NEAFS Newsletter

Volume 48, Issue 1 Spring 2023

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Massachusetts State Police Crime Lab
124 Acton Street
Maynard, MA 01754

president@neafs.org

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Regional Associations Committee Representative: Beth Saucier Goodspeed
Massachusetts State Police Crime Lab
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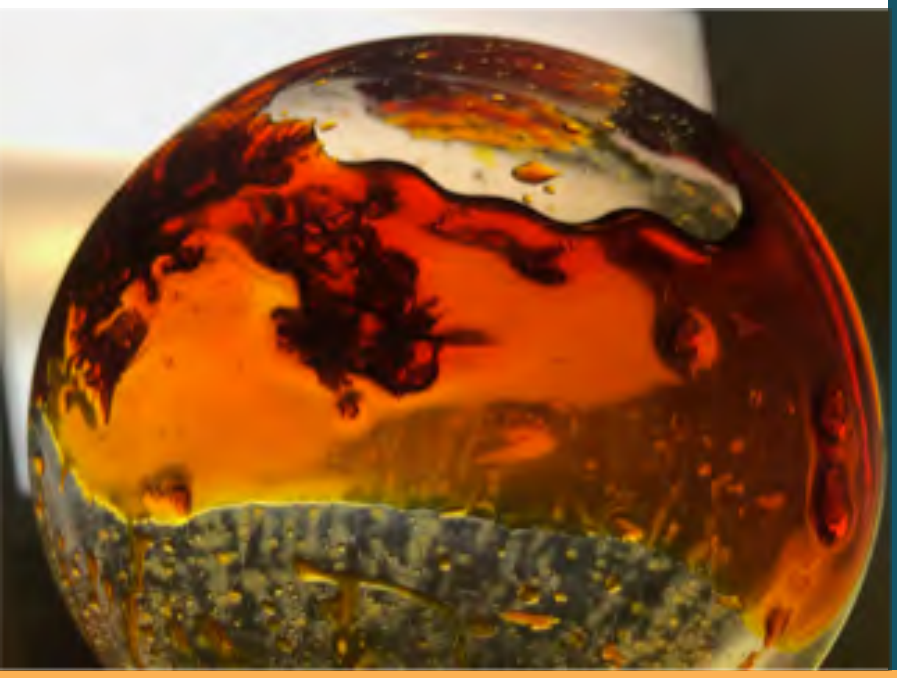
Maynard, MA 01754
978-451-3504
rac@neafs.org

SYNTHCON



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MEET THE 2023 BOD

Elizabeth Duval – President
Massachusetts State Police Crime Laboratory since 2009
Forensic Scientist III, DNA Unit Supervisor - 2019 – present
BS Genetics, Texas A&M University
BS in Forensic Science, University of New Haven

Stephanie Minero– President-elect
Nassau County Office of the Medical Examiner, Division of Forensic Service, Controlled Substance Analysis 2011-present
NYPD Police Laboratory, Controlled Substance Analysis 2008-2011
BS in Forensic Science- Long Island University/CW Post
MS in Biology- Long Island University/CW Post

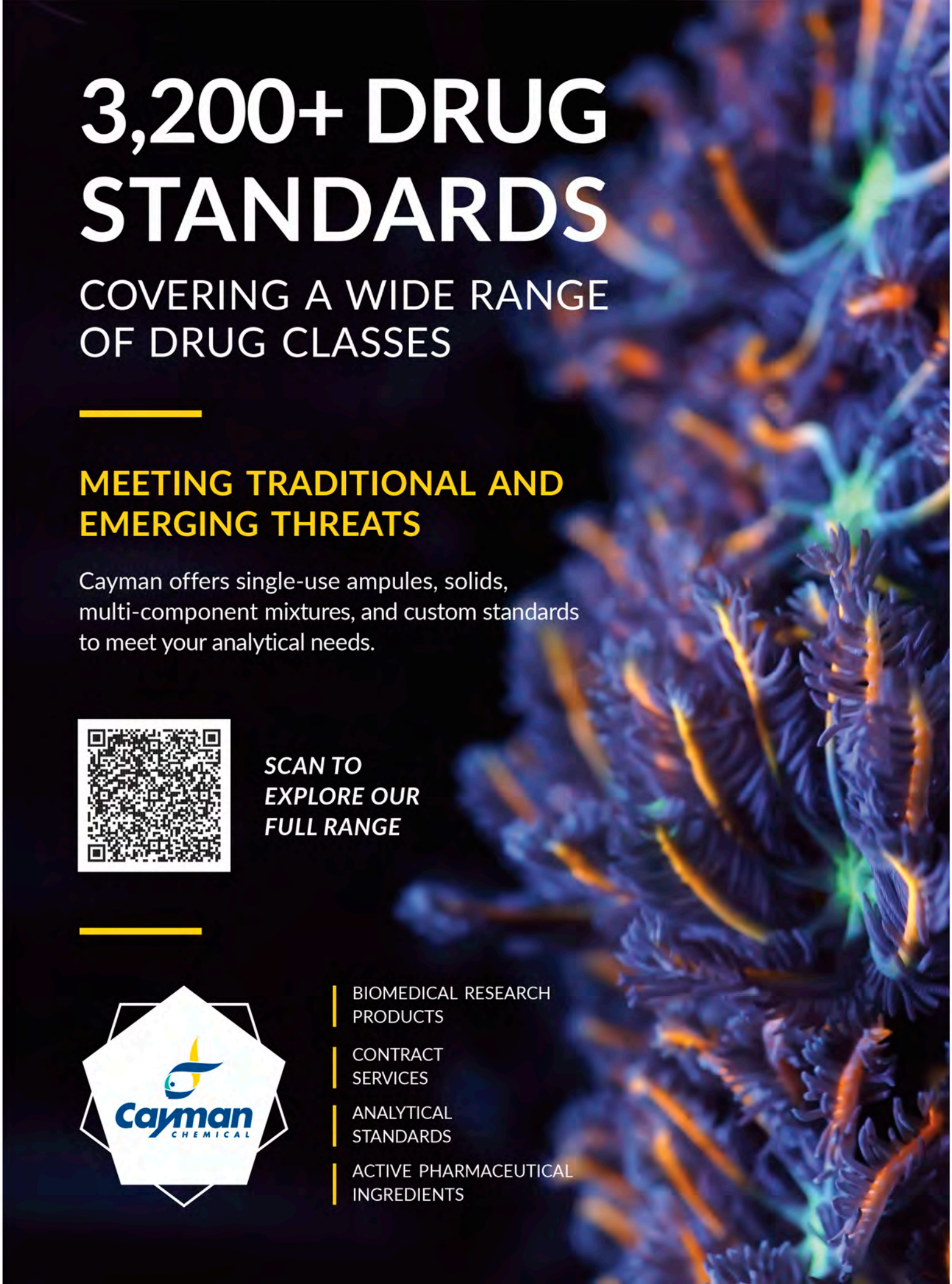
Alanna Laureano- Secretary
Westchester County Department of Labs & Research, Division of Forensic Sciences Since 2007
Senior Forensic Scientist and DNA Technical Leader
BS in Molecular Biology and Biochemistry- University at Albany, SUNY
MS in Forensic Biology- University at Albany, SUNY

Matthew Marino - Treasurer
New Jersey State Police Office of Forensic Sciences, East Regional Laboratory from November 2011 to present
Forensic Scientist 2 in the Drug Unit, Criminalistics Unit and Quality Assurance Unit
Forensic Technician, Westchester County, NY Forensic Laboratory from July 2007 to September 2011
BS in Natural Sciences with a concentration in Chemistry-St. Thomas Aquinas College

Amanda White - Director
New York State Police Crime Laboratory, FS III- Controlled Substance Analysis from 2019-Present
Westchester County Department of Labs & Research, Controlled Substance Analysis 2016-2019
NYPD Police Laboratory, Controlled Substance Analysis/Latent Print Development 2011-2016
MS Biomedical Forensic Science, Boston University
BS Biology & Anthropology, SUNY Oneonta

Anisha Paul M.S.F.S, D-ABFT-FT - Director
Vermont Forensic Laboratory, Department of Public Safety - Forensic Chemist Toxicology division since 2017
Adjunct professor at Champlain College since 2017
Masters of Science in Forensic Science from Arcadia University
Certified as a Diplomate by the ABFT in the field of Forensic Toxicology

Sarah Roseman - Director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NEAFS President's Message

ELIZABETH (BETSY) DUVAL

Hello Fellow NEAFS Family Members!

It is my distinct honor to be sending out my first official address as your organization's 2023 President. When I joined NEAFS as an eager reinvented "young" professional back in 2009 I never imagined myself here. I'd be remiss to not thank all of you who came before me and supported me as well as all those who will follow by making this a wonderful year of growth and change for NEAFS.

I want to send an extra shout out in deepest gratitude, once again, to the amazing Board of Directors, Staff and Committee Chairs, Session Chairs and Co-Chairs, Workshop Coordinator and Moderators, student volunteers, Presenters, Sponsors, Vendors, and everyone that worked tirelessly to make last year's meeting a success while selflessly sacrificing either some sort of compensation or perk for the financial stability of this fantastic organization. It was amazing to see everyone come together in the spirit of collegiality to celebrate the field of forensic science we all love!

As a voluntary organization we are only as good as the sum of our parts (membership). As your President, along with your fellow BOD and staff, we are continuously thinking about a sustainable future for NEAFS. Our hope and goal are to empower NEAFS and each other by building and advancing our role as a successful regional forensic organization. From its incorporation in 1976 to today, NEAFS has grown from ~ 200 to 534 members. From the first award given in 1998, NEAFS has also supported the education of future practitioners over the years by granting more than 70 scholarships and research grants. Finally, NEAFS has promoted forensic research, continuing education and professional development/networking for tens of thousands of practitioners, legal professionals, and students through many avenues including our Annual Meetings.

We want to continue this positive trajectory of growth through potential membership advancements and future outreach opportunity partnerships which we hope to make happen in 2023.

In addition, I am also eagerly looking forward to this year's upcoming 49th Annual Conference in the beautiful and picturesque Groton (Mystic), CT. Your meeting is being crafted by the brilliant mind of your Program Chair and President-Elect, Stephanie Minero, and I know it is going to provide us with both a thoughtful and thought-provoking experience! All her preparations are being made with meticulous care and purpose (for our benefit), consummate professionalism (with everyone involved), and a (simply the best) positive attitude!

So, with all this to look forward to, I'd like to leave us all with the following to consider:

Is it enough to **BECOME** a NEAFS member or is it equally important **BEING** a NEAFS member?

What does it mean BEING a member? You simply participate, in any way you can. You can promote NEAFS to potential members. You can participate in vetting/voting on proposed changes to your organization. You can volunteer to help on a committee or for an upcoming conference. You can take advantage of the Training Scholarship Fund or help in getting NEAFS funded training at your agency. You can attend the meeting and enjoy learning and connecting with fellow members and new faces!!

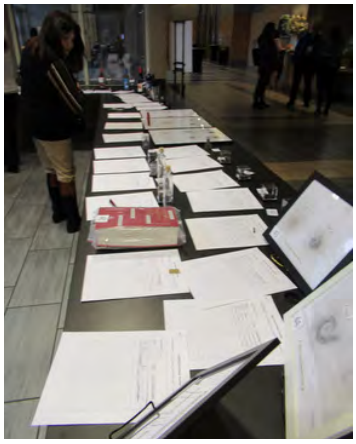
How can we all help? It's easy. Please just take a few minutes to visit our website at www.neafs.org and check out what's going on. Follow us on Facebook, Twitter or Instagram. Enjoy the quarterly newsletter, open and read your membership emails, answer membership requests (voting/sponsorship/outreach opportunities), register for the meeting, perhaps present at a meeting and finally, **YOU** can help NEAFS by simply sharing what NEAFS is with others!

The future of this amazing association depends on everyone's willingness to engage. Small or large these efforts can make difference, not just for NEAFS but to advance the field of Forensic Science as a whole.

Elizabeth (Betsy) Duval
President, NEAFS 2023


Sheraton At The Falls
Welcome, we've been expecting you.
Bienvenue ようこそ Bienvenido REGISTRATION 歡迎 Willkommen ברוך הבא


CONVENTION
CENTER




minimalism
CONVENTION
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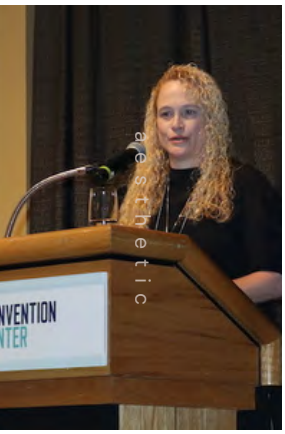
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Adam S. Hall


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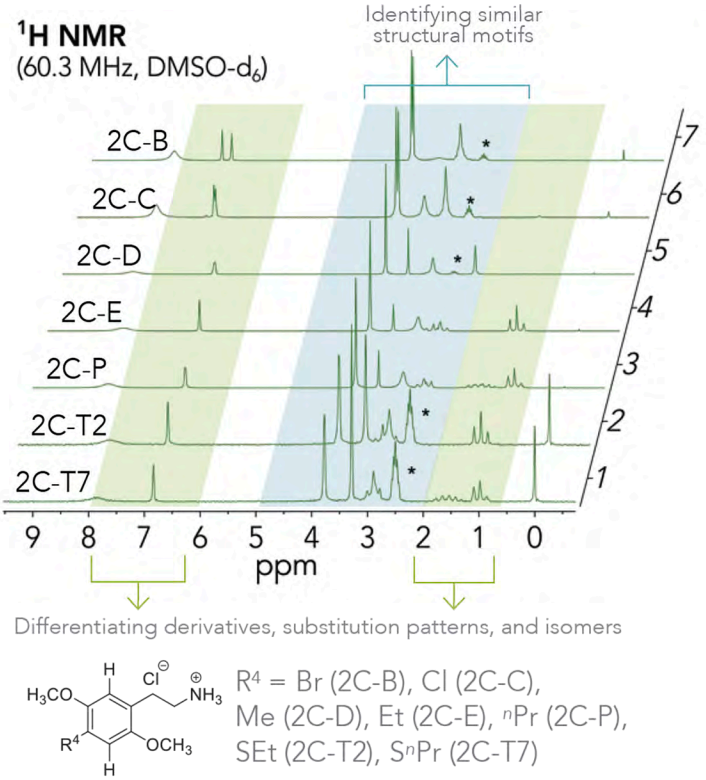
Case Study #1

Autotmated Identification of Designer Drugs

without reference materials

Why benchtop NMR?

- Provides structural information and capabilities of flagging new designer drugs
- Is an inherently quantitative method and certified reference material is not required unlike for other common forensic analytical methods
- Non-targeted, i.e., it does not require standards for identification
- Small footprint allows instrument to be placed at the point of need
  e.g., crime scene, small lab
- No maintenance
- Accessible. Affordable. Automatable



R^4 = Br (2C-B), Cl (2C-C), Me (2C-D), Et (2C-E), ^{n}Pr (2C-P), SEt (2C-T2), S^{n}Pr (2C-T7)

2C-X series are potent hallucinogenic and stimulant drugs that can be easily synthesized and structurally modified. However, we have shown[1] that they can be clearly differentiated by their 60 MHz ^{1}H NMR spectra.

[1] Araneda, J. F.; Baumgarte, M.; Lange, M.; Maier, A. F. G.; Riegel, S. D. *Magn. Reson. Chem.* **2021**, 1.

