Field Forward Forensics: Triaging the Detection Challenge

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ABSTRACT

For the defense community trying to develop detection capabilities for military, security, and emergency response forces, the current and future strategic environment means that there are literally thousands of lethal materials that can be used as weapons. The sensing of chemical threats is important to obtain "real-time" answers that allow actionable decisions to be made on-the-spot; to reduce the logistical burden by moving the analysis closer to the source of the sample; to rapidly screen materials to identify samples that need to be sent to a lab for additional analysis and minimize the number of these samples; and to nondestructively analyze large, valuable, or immovable objects for which excising samples is not possible.

This work details a multi-team approach to triaging chemically contaminated surfaces through rapid orthogonal technologies to ascertain forensic level information, closer to the point of origin. Discussed will be the development of a portable Raman microscopy system allowing the non-invasive, non-destructive analysis of various types of surfaces and samplers for initial contamination determination. Samplers could consist of consumables already associated with other techniques, for example, mass spectrometry or ion-mobility swabs utilized at checkpoints, screening locations, and sensitive site exploitations. The swabs can be used in typical fashion to swipe a surface, but before being inserted into the mass spectrometry (MS) or ion mobility spectrometry (IMS), which are chemically destructive techniques, Raman microscopy can be performed to determine initial chemical contamination. Samplers could also consist of modified paper-based substrates which contain chemical coatings to enhance interaction of the potential threat. In this scenario, a surface-enhanced Raman spectroscopy (SERS) substrate coated with gold or silver nanoparticles, can be analyzed by a portable Raman system. The same substrate could then be analyzed by the portable Raman microscope for additional information, and finally analyzed by a portable mass spectrometer yielding three individual data points within a matter of minutes all on the same sample.

This approach enables rapid analysis of trace level contamination of surfaces and samples demonstrating multiple technologies, multiple personnel, coming together to detect the threat to increase situational awareness of a chemically contaminated environment.

1.0 BACKGROUND

The ability to detect trace quantities of hazardous materials, including explosives, narcotics, toxins, chemical and biological materials has become important in the wake of increased terrorist attacks around the world and the continued rise of drug trafficking, particularly in regard to prescription and illegal opioids [1]-[3]. In the field, the need to rapidly determine the potential hazardous materials on contaminated surfaces, is desirable, in order to provide rapid, actionable information to military commanders in support of counter-

terrorism operations as well as domestically to first responders and field analysis operators. Current techniques for detecting and conducting forensic analysis still rely heavily on a combination of factors including the need to transport samples over long distances to a field laboratory with requisite analytical equipment which can delay information. Furthermore, confirmation analysis techniques such as mass spectrometry and high performance liquid chromatography while ideal for true identification purposes are destructive in nature and will degrade the integrity of the sample. New techniques under development are exploring non-invasive, non-destructive methodologies to provide earlier presumptive information of a potential hazard which maintains the sample integrity for further confirmational analysis.

Historically, bulk level detection of solid and liquid hazardous threats has been accomplished through a variety of handheld optical and non-optical technologies such as Raman spectroscopy, infrared spectroscopy, and colorimetrics. [1]-[6] However, the challenge, as the concentration or mass of the threat on the contaminated surface becomes lower and lower, is not only detecting the threat, but locating the threat as well. Figure 1 captures this challenge space in how the bulk material eventually disappears from 10's of milligrams to visually undetectable nanogram levels. However, the threat is still there, and is revealed in this example with grazing-angle illumination technique highlighting the potential contamination from the residual fingerprint.

