Microanalytical Methods for Bio-Forensics Investigations

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1: Sandia National Laboratories
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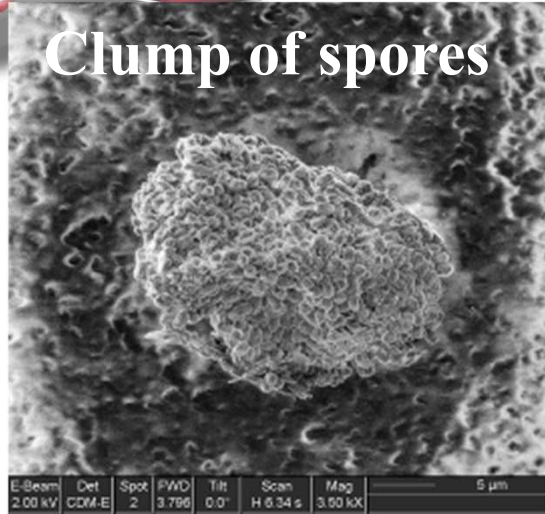
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Goal of Bioforensics

Clump of spores

Vial of spores

We want to know…
How "they" made it?
Where "they" made it?
When "they" made it?
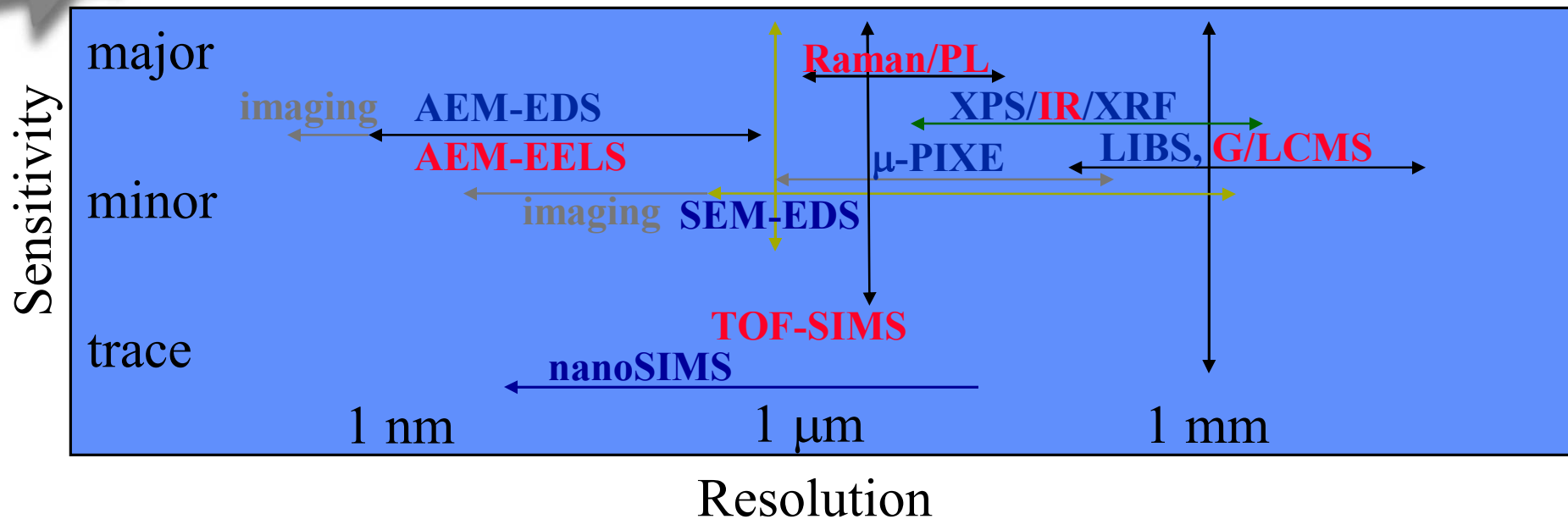
Who made it??

Assess ability of analytical approach for:

- Discrimination. Are the samples different? (differentiable signals from a spectrometer)

- Attribution. How are they different? (the iron from sample Q is from $FeCl_2$, while the iron in sample P is from steel)

- Quantification. How different are they? (Sample R is 5 vol% FeCl2 and is statistically different from sample Q at 10 vol%)

Microanalytical Methods for Bioforensics

elemental, **molecular**

major	**Raman/PL**
imaging AEM-EDS AEM-EELS	XPS/IR/XRF μ-PIXE LIBS, G/LCMS
minor	imaging SEM-EDS
	TOF-SIMS
trace	nanoSIMS

Sensitivity

1 nm   1 μm   1 mm

Resolution

As part of a larger, national laboratory team (LLNL, LANL, ORNL, PNNL, and SNL),

We have explored a large number of analytical techniques that span this space.

Homeland Security

Sandia National Laboratories

Microanalytical Methods for Bioforensics
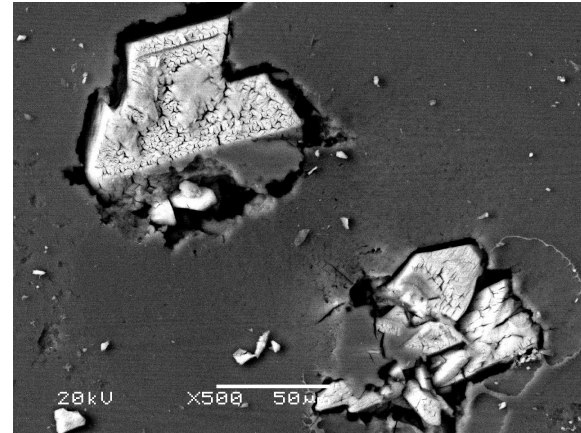
What useful signatures can be generated?

What can be learned from the matrix material (everything but the organism)?

What can be learned from the organism itself?

This presentation surveys results from a set of bio-weapon simulants using B. thuringiensis.

