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Coupling Raman Spectroscopy with Ambient Sampling, Portable Mass Spectrometry for On-site, High-Throughput Evidence Confirmation on a Single Instrumental Platform

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Final Research Report

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Project Summary

Major Goals and Objectives

Forensic evidentiary backlogs[1] are indicative of the growing need for cost-effective, high-throughput chemical identification methods, and next generation approaches are required to tackle both the complexity and sheer magnitude of samples. The primary goal of this integrative project was to develop a portable resource for collecting prosecutorial evidentiary data from illicit drugs by leveraging two instrumental techniques that are widely utilized in the forensic sciences, Raman spectroscopy and mass spectrometry (MS). Both Raman and MS are regarded for their discriminating power, denoted by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)[2] as Category A techniques. While portable instrumentation is available for both of these techniques, enabling rapid screening of evidence in the field setting to assist in investigative decision-making, neither individually fulfills the two-tiered identification guidelines recommended by SWGDRUG for generating prosecutorial data. However, development of a portable instrument that integrates two independent, validated techniques would enable field-based, yet court admissible, evidence identification, potentially circumventing the need for off-site laboratory processing and significantly reducing the influx of casework into publicly-funded forensic labs.

Through National Institute of Justice funding (NIJ Grant No. 2017-R2-CX-0022), an interdisciplinary team of researchers developed rapid evidence identification methods by integrating Raman spectroscopy with a commercially-available, portable mass spectrometer capable of paper spray ionization-mass spectrometry (PSI-MS) analysis.[3-4] Proof-of-principle investigations conducted on a prototypical, fieldable testbed suggest that this integrated methodology could be simplistic in operation and robust to the needs of today's forensic and law enforcement practitioners, yet capable of yielding prosecutorial chemical information from a single examination of drug evidence in question. To extend the applicability of Raman spectroscopy to trace evidence, modification of paper substrates with novel metallic nanoparticles was examined to enable trace detection via surface-enhanced Raman scattering (SERS) processes[5] while minimizing interferences with PSI-MS spectral interpretation. This approach leveraged unique physical

properties of novel nanoparticles for forensic applications, aligning with the U.S. National Nanotechnology Initiative to harness nanoscale science in solving important societal problems.

Research Questions

Principle research questions that were addressed in order to establish proof-of-principle, performance, and impact of the proposed integrated mass spectrometric and Raman spectroscopic analysis (referred to as SERS-PSI-MS herein) included:

(i) can a nanoparticle-modified paper substrate enable trace detection via both SERS and PSI-MS?

(ii) is the analytical performance of this technology on par with currently-established methods for forensic evidence processing?

(iii) can the proposed technology be used in the field to collect court-admissible data to circumvent traditional laboratory processing?

(iv) can the proposed technology be adapted for routine and reliable usage by non-technical operators?

Research Design, Methods, and Analytical Techniques

Screening of contraband, particularly routine drug evidence, on-site has the potential to streamline the front end of the forensic science process by rapidly assessing the probative value of chemical evidence directly at the crime scene or policing activity, requiring only pertinent samples to be sent to off-site laboratories for confirmation, if need arises. Field screening could assist in determining whether a criminal investigation is needed and provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial. However, initial approaches designed for field processing of chemical evidence do not meet the standards for prosecutorial information, still necessitating the role of the traditional forensic laboratory for court-admissible confirmation. Next generation approaches that not only maintain portability, simplicity, and flexibility of existing methodologies, but also integrate multiple independent analyses to validate positive samples (i.e. SERS-PSI-MS) could have a multifaceted positive impact on the criminal justice system.

To effectively address these broad research directions required to establish proof-of-principle of SERS-PSI-MS, an interdisciplinary approach was taken, breaking down objectives into three main streams: *development and optimization of nanoparticle-modified paper substrates*, *design and prototyping of an integrated SERS-PSI-MS source*, and *assessment of analytical performance towards illicit drug analytes.* A variety of methodologies were employed to conduct the research in question, including instrumental techniques utilizing MS and Raman spectroscopy, materials chemistry, and analytical validation, topically discussed below.

