NEAFS Newsletter

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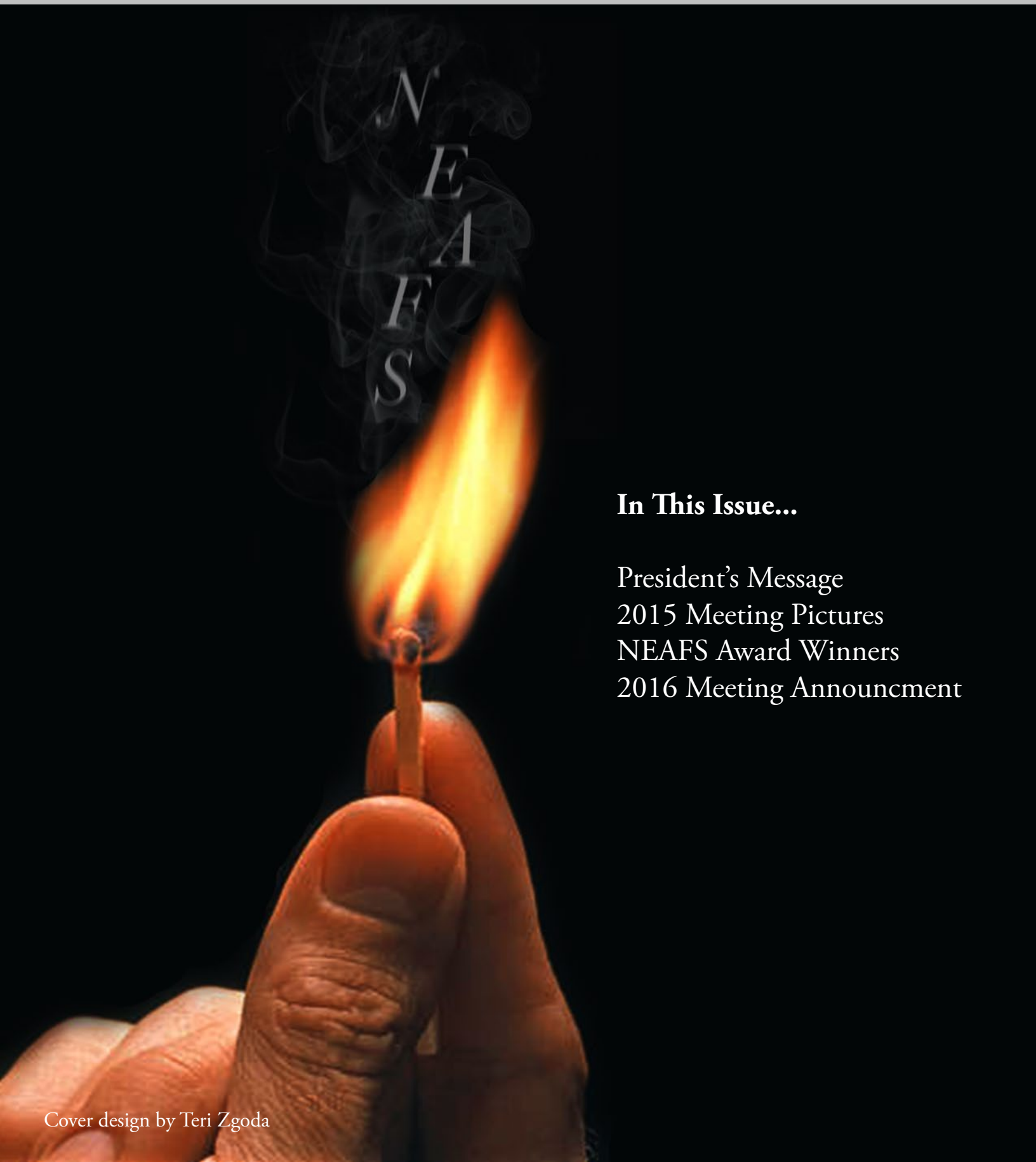

In This Issue...

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President's Message
The Year In Review

It has been a great year. Our membership increased, we developed a new partnership with the Green Mountain DNA conference, and we instituted a Visiting Scientist program. NEAFS will be handling many of the logistical issues associated with the Green Mountain DNA Conference and six NEAFS members are guaranteed conference registration. The Green Mountain DNA conference which occurs each summer in Burlington, Vermont limits registration to 75 individuals and those spots fills very quickly. The Visiting Scientist program is designed to help laboratories with training and technical assistance particularly in situations where travel for laboratory personnel may not an option. Any private or forensic science laboratory in the NEAFS geographical area needing technical, scientific, or training assistance may apply. Interested laboratories can simply fill out an application stating their request and NEAFS will make a good faith effort to find a qualified individual or individuals to come to the laboratory and provide the assistance. The person selected will be agreed upon between NEAFS and the laboratory. NEAFS will subsidize the visit which can be up to 5 working days in duration. Travel logistics will be arranged by the laboratory in conjunction with both the visiting scientist(s) and NEAFS. Applications are available under the Training link on the NEAFS website. As part of our commitment to serving our members and the forensic science community, NEAFS also sponsored a probabilistic DNA mixture interpretation workshop held at Cedar Crest College in September where NEAFS members attended for free.

You may notice a powerpoint presentation titled "learn what NEAFS can do for you" on the home page of the NEAFS website. I urge all of you to share this with your colleagues and students who are not members. Perhaps if they know the benefits that come with membership, they will apply for membership themselves. As can be learned by the presentation, there is no reason not to.

We had an outstanding annual meeting held in October in Hyannis. Erica Nadeau put together an outstanding scientific program that included an excellent General Session program on Cognitive and Human Factors in Forensic Science presented by Dr. Itiel Dror and a Plenary Session on the Maricopa County Sheriff's Mail Bomb Case presented in part by our very own Vincent Desiderio. In addition, congratulations are in order to both the undergraduate forensic science program at Western New England University and the graduate forensic science program at Boston University who won this year's Kirk Cup. I hope that more academic programs will participate in the future for what is becoming an annual event. Finally, Dave San Pietro was presented with the 2015 Meritorious Service Award, a well-deserved honor for my long-time friend and colleague.

Next year's meeting will hopefully be even better. Beth Goodspeed is already planning for our third visit to Atlantic City. To encourage even more participation from our membership, free registration will be given to any member or active applicant for membership who submits an abstract.

I have already said my goodbyes at the annual meeting. Simply said, it was an honor. Thank you for the opportunity to lead this organization. Good luck Erica! I could not ask for a better person to succeed me.

