Microanalytical Methods for Bio-Forensics Investigations

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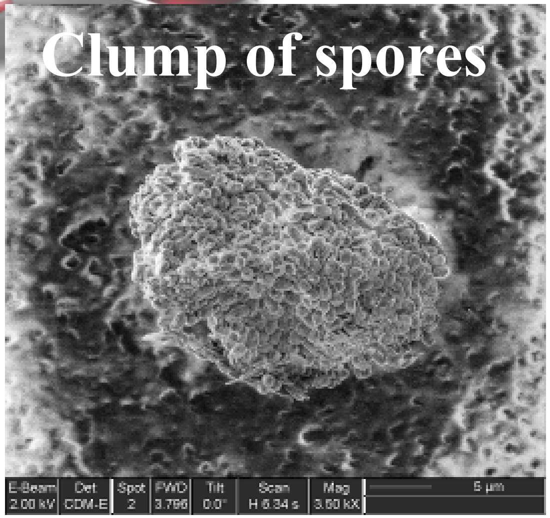
Goal of Bioforensics



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? (The iron signal from our spectrometer indicates that samples Q and P are different)
- 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%)

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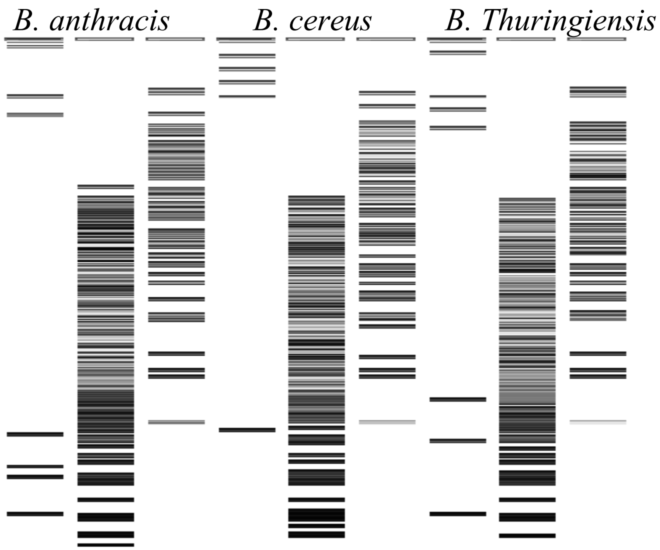
Approaches to Bio-forensic Attribution

Molecular Genomics



Courtesy of B. Marrone, LANL

Standard biological approaches crucial for identifying organism

Genomic and phenotypic information may provide some clues about production, but…

Once I know the organism is B. cereus, do I have any clues about how or where it was made??

What further information can be gained by applying microanalytical techniques from materials science, geology, surface science, etc.?

How would a Materials Science Lab become involved in Bio-forensics?

Ask the right questions with the right capabilities at the right time.

Microanalytical Capabilities

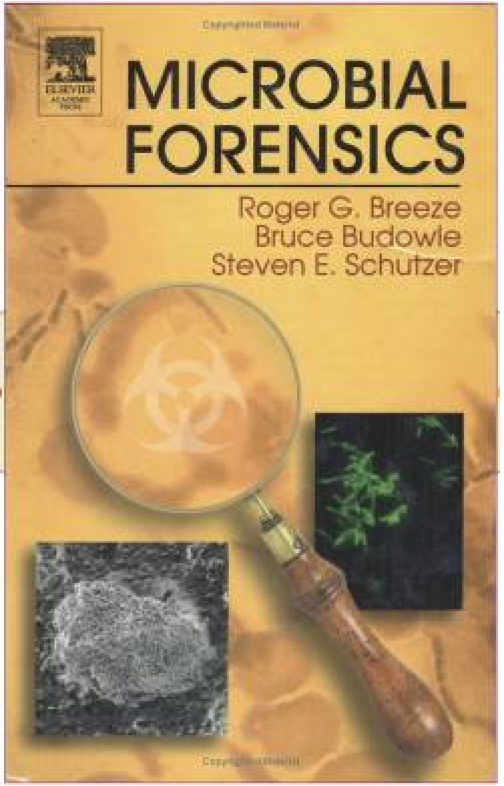
FEI F30 TEM/STEM

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

Previous experience with materials forensics problems

- USS Iowa
- Exploding glocks
- Ruptured gas lines
- FA on microelectronic devices
- Microanalytical methods developed for NW materials development and problem solving

National interest in advancing bio-forensics methods



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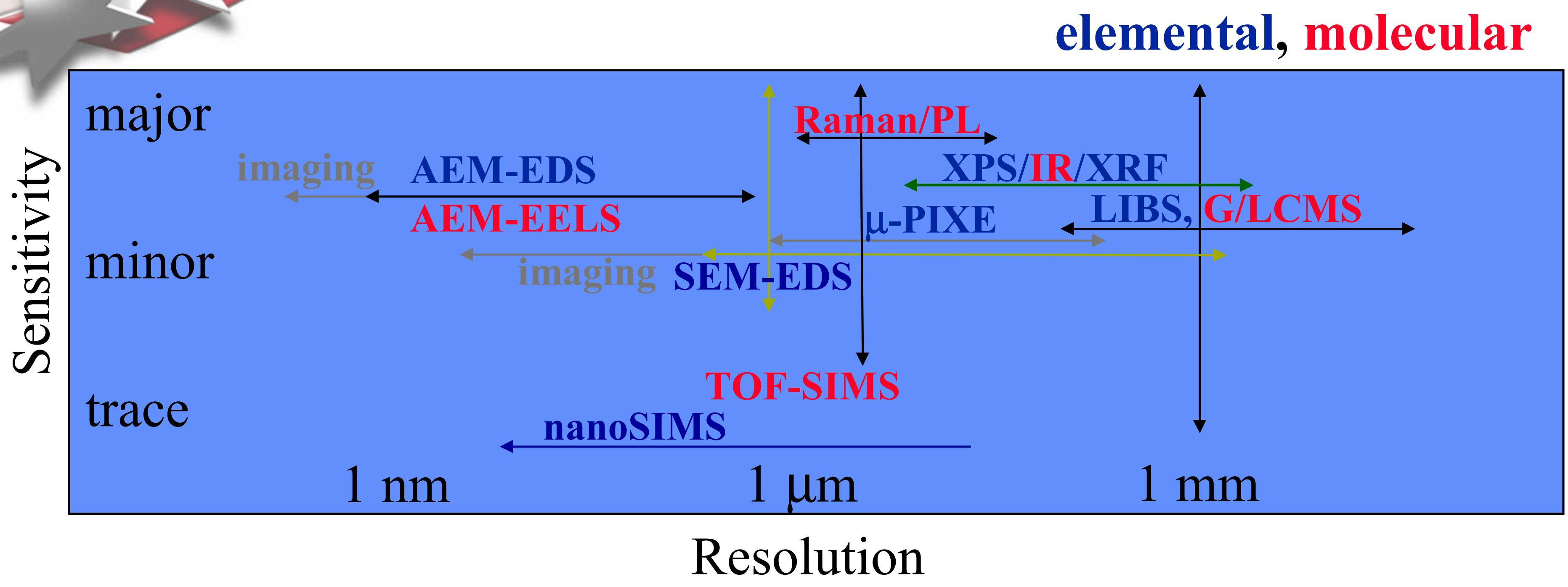
Microanalytical Methods for Bioforensics



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.

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Microanalytical Methods for Bioforensics

What useful signatures can be generated?

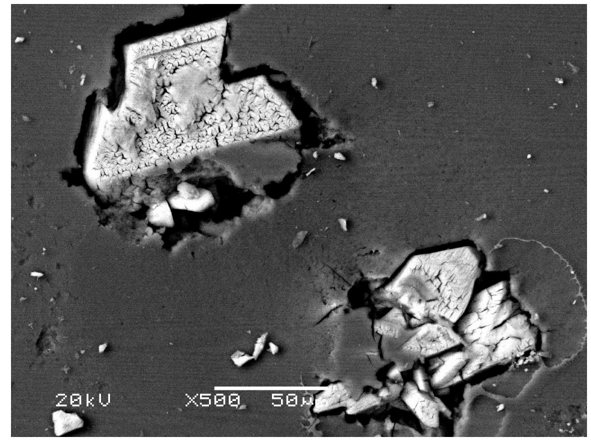
What can be learned from the matrix material (everything but the organism)?

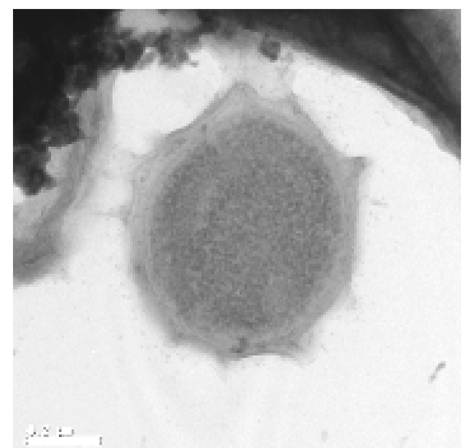
What can be learned from the organism itself?

