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13. ABSTRACT (Maximum 200 words)

ChemImage Biothreat LLC (ChemImage), in collaboration with the Edgewood Chemical Biological Center (ECBC), and the Armed Forces Institute of Pathology (AFIP) have teamed on a 1 year project entitled, “Optical Detection of Biological and Chemical Threats in Water and Food,” to develop and validate waterborne pathogen detection technology in support of the Joint Services Advanced Water Monitoring (JSAWM) Program. The optical detection of waterborne threats project integrates proven Raman Chemical Imaging (RCI) and sample pre-concentration technology to collect, enrich, detect and identify CB threats in water and food.
The objective of this program has been to advance the state-of-the-art in detection and identification of biological and chemical threat materials in water and food. Improved collection & concentration focused on the use of tangential flow filtration (TFF) coupled with centrifugation. Biological materials used in this program were supplied by AFIP. AFIP also operated RCI equipment in their BSL-2 laboratory for limited sample quality evaluation. Hazardous chemical materials used in this program were supplied by ECBC for analysis within their secure facilities. Additionally, ECBC personnel worked with ChemImage personnel in developing protocols for the analysis of water samples, including the construction of receiver operator characteristic (ROC) curves.

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Foreward

ChemImage Biothreat LLC (ChemImage), in collaboration with the Edgewood Chemical Biological Center (ECBC), and the Armed Forces Institute of Pathology (AFIP) have teamed on a 1 year project entitled, "Optical Detection of Biological and Chemical Threats in Water and Food," to develop and validate waterborne pathogen detection technology in support of the Joint Services Advanced Water Monitoring (JSAWM) Program. The optical detection of waterborne threats project integrates proven Raman Chemical Imaging (RCI) and sample pre-concentration technology to collect, enrich, detect and identify CB threats in water and food.

The objective of this program has been to advance the state-of-the-art in detection and identification of biological and chemical threat materials in water and food. Our focus has been to detect biological threat materials in water by integrating advanced technology for collection and concentration with RCI technology for detection and identification. Improved collection & concentration focused on the use of tangential flow filtration (TFF) coupled with centrifugation. Biological materials used in this program were supplied by AFIP. AFIP also operated RCI equipment in their BSL-2 laboratory for limited sample quality evaluation. Hazardous chemical materials used in this program were supplied by ECBC for analysis within their secure facilities. Additionally, ECBC personnel worked with ChemImage personnel in developing protocols for the analysis of water samples, including the construction of receiver operator characteristic (ROC) curves.

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List of Appendixes

None.

Statement of Problem Studied

ChemImage Biothreat LLC (ChemImage), in collaboration with the Edgewood Chemical Biological Center (ECBC) and the Armed Forces Institute of Pathology (AFIP) have teamed on a project entitled, “Optical Detection of Biological and Chemical Threats in Water and Food,” to develop and validate waterborne pathogen detection technology in support of the Joint Services Advanced Water Monitoring (JSAWM) Program. The optical detection of waterborne threats project integrates proven Raman Chemical Imaging (RCI) and sample pre-concentration technology to collect, enrich, detect and identify CB threats in water and food.

RCI combines digital optical microscopy and fluorescence imaging for targeting, with Raman spectroscopy and imaging for identification. ChemImage has demonstrated the ability of RCI to detect *Bacillus anthracis* (Ba) and other select biothreat agents. Detection has been demonstrated in the presence of complex background matrices that are an inherent feature of the chemical imaging technique. Concentration-dependent ROC curves have been generated using AFIP-supplied *B. globigii* and commercially available ovalbumin in tap and chlorinated reverse osmosis water recipes.

In this project, we have demonstrated a hierarchical approach to applying RCI as a rapid, reagentless solution, which improves over reagent-based solutions for threat detection in water. The optical detection of waterborne threats project is a follow-on to the previous feasibility study, and will test the limits of Raman-based optical technology by establishing the sensitivity, selectivity and limits of detection (LODs) for select agents in complex aqueous environments.

This project was guided by the following JSAWM Program objectives:

- To detect and identify CB contamination from source, treated, stored and distributed water
- Ten-minute detection times with limited false alarms
- Real time, autonomous and batch detection
- High specificity and high sensitivity (~100 spores/L)
- Man-portable, small, compact, environmentally friendly, low or no power

Summary of Results

During the optical detection of waterborne threats project, the team demonstrated, using FALCON II™ and EAGLE™ Molecular Chemical Imaging Systems at ECBC, AFIP and ChemImage, the high sensitivity and low false alarm potential of the technology for waterborne CB contamination detection. Sample pretreatment to reduce background clutter facilitated ChemImage's optical detection technology and allow the team to generate component-level receiver operator characteristics (ROC) curves. The effort in Year 1 concluded with a preliminary design review of a reagentless waterborne threat sensor system.