Larry Quarino

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2015 NEAFS Meritorious Service Award
David San Pietro

I am pleased to nominate David San Pietro for the 2015 NEAFS Meritorious Service Award.

Dave began making contributions toward the field of Forensic Science when he started working at the New York City Office of Chief Medical Examiner in 1992 after receiving his Master's Degree from Hunter College. He continues to make contributions to the field to this day. He is currently working towards his Doctorate Degree from the University of Verona, Italy.

I have had the honor of working with Dave at the Westchester County Forensic Laboratory for close to fourteen years. During this period, I have witnessed his enormous dedication to the field. He is a wealth of knowledge and is willing to share his knowledge with others. Dave is a big supporter of not "reinventing the wheel" and uses this philosophy to help our laboratory adapt to the changing climate of our field. He constantly keeps abreast of the continual changes and offers new recommendations.

Dave works in the disciplines of Serology, DNA and Bloodstain Pattern Analysis. He also is a core member of our Crime Scene Investigation unit which he was instrumental in creating. In 2001 the Westchester County Forensic Laboratory was one of the first two laboratories in the country to obtain accreditation from ASCLD/LAB for Crime Scene Investigation. I believe this would not have been possible without Dave's knowledge and dedication.

Dave is a Past President of NEAFS and has held several positions in NEAFS through the years working his way up from the Membership Committee. He has also been session chairs at several NEAFS meetings as well as a presenter (for example he presented "An Assessment of in vitro DNA Repair Mechanisms as Related to Damaged Forensic Specimens," at the 2014 Meeting). One of the key moments that define Dave is that during the 2009 NEAFS meeting at which time he was president, he was set to be the moderator for the DNA Literature Review & Mixture Interpretation Workshop for instructor Dr. John Butler. Unfortunately, at the last minute, Dr. Butler had to cancel. Instead of canceling, Dave took it upon himself to run the workshop and worked with Dr. Butler to set up a remote call in so that the members taking the workshop would not miss out on the great opportunity.

Dave is also a member of the American Academy of Forensic Science and the International Association of Identification. He is an adjunct professor at the University of New Haven were he helps mold the minds of future Forensic Scientists. He also actively teaches detectives, other scientists and future scientists in Bloodstain Pattern Analysis (at places such as Cedar Crest College and NEAFS meetings), sharing his expertise yet again in striving to continue to keep our field at its best.

Dave is one of the only individuals I know that will bring a DNA book home for personal reading. I can picture him sitting in a recliner chair with a trusted bottle of Diet Pepsi next to him ready to take on the next exciting chapter in Forensic DNA Typing by John Butler Ph.D. Or maybe he is struggling with deciding between titles such as Advanced Topics in Forensic DNA Typing Methodology or Advanced Topics in Forensic DNA Typing Interpretation? Not only does he read books in his chosen disciplines on his own time, his library is full of books on other Forensic disciplines that he does not even perform casework in such as The Science of Fingerprints or Footwear Impression Evidence. His passion and holistic approach to the field should be an approach shared by more scientists.
Another one of Dave's greatest traits is to bring a sense of humor to the laboratory. His ability to see the lighter side of things allows for stress levels to be lowered and to diffuse difficult situations. He has a light-hearted attitude and has a higher level of self-acceptance than most.

It is a privilege to know this dedicated scientist, teacher, student, colleague and friend. I can think of no one more deserving of this award than Dave. I nominate him and ask that you please call on me if I can further assist you in this regard.
Brandi Clark
Forensic Scientist - Westchester County Forensic Laboratory

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George W. Neighbor Jr. Memorial Scholarship Winner
Kaitlyn Hess
Cedar Crest College – Graduate Student

My interest and enthusiasm for science and its teachings has always, and still is present. At Lehigh University I began my studies and embraced my passion in the sciences by pursuing a degree in Biochemistry. During my time at Lehigh I completed challenging lecture courses in calculus, physics, biology, chemistry, and biochemistry while excelling in the demanding laboratory courses. Additionally I served as a research assistant, working to develop a rapid immunoassay technique for the presence of tuberculosis in human serum, and was recognized for the time and hard work I devoted to the study with a publication. I remained in good academic standing during my time at Lehigh and held several merit scholarships. I graduated from Lehigh in May 2014, after only three years, with a Bachelor of Science in Biochemistry, with honors, proving my ability to work hard and persevere. I chose to attend Cedar Crest College to pursue a Master of Science in Forensic Science degree to fulfill my interest in the field. The courses and faculty at Cedar Crest College have challenged me to not only manage my time more efficiently, but also have encouraged me to commit to a practice of excellence. I now ensure that every assignment, lab exercise, or public appearance is the best work I can produce and above all, professional. I have begun my graduate thesis research where I will be looking to simultaneously detect many illicit substances in urine using a BioSPME and LC-MS/MS method. I also have served as a graduate assistant, preparing and helping undergraduate students in biochemistry lab in fall 2014 and performing quality control testing on laboratory instruments in spring 2015. I hope to present my research at professional conferences throughout the upcoming academic year and to publish in a peer-reviewed journal as well. Following completion of my degree at Cedar Crest College I aspire to obtain a position in either the toxicology section or the drug chemistry section of a crime laboratory and to eventually work for a Medical Examiner's office. I would like to receive the George W. Neighbor Jr. Memorial Scholarship because I want to continue George W. Neighbor Jr.'s legacy of teaching and mentoring younger scientists. The success of future generations relies heavily on the instruction and knowledge given to the upcoming generations. It is incredibly important to advocate for the education of younger students since they will be our successors, hopefully presenting valuable insight, in the scientific community. Recently, I participated in an outreach program with Delta Delta Epsilon in which we engaged middle school students in forensic science related activities. Watching them learn and become excited with the activities we presented them was truly inspiring. I believe I have proven myself to be a hardworking, driven student, who has a sincere interest in helping teach and engage others. Furthermore, I am ambitious, motivated, and eager to begin diligently working on my thesis research such that I can teach my findings and make a meaningful contribution to the forensic science community.

