The Research After *Eureka*! – How NIJ Funded Research Supports the Science of Forensics

2013 Forensic Science R&D Grantees Meeting Agenda

Tuesday, February 19, 2013

Welcome and Opening Remarks
8:20-8:30 am

Dr. Greg Ridgeway
Acting Director
National Institute of Justice

Morning Session I – Straight to the Bone: Advances in Forensic Anthropology
8:30-10:00 am

8:30 am	***A Subadult Radiographic Database for Forensic Anthropology*** Stephen Ousley, Mercyhurst University
9:00 am	***Improving Sex Estimation from Crania using 3-dimensional CT Scans*** Natalie Shirley, Lincoln Memorial University, University of Tennessee
9:30 am	***Independent Validation Test of Microscopic Saw Mark Analysis*** Jennifer Love, Harris County Institute of Forensic Sciences

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Break
10:00 – 10:15 am

Morning Session II – Falling Into Decay: Postmortem Interval and Molecular Autopsy
10:15 am - 12:15 pm

10:15 am	***Microbial Community Change Associated with Decomposing Corpses*** Rob Knight, The Regents of the University of Colorado
10:45 am	***Using Differential RNA Degradation to Estimate an Extended Postmortem Interval*** Clifton Bishop, West Virginia University
11:15 am	***Applications of Curvilinear Development Modeling for the Blow Fly Lucilia sericata (Meigen)*** Leon Higley, University of Nebraska-Lincoln
11:45 am	***Investigating Unexplained Deaths through Molecular Autopsies*** Yingying Tang, New York City Office of the Chief Medical Examiner

Lunch Break – On Your Own
12:15 pm-1:15 pm

Afternoon Session I – Tarnished Gold Standard: Limited Quantity and Degraded DNA
1:15-3:15 pm

1:15 pm ***What I Thought I Knew about Ancient and Degraded DNA, but Have Come To Learn***
Brian Kemp, Washington State University

1:45 pm ***Addressing the Quality and Quantity: the role of DNA repair and Whole genome Application in Forensically Relevant Samples***
Bruce Budowle, University of North Texas

2:15 pm ***Simultaneous Detection of mtDNA and a Nuclear Pseudogene Insert using Common Forensic mtDNA Primer Sets and Deep Sequencing***
Mark Wilson, Western Carolina University

2:45 pm ***Towards Genotyping Single DNA Molecules without PCR***
Matthew Antonik, The University of Kansas Center for Research, Inc.

Break
3:15-3:30 pm

Afternoon Session II – Pills and Particles: Toxicology and Linking Trace Evidence
3:30-5:00 pm

3:30 pm ***In vitro Inhibition of Oxycodone Oxidative Metabolism by H2-antagonists and Proton Pump Inhibitors***
David Moody, University of Utah

4:00 pm ***Pattern Recognition Assisted Infrared Library Searching to Enhance Investigative Lead Information for Automotive Paints***
Barry Lavine, Oklahoma State University

4:30 pm ***Exploitation of Very Small Particles to Enhance the Probative Value of Carpet Fibers***
David Stoney, Stoney Forensic Inc.

Wrap-up & Adjourn
5:00 pm

2013 Forensic Science R&D Grantees Meeting
Grantee Presenter Abstracts

A Subadult Radiographic Database for Forensic Anthropology

Stephen Ousley

Mercyhurst University (2008-DN-BX-K185)

Current techniques in forensic anthropology for estimating age at death in subadults are of questionable validity due to a lack of data from modern and diverse groups and appropriate statistical methods. Age estimation in subadults has been based on data from clinical studies undertaken to assess whether children of known age, many born in the 1930's, showed normal bone growth and development. In forensic analysis, however, the unknown age is to be estimated from known (measured) bone lengths or developmental status. Modern children are tall for age and mature faster skeletally, dentally, and sexually than before, effects known as secular changes. Radiographs obtained from Medical Examiner's and Coroner's Offices (MECOs) provide anthropologists with a means of assembling large amounts of data from modern subadults with known age, sex, ancestry, date of birth, and other demographic information. Such a radiographic database fills a need for data that are not included in the well-known Forensic Data Bank at the University of Tennessee. NIJ grant award 2008-DN-BX-K152 established a database of digital radiographs and demographic data from modern American subadults.

In all research involving growth, it is essential to obtain large samples by age, ancestry/ethnicity and sex, and not merely a large sample in total. Radiographs from a clinical setting provide samples from ages that are poorly represented in MECOs, which have very few cases involving children between five and 14 years of age. Basic information to be gleaned from radiographs includes appearance and fusion of epiphyses and long bone lengths.

