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Symposium on Safe Handling of Engineered Nanoscale Materials

Exposure Assessment: Advance Analytical Techniques

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July 8, 2008

What are the goals?

- To provide guidance that will help the NSRCs (and industry) develop site-specific controls that will protect workers and the environment.
- Offer reasonable guidance for managing the uncertainly associated with nanomaterials whose hazards have not been determined.
- Reduce to an acceptable level the risk of worker injury, worker ill-health and negative environmental impacts.

What are the issues with respect to nanoparticle measurement?

- Sampling and analysis protocols have not been established for nanoparticles.
- Nanoparticle measurement is evolving.
- New analytical technology is available.
- Can these new technologies be used in a cost effective manner?
- How do we guide the NSRCs (and industry) with respect to monitoring nanoparticles the workplace environment?

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"It is very easy to answer many of these fundamental biological questions; you just look at the thing!"

--- Richard P. Feynman – 1959
"There's Plenty of Room at the Bottom"

How do we proceed forward?

- Learn from previous experiences
  - If we don't pay attention to the past, we are likely to make the same mistakes in the future
- The ambient air sampling experience
  - The evolution from TSP to $PM_{2.5}$ and beyond
- The asbestos experience
  - Single crystal ceramic whiskers
- Base initial nanoparticle sampling and analysis protocols on established methodologies
  - Modify as new information becomes available
  - Realize that this is an evolutionary process

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TSP

PM_{10}

Speciation Sampler

Mini-Vol

E-bam Continuous $PM_{2.5}$ Sampler

UNC Passive Sampler

The Asbestos Experience

- Use asbestos sampling and analysis protocols as a guide in the development of nanoparticle sampling and analysis methodologies
  - Asbestos is the first industrial “nano” particle
    - Asbestos fiber diameters are typically < 50 nm
- Sampling and analysis methodologies have evolved throughout the years as technology advanced and more information became available.

Asbestos Analyses

- Measurements have been made of asbestos exposure for over 80 years
- Initial Samples Collected using impingers, konimeters
  - Relied on hand operated pumps
  - Used a water or alcohol bath to trap airborne particles
    - Analyses of collected samples performed using light microscopes
      - Counted visible particles, not necessarily fibers
      - Could not differentiate asbestos from other fibers

Asbestos Analyses

- To improve the robustness of asbestos analyses, two developments occurred in the 1950's and 60's
  - Safe, light-weight battery operated pumps were developed
  - Efficient filter media were created and tested
- Asbestos analytical protocols were standardized in the late 1960's and early 1970's with the development of the phase contrast microscopy (PCM) procedure
  - Precision of the analysis standardized by restricting the size of fibers to be counted
  - Precision improved by standardizing the specification of the PCM (resolution, magnification, etc.)

Asbestos Analyses

- Initial asbestos testing occurred in industries where commercial asbestos was used (textile, cement, friction products, etc.)
  - Errors associated with assuming any counted fiber to be asbestos were minor
- Later testing in other industries where asbestos was a minor component of the airborne particulate led to the development of electron microscopy methods

Asbestos Analyses

- Environmental testing in the late 1970's was performed using non-standardized transmission electron microscopy (TEM) techniques
  - Sample preparation procedures at one lab differed from another lab
  - The size of counted particles and the degree to which they were identified also varied from lab to lab

TEM Asbestos Analyses

- TEM procedures were standardized beginning in 1978 (US EPA) with later modifications in 1984
- The EPA promulgated a “cleanliness” procedure in 1987 (AHERA)
- OSHA issued their version in 1991 (NIOSH in 1989)

TEM Asbestos Methods

- The TEM methods specify:
  - Analytical magnification
  - Filter area to be analyzed
  - Acceptable sample preparation procedures
  - How and when to count a “fiber”
  - Minimum rules to define what is and what is not asbestos

Asbestos Analytical Methods

- All asbestos analyses produce an index of exposure
  - No method counts all asbestos fibers
  - Every method has internal limitations to improve the precision of the analysis
    - All have some fiber dimension limitation
    - Some require counting based on statistical sampling "frames" – other do not
    - Some require detailed counting of complex structures – for others, each complex structure counts as "1" fiber.

Asbestos analyses – what have we learned?