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George W. Neighbor Jr. Memorial Scholarship Winner
Erica Johnson
Arcadia University – Graduate Student

It would be a great honor to receive this award in remembrance of someone so monumental in the field of forensic science. I am currently enrolled in Arcadia University's five year accelerated program in which I am completing my Bachelor of Arts in Biology and Master of Science in Forensic Science. In this program, I have been exposed to all disciplines of forensics such as pattern analysis, forensic chemistry, toxicology, serology, quality management in a forensics lab, and crime scene analysis. This past semester I have balanced graduate courses and my senior capstone project while earning a 3.79 GPA and the Dean's Honor List. My personal goals preceding completion of the program include working for a high-profile government lab as a forensic toxicologist. I also aspire to open my own lab overseas and incorporate quality management skills I have learned in my university curriculum. My graduate research is a toxological study on the detection and quantification of the drug ketamine and its metabolites in bones of remains that have been buried and exhumed at different stages of decomposition. With this research I have applied many new topics outside of the curriculum such as accumulated degree days, a method to score remains to determine 'total body score', postmortem interval calculations, and drug extraction techniques. This summer I will be participating in a five-week trace evidence internship at the New Jersey State Police Office of Forensic Sciences to expand my knowledge in a forensic discipline I have not yet explored.

Outside of the forensic program I am actively involved on campus and in the community. I am currently my Senior Class Treasurer in which I plan campus events and monitor all budgetary matters. Additionally, I am the president of Arcadia's feminist club, For the Women, in which I hold weekly meetings and host several events that fundraise for local organizations. This past February I was able to donate $1500 to a local organization called "Women in Transition" which helps women that are transitioning out of abusive relationships or other traumatic life chapters. Working with a club like this is another reason I am so interested in forensic science. I know that as a professional of this field I can help victims of violence. Outside of school, I waitress long nights every weekend to support myself and pay for the program tuition. Awarding me this scholarship would provide tremendous help in allowing me to reach my career goals.

I have worked very hard to learn and excel in all fields of forensic science, and I am excited to begin my career in this area of science.

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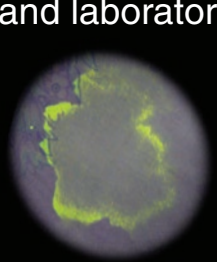

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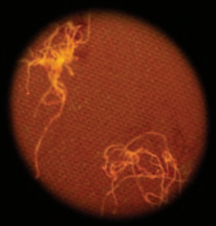

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Carol De Forest Forensic Science Research Grant Winner
Ashton Lesiak
University at Albany, SUNY – PhD Candidate

I am a fourth year Ph.D. candidate in the Department of Chemistry at the University at Albany, SUNY and I work in the research laboratory of Professor Rabi A. Musah. Based on my: (1) academic record, including success in coursework and research; (2) strength of character; and (3) future aspirations, I believe I am a strong applicant for the Carol De Forest Research Grant. Some details regarding my scholastic achievements, research success, character and future aspirations are outlined below.

I have excelled in my graduate coursework and have earned an overall GPA of 3.81. Classes that I have taken include: Advanced Forensic Chemistry, Comprehensive Biochemistry, Experimental Methods of Organic Structure Elucidation, and Special Topics in Mass Spectrometry. I received the Chemistry Department's Harry L. Frisch Award in 2013 for academic excellence. I was also a recipient of the George M. Neighbor Memorial Scholarship from NEAFS in 2013 for academic excellence and promise of success in the field of forensic science. In the Department of Chemistry, I have served as a teaching assistant for General Chemistry and Advanced Forensic Chemistry Laboratory courses. I have performed well as a teaching assistant (TA) and this was acknowledged by the Chemistry Department with an Arthur O. Long Department Teaching Award in 2013. I currently am the lead TA in the Advanced Forensic Chemistry Lab course, and I oversee the instruction of junior and senior-level undergraduate students in laboratory techniques and instrument operation. I also guide another Forensic Chemistry teaching assistant on laboratory protocols.

In addition to coursework, I have been successful in research pursued here at UAlbany and as a Ph.D. candidate. I have demonstrated that I can successfully advance a sound research proposal, develop and execute a plan for its implementation, and interpret and present the data obtained to the scientific community. I have worked on analysis of mind-altering synthetic street drugs and development of rapid novel drug detection methodologies for use in forensic laboratories. As outline in my appended research proposal, these studies have been amplified to include identifying and characterizing plant-based alternatives to drugs of abuse, and developing a statistical classification system to create a database of plant materials of abuse that can be adopted by government crime labs. I am first author on five peer- reviewed research articles, as well as a contributing author on two more, in venues that include the *Journal of Forensic Science, Forensic Science International, Rapid Communications in Mass Spectrometry,* and the *Analyst*. I am also contributing author on three submitted manuscripts and a fourth that is in preparation. I have disseminated my research findings in oral presentations at the NEAFS meetings in 2012 and 2014 and given poster presentations at the American Society of Mass Spectrometry (ASMS) International Annual Meeting in 2014 and the ASMS Sanibel Conference in 2015. Recently, I was selected to give an oral presentation on my research on natural alternatives to drugs of abuse at the international ASMS Annual Meeting in June 2015.

My success in research has not been without challenges. During my second year in the graduate program, I felt that my training and career aspirations would be best served by transitioning to a research laboratory different from the one in which I found myself. I was extremely frightened and uncertain about the consequences that such a change might have on my career both as a graduate student and a professional. I had to find within myself the courage to make the transition on my own volition, and risk losing all that I had worked towards, including my research project. I made the transition and I am now in a research laboratory where I am receiving the education and training to be highly successful in my chosen field. Furthermore, with my new advisor Professor Musah, I have been able to successfully develop, implement, and publish my research work. Facing and overcoming the challenge I faced in switching research labs midway through my graduate career has given me the confidence to know that I can and will accomplish anything to which I set my mind, and it is a tangible demonstration that my character is strong enough to overcome challenges I may face.

In its 2009 status of forensic science report, the National Academy of Sciences highlighted the need for the development of novel analytical and robust reporting methods that readily adapt to the rapidly changing drug abuse landscape. I aspire to make seminal contributions to that effort through development of cutting edge mass spectrometric and statistical analysis methods that will overcome many of the deficiencies of conventional

methods. I believe I can do so by serving as a Forensic Chemist at either the Drug Enforcement Administration Special Testing Laboratory or the Federal Bureau of Investigation Forensic Laboratory. I plan on applying for either a post-doctoral position in one of these laboratories or a position as a forensic scientist at a state or federal laboratory upon receiving my doctorate.

My accomplishments listed above demonstrate that I am a strong candidate for the Carol De Forest Research Grant. I am a qualified student and have proven success in my research in the forensic field. I have experience in developing and executing a research project, as well as in disseminating scientific results to the broader community. I firmly believe that I have the fortitude, passion and drive required to pursue a career as a forensic chemist and in this regard, I plan to eventually serve in a leadership role. I have a tremendous interest in casework, as well as in developing, improving and validating methodologies that can enhance and extend to ability of law enforcement to solve crimes and inform the drafting of relevant legislation. The grant proposal I have put forward is one step to contribute to the scientific community in that manner. With my strong academic background, breadth of training in research, personal fortitude and ambitious career goals, I believe that I can serve as an accomplished representative of NEAFS, work diligently to achieve success in my proposed grant project, and be an asset to the greater scientific and law enforcement communities as whole.