Portable MS System and Paper Spray Ionization – Mass Spectrometry (PSI-MS)

The portable MS system employed for this research was the FLIR Systems AI-MS 1.2 cylindrical ion trap mass spectrometer (Figure 1), which offers both ruggedness towards field conditions and tandem MS analysis for increased selectivity of chemical identification. Through past NIJ funding (NIJ Grant No. 2011-DN-BX-K552, end date: 12/31/2014),[6] this system was shown to be applicable to a myriad of forensic chemicals and evidence types, with particular proficiency in illicit drug identification.[7-8] Further, the AI-MS 1.2 allows for "red light/green light" operation, alleviating the need for user-based data analysis by employing automated chemical identification based upon an on-board spectral database.[4]

This system can also be coupled with ambient ionization methods, which in turn allows the analysis of forensic evidence in its native state, with little to no preparation necessary; alleviating sample preparation increases both the sample throughput and simplicity of operation by non-technical operators. Past grantwork showed that paper spray ionization (PSI) had especially high proficiency for common and emerging drug evidence types (NIJ Award No. NIJ 2015-IJ-CX-K011, end date: 12/31/2017),[3,9] allowing the quick screening of both bulk illicit substances and trace residues via surface transfer swabbing. Shown in Figure 2, PSI utilizes a paper substrate as both the sampling apparatus and the disposable ionization source. Simple application of solvent and high voltage allows the analysis of surface-bound analytes.

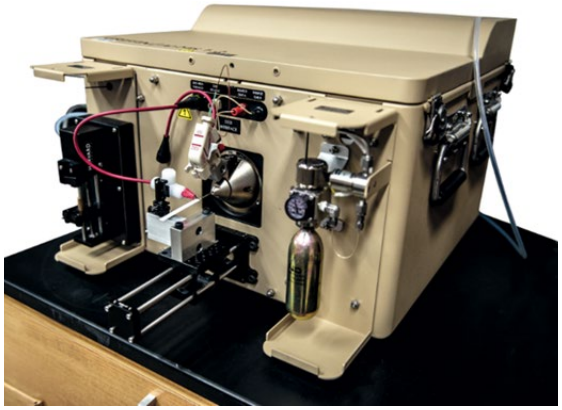

Figure 1. FLIR Systems AI-MS 1.2 portable, ambient sampling mass spectrometer (MS). This simplified, ruggedized instrument allows users to perform mass analysis and automated chemical detection directly from surface swabs in the form of paper spray ionization (PSI).

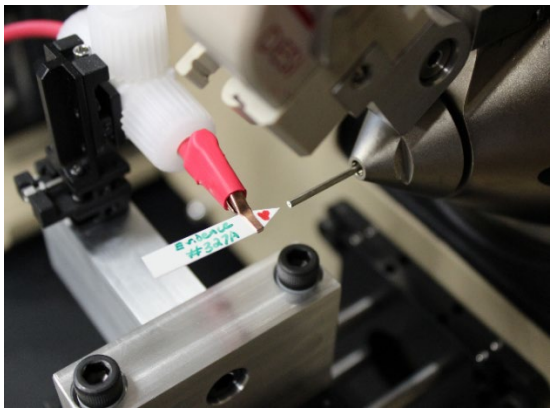

Figure 2. Simplified paper spray ionization (PSI) source implemented on the AI-MS 1.2 portable MS system. Chemical analysis is performed directly from a triangular paper swab after addition of solvent and voltage. Said swab can be used to dip-test forensic evidence or implemented as a surface swab.

Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS)

Raman spectroscopy, a non-destructive, laser-based methodology, provides molecular information about the chemical makeup of a sample, and the subtle changes in the frequency of a particular functional group's vibration (e.g. group frequency) can provide additional details of chemical structure, local environment surrounding the bond, bond angle, length, geometry, and conformation - these attributes have led to the development of chemical identification approaches based on these Raman spectral fingerprints that require little to no sample pretreatment. Of importance to this project, numerous low-cost portable Raman spectrometers are commercially available, and have been demonstrated for efficient screening of contraband in the bulk phase (e.g. counterfeit pharmaceuticals,[10-12] powder-based illicit drugs,[13-15] etc.).

Methods to enhance the Raman signal such that trace level detection is possible are critical to realize the full potential for chemical identification of illicit drugs, and surface-enhanced Raman spectroscopy (SERS) is one approach poised to achieve that goal. SERS is a technique in which the Raman signal of a sample is significantly amplified via adsorption onto a nanoscale metal particle possessing a strong surface plasmon resonance; [5,16] SERS offers the benefits of traditional Raman, namely, chemical and structural specificity, while providing a markedly improved sensitivity.[17] Currently, a concerted effort within the SERS community is to move toward paper-based SERS substrates.[18,19] Paper-based materials offer the

potential for reproducible mass production of SERS substrates without the need for expensive fabrication equipment, and of importance to these efforts, offers the capability of integration of PSI-MS by developing dual use substrates. Figure 3 depicts filter paper substrates modified with gold nanoparticles (AuNPs) of both spherical and anisotropic shape, producing considerable enhancements to the analyte-specific Raman fingerprint.