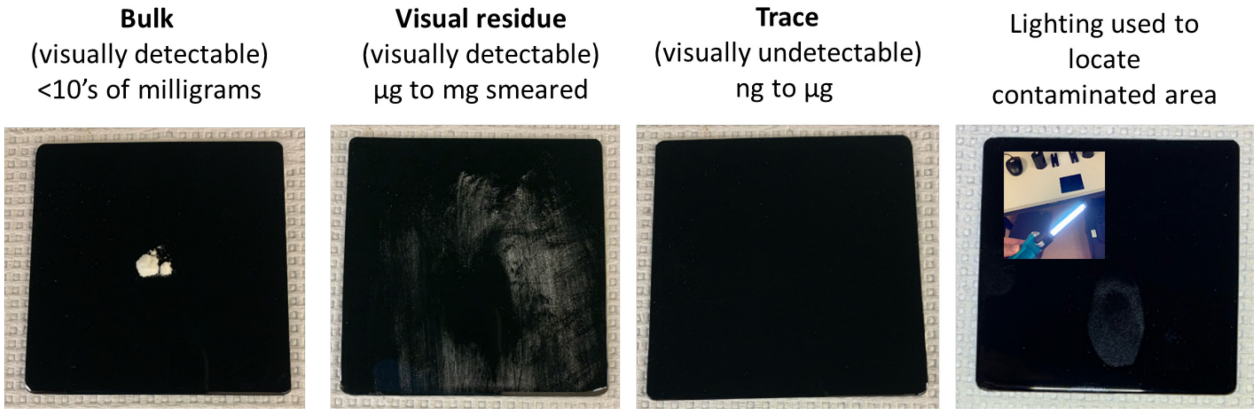

Figure 1: Bulk contamination versus trace level contamination on surfaces.

Under the U.S. Army Explosive Forensics program, research has been ongoing to develop and augment technologies to locate, detect, and identify trace amounts of hazardous materials on contaminated surfaces. One technological development is the Portable Microscopy Chemical Detection System (PMCDS) which was designed to enable the non-visible trace detection of threat material located on surfaces from fingerprints, palm strikes, or residual materials left over from bulk contamination [7]-[9]. This paper describes recent work utilizing the PMCDS in various operational spaces and combined with established methodologies to demonstrate the potential to transform a laboratory-based technique (Raman microscopy), to a portable device, ultimately leading to the development of next generation of expeditionary systems for military forensic analysis in the field.

2.0 PORTABLE RAMAN MICROSCOPY DEVELOPMENT

The exploration of Raman microscopy for forensics analysis has been pursued by DEVCOM CBC for over a decade. Initially proof of concept experiments were performed to demonstrate that Raman spectroscopy could non-invasively detect trace level of solid contaminants found in the minutiae of residual fingerprints [7]. Originally performed with a research grade Lab based-Raman Chemical Imaging (L-RCIM) system, the ChemImage Falcon II Raman Chemical Imaging system. We demonstrated that with the L-RCIM, micron

size trace level solid particles could be located and detected from chemically contaminated residual fingerprints in a complex field of view. Analysis times however were in the 100's of hours for complete analysis of a one-inch squared area (about the size of a fingerprint). Through additional research and algorithm development along with imaging advancements (not described in this paper), with the purpose of optically detecting trace particulates and with Raman microscopy, chemically characterizing the detected particulates, we were able to reduce the time to 10's of minutes without a loss of detection performance. Information learned during this initial phase of research enabled the prototyping of a first-generation Chemical Fingerprint Identification System (CFIS) in 2017. The system, produced by ChemImage Corporation greatly reduced the SWAP from 150 lbs to 80 lbs and decreased the software complexity of the research grade L-RCIM down to a simple user interface. However, performance characteristics limited the CFIS's functionality to analyze a variety of surfaces for trace level contamination detection. During the assessment period of the CFIS and utilizing the L-RCIM, further research was performed on the concept of portable microscopy by developing methodologies to image curved surfaces and increasing the robustness of the algorithms and speed of analysis which decreased detection times down to seconds. This work and research lead to development of the Portable Microscopy Chemical Detection System (PMCDS) delivered in 2020, built by Pendar Technologies. The PMCDS is considerably smaller in size and weight (~10 lbs.) than the CFIS, while including all of the primary characteristics of the original L-RCIM.