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

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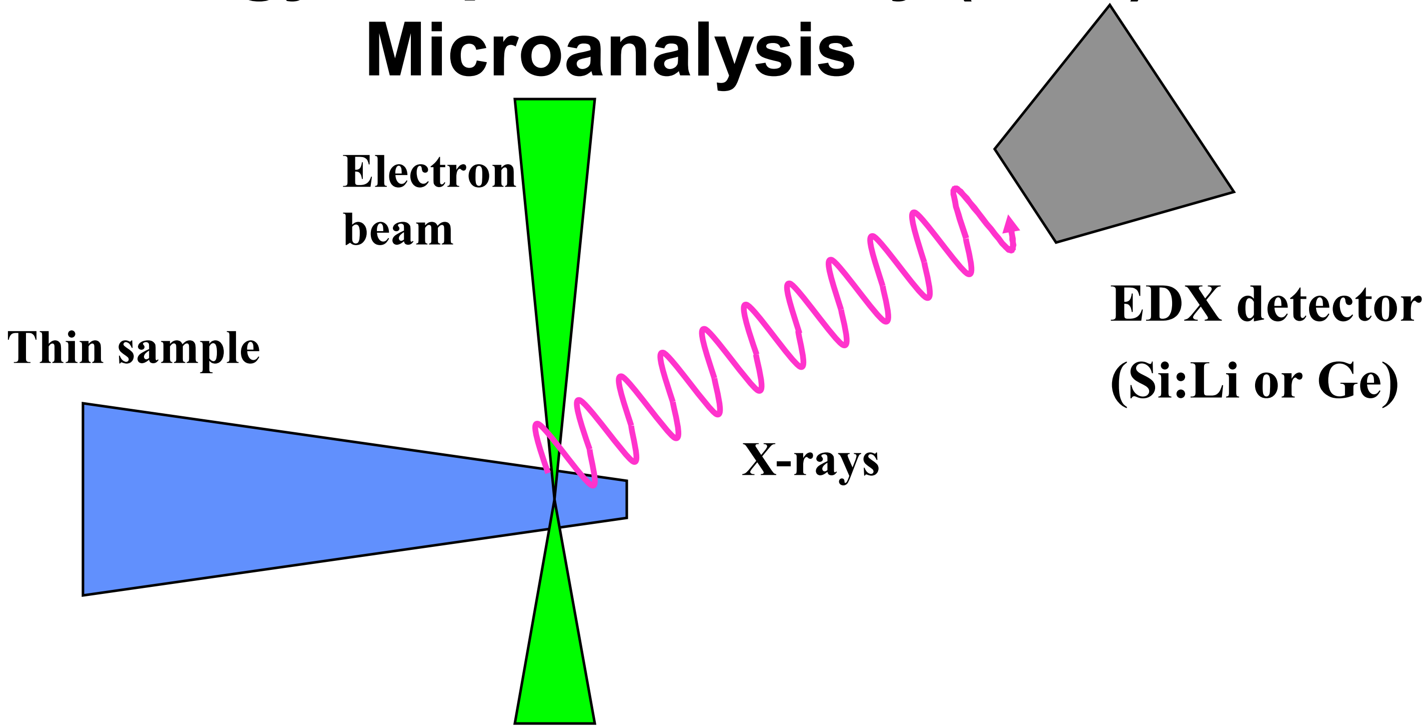
Energy Dispersive X-ray (EDX) Microanalysis



- The high energy beam (100-300keV STEM or <30keV SEM) generates characteristic x-rays based upon the element present in the sample
- For thin samples (STEM) high spatial resolution achieveable-~5nm
- For thick samples (SEM) much less spatial resolution (>1μm)
- Each point on the sample results in an EDX spectrum with peaks from the elements present at that point

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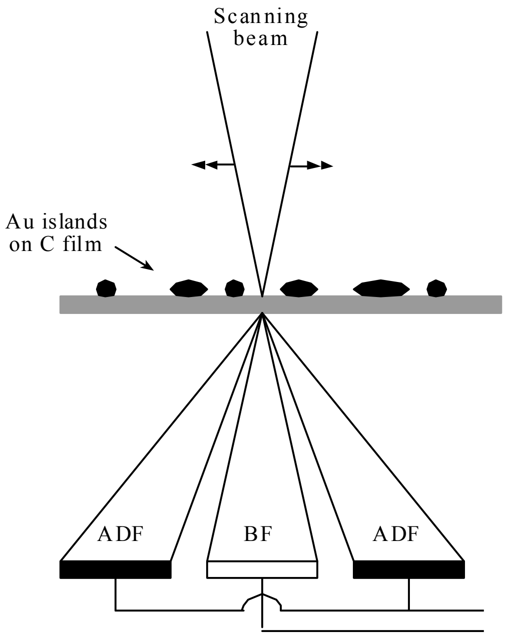
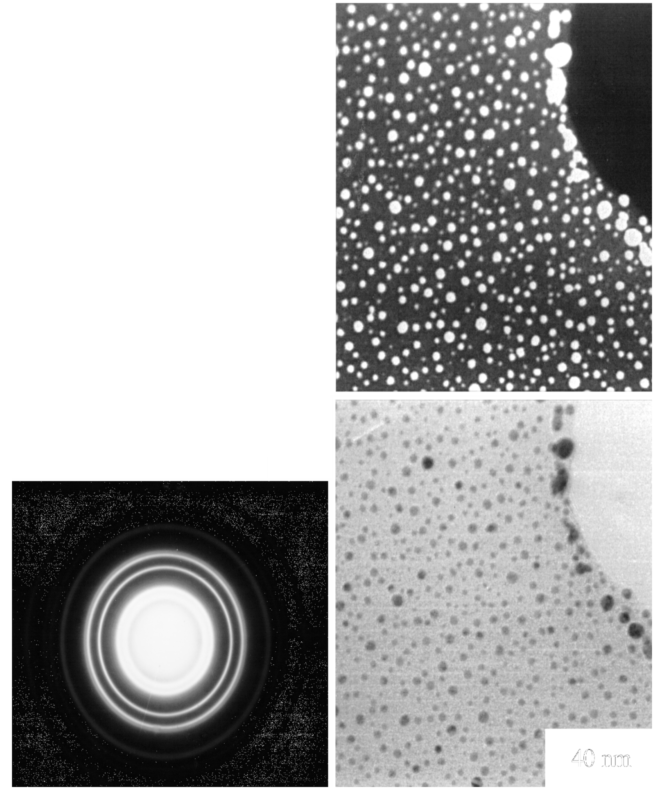
Annular Dark Field Imaging—”Z contrast”





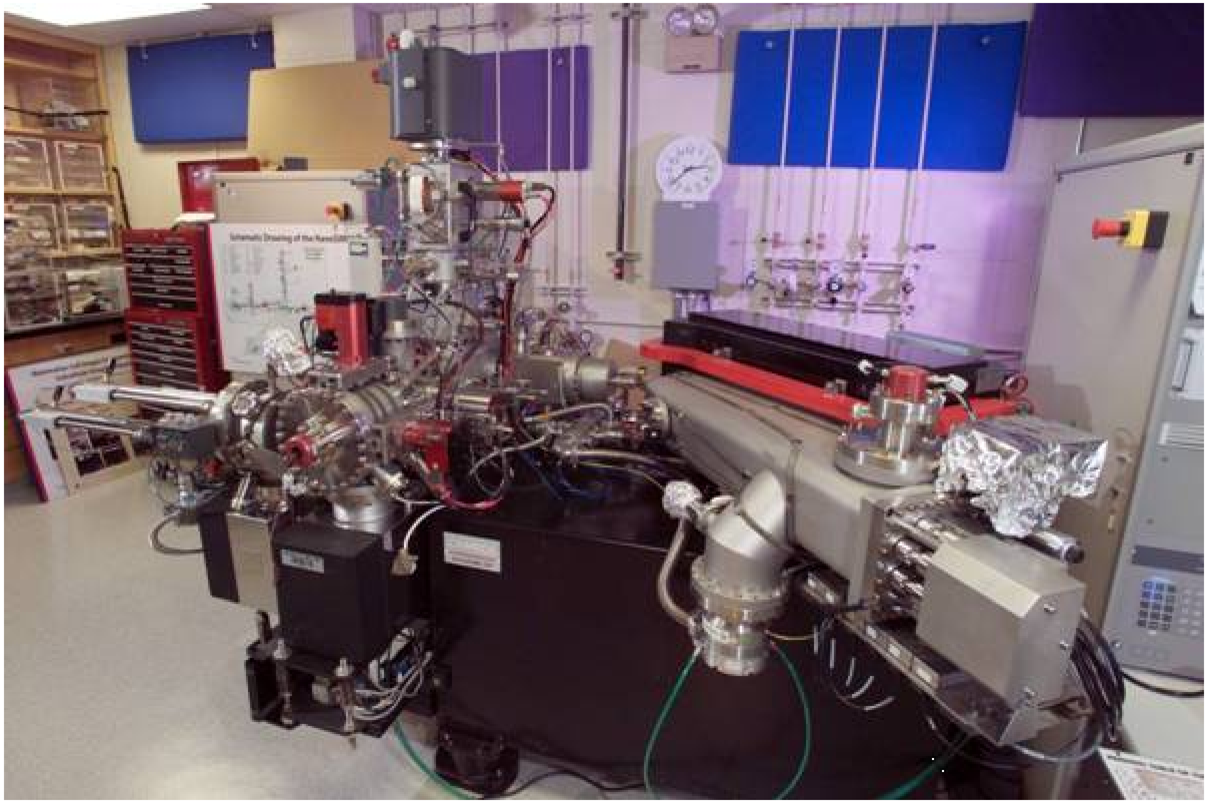
DF

BF

- Electrons that are scattered out to high angles (>50mrad) are “incoherently” scattered—scattering depends upon the Z of the material
- A high-angle annular dark field detector is used (HAADF)
- This is a most useful signal for STEM, because the interpretation of bright and dark is quite straightforward