IDENTIFICATION AND CLASSIFICATION OF FORENSIC BOTANICAL EVIDENCE USING DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY (DART-MS): DEVELOPMENT OF CHEMOTAXONOMIC PROFILING BY MASS SPECTRAL ANALYSIS

A. INTRODUCTION

The purpose of the research described in this application is to investigate the utility of an ambient soft ionization mass spectral technique, coupled with chemometric methods, in the analysis of plant based substances of abuse. This project will explore the idea that the chemical composition of botanical evidence can be characterized by its mass spectral profile and that these "chemical fingerprints" can be used for identification and classification in a botanical forensic evidence database. It is anticipated that successful accomplishment of this goal will address and circumvent major challenges encountered with more conventional forensic chemistry analytical methods used in natural products of abuse identification. These postulates will be explored through pursuit of the following three specific aims:

- *Specific Aim I*: Investigation of whether chemometric analysis of mass spectral profiles of forensically relevant plant matter can be used to identify and discriminate one plant based psychotrope from another.
- *Specific Aim II*: Determination of the experimental parameters required to reproducibly generate data that can be used in Specific Aim I.
- *Specific Aim III*: Creation of a classification system for forensically relevant plant evidence based on chemometric analysis of mass spectral data.

B. BACKGROUND AND SIGNIFICANCE

In current forensic drug testing, a new paradigm is emerging where the drugs that are being submitted to crime labs for analysis are no longer restricted to well-characterized abused substances such as cocaine, heroin, tetrahydrocannabinol (THC) and prescription drugs among many other substances. The United Nations Office on Drug and Crime has compiled a list of 20 mind altering plants of high concern, as they are readily available for purchase on the internet and remain unscheduled in most countries [1]. The advent of these natural products-based psychotropics has called into question the utility of the use of well-established conventional techniques in the analysis of novel and ever changing drug formulations. There are few standard operating protocols (SOPs) available to analyze these complex synthetic mixtures and the natural products contained in the complex plant matrices. Furthermore, the ever-shifting landscape in terms of the variety of plant drugs available makes method development and validation based on previously established rubrics highly impractical. This problem has made it exceedingly difficult for law enforcement to analyze and identify plant-based drugs of abuse, and has rendered the judicial system impotent in dealing with this problem which has reached epidemic proportions.

Botanical forensic evidence is particularly challenging for forensic analysts, as its physical form can vary from whole, live plants to small amounts of crushed powder. Because of the diversity of incarnations of plant material, a forensic analyst must use a variety of techniques to identify the evidence. For live plant analysis, the expertise of forensic botanists is often utilized to characterize the morphological features to identify the species of plant. However, if the plant is missing its flowers, fruits or seeds, it can be difficult to determine the species. Furthermore, since the field of forensic botany is so specialized, many forensic crime labs do not have access to certified botanists for routine analyses.

In the absence of the assistance of a forensic botanist, many forensic chemists use microscopy to identify well known physical features of plant material, along with color tests to identify psychoactive components in the plant material. However, if the plant material is too well pulverized, these physical features are absent, making species identification difficult or impossible. With regard to microscopic analysis of other plant-based materials, there are often few distinguishing physical features to facilitate discrimination of one species of plant from another. Color tests are also used in identifying botanical evidence. However in general, these analyses only permit identification of drug classes, and are plagued with relatively high false positive/negative rates, especially with regard to new psychoactive material. Thus, the utility of such tests is limited and by SWGDRUG guidelines, the analysis needs to be augmented with other more confirmatory tests.

Hyphenated techniques, most commonly GC-MS, are used to confirm the presence of psychoactive compounds in botanical evidence. The compounds are extracted into an appropriate organic solvent and then derivatized if the compounds are non-volatile. Following solubilization and any other sample preparation steps, the material is analyzed by

GC-MS, using a method that often includes a long temperature program to ensure resolution of chromatographic peaks. The retention times of the various separated components, along with their respective mass spectral fragmentation patterns, are then compared to those of authentic standards in order to make a positive identification. These protocols are time consuming and can be expensive, particularly if sample derivatization is required. In the case of analysis of plant material that is different from well-known species such as cannabis or opium poppy, resource intensive development of new analysis protocols must be conducted, and these protocols must be tested, standardized and widely disseminated to be useful. These factors make forensic identification of plants of abuse difficult and ineffectual by the most commonly used conventional methods.

To compound this issue, another long-standing problem highlighted in the 2009 National Academy of Sciences (NAS) report [2] is the absence of statistical analysis in the reporting of results in all forensic areas except DNA analysis. In principle, this problem could be addressed using multivariate statistical analysis of the chromatographic data that is produced in gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) experiments. However, this approach is impractical for two reasons: (1) hundreds if not thousands of sample analyses would be required for statistical validation; and (2) the analyses are time-consuming, with the processing of individual samples taking upwards of two hours from start to finish in many cases. Furthermore, there are a lack of standard operating procedures nationwide for chromatographic temperature programs, and the operating protocols must be standardized across local, state and federal laboratories in order to perform reliable and accurate statistical analysis

A new approach to address the shifting paradigm in forensic drug analysis is needed. The advent of modern techniques such as ambient ionization mass spectrometry could in principle circumvent many of the aforementioned challenges facing forensic labs today. Ambient ionization mass spectrometry is a development of the past decade that allows for the ionization of samples without the need for reduced pressure. Desorption electrospray ionization (DESI) and direct analysis in real time (DART) are the two most well studied ambient ionization techniques. In both cases, a stream of ionizing medium is directed at a sample, and the analyte ions are desorbed [3,4]. The benefits of DESI and DART over other techniques are that: (1) the analyses are performed under ambient conditions; and (2) sampling can be performed in an easily accessible open area. This benefit permits many different surfaces including pills, TLC plates, reaction pots and plant material to be analyzed directly.

The DART ion source is frequently interfaced with a time-of-flight (TOF) mass analyzer, which provides high resolution (HR) mass measurements. As a soft ionization method, DART predominately forms protonated molecules $[M+H]^+$ with little to no fragmentation of the analytes. An added benefit of the DART ion source is the capability of in-source collision induced dissociation (CID), which causes molecule fragmentation, the results of which can be a rich source of structural information. DART-TOF-MS has already been shown to enable successful analysis of a wide variety of substances of abuse [5-10] due not only to the ability to perform high resolution mass measurements, but also the in-source CID capability and rapid analysis time. These distinct advantages of HR-DART-TOF-MS over current conventional mass spectrometric methods can be exploited to address drug analysis challenges that are an unfortunate reality in many forensic chemistry labs.