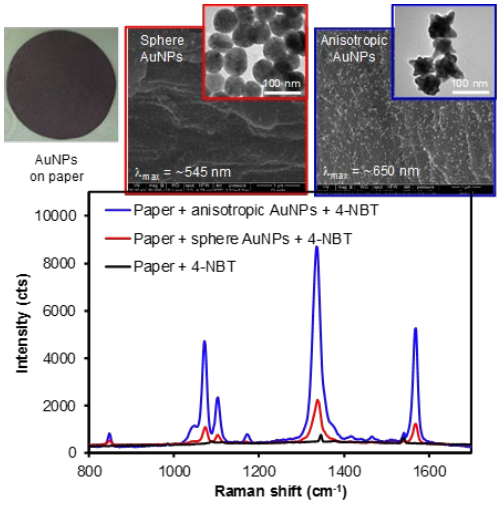

Figure 3. Digital photo and SEM images of gold nanoparticles of diverse shape loaded onto paper substrates. SERS spectra (below) show considerable enhancement for a test compound (4-NBT).

Analytical Classification, Court Admissibility, and Method Validation

Gatekeepers like SWGDRUG[2] seek to provide minimum standards to which forensic practitioners should adhere in order to reduce or remove uncertainty in evidentiary identifications, as well as general classification of instrumental methods in terms of their discriminating power. Both MS and Raman are regarded as "Category A" techniques for forensic analysis, which is only conferred to the most discerning methods. Public labs are expected to utilize multiple techniques to ensure accurate identification of evidence, ideally involving at least one of Category A status. This, in turn, yields prosecutorial chemical information that meets applicable admissibility standards in the context of the Daubert standard.[20,21] Significant effort went into establishing the analytical performance of the developed SERS-PSI-MS methodology towards identification of common and emerging drugs, employing validation categories such as method robustness, limit of detection (LOD), reliability in terms of false positive/false negative rates for statistically-relevant sample populations, and inter/intra-day reproducibility of spectral signatures.

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Expected Applicability of the Research

Considering the broad applicability previously demonstrated for PSI-MS through prior NIJ grantwork (NIJ Award No. NIJ 2015-IJ-CX-K011, PI Mulligan and co-workers)[6] and the current employment of portable Raman devices for presumptive testing of contraband,[13,22] implementing a portable SERS-PSI-MS technology could modernize policing of drug-related crime and traffic violations. Initial data collected through these efforts suggest that this integrated technique has the potential to accommodate chemical mixtures (e.g. street drugs, cutting agents, adulterants, etc., afforded by PSI-MS and MS/MS fragmentation pattern recognition) and speciation of select isomeric drug classes (afforded by the functional specificity of SERS fingerprints). If field collected data does indeed prove to be prosecutorial, processing of collected evidence on-site would lead to a subsequent expedition of criminal investigations, casework, judicial proceedings, and potential plea bargaining. The portable nature of the instrumentation could also find use as a rotating resource for rural communities or correctional facilities in need of periodic drug evidence testing, and the simplified nature of the SERS-PSI-MS method has a strong chance of maturing into non-technical operation; this, in turn, could initiate a paradigm shift in forensic science by potentially merging the law enforcement and forensic analyst roles.

Participants and Other Collaborating Organizations

Research work through NIJ Grant No. 2017-R2-CX-0022 was conducted solely by project personnel at Illinois State University in the Departments of Chemistry (CHE) and Management and Quantitative Methods (MQM). Dr. Christopher C. Mulligan (CHE) served as the lead PI for the research team, primary advisor for all MS-based research, and technical contact to the NIJ. Dr. Jeremy D. Driskell (co-PI, CHE) served as the primary advisor for all SERS/Raman-based research and assisted in characterizing nanoparticle-modified paper candidates. Dr. Jun-Hyun Kim (co-PI, CHE) served as the primary advisor for all research involving the design and characterization of SERS-active nanoparticles, as well as the production of nanoparticle paper substrates for analytical validation efforts. Dr. Jamie R. Wieland (co-PI, MQM) led all design of experiments (DOE) efforts for assessing reliability of developed techniques via

error rate determination and oversaw statistical protocols. The broader research team was comprised of 8 undergraduate/graduate student researchers from ISU CHE and Dr. Jean Standard (CHE), who assisted with density functional theory (DFT) calculations for target drug analytes.