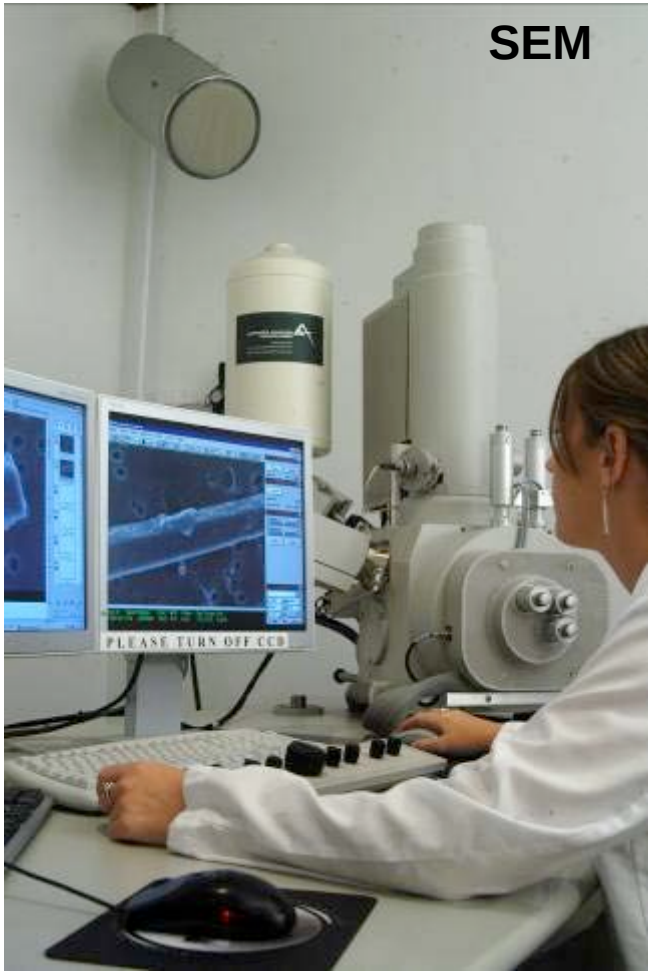
- Asbestos issues have improved the science of industrial hygiene
  - Improved sample pumps
    - Hand pumps to battery operated pumps to passive monitors
  - Improved sample collection media
    - Water/alcohol impingers to filters
  - Improved sample analysis
    - Optical and electron microscopy
  - Provided guidance in the sampling and analysis of other materials

Advance Analytical Techniques

Electron Microscopy

- Scanning electron microscopy
  - Field-emission SEM (FESEM)
- Computer-controlled SEM (CCSEM)
- Transmission electron microscopy (TEM)
  - Field-emission TEM
    - High resolution (HRTEM)
- Hybrid SEM/TEM
  - Hitachi S-5500
- Energy dispersive spectroscopy (EDS)
  - Silicon drift detectors (SDD)

Complementary TEM and SEM Techniques



Complementary TEM and SEM Techniques

TEM

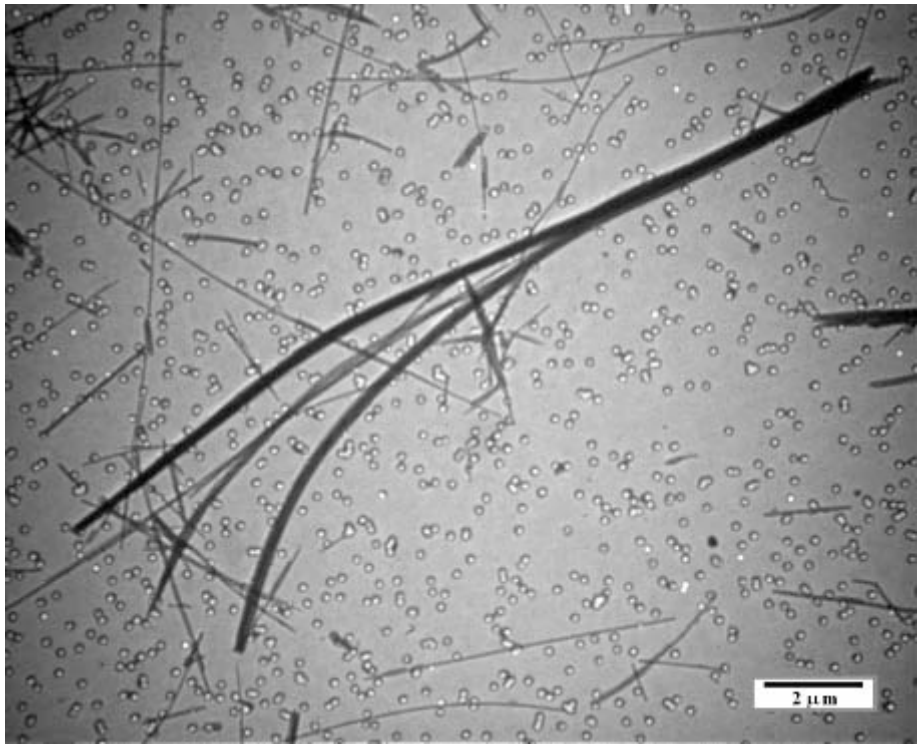
- Technique called-for under current asbestos regulations
- Provides projection image (no surface details and limited morphology)
- Provides chemistry (EDS)
- Provides crystallography (SAED)