Task	Deliverable	% Complete
1	Report of Site Acceptance Test for FALCON II at ECBC Report of Site Acceptance Test for FALCON II at CI Report of Training on FALCON II at ECBC	100
2	Report of Laboratory Set up Plan Report of Laboratory Set up Report of Site Acceptance Test for EAGLE at ECBC Report of Training on EAGLE at ECBC	100
3	Report of Raman Analysis	100
4	Report of Validated Generation I Collection System	100
5	Report of Combined Generation I Collection and Analysis System	100
6	Report of Proof of Concept Generation II Collection System	100
7	Report of Preliminary Integrated System Design	100

Task 1: Deliver FALCON II's to ECBC and ChemImage.; Train ECBC personnel on FALCON

As part of the Task 1 Package, ChemImage delivered to ECBC and ARO on June 15, 2005 the following: 1) A copy of the Site Acceptance Test for the FALCON II at ECBC, 2) a copy of the Site Acceptance Test for the FALCON II at ChemImage, and 3) a report documenting the training.

Task 2: Set up ChemImage water analysis laboratory; Deliver EAGLE to ECBC and train personnel

As part of the Task 2 Package, ChemImage delivered to ECBC and ARO on November 28, 2005 the following: 1) A copy of the Laboratory Setup Plan, 2) a report of the laboratory setup, 3) a copy of the Site Acceptance Test for the EAGLE at ECBC and 4) a report documenting the training.

Task 3: Generate Reference Spectra to Validate Raman Analysis of Bioagents

As part of the Task 3 Package, ChemImage delivered to ECBC and ARO a report of Raman analysis validation on February 17, 2005.

Significant results from Task 3 included the generation of threat agent reference spectra to validate Raman analysis. A successful inter-laboratory validation study was conducted between ECBC, AFIP and ChemImage. The validation criteria included Raman expert analysis and statistical analysis. Raman experts at ECBC (Steve Christesen), AFIP (Kathy Kalasinsky) and ChemImage (Patrick Treado) agreed that visual assessment of Raman signatures indicated the Raman signatures collected in each of the three laboratories are comparable. Statistical analysis based on Mahalanobis Distance (MD) based identification confirms Raman signatures measured at ECBC map successfully to the joint AFIP/ChemImage pathogen Raman signature library.

Task 4: Validate Generation I Collection System

As part of the Task 4 Package, ChemImage delivered to ECBC and ARO a report of the validated Generation I collection system on January 24, 2006.

Significant results in Task 4 include the validation of a Tangential Flow Filtration (TFF) Generation I Sample Collection System. We employed fluorescent polystyrene microspheres as a reference material suitable for qualification of the TFF system. In performing this work, we evaluated TFF microsphere recovery efficiency from deionized (DI) and tap water recipe source waters over a range of analyte concentrations as a key subsystem performance metric. Recovery efficiencies ranging from 10 to >50% have been shown. The mean recovery efficiency in DI water is 18% +/- 11%, and the mean recovery efficiency in tap water is 39% +/- 31%.

Pre-concentrator recovery efficiency is a critical parameter in a developing Raman Water Monitor performance model. By carefully considering the TFF recovery efficiency, the performance requirements of a Generation II (GenII) preconcentrator was established, as part of an overall Raman Water Monitor System. Preliminary target specifications for this GenII preconcentrator were presented in the Task 4 report. By exercising a Notional Raman Water Monitor System performance model, we identified an operational approach that has the potential to deliver reagentless detection of waterborne pathogens within the required time to detect at necessary detection limits.

Task 5: Validate Combined Generation I Collection and Analysis System

As part of the Task 5 Package, ChemImage delivered to ECBC and ARO a report of the combined generation I collection and analysis system on April 3, 2006.

Significant results in Task 5 included the analysis of negative controls (i.e., chlorinated reverse osmosis (RO-Cl) and synthetic tap water recipes and aluminum-coated microscope slides) and analyte source materials (AFIP-supplied *B. globigii* and ovalbumin). Concentration-dependent studies were conducted of the analyte source materials in negative controls. ROC Curves and accumulated confidence factor-area under ROC curves were generated from the data obtained in the concentration-dependent studies.