Changes in Approach from Original Design

No major problems were encountered while conducting the proposed workplan for NIJ Grant No. 2017-R2-CX-0022, necessitating no appreciable changes over the life of the grant. Project personnel did observe chemical noise present in either Raman spectroscopic data or PSI mass spectra in some iterations of nanoparticle-paper combinations, and this was considered in selecting the most viable nanoparticle-modified paper substrate for the combined SERS-PSI-MS method that was demonstrated and validated through this work. This was shown to be a navigable issue and is not thought to have any long-term effect on the sufficiency of SERS-PSI-MS when implemented in field environments.

Outcomes

Activities and Accomplishments

Interdisciplinary research objectives were broken into three main streams: *development and optimization of nanoparticle-modified paper substrates, design and prototyping of an integrated SERS-PSI-MS source,* and *assessment of analytical performance towards illicit drug analytes.* Within these streams, the following accomplishments were realized:

- Fabrication of an optimized nanoparticle-modified paper substrate for integrated SERS-PSI-MS analysis
- Demonstration of two-dimensional confirmatory analysis with a prototype SERS-PSI-MS source
- Assessment of analytical performance of SERS-PSI-MS toward illicit drug confirmation
- Delivery of project-related deliverables and technical reports to the NIJ

Results and Findings

Development and Optimization of Nanoparticle-Modified Paper Substrates

Several varieties of SERS-active nanoparticles (NPs) of 50-60 nm in diameter were designed, synthesized, and characterized after deposition onto PSI-compatible paper substrates (e.g. MQuant blank testing strips (Millipore-Sigma), Whatman filter paper, etc.). Figure 4 shows representative scanning electron microscopy (SEM) images of silver (Ag), gold (Au), Ag-Au core-shell, and anisotropic Au nanoparticles, with their corresponding SERS signal enhancements observed in Figure 5. Anisotropic NPs are marked by having irregular shapes, allowing control of the accessible surface area and optical properties. Among the candidate substrates, anisotropic AuNPs on filter paper exhibited the highest responses, which were typically 2-4 times greater than other candidates due to the increased surface area and the broad surface plasmon resonance partially covering the Raman laser excitation wavelength. Novel paper loading strategies were also investigated, including dip-coating, spray-coating, and sputtered metal topcoating, as well as a "sandwiched" AuNP layering method that was recently published.[23]

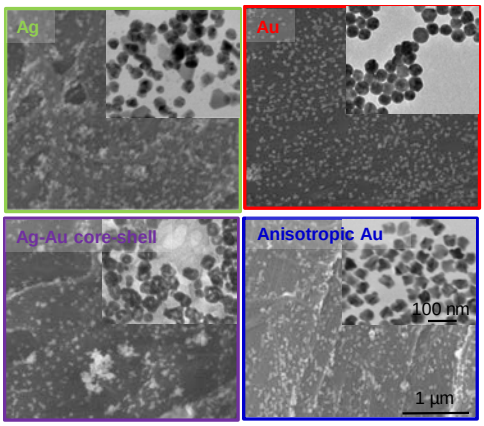

Figure 4. Four types of SERS-active nanoparticles synthesized to date, including spherical silver (Ag), spherical gold (Au), mixed metals (Ag-Au core shell), and anisotropic Au structures.

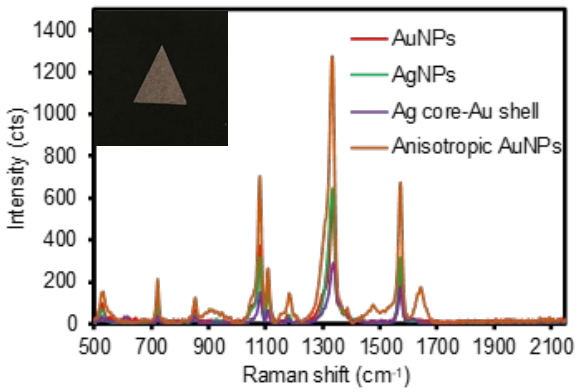

Figure 5. Representative SERS spectra with relative enhancement for differing nanoparticle-modified paper substrates. Example SER-PSI-MS triangular substrate utilized for dual investigation inset.