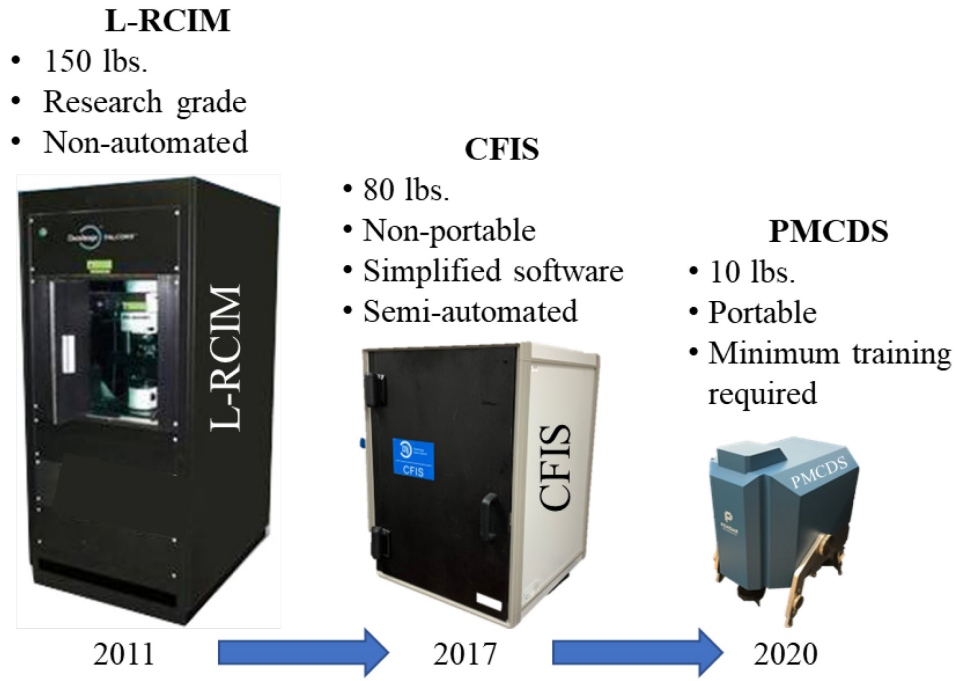

Figure 2: Portable microscopy system development cycle.

3.0 PMCDS MODES OF OPERATION

In order to develop next generation expeditionary systems for military forensic analysis in the field, technology has to be easy to use with minimal training while providing novel detection information to the user. The intent of the PMCDS is to enable a scan of an area of interest where residual solid chemical contamination may be present on a surface, locate particles of interest in that area, and report detection occurrences to the operator. Figure 3 pictorially describes the mode of operation of the current PMCDS. Initially a sample is placed under the instrument (in this example, a green cloth with potassium chlorate and RDX particles). The system acquires a bright field image of the area (Figure 3, C), and automatically locates

any particles of interest in the field of view (green dots, Figure 3, D) while simultaneously recording x, y, and z, coordinates. The particles of interest are categorized by the system by pixel intensity and numbered one through n (number of particles recorded in the field of view). The system then reads the x, y, z coordinates of the first particle, maneuvers the microscope objective to the coordinate (Figure 3, E), acquires a Raman spectrum, and reports the results (Figure 3, F) from a library matching algorithm to the user.

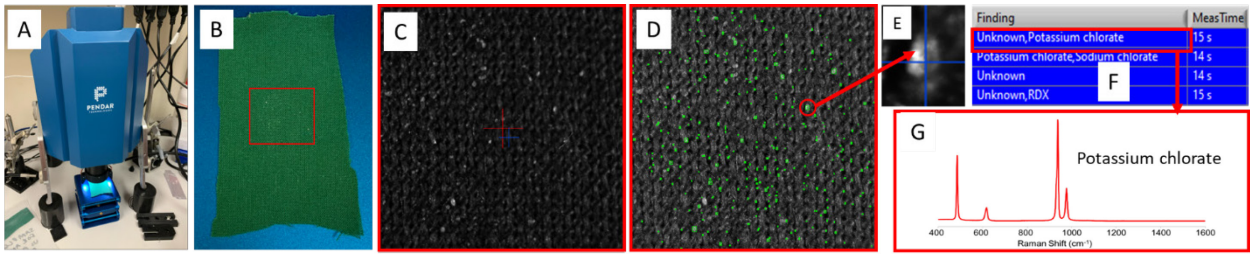

Figure 3: PMCDS operation example for PC/RDX on green cloth. A) PMCDS setup, B) green cloth with area of analysis highlighted, C) brightfield image, D) particles for analysis located and coordinates recorded, E) ~25-micron size particles, F) results indicating presence of potassium chlorate via onboard library matching, and G) resultant potassium chlorate Raman spectrum.