Negative Controls Characterization

Optical and spectroscopic negative control studies of water sources and substrate materials were successfully performed to gain an understanding of the source of potential background interference. RO-Cl produced a low contribution to interfering fluorescence background, exhibited relatively few interfering Raman spectral bands and had morphological features quite different from Bg spores or ovalbumin. Such characteristics made RO-Cl a somewhat idealistic water source for spore and toxin analysis. Tap water recipe on the other hand exhibited high contribution to interfering fluorescence background both optically and spectroscopically and had residual particulate resembling the morphology of bacterial spores. These factors made analysis in tap water recipe a much greater challenge than the RO-Cl water source. An aluminum-coated slide was also characterized optically and spectroscopically since it was used as the substrate upon which all materials were deposited. The aluminum-coated slide provided little to no background contribution was optically flat, resistant to photodamage and relatively Raman inactive, making it an ideal substrate for spore and toxin Raman spectroscopy and Raman Chemical Imaging analysis.

Analyte Source Materials Characterization

Optical (i.e., brightfield reflectance, polarized light, differential interference contrast and autofluorescence imaging) and spectroscopic (i.e., dispersive Raman, liquid crystal (LC) / fiber array spectral translation (FAST)-based Raman Chemical Imaging) studies of source materials were successfully performed to provide the morphological and spectroscopic signatures to be assessed throughout the Task. High SNR Raman data was obtained from AFIP-supplied *B. globigii*. Spectral signatures were consistent with library Bg and had morphological features consistent with bacterial spores. Consistent Raman spectra were obtained from two lots of Bg provided by AFIP on December 8, 2005 and February 7, 2006, respectively. Ovalbumin obtained from Sigma-Aldrich produced characteristic high SNR data.

Concentration-Dependent Bg Characterization

Optical (i.e., brightfield reflectance, polarized light, differential interference contrast and autofluorescence imaging) and spectroscopic (i.e., dispersive Raman, LC/FAST-based Raman Chemical Imaging) studies were successfully performed on Bg samples prepared in RO-Cl and tap water recipe sources, respectively. With TFF preconcentration, Bg was detected and characterized at two log steps from 10^{10} down to 10^{6} cfu/L. Liquid crystal-based Raman imaging was used to generate ROC curves in this study provided advantages in terms of statistical large numbers of spectra and providing high fidelity imaging. Practical speed of acquisition limitations limited the analysis to the C-H stretching region of the spectrum. It is believed that a future implementation of the technology will require FAST Raman Chemical Imaging to address the time to detect requirements of the program. This method produces a low fidelity Raman image with full spectral range coverage and speeds that rival ordinary dispersive Raman spectroscopy. Preliminary results show great promise for FAST as a method for waterborne pathogen detection.

Concentration-Dependent Ovalbumin Characterization

Optical (i.e., brightfield reflectance, polarized light, differential interference contrast and autofluorescence imaging) and spectroscopic (i.e., dispersive Raman, LC/FAST-based Raman Chemical Imaging) studies were successfully performed on ovalbumin samples prepared in RO-Cl water source. Using TFF preconcentration, ovalbumin was detected and characterized at two log steps from 10^{8} down to 10^{6} ng/L. Like Bg, liquid crystal-based Raman imaging was used to generate ROC curves in this study.

Concentration-Dependent ROC Curves and Accumulated Confidence Factor-Area Under ROC Curves

ROC curves were successfully generated for Bg in RO-Cl water, Bg in tap water recipe and ovalbumin in RO-Cl water, respectively. ROC curves were produced and compared using cosine correlation analysis (CCA) and Mahalanobis distance (MD) methods in both an untargeted and targeted fashion. Area under the curve was calculated for each ROC curve as a measure of the quality of the ROC curve. Areas under the ROC curve results were summarized using bar charts. Briefly, bar charts results were shown to dramatically improve when using the targeting approach as opposed to an untargeted approach. In general, the area under the ROC curve is greater when using the CCA methods compared to the MD approach. On average the area under the ROC curve is greater for the RO-Cl runs in comparison to the tap water runs. Due to the lower amount of background interferent in the RO-Cl water compared to the tap water recipe. Ovalbumin ROC curves were closer to ideal than comparable Bg ROC curves due likely to the higher Raman signal strength of the data associated with the ovalbumin when compared to Bg.

Task 6: Proof of Concept Generation II Collection System

As part of the Task 6 Package, ChemImage delivered to ECBC and ARO a report of the proof of concept generation II collection system on April 25, 2006.

Significant results in Task 6 included the demonstration of Proof of Concept of a Generation II Collection System. We employed fluorescent polystyrene microspheres as a reference material suitable for qualification of direct flow filtration (DFF)[1] and centrifuge systems. In performing this work, we evaluated DFF and centrifuge microsphere recovery efficiency from ultrapurified water over a range of analyte concentrations as a key subsystem performance metric. Recovery efficiencies ranging from 12% to >68% have been shown. The mean recovery efficiency in ultrapurified water was 37% for DFF and 38% for centrifugation.

