr) analysis of human hair to determine an individual’s travel histories and region-of-origin. It has been previously established that 87Sr/86Sr ratios of internal tissues (e.g., bones and teeth) relate to geography, however human hair differs from other Sr-containing human tissues in that hair it is primarily influenced by exogenous, rather than endogenous, Sr contributions. Each distinct Sr-source imparts a unique Sr isotope signature to hair that is of forensic use and each source has the potential to be an ideal forensic tool for law enforcement personnel to assist in the reconstruction of an individual’s geographic-movement histories. Until very recently, technological limitation did not allow these two Sr-source signals to be separated. Here, we propose to analyze strontium isotope ratios of (a) 200+ in-house archived hair samples previously collected from throughout the United States and originating from regions with different soil strontium isotope ratio values and (b) new intra-city collections needed to differentiate exogenous from endogenous strontium contributions. We will measure the strontium isotope values to understand how hair 87Sr/86Sr values relate to geography. We will then build a geospatial model and map of hair 87Sr/86Sr values across the U.S. and develop mechanistic models to describe the exogenous Sr-signal incorporation to hair. In addition, all inhouse hair samples to be used in this study have been previously analyzed for hydrogen and oxygen isotope ratios. The proposed hair 87Sr/86Sr model/map will be combined with previously developed hair hydrogen and oxygen isotope models/maps to create a multi-proxy and high fidelity geo-location tool of forensic relevance. Applications of this product are diverse and include reconstructing travel histories of unidentified murder victims, reconstructing monthly movements of trans-nationals associated with crimes and having uncertain origins, and reconstructing the region-of-origin of exploited individuals transported across state and/or national boundaries.

NIJ Point-of-Contact

Program Manager: Gerald Laporte