The initial steps of placing the sample underneath the system (or placing the system over the sample), performing a macro focus of the area as well as setting up particle parameters and time of analysis in the software still require user input. However, upon those operations, all other functions are autonomous, and the system will operate until either stopped by the user or the analysis of preset number of particles in the field of view are complete.

4.0 TRIAGING THE DETECTION CHALLENGE

Previous work demonstrated the PMCDS as a standalone detection device for various use cases of operation, such as entry control points, sensitive exploitation, and mobile lab operations [10]. In the case of the entry control point operation the PMCDS can be used to analyze spectrally benign surfaces (no background Raman signature) to detect the presence of trace chemical materials left from a intentionally placed thumbprints/fingerprints during a check point processing scenario. Operating with a known background that does not interfere takes full advantage of microscopy in that high quality individual detections of particles can be made even when two particles are located less than 50 microns apart (Figure 4, A). This demonstrated that complex samples or mixtures are not as problematic for Raman field microscopy as opposed to traditional handheld units that analyze larger amounts of materials (bulk) and must rely on demixing algorithms to target residual materials in the field of view. Additional testing was performed on more complex surfaces which may be more realistic of what would be encountered in the field in clandestine labs, or mobile laboratory operations. In these examples, illicit pills were analyzed for trace amounts of synthetic opioids (Figure 4, B), curved surfaces with residual fingerprints (Figure 4, C) and black car panels (Figure 4, D) mimicking trace contamination left on a car or metallic surface. In this standalone operation, continued testing and evaluation of the PMCDS system is ongoing to understand functionality against a wide variety of contaminated surfaces and ground samples.

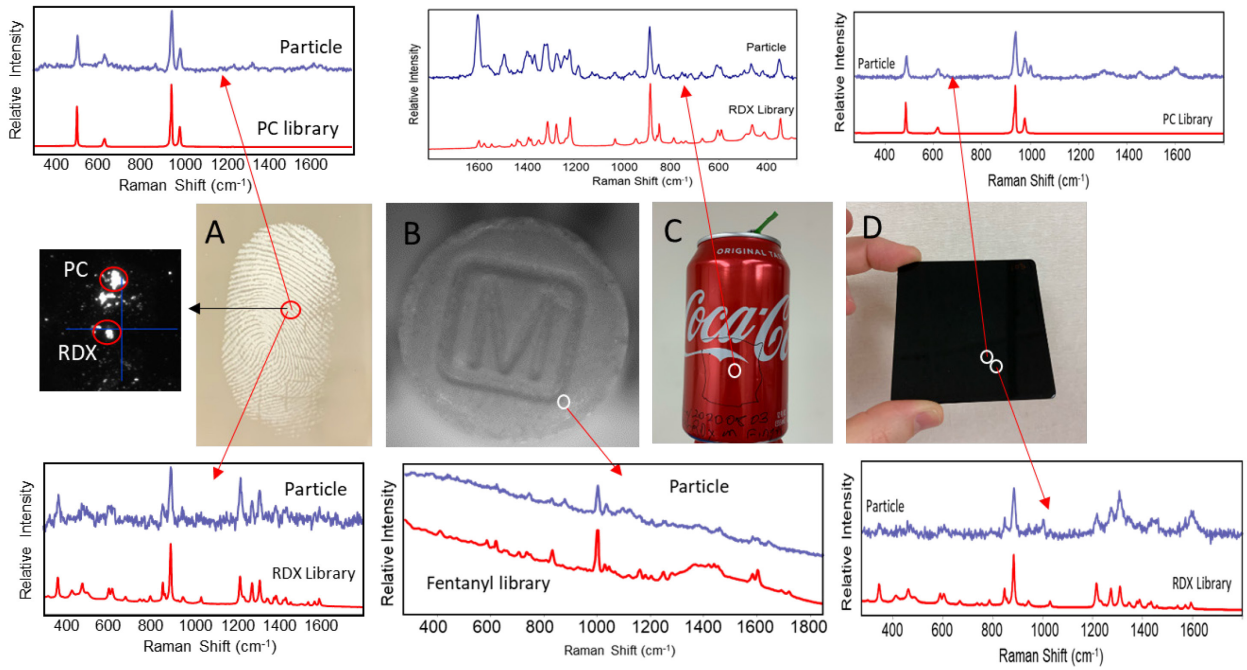

Figure 4: Examples of PMCDS detecting trace hazardous materials on A) deposited fingerprints, B) illicit pills, C) curved surfaces, and D) painted car panels.