Pre-concentrator recovery efficiency is a critical parameter in a developing Raman Water Monitor performance model. By carefully considering the DFF and centrifuge recovery efficiencies, the performance requirements of a Generation II (Gen II) pre-concentrator was established, as part of an overall Raman Water Monitor System. Preliminary target specifications for this Gen II pre-concentrator were presented in a Trade Study among known pre-concentration technologies. By exercising a Notional Raman Water Monitor System performance model, we identified an operational approach that has the potential to deliver reagentless detection of waterborne pathogens within the required time to detect at necessary detection limits.

Task 7: Preliminary Design of Integrated System

As part of the Task 7 Package, ChemImage delivered to ECBC and ARO a report of the preliminary design of the integrated system on June 26, 2006.

Significant results in Task 7 included a preliminary design of an integrated Raman water monitor system. The preliminary design includes four (4) modules: concentration, deposition[2], detection and analysis. The time to detect per sample is estimated at 25 minutes based on PDR.

Listing of Publications

Peer Reviewed Journals

None.

Non-peered Reviewed Journals or conference proceedings

None.

Presented at meetings, but not published in conference proceedings

Treado, P. J., Nelson, M. P., Maier, J., Stewart, S., Tripathi, A., Jabbour, R., Jenson, J., Samuels, A., Christesen, S., Kalasinski, K. "Reagentless Raman Detection of Waterborne Threats." *The SPIE Defense and Security Symposium*, Orlando, FL, April 17-21, 2006.

Treado, P. J., Nelson, M. P. "Sensing Chemical and Biological Warfare Agents in Compex Air, Water and Food Background Matrices Using Chemical Imaging." *The Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy*, Orlando, FL, March 12-17, 2006.

Treado, P. J., Neiss, J., Nelson, M. P., Roskovensky, R., Kalasinsky, K., Christesen, S. D., Tripathi, A., Jabbour, R., Snyder, P., Jensen, J. "Reagentless Detection of Waterborne Bioagents Using Raman Chemical Imaging Spectroscopy (RCIS)." *ISSSR*, Bar Harbor, ME, May 29th-June 2nd, 2006.

Manuscripts submitted, but not published

Tripathi, A., Jabbour, R. E., Treado, P. J. "Biological Substance Characterization in Water Matries with Raman Microspectroscopy." (In preparation to be submitted to *Appl. Spectrosc.*, September, 2006).

Technical Reports submitted to ARO

M.P. Nelson, "Report of Site Acceptance Test for FALCON II at ECBC", in support of contract # W911NF-05-C-0052, June 7, 2005.

M.P. Nelson, "Report of Site Acceptance Test for FALCON II at CI", in support of contract #W911NF-05-0052, June 7, 2005.

M.P. Nelson, "Report of ECBC Training on FALCON II" in support of contract #W911NF-05-0052, June 7, 2005.

M.P. Nelson, T.S.Powers, P.J. Treado, "ECBC Laboratory Set-up Plan", in support of contract #W911NF-05-0052, November 28, 2005.

M.P. Nelson, T.S.Powers, P.J. Treado, "ECBC Laboratory Set-up Report", in support of contract #W911NF-05-0052, November 28, 2005.

M.P. Nelson, C.W.Gardner, P.J. Treado, "ECBC EAGLE SAT Report", in support of contract #W911NF-05-0052, November 28, 2005.

M.P. Nelson, C.W.Gardner, P.J. Treado, "ECBC EAGLE Training Report", in support of contract #W911NF-05-0052, November 28, 2005.

M.P. Nelson, P.J.Treado, "Report of Generation 1 Collection System", in support of contract #W911NF-05-0052, January 24, 2006.

M.P. Nelson, P.J.Treado, "Report of Raman Analysis Validation", in support of contract #W911NF-05-0052, February 16, 2006.

M.P. Nelson, P.J.Treado, "Report of Combined Generation 1 Collection and Analysis System", in support of contract #W911NF-05-0052, April 15, 2006.

M.P. Nelson, P.J.Treado, "Report of Preliminary Design of Integrated System", in support of contract #W911NF-05-0052, June 26, 2006.

List of Personnel earning advanced degrees while on project

None.

Report of Inventions

None.

Bibliography

[1] "A Field Sample Concentration Method for Rapid Response to Security Incidents", Stanley States[a], Jennifer Wichterman[a], Georgina Cyprych[a], John Kuchta[a] and Leonard Casson[b]; [a]Pittsburgh Water and Sewer Authority; [b]Department of Civil and Environmental Engineering University of Pittsburgh.

[2] "Sequential Injection: a New concept for Chemical Sensors, Process Analysis and Laboratory Assays", Ruzicka, J., Marshall, G.D., Anal. Chim. Acta, 237 (1990), 329 – 343.

Appendixes

None.