In order to identify the optimal paper substrate for dual purpose testing, all aspects of the SERS-PSI-MS method were considered, including analyte signal enhancements, presence of interfering signatures from chemical noise, robustness to the PSI-MS spray mechanism, robustness to surface transfer swabbing, and overall chemical hygiene of the MS inlet system. When incorporating all these aspects, project PIs

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identified *Grade 1 Whatman filter papers modified with spherical AuNPs* as the target substrate for analytical validation efforts. Explicit detail regarding the synthetic protocols utilized were included as an end-of-project deliverable.

Design and Prototyping of an Integrated SERS-PSI-MS Source

A prototypical mounting system that allows both simultaneous and sequential SERS-PSI-MS scanning modes was completed and tested on the FLIR Systems AI-MS 1.2 portable mass spectrometer. Utilizing commercially-available parts and polypropylene 3D printing, a cost-effective source was crafted to offer easy optimization of substrate positioning and quick transition between SERS and PSI-MS analyses. 3D-printed cartridges with embedded copper-based electrodes (seen in Figure 6A) were fabricated to hold the triangularly-shaped, AuNP-modified paper substrates for both analysis events.

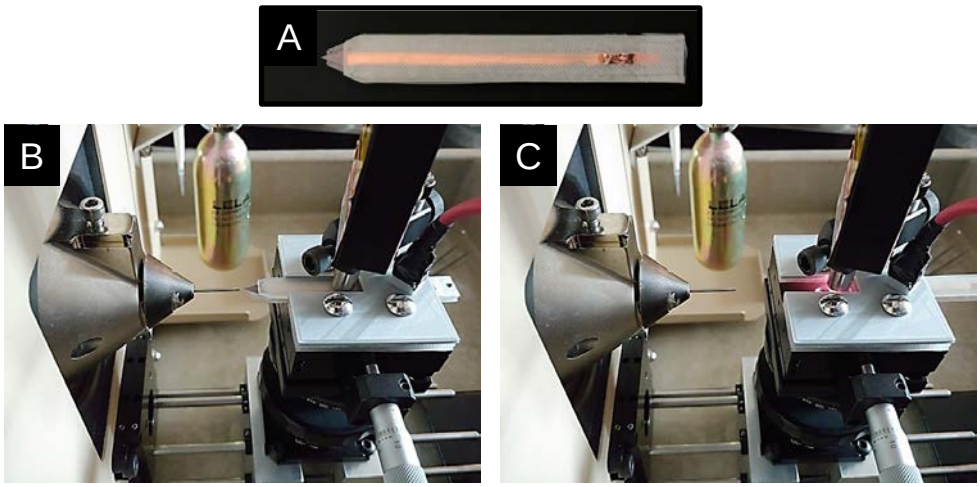

Figure 6. (A) Prototype, 3D-printed sample cartridge for housing AuNP-modified paper substrates for SERS-PSI-MS. (B) Optimized SERS-PSI-MS mount, supplemented with 3D-printed components. The sample cartridge is slid forward (relative to the MS inlet), initiating electrical contact and – upon addition of solvent – starts the PSI investigation (C) The sample cartridge is slid back for collection of SERS spectra via the Raman laser probe.

This sample cartridge slides into the central channel of the sample stage to facilitate accurate positioning in the x-y-z translational planes. This platform allows 2-point positioning of the paper relative to the MS inlet and the Raman probe. These two discrete positions were designed to optimize both sample introduction into the MS and laser focusing, thereby maximizing the analytical signals. The cartridge is completely slid into the mount to position the paper at the MS inlet for PSI-MS analysis (Figure 6B) and

slid out of the mount to position the paper below the focused laser for SERS analysis (Figure 6C). The fine adjustment afforded by the constructed SERS-PSI-MS prototypical source allowed method robustness to be assessed, including the examination of SERS probe and PSI-MS source positioning, efficacy of simultaneous vs. sequential SERS-PSI-MS scan modes, and spectral quality effects as a function of laser wavelength and power. Of note, it was observed across several illicit drug analytes that SERS spectral quality was largely improved when collected after PSI-MS was conducted during a sequential scan mode.