With regard to natural alternatives to scheduled drugs, HR-DART-TOF-MS provides an opportunity to characterize the chemical composition of the entire plant with minimal if any destruction of the evidence. Roots, seeds, and aerial parts of the plants can all be tested directly by DART-MS without the need for lengthy sample preparation steps. The psychoactive substances contained within the plant material can easily be identified from the high resolution data generated under soft ionization conditions, and tentative structural assignments can be confirmed through the use of CID and comparison of the results with those of CID conducted using authentic standards. Moreover, the complete mass spectral profile, which includes any constituent psychoactive components, can provide a diagnostic fingerprint signature of the plant which may be unique enough to enable species identification and differentiation.

The research proposed here will explore the idea that forensic botanical evidence analyzed by Direct Analysis in Real Time-Mass Spectrometry, will generate data that can be rapidly used to identify and classify evidence, and that this can be conducted in a manner that circumvents some of the major challenges encountered with conventional analytical methods. Furthermore, the hypothesis that the chemical fingerprint of forensic evidence can be determined by its mass spectral profile, and that multivariate statistical analysis processing of this data can enable identification of species will be investigated.

C. EXPERIMENTAL PROCEDURES

Model plants, plant material and products will be purchased from various online vendors and will include such substances as Kratom, *Salvia divinorum*, kava kava powder, *Datura stramonium* seeds, *Amanita muscaria* mushrooms, Ayahuasca plants, Wild Dagga plants, and Iboga powders, among others. Authentic chemical standards for chemotaxonomic markers will be purchased from Sigma Aldrich (St. Louis, MO), Cayman Chemical (Ann Arbor, MI) and Cerilliant Corporation (Round Rock, TX).

Analysis of the plant material and standards will be performed on a JEOL AccuTOF© time-of-flight mass spectrometer (JEOL USA, Peabody, MA) coupled to a Direct Analysis in Real Time (DART)-SVP ion source (IonSense, Saugus, MA). The parameters of the instrument will be modified accordingly in order to optimize the reproducibility of the data. Due to the variation of the physical form of analyzed plant material (e.g. seeds, leaves, etc.) the optimal sampling technique for plant material will be determined. Sampling of plant material will be conducted in three ways: analysis of material in its native form (e.g. whole seed, whole leaf); analysis of native material as slices or cuttings; and extract analysis. For extract analysis, multiple solvents, including ethanol, ethyl acetate, hexane, dichloromethane, and water, will be used to determine the optimal solvent or solvent system for observation of the most comprehensive fingerprint profile. Initial parameters will be to analyze the plant material directly at 350 °C for ionization of psychoactive compounds.

Classification of forensic evidence will be developed using multivariate statistical analysis using appropriate software, including Mass Mountaineer and Solo software systems. Upon collection, spectra will be imported into the software and a guided classification system will be chosen. For statistical analysis, peaks corresponding to compounds unique to a particular species of plant should provide the most discriminating power between species. However, many plants within the same genus have the same or similar biomarkers. Therefore, two approaches for peak selection will be taken. First, a guided classification setup will be created using major, identifiable biomarker peaks from the spectra produced from plant material analysis. Peaks that are unique to each species will then be added to the classification and the discrimination will be tested. After choosing the statistical model and peaks which allow for the best classification of species, validation will be achieved through leave one out cross-validation (LOOCV) and the classification system will be tested with the use of unknown samples. Secondly, an unsupervised classification system using hierarchical clustering will be used for analysis. Unsupervised classification does not rely on the analyst to choose the parameters for classification, but instead uses the entire available data set. By using the entire mass spectrum (rendered as a heat map) for unsupervised classification, the software is able to classify based on the entire data set, and no "analyst bias" is introduced. Mass Mountaineer software will be used to produce heat maps of the relevant data and the heat map data will then be imported into Solo software and Cluster 3.0, a genome clustering program, to conduct single linkage hierarchical clustering.

D. PRELIMINARY RESULTS

Preliminary data was generated and assembled in support of the proposed project. However, it should be noted that this project is not supported by other funding sources, nor does it represent a continuation of an existing project. The ability of HR-DART-TOF-MS to generate fingerprint profiles that can be used to identify plant tissue was investigated through the analysis of four currently unscheduled products identified by UNODC: Kratom leaves, *D. stramonium* seeds, *S. divinorum* leaves and Kava powder. Culinary sage (*S. officinalis*) served as a non-psychoactive control. These four products are ideal models, as they have well documented psychoactive components and have been previously characterized by conventional methods, including NMR, GC-MS and LC-MS [11-14]. Thus, the methodology developed here can be compared to other more traditional forms of analysis. Additionally, there is significant available information about the small molecule profiles of these plants. The results of DART-MS analysis and chemometric processing of the observed data are shown in Figure 1. Panels a-e show representative DART-MS spectra of the analyzed plant material. Each analysis was achieved in 3-5 seconds and data processing was completed in under five min. Chemotaxonomic markers, as well as the main psychoactive components are highlighted in each spectrum. Of note is that the profiles for each species are unique and can be visually distinguished from those of other species. The ability to differentiate between plants of interest was tested by partial least squares discriminant analysis (PLSDA), with the results shown in Panel f. The well-resolved, tight clustering of the data indicates that discrimination between species was successful. The preliminary classification system was tested using unknown plant material (Panel g). The results demonstrate that the unknown material (grey circles) clusters with *S. divinorum* (blue triangles). This result was further supported by the "Most Probable Predicted Class" analysis, shown in Panel h. There were no missed classifications or multiple class assignments.

In summary, the preliminary data validate the hypothesis that the fingerprint of the psychotropic plant material can be used as the basis for identification of plants of abuse. Furthermore, the success of differentiating between species implies that a forensic botanical database of abused plants could be developed using mass spectral profiles.