Samples will be identified as "A" through "I"
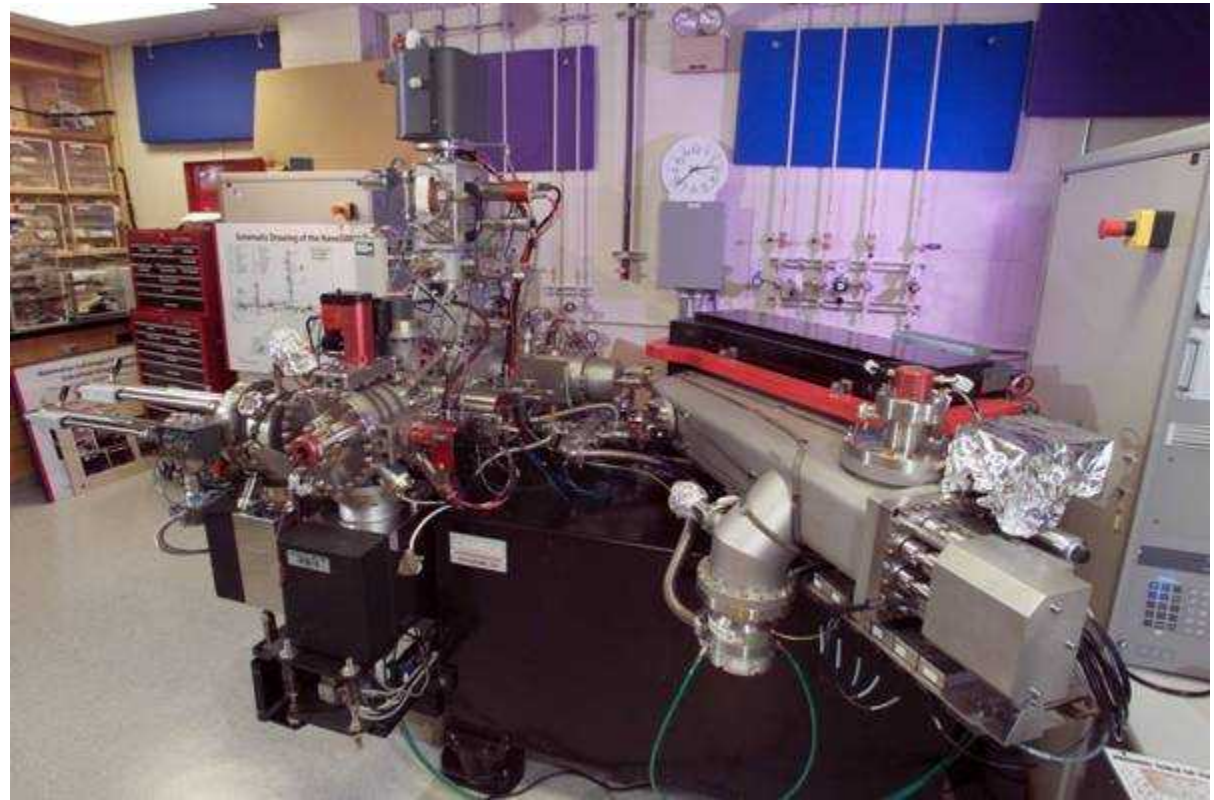
Matrix Material
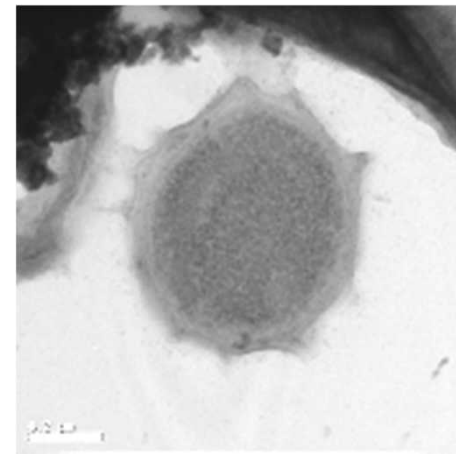
Phenotypic Observations

NanoSIMS—combining high spatial resolution with high elemental sensitivity

Trace element and isotopic characterization at sub-micron scale

Magnetic sector with fixed detectors

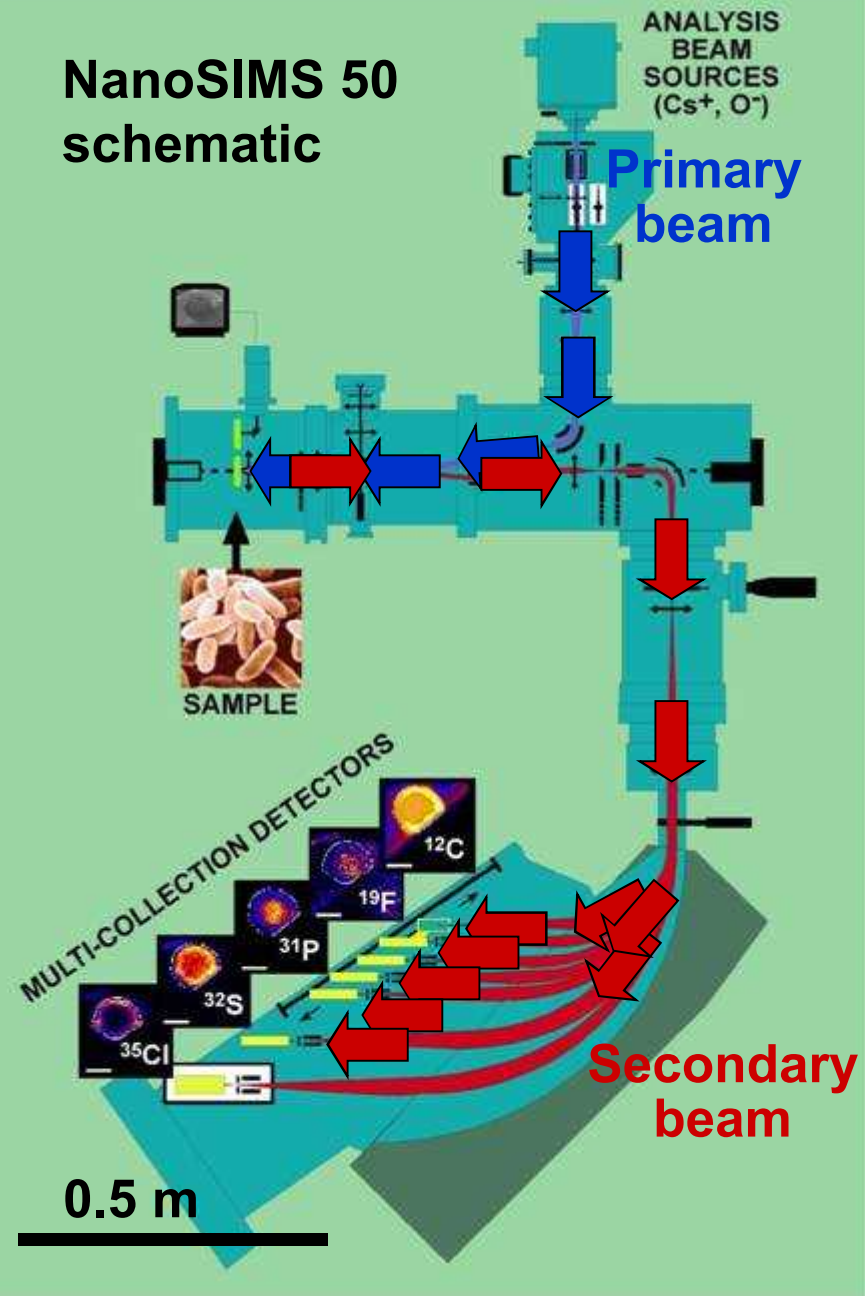**Target specific species for quantification**