Eighteen MECOs and two clinical offices were visited during the course of the grant. Information regarding age, sex, stature, ancestry, birth date, anthropometric data such as stature or crown-heel length, weight, and manner of death were recorded when available. Clinical radiographs were obtained from two locations. A total of 44,220 radiographic images were assembled from 9,709 different individuals, almost 4,000 of which were x-rayed on multiple occasions at the clinical offices. Of the 9,709 individuals, 4,061 are females, 5,610 are males, and 38 are of unrecorded sex. All major ethnic groups and ancestries in the US are reasonably well represented.

Four Master's students at Mercyhurst University have utilized the radiographic scans in their thesis work so far and their results confirm and illustrate that children today are taller for a given age and are maturing faster skeletally than suggested by the currently used forensic and clinical standards. The radiographic database will provide the basis for more accurate and unbiased age estimation in modern subadults. The collection has tremendous potential for further research in age, sex, and ancestry estimation methods, trauma analysis, and bone healing rates, and the database is expected to grow further.

This grant fits with theme of the 2013 AAFS Meeting, “The Forensic Sciences: Founded on Observation and Experience, Improved by Education and Research” because experience and observation must always be informed by up-to-date research.

Improving Sex Estimation from Crania using 3-dimensional CT Scans

Natalie Shirley

University of Tennessee (2008-DN-BX-K182)

The goal of this research was to re-evaluate sex estimation from the cranium and improve it beyond what has been achieved with traditional methods. Traditional methods involve either a subjective assessment of gross morphological features such as the glabellar region, nuchal region, mental eminence, orbital margins, and mastoid process, or a metric assessment using a standard set of ectocranial measurements and discriminant functions. Accuracy rates of these methods do not normally exceed 90%, which compares unfavorably with postcranial long bones, which can often achieve 95%. Since crania are over represented among forensic skeletal remains, the importance of better sexing techniques becomes obvious.

We used CT scans of the William Bass donated collection at the University of Tennessee to comprehensively search for the most sexually dimorphic regions of the cranium. Three-dimensional models of the crania were rendered from the CT scans and used to develop statistical bone atlases that capture the primary shape variation of the skeletal element. A statistical treatment combining principal components analysis and Fisher's discriminant ratio was used to pinpoint regions of high dimorphism. Computer-automated endo- and ectocranial measurements were taken in these regions and subjected to linear discriminant analysis with variable selection. The most powerful discriminators were then translated into measurements that in some cases can be taken with ordinary instruments, and in some cases require measurements from radiographs.

The most dimorphic region of the skull is the glabellar region of the frontal bone. The projection of glabella beyond the nasion-supraglabella line was the single most dimorphic dimension. Beyond that, high sex dimorphism in bizygomatic breadth was confirmed, as was cranial base length and mastoid length. Perhaps the most surprising result was the role played by vault thickness at metopion. Unlike all other vault thicknesses, females exceed males at this site.

Using as few as four dimensions, we are able to achieve sexing accuracies of 95% or better. These results put the cranium on a par with postcranial dimensions. They also confirm, consistent with our history of observation and experience, the importance of specific regions of the cranium, in particular the frontal, in estimating sex, but improve it by quantification rather than merely observing or scoring visually. The results also yield additional insight into the nature of sex dimorphism. Dimorphism in the frontal is likely the result of its responsiveness to sex hormones that develop during adolescence. Cranial base dimorphism is a growth phenomenon resulting from earlier cessation of growth in females. Occipital dimorphism is primarily functional, resulting from greater muscle attachment sites in males.

This research relates to the 2013 AAFS meeting theme, as we used innovative three-dimensional modeling to locate regions of significant cranial sex dimorphism, quantified this dimorphism with a combination of traditional and novel measurements, and improved the accuracy of sex estimation from the cranium using a minimum number of measurements. The most dimorphic regions agreed with many of the regions that are used to make subjective sex assessments from the cranium, and new information about cranial dimorphism was revealed as well.