NanoSIMS—combining high spatial resolution with high elemental sensitivity

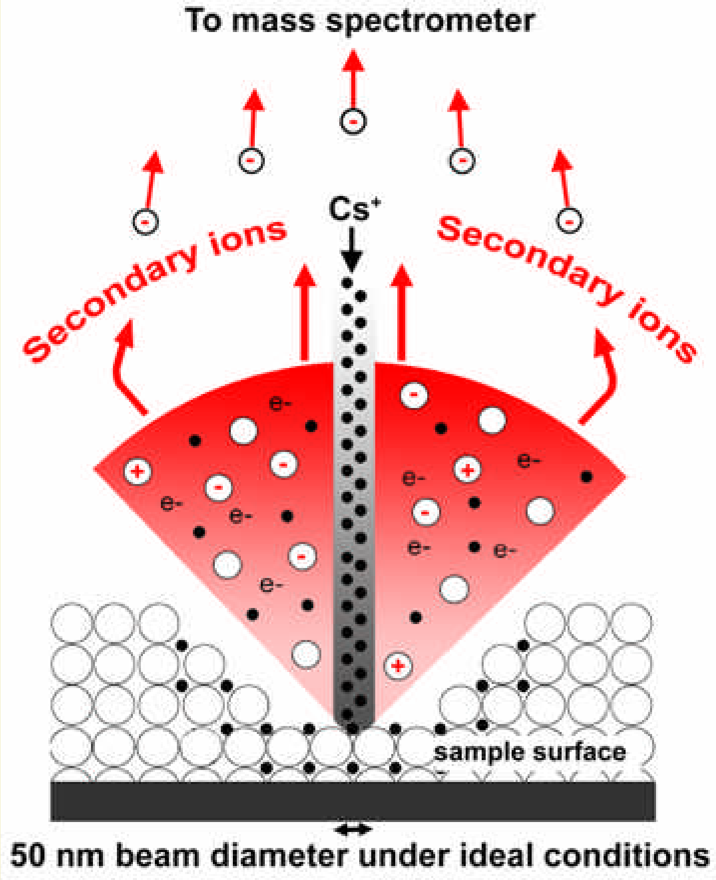
- Trace element and isotopic characterization at sub-micron scale
- Magnetic sector with fixed detectors
- Target specific species for quantification

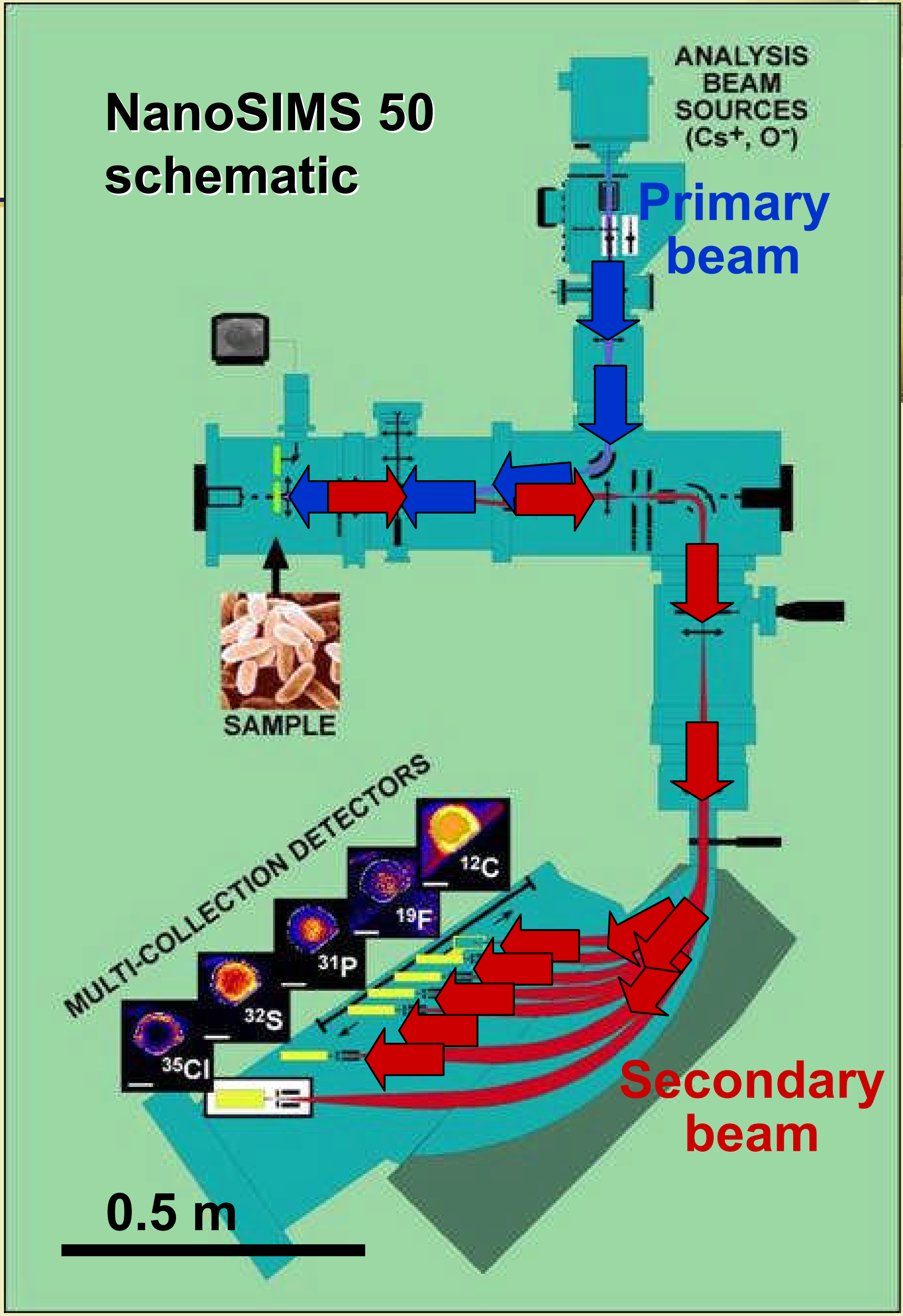


LLNL NanoSIMS Laboratory

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





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

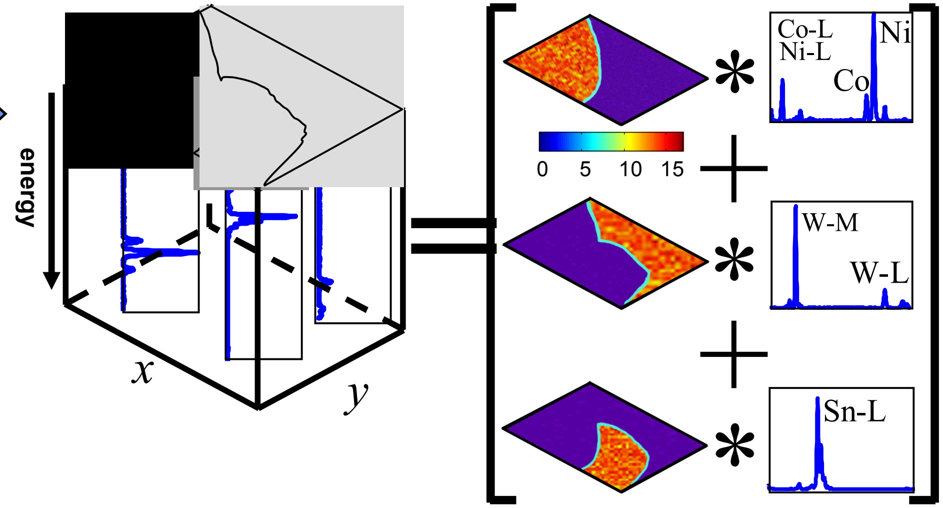
Spectrum imaging-capable instrumentation

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

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Automated method for spectral image analysis†

- **Assume the data follow a linear additive model**

  $D = CS^T$

  D is an *m*-pixel × *n*-channel spectral data matrix

  S is an $n \times p$ matrix containing the *p* pure-component spectra

  C is an $m \times p$ matrix containing their spatial distributions

- **Scale the data matrix to account for non-uniform noise (e.g. counting statistics)***
- **Perform an eigenanalysis to determine the number of components *p* to retain in the model**
- **Determine the scaled C and S using MCR-ALS**
- **Inverse scale C and S**

† P.G. Kotula, M.R. Keenan and J.R. Michael, *Microsc. Microanal.* **9**[1] (2003) 1-17, M.R. Keenan and P.G. Kotula, **U.S. patents #6,584,413 and 6,675,106**

* M.R. Keenan and P.G. Kotula, *Surf. Int. Anal.* **36** (2004) 203-212.

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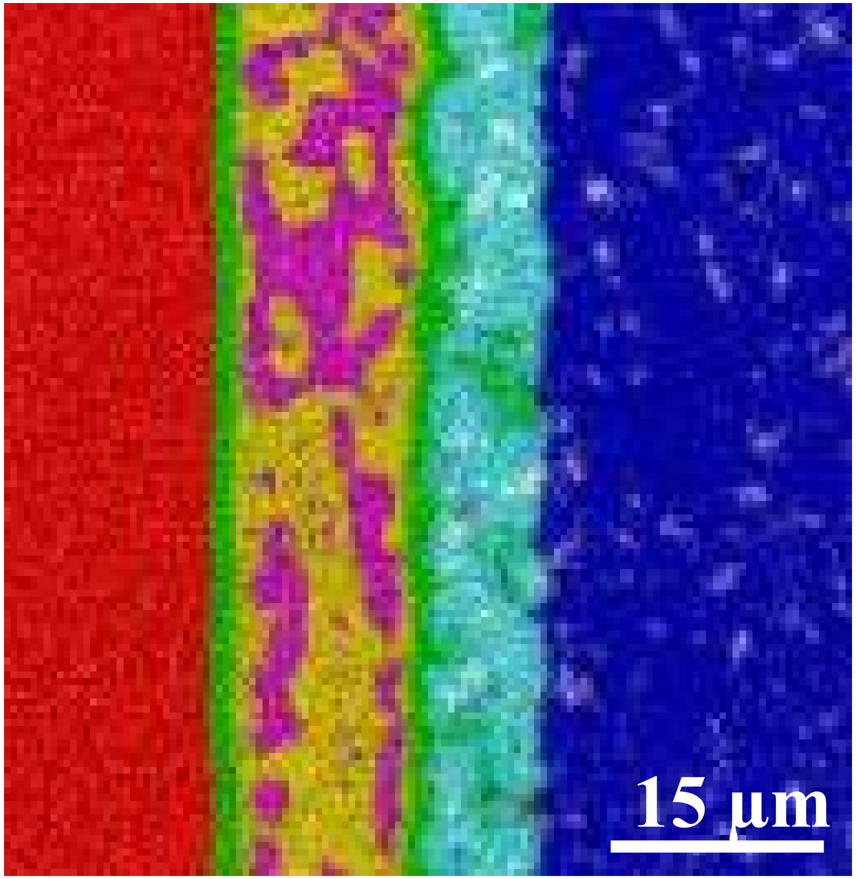
Automated analysis of a Braze: RGB composite