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NEAFS 2023

ANNUAL MEETING

NOVEMBER 6TH, 2023 - NOVEMBER 10TH, 2023

MYSTIC MARRIOTT, GROTON, CT

2023 ANNUAL MEETING PRELIMINARY SCHEDULE

MONDAY, NOVEMBER 6TH

2:30pm - 4:30pm
Board of Directors and Staff Outing
6:30pm - 9:30pm
Board of Directors and Staff Dinner

TUESDAY, NOVEMBER 7TH

7:30am - 9:15am
Registration
7:30am - 9:00am
Breakfast
9:00am - 5:00pm
Full Day Workshops
9:00am - 12:30pm
Half Day AM Workshops
10:30am - 10:45am
Morning Break
12:30pm - 1:45pm
Registration
12:30pm - 1:30pm
Lunch on your own
1:30pm - 5:00pm
Half Day PM Workshops
1:30pm - 4:30pm
Student Forum
3:00pm - 3:15pm
Afternoon Break
5:00pm - 8:00pm
Exhibits Set-Up
5:00pm - 6:00pm
Registration
6:00pm - 8:00pm
Educators' Forum

WEDNESDAY, NOVEMBER 8TH

7:30am - 9:30am
Registration
7:30am - 9:00am
Breakfast
8:00am - 8:00pm
Exhibits
9:00am - 5:15pm
Scientific Sessions
10:30am - 10:45am
Morning Break
12:30pm - 2:00pm
Annual Business Lunch
3:15pm - 3:30pm
Afternoon Break
5:30pm - 7:30pm
Welcome Reception and Poster Session
6:30pm - 7:30pm
Registration
7:30pm - 9:30pm
Evening Plenary Session

THURSDAY, NOVEMBER 9TH

7:30am - 9:15am
Registration
7:30am - 9:00am
Breakfast
8:00am - 11:30am
Exhibits
9:00am - 11:30am
Morning Plenary Session
10:15am - 10:30am
Morning Break
11:30am - 1:30pm
Exhibits Break-Down
12:00pm - 2:00pm
Annual President's Award Luncheon
2:30pm - 5:00pm
Afternoon Plenary Session
3:30pm - 3:45pm
Afternoon Break
5:30pm - 6:30pm
George W. Chin Cup Competition
7:00pm - 11:00pm
President's Reception

FRIDAY, NOVEMBER 10TH

7:30am - 9:00am
Breakfast
9:00am - 1:00pm
ABC Exams
9:00am - 12:00pm
Outreach Event

www.neafs.org/preliminary-schedule

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS
49 TH ANNUAL MEETING
NOVEMBER 6 TH -10 TH , 2023
MYSTIC MARRIOTT
625 NORTH ROAD
GROTON, CT

CALL FOR PAPERS AND POSTERS

MEMBERS AND ACTIVE APPLICANTS

ELIGIBLE FOR $75 REIMBURSEMENT IF SUBMITTED PRIOR TO AUGUST 15 TH

DEADLINE: SEPTEMBER 15 TH , 2023

Note: All presenters must register for the meeting. Request for reimbursement must be submitted after presentation has been given and submitted to the NEAFS Treasurer using the electronic Travel and Expenses form. Must include proof of registration, payment, and listing in program booklet to qualify.

Note: Author designations, associations, and presentation titles will be printed in the meeting booklet and proceedings **as submitted**. Contact individual session chairs with revision requests.

TAKE YE TO THE ABSTRACT FORM

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Documents examination
- Document examination
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Chemical Criminalistics
- Personal lubricant examination
- Glass examination
- Fibres examination
- Ignitable fluid residue analysis
- Automotive paint examination
- Pepper spray
- Ink analysis

Fingerprint examination
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- Enhancement, detection & identification

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- Small media examination
- Mobile phone forensics

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NOVEMBER 6TH - 10TH, 2023

Room Rate Per Night Total $160 (Plus state and local taxes)
Group Rate Start Date: Sunday, November 5, 2023
Group Rate End Date: Saturday, November 11, 2023
Last Day to Book: Friday, October 6, 2023

Why is it important to book in the block? NEAFS has secured a room block and is providing discounted rates to NEAFS 2023 attendees based on a guaranteed percentage of attendees staying in the official conference hotel. Booking a hotel room outside of NEAFS's block impacts NEAFS meeting space, dates and rates in future years. Guaranteeing room blocks gives associations the opportunity to negotiate concessions, such as better room rates, free Internet, less expensive food and beverage, number of meeting rooms, affiliate meeting space, gym access, etc. Housing is a key component in how this leverage is measured, and booking outside of the contracted block decreases NEAFS's negotiating power–ultimately making the meeting more expensive and forcing future registration and hotel prices to increase! We know none of us wants to see higher attendance costs.

BOOK FOR 2023 NEAFS CONFERENCE

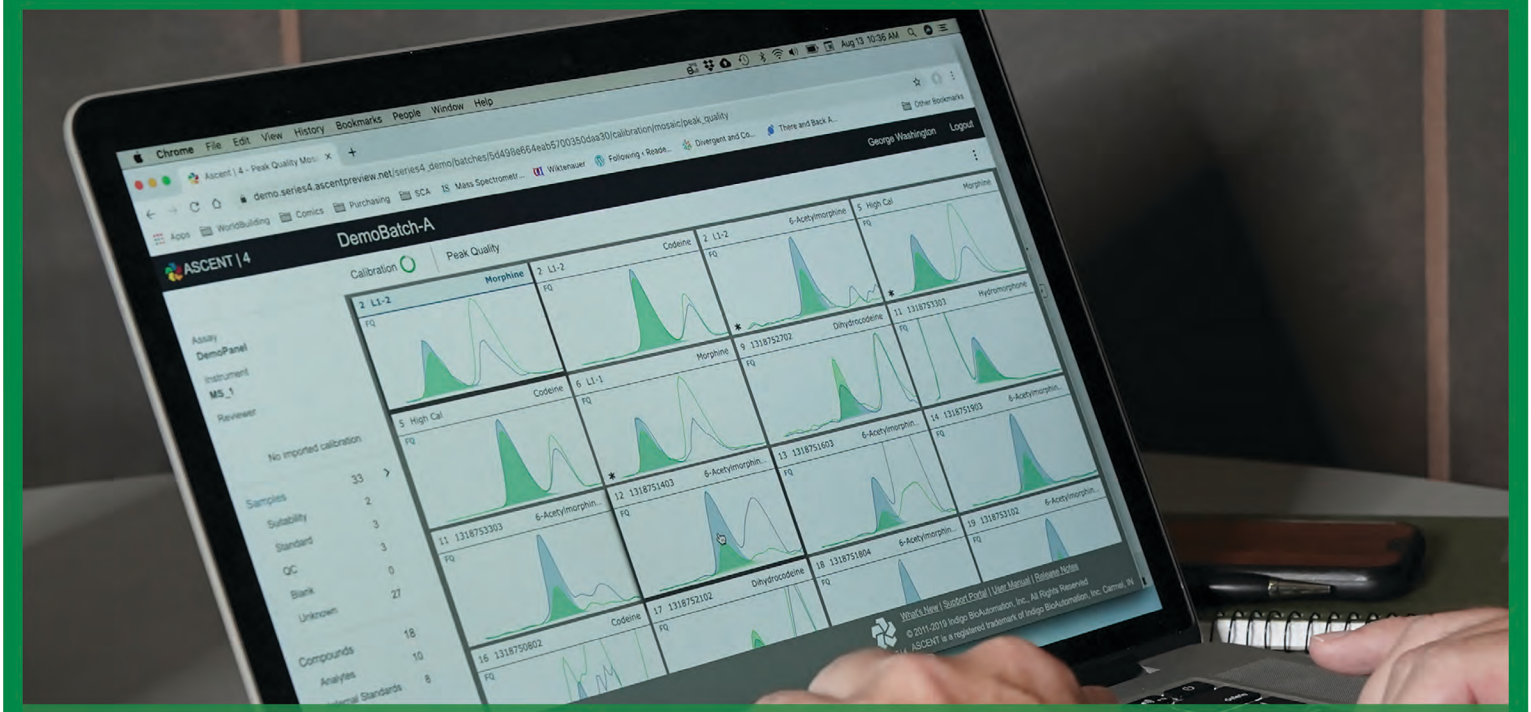
TSHIRT

DESIGN CONTEST

Free meeting registration to the winner of the contest.

Send your design to merchandise@neafs.org by April 10th
Theme ideas include NEAFS Years Eve (President's Receiption), and Mystic CT (location)

www.neafs.org



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NEAFS 2023
WORKSHOPS

11/07

Full Day

Leadership Training for New Supervisors
Laura Tramontin, M.S., CFM-I, LAT Forensics, LLC

Scanning Electron Microscopy
Jens Breffke, Ph.D., JEOL

TBD ("Stay Tuned!")

Half Day

LC/MS Fundamentals
Agilent Technologies

Next Generation Sequencing & Beyond
Verogen

Emerging Trends in Drug Chemistry
NMS Labs

Future Trends in Forensic DNA Technology
Thermofisher Scientific

Identification of Fentanyl Analogs by LC/QTOF
Agilent Technologies

Understanding the Analytical Balance, Calibrations, and Quality Control
Mettler Toledo

STUDENT FORUM

7 NOV,2023 | 1:30-4:30 PM | NEW DATE NEW TIME

EDUCATORS FORUM

TUESDAY, NOVEMBER 7TH | 6-8 PM

2023 NEAFS Annual Meeting

NEW DATE , NEW TIME

NEAFS 2023 ANNUAL MEETING

SPEAKERS

WEDNESDAY,NOVEMBER 8TH

7:30PM - 9:30PM

Evening Session : Det. Malcom Reiman (as seen on Netflix's the Time Square Killer) of the NYPD Bronx Homicide Squad (retired).

THURSDAY,NOVEMBER 9TH

9:00AM - 11:30AM

AM Plenary : Dr. Itiel Dror (Cognitive Consultants International)

12:00PM - 2:00PM

Luncheon : Mark Desire (Assistant Director, NYC OCME) will speak about his time at Ground Zero and the advancements of the OCME 9/11 Victim Identification Program as technology has evolved over the last 22 years.

2:30PM - 5:00PM

PM Plenary : Tiffany Roy, MSFS, JD (ForensicAid) and Brandon Garrett, JD (Duke University School of Law)* Panel discussion

*presenter may be virtual

www.neafs.org

Chin Cup Competition

THURSDAY
NOVEMBER 9TH
5:30PM - 6:30PM

NEAFS 2023
ANNUAL MEETING

NEW DATE
NEW TIME

COME OUT AND SUPPORT YOUR TEAM

and then celebrate at the
President's Reception!

NEAFS PRESENTS A

NEAFS YEARS EVE

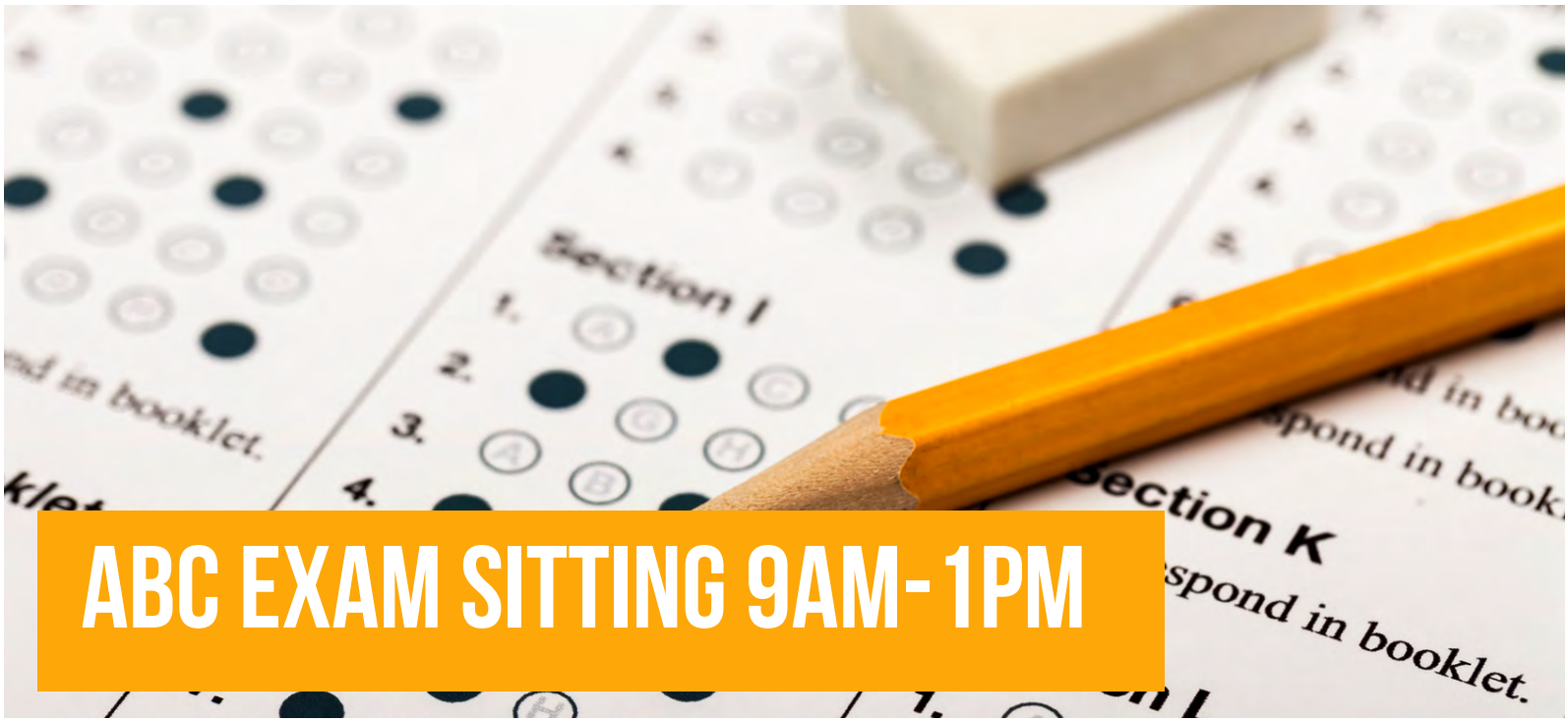
Presidents Reception

9 NOVEMBER

AT 7.00 PM

NEAFS 2023
Annual Meeting

LIVE MUSIC
DRINKS



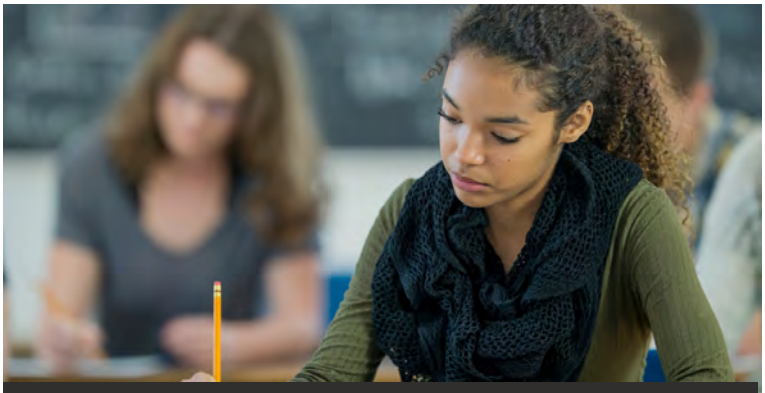
ABC EXAM SITTING 9AM-1PM

The Forensic Science community has an obligation to:

- Establish professional levels of knowledge, skills and abilities;
- Define a mechanism for achieving these levels;
- Recognize those who have demonstrated attainment of these levels;
- Promote growth within the profession.

CERTIFICATION

Certification is a voluntary process of peer review by which a practitioner is recognized as having attained the professional qualifications necessary to practice in one or more disciplines of criminalistics. The ABC offers a certifications in biological evidence screening, forensic DNA, molecular biology, drug chemistry, and comprehensive criminalistics.



HTTPS://WWW.CRIMINALISTICS.COM/

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS

Certification Reimbursement

The NEAFS Board of Directors has voted to reimburse the American Board of Criminalistics and International Association for Identification exam sitting fees for five NEAFS members (regular or associate) in good standing who pass the ABC or IAI exam. This offer is for any exam completed during the current year. After passing the examination, please fill out the Certification Reimbursement Form (https://www.neafs.org/certification). The reimbursement is based on a first come first served basis. Remember you must pass the ABC or IAI exam to be considered for reimbursement.

For more information about the examination sitting, please contact...

Peter Diaczuk
certification@neafs.org

For more information about certification with the ABC, please visit...

American Board of Criminalistics
http://www.criminalistics.com

For more information about certification with the IAI, please visit...