LLNL NanoSIMS Laboratory

A surface sputtering technique

- **Primary beam scans sample surface to produce secondary ions**
- **Secondary ions detected to produce quantitative digital images**
- **Simultaneous detection of 5 species**
- **High sensitivity: → 5% useful yield**

To mass spectrometer

Secondary ions

Cs+

Secondary ions

e-

sample surface

50 nm beam diameter under ideal conditions

NanoSIMS 50 schematic

ANALYSIS BEAM SOURCES (Cs+, O-)

Primary beam

SAMPLE

MULTI-COLLECTION DETECTORS

12C
19F
31P
32S
35Cl

Secondary beam

0.5 m
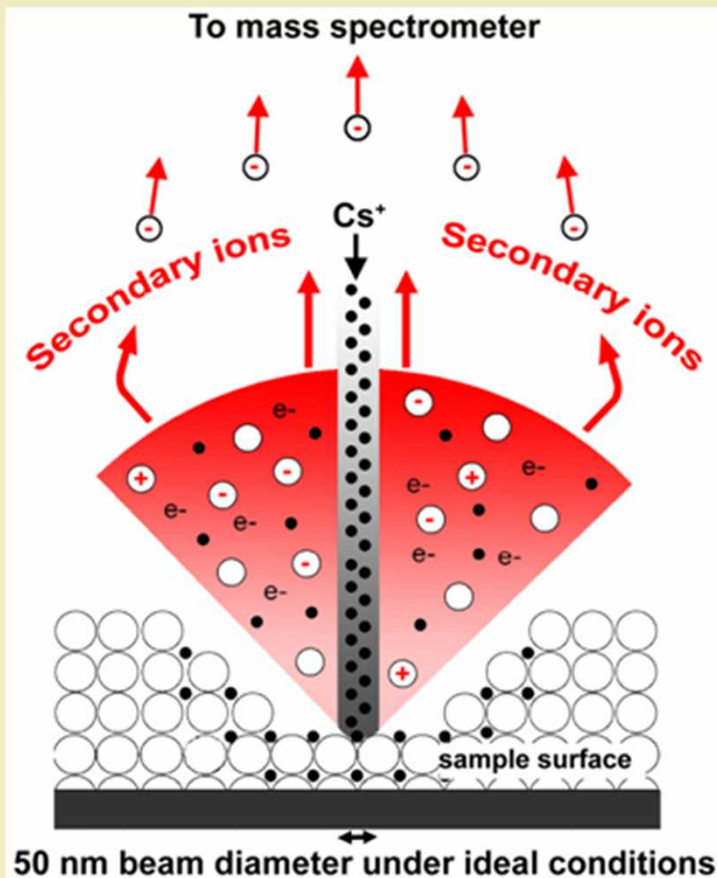
What do we do with all of this data??: Multivariate Tools for Bio-forensics

Spectrum imaging-capable instrumentation

FEI F30 TEM/STEM

- Microanalysis
  - SEM
  - STEM
  - XRF
- Surface analysis
  - XPS
  - TOF-SIMS

Multivariate Statistical Analysis Tools
AXSIA software suite for analysis
MCR, spatial simplicity calculations
(P. Kotula & M. Keenan, SNL)
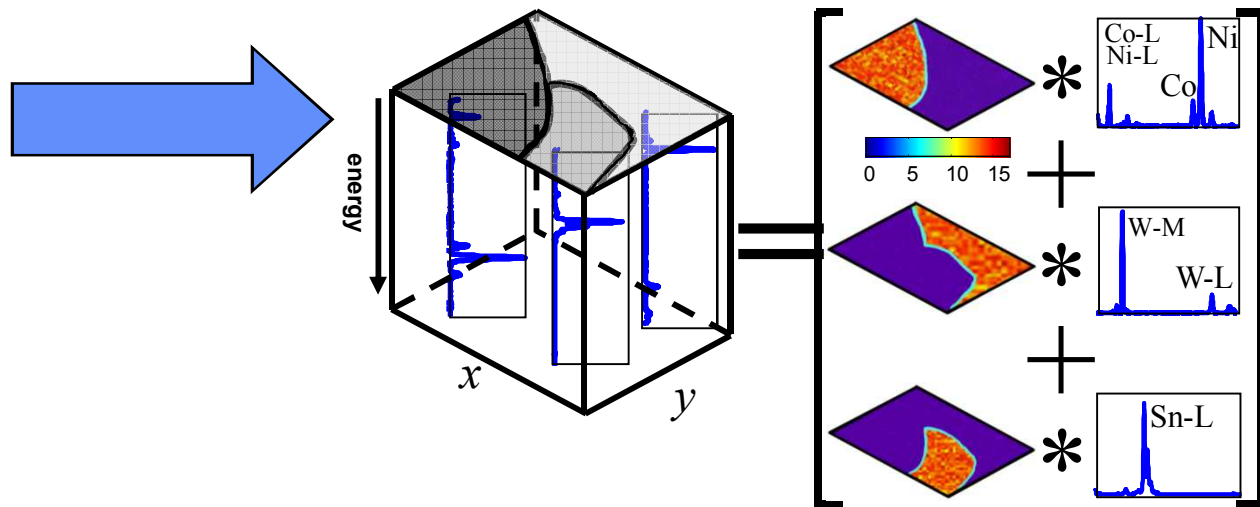
- Rapid decomposition of huge data sets
- Unbiased—no input guesses needed
- Ability to find "needle in haystack"
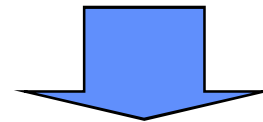
Comprehensive Forensics Analysis