Cu = red
Ni-P = green
Al_2O_3 = blue
Bi = magenta
Sn = yellow
Mo/Pb = cyan
glass = white



10 minute acquisition
5 second calculation

Data courtesy of David Rohde, Thermo NORAN

NORAN Vantage DSI (Digital Imaging with Spectral Imaging)
JEOL JSM-840 W filament, 20kV, $10mm^2$ detector (138eV),
128x128 pixels, 36 frames@1msec/pixel, 50% Dead time

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Courtesy of P. Kotula

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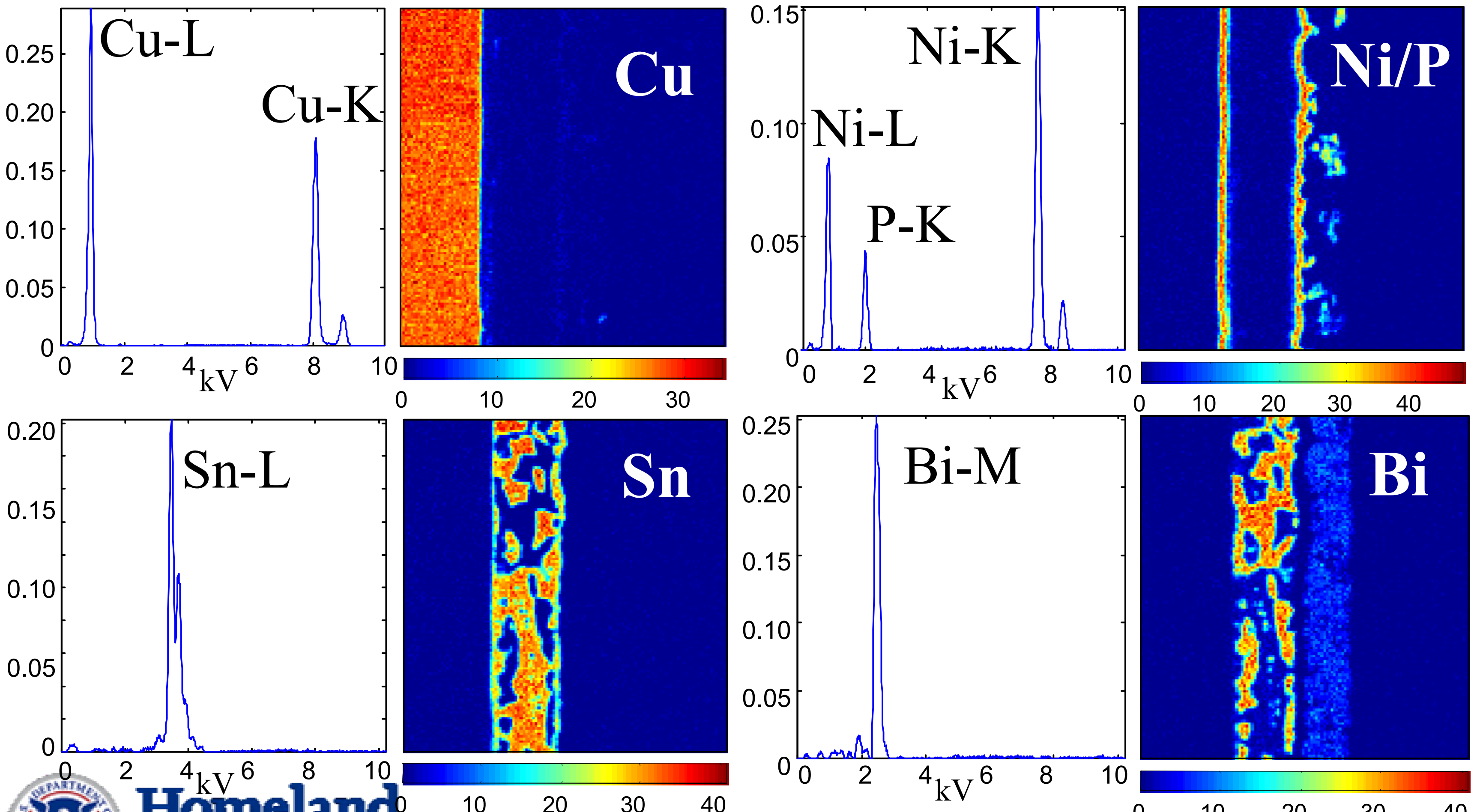
Automated analysis of a Braze Interface: Pure component images and spectra



Courtesy of P. Kotula

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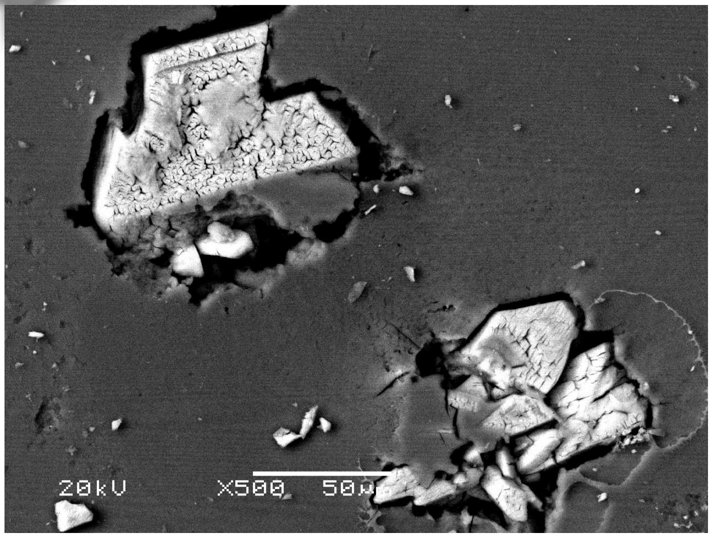
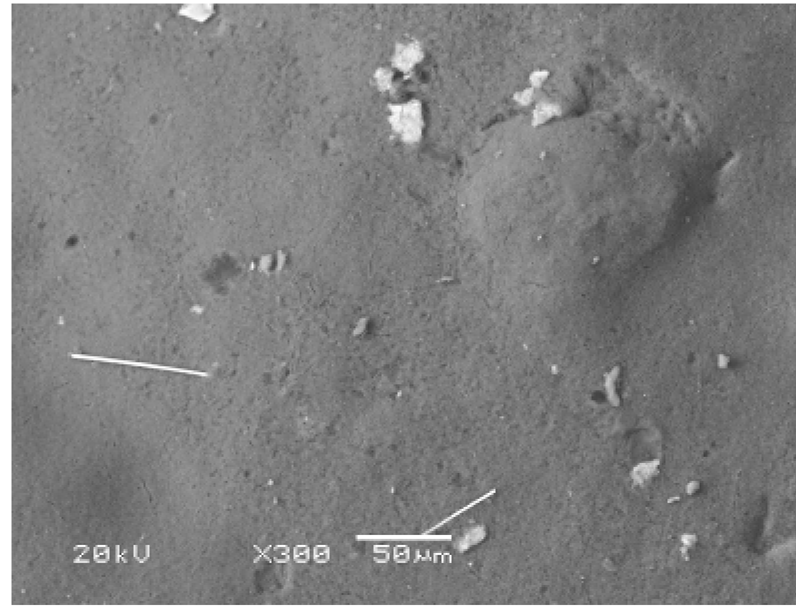
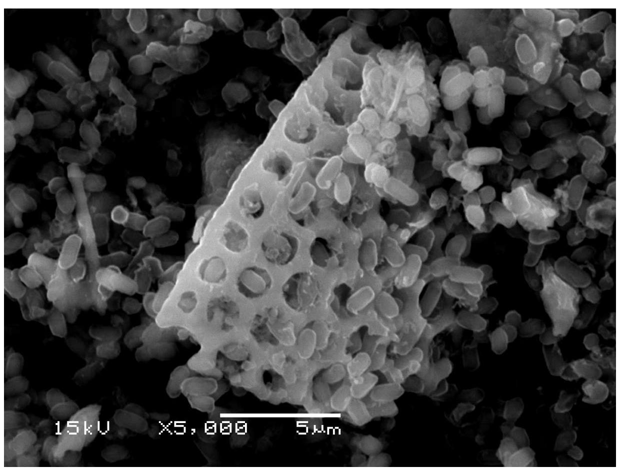
Microanalytical Signatures from Matrix Material

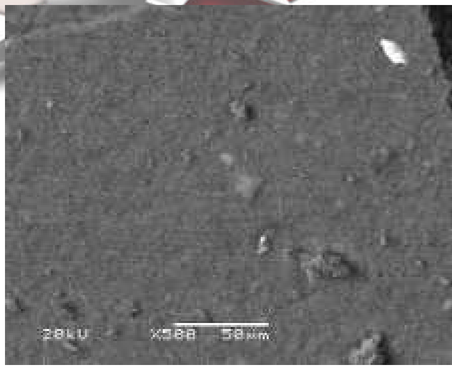


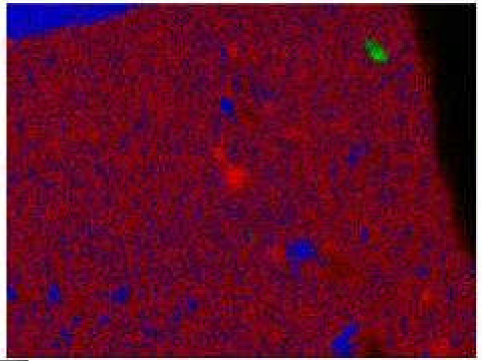


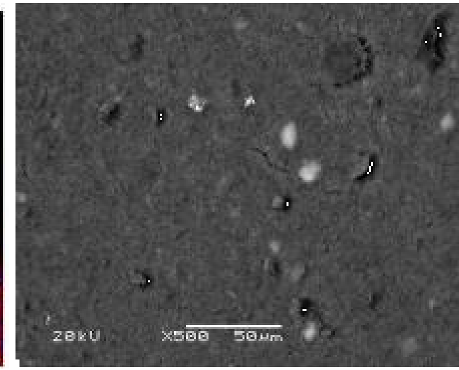


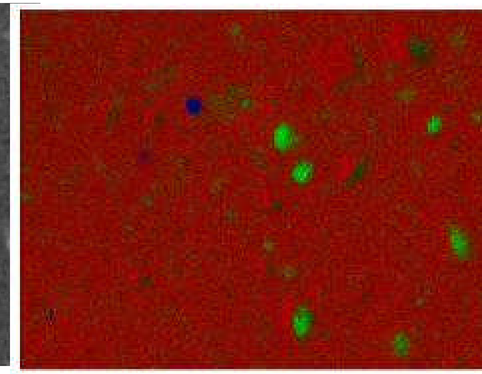
What do these “bits and pieces” tell us about sample production?