Independent Validation Test of Microscopic Saw Mark Analysis

Jennifer Love

Harris County Institute of Forensic Sciences (2010-DN-BX-K235)

Independent validation is a critical step of science through which methods are strengthened and error rates defined. However, little research is aimed at simply validating established methods, especially in the field of forensic anthropology. Often a method is published and, if lucky, anthropologists see merit in the method, have confidence in the reported uncertainty and adopt it. Most often the next step is to improve the method, seeking and testing additional variable using new technologies. This approach to research is well-exemplified in the area of tool mark analysis on cartilage and bone. For example, three seminal articles established the method of microscopic saw mark analysis on bone, none of which defined an error rate inherent to the method (Bronte 1975, Andahl 1978, Symes 1992). This research was followed by an attempt to increase the discriminatory power of the method (Saville et al 2007) and then to apply it to worn saws (Freas 2010) and burned remains (Pope and Smith 2004; Marciniak 2009). Despite the attention given to the method, to date no one has published an independent validation of the original method or an associated error rate. Given this void in the research, Harris County Institute of Forensic Sciences through grant funding (2008-NIJ-1735 and 2010-DN-BX-K235) designed two studies to validate the generally accepted method of microscopic saw mark and knife mark analysis in bone and costal cartilage.

The first study (2008-NIJ-1735) was designed to validate microscopic knife mark analysis. For the study, three knives with different blade types were used to make experimental cut marks in costal cartilage of pigs. Each cut surface was cast and each cast was examined by three analysts working independently. Presence of striations, regularity of striations and presence of a primary and secondary striation pattern were recorded for each cast. The distance between each striation was measured. The results showed that striations were inconsistently impressed on the cut surface by the blade's cutting edge. Also, blade type classification by presence or absence of striations led to a 65% misclassification rate.

The second study (2010-DN-BX-K235) was designed to validate microscopic saw mark analysis. Four morphologically different saws were used to make experimental saw marks in human femurs. The saw marks were examined independently by three doctoral level anthropologists using a digital microscope. Eleven variables were documented for each saw mark. The rate of saw type misclassification ranged from 8.62% to 17.82%.

In both studies, classification tree modeling was used to statistically establish a ranking of multiple qualitative and quantitative variables. The end product, a decision tree, was generated to guide the analyst through the interpretation process to reach a conclusion based on sound science with an associated error rate. Presentation of these studies directly relates to the theme of the 2013 AAFS Meeting, "*The Forensic Sciences: Founded on Observation and Experience, Improved by Education and Research*," through providing a model to reexamine historically accepted, qualitative methods.

1. Andahl RO. (1978) The examination of saw marks. *Journal of Forensic Sciences Society* 18:31-46.

2. Bronte W. (1975) Tool marks in bones and cartilage. *Journal of Forensic Sciences* 20(2):315-25.
3. Freas, LE (2010) Assessment of wear-related features of the kerf wall from saw marks in bone. *JFS* 55(6):1561-1569.
4. Marciniak SM (2009) A preliminary assessment of the identification of saw marks on burned bone. *Journal of Forensic Sciences* 54(4): 779-785.
5. Pope EJ, Smith OC (2004) Identification of traumatic injury in burned cranial bone: an experimental approach. *JFS* 49(3):431-440.
6. Symes SA, (1992) *Morphology of Saw Marks in Human Bone: Identification of Class Characteristic.* Dissertation at The University of Tennessee, Knoxville.

Microbial Community Change Associated with Decomposing Corpses

Rob Knight

The Regents of the University of Colorado (2011-DN-BX-K533)

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 grave soil, but no method is successful in every scenario. Therefore, the development of new forensic tools is important. We live in a world dominated by microbes. Microorganisms dominate the diversity of most—perhaps all—environments, from soils to oceans to animal intestines. This ubiquity coupled with recent knowledge gained from high-throughput sequencing studies about their diversity makes microbial ecology a promising field to search for new forensic tools. Two characteristics of microbial diversity make them appealing as a potential forensic tool 1) as in other environments, the assembly and succession of microbial communities associated with decomposing corpses is likely predictable over time 2) microbial communities in different environments (including both animal-associated and soil-associated environments) are quantifiably distinct. Our goal is to explore the potential for using microbial communities associated with corpses to estimate time since death and to locate possible grave sites. Although most culture-independent microbiome research has focused on bacteria communities, similar research is now being done on microbial eukaryotic communities, including fungal communities. In our NIJ-funded research, we explore the use of 16S ribosomal RNA (universal bacterial), 18S ribosomal RNA (universal eukaryotic), and Internal Transcribed Spacer (ITS, fungal specific) high-throughput sequence data of corpse-associated microbial communities for forensic science. We conducted laboratory experiments in which mice corpses were allowed to decompose under a variety of soil conditions. Samples from the abdominal cavity, skin and gravesoil were collected at regular time intervals from 5 corpses per treatment group. Genes were sequenced using Illumina HiSeq deep sequencing technology and computational pipelines were used to characterize the succession of bacterial and eukaryotic communities during the decomposition process. Microbial communities associated with decomposing corpses changed progressively over the time course of decomposition and became highly differentiated from starting communities at each site (soil, abdominal, or skin). Furthermore, microbial communities changed fairly consistently across replicates within each time point at each site. Microbial taxa abundance changes over time often made excellent biological sense. For example, our results lend support to the long-held hypothesis that decomposition in the abdominal cavity shifts from anaerobes (e.g. *Lachnospiraceae*) to aerobes or facultative anaerobes (e.g. *Rhizobiales*) after corpse rupture occurs. Additionally, we discovered that a bacteria-eating nematode in the family *Rhabditinae* becomes abundant at all sites during the late stage of decomposition. We demonstrate that microbial communities hold great promise as a forensic tool as there is a predictable succession of taxa over time. Furthermore, our work relates to the 2013 AAFS Meeting theme by highlighting a new direction of research for forensic science.