SEM

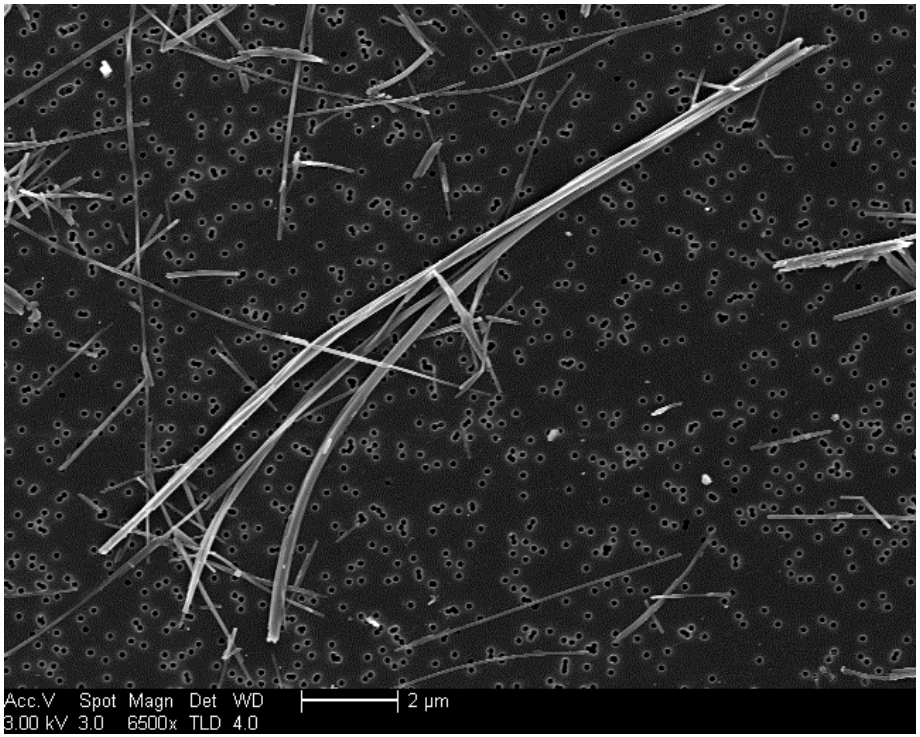
- FESEM provides comparable magnifications to TEM
- Allows observation of unique morphological features not seen in TEM
- Provides chemistry (EDS)
- Crystallography only with specialized equipment