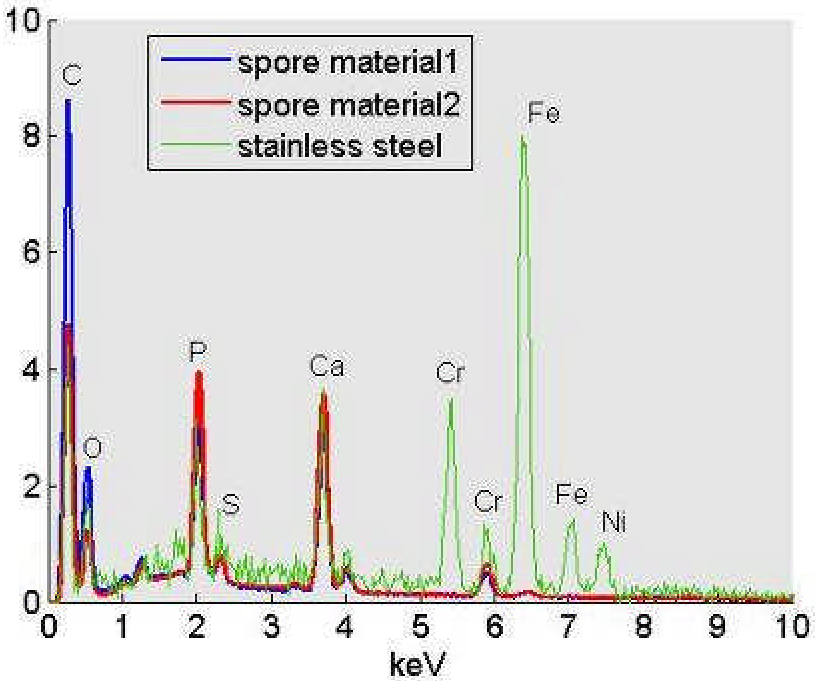
Transition Metal Signatures from the matrix material

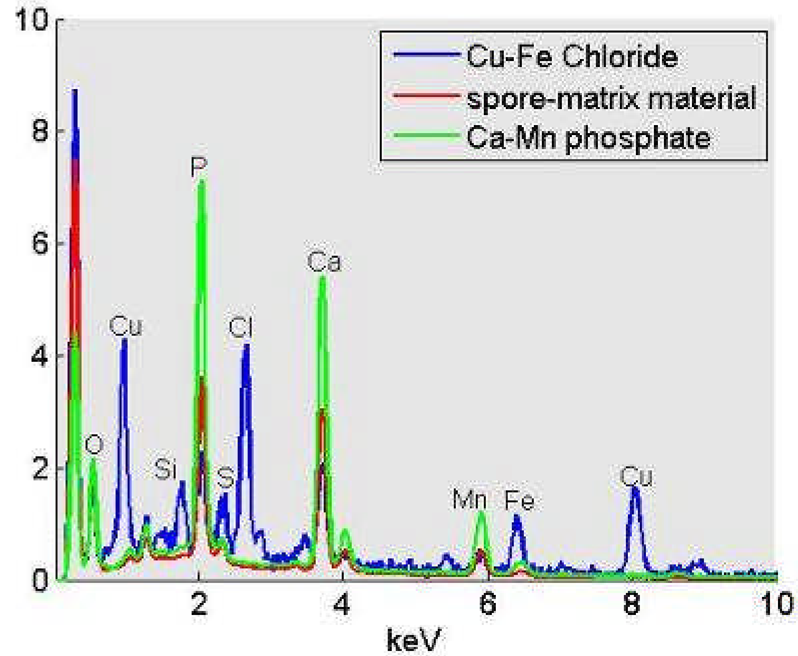












Identification of stainless steel particles and metal chlorides can provide information about the processing equipment and chemicals.

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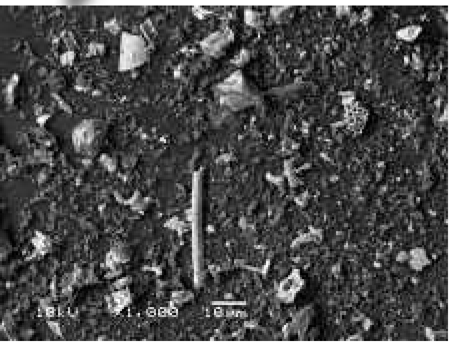
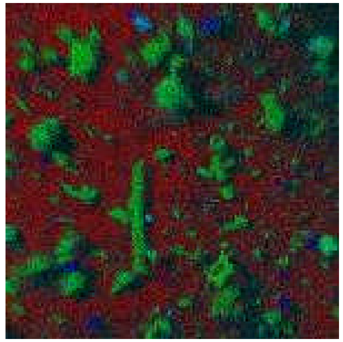
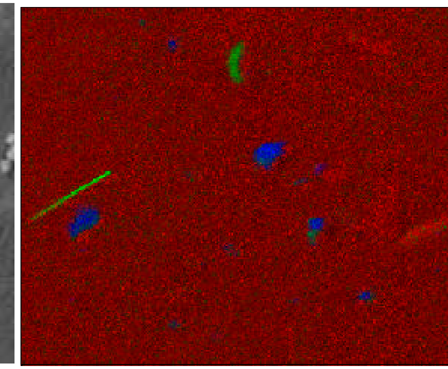
Si signatures from matrix material

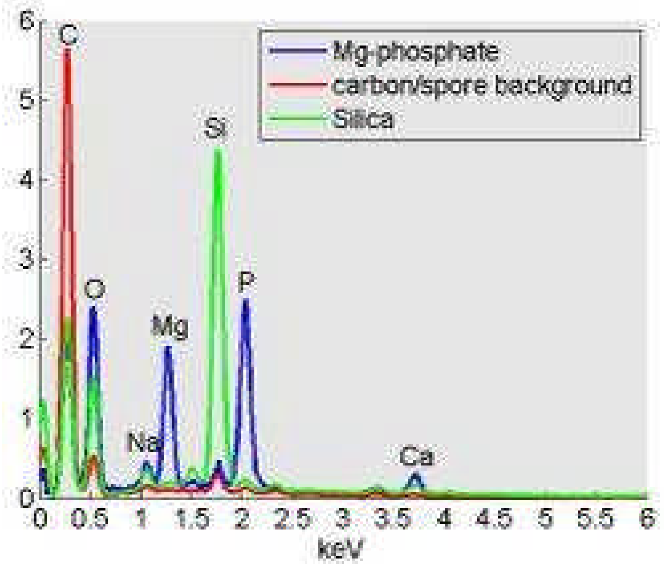
Sample "J"

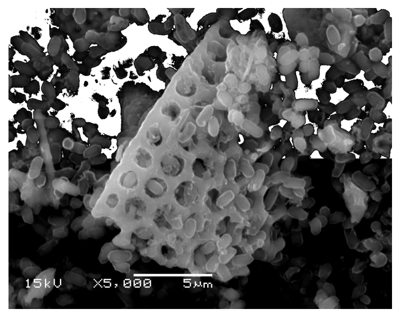


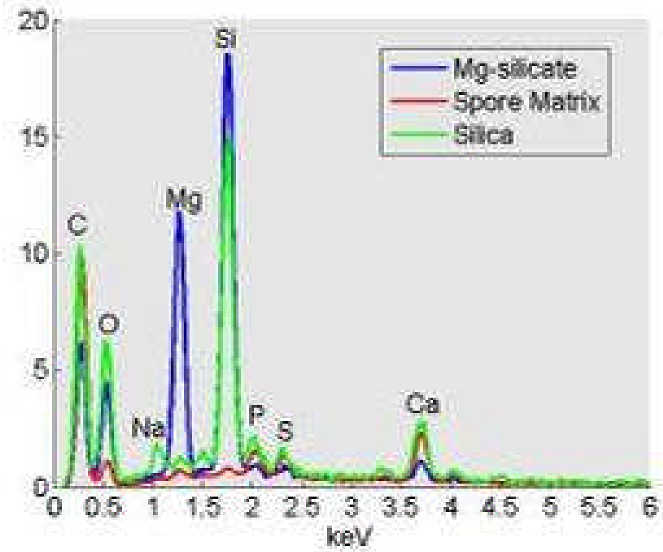


Sample "G"