Using Differential RNA Degradation to Estimate an Extended Postmortem Interval

Clifton Bishop

West Virginia University (2010-DN-BX-K180)

Time and temperature dependent differential decay rates of selected mRNAs and rRNAs are used to estimate an extended time since death or postmortem interval (PMI). RNA for these studies is isolated from tooth pulp and subjected to qPCR analysis. Studies using tooth pulp provide several advantages: teeth are one of the last parts of a body to decay, the pulp is resistant to predation/scavengers, and the hard enamel provides protection from external factors (including humidity). Our results indicate that useful data can be obtained from tooth pulp long after complete skeletonization has occurred, extending the time frame over which PMI estimates can be obtained using conventional forensic entomology. Additionally, the molecular approach presented here can be applied to samples isolated anywhere in the world, without specialized knowledge of local insect fauna.

Applications of Curvilinear Development Modeling for the Blow Fly Lucilia sericata (Meigen)

Leon Higley

University of Nebraska-Lincoln (2010-DN-BX-K231)

The blow fly *Lucilia sericata* is a ubiquitous fly that can quickly locate and colonize decomposing remains. The developing stages of *L. sericata* can be used in human death investigations for estimating time since death or postmortem interval (PMI). The PMI estimation can be crucial in the reconstruction of crime scene events, such as: movement of the body, toxicology, suspects, and testimony corroboration. A series of experiments were conducted to create a curvilinear development model for *L. sericata*. The study was comprised of eleven temperatures (7.5 ˚C, 10 ˚C, 12.5 ˚C, 15 ˚C, 17.5 ˚C, 20 ˚C, 22.5 ˚C, 25 ˚C, 27.5 ˚C, 30 ˚C, and 32.5 ˚C) with a light:dark cycle of 16:8. Twenty eggs (collected within 30 minutes of oviposition) were placed on 10 g of beef liver that was in a 29.5 mL plastic cup. The cup was placed in a 7 cm x 7 cm x 10 cm plastic container that had 2.5 cm of wood shavings in the bottom. The container was then placed randomly in a chamber. Each life stage (egg-1st stage, 1st stage-2nd stage, 2nd stage-3rd stage, 3rd stage-3rd migratory, 3rd migratory-pupation, pupation-adult) was calculated from accumulated degree hours (ADH) and divided equally into five sampling times. Each sample was replicated four times, for a total of 20 measurements per life stage. Results indicated that times of transition between maggot life stages are much more protracted than previously thought, and provide an basis for establishing indications of variability for PMI estimates. Comparisons of PMI estimates with data here versus previous (smaller) data sources, demonstrate the practical importance of improving the biological basis for determining PMI.

Investigating Unexplained Deaths through Molecular Autopsies

Yingying Tang

New York City Office of the Chief Medical Examiner (2011-DN-BX-K535)

Sudden unexplained death syndrome (**SUDS**) in apparently healthy individuals is not only concerning for the families but it also presents vexing challenges for medical examiners, law enforcement, and the public health of society. Even after a thorough autopsy, comprehensive laboratory tests such as toxicology, microbiology, metabolic screening, review of clinical history, and scene investigation, the cause of death often remains unknown. More than 4,500 infants die suddenly each year in the United States with no immediate or obvious cause of death; half of those deaths remain unexplained after a forensic investigation. Furthermore, the prevalence of sudden unexplained deaths above infancy age (1-22 years old) is also estimated to be greater than 4,000 annually in the United States. Despite the SUDS rate decreasing over the past decade, the rates are still disproportionately high amongst certain population groups, thus, remaining an important public health priority. A sudden unexplained death leaves medical examiners and law enforcement personnel to make difficult decisions.