The ultimate goal of our research, however, is twofold: First to push forensics technologies out of the lab and closer to the threat in the field thus minimizing the time between analysis and reporting the information to the operators and second, to preserve the analyzed sample for orthogonal technique confirmation (such as MS, IMS etc). In order to accomplish this, new technologies like the PMCDS need to be developed as well as potential reliance on established technologies that could be used sequentially. While the PMCDS can operate as standalone detection device a triaging concept is being developed where the system can be used to non-destructively and rapidly scan an unknown surface sample for presumptive chemical information while maintaining the integrity of the sample for further analysis by supplementary techniques. Figure 5 shows this concept decision tree. If an unknown sample is encountered, and there is bulk material present, then current handheld systems can be utilized to determine chemical information. If there is suspected material present, but visually undetectable or residue is probable, then the PMCDS can be utilized to determine presumptive chemical information and in the future potentially biological/toxin information. The sample can then be transported/transitioned to another higher fidelity technique for confirmation and identification (mass spectrometry, high performance liquid chromatography, and in the case of biological or toxin potential, proteomics or other identification methodology).

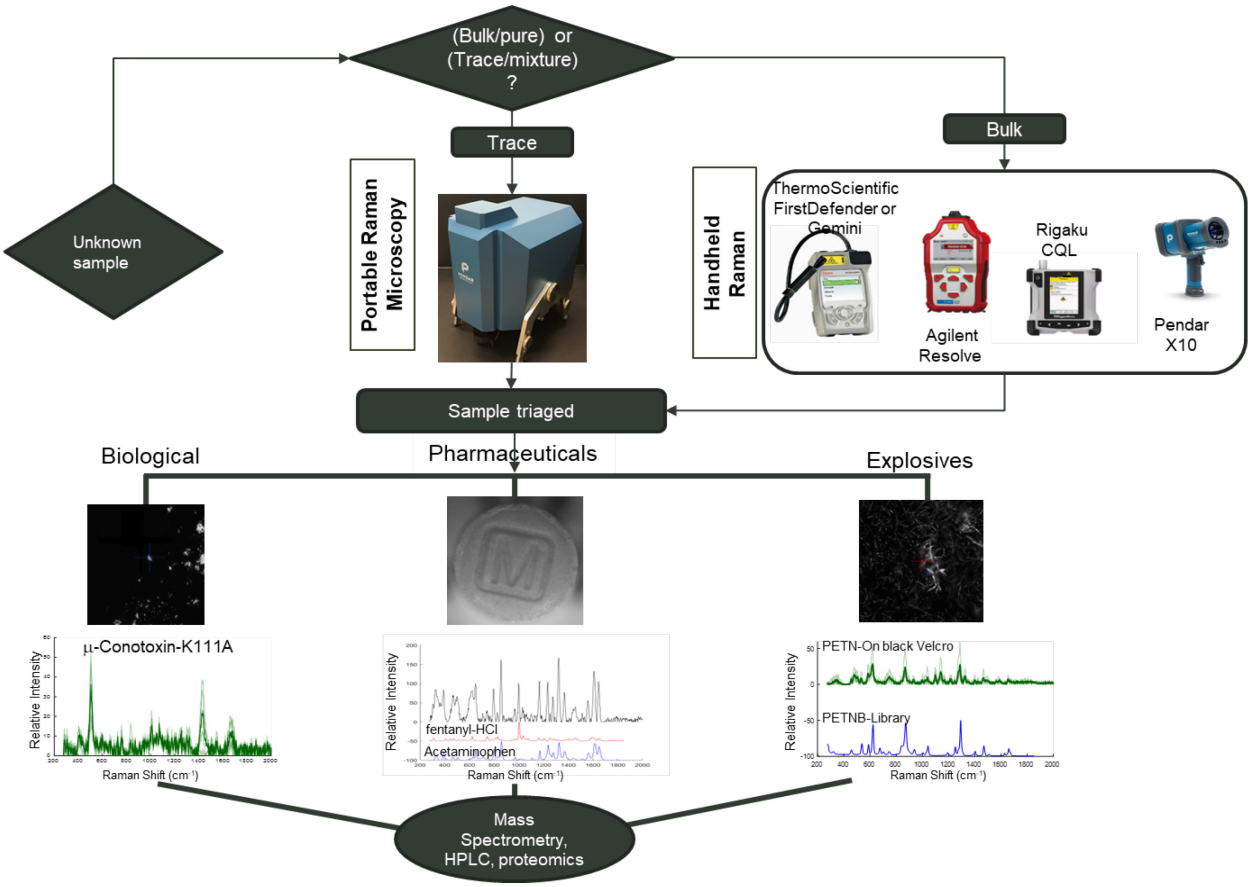

Figure 5: Triaging the detection challenge decision tree.