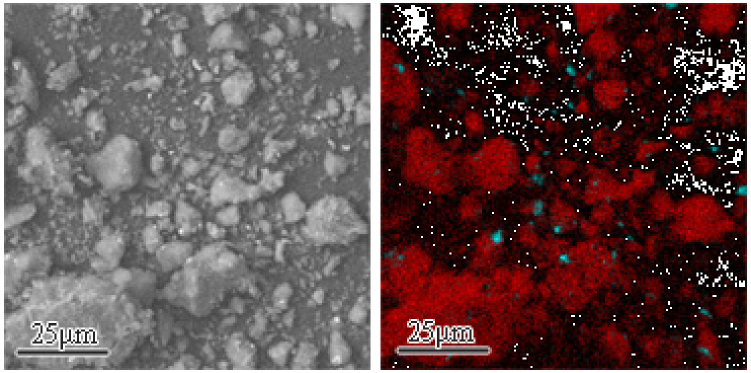


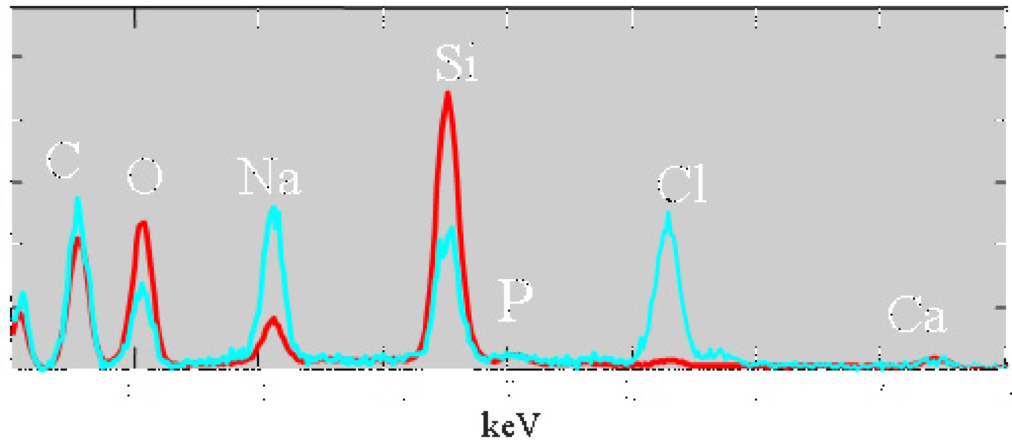




Silicates present themselves in many different forms in the matrix material.

The importance of length scale in describing the matrix material…





Sample K

- Si signal appears to be everywhere in SEM data.
- Discrete NaCl particles visible
- Ca and P signals (spore and buffer signatures) relatively minor
- Preliminary conclusion…we have NaCl particles but we can't say anything about the nature of SiO_2—"it's there"

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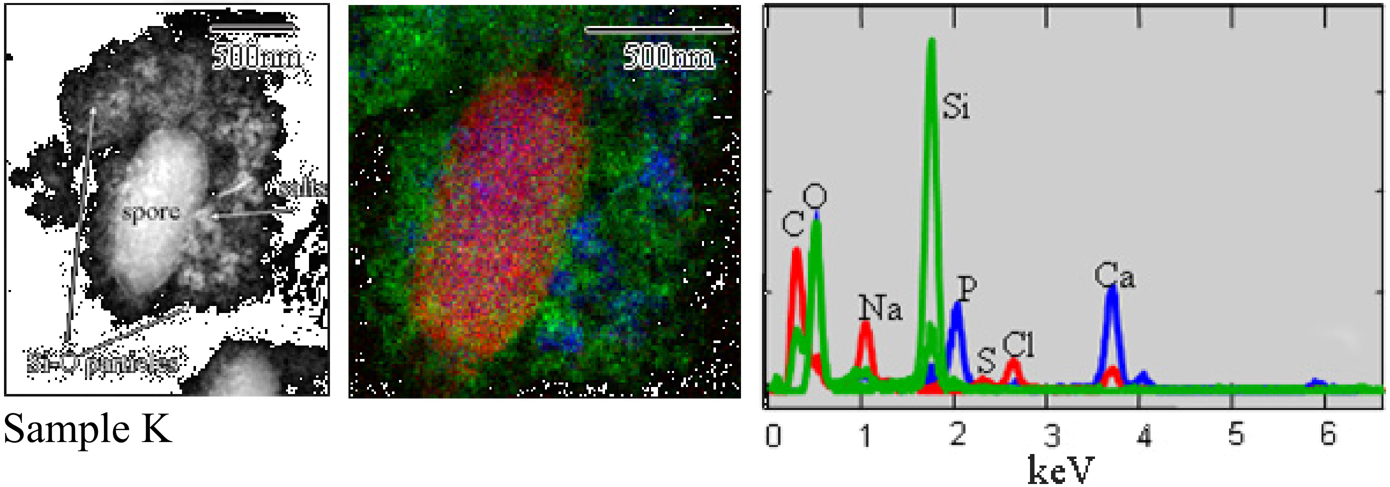
Matrix material at the nanometer length scale (STEM-EDX)

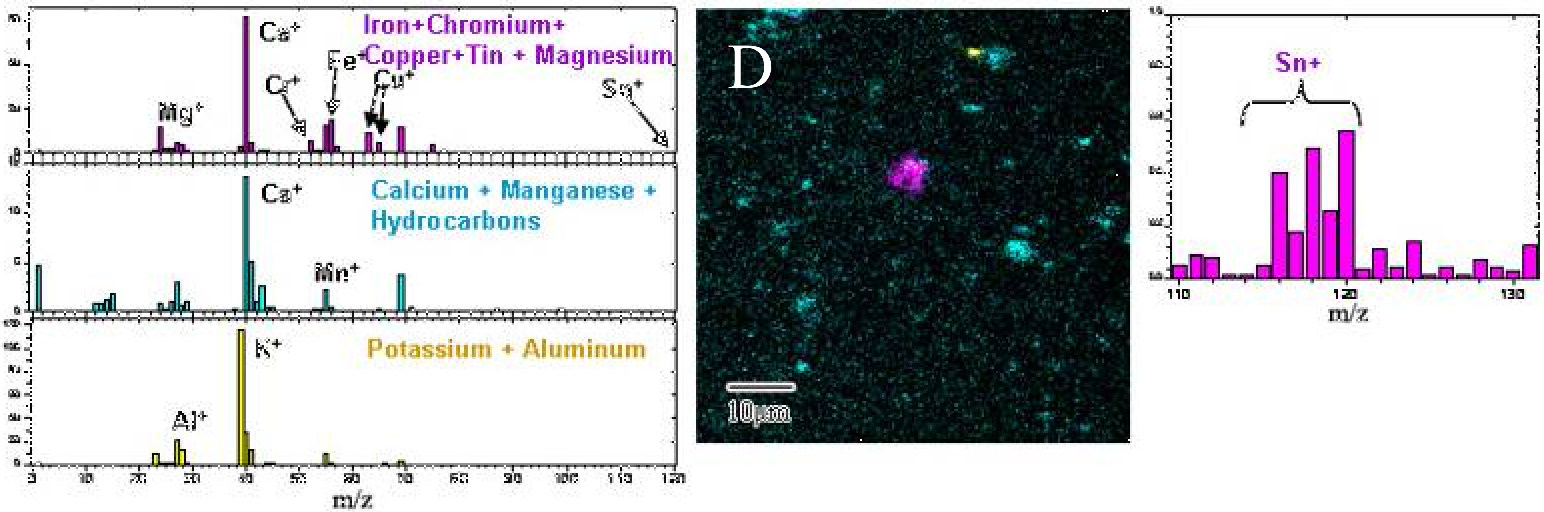


Sample K

- We now see discrete SiO_2 particles—well below 100nm
- Discrete Ca-phosphate particles—not seen in SEM data at all.
- Na and Cl in elemental signature but associated with spore
- The particles we identify depends upon which data set we are using

Length scale is really important—wrong conclusions can be drawn by neglecting analyses at either micro and nano length scales

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…)

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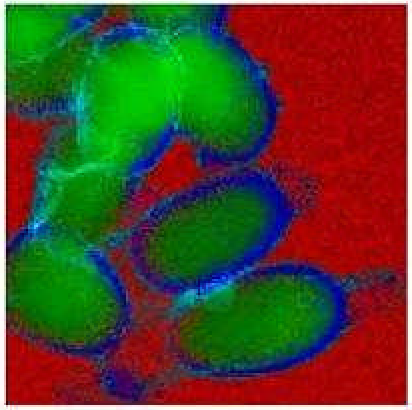
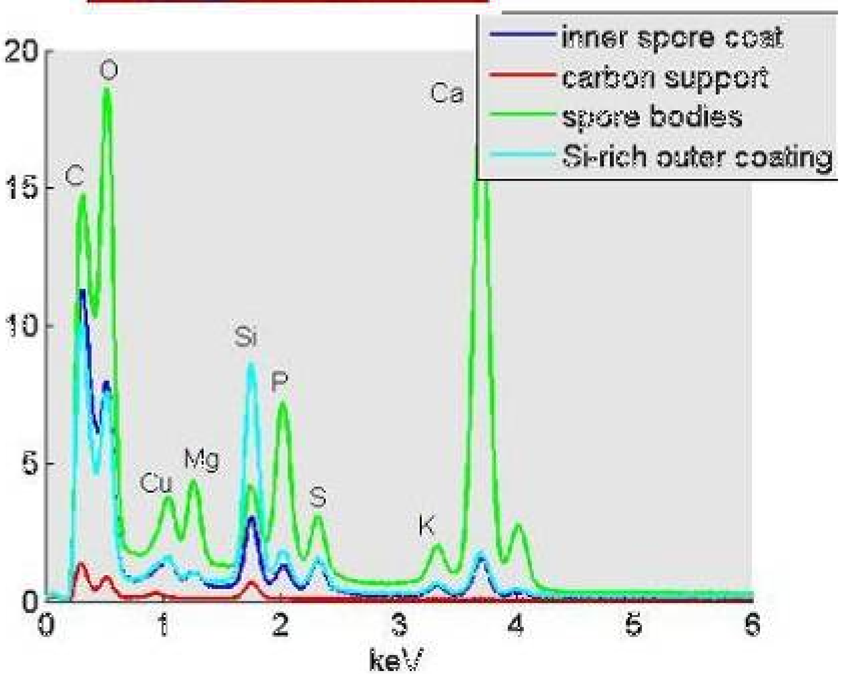
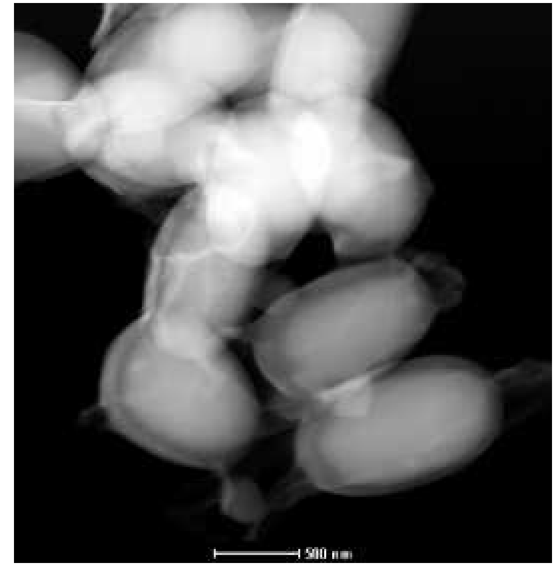
Elemental Signatures from the organisms