E. EXPECTED RESULTS AND CONTRIBUTION TO FORENSIC SCIENCE

It is expected that expanding the research by including multiple types of natural products of abuse, including *Datura* spp., *S. divinorum*, ayahuasca, and kava powders, among others, will produce high resolution spectra that can be used for discriminatory purposes. The ability to analyze a wide variety of plant materials, including seeds, leaves, aerial parts and roots, without the need for lengthy extraction protocols, allows for high throughput analysis of complex matrices. Furthermore, various types of plant materials of abuse have psychotropic compounds and chemotaxonomic markers that can be readily identified using HR-DART-TOF-MS. This method has the potential to facilitate almost immediate identification of the plant material. The high throughput capability of

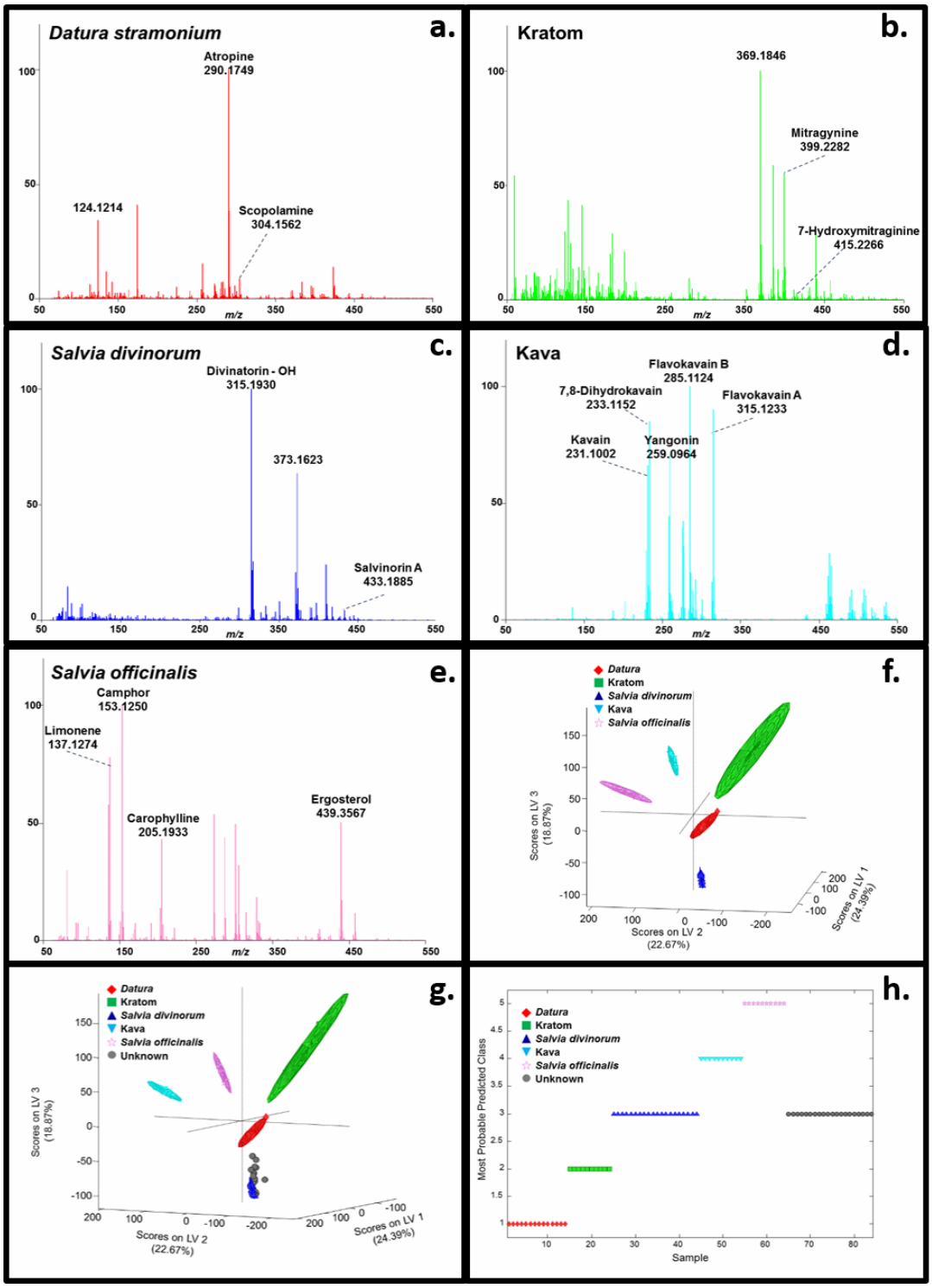

Figure 1: Mass spectral data of psychoactive plants of abuse. Panel a: A representative DART-TOF-MS mass spectrum of *D. stramonium* seed. Panel b: A representative DART-TOF-MS mass spectrum of a Kratom leaf clipping. Panel c: A representative DART-TOF-MS mass spectrum of an *S.divinorum* leaf clipping. Panel d: A representative DART-TOF-MS mass spectrum of Kava powder. Panel e: A representative DART-TOF-MS mass spectrum of a *S. officinalis* leaf clipping. Panel f: Partial least squares discriminant analysis plot of mass spectral data of *Datura* species (red diamonds), Kratom (green squares), *S. divinorum* (blue triangles), Kava (teal triangles) and *S. officinalis* (pink stars). Panel g: Partial least squares discriminant analysis plot of mass spectral data of *Datura* species, Kratom, *S. divinorum,* Kava, *S. officinalis* and unknown plant material (grey circle). The unknown plant material (grey circles) clusters with the *S. divinorum (*blue triangles), indicating a possible identification. Panel h: Most predictable class assignment of mass spectral data of *Datura* species, Kratom, *S. divinorum,* Kava and *S. officinalis.* The unknown plant material was correctly identified as *S. divinorum* in all test cases with no missed classifications or multiple class assignments.

DART-MS would allow chemometric processing of a wide variety and large number of samples towards the creation of a natural products of abuse database that could be readily used by forensic labs, thereby circumventing time-consuming analytical methods.

This project will contribute greatly to the field of forensic science, especially in the forensic analysis of mind-altering plants, by developing needed innovations. These are (1) **Rapid Analysis and Streamlined Protocols** through the use of ambient ionization mass spectrometry. The reduction in sample preparation and extraction steps will not only increase the rapidity of analysis, but also contribute to the alleviation of casework backlogs; (2) **Class and Species Identification** for plants of abuse, which has yet to be accomplished; (3) **Statistical Analysis** using the DART-MS spectra obtained from drug analyses. The increased number of samples tested per unit time would allow application of multivariate statistical analysis methods. This is not currently possible with conventional, hyphenated techniques because data generation is too slow; and (4) **Creation of an** "**Abused Plant Database"** analogous to controlled substance databases, against which unknowns can be screened and rapidly identified with a defined level of confidence. These four developments will have both immediate and long-term impacts on forensic science practice and eventually, the legislation of mind-altering psychotropics. Moreover, the research proposed here could in principle be applied to seized drugs and synthetic drug alternatives.