Microanalytical Signatures from Matrix Material
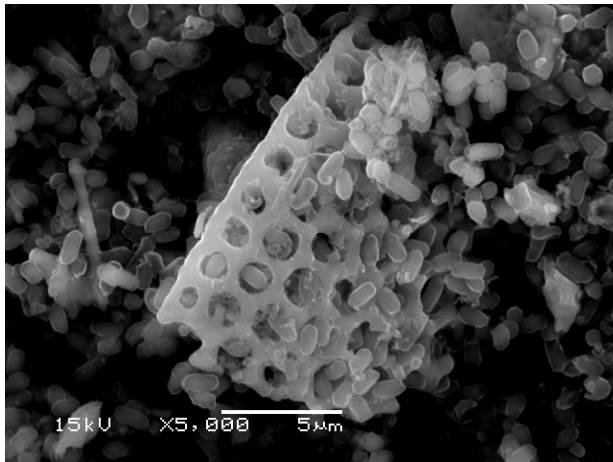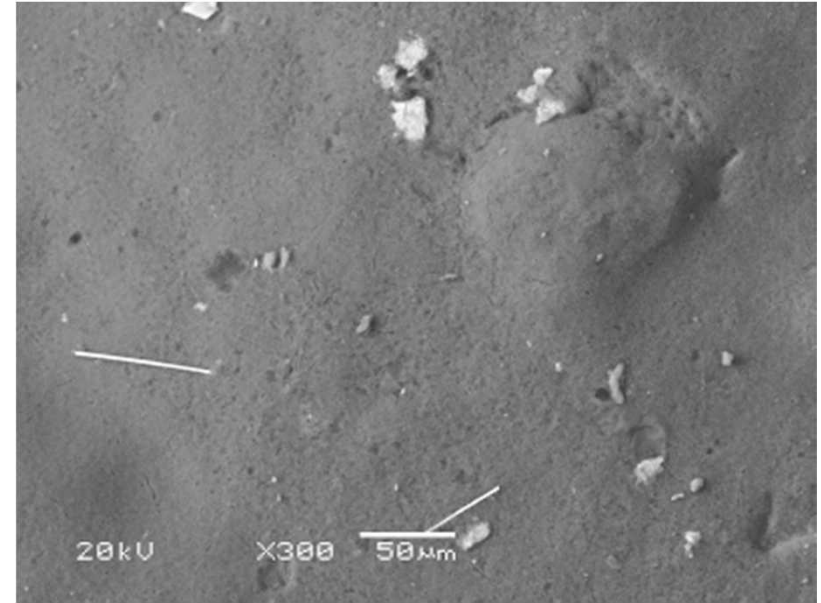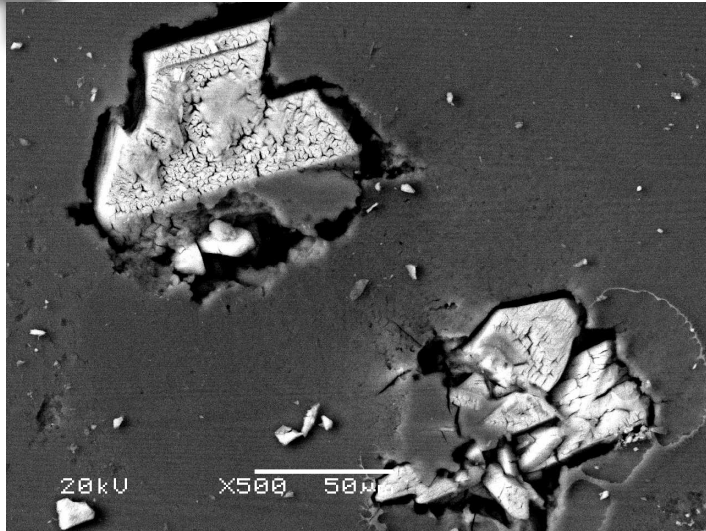
What do these "bits and pieces" tell us about sample production?
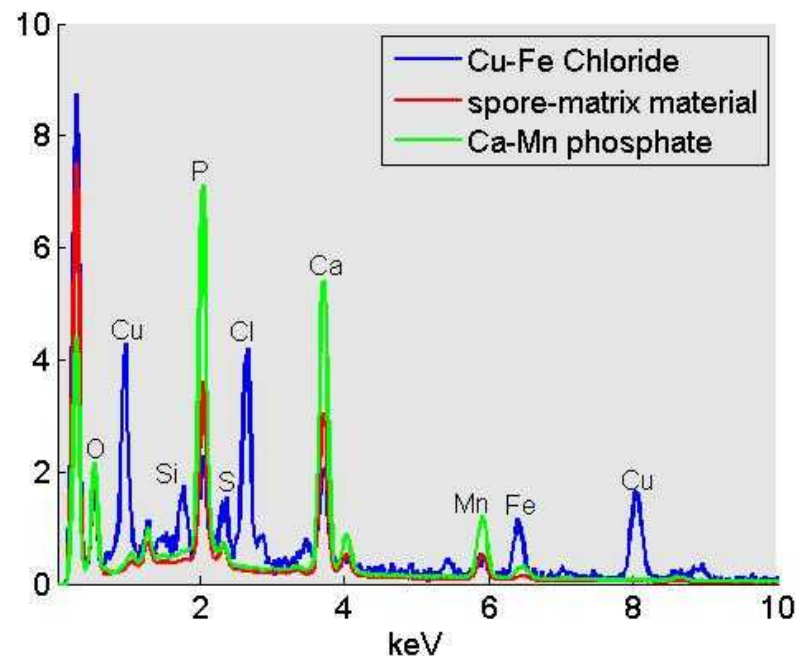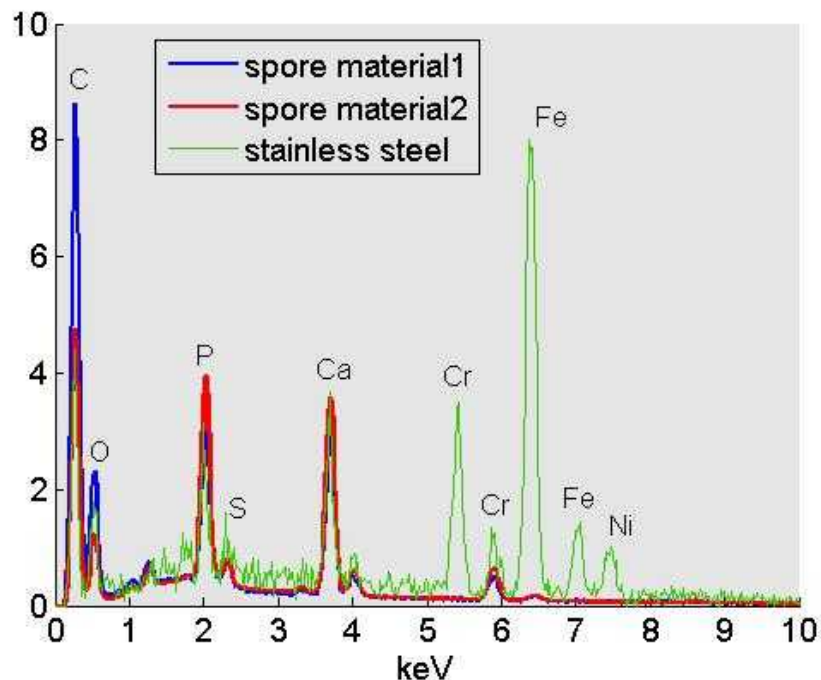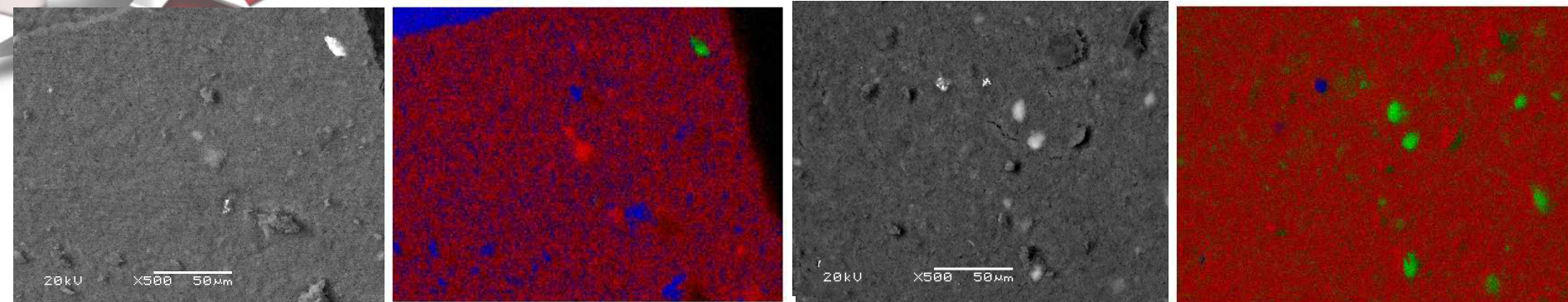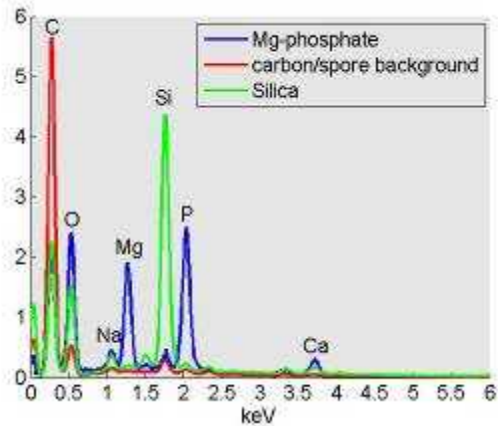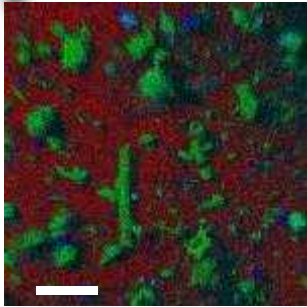
Identification of stainless steel particles and metal chlorides can provide information about the processing equipment and chemicals.