Complementary TEM and FESEM Characterization

TEM Image



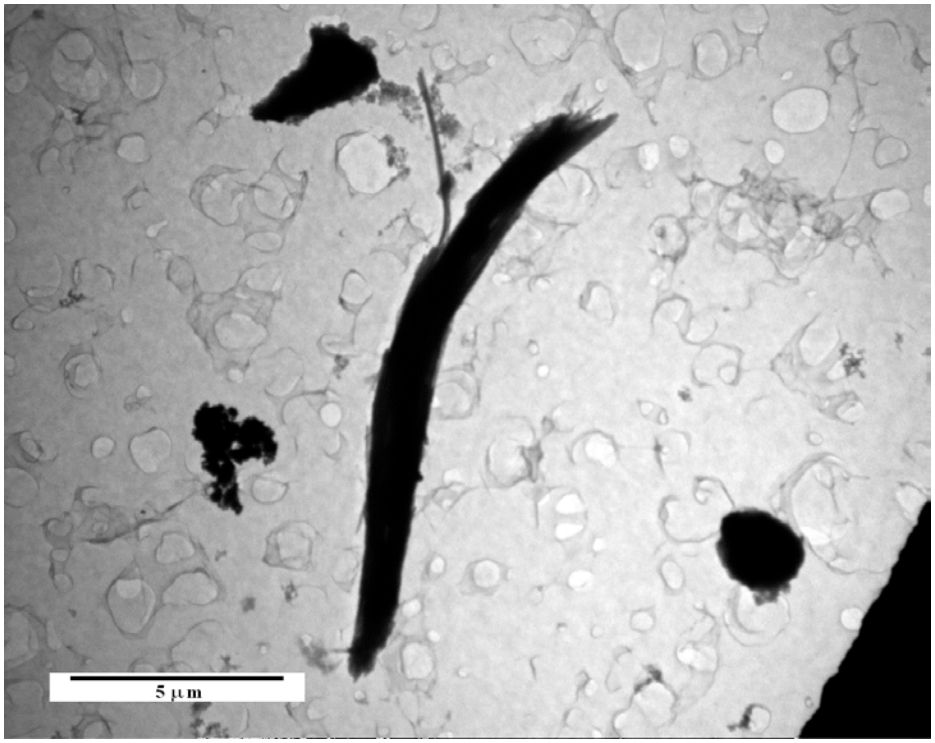
FESEM Image



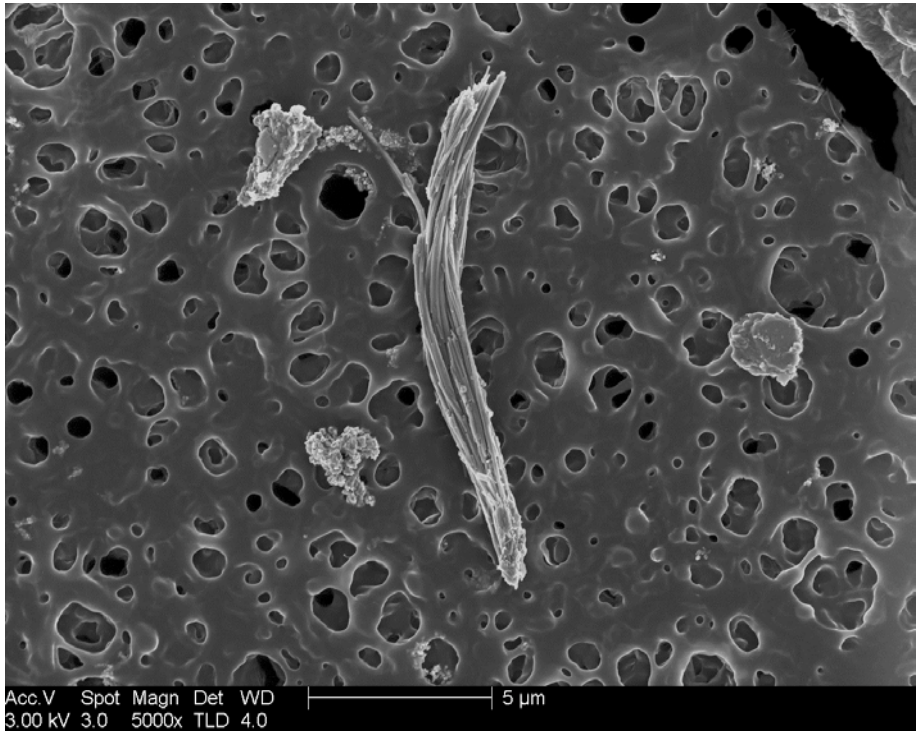
Comparison of TEM and FESEM images for a bundle of asbestos fibers