The International Association for Identification
https://www.theiai.org/certifications.php

Become a Certified Footwear Examiner

Are you a practicing footwear examiner looking to elevate your credentials to the next level? The International Association for Identification (IAI) offers certification specifically to meet the needs of the footwear discipline.

The footwear certification program is designed for those individuals actively engaged in comparing footwear impression evidence. To qualify for test participation, individuals must meet specific criteria. Years of experience as a footwear examiner, combined with formal education and documented training, will be the primary focus when determining an individual's eligibility for participating in the examination process.

The certification process involves completing our application, fee submission, and the successful completion of both written and practical examinations. More information regarding cost, requirments, and application can be found on the IAI website, footwear certification page (www.theiai.org/footwear.php).

Inquiries can be made to the IAI Footwear Certification Board Secretary at: Footwear.Cert.Secretary@gmail.com

FORENSIC CERTIFICATION MANAGEMENT BOARD

International Association for Identification

Welcome

NEW MEMBERS

BRIANNA GREGORY

CEDAR CREST COLLEGE

BRIANNA ROBINSON

DIVISION OF CRIMINAL JUSTICE SERVICES

KARA KOVACH. KEITH KRANTZ. MICHELLE SCOTLAND. STEPHEN SCHUTTA

ERIE COUNTY CPS FORENSIC LABORATORY

JESSICA HARESIGN

INDUSTRIAL UI SERVICES

EMILY PALMER. JULIE PIESLAK

MASSACHUSETTS STATE POLICE CRIME LABORATORY

EMILY PALMER. JULIE PIESLAK

CAITLYN TAVEIRA

JENNIFER FARLEY. KATHERINE FERRARI

NEW HAMPSHIRE STATE POLICE FORENSIC LABORATORY

ALEXANDRA ROTHAAR. SAMANTHA JARVIS. VICTORIA DEPRIMO

NEW YORK CITY POLICE DEPARTMENT

Welcome

NEW MEMBERS

FALYN VEGA, KEVIN MCKENNA, LAUREN CHWATT
OFFICE OF CHIEF MEDICAL EXAMINER

KATHLEEN CORRADO
ONONDAGA COUNTY CENTER FOR FORENSIC SCIENCES

KIRSTEN PATRICK, MORGAN WIERNUSZ
PENNSYLVANIA STATE POLICE LIMA REGIONAL LABORATORY

BIANCA BRANDON
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ELISE MCINNIS, HALEY CROOKS
SYRACUSE UNIVERSITY

GRACE CHEONG
UNITED STATES CUSTOMS AND BORDER PROTECTION

ALEXA FIGUEROA, BENEDETTA GAROSI
UNIVERSITY OF ALBANY

JULIA DOLLEN
UNIVERSITY OF NEW HAVEN

LAURA MILLEVILLE
VERMONT FORENSIC LABORATORY

Welcome

NEW MEMBERS

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SWGDRUG Bulletin

January 2023

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) proudly supports the forensic seized drug community by providing guidance and resources for a broad breadth of analytical and quality management challenges. The pace of change within our discipline has never been faster, and laboratories require steadfast improvement and adaptation to successfully address these dynamic issues.

Due to pandemic-related international travel restrictions, the SWGDRUG core committee met in August 2022 for the first time in three years. The committee wrapped up old business regarding documents that had been out for public comment and then got right to work on improving numerous recommendations and resources. We're looking forward to meeting again in 2023 and continuing our efforts!

Tools

The SWGDRUG website is a central repository for all sorts of tools, links, and resources for the forensic seized drug community. A short example list includes:

- Mass Spectral Library (v3.12, 20230116)
- FTIR Spectra Library (v2.1, 20190827)
- Opioid Crisis Response Landing Page
- Qualitative and Quantitative Sampling Calculators
- Drug Monographs, sortable by name or EI-MS base peak (611 and counting!)
- Worldwide forensic organization links
- Supplemental Documents for applying concepts of method validation, measurement uncertainty, and reporting
- ENFSI Education and Training Outline and example questions for each topic

2022 SWGDRUG Core Committee

Recently Approved Documents

- Revisions to the Core Recommendations Part IVA, Quality Assurance/General Practices
- Revised Supplemental Document, SD-5, Reporting Examples

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS

TRAINING SCHOLARSHIP FUND

OPEN APPLICATION PERIOD
JANUARY 1st to DECEMBER 31st
OF THE CURRENT YEAR

APPLICATION REQUIREMENTS

The Northeastern Association of Forensic Scientists(NEAFS) is proud to offer its members a Training Scholarship Fund (TSF). Members in good standing are eligible to receive up to $400 towards training, workshop or non-NEAFS meeting registration and travel expenses. Individuals will only be allowed reimbursement once per application period. Any NEAFS Annual Meeting expenses are ineligible to receive funding. Reimbursement will occur upon receipt of a certificate showing successful attendance and completion of the course along with an article summarizing the course for the NEAFS newsletter.

APPLICATION INSTRUCTIONS

Applicants must submit a Pre-Approval Application prior to attending the training for which they wish to obtain funding. For additional instructions, requirements and forms visit the NEAFS website.

https://www.neafs.org/trainingscholarshipfund

TRAINING SCHOLARSHIP FUND

BETH SAUCIER GOODSPEED
75TH AAFS ANNUAL SCIENTIFIC MEETING, ORLANDO, FL

Beth took advantage of the training scholarship fund and so can you.
Visit https://www.neafs.org/trainingscholarshipfund

Beth Saucier Goodspeed
Training Scholarship Fund Write-Up
75th AAFS Annual Scientific Meeting, Orlando, FL

During the week of February 13, 2023, I attended the American Academy of Forensic Sciences Annual Meeting in Orlando, Florida. While there, I attended two workshops, scientific sessions lectures and evening sessions. I also served as a judge and a Criminalistics section moderator at the meeting as well as filling the role of abstract reviewer before the conference began. I enjoy learning about new techniques and seeing new technologies at the vendor booths and I always return home with new information in the field of forensic science.

The first workshop that I attended was entitled "How Science Works to Identify Unknown Decedents Decades After Death". This workshop described the process on how archaeology, anthropology, odontology, material evidence examinations, chest radiograph comparisons, isotope testing and DNA helped to identify two decedents located in Vietnam and Korea. The first case involved a Chinook helicopter that crashed in Vietnam in 1971. There were 10 people aboard the helicopter and there were bodies that were not initially recovered. The second case involved a battle that occurred in 1950 during the Korean War.A field battle occurred, and several unidentified remains were located and not identified. Most of the presenters worked for the Defense POW/MIA Accounting Agency. Their job is to try to find all missing persons in the military. The workshop began with the topic of how science starts in the field. The process of digging for remains follows the scientific process, in the same manner that scientists follow this process in the lab.An interesting fact that I learned is that all unidentified skeletal and other remains of military personnel belong to the federal government and testing of these remains can be conducted without permissions or approvals. All work conducted at this agency is completed "in the blind". The assigned analyst is given the minimum amount of information needed to conduct their jobs. Another analyst or supervisor does not work "in the blind" and filters the information to

the case working analyst performing the work. During a presentation on chest radiograph comparisons, I learned that the clavicle of the human skeleton is unique to a person, much like fingerprints. The size, shape and other characteristics of the clavicle are used to help identify a person. At the end of the workshop, the testing that was completed in each field was summarized and explained how a positive identification was made of the deceased individual in each case.

The second workshop that I attended was entitled “Transfer and Persistence of Physical Evidence: Deciphering Implications”. This workshop described how trace evidence, in many different forms, will transfer during an event and how their collection, identification and assessment could assist in event reconstruction. Trace evidence such as DNA, fibers, drugs, and ignitable liquids were discussed. Trace evidence does not necessarily mean that the evidence is small in size – it can be visible to the naked eye. The term “traces” was discussed and was defined by Brook W. Kammrath, PhD, as “remnants or signs of past activities or vestiges of the event”. Trace evidence can come in the form of pattern traces as well as material traces. The transfer of material can occur through contact as well as contactless transfer. I agreed with Dr. Kammrath’s statement of “The crime scene is a recording medium and evidence is the elements of the record”. In one fiber transfer study that she conducted with a student, she found that planted fibers will transfer during a washing machine cycle.She noted that fibers seem to stick to the seams after washing. This was very interesting and something that I will keep in mind when I examine clothing items in the lab. During this workshop, a demonstration was conducted by an employee of Noble, of an instrument similar to a Rapid DNA instrument. It was very informative to see how easy it was to use in the field.An exercise was also conducted which demonstrated how trace evidence can persist after time passes. The exercise involved placing a volunteer’s finger in a petri dish of trace materials that included different shapes and sizes of glitter and nitrate particles. A series of 20 fingerprints were made on glass slides. Observations were made of each fingerprint to see how much of the trace material transferred and persisted along the series of fingerprints.A Smiths Detection IonScan 600 was then used to test a fingerprint to determine if the nitrates could be detected in the fingerprint. Elaine Pagliaro, JD, also spoke about proposed changes to Rule 702: Testimony of Expert Witnesses as well as Rule 603 and 703.

A Luncheon Seminar entitled “The Lindbergh Kidnapping Suspect No. 1- The Man Who Got Away” was also very interesting. This presentation was conducted by Hon. Lise A. Pearlman, JD who wrote a book with the same title. I had heard of this case but did not know the specific details of what occurred during the crime as well as the aftermath. Pearlman described the history of the Lindbergh family as well as the details of the crime itself and the evidence that was left at the scene. She gave her opinion as to what she believed happened in the case as well as how she believes that an innocent man was put to death for the kidnapping and murder of Charles Lindbergh, Jr.

During the Last Word Society session, I attended a presentation about the Somerton Man – an unknown deceased male that was found on the beach in Australia. This was a case that I had heard on a podcast, and it always intrigued me. The presentation was given by Colleen M. Fitzpatrick, PhD and she discussed how she used Forensic Genetic Genealogy to identify this man after many years. She also presented a case where she used Forensic Genetic Genealogy to identify a hitchhiker from 1961.

Once again, the AAFS annual meeting was excellent. I always leave the meeting with more knowledge that I arrived with and that I can use in my current position with the MA State Police Crime Laboratory.Thank you to NEAFS for affording me the opportunity to attend the AAFS meeting by helping me defray some of the costs to attend.

Beth Saucier
Goodspeed

2024 ANNUAL **MEETING**

October 21st, 2024 - October 24th, 2024

Atlantic City, NJ

Contact Info:
Program Chair
Alanna Laureano
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NORTHEASTERN ASSOCIATION OFFORENSIC SCIENTISTS

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Twelve Month Update—Forensic Entomotoxicology in the Modern Age: Application of Direct Analysis in Real Time-High Resolution Mass Spectrometry in the Analysis of Insect Evidence

Research Overview:

The traditional role of entomological evidence in death investigations is to facilitate determination of the postmortem interval (PMI) or time since death. This is possible due to the well-documented correlation between the extent of decomposition, the colonization of the remains by insects, and the order in which various species of insects appear. However, it is increasingly being recognized that insects retrieved from decomposing remains can provide much more information of relevance to a cause of death investigation than PMI estimation. For example, when decomposition is advanced enough or when the remains have been scavenged by animals or destroyed through exposure to the elements, conventional methods of PMI estimation, such as examination of internal organs, or analysis of urine and blood for postmortem drug detection can no longer be employed. In such cases, it has been suggested that entomological evidence can offer a solution through the toxicological analysis of insects that have fed on the remains. When flies colonize a corpse following death, the maggots ingest the tissue along with any xenobiotics contained therein. Thus, one way to retrieve information on the cause of death, particularly when it involves drugs or toxins, is to analyze the larvae that have fed on the tissues, with the aim of identifying within the larvae, the xenobiotics (or their metabolites) that were in the tissues of the deceased.

The hypothesis explored in this body of work is that Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) can be used as a new method for the rapid toxicological analysis of insects recovered in the course of forensic investigations, based on its utilization in both drug analysis[1-3] (in an ever-increasing number of crime labs), and forensic entomology.[4-6] This hypothesis was explored through the pursuit of the following specific aims:

Specific Aim I: Demonstration of the ability of DART-HRMS as a presumptive test that can be used for the rapid detection of drugs and their metabolites in insects that have fed on decomposing tissue that contains drugs.

Specific Aim II: Development of optimal procedures for forensic entomotoxicological analysis using DART-HRMS and statistical analysis processing of the acquired data.

Research Results-To-Date:

In order to test the stated hypothesis, adult flies of the necrophagous species *L. sericata* were presented with acetyl norfentanyl (ANF)-laced liver, furanyl norfentanyl (FNF)-laced liver, or un-spiked (control) liver on which to lay their eggs. The larvae that hatched from these eggs were reared to adulthood following feeding and samples from each of the various life stages through which they transitioned (i.e., larvae (1st, 2nd and 3rd instar), pupae, and adult) were collected and preserved in 70% aqueous ethanol until analysis. Whole specimens were then analyzed by DART-HRMS in the pursuit of the specific aims. It was previously reported that while the drugs (ANF and FNF) were not detectable by DART-HRMS in the tissue of the of the larvae that ingested laced liver, it was nevertheless possible to differentiate between insects that fed on drug-laced versus control liver samples through Kernel Discriminate Analysis (KDA) and Discriminate Analysis of Principle Components (DAPC) of the data generated from DART-HRMS analysis of their tissues. Further, this differentiation appears to be possible for all of the insect life stages (1st instar larvae, 2nd instar larvae, 3rd instar larvae, pupae (including puparia casings), and adults), although the accuracy of the results was found to be a function of the life stage (data not shown). The life stages which enabled the most accurate differentiation between the classes (i.e., between drug exposure versus control) were the pupae, their puparia casings, and adult flies. However, the DART-HRMS analysis of the whole sample specimens is a destructive process, which is not ideal when considering current forensic practices, and the importance of the preservation of evidence. In deference to the preference for non-destructive analysis techniques,

an alternative approach that utilized the 70% ethanol solution within which the samples had been stored, as opposed to analysis of the insect life stages themselves, was devised.

As with the statistical analysis of the spectra obtained from the whole specimens, both KDA and DAPC models were generated for the differentiation of insects which had fed on drug-laced liver as maggots (labeled "Drugs") and those which fed on un-spiked liver (labeled "No Drugs") (see **Figure 1**). The visually apparent clustering reveals that inherent in the data are: (a) similarities between the chemical profiles of *L. sericata* samples that have been exposed to the drugs, independent of the identity of the drugs themselves; and (b) the chemical

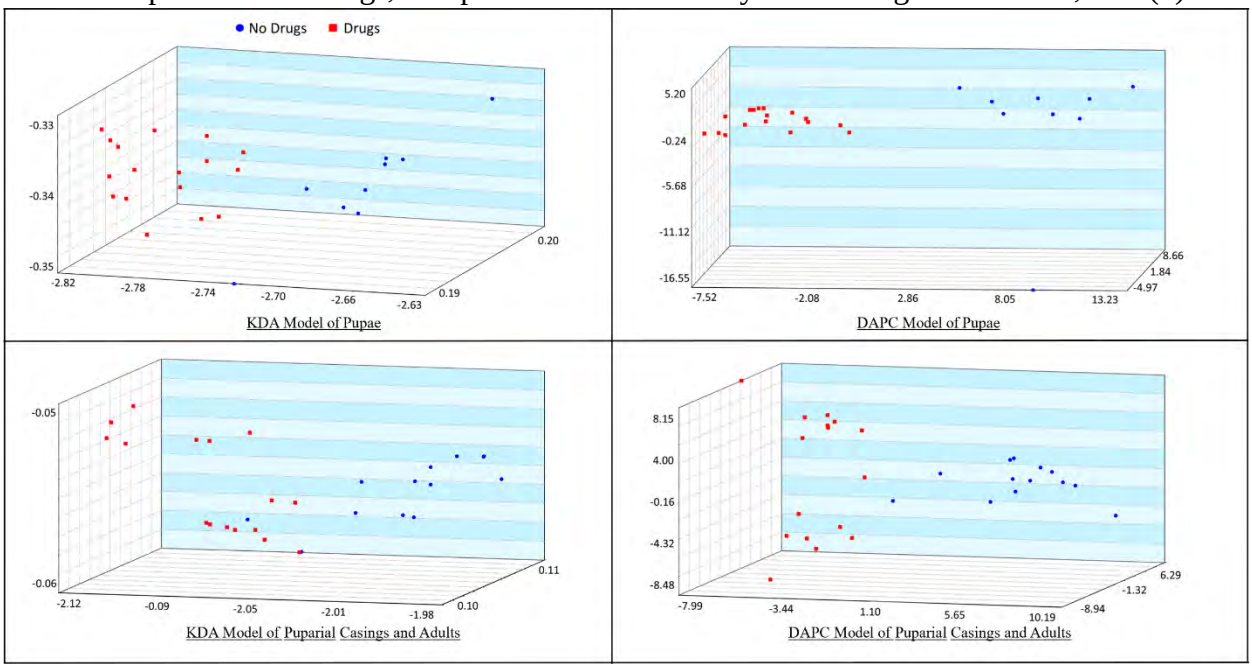

Figure 1. KDA (left) and DAPC (Right) prediction models generated from the DART-HRMS analysis of the 70% ethanol preservation solutions in which the indicated samples were stored following ingestion by *L. sericata* larvae and the rearing of the insects to adulthood. The top panel shows the results for pupae, and the bottom, the puparia casings and adults. Blue circles represent the specimens which fed on un-spiked liver and the red squares represent specimens which fed on one of the two drug-laced livers.

profile of the tissue of larvae that fed on un-spiked samples, and the subsequent life stages, is distinct from that of those derived from drug-laced samples. The accuracy of the models was evaluated using Leave-One-Out Cross Validation (LOOCV). It was found that the KDA and DAPC models performed similarly for the pupae, with a LOOCV of 96.15%. The puparia casing and adult specimens could not be evaluated separately as they were stored in the same solution. As such, the results for the casings and adults are displayed together. The KDA and DAPC models had LOOCVs of 92.59% and 85.19%, respectively. These observations were very similar to those of the models that were generated from the whole specimens, as shown in **Table 1**. This finding reveals that DART-HRMS analysis of the ethanol storage solutions suffices, and that it is unnecessary to subject whole samples to destructive analysis.