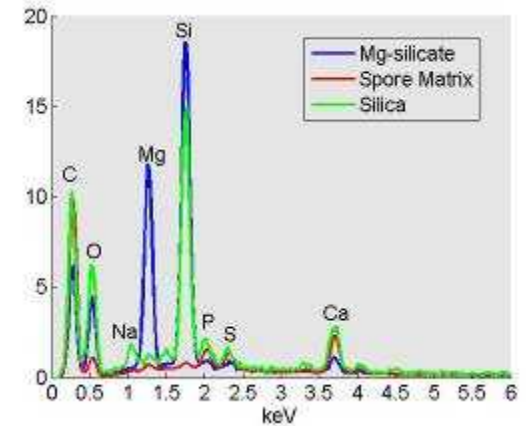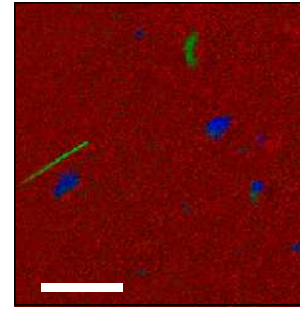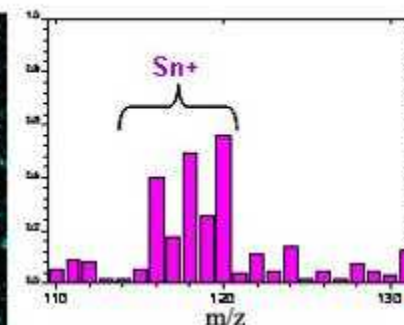# Si signatures from matrix material

Sample "J"
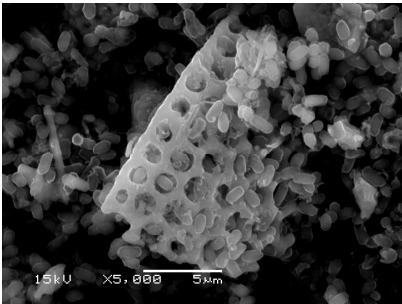
Sample "G"
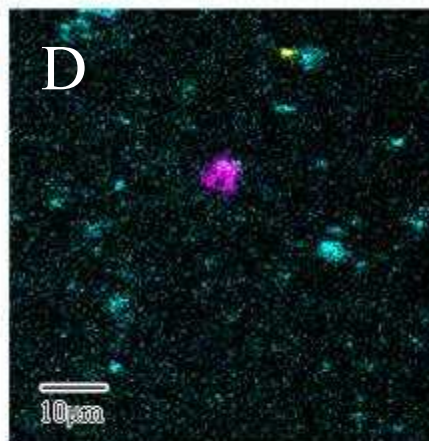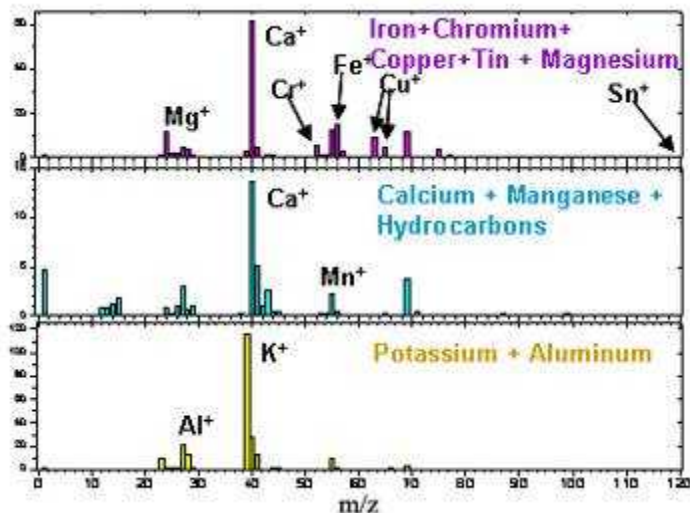