Assessment of Analytical Performance towards Illicit Drug Analytes

The validation plan implemented for SERS-PSI-MS was constructed to include performance characteristics delineated in recent SWGDRUG recommendations[2] for seized drug analysis methods. Specific categories incorporated were *method robustness* of evidence screening on the FLIR AI-MS 1.2 (discussed previously), *selectivity* of analyte identification, *detection limit* for trace residues, *accuracy/precision* of chemical detection (*i.e.*, reliability via error rate assessment, intra/inter-day variability), and *sample throughput.*

Selectivity of analyte identification was assessed by the examination of common and emerging drugs, with particular interest toward discrete examples of structural isomers amongst novel psychoactive substance (NPS) classes. SERS-PSI-MS was also shown capable of collecting characteristic spectra from authentic drug seizures (e.g. crystal methamphetamine) examined through interactions with local police practitioners. Data collected during these efforts were crafted into a deliverable spectral database of PSI-MS, MS/MS and SERS spectra. Limits of detection (LODs) for target drugs via SERS-PSI-MS utilizing spherical AuNP-modified paper substrates were determined, implementing both direct deposition of drugs, as well as surface swabbing protocols. Generally, LODs collected for the target drugs were in the low to mid-nanogram range, even from surfaces of interest to forensic investigations. To assess reliability in terms of false positive/false negative error rates for drug analyte confirmation via field-portable SERS-PSI-MS, a comprehensive, blinded experiment was designed and initiated. As part of the study, 500 user-blinded samples were generated and analyzed. Samples included randomly-assigned, blinded, positive drug controls and blinded negative controls. All drug standards were present at a deposited mass less than 200 ng to

represent trace residues. Samples were assigned in groups over 25 daily analysis sessions to also allow determination of inter/intra-day variability, sample throughput, and user proficiency as experience was gained. Of note, no false positives and a marginal false negative rate of 0.17% were observed during the study. Sample throughput assessments (including both the SERS-PSI-MS examination of a sample, but also a subsequent analysis of a blank sample to ensure instrument hygiene) averaged to 6.84 min/sample over novice user training phases, but decreased to 4.87 min/sample as more and more experience and comfort with the technology was gained.

While recent advances in the fields of ambient MS[3] and portable Raman instrumentation have intrinsic value in combatting the growing forensic evidentiary backlog, this work represents the first multi-category analytical validation of a field-ready system capable of the proposed SERS-PSI-MS two-tiered drug identification method. Validation categories examined, particularly error rate and reproducibility, will assist in meeting the demands of the Daubert[20] and Frye[24] standard for future admissibility of field-collected Raman and MS data.

Limitations

As this project encompasses proof-of-principle studies and utilizes prototypical source designs created by project PIs at Illinois State University, the data, methods, validation and deliverables serve to establish the potential and practicality of the SERS-PSI-MS in generating prosecutorial evidentiary data in the field setting. Currently, the methodology is not commercialized, which is a critical step for any broad implementation efforts. It is probable, however, that a commercial solution could be engineered, given that fieldable MS and Raman systems are currently on the market. Similar constraints apply to the novel substrates utilized for the work, which were developed and manufactured in-house for the reported experiments – purchasable, ready-to-use consumables will be key for practical usage by law enforcement. Once commercial solutions are in place, stringent validation efforts utilizing authentic controlled substances comprised of street-cut compositions (e.g. cutting agents, adulterants, etc.) and isomeric drugs of current concern will be prudent, as well as pilot testing programs with forensic and law enforcement agencies before broad implementation.

Artifacts

List of Products Stemming from Grant Funding

Publications – 3 Total

(3) Burr, D.; Fatigante, W. L.; Lartey, J. A.; Stelmack, A. R.; Standard, J.; Wieland, J. R.; Kim, J.-H.; Mulligan, C. C.; Driskell, J. D. Integrating SERS and PSI-MS with Dual Purpose Plasmonic Paper Substrates for On-Site Illicit Drug Confirmation. *Anal. Chem.* **2019**, *manuscript in preparation.*

(2) Fatigante, W. L.; Mukta, S.; Lawton, Z. E.; Bruno, A. M.; Traub, A.; Gasa, A. J.; Stelmack, A. R.; Wilson-Frank, C. R.; Mulligan, C. C. Filter Cone Spray Ionization Coupled to a Portable MS System: Application to On-Site Forensic Evidence and Environmental Sample Analysis. *J. Am. Soc. Mass Spectrom.*, **2019**, *accepted for publication (Manuscript No. js-2019-00098w).*