Table 1. Comparison of the LOOCV Results for Statistical Models Generated From Whole Specimens and their Ethanol Storage Solutions for the Drug verses Non-drug Classes

Model	KDA	DAPC
Pupae (whole)	92.31%	92.31%
Pupae (ethanol)	96.15%	96.15%
Casings (whole)	92.86%	100%
Adults (whole)	100%	100%
Casings and Adults (ethanol)	92.59%	85.19%

It was also found that within the "Drug" class, the AN and FN sub-classes could be distinguished from one another. This is illustrated in the KDA and DAPC models displayed in **Figure 2**.

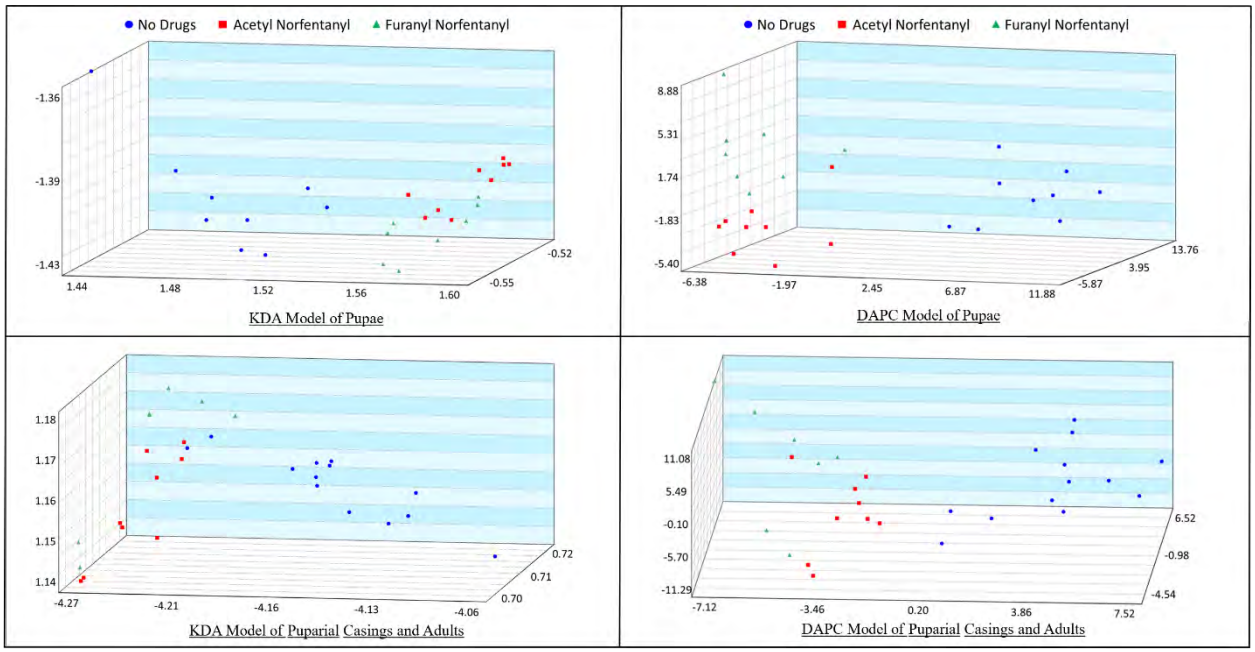

Figure 2. KDA (left) and DAPC (right) prediction models are displayed to show the differentiations of pupa (top), puparia casing and adult (bottom) specimens which fed on un-spiked liver (blue circles), acetyl norfentanyl (ANF)-laced liver (red squares) or furanyl norfentanyl (FNF)-laced liver (green triangles) based on the analysis of the 70% aqueous ethanol solutions used to preserve them.

Just as with the analysis of the whole specimens, the pupae models had poorer performance than the models consisting of puparia casing and adult samples. The pupae models had LOOCVs of 73.08% and 76.92% for the KDA and DAPC model, respectively. The KDA model of the puparia casing and adults samples had a LOOCV of 82.76%, and the DAPC model had a LOOCV of 82.76%. Again, a comparison of these results to those obtained from the LOOCV analysis of the KDA and DAPC models generated from the whole specimen analysis is displayed in **Table 2**.

Table 2. Comparison of the LOOCV Results for Statistical Models Generated From Whole Specimens and their Ethanol Storage Solutions for the Drug verses Non-drug Classes

Model	KDA	DAPC
Pupae (whole)	76.92%	88.46%
Pupae (ethanol)	73.08%	76.92%
Casings (whole)	86.21%	89.66%
Adults (whole)	72.00%	96.00%
Casings and Adults (ethanol)	82.76%	82.76%

Conclusions

DART-HRMS can be utilized for the toxicological examination of entomological evidence in forensic investigations through the collection of the insect chemical signatures. Further, it has been determined that the potentially destructive direct analysis of whole insect specimens is not necessary in order to collect these chemical signatures. The 70% aqueous ethanol which is used to preserve the insects acts as an appropriate intermediary which can be used in place of the whole specimens. Though the insect chemical signatures do not reveal *m/z*

values reflective of drugs consumed by the insects, the presence of the drugs can still be inferred through the consistent changes in the insect chemical signatures as compared to insects that were not exposed to drugs. Statistical analysis algorithms such as Kernel Discriminant Analysis and Discriminant Analysis of Principle Components can exploit these distinctions to differentiate between insect chemical signatures, and screen for possible drug consumption by the insects.

Future Work

Any remaining future work that is completed on this project will be included in the graduate thesis of the grant principle investigator.

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Development of a spectroscopic screening tool to determine optimal sampling sites for DNA recovery from human skeletal remains

Annual Report

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08 Jan 2023

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Development of a Spectroscopic Screening Tool to Determine Optimal Sampling Sites for DNA Recovery from Human Skeletal Remains

A. INTRODUCTION

There are a variety of scenarios in which unidentified human remains (UHRs) may be submitted for DNA testing such as those obtained from homicides, fires/explosions, natural disasters, terrorist attacks, war conflicts, mass graves, and aviation accidents. However, numerous challenges exist with providing identification to UHRs due to non-uniform bone diagenesis, DNA degradation and damage, and PCR inhibitors [1]. Additionally, forensic genetic testing of bones and teeth is a time-consuming, labor-intensive, and destructive process. Ultimately, there is a need for an improved and streamlined method for forensic genetic investigation of UHRs. Development of a screening tool to determine optimal sampling sites for maximum DNA recovery from human skeletal remains could provide a solution. Raman spectroscopy was evaluated as a screening tool to obtain sufficient information about bone microstructure and the stage of diagenesis, which could be informative regarding the quantity and quality of endogenous DNA in a particular section of bone.

Previous studies demonstrate that teeth (specifically molar teeth), and weight-bearing long bones (e.g., femur, tibia) have the highest DNA preservation rates due to the rigidity of the tissues of which they are composed. This provides protection from the environment for the endogenous DNA. In weight-bearing long bones, the epiphyses (ends) are made up of spongy, porous bone whereas the diaphysis (shaft) of the long bone is composed of dense, compact bone. Because of the structure of the diaphysis, it can resist the process of diagenesis and simultaneously preserves DNA most efficiently [2].

The main two components of bone are calcium hydroxyapatite and collagen. Diagenesis, or the process of degradation of the bone microstructure during postmortem decomposition, correlates directly to DNA quantity and quality. Diagenesis involves the alteration and leaching of hydroxyapatite, as well as the breakdown and leaching of collagen. In a bone of a living human, the negatively charged DNA backbone is bound to the positively charged calcium residues in the hydroxyapatite and this interaction provides protection to DNA from environmental insults. However, in the bone of a deceased human, as diagenesis of the hydroxyapatite progresses, ionic substitution occurs, and calcium residues are displaced. This in turn causes the DNA to dissociate from the mineral matrix thus making it more vulnerable to damage. Collagen breakdown and leaching also occurs because of increased bone porosity, and this collagen degradation also decreases the stability of the mineral matrix [3].

Diagenesis progresses in a heterogeneous, non-uniform manner along the diaphysis of a long bone, but its effects are not visible to the naked eye. Although standard forensic practice involves taking a cutting from the diaphysis for DNA testing, the precise location along the shaft to cut is a blind process. If a cutting is taken from an area of advanced diagenesis (i.e., an area with highly degraded microstructure), the resultant DNA recovery is often insufficient. Additional cuttings must be taken and tested until enough genetic material is recovered to generate a DNA profile. This is a destructive process and consumes the bone cuttings during testing, thus decreasing the amount of skeletal material that is available to return to the family of a decedent. A method for screening the state of the bone microstructure along the shaft of a long bone, and thus identifying the most preserved, non- degraded region of the bone, is needed.

Therefore, the purpose of this research was to develop an effective screening method using Raman spectroscopy to locate and predict the optimal sampling sites along the diaphysis of a long bone containing the most intact microstructure, which should then correlate to obtaining the highest DNA quantity and best DNA quality. Additionally, this proposed method will potentially reduce the amount of time and labor necessary for processing, lower the costs of DNA testing, and ultimately minimize destructive sampling and bone consumption. In this research, the capabilities and limitations of Raman spectroscopy and hyperspectral imaging as a screening tool for DNA recovery from human skeletal remains was evaluated.

B. METHODOLOGY

Three sets of weight-bearing long bones from skeletonized human remains of varying postmortem intervals (PMIs), and thus varying degrees of diagenesis, were collected. The first set of remains, exhumed from a cemetery in 2020 partially skeletonized, includes a femur and possesses a 9-month PMI. It is expected that minimal-to-no bone diagenesis will be present in the diaphysis of this weight-bearing long bone. The second set of remains, discovered in 2019 fully skeletonized, also is a weight-bearing long bone and has a minimum PMI of 3 years (although a PMI of 5 years is more likely). It is expected that an intermediate degree bone diagenesis will be present in the diaphysis of this long bone. The third set of remains, discovered in 1974 fully skeletonized, has a minimum PMI of 47 years (but could likely be in the 50-year range). It is expected that there would be advanced stage of bone diagenesis present in this sample.

The HORIBA XploRA PLUS Particle Analyzer Confocal Raman Microscope was used to collect spectral data. Method development focused on varying the instrument parameters including laser strength, wavelength, frequency, and exposure times. The chosen, optimal parameters were tested on a fresh mammal (bovine) bone without any degree of diagenesis to provide baseline spectra and sufficient bone compositional data before proceeding with the human bones of varying PMIs.

To assess if exposure to Raman caused degradation to DNA, two buccal (cheek) swabs were collected from 3 different individuals (for a total of six samples). DNA extractions were performed with the QIAamp DNA Investigator Kit (Qiagen Corporation, Germantown, Maryland USA) using the "Isolation of Total DNA from Buccal Swabs" protocol (50-µl elution volume), according to the manufacturer's recommendations.

Human DNA quantities recovered from each sample (six buccal swabs) were determined using the Quantifiler™ Trio Human DNA Quantification Kit (Thermo Fisher Scientific, Waltham, Massachusetts USA) and the QuantStudio™ 5 Real-time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts USA), according to the manufacturer recommendations. The Quantifiler™ Trio assay provides information regarding both the quantity and quality of DNA recovered. A Degradation Index (DI) is generated for each DNA sample, indicating the degree of DNA degradation/damage present. This information is useful in selecting appropriate downstream testing approaches to maximize chances of profiling success.

After quantification, all samples were subjected to Raman exposure using the previously optimized Raman parameters. The following procedure was conducted by Silverman:

1. Six DNA samples (extracted from buccal swabs) and the associated reagent blank were removed from the freezer and thawed.
2. The HORIBA XploRA PLUS particle Analyzer Confocal Raman Microscope was set to the optimized parameters determined during analysis of the known standards and the mammal (bovine) bone.
3. The area surrounding the instrument and all materials were sterilized (using 20% bleach and 100% ethanol solutions) before processing the extracted DNA samples.
4. Using sterilized scissors, the cap was removed from one microcentrifuge tube. Using a micropipette, the entire 50-µl of extracted DNA was transferred from inside the microcentrifuge tube into the holding well of the cap.
5. The cap from one sample was placed on a sterilized quartz microscope slide. DNA samples were processed one at a time to mitigate cross-contamination between samples, as well as to try to prevent contamination from the laboratory environment.
6. The cap containing the extracted DNA solution was exposed to the optimized Raman parameters (532 nm wavelength, 50% filter for the laser power, a spectrum range of 300-2000 cm^{-1}, and an objective lens with 100x magnification).
7. After Raman exposure, the cap was then placed back onto the microcentrifuge tube (by inverting the tube onto the cap and snapping back into place).
8. Steps 4-8 were repeated for all extracted DNA samples, changing gloves and sterilizing equipment between each sample.
9. Microcentrifuge tubes were then briefly centrifuged to remove drops from inside the lid and placed back into the freezer for storage at -20°C.

The DNA samples were then re-quantified using the same real-time qPCR procedure previously described to assess the effects of Raman exposure, specifically the differences in DNA quantity and DI. For the Raman microscope to be an effective screening tool, the comparison between the quantification results before and after Raman exposure should be the same (or with minimal change). Since forensic casework samples typically are already of limited quantity and are often highly degraded (both of which reduce the probability of DNA profiling success), a pre-screening methodology prior to DNA testing should not cause additional damage or loss of DNA.

Following Silverman's previously described step-by-step procedure, 59 buccal swabs (from 3 different individuals) were collected. DNA extractions were performed with the QIAamp DNA Investigator Kit (Qiagen Corporation, Germantown, Maryland USA) following the "Isolation of Total DNA from Buccal Swabs" protocol using a 50-µl elution volume. DNA quantities recovered were determined using the Quantifiler™ Trio Human DNA Quantification Kit (Thermo Fisher Scientific, Waltham, Massachusetts USA) and the QuantStudio 5 Real-time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts USA). The Quantifiler™ Trio assay provides information regarding both the quantity and quality of DNA recovered. After quantification, the DNA samples were subjected to Raman exposure using Silverman's previously described step-by-step procedure. However, for Step 2 and Step 6, Smith's optimized parameters were used instead (i.e., 532 nm wavelength, 25% filter for the laser power, 10x objective, 1200 (750 nm) grating, 2s acquisition time, 2 accumulations, 4s RTD time). Raman hyperspectral images of the diaphysis of the human femur with a PMI of approximately 3 years were also collected (using Smith's parameters) which demonstrated the non-uniform diagenesis that progresses across the diaphysis.