In recent years, advances in molecular genetics have shed light on how genetic factors play a role in sudden deaths. Since 2008 our Molecular Genetics Laboratory (accredited by American College of Pathologists) in the New York City Office of Chief Medical Examiner (**NYC OCME**) has been providing molecular testing routinely in a SUDS investigation, even though molecular autopsies are not yet regularly performed nationally. Currently, however, our laboratory tests six major cardiac arrhythmia genes that account for only 10 - 15% of SUDS, indicating that more candidate genes need to be evaluated.

In our grant awarded project, we proposed to identify and significantly expand the number of disease-causing mutations that contribute to SUDS. To achieve this goal, we proposed to test 80 candidate genes in a large study cohort (250 cases), which have undergone a highly selective process, using next-generation sequencing technology from Illumina. This grant will facilitate our lab in the discovery of new genes and help us better comprehend sudden unexplained deaths.

We also recognize that understanding genetics in SUDS requires a multidisciplinary approach, where forensic laboratory geneticists, medical examiners, bioinformatician and biostatistician, academic researchers, and clinicians must work together. We have embraced this approach and have published the results of such collaborative efforts. This research project fits with the theme of the 2013 AAFS meeting, "*The Forensic Sciences: Founded on Observation and Experience, Improved by Education and Research*" because we will expand our knowledge on various genes and their mutations that can cause SUDS. Through this grant we will further our education and expand our research to aid the medical examiners in determining cause of death and assist law enforcement officials in their investigation. In addition, our research can contribute towards the common goal of improving public health for the population groups with high risk of SUDS and affected families by equipping the clinicians with another tool in providing personalized preventive treatment options.

What I Thought I Knew about Ancient and Degraded DNA, but Have Come To Learn

Brian Kemp

Washington State University (2008-DN-BX-K008)

The ability to analyze DNA from plants, animals, and microorganisms that have been long since dead (or are now even extinct) opens up a tremendous potential for studying evolution. However, the inherent power of ancient DNA evidence is tempered by the challenging nature of its retrieval. Proposed criteria for the authentication of ancient DNA results take advantage of observed "behavioral" differences of ancient DNA from that of modern DNA. Yet, our knowledge about of these differences relies strongly on the experiments designed to detect them.

With grant support from the United States National Institute of Justice, various experiments were conducted to explore a number of the basic qualities of degraded and low copy number DNA samples. The results enhance our basic understanding of the characteristics of degraded DNA molecules and are bound to improve future studies of ancient and/or forensic DNA.

Specifically, in this presentation will be detailed quantitative PCR (qPCR) experiments used to evaluate the efficacy of sodium hypochlorite in the removal of contaminating DNA from bone surfaces. While our findings are consistent with previous studies that found sodium hypochlorite to be highly efficient (~81-99%) at contamination removal, there emerged no treatment that removed 100% of the contamination across all of the experiments. Furthermore, this study suggests that previous claims that sodium hypochlorite is particularly damaging to endogenous ancient DNA molecules are inaccurate. Experiments conducted during this phase of the grant period led to two additional relevant observations. First, mitochondrial DNA (mtDNA) preservation across individual bones was determined to be highly variable and not related to the density of the bone material, despite previous belief of such a relationship. Secondly, utilizing qPCR and a synthesized "standards" approach to measure the efficiency of some common DNA extraction methods for degraded skeletal samples, all methods were determined to perform poorly in retaining short segments of DNA. These findings challenge low copy number expectations, suggesting that ancient and forensic specimens may contain far more preserved genetic material than previously recognized.

In line with the theme of the 2013 AAFS meeting, this presentation will emphasize the importance of observations over intuition in continuing to build expectations for working with highly degraded DNA samples. It further highlights the role of serious academic scholarship in unraveling how we come to know what we think we know.

Addressing the Quality and Quantity: the role of DNA repair and Whole genome Application in Forensically Relevant Samples

Bruce Budowle

University of North Texas (2010-DN-BX-K227)

A variety of solutions which address the problems encountered when profiling forensic samples that contain a limited quantity of low quality DNA have been pursued. These include *in vitro* DNA repair and whole genome amplification (WGA). Neither of these methods has been implemented on an operational scale. Adoption of the methods by casework laboratories may be low due to the perceived risk/benefit ratio for these methods and may present a barrier for their widespread adoption. Available data on the downstream effects of template generation in this manner is not sufficient to warrant effort by the forensic community to invest in the technologies. It is not known how much additional information or success can be gained and the number or type of cases/samples that would be impacted remains undefined. To employ these methods, it is necessary to determine if DNA repair or WGA introduce errors that affect the reliability of DNA typing results. The goal of this proposal is to develop and identify the optimal approach(es) to DNA repair and WGA and determine if they are suitable for use with commonly encountered sources of degraded and low copy DNA.