Prevalence of Si-based particulates a clear signature

White scale bars represent 50μm

# Higher elemental sensitivity signatures from matrix material (TOF-SIMS)

- The cyan and magenta components are similar to what has been observed previously in SEM-EDX
- K, Al, and Sn not observed in SEM-EDX and yet potentially quite important.
- Sn signal too low to be identified by operator without multivariate statistical analysis. (unless they were looking for it…)

Elemental Signatures from the organisms

Sample "H"

inner spore coat
carbon support
spore bodies
Si-rich outer coating
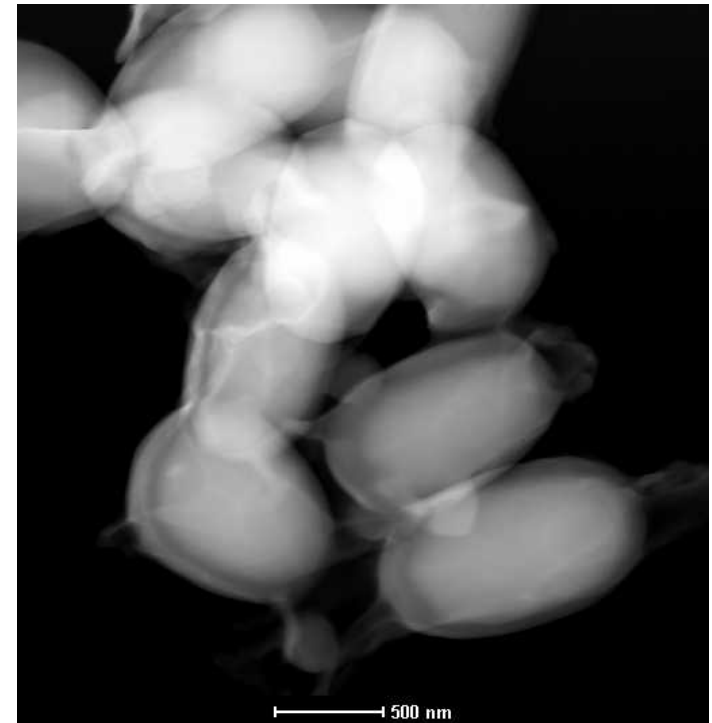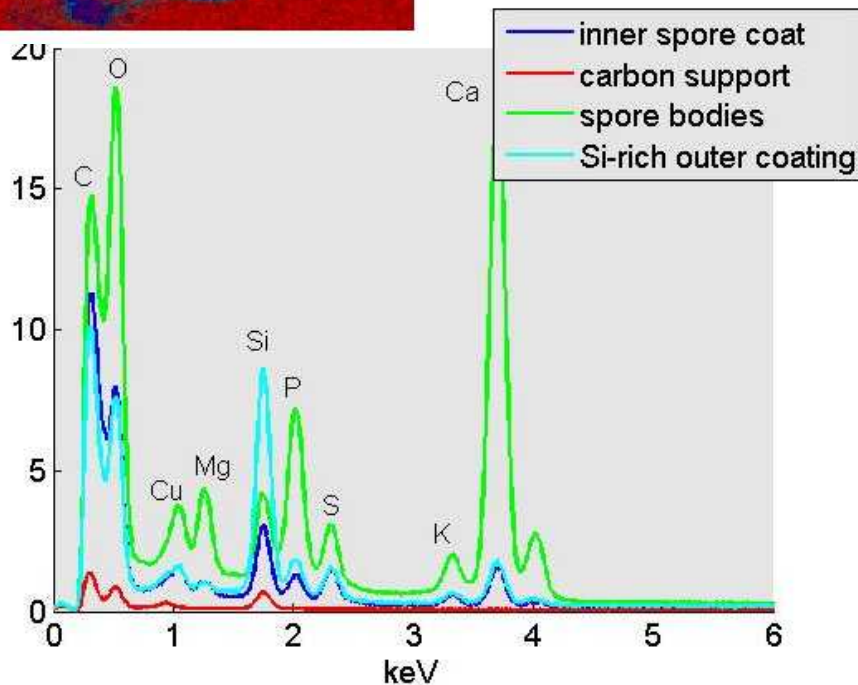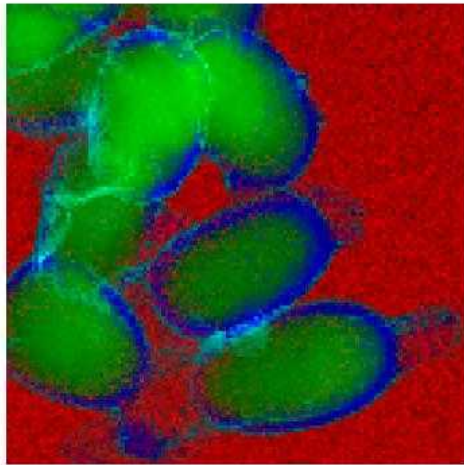
Si manifested both inside and outside the agent itself…

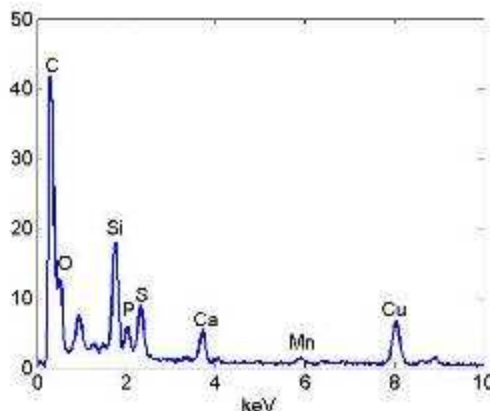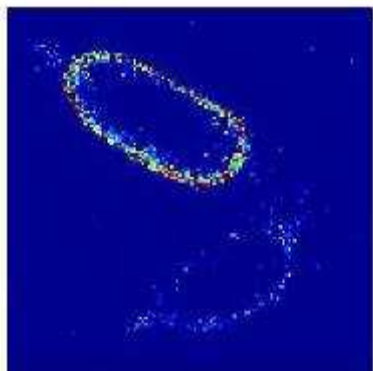Note the difference in elemental ratios in coatings of H and F.