This concept has been explored recently by pairing the operation of the PMCDS with various established technologies currently in use in the field and laboratories to include MS and IMS techniques. IMS has traditionally been used in screening for trace explosives applications from airports for both personnel and luggage to mobile labs. However, IMS is a destructive technique in that the sample is essentially degraded upon analysis. We acquired a representative IMS swab and prepared a mixture of 50:50 w/w of potassium chlorate and RDX. A sample was loaded by depressing a finger into the mixture, then subsequently wiping off the finger to ensure no visual powder remained. The finger was then swabbed with the IMS sampler and analyzed with the PMCDS. Figure 5 shows the results of this analysis. The analysis time took approximately 5 minutes total to locate and detect over 100 particles in the field of view, however, the first reported detection of material (potassium chlorate) occurred within 60 seconds, followed by RDX seconds later. The non-invasive nature of the analysis would allow the IMS swab to be used sequentially in the appropriate IMS system. This was demonstrated on a secondary IMS swab (not pictured) contaminated with only RDX in which the PMCDS successfully detected the material followed by successful detection by the IMS device. Interesting to note, the PMCDS device potentially gives the user additional information that the IMS may not be able to detect. For the example included here, while RDX can be easily detected with IMS device, potassium chlorate needs to be heated to 400 C to decompose to a vapor mixture comprised of chlorine dioxide, chlorine gas and oxygen. Currently, IMS based fielded detection devices are not set to reach this temperature. Same constraints can be true for any low-vapor pressure oxidizer/energetic compounds.

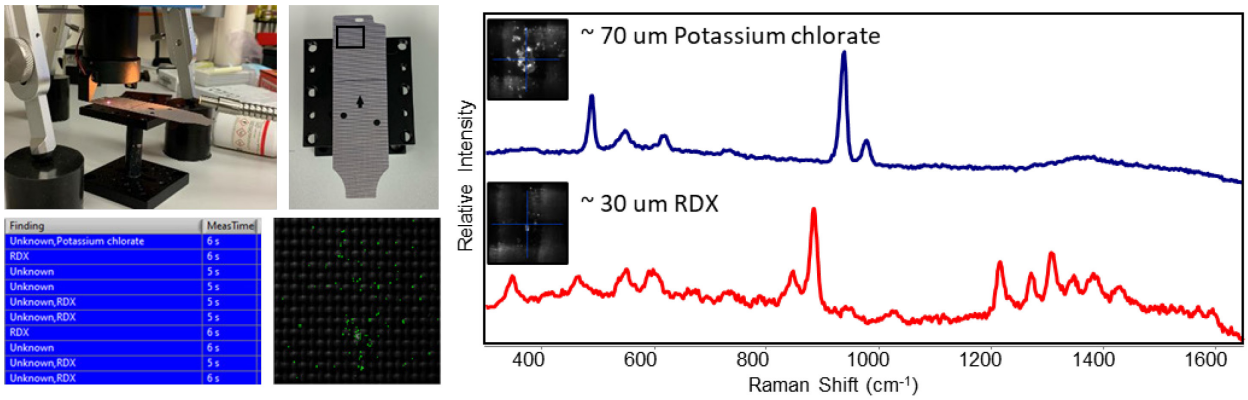

Figure 6: PMCDS non-destructively analyzing IMS swab.