Complementary TEM and FESEM Characterization

TEM Image



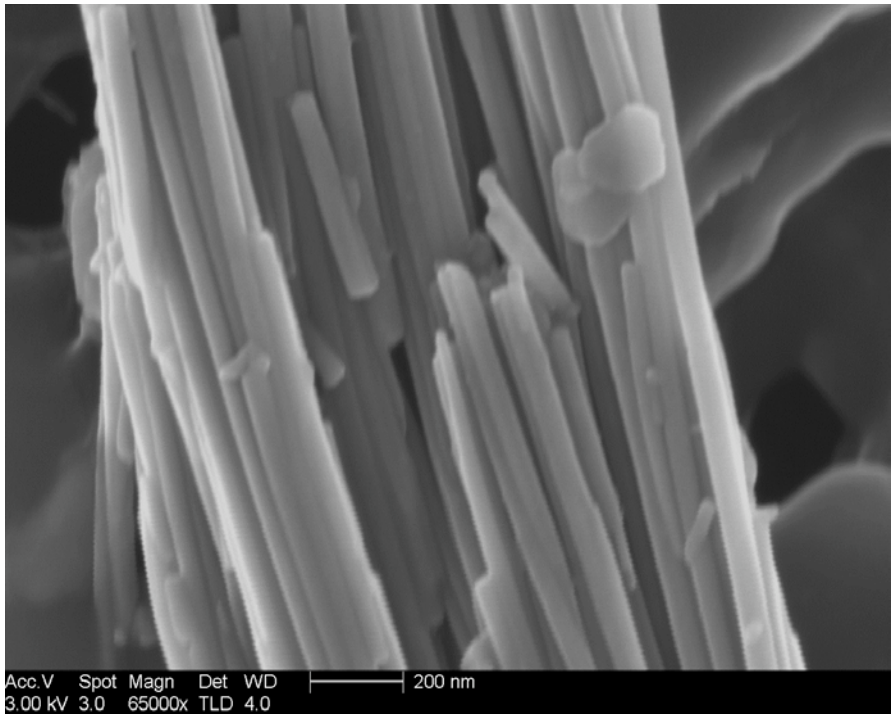
FESEM Image



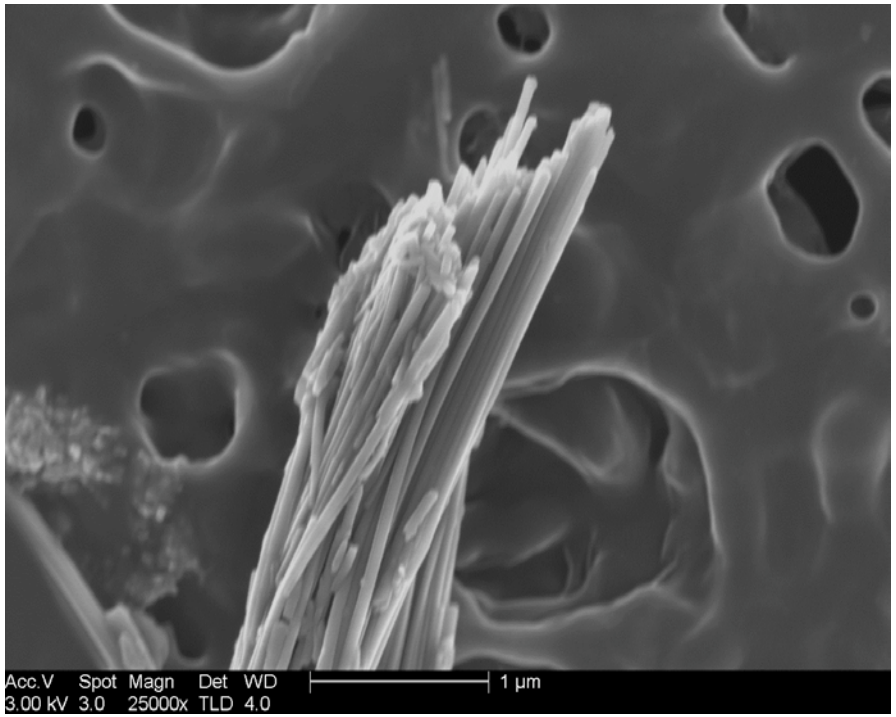
Comparison of TEM and FESEM images obtained for a bundle of chrysotile asbestos fibers found in an air sample.

The Advantage of FESEM Characterization

Particle Surface