(1) Lartey, J. A.; Harms, J. P.; Frimpong, R.; Mulligan, C. C.; Driskell, J. D.; Kim, J.-H. Sandwiching Analytes with Structurally Diverse Plasmonic Nanoparticles on Paper Substrates for Surface Enhanced Raman Spectroscopy. *RSC Adv.* **2019**, *9*, 32535-32543. [Open Access]

Presentations and Conference Proceedings – 19 Total

(19) Driskell, J. D.; Kim, J.-H.; Mulligan, C. C.; Wieland, J. R.; Burr, D. S.; Fatigante, W.; Lartey, J. Portable SERS-PSI-MS Dual Analysis Platform Using Gold Nanoparticle-Embedded Paper for Trace Detection of Illegal Drugs. *Invited Address at the "NIJ Forensic Science Research and Development" Symposium at the 72nd Annual Scientific Meeting of the American Academy of Forensic Sciences (AAFS), Anaheim, CA*, **2020**.

(18) Mulligan, C. C.; Driskell, J. D.; Kim, J.-H.; Wieland, J. R. Fatigante, W.; Burr, D. S.; Lartey, J. Developing a Fieldable SERS-PSI-MS Platform for On-Site, High Throughput Drug Evidence Confirmation. *Invited Address at the "NIJ – Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology" Symposium the 71th Pittsburgh Conference on Analytical Chemistry NIJ Poster Session, Chicago, IL,* **2020.**

(17) Lartey, J. A.; Harms, J.; Frimpong, R.; Mulligan, C. C.; Driskell, J. D.; Kim, J.-H. Sandwiching Target Analytes with Structurally Diverse Plasmonic Nanoparticles for Surface Enhanced Raman Spectroscopy Sensing. *Presentation at the "NIJ Forensic Science Symposium" Poster Session at the 71th Pittsburgh Conference on Analytical Chemistry, Chicago, IL,* **2020.**

(16) Driskell, J. D.; Mulligan, C. C.; Kim, J.-H.; Fatigante, W.; Burr, D. S.; Lartey, J. Integrated SERS-PSI-MS Platform Using Gold Nanoparticle-embedded Paper for Trace Detection of Illegal Drugs. *SciX 2019 – The Great Scientific Exchange for the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS), Palm Springs, CA,* **2019.**

(15) Mulligan, C. C.; Fatigante, W.; Stelmack, A. R.; Burr, D. S.; Harms, J. P.; Kim, J.-H.; Driskell, J. D.; Wieland, J. R. Towards On-Site Drug Evidence Confirmation Using Ambient Sampling, Portable Mass Spectrometry. *Invited Address at the "Frontiers in Forensic Mass Spectrometry" Symposium at the 257th American Chemical Society (ACS) National Meeting, Orlando, FL,* **2019.**

(14) Mulligan, C. C.; Driskell, J. D.; Kim, J.-H.; Wieland, J. R. Coupling Raman Spectroscopy with Ambient Sampling, Portable Mass Spectrometry for On-Site, High-Throughput Evidence Confirmation. *Invited Address for the 2019 "Emerging Forensic Research" Webinar Series by the Forensic Technology Center for Excellence (FTCoE),* **2019. (LINK)**

(13) Fatigante, W.; Stelmack, A. R.; Burr, D. S.; Harms, J. P.; Driskell, J. D.; Kim, J.-H.; Wieland, J. R.; Mulligan, C. C. Towards On-Site Drug Evidence Confirmation via Surface-Enhanced Raman Spectroscopy and Paper Spray Ionization Employed on Portable Instrumentation. *Address at the "Forensics: Innovations and Applications" Session at the 67th ASMS Conference on Mass Spectrometry and Applied Topics, Atlanta, GA.* **2019**.

(12) McDaniel, T. J.; McClurg, N. W.; Fatigante, W.; Kim, J.-H.; Driskell, J. D.; Mulligan, C. C. The Performance of Nanoparticle-Modified Paper Substrates Employed as Surface Transfer Swabs for Combined SERS and PSI-MS Investigation. *67th ASMS Conference on Mass Spectrometry and Applied Topics, Atlanta, GA.* **2019**.

(11) Stelmack, A. R.; Fatigante, W. L.; Mukta, S.; Mulligan, C. C. Rapid Profiling of Authentic Forensic Evidence via Paper Cone Spray Ionization Employed on Portable MS Instrumentation. *67th ASMS Conference on Mass Spectrometry and Applied Topics, Atlanta, GA.* **2019**.