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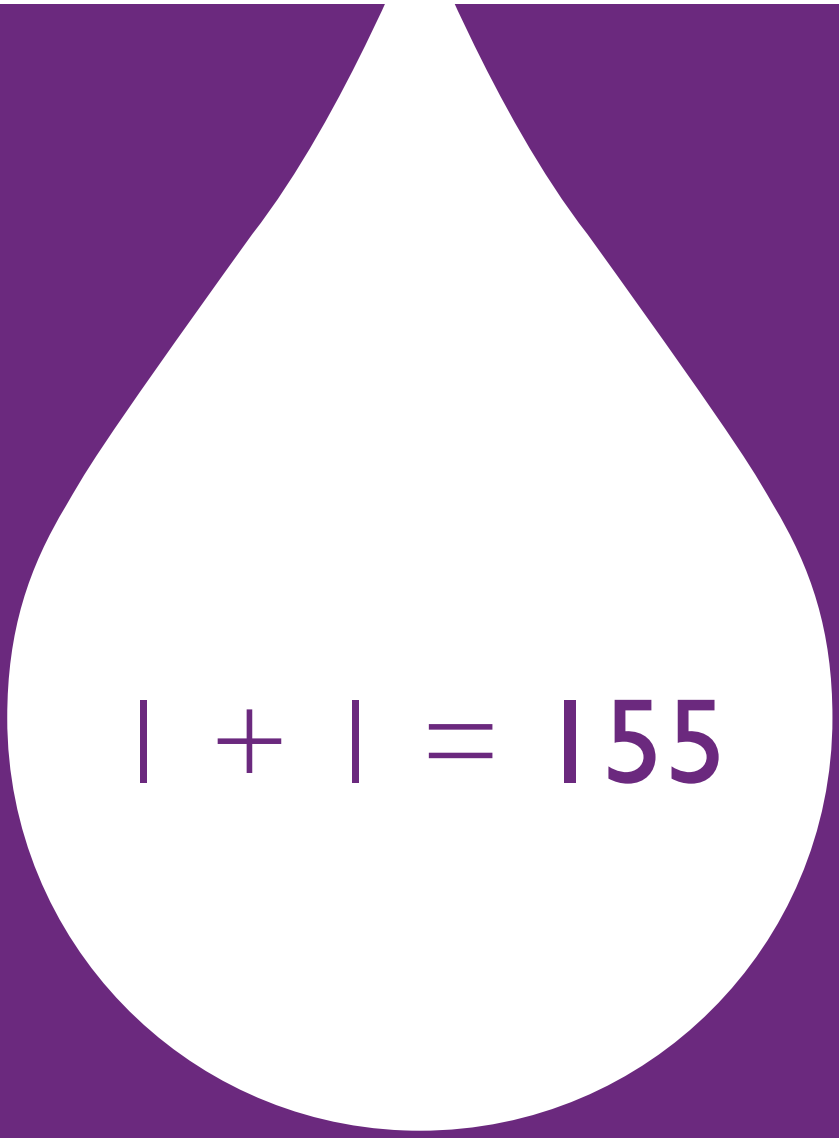
The session in which Dr Reffner received his award took place on Monday 16-November. John invited several prominent scientists to join in this outstanding experience (see photo), who all gave memorable presentations, capped off with John's moving and informative presentation that included milestones in his career.



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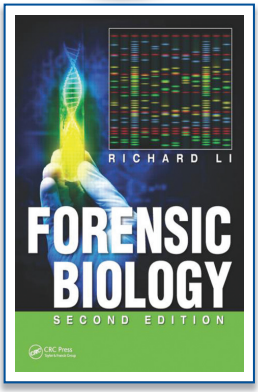

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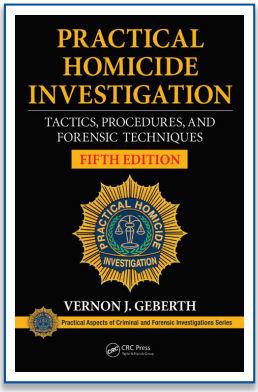

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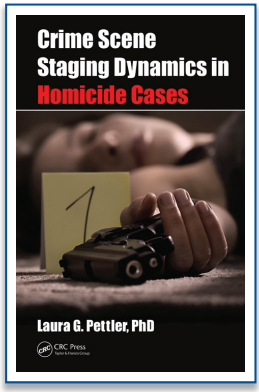

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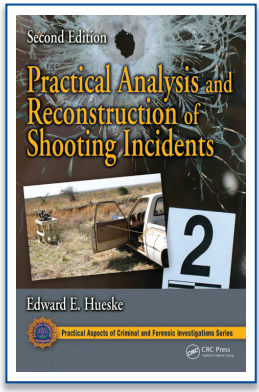

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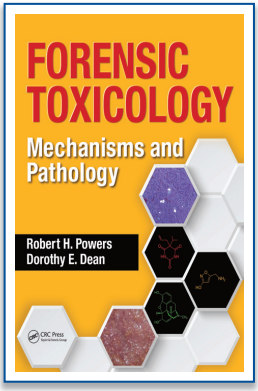

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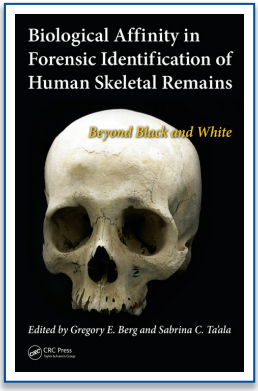

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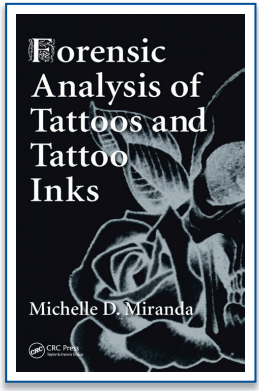

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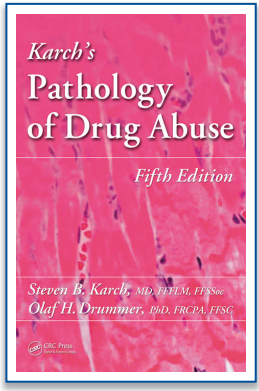

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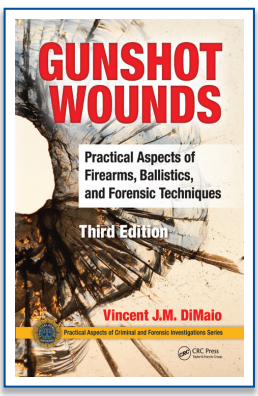

