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L. N. Brewer, P. K. Weber, R. P. Grant, S. Ghosal, J. R. Michael

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Microanalytical Methods for Bio-Forensics Investigations.

L.N. Brewer[1], P. K. Weber[2], R.P. Grant[1], S. Ghosal[2], J.R. Michael[1]

[1]Sandia National Laboratories, Materials Characterization Dept., P.O. Box 5800-MS 1411 Albuquerque, NM 87185-1411

[2]Lawrence Livermore National Laboratory, 7000 East Ave, L-231, Livermore, CA 94550

Forensics investigations of bio-crime or bio-terrorism incidents require careful analysis of collected evidentiary material. Although the biological markers in the evidentiary material are important (e.g. genomic signatures, protein markers), the elemental make-up of the organisms themselves and the surrounding non-biological material is extremely useful for attributing a specific process and, perhaps, specific persons to the production of the biological agent.

This talk will describe the coordinated use of microanalytical techniques such as SEM-EDX, STEM-EDX, and NanoSIMS for generating compositional signatures for bio-forensics investigations. These analytical techniques span length scales from the 50μm range to the 5nm range. The range of analytical sensitivities spans from ~.5wt% for EDX down to parts per billion for SIMS techniques.

In addition, we will discuss the use of spectrum imaging techniques for rapidly extracting the key elemental signatures from large scale data sets. Spectrum imaging techniques combined with multivariate statistical analysis allow for the collection and interrogation or enormous quantities of data without pre-biasing the answer.[1] Spectrum imaging has been used successfully in EDX microanalysis[1] (both in the SEM and TEM) and TOF-SIMS[2].

In this study, a set of test biological agents, γ-irradiated Bacillus thuringiensis (Bt), were examined using the aforementioned microanalytical techniques. The sample set included a number of processing conditions to gauge the ability of these techniques to identify the production methods of these simulated agents.

Complementary but distinct forensic signatures were obtained by all three analytical techniques. Figure 1 shows two types of silicate particles observed among the spore material itself. At this length scale, the spores themselves cannot be resolved, but the presence of these silicates is key marker for distinguishing this production route. A STEM-EDX spectrum image from the same material does not show these large silicates but instead shows the segregation of elements such as sulfur and silicon to the extra-cellular material between spores, again a result of the specific process usedto produce this simulated agent (Figure 2). NanoSIMS data from the same material also shows the segregation of Si in this preparation. The NanoSIMS data also displays and quantifies the distribution of elements such as fluorine at levels which were not detectable in the STEM-EDX measurements (Figure 3).

1. P. G. Kotula, et al., Microscopy and Microanalysis. **9**(1):(2003) p. 1-17.
2. J. A. Ohlhausen, et al., Applied Surface Science. **231-232**:(2004) p. 230-234.
3. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United Stated Department of Energy (DOE) under contract DE-AC0494AL85000. LLNL work performed under the auspices of the U.S. DOE by the University of California, LLNL under Contract W-7405-Eng-48.

Figure 1. SEM-EDX spectrum image of Bt spore preparation. Note: colors in spectrum image correspond to colors in EDX spectra. A.) BSE image of spore material, B.) Spectrum Image, C.) EDX spectral components.

A.) B.) C.)

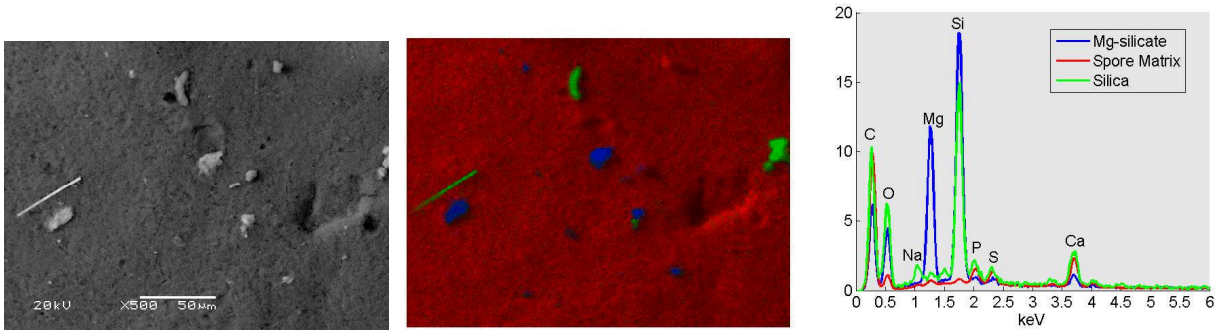

Figure 2. STEM-EDX spectrum image of Bt spore preparation. Note: colors in spectrum image correspond to colors in EDX spectra. A.) Annular dark field image of spore material, B.) Spectrum Image, C.) EDX spectral components. The high Ga signal in the cyan component is due to redeposition during FIB sample preparation.

A.) B.) C.)

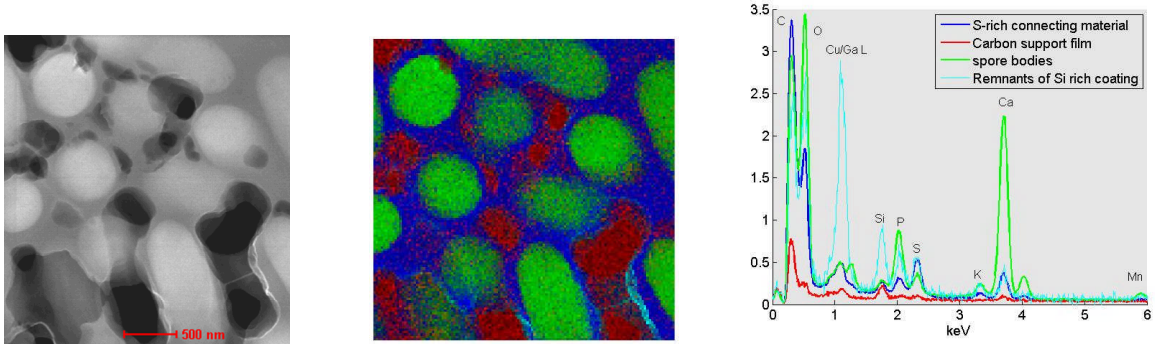

Figure 3. NanoSIMS secondary electron (SE) and quantitative digital ion images of two sectioned Bt spores. The color scale bars indicate relative total counts of each species. Spatial resolution is ~50 nm. Note that here, the spore sample is on a Ge wafer that contained some Si. Scale bar = 500 nm.