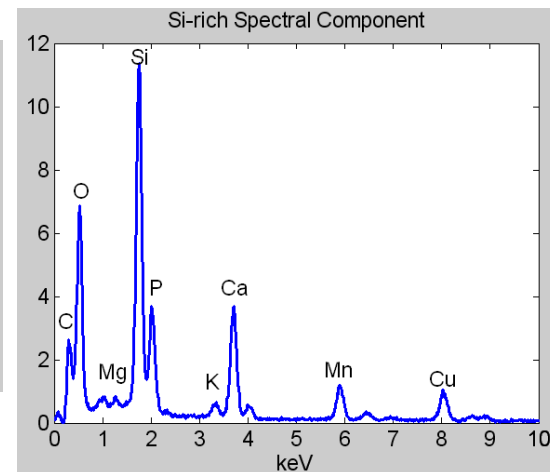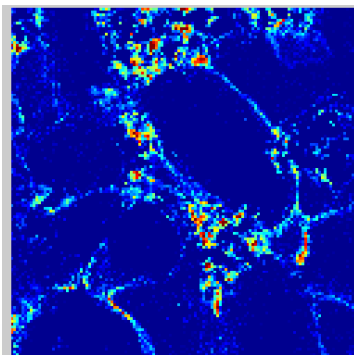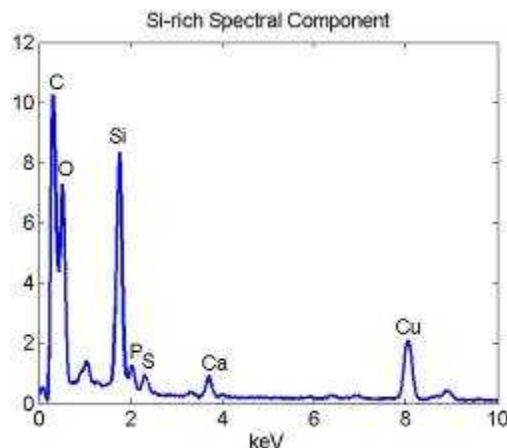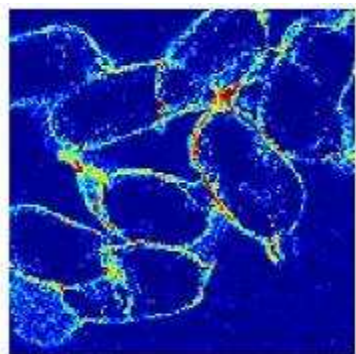# Comparison of Si-rich Coating Component Spectra

A
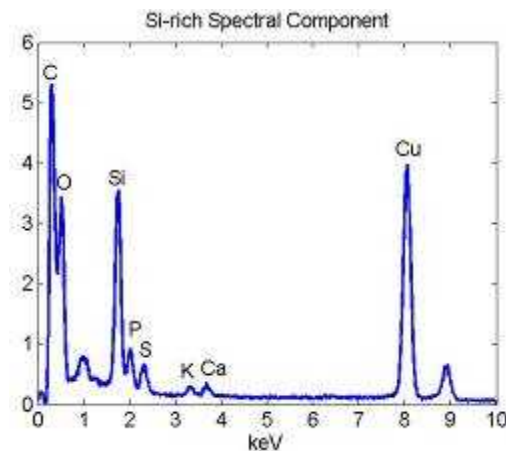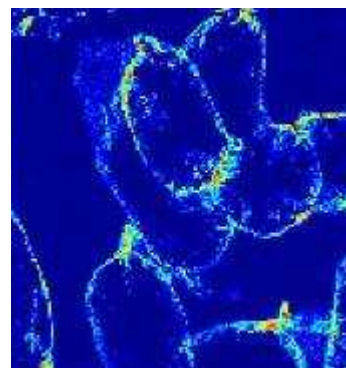

E

F

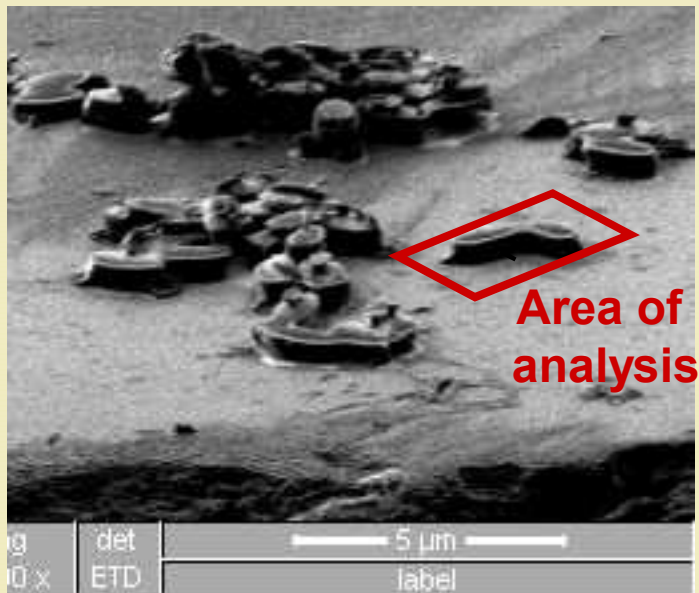H

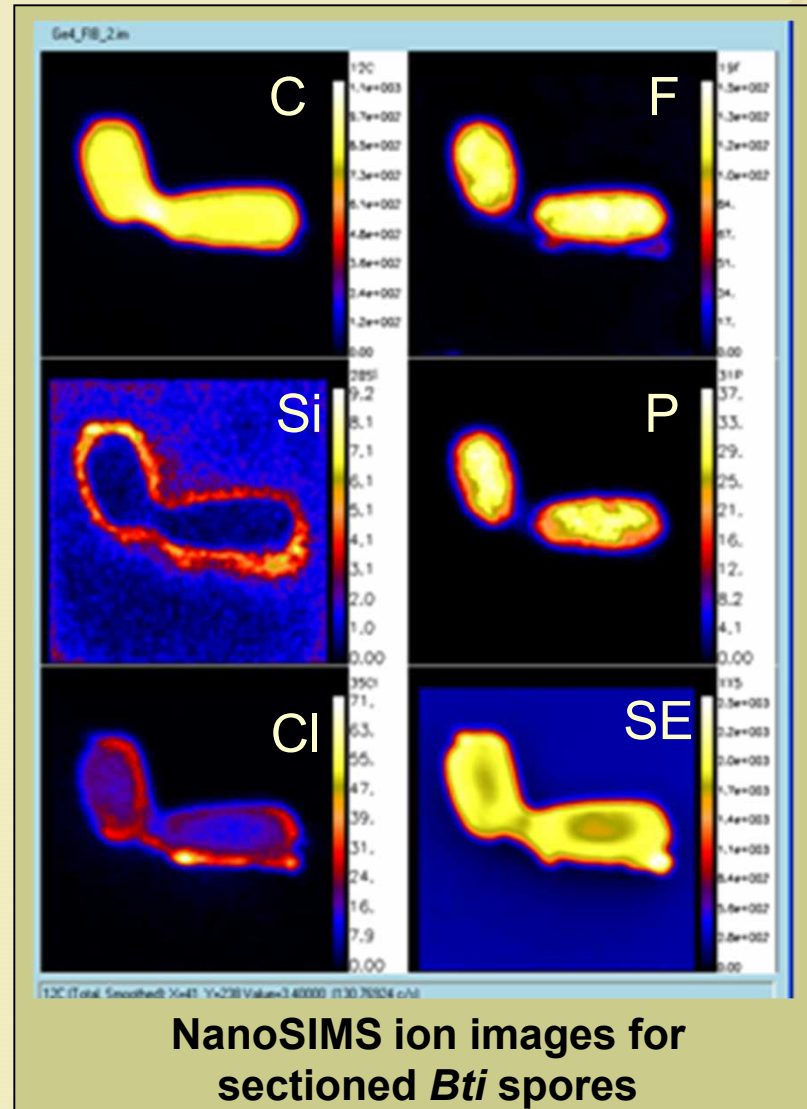Note that all of these processes generate Si-rich coatings of <u>different compositions…</u>