Simultaneous Detection of mtDNA and a Nuclear Pseudogene Insert using Common Forensic mtDNA Primer Sets and Deep Sequencing

Mark Wilson

Western Carolina University (2010-DN-BX-K171)

Challenging forensic DNA samples extracted from, for instance, bones and hair can be degraded and/or contain very little DNA. Because of it's high sensitivity, mitochondrial DNA (mtDNA) analysis is often utilized on these kinds of samples in forensic casework. Studies employing newly emerging DNA sequencing technologies are designed to interrogate targets down to the single molecule level. As expected, such studies have shown tissue differences (heteroplasmy) within individuals, and have lead to recent public calls for a re-evaluation of current interpretational approaches to forensic mtDNA comparisons.

Human mitochondrial DNA analysis, in a forensic setting, is currently limited in both breadth (the amount of sequence data obtained) and depth (the ability to detect minor variants arising from mutations but present at very low levels). Using emerging technologies, an extension of the breadth of sequence data obtained from a forensic sample can extend to the entirety of the human mtDNA genome. However, certain technical limitations related to the paucity of DNA present in the DNA extract must be addressed before an increase in sequence breadth can be assured. Extension in the complementary dimension (depth) is expected to reveal subtle mixtures that are currently not detected by forensic DNA laboratories using Sanger di-deoxy terminator chemistries. Hence, new DNA sequencing technologies have the promise of providing information in both of these dimensions and thereby expanding the utility of mtDNA analysis in forensic science.

We have determined the minor variant detection threshold of the Roche GS Junior instrument using mixtures of human mtDNA control region amplicons with known sequences. In addition to the expected variants originally obtained using dideoxy terminator sequencing, we also detected a set of unexpected SNP variants in HV1b reads at a level of approximately 1%. These variants are reproducible in nuclear-rich tissues and are always detected as a set within individual reads of HV1b amplicons provided sufficient DNA is present for amplification. The total depth of coverage did not appear to affect the level at which the unexpected variants were detected.

A standard nucleotide BLAST search of the minor variant sequence was performed and was identical to a 611 bp nuclear mitochondrial pseudogene (NumtS) originally reported in 1995 by Zischler et al. This NumtS is an insertion of the mitochondrial control region (bases 16,089 – 59) on the short arm of chromosome 17, spanning the primer binding sites of the targeted HV1b region. Nuclear DNA specific primers flanking the insertion were used to amplify the pseudogene from buccal extracts without amplifying DNA from the mitochondrial control region. This amplification strategy confirmed the presence of the NumtS in nineteen out of twenty donors, with one donor being homozygous negative for the insertion. Dideoxy terminator sequencing was used to successfully confirm the presence of the variant sequence in the amplified NumtS from the donors positive for the insertion. This identification furthers our understanding of human mtDNA variants and is expected to have a positive effect on the interpretation of mtDNA profiles using deep sequencing methods in forensic casework.

Towards Genotyping Single DNA Molecules without PCR

Matthew Antonik

The University of Kansas Center for Research, Inc. (2011-DN-BX-K542)

In attempting to PCR low copy number DNA samples, stochastic variations in early cycles are amplified along with the signal, yielding results which are difficult to interpret. In our research, we are attempting to develop methods that bypass this problem by avoiding PCR and instead analyze single DNA molecules using a combination of fluorescence and scanning probe techniques. Although these single molecule approaches will likely be more time consuming and expensive than PCR analysis, they will be useful in the typing of high-value DNA evidence in selected cases.

The single molecule approaches that we are developing consist of a two step procedure where rare DNA molecules are localized to a surface and subsequently genotyped. A variety of ligands are currently being tested to selectively bind DNA to the surface while simultaneously indicating their presence via fluorescence. By selectively trapping DNA on the surface, purification and quantification of the amount of DNA present are simultaneously achieved, and a more concentrated DNA sample may be yielded. Following attachment, DNA strands are reversibly labelled with fluorescence markers and individually genotyped. Genotyping is performed by counting fluorescence markers (one per STR), measuring a fluorescence intensity which is proportional to STR repeat length, or measuring the DNA length via fluorescence resonance energy transfer, two color localization experiments, or scanning probe techniques.