A second example of sequential technologies being used can be seen in Figure 7 demonstrating the detection of 1% fentanyl in the presence of 99% acetaminophen. The mixture was prepared and swabbed with commercially available SERS substrate from Metrohm Raman (Ag-P-SERS). The substrate was initially analyzed by the PMCDS (upon wetting of the substrate), followed by analysis on a commercial handheld Raman spectrometer (Metrohm Raman MIRA DS), followed by analysis on a modified portable mass spectrometer prototype incorporating an atmospheric pressure chemical ionization (APCI), and finally a liquid-chromatography mass spectrometer (LC-MS) for confirmational analysis. The PMCDS successfully detected fentanyl followed by the handheld detection system giving an alarm to Methamphetamine/Fentanyl Compound. The sample was then inserted into the portable mass spec APCI adapter (Figure 7, C) and within a few seconds, alarmed to fentanyl. The first three analysis methods took approximately three minutes to complete. The novelty of the developing mass spectrometer system is the technique only interrogates (destroys) a very small spot on the substrate, leaving additional areas to be analyzed. The sample was then transported to a different laboratory where the sample was completely destroyed through solvent extraction for final LC-MS analysis which confirmed and determined that the sample contained fentanyl and acetaminophen. The operating principal for triaging the sample would include, uploading a user developed preset routine for detection with portable mass-spectrometer systems. For example, in the case of fentanyl and acetaminophen, MS/MS setting would include looking for fentanyl parent ion of m/z 337 in positive ionization mode. Conversely, if TNT is detected, MS/MS would be performed on m/z 227 in negative ionization mode. Thus, PMCDS can triage a sample for MS analysis.

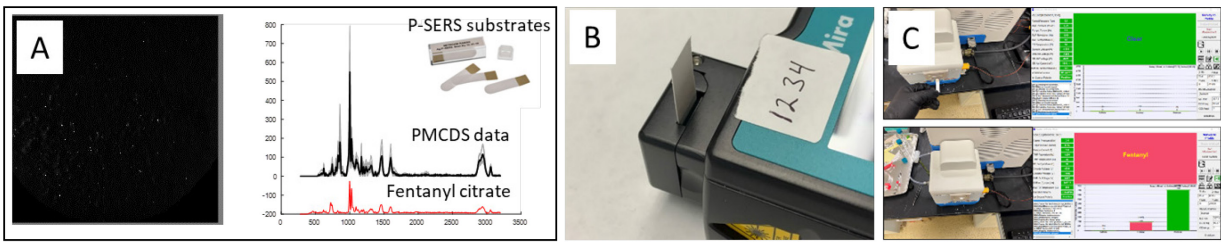

Figure 7: Sequential analysis of SERS substrate with PMCDS, Metrohm Raman MIRA DS, portable mass spectrometer and LC-MS.

The third example of threat category is bio-toxins. To determine whether the current version of PMCDS can be used to detect bio-toxins, we used an opportunity presented by a limited study of high purity toxin protein samples with multilaser-Raman microscopy. We were provided nine types of toxins. These samples were in solid lyophilized form (not exceeding 5 mg) or in solution form (not exceeding 1mg/ml and 2ml volume). Each of these samples were deposited on separate aluminium-coated microscopy slides. The

aluminium-coated slides laden with toxin samples were placed under the lab-based three-excitation laser (532, 633, and 785 nm) Raman chemical imaging microscope (WITec Apyron). These samples were also analyzed with PMCDS. Figure 8 shows Raman spectral data obtained from five different toxins and comparison of spectra between those obtained with the lab-based WITec Raman microscope with 785 nm excitation and PMCDS. There is a remarkable agreement between the spectral data obtained from the two instruments. This suggests that there is a possibility of detecting toxins with PMCDS. In a triage methodology, if presence of toxins is determined by PMCDS, the confirmatory analysis will be performed with a proteomics-based MS technique.