C. RESULTS AND DISCUSSION

a. Raman Spectral Analysis

There have been many previous research studies that have used Raman for analysis of bone composition; however, the parameters that were used varied widely [4-7]. The main parameters of focus were the excitation wavelength, laser power/intensity, spectrum range, and magnification. To optimize the Raman parameters, known calcium hydroxyapatite and collagen standards were tested with a variety of parameters. The Raman analysis was optimized using traditional figures of merit, with a prioritization on high intensity peaks and good resolution. The parameters that gave the best results **(Figures 1 and 2)** were determined in the initial phase of this research to be 532 nm laser excitation wavelength, 50% filter for the laser power, and an objective lens with a 100x magnification.

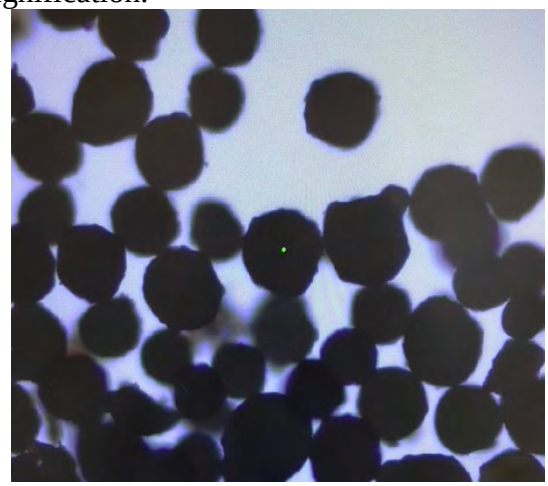
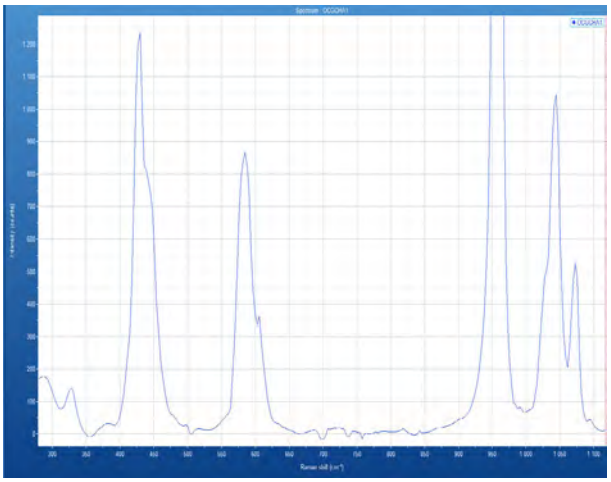
(A)  (B) 

Figure 1: *(A) Calcium hydroxyapatite crystals viewed using the Raman microscope 10x objective, and (B) its Raman spectrum collected with initially optimized Raman parameters (Silverman)*

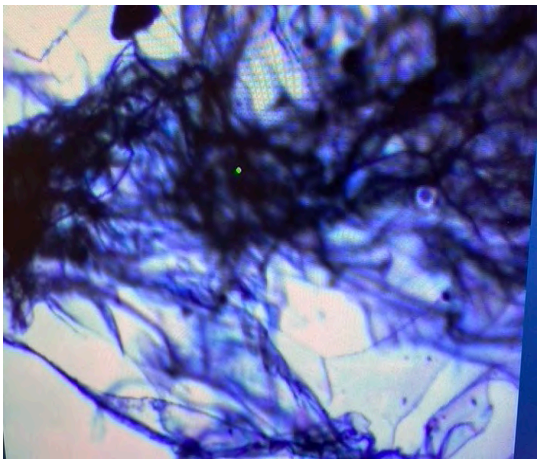
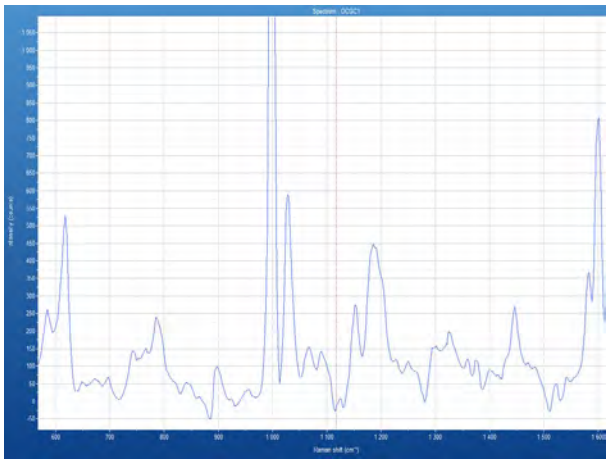
(A)  (B) 

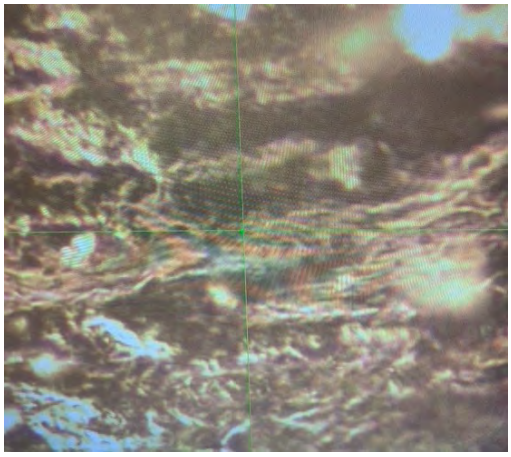
Figure 2: *(A) Collagen fibers viewed using the Raman microscope 50x objective, and (B) its Raman spectrum collected with optimized Raman parameters (Silverman)*

Since Raman spectroscopy had never been used in association with DNA extraction from bone, it was unknown if the strength of the Raman lasers could degrade the DNA. For this reason, fresh mammal bone was initially used instead of human skeletal remains (as proof-of-concept). To prepare the bone, first the soft tissue needed to be debrided from the bone surface **(Figure 3A)**. The bone was subsequently soaked in a Tergazyme™ solution to remove the periosteum, a layer of thin tissue that covers the bone surface. Next, the debrided bone was allowed to air-dry in a dead-air laminar flow hood. At this point, the bone was cleaned and almost ready to be used for Raman analysis **(Figure 3B)**. Since the entire bone cannot be placed in the microscope, a sectioned "window" had to be cut from the diaphysis of the bone **(Figure 3C).** The window was placed under the Raman microscope **(Figure 4A)** and a Raman spectrum was then collected using the optimized parameters determined from the known standards (i.e., calcium hydroxyapatite crystals and the collagen fiber). A quality (expected) spectrum was able to be collected based on the optimized parameters from the standards **(Figure 4B)**.

(A)  (B) 

(C) 

Figure 3: *Preparation of mammal bone for Raman spectral analysis: (A) debridement of soft tissue from the bone surface using a small razor blade and performed inside a hood, followed by (B) submersion in a Tergazyme™ solution to remove the periosteum and then air-drying in a dead-air laminar flow hood, and (C) sectioning of a "window" from the diaphysis using a bone saw to enable it to fit under our Raman microscope.*

(A) 

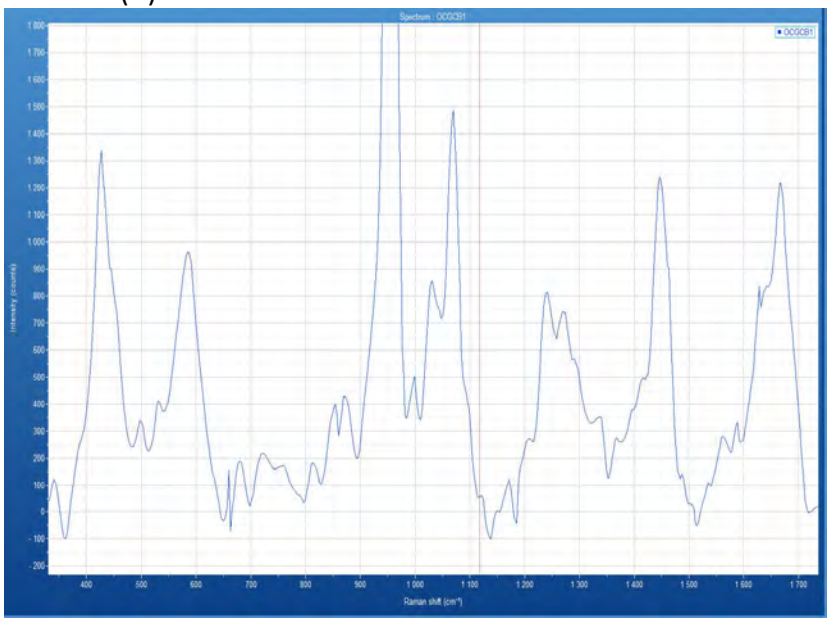
(B) 

Figure 4: *(A) Mammal bone viewed* using the Raman microscope 100x objective*, and (B) its corresponding Raman spectrum collected with optimized Raman parameters (Silverman)*

Using the parameters mentioned above, it was observed that the mammal bone had been burnt (charred) during Raman analysis, which negates the concept of this method being used for non-destructive screening before DNA extraction. Therefore, exploration of optimal Raman parameters had to be further explored, specifically reducing the laser power. The parameters that yielded the best results included using a 532 nm wavelength, 25% filter for the laser power, 10x objective, 1200 (750 nm) grating, 2s acquisition time, 2 accumulations, 4s real-time display (RTD) time, and using a 20 X 20 step size for the hyperspectral imaging mosaic map acquisition (Smith). Using the optimized parameters, Raman spectra for standard calcium hydroxyapatite crystals **(Figure 5)** and standard collagen fibers **(Figure 6)** were obtained. A Raman spectrum was collected using the optimized parameters for the mammal (bovine) bone **(Figure 7)**, and a Raman hyperspectral imaging mosaic map acquisition was collected for the mammal (bovine) bone **(Figure 8)**.

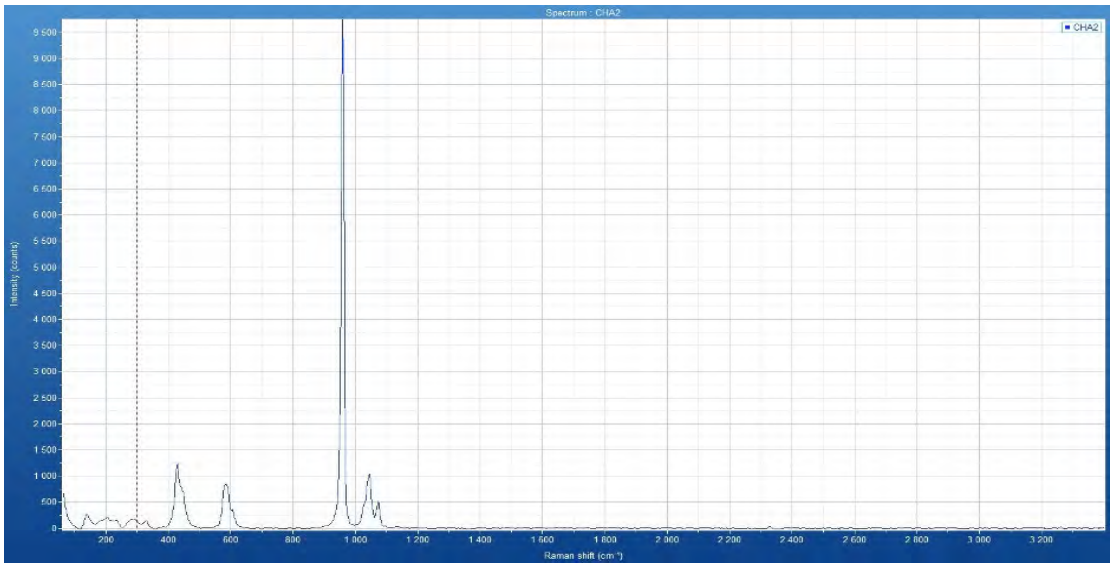


Figure 5. *A Raman spectrum for the mammalian (bovine) bone was re-collected using the optimized parameters (Smith) for standard calcium hydroxyapatite crystals.*

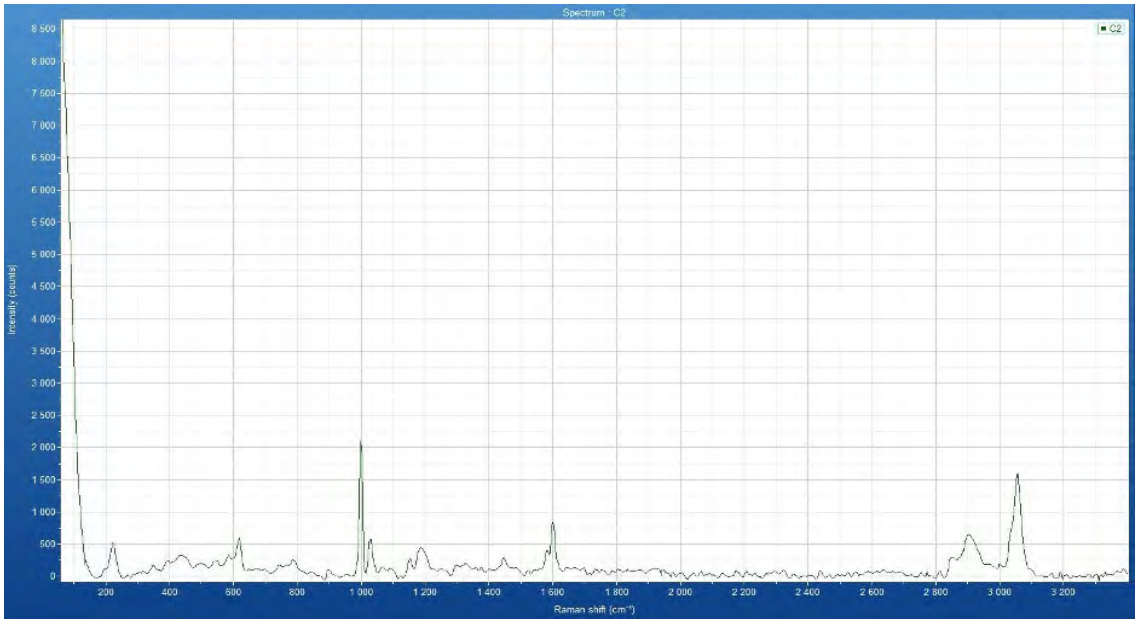


Figure 6. *A Raman spectrum for the mammalian (bovine) bone was collected using the optimized parameters (Smith) for standard collagen fibers.*

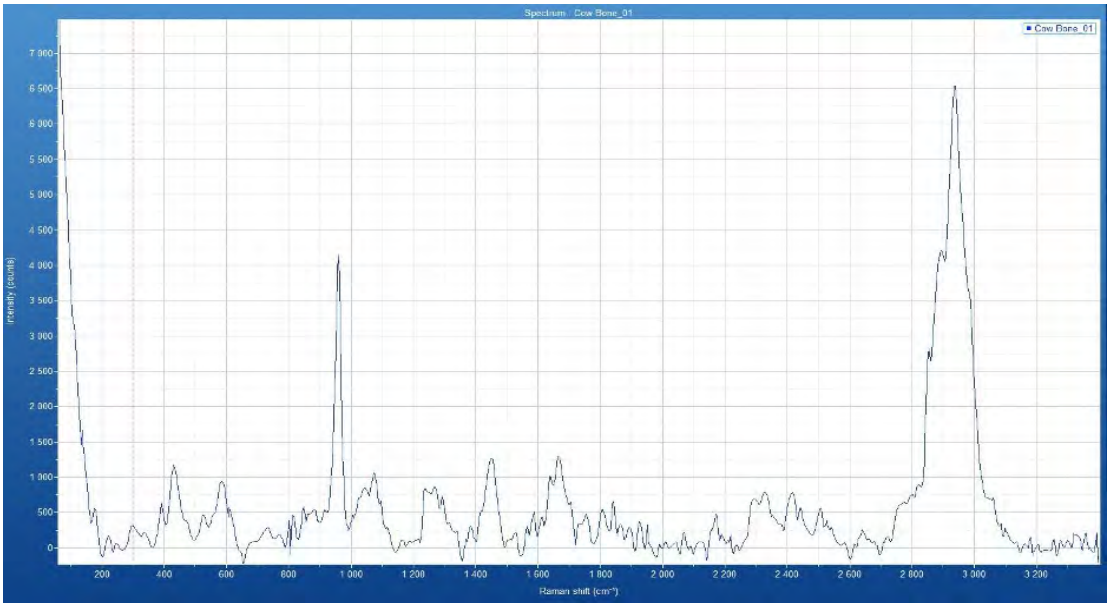


Figure 7. *A Raman spectrum was collected using the optimized parameters (Smith) for the mammal (bovine) bone.*