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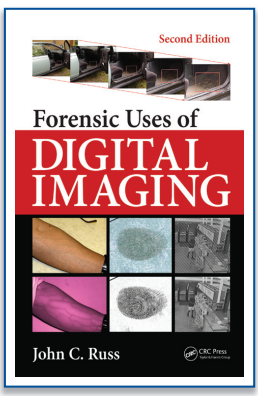

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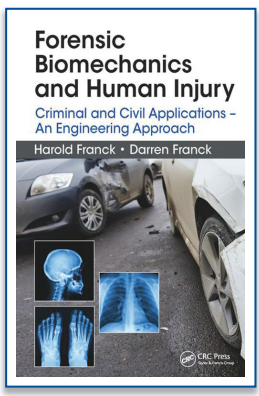

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2015 Training Scholarship Fund

The Northeastern Association of Forensic Scientists (NEAFS) is proud to offer its members a 2015 Training Scholarship Fund. Regular members, in good standing, are eligible to receive up to $200 towards training, workshop or non-NEAFS meeting registration expenses. Detailed instructions and application forms are available on the NEAFS website. Simply click the "Training" link at the top of the screen and scroll down to the "NEAFS Training Scholarship Forms".

The current application period is January 1st, 2015 to December 31st, 2015. Reimbursements will be issued on a first come, first serve basis and funding is limited. If you plan to attend a non-NEAFS meeting workshop, training or course during this application period and will not be funded by your agency or any other non-NEAFS related entity, we highly encourage your swift application for the 2015 Training Scholarship Fund.

Please visit the NEAFS training website to take advantage of this great NEAFS opportunity and to view upcoming training opportunities!

Upcoming Training

Midwestern Association of Forensic Scientists' Annual Meeting
October 3-7, 2016
Branson, Missouri

Hilton Branson Convention Center
conventioncenter.hiltonsofbranson.com
(Group Code: MAFSMO)

Hosted by The Missouri State Highway Patrol
Contact: Abigail Lehman 573-526-6134 x2529
http://www.mafs.net/news-feeds-1/mafs-2016-meeting

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Bullet

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Is comprised of numerous Forensic Biology Laboratories united in the creation of tools relevant and dedicated to the Forensic Biology and HID industries.

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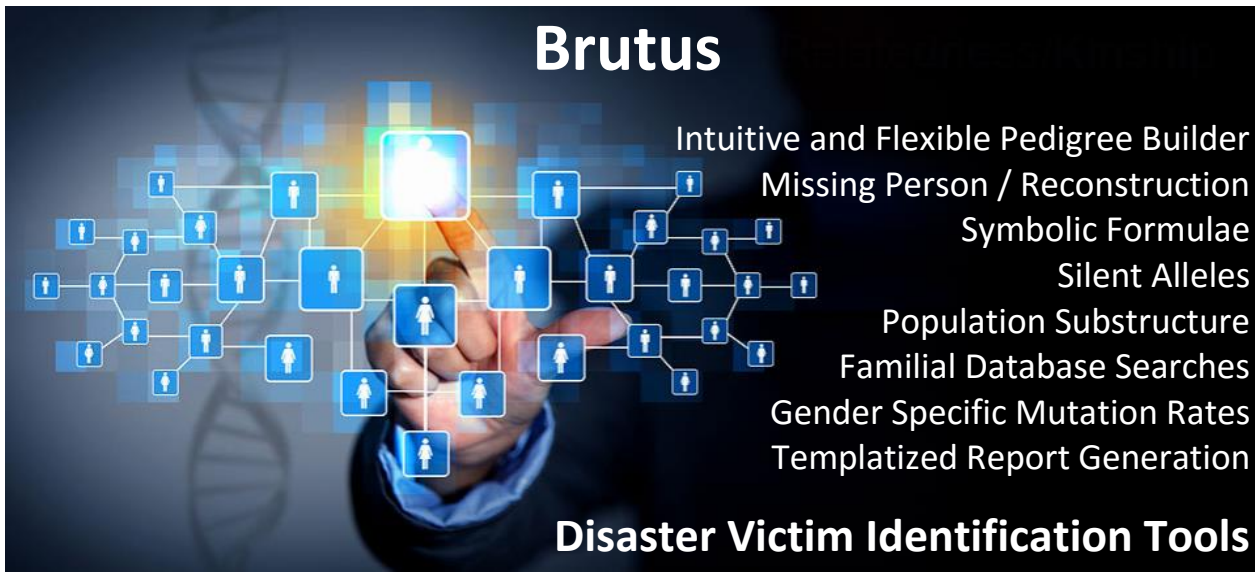

eDNA©

We invite you to contact us for a free live demo

877-451-4363

www.eDNALIMS.com

ABC Reimbursement

The NEAFS Board of Directors has voted to reimburse the American Board of Criminalistics exam sitting fees for five NEAFS members (regular or associate) in good standing who pass the ABC exam. This offer is for any ABC exam taken in 2015. There will be an exam offered at the NEAFS Annual meeting in Hershey. After passing the examination, please fill out the ABC Examination Reimbursement Form (www.neafs.org) and email the completed form with proof of passing the exam to the NEAFS Certification Chair Peter Diaczuk at certification@neafs.org. The reimbursement is based on a first come first served basis. Remember you must pass the ABC exam to be considered for reimbursement.

Education

Moving forward in the areas of education/training, I have been working with Keith Mancini to possibly utilize the NEAFS site to organize and store educational/training documents including SOP's. Organized by discipline, this would allow us to post useful documents, making them available to all of our members. These could then be used to develop and enhance both employee training as well as our testing protocols. In Drug Chemistry for instance, we could post unknown spectra for review and comments. Our lab has a narcotic tablet database sorted by imprint that is used by law enforcement and DA's to determine possible drug charges. We also would be willing to post our Duquenois Levine validation spreadsheet that contains over 100 tested herbs and spices along with the reaction pictures.

Every lab has some great documents that would benefit other labs. By working together and combining our talents we improve our own lab, the profession as a whole, and NEAFS as an organization.

If you are interested in contributing please contact me at JWesley@monroecounty.gov.

Jim Wesley

Missing Something

Be sure to check the NEAFS website for the latest **Job Opportunities.**

B.O.D. Meeting Minutes and Financial Statements will now be placed in the Member Area of the NEAFS website. If you have trouble logging in please contact the web master, webmaster@neafs.org.

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