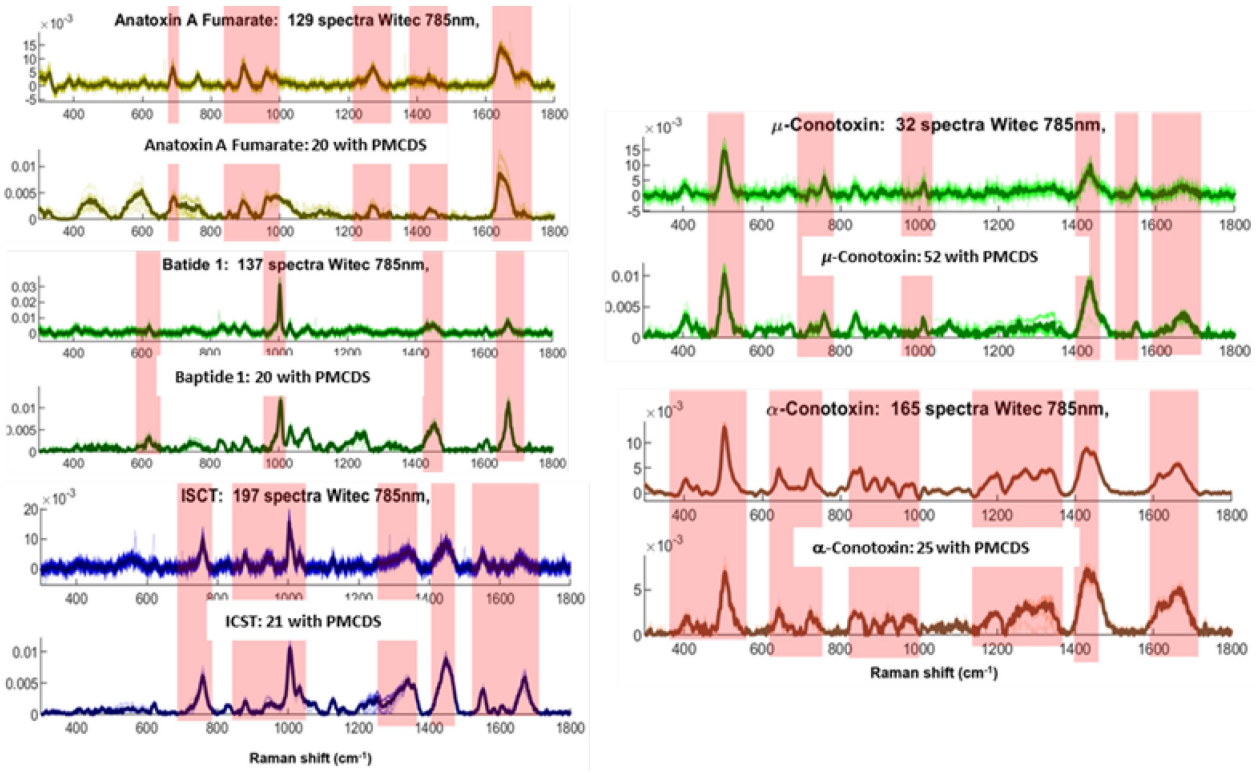

Figure 8: Raman spectral data of five different toxins obtained with lab-based Raman microscope and PMCDS.

5.0 CONCLUSION

The development of the PMCDS has demonstrated the potential ability to move traditionally lab-based techniques for trace level forensic chemical analysis more forward into the field. PMCDS can be used to triage the unknown sample non-destructively. A high-confidence initial determination of trace sample can be provided with PMCDS. The class of sample: narcotics, energetics and/or toxin, initially can be determined. This can be helpful in determining subsequent actionable steps. Furthermore, a Raman based presumptive identification of samples can help in determining the most appropriate route for confirmatory analysis. This could help determine MS analysis parameters to include, ionization modes, liquid chromatography vs gas chromatography analysis, etc.; thus, reducing the time-to-determination and sample throughput.

6.0 DISCLAIMER

The opinions, interpretations, conclusions, and recommendation are those of the authors and are not necessarily endorsed by the United States Government. Funding was provided by the U.S. Army DEVCOM Chemical Biological Center Explosive Forensics Program.

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