The absence of PCR in the analysis avoids commonly encountered artifacts such as stutter, allele drop out, pull up, etc. Single molecule artifacts, including incomplete labeling and Poisson counting fluctuations, are addressed by repeated measurements of the same molecule, which is made possible by the reversible fluorescence labeling. These approaches have the potential to be non-destructive to the DNA evidence, allowing subsequent analysis using other techniques. Further development is targeted towards isolating DNA from a single cell and genotyping the 13 CODIS STR loci. In addition to permitting genotyping of low copy number DNA evidence, our techniques should also be useful in resolving mixtures and are applicable to DNA samples which are normally not amenable to currently accepted protocols and PCR based analysis (e.g., cell-free DNA evidence, trace DNA, and/or damaged, degraded, or contaminated DNA samples). Moreover, the purification and quantification of the DNA evidence may sufficiently improve its quality for traditional PCR genotyping procedures.

Single molecule techniques have been highly developed over the last two decades to permit accurate, quantitative, and robust characterization of molecular species. To date, however, these approaches have not been widely applied to address the challenges faced in the forensics community. Whereas most single molecule research establishes confidence in the measurements by interrogating many individual molecules once each, this research seeks to retool the techniques to perform multiple measurements on a single molecule. This work is guided by interactions with experts in the forensics community who highlight particular capabilities which would be advantageous in the context of their routine analysis. This project is therefore basic research guided by the experience of our collaborators.

In vitro Inhibition of Oxycodone Oxidative Metabolism by H2-antagonists and Proton Pump Inhibitors

David Moody

University of Utah (2011-DN-BX-K532)

The aim of the NIJ award is to study the potential for inhibition of the metabolism of oxycodone, buprenorphine and methadone in human liver microsomes (HLM) and the appropriate cDNA-expressed cytochrome P450s (rCYP) by a number of diverse drugs. The goal is to provide the forensic science community with information on when the presence of other drugs along with these opioids may offer alternative explanations for opioid concentration in the matrix, and as a warning to the general community as to which drugs may be dangerous to take along with these opioids. Oxycodone is metabolized to the inactive noroxycodone by CYP3A4 and 2C18 and to the equally, or more, active oxymorphone by CYP2D6. Inhibition of the first pathway may lead to more active drug and metabolite, inhibition of the second pathway may increase parent drug but decrease active metabolite. Here we present our basic approach and findings for oxycodone and the H_2-receptor antagonists and proton pump inhibitors (PPI). Oxycodone was first incubated with the potential inhibitors, HLM and an NADPH-generating system. Inhibitors were added at 3 concentrations, 10, 300 and 1000 µM for the H_2-receptor antagonists and 1, 30 and 200 µM for the PPIs. In one set the reaction was initiated after HLM, substrate and inhibitors had been added, in a second set the inhibitors, HLM and NADPH-generating system were pre-incubated for 15 minutes before adding substrate as a test for mechanism based inhibition. Following incubation for a set period of time reactions were stopped by addition of methanol and freezing. Samples were subsequently analyzed following liquid-liquid extraction by liquid chromatography-tandem mass spectrometry. The H_2-receptor antagonists, cimetidine and famotidine caused significant inhibition of both pathways with no effect from pre-incubation. Ranitidine and nizatidine inhibition did not exceed 50% at 1000 µM. The respective IC_{50}s (µM) for the rCYPs with cimetidine and famotidine were: for noroxycodone formation by CYP3A4, 65 and 190; by CYP2C18, 1000 and > 2000; for oxymorphone formation by CYP2D6, 120 and 300 µM. All five PPIs caused significant inhibition of noroxycodone formation with no effect from pre-incubation. The respective IC_{50}s (µM) for noroxycodone formation by CYP3A4 and CYP2C18 were: esomeprazole, 0.45 and 18; omeprazole, 0.80 and 12; lansoprazole, 1.2 and 25; rabeprazole 3.5 and 15; and pantoprazole, 1.0 and 60. Only lansoprazole and rabeprazole caused greater than 50% inhibition of oxymorphone formation in HLM. Their IC_{50}s (µM) for oxymorphone formation by CYP2D6 were 0.3 and 15 µM. The PPIs were more potent inhibitors than the H_2-receptor antagonists. The effect of omeprazole and esomeprazole on CYP3A4 and lansoprazole on CYP2D6 are notable. We are currently gathering similar data on the metabolism of R- and S-methadone to R- and S-EDDP and buprenorphine to norbuprenorphine.