Sample "H"



•Different portions of the spore have different compositions.

•Note the high level of Si in the outermost coating.

•We can use changes in these compositions to characterize changes in process.

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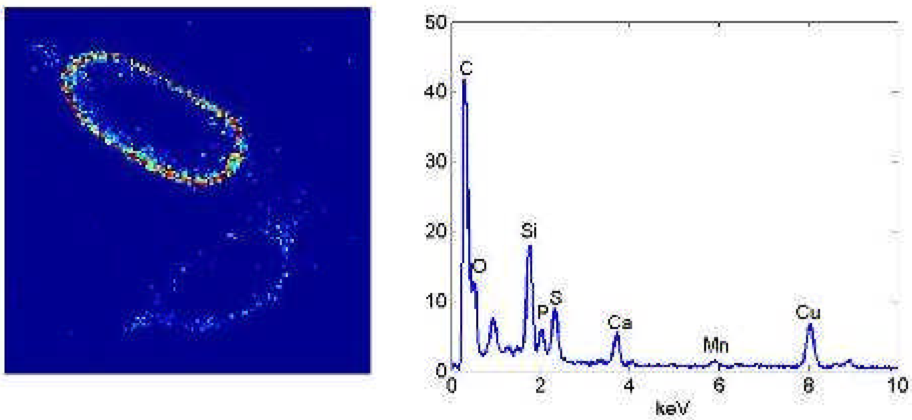
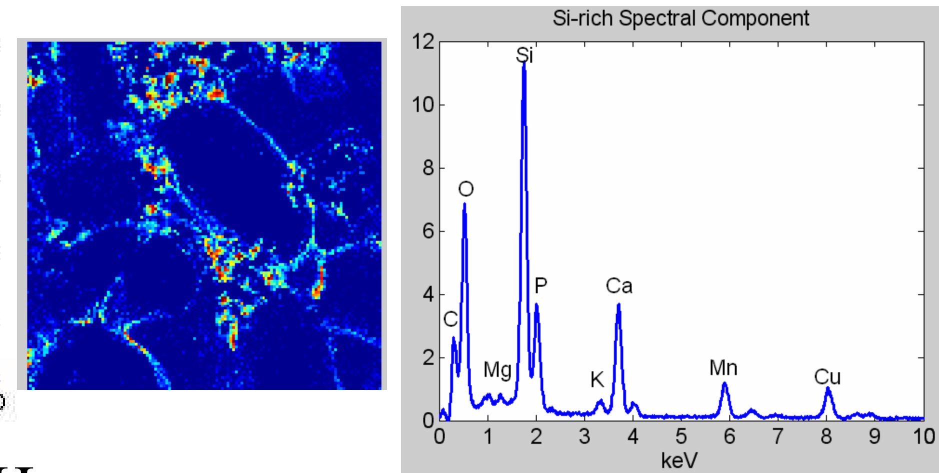
Comparison of Si-rich Coating Component Spectra

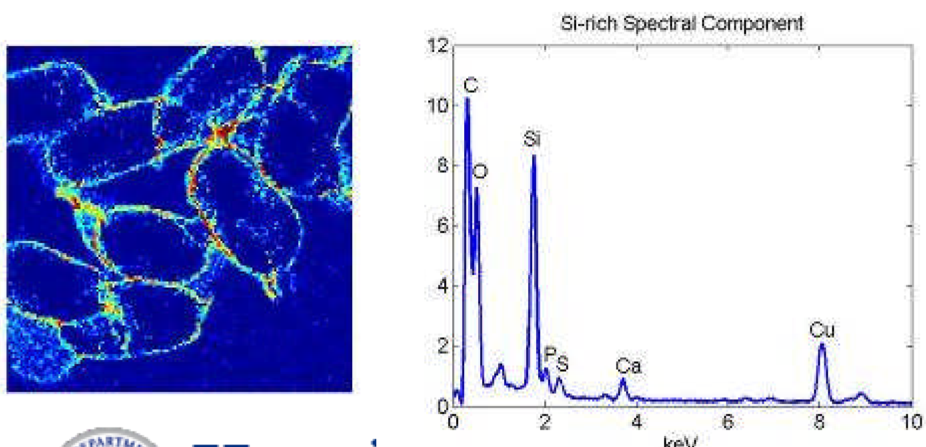
A



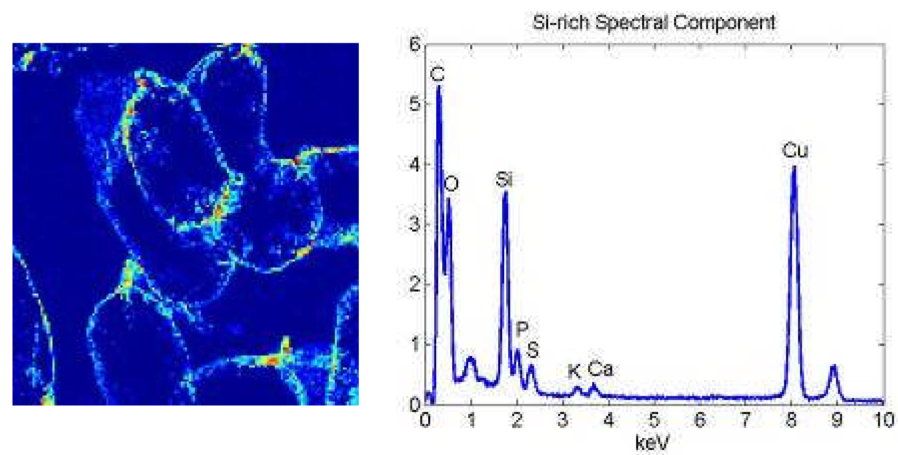
E



F



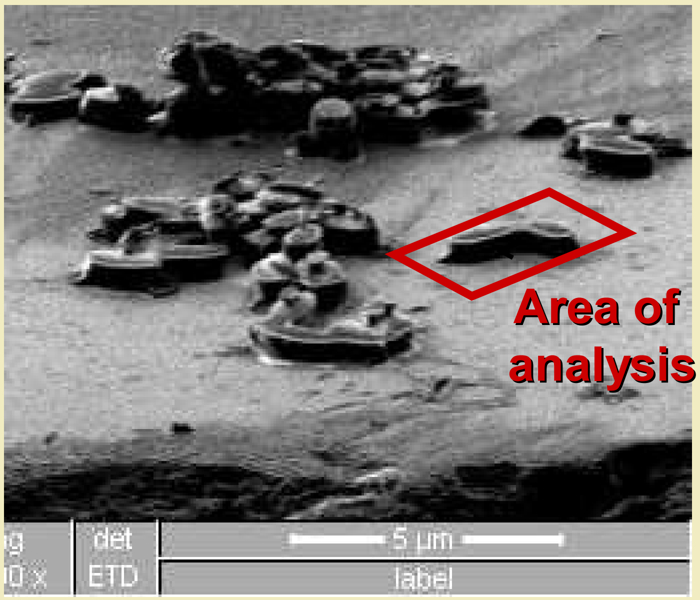
H



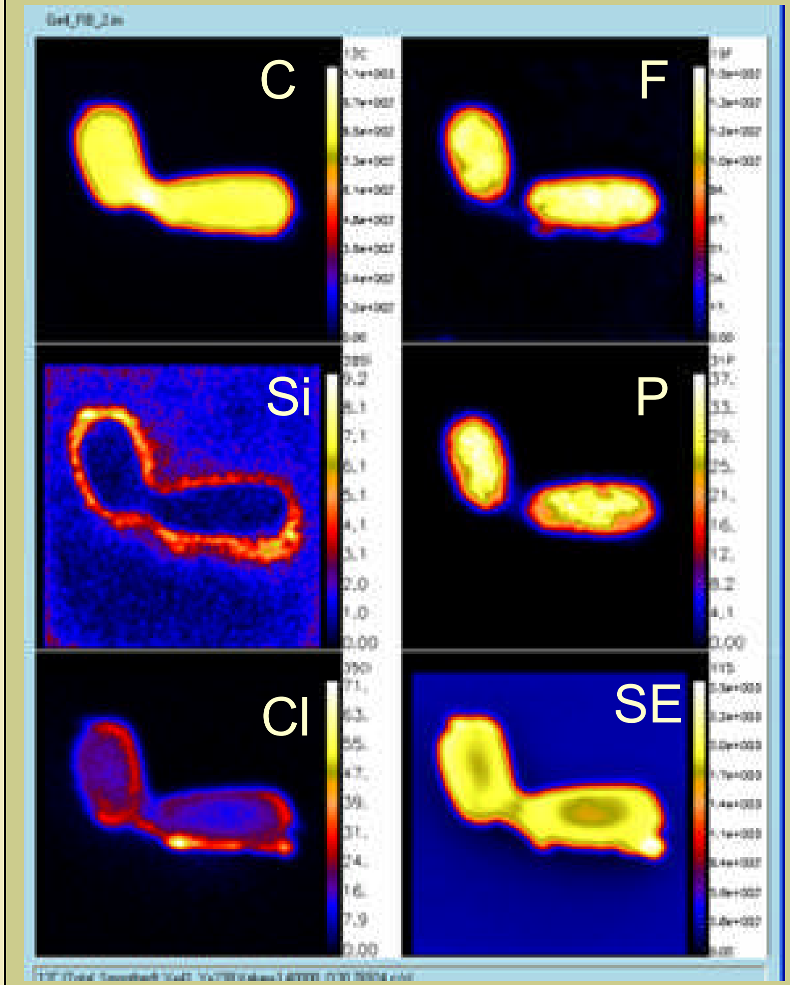
Note that all of these processes generate Si-rich coatings of <u>different compositions…</u>