NanoSIMS Results: Quantitative Digital Images
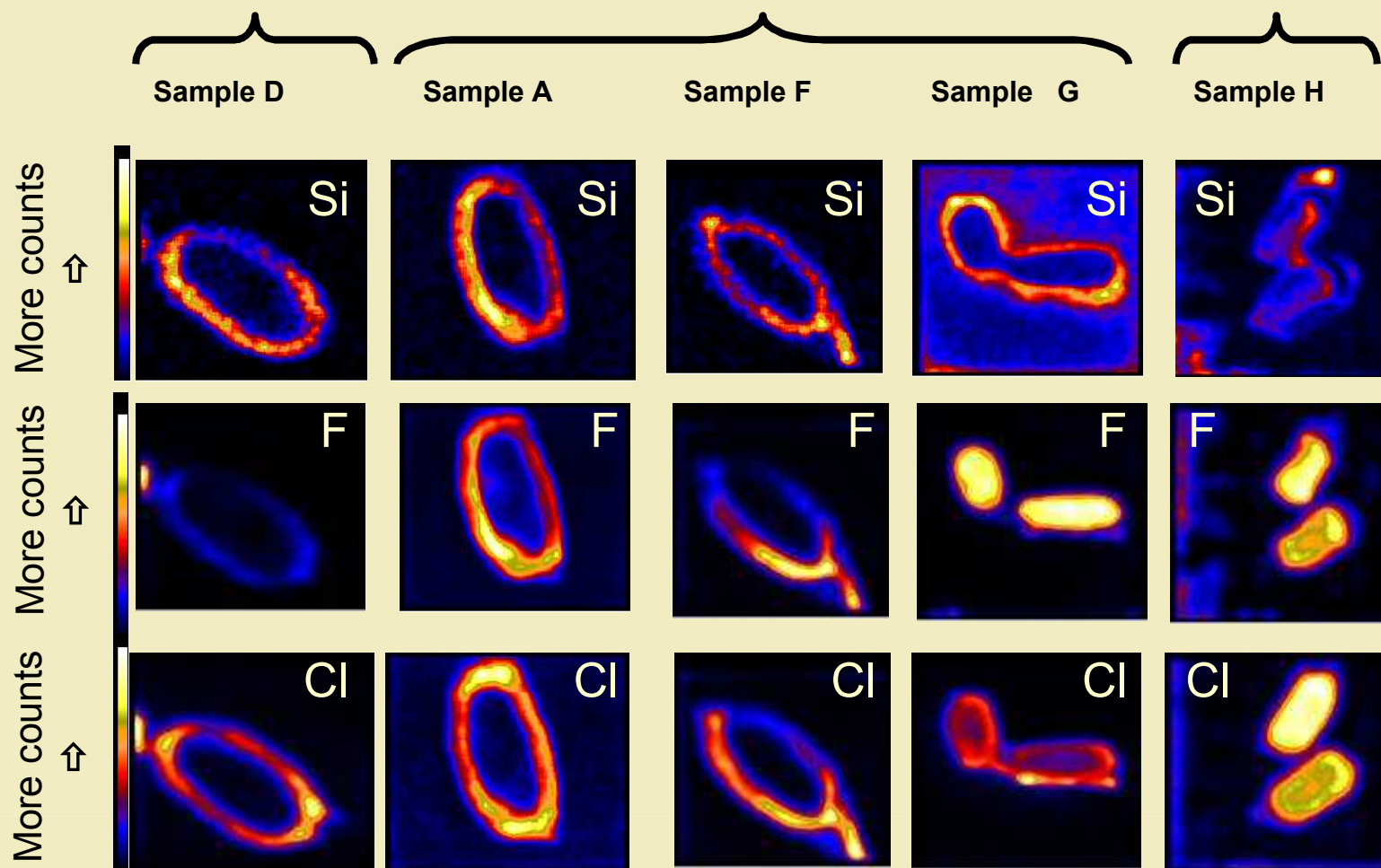
- **Data processed pixel by pixel with custom software**

Oblique view of FIB section of spores

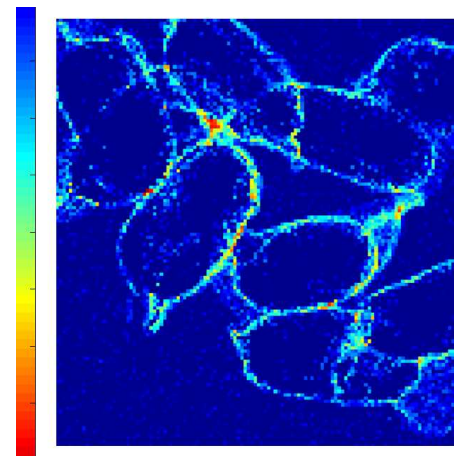**NanoSIMS ion images for sectioned *Bti* spores**

Elemental distributions differ with production factors

Summary

- Microanalysis applied to bioforensics is
  - Multipronged: coordinated analyses by several analytical techniques
  - Multivariate: Spectrum imaging is key to analytical forensics analysis
- Analyses at multiple length scales and analytical sensitivities required for <u>comprehensive</u> characterization of unknown
- MVSA combined with spectrum imaging has important analytical advantages
  - Un-biased identification of separate, microstructural features
  - Component signatures include the relationships between several elements
  - Higher feature contrast with less total signal