(10) Poehls, A. M.; Mukta, S.; Mulligan, C. C. Analysis of Cosmetic Products for Evidentiary Value via Paper Spray and Paper Cone Spray Ionization-Mass Spectrometry. *67th ASMS Conference on Mass Spectrometry and Applied Topics, Atlanta, GA.* **2019**.

(9) Eyimegwu, P. N.; Harms, J. P.; Burr, D. S.; Fatigante, W.; Mulligan, C. C.; Driskell, J. D.; Kim, J.-H. Sandwich Structure of Plasmonic Paper for Surface Enhanced Raman Spectroscopy. *257th American Chemical Society (ACS) National Meeting, Orlando, FL,* **2019**.

(8) Fatigante, W.; Mukta, S.; Stelmack, A. R.; Lawton, Z. E.; Wieland, J. R.; Gizzi, M. C.; Mulligan, C. C. Optimization of Plasmonic Paper for Two-Tiered Drug Analysis on a Portable SERS-PSI-MS Platform. *Invited Address in the "NIJ – Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology" Symposium at the 70th Pittsburgh Conference on Analytical Chemistry, Philadelphia, PA,* **2019.**

(7) Burr, D. S.; Fatigante, W.; Harms, J. P.; Kim, J.-H.; Driskell, J. D.; Mulligan, C. C. Optimization of Plasmonic Paper for Two-Tiered Drug Analysis on a Portable SERS-PSI-MS Platform. *Invited Poster Presentation at the 70th Pittsburgh Conference on Analytical Chemistry NIJ Poster Session, Philadelphia, PA,* **2019.**

(6) Mulligan, C. C.; Fatigante, W.; Mukta, S.; Stelmack, A. R.; Lawton, Z. E.; Evans-Nyugen, K. E. Characterizing a Portable MS System Featuring Interchangeable, Ambient Ionization Sources for Routine Forensic Evidence Screening. *Invited Address at the "Ambient Ionization and Forensic Science" Symposium at the 255th American Chemical Society (ACS) National Meeting, New Orleans, LA,* **2018**.

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Technologies or Techniques

Through project funding, PIs and project personnel have prototyped and demonstrated the use of a combined portable technology that integrates PSI-MS screening and non-destructive Raman/SERS analysis on a portable mass spectrometer (FLIR Systems AI-MS 1.2 system). The integrated technology targets the existing need for field-based, confirmatory analysis of drug evidence by law enforcement and forensic practitioners. Novel metallic nanoparticle synthetic techniques have been developed through this grantwork, as well as new strategies for deposition/loading of said nanoparticles onto paper-based substrates. Such efforts support the U.S. National Nanotechnology Initiative.

Data Sets Generated Through Grant Activities

Grant No. 2017-R2-CX-0022 allowed the development of novel, diverse data sets that were delivered to the NIJ, including: (i) an optimized protocol for developing gold nanoparticle-modified paper substrates for dual usage with PSI-MS and SERS, (ii) design considerations, parts lists, and robustness data of prototypical SERS-PSI-MS source, (iii) analytical performance data for the prototypical SERS-PSI-MS source (including detection limit, false positive/false negative rates, inter/intra-day variability, and sample throughput), and (iv) a spectral database containing representative PSI-MS, MS/MS, and SERS spectra for common and emerging drugs of abuse.

Dissemination Activities

Dissemination of project results for NIJ Grant No. 2017-R2-CX-0022 was accomplished via publications in leading chemistry journals and presentations at a myriad of national/international and regional scientific conferences, including meetings of the American Chemical Society (ACS), American Society for Mass Spectrometry (ASMS), the Pittsburgh Conference on Analytical Chemistry (PittCon), the Great Scientific Exchange (SciX) for the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS), and the Midwestern Association of Forensic Scientists (MAFS). PI Mulligan took part in the Spring 2019 Webinar Series entitled "Emerging Research: Toxicology and Drugs" through the Forensic Technology Center of Excellence (FTCoE), where he discussed aspects of this grant work.

Project personnel had the opportunity to disseminate project results and share capabilities of our proposed methods with local law enforcement practitioners and vice squad officers, as well as perform PSI-MS and Raman spectroscopic investigations on authentic drug evidentiary seizures to demonstrate proof-of-principle.

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