NanoSIMS Results: Quantitative Digital Images

- Data processed pixel by pixel with custom software

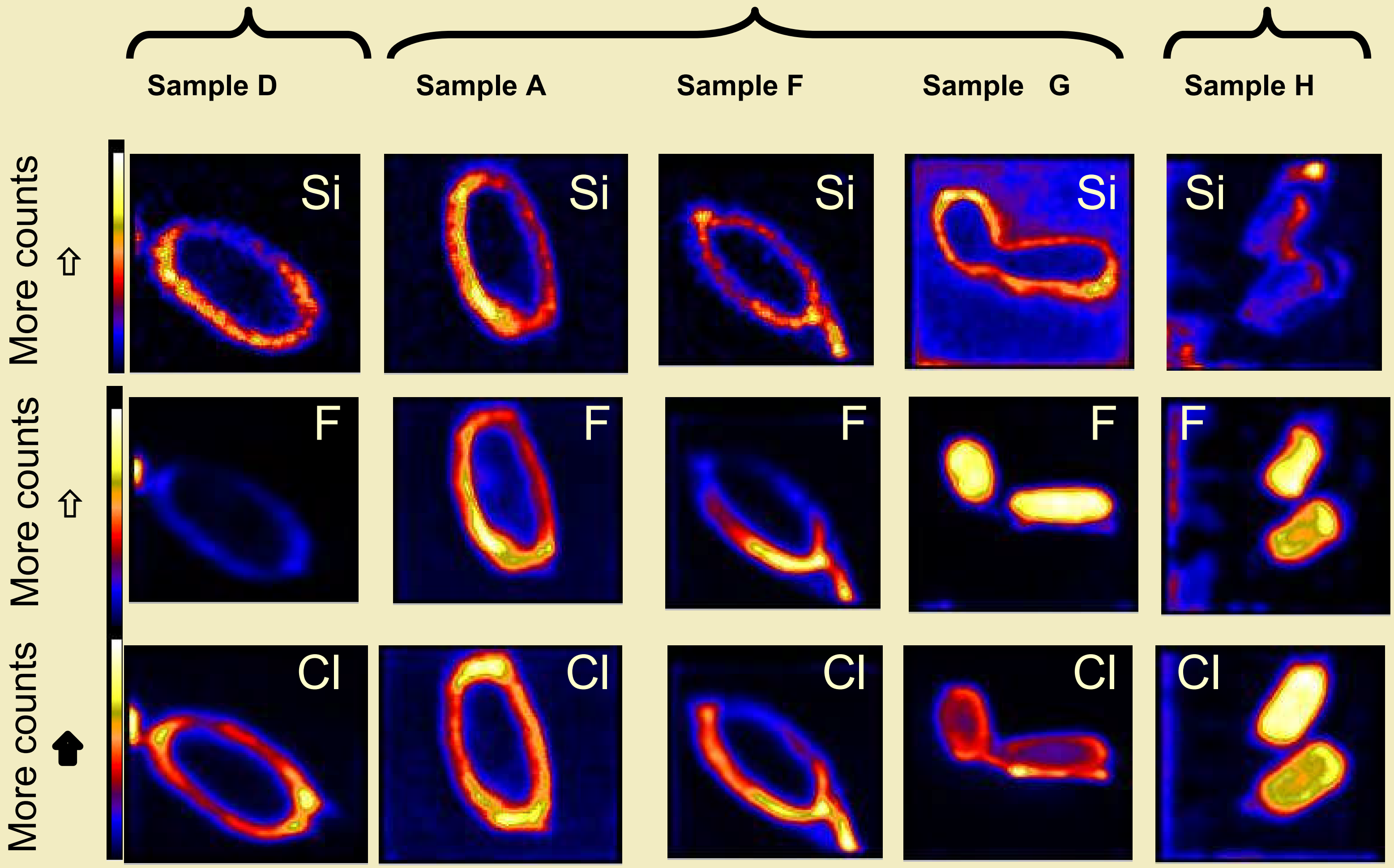


Oblique view of FIB section of spores



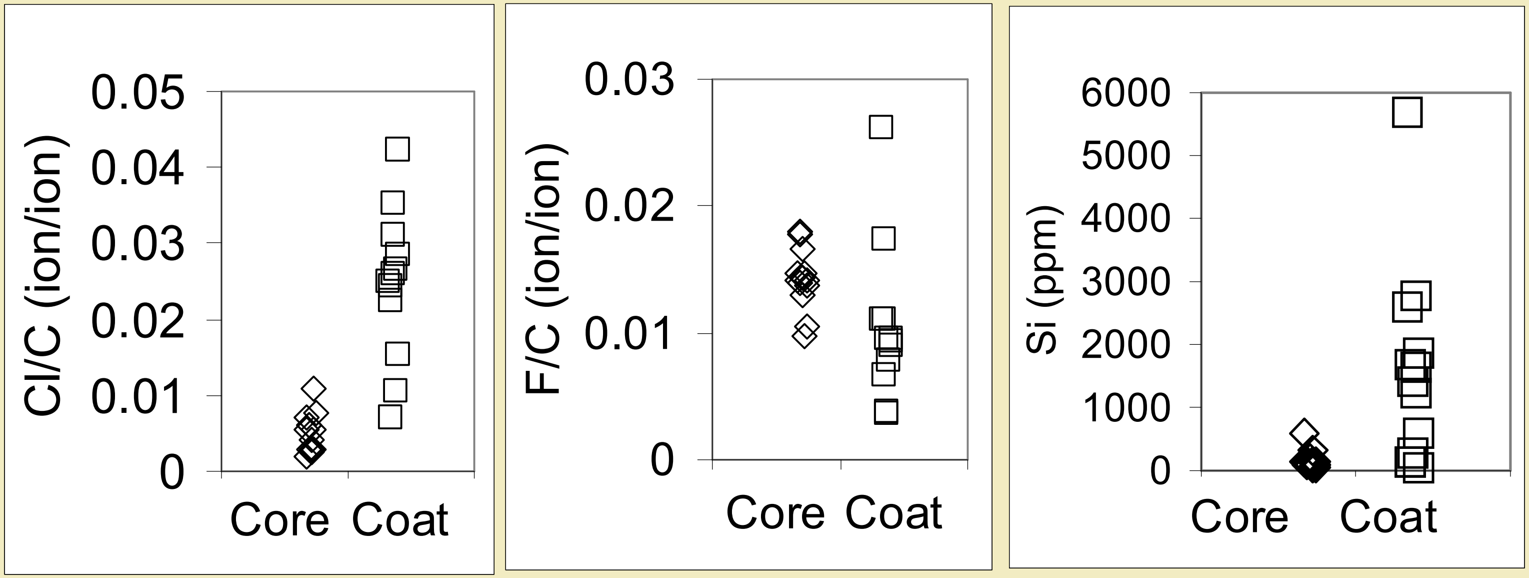
NanoSIMS ion images for sectioned *Bti* spores

Elemental distributions differ with production factors



NanoSIMS provides a quantitative basis for sample comparison

- **High sensitivity of NanoSIMS provides a clear picture of microstructural signature variability**



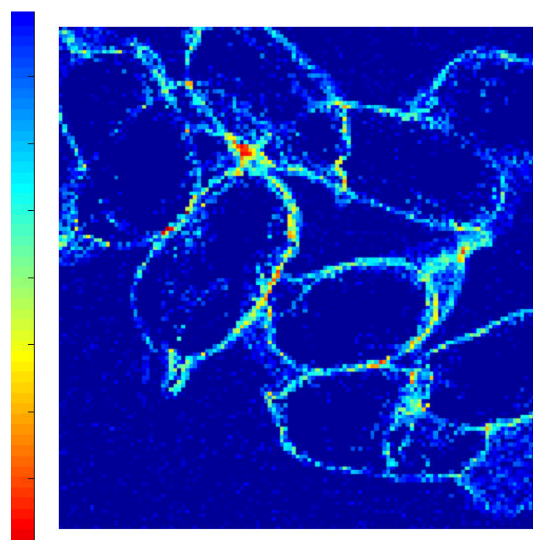
RSD = 50%, 40% **RSD = 20%, 60%** **RSD = 90%, 100%**

Elemental heterogeneity within a single sample of spores (G)

Note: Cl and F data are raw ion yield; Si concentration calculated based on RSF.

Summary

- Microanalytical techniques from the physical sciences are potentially very useful in combination with biological data for bioforensics investigations.
- Important elemental signatures can be obtained from both the matrix material and from the organisms themselves
- Multivariate statistical analysis plays an important role in rapidly analysing large amounts of data
- For critical analyses, acquiring data at multiple length scales and sensitivity levels is crucial.



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