Pattern Recognition Assisted Infrared Library Searching to Enhance Investigative Lead Information for Automotive Paints

Barry Lavine

Oklahoma State University (2010-DN-BX-K217)

New techniques to search IR library spectra from the Paint Data Query (PDQ) database have been developed to determine the model and year of an automobile from a clear coat paint smear that all too often is the only layer of paint left at the crime scene. In these cases, the text based portion of the PDQ database cannot identify the motor vehicle as to manufacturer and model. However, crucial investigative lead information can be extracted from IR spectra of clear coat paint smears using search prefilters. Wavelengths in the fingerprint region of the IR spectra indicative of the manufacturing plant were identified using a genetic algorithm for wavelength selection and classification. These wavelengths were formulated into search prefilters. Utilizing search prefilters configured in a hierarchical structure, many of the problems encountered in library searching of IR spectra have been addressed.

Most spectral comparisons performed during a search are of little use because the spectra in question are dissimilar. A prefilter can identify dissimilar spectra, thereby avoiding a complete spectral comparison. Prefilters also allow for more sophisticated and more time-consuming algorithms to be used for spectral matching since the size of the library is culled down for a specific match. Even in challenging trails where the samples evaluated were all the same model (General Motors) with a limited production year range, the respective manufacturing plants could be correctly identified. Furthermore, successful validation of all search prefilters was performed using blind samples. All search prefilters in this study developed using spectral data from one instrument could correctly identify spectra of paint samples collected on an instrument from a different manufacturer. An approach based on instrumental line functions was used to transfer the search prefilters (i.e., classificationmodels) between instruments.

Searches currently performed using the PDQ database often generate a large number of hits because the chemical information in the current PDQ database is only described in terms of generic chemical formulations. The major advantage of using the pattern recognition approach to identify paint samples is an increase in search accuracy because spectra from the entire database are searched. Improving discrimination capability between spectra in the database using the wavelet packet transform for spectral preprocessing and the pattern recognition GA to identify informative coefficients permits inter-comparison of original equipment material (OEM) automotive paint layer systems using the infrared spectra alone, This allows comparison of all possible pairs in the database, reducing dependence on the text-based portion of the database, resulting in improved ease of use and fewer errors.

Exploitation of Very Small Particles to Enhance the Probative Value of Carpet Fibers

David Stoney

Stoney Forensic Inc. (2012-DN-BX-K041)

Very small particles (VSP) are ubiquitous in our environment and are virtually ignored by forensic science (gunshot residue being a notable exception). These particles range in size from an order of magnitude smaller than conventional trace evidence, down to the molecular level (now routinely exploited through DNA analysis). We move around in a soup that is a combination of these VSP. They are present in and on other items of evidence and are an extraordinary, largely untapped resource that can be used to test associations and enhance probative value. By means of adhering VSP, evidence that is limited to class associations can be enhanced to highly individual, testable associations, akin to those arising from multiple-transfers of uncorrelated traces, or the co-occurrence of independent, highly variable events.

The combinations of VSP are so complex that until recently there was no practical method to identify and interpret these combinations. Particle combination analysis (PCA) is a new capability that focuses on these methods. This project involves the application of PCA as a means to objectively verify and improve traditional trace evidence analysis of fibers. VSP particles on the surfaces of transferred carpet fibers are used to test and enhance class associations of these fibers to reference (crime scene) carpets.

Methods have been developed to remove VSP from carpet fibers and computer-assisted SEM/EDS methods have been used to quantitatively characterize the recovered particle population. Hundreds to thousands of VSP have been harvested from the surfaces of individual carpet fibers and are sufficient to associate these fibers with their carpet area of origin. Carpets vary widely in the types and quantities of VSP adhering to their fiber surfaces. Highly characteristic patterns of particle types found in target particle profiles were consistently represented in the particle distributions from individual test fibers from the same carpet, and consistently absent among those from different carpets. Particle distributions found on carpet fibers can therefore contribute substantially to the weight of evidence linking fibers to a specific carpet.

Current research is focused on: (1) application and testing of the method under realistic conditions of carpet fiber transfer and crime scene sampling (collection by crime scene practitioners in jurisdictions across the United States); (2) expanding and improving VSP target particle type classification criteria (using statistical analysis of between-carpet and within-carpet data); (3) development of methods for the measurement of the degree of correspondence between two VSP profiles (based on well-defined statistical approaches and explicit assumptions); and (4) development of quantitative measures of the probative value of this degree of correspondence (based on estimates of population parameters for the correspondence measures among mated (same source) pairs and among unrelated (different source) pairs).

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