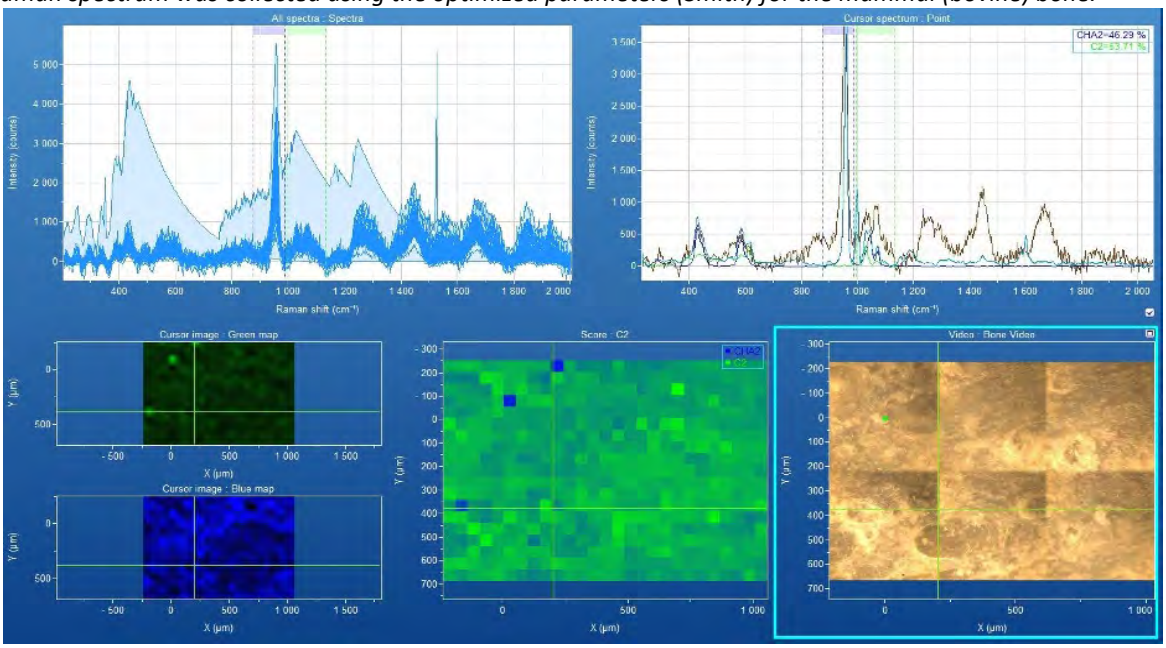


Figure 8. *A Raman hyperspectral imaging mosaic map acquisition was collected using the optimized parameters (Smith) for the mammalian (bovine) bone.*

In order to prepare the male human bone with a PMI of approximately 3 years **(Figure 9A)** for Raman analysis, a thin "window" was sectioned from the diaphysis using a Stryker® autopsy saw **(Figure 9B).** This sectioned "window" was then surface-sanded using a Dremel® Rotary Tool with an aluminum oxide grinding stone attachment **(Figure 9C-D).** Sanding the bone removes soil inhibitors and other environmental contaminants from the bone surface (e.g., humic acids and fulvic acids) that could interfere with both Raman and DNA analysis.

A Raman spectrum was obtained using the optimized (Smith) parameters for the human skeletal remains (PMI ~3 years) **(Figure 10)**, and a Raman hyperspectral imaging mosaic map acquisition was collected **(Figure 11)**.

(A)

(B)

(C)

(D)

Figure 9: *Preparation of human bone for Raman spectral analysis: (A) male human bone (PMI of approximately 3 years) as received; (B) removal of a "window" from the diaphysis using a Stryker® Autopsy Saw; and (C-D) surface-sanding of the sectioned "window" using a Dremel® Rotary Tool with an aluminum oxide grinding stone attachment.*

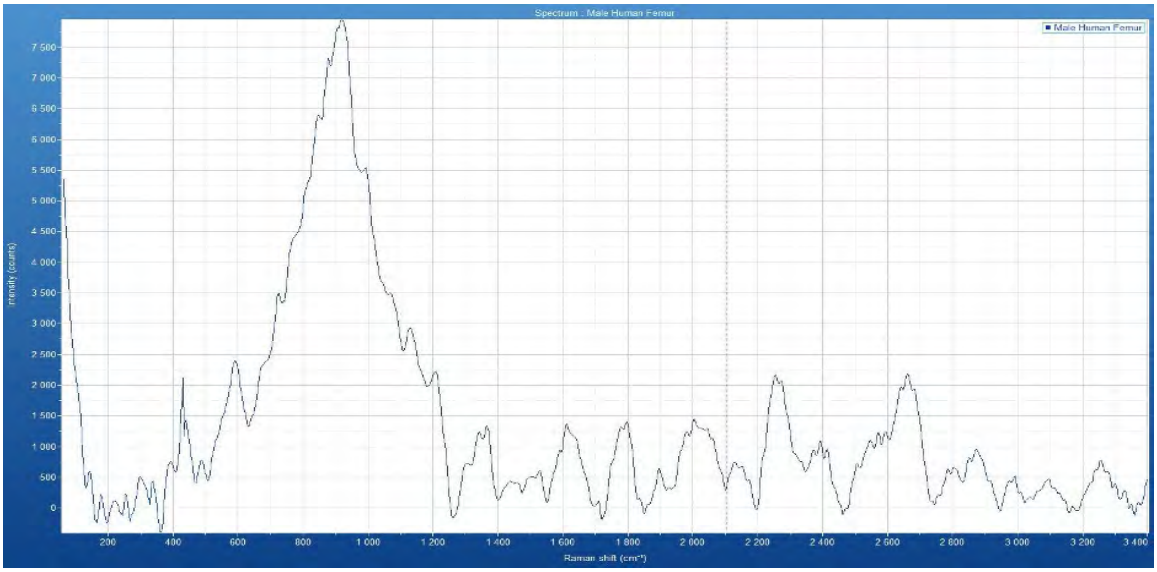


Figure 10. *Raman spectrum obtained for human skeletal remains with PMI ~3 years using the optimized parameters (Smith).*

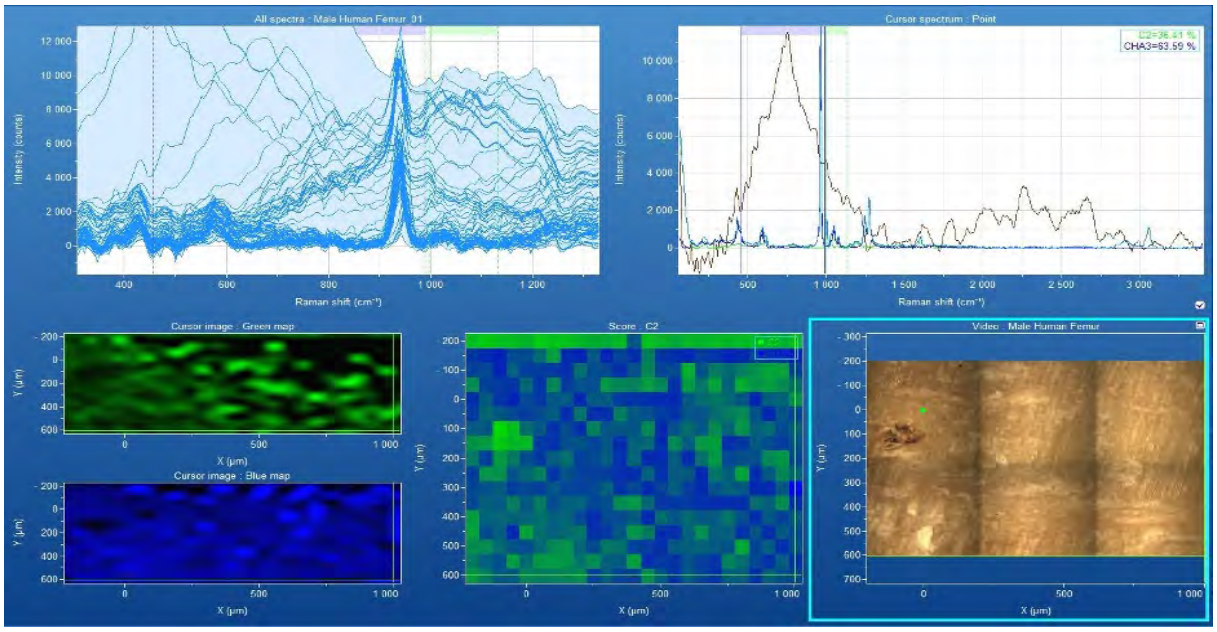
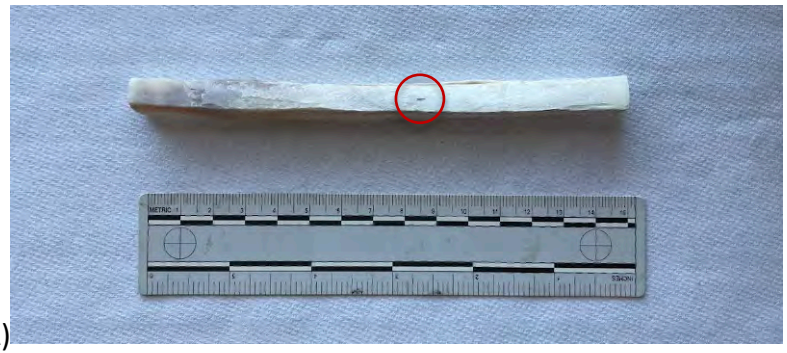


Figure 11. *Raman hyperspectral imaging mosaic map acquisition for human skeletal remains (PMI ~3 years) using the optimized parameters (Smith).*

Although sufficient data regarding the calcium hydroxyapatite and collagen content present in that section of bone was obtained **(Figures 10-11)**, a major issue was observed. The Raman laser had burned (charred) the bone sample during scanning and data acquisition **(Figure 12)**. Although this research is ongoing, these observations suggest that Raman spectroscopy should not be used as a screening tool for samples intended for DNA testing because the Raman parameters required to collect quality spectra is destructive to the bone surface. Burning (heat) is one of the most destructive forces on the molecular structure of DNA, so this is a concerning observation that may preclude the use of Raman spectroscopy as a pre-screening tool for forensic casework samples. Moreover, numerous studies have established and documented that heat (burning) damages both the macrostructure and microstructure of bone – and preservation of DNA is directly correlated to the quality of bone microstructure (specifically the hydroxyapatite mineral matrix). DNA molecularly binds to hydroxyapatite *in vivo* and is protected from damage while bound; however, as change to the hydroxyapatite matrix occurs, the DNA dissociates and becomes susceptible to damage and loss. Amplifiable DNA is often not obtainable from charred bone and, hence, these types of samples typically do not produce sufficient quality or quantity DNA data to be used for human identification [8].

The heat generated during hyperspectral imaging burned/charred both the bovine and human bone samples **(Figure 12)**. Although both the optimized parameters by Silverman and the refined (updated) parameters by Smith consistently produced quality spectral data, it was unexpected that the heat generated would actually visibly burn the bone surface during scanning and data acquisition. In forensic casework, skeletal remains typically arrive in the lab already degraded and significantly compromised by environmental exposure. Therefore, it would not be prudent to expose the skeletal samples to any methodology that could further degrade and damage the endogenous DNA (or the bone microstructure which, again, is directly correlated to DNA preservation).

(A)



(B)

Figure 12: *Photos depicting burnt/charred areas (circled in red) on (A) bovine bone and (B) human bone after Raman exposure using the optimized parameters (Smith) that yielded the best spectra results. Burning (heat) is one of the most destructive forces on the molecular structure of DNA, so this is a concerning observation that may preclude the use of Raman as a pre-screening tool for forensic casework samples.*

b. Evaluation of DNA Degradation Caused by Raman Spectral Analysis

DNA extracted from buccal swabs is "naked" DNA, and therefore is not protected by the normal cellular milieu. “Native” DNA (still encompassed within a cell) is protected by both the nuclear and cellular membranes, as well as histone proteins that help condense DNA into chromosomes. It is feasible to expect that there would be less effect on native DNA than naked DNA using a screening tool such as Raman spectroscopy. For example, DNA encased within bone is not only protected from damage by the cellular/nuclear membranes and histone proteins, but also by the rigid microstructural components of the bone itself (i.e., the hydroxyapatite mineral matrix and the collagen protein). If there are no statistically significant differences in DNA quantity and DNA quality when exposing naked DNA to the optimized Raman parameters, it could be presumed that this Raman screening method likely would not induce damage in native DNA encompassed within and protected by the bone matrix itself.

To assess if Raman spectroscopy had the capacity to degrade naked DNA, two buccal swabs were collected from three different individuals (for a total of six samples). DNA was extracted and quantified before Raman exposure **(Table 1)**, and then subsequently re-quantified after exposure to Raman at the optimized parameters **(Table 2)**. A Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio 5 Real-time PCR System provided information regarding both the quantity and quality of DNA recovered (based on comparison to five known DNA standards and generation of a passing standard curve). A Degradation Index (DI) was calculated for each DNA sample, indicating the degree of DNA degradation/damage present.

Table 1. *DNA quantities recovered after extraction and before Raman exposure. DNA quantity was determined using a Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio 5 Real-Time PCR System, based on comparison to a standard curve generated with known DNA standards. A Degradation Index (DI) was calculated based on the Large Autosomal (LA) and Small Autosomal (SA) quantities (NA = not applicable; undetermined = no DNA detected).*

Well	Sample Name	Target Locus	Degradation Index (Before Raman)	Total DNA (ng) (Before Raman)
D1	CS buccal #1	T.Large Autosomal	0.935	480
D1	CS buccal #1	T.Small Autosomal	0.935	448
D1	CS buccal #1	T.Y	NA	484
D2	CS buccal #2	T.Large Autosomal	0.798	462
D2	CS buccal #2	T.Small Autosomal	0.798	369
D2	CS buccal #2	T.Y	NA	418
D3	AA buccal #1	T.Large Autosomal	0.621	999
D3	AA buccal #1	T.Small Autosomal	0.621	621
D3	AA buccal #1	T.Y	NA	Undetermined
D4	AA buccal #2	T.Large Autosomal	0.647	1510
D4	AA buccal #2	T.Small Autosomal	0.647	977
D4	AA buccal #2	T.Y	NA	Undetermined
D5	BK buccal #1	T.Large Autosomal	0.699	489
D5	BK buccal #1	T.Small Autosomal	0.699	342
D5	BK buccal #1	T.Y	NA	Undetermined
D6	BK buccal #2	T.Large Autosomal	0.690	401
D6	BK buccal #2	T.Small Autosomal	0.690	277
D6	BK buccal #2	T.Y	NA	Undetermined

Table 2. DNA quantities after Raman exposure (using optimized parameters). The *DNA extracted from each buccal swab was re-quantified with the Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio™ 5 Real-Time PCR System. A Degradation Index (DI) was calculated based on the Large Autosomal (LA) and Small Autosomal (SA) quantities (NA = not applicable; undetermined = no DNA detected).*

Well	Sample Name	Target Locus	Degradation Index (After Raman)	Total DNA (ng) (After Raman)
D1	CS buccal #1	T.Large Autosomal	0.765	528
D1	CS buccal #1	T.Small Autosomal	0.765	404
D1	CS buccal #1	T.Y	NA	431
D2	CS buccal #2	T.Large Autosomal	0.743	524
D2	CS buccal #2	T.Small Autosomal	0.743	389
D2	CS buccal #2	T.Y	NA	440
D3	AA buccal #1	T.Large Autosomal	0.630	950
D3	AA buccal #1	T.Small Autosomal	0.630	599
D3	AA buccal #1	T.Y	NA	Undetermined
D4	AA buccal #2	T.Large Autosomal	0.640	1279
D4	AA buccal #2	T.Small Autosomal	0.640	818
D4	AA buccal #2	T.Y	NA	Undetermined
D5	BK buccal #1	T.Large Autosomal	0.680	546
D5	BK buccal #1	T.Small Autosomal	0.680	371
D5	BK buccal #1	T.Y	NA	Undetermined
D6	BK buccal #2	T.Large Autosomal	0.912	292
D6	BK buccal #2	T.Small Autosomal	0.912	266
D6	BK buccal #2	T.Y	NA	undetermined

The results of **Table 1** were compared with the results of **Table 2**. As mentioned previously, for the Raman microscope to be an effective screening tool, the comparison between the quantification results before

Raman exposure **(Table 1)** and after Raman exposure **(Table 2)** should be relatively the same in order to indicate that DNA damage and loss is not occurring. A percent loss/gain for total DNA using "IF" statements was calculated to compare the total DNA between both tables. The data was denoted as an overall "success" or failure with this comparison **(Table 3)**. For a potential "success," the Large Autosomal (LA) and Small Autosomal (SA) DNA quantities after Raman had to be the same or less than before Raman. If the Large Autosomal (LA) and Small Autosomal (SA) DNA quantities increased after Raman exposure, this suggests that contamination is present, which could be a consequence of the sensitivity of modern DNA technology combined with the fact that the Raman instrument is housed in a location that does not meet the "contamination prevention criteria" necessary for forensic DNA casework operations. If this method is to be implemented in an operational forensic casework context, there would need to be a dedicated Raman instrument inside a laboratory space that meets these strict contamination prevention requirements.

Table 3. *Overall "success" or failure of the DNA samples tested. According to the IF statement created, a gain immediately is assigned an overall failure, because it is impossible for the total DNA to increase, unless there is contamination present. If there is a loss, then the loss percentage is displayed. The first number in each grouping represents the loss percentage of the large autosomal (LA) DNA locus; the second number represents the loss percentage of the small autosomal (SA) DNA locus; and the third number represents the loss percentage of the Y-chromosome locus (only for CS, since this was the only male sample tested). If both fragments (or the Y-chromosome) show a decrease, then there was an overall "success" designation assigned, although it should be noted that any method that results in a decrease in DNA quantity and/or DNA quality would not be considered for use in unidentified human remains (UHR) casework.*

IF Statement Gain/Loss	Overall Success/Failure
FAILURE	FAILURE- CS buccal #1
-9.95%	
-10.93%	
FAILURE	FAILURE- CS buccal #2
FAILURE	
FAILURE	
-4.93%	"SUCCESS" - AA buccal #1
-3.53%	
-15.32%	"SUCCESS" - AA buccal #2
-16.24%	
FAILURE	FAILURE- BK buccal #1
FAILURE	
-27.19%	"SUCCESS" - BK buccal #2
-3.79%	

Based on the IF statement, three out of the six samples were designated "successful" (i.e., AA buccal #1, AA buccal #2, and BK buccal #2). As mentioned previously, six DNA samples were quantified, with a quantification value provided for both a small autosomal (SA) and a large autosomal (LA) locus. To simplify **Tables 1-2**, the data were tabulated for only the designated "successful" samples without contamination, for both the total DNA (ng) recovered and the degradation index (DI) *before* and *after* Raman exposure **(Tables 4-5)**. In these tables, the data for AA buccal #1, AA buccal #2, and BK buccal #2 are presented, which only represents half of the data set. The reason for this was DNA contamination issues, which precludes the use of the data in a simulated or real casework context. First, the analysis was focused on the three "successful" samples and then the issue of contamination was explored for the other three samples. In **Table 4**, the total DNA (ng) after Raman exposure decreased for the three "successful" samples, which does not support the use

of this screening tool for skeletal remains samples (which already typically exhibit low DNA quantities). In **Table 5**, the DI after Raman exposure increased for the three "successful" samples, also which does not lend support for the use of this screening tool for skeletal remains cases because casework bone samples often inherently contain low-quality (degraded) DNA due to extended environmental exposure.

Table 4. *DNA quantification of total DNA (ng) before Raman compared to total DNA (ng) recovered after Raman for the three designated "successful" samples.*

Sample Name	Target Locus	Total DNA (ng) Before Raman	Total DNA (ng) After Raman
AA buccal #1	T.Large Autosomal	999	950
AA buccal #1	T.Small Autosomal	621	599
AA buccal #2	T.Large Autosomal	1510	1279
AA buccal #2	T.Small Autosomal	977	818
BK buccal #2	T.Large Autosomal	401	292
BK buccal #2	T.Small Autosomal	277	266

Table 5. *Degradation indices (DIs) before Raman compared to degradation indices (DIs) after Raman for the three designated "successful" samples.*

Sample Name	Degradation Index (DI) Before Raman	Degradation Index (DI) After Raman
AA buccal #1	0.621	0.630
AA buccal #2	0.647	0.640
BK buccal #2	0.690	0.912

The average total DNA before Raman exposure and after Raman exposure were compared **(Table 6).** It is known that larger DNA fragments degrade more readily than small fragments, so it is expected to see greater changes in the large autosomal target than the small autosomal target. This is indeed what the data is showing, as there is a decrease of 13.4% for the quantity of large autosomal (LA) fragments present and a decrease of 10.2% for the quantity of small autosomal (SA) fragments present. A decrease in recoverable DNA after Raman exposure contraindicates support for the use of this as a screening method in degraded skeletal remains casework. The DI before and after Raman exposure were also compared for the three designated "successful" samples **(Table 7)**. A DI of 1 (or less than 1) generally indicates that the DNA sample is of good quality (i.e., not degraded). The higher the DI, the greater the degree of degradation is present. For the three designated "successful" samples, there was an average 11.4% increase in the DI after Raman exposure. Although none of the three samples were degraded beyond the point of viability to obtain a probative DNA profile (which is expected, given that they are freshly-collected, high-quality, nondegraded buccal samples), an 11.4% increase in DNA degradation in a true casework sample (particularly skeletal remains) very likely would have a significant effect on downstream DNA testing success, as well as in the quality of DNA data that could be obtained for use in the identification process. Hence, this difference (increase) in DI needs to be further investigated with a larger sample set. If the trend of increased DI continues, the data would preclude any recommendations for use of Raman as a screening tool for skeletal remains cases (or with any type of degraded/challenged forensic sample).

Table 6. *Average total DNA (ng) recovered before Raman compared to average total DNA (ng) recovered after Raman for the three designated "successful" samples. A percent loss was calculated for the large autosomal (LA) DNA fragments and the small autosomal (SA) DNA fragments.*

Target Locus	Average Total DNA (ng) Before Raman	Average Total DNA (ng) After Raman	Percent DNA Loss
T.Large Autosomal	970	840	-13.4%
T.Small Autosomal	625	561	-10.2%

Table 7. *Average degradation index (DI) before Raman and after Raman for the three designated "successful" samples. A percent increase in DI is reported.*

Average Degradation Index (DI) Before Raman	Average Degradation Index (DI) After Raman	Percent Increase in Degradation Index (DI)
0.653	0.727	+11.4%

To determine the statistical significance between the three designated "successful" samples, a t-test for a paired two-sample for means test was run to compare the total DNA and the DI. Using a t-test for a paired two-sample for means test, there is significant evidence at a 95% confidence level, that the two sample means of Total DNA (*before* Raman) and Total DNA (*after* Raman) are significantly different from each other (i.e., t-stat = 2.553 is greater than 1.96). This means that there is a significant difference between the total DNA measured *before* and *after* Raman exposure. These results do not lend support for the use of Raman as a pre-screening tool, because it may significantly damage autosomal DNA fragments, decreasing the overall intact total DNA that is recoverable for downstream analysis. However, this data represents preliminary results with a very small sample size; hence, further investigation using a larger number (and greater variety) of samples is warranted before making any definitive recommendations or drawing formal conclusions. Using a t-test for a paired two-sample for means test, there is not significant evidence at a 95% confidence level that the two sample means of DI (before Raman) and DI (after Raman) are significantly different from each other (i.e., t-stat = 0.9387 is not greater than 1.96). Although a statistical t-test here does not reveal significance in DIs before and after Raman exposure, it is well-established in forensic DNA casework that even small changes in the quantity or quality of DNA recovered can have a disproportionately negative impact DNA testing success. The observation that Raman exposure both *decreases* DNA quantity and *increases* the degree of degradation in samples is cause for concern, as low DNA quantity or poor DNA quality in isolation do not affect typing success nearly as much as when both conditions are present in concert with each other. However, again, further exploration using a larger sample size is worthy of investigation.

Table 8. *Degradation Indices (DIs) and total DNA (ng) recovered before Raman exposure for both the small autosomal (SA) and large autosomal (LA) loci. DNA was quantified using a Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio™ 5 Real-Time PCR System.*

Sample Name	Degradation Index (DI) Before Raman	Total DNA (ng) Small Autosomal (SA) locus Before Raman	Total DNA (ng) Large Autosomal (LA) locus Before Raman
AA Buccal #1	0.809	1766	2181
AA Buccal #2	0.877	1422	1621
AA Buccal #3	0.689	1486	2157
AA Buccal #4	0.766	1134	1480
AA Buccal #5	0.796	1958	2461
AA Buccal #6	0.863	1041	1205
AA Buccal #7	0.814	1336	1641
AA Buccal #8	0.713	1150	1614

AA Buccal #9	0.691	835	1208
AA Buccal #10	0.736	1106	1504
KS Buccal #1	0.781	1948	2493
KS Buccal #2	0.859	1123	1307
KS Buccal #3	0.718	580	808
KS Buccal #4	0.915	912	996
KS Buccal #5	0.657	853	1298
KS Buccal #6	0.721	771	1069
KS Buccal #7	0.760	822	1082
KS Buccal #8	0.717	634	884
KS Buccal #9	0.753	1021	1356
KS Buccal #10	0.863	750	869
KS Buccal #11	0.888	712	801
KS Buccal #12	0.866	652	753
KS Buccal #13	0.712	604	847
KS Buccal #14	0.620	1427	2303
KS Buccal #15	0.697	1583	2272
KS Buccal #16	0.672	1466	2182
KS Buccal #17	0.797	1122	1408
KS Buccal #18	0.860	840	977
KS Buccal #19	0.760	1715	2257
KS Buccal #20	0.705	1670	2368
KS Buccal #21	0.756	723	957
KS Buccal #22	0.765	2195	2868
KS Buccal #23	0.734	1832	2495
KS Buccal #24	0.784	898	1146
KS Buccal #25	0.658	1865	2835
KS Buccal #26	0.745	2212	2970
KS Buccal #27	0.815	1294	1586
KS Buccal #28	0.841	1102	1311
KS Buccal #29	0.907	874	964
KS Buccal #30	0.770	1910	2480
KS Buccal #31	0.710	1053	1484
KS Buccal #32	1.011	2595	2575
KS Buccal #33	1.105	3897	3529
KS Buccal #34	1.029	2898	2817
KS Buccal #35	1.120	1554	1387
KS Buccal #36	1.117	1692	1514
KS Buccal #37	1.063	5378	5059
BK Buccal #1	0.673	826	1227
BK Buccal #2	0.722	558	773
BK Buccal #3	0.677	528	780
BK Buccal #4	0.678	252	371
BK Buccal #5	0.752	634	842
BK Buccal #6	0.800	538	673
BK Buccal #7	0.682	392	575
BK Buccal #8	0.749	702	938
BK Buccal #9	0.728	749	1028
BK Buccal #10	0.770	831	1079

BK Buccal #11	0.792	981	1239
BK Buccal #12	0.775	854	1102

Table 9. *Degradation Indices (DIs) and total DNA (ng) recovered after Raman exposure for both the small autosomal (SA) and large autosomal (LA) loci. DNA was quantified using a Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio™ 5 Real-Time PCR System.*

Sample Name	Degradation Index (DI) After Raman	Total DNA (ng) Small Autosomal (SA) locus After Raman	Total DNA (ng) Large Autosomal (LA) locus After Raman
AA Buccal #1	0.729	1459	2000
AA Buccal #2	0.772	1311	1697
AA Buccal #3	0.689	1308	1899
AA Buccal #4	0.639	905	1416
AA Buccal #5	0.590	1231	2088
AA Buccal #6	0.770	1606	2086
AA Buccal #7	0.753	2048	2721
AA Buccal #8	0.735	2064	2809
AA Buccal #9	0.635	1216	1915
AA Buccal #10	0.705	1297	1840
KS Buccal #1	0.640	1061	1657
KS Buccal #2	0.869	866	996
KS Buccal #3	0.782	558	714
KS Buccal #4	0.823	612	744
KS Buccal #5	0.801	1444	1802
KS Buccal #6	0.696	1058	1519
KS Buccal #7	0.631	1009	1600
KS Buccal #8	0.740	1015	1372
KS Buccal #9	0.812	1794	2210
KS Buccal #10	0.730	1061	1454
KS Buccal #11	0.810	1341	1655
KS Buccal #12	0.667	894	1340
KS Buccal #13	0.778	1103	1418
KS Buccal #14	0.660	1895	2872
KS Buccal #15	0.774	2306	2979
KS Buccal #16	0.851	2381	2800
KS Buccal #17	0.780	1553	1990
KS Buccal #18	0.755	1271	1684
KS Buccal #19	0.715	1754	2452
KS Buccal #20	0.739	1565	2118
KS Buccal #21	0.924	6782	7342
KS Buccal #22	0.850	3753	4413
KS Buccal #23	0.813	3053	3755
KS Buccal #24	0.725	1223	1687
KS Buccal #25	0.802	3087	3850
KS Buccal #26	0.938	3243	3458
KS Buccal #27	0.940	2060	2191
KS Buccal #28	0.736	1320	1794

KS Buccal #29	0.649	998	1536
KS Buccal #30	0.784	2811	3586
KS Buccal #31	0.716	1472	2055
KS Buccal #32	0.784	2402	3063
KS Buccal #33	0.831	4037	4859
KS Buccal #34	0.851	3050	3583
KS Buccal #35	0.803	1513	1884
KS Buccal #36	0.848	1507	1777
KS Buccal #37	0.942	6212	6593
BK Buccal #1	0.612	1040	1700
BK Buccal #2	0.713	711	997
BK Buccal #3	0.683	582	852
BK Buccal #4	0.673	326	483
BK Buccal #5	0.716	899	1256
BK Buccal #6	0.629	689	1096
BK Buccal #7	0.610	546	894
BK Buccal #8	0.735	1001	1362
BK Buccal #9	0.602	977	1622
BK Buccal #10	0.672	1253	1864
BK Buccal #11	0.674	1303	1933
BK Buccal #12	0.647	1043	1612

Table 10. *Average total DNA (ng) recovered before Raman compared to average total DNA (ng) recovered after Raman for 59 buccal DNA samples. A percent change was calculated for the large autosomal (LA) DNA fragments and the small autosomal (SA) DNA fragments. An increase in DNA recovery was observed after Raman exposure, which is unexpected and indicates that DNA contamination was present. As observed previously in the initial (earlier) sample set, this contamination issue is likely a consequence of the sensitivity of modern DNA technology combined with the fact that the Raman instrument is housed in a location that does not meet the "contamination prevention criteria" necessary for forensic DNA casework operations. If this method is to be implemented in an operational forensic casework context, there would need to be a dedicated Raman instrument inside a laboratory space that meets these strict contamination prevention requirements.*

Target Locus	Average Total DNA (ng) Before Raman	Average Total DNA (ng) After Raman	Percent Change in Total DNA recovered
T.Small Autosomal	1292	1693	+30.1%
T.Large Autosomal	1593	2185	+37.2%

Table 11. *Average degradation index (DI) before Raman and after Raman for 59 buccal DNA samples. A percent decrease in DI is reported.*

Average Degradation Index (DI) Before Raman	Average Degradation Index (DI) After Raman	Percent Change in Degradation Index (DI)
0.793	0.745	-6.04%

The results reported in **Table 8** were compared with the **Table 9** results to assess differences in DNA recovery before Raman exposure (**Table 8**) and after Raman exposure (**Table 9**). Results include degradation indices (DIs), which indicate the degree of DNA degradation present, as well as DNA quantities (ng) recovered for both the Small Autosomal (SA) and Large Autosomal (LA) targets. **Table 10** reports the average total DNA quantity (ng) for the Small Autosomal (SA) and the Large Autosomal (LA) targets for all 59 samples both before and after Raman exposure. The average DNA quantity (ng) increased by 30.1% for the Small Autosomal (SA) locus and by 37.2% for the Large Autosomal (LA) locus after Raman exposure. This increase in DNA recovery could potentially be explained by one of two factors: 1) DNA contamination issues relating to the high sensitivity of modern DNA detection technology combined with the non-optimal location and isolation of the Raman instrument (i.e., a non-controlled environment not conducive to the strict contamination prevention measures necessary for forensic DNA casework), or 2) evaporation of liquid from the exposed microcentrifuge cap due to the heat generated during Raman laser scanning (which would alter the calculation of total DNA recovered and falsely implicate the presence of DNA contamination). It is certainly possible (and perhaps likely) that both of these factors collectively (in concert with each other) contributed to the results obtained.

Concerns arose during the *experimental design* phase of this research regarding the potential for DNA contamination during Raman screening. Not only is the Raman instrument housed in a separate laboratory not properly designed for DNA testing, but the Raman screening process itself requires the microcentrifuge tubes containing extracted (naked) DNA to be open and exposed during scanning. Open tubes are typically a contributing factor in observed incidences of contamination. Additionally, excitation of exposed (uncovered) DNA molecules by the Raman laser could have contributed to DNA carryover or cross-contamination between samples. In other words, contamination may have occurred during the Raman exposure process in which the DNA in the open cap was scattered by the Raman laser, and cleaning the instrument and surrounding areas between samples was insufficient.

In considering these possibilities, careful cleaning and pre-sterilization of the workspace and Raman instrument continued to be incorporated. However, it was a challenge in this experiment to overcome contamination due to the necessary handling of samples to obtain Raman data and the opening of tubes in a non-sterile environment (i.e., non-DNA laboratory).

Alternatively, the heat generated by the Raman laser could be another possible reason for the higher DNA quantities observed after Raman exposure compared to pre-Raman scanning. The total quantity of DNA (ng) in each microcentrifuge tube cap was calculated based on the initial elution volume used during the DNA extraction process (i.e., 50 µl). To explain, total DNA quantity (ng) calculations were performed by multiplying the pre-Raman DNA concentration (ng/µl) determined by the QuantStudio 5 Real-Time PCR instrument by 50 µl (i.e., the amount of elution buffer used in the original DNA extraction procedure). If some of the originally eluted volume of liquid does actually evaporate during Raman laser exposure, the post-Raman total DNA quantities (ng) would be falsely inflated.

Table 11 depicts the degradation index (DI) for each of the 59 samples tested before Raman compared to after Raman exposure. The DI is the ratio of the Small Autosomal (SA) quantity to the Large Autosomal (LA) quantity. The DI for high-quality (non-degraded) DNA typically falls close to 1, since the concentration of small and large DNA fragments are approximately equal in a non-degraded sample. The higher the DI above 1, the more degraded the DNA sample is. The observed decrease in DI with the current dataset could possibly be attributed to pipetting variation (or pipetting error) during preparation of the pre-Raman and post-Raman quantification runs. Further exploration of this observation is being pursued.

D. FUTURE DIRECTIONS

This research is ongoing and is important because of the repeatedly observed occurrence of varying quantities and quality of DNA recovered from different regions along the shaft of the same long bone in a human

skeleton that has been subjected to long-term exposure to environmental insults (such as would be encountered in forensic casework). These observed differences in DNA quantity and quality are due to the non-uniform, heterogeneous progress of diagenesis in bone microstructure. Therefore, continued research data that demonstrates this phenomenon can inform on and support the recommendation for casework labs to consider taking more than one cutting from the diaphysis of a long bone when testing for DNA. For example, if a cutting is taken from an area along the shaft with advanced diagenesis (i.e., an area with highly degraded bone microstructure), the quantity and quality of recoverable DNA may not be sufficient to generate a profile. However, areas adjacent to the original cutting may be in a less-advanced stage of diagenesis, and therefore would contain DNA of higher quality and quantity (potentially yielding a partial or complete DNA profile). For this reason, even though current data does not support the use of Raman spectroscopy as a pre-screening tool for bone *prior to DNA testing* (i.e., because it is destructive to DNA), spectral data obtained from various regions along the shaft of the *same* long bone would be useful in providing further support for the recommendation to sample from more than one area/region of the diaphysis (notably if the spectral scans demonstrate the non-uniform, heterogeneous process of diagenesis).

With regards to continuing experimentation and additional trouble-shooting, 50 new buccal swab samples have been extracted for DNA and quantified. One goal of this subsequent testing is to assess and determine whether the previously observed increase in DNA quantities after Raman exposure is due to evaporation (i.e., via heat generation from the Raman laser) or contamination. With the current batch of DNA samples, the total volume (µl) remaining in the microcentrifuge tube cap after exposure to the Raman laser will be carefully measured and calculated via incremental pipetting using a small-volume pipette until all liquid has been recovered from the cap. This process will be repeated for each individual DNA sample exposed to the Raman laser (as the degree of evaporation may vary between samples). These carefully measured volumes of post-Raman DNA will then be used to calculate the total DNA recovered after Raman exposure (instead of the originally measured 50-µl elution volume used during the extraction process).

Although the Raman spectral analysis explored thus far visually burned/charred the human bone (negating its application in DNA testing applications), there are questions we still intend to investigate. Specifically, this research will be continued in an attempt to demonstrate the process of non-uniform, heterogeneous diagenesis along the diaphysis of a single long bone. With the same optimized parameters discussed previously, “windows” cut from the diaphysis of three human long bones of varying PMIs (9 months, ~3 years, ~47 years) will be scanned with the Raman instrument. Hyperspectral imaging data for each region along the shaft of each long bone will be converted to color-coded “maps” that visually represent the degree of bone microstructure (i.e., hydroxyapatite, collagen) diagenesis present. It is hypothesized that the degree and variation of the stages of diagenesis present will increase with the age (PMI) of the skeletal sample. All three skeletal samples used in the current study may exhibit non-uniform diagenesis along the shaft, which would support the recommendation of processing multiple bone cuttings for DNA to increase chances of typing success.

However, computing power and memory present a meaningful limitation to this research. The WiRE Particle Analysis supports images up to ~530 MP in size and the file size is currently too large to accomplish scanning the entire diaphysis of the long bone. More specifically, the issue is that when using a 10x objective (which is the minimum magnification needed to achieve adequate spatial resolution to detect the microchemistry of the bone), the maximum tiled image size for a standard Raman instrument is 34 X 22 (Tim Prusnick, Renishaw). The single image field-of-view (FOV) for a 10x objective is 900 X 588 microns; thus, the largest Raman hyperspectral image that can possibly be collected is 3.06 X 1.2 cm. Therefore, adjacent areas along the diaphysis of each long bone will be sequentially quadranted using a Sharpie marker before Raman scanning. In this manner, multiple hyperspectral images (each representing a different quadrant of the shaft) can be pieced together to illustrate the non-uniform, heterogeneous nature of bone diagenesis.

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EMPLOYMENT OPPORTUNITIES

RI Department of Health, Providence, RI
Principal Forensic Scientist (Toxicology)

To be responsible for the technical operations of a forensic laboratory in the area of specialization indicated by the title of the class of position: the analysis of post-mortem biological specimens for drugs and poisons contributing to cause of death; analysis of biological specimens for levels of alcohol and drugs capable of impairing motor vehicle operation; and other forensic toxicological examinations as required; This position will serve as the technical lead in the Forensic Toxicology laboratory under the direction of the unit supervisor. The preferred applicant will possess an advanced degree in chemistry, have 5+ years' experience within a forensic laboratory, extensive knowledge in mass spectrometry (LC-MS/MS preferred) including method development, validation and troubleshooting. Knowledge regarding QA/QC requirements and experience leading a small group of interdisciplinary scientists is also preferred. Priority will be given to applicants in possession of an ABFT certification with Diplomat status. Please see posting for more information.

To apply, visit: https://www.governmentjobs.com/careers/rhodeisland/jobs/3943057/principal-forensic-scientist-toxicology?pagetype=jobOpportunitiesJobs

Closes: 04/15/2023

University of New Haven, West Haven, CT
Forensic Science Non-Tenure Track Faculty, Department of Forensic Science

The University of New Haven is seeking a Non-Tenure Track faculty member to teach course work in both the undergraduate and graduate forensic science programs. Some courses will be consistent with the faculty member's area of expertise, and others will be general courses such as Survey of Forensic Science. In addition, this candidate must be competent in advising and mentoring students at various levels including both undergraduate and graduate level thesis projects. Research and professional development are key elements of this position as well.

The minimum requirement is an MS in natural science, or some closely related forensic science discipline. Preferred area of interests are crime scene investigation and crime scene reconstruction. Practical experience and courtroom testimony is key for this position.

The successful candidate must have significant knowledge of crime scene investigation techniques both in a field and laboratory setting, as well as knowledge of crime scene reconstruction techniques. Previous teaching experience in higher education is preferred but not required. The successful candidate must be willing to teach nights and weekends as needed.

Position URL apply.interfolio.com/106354

Closes: 04/15/2023

Sciex (New York, NY)
LC-MS/MS Sales Representative

- Bachelor's degree (B.S./B.A.) In Life Sciences or similar subject area.
- Professional and / or educational experience that provides knowledge and exposure to fundamental theories, principles and concepts of LC/MS/MS Chromatography
- Ability to travel up to 50 % with valid driver's license and work remotely from home office
- Chosen candidate must live near and service the greater NYC area. Relocation can be considered

For more information and to apply please visit: https://jobs.danaher.com/global/en/job/R1228408/Mass-Spectrometry-Territory-Sales-Executive-greater-NYC

University of New Haven, West Haven, CT
Forensic Science Lecturer (Non-Tenure track), Department of Forensic Science

The University of New Haven is seeking a Forensic Science Lecturer to teach coursework in the undergraduate and graduate forensic science programs at University of New Haven. Some of these courses will be consistent with the faculty member's area of expertise, and other courses will be more generalized, such as Survey of Forensic Science. In addition, this candidate must be competent in advising and mentoring students at various levels, including undergraduate and graduate level thesis projects. Research and professional development are key elements of this position as well.

The minimum requirement is an MS in a natural science, or some closely related forensic science discipline. Preferred areas of interest are crime scene investigation and crime scene reconstruction. Strong candidates with Biology or Chemistry backgrounds will be considered. Practical and courtroom testimony experience are important qualifications for the position.

The successful candidate must have significant knowledge of crime scene investigation techniques (both in a field and laboratory setting) and knowledge of crime scene reconstruction methodologies. Previous teaching experience in higher education is preferred but not required. The successful candidate must be willing to teach nights and weekends as needed.

Position URL apply.interfolio.com/115284

Closes: 04/15/2023

Onondaga County Health Department Forensic Laboratories, Syracuse, NY
Latent Print Examiner I, II or III
Salary: $59,484-$77,699 DOE

Hours of work: 35 hours per week, Optional 4-Day Work Week Available

The work involves responsibility for processing items of physical evidence for the purposes of developing latent print friction ridge detail (fingerprints, footprints, palm prints) using various physical and chemical methods. These latent prints are permanently recorded through the use of digital photography. An employee in this class determines the best technique to develop each specific print and may, if appropriate, conduct comparisons of latent or patent prints to known prints. In addition, the work involves explaining analysis to a jury and providing demonstrations/visual displays. General supervision is received from the Senior Latent Print Examiner. Does related work as required. All work is performed in accordance with Federal and State accreditation requirements and departmental procedure manuals and guidelines.

Please email a cover letter, curriculum vitae and transcript(s) to: Lauren Pyland, Director of Operations at laurenpyland@ongov.net

Deadline for applications: April 20, 2023

Official job description, including minimum qualifications, can be found on the Personnel page at Onondaga County Job Descriptions.

Emporia State University
Instructor/Assistant/Associate Professor Forensic Chemistry

View full ad here - https://sites.google.com/g.emporia.edu/human-resources/faculty-staff-open-positions/1133-instructor-or-assistantassociate-professor

Application review will begin January 30th and continue until the position is filled. Salary is commensurate with experience and the position is benefits eligible. Please contact the search committee chair, Dr. Melissa Bailey (mbailey4@emporia.edu) with any questions.

New York Police Department Laboratory, Queens, NY
Deputy Director, Police Laboratory
Salary Range: $77,000 to $180,000

The Managerial Criminalist, M-III, designated Deputy Director, Police Laboratory will be responsible for the following:

- Assist in the leadership of a professional scientific and administrative support staff engaged in forensic examinations and analyses of physical evidence, administrative tasks related to criminal investigations, and providing expert testimony with respect to physical evidence in legal proceedings;
- Ensure all laboratory work performed is in accordance with Department procedures, federal and state regulations, and accreditation standards of the American National Standards Institute (ANSI) National Accreditation Board (ANAB) and the New York State Commission of Forensic Science (NYSCOFS);
- Assist the Director in formulating and implementing short and long-term goals and in determining future staffing requirements, equipment needs, physical building improvements, and other capital expense requirements for the administration of the laboratory's total budget of approximately $62 million;
- Serve as an advisor to the Director, members of the Police Laboratory, and other executive level personnel in the Forensic Investigations Unit and make recommendations regarding the formulation and implementation of programs, policies, and procedures directly affecting the daily operation of the Police Laboratory;
- Oversee major cases submitted to the laboratory for examination, meet with Section Assistant Directors/ Commanding Officers, Section Supervisors and Criminalists/ Examiners to monitor progress and report the status of such cases to the Director.
- Facilitate communication between key laboratory staff and other governmental agencies to ensure the efficient, relevant, and competent examination of physical evidence.

Requirements to Qualify
A baccalaureate degree from an accredited college with specialization in criminalistics, forensic science, chemistry, biology, physics, biochemistry, molecular biology, or a closely related scientific or engineering field, and five (5) years of full-time professional experience working in the area of criminalistics, forensic science, or analytical chemistry, eighteen (18) months of which must have been in a managerial, supervisory, or administrative capacity performing significant managerial or supervisory duties in a forensic laboratory with a substantial case load and a staff of proficiency tested examiners/analysts. Education beyond a baccalaureate degree in one of the specialization areas listed above may be substituted at the rate of two (2) years' experience for a Master's Degree and an additional year for a doctoral degree. However, all candidates must have the eighteen (18) months of managerial, supervisory, or administrative experience as detailed above.
NYC Residency is NOT REQUIRED.

Email resume and cover letter to Yonette.grahamdecaul@nypd.org

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San Diego Police Department
Multiple Criminalist I/II positions, likely in the Forensic Biology/DNA Unit

Criminalist I positions perform chemical, biological and physical analyses on narcotics, firearms, organic material and other substances involved in police investigations; prepare evidence for presentation in court; testify as expert witnesses; and perform other duties as assigned.

Link for Criminalist I position: https://www.governmentjobs.com/careers/sandiego/jobs/3846296/criminalist-i-t10930-202301

Criminalist II positions perform complex chemical, biological and physical analyses on narcotics, firearms, organic material and other evidence involved in police investigations; conduct crime scene reconstructions; prepare evidence for presentation in court; testify as an expert witness; and perform other duties as assigned.

Link for Criminalist II position: https://www.governmentjobs.com/careers/sandiego/jobs/3847057/criminalist-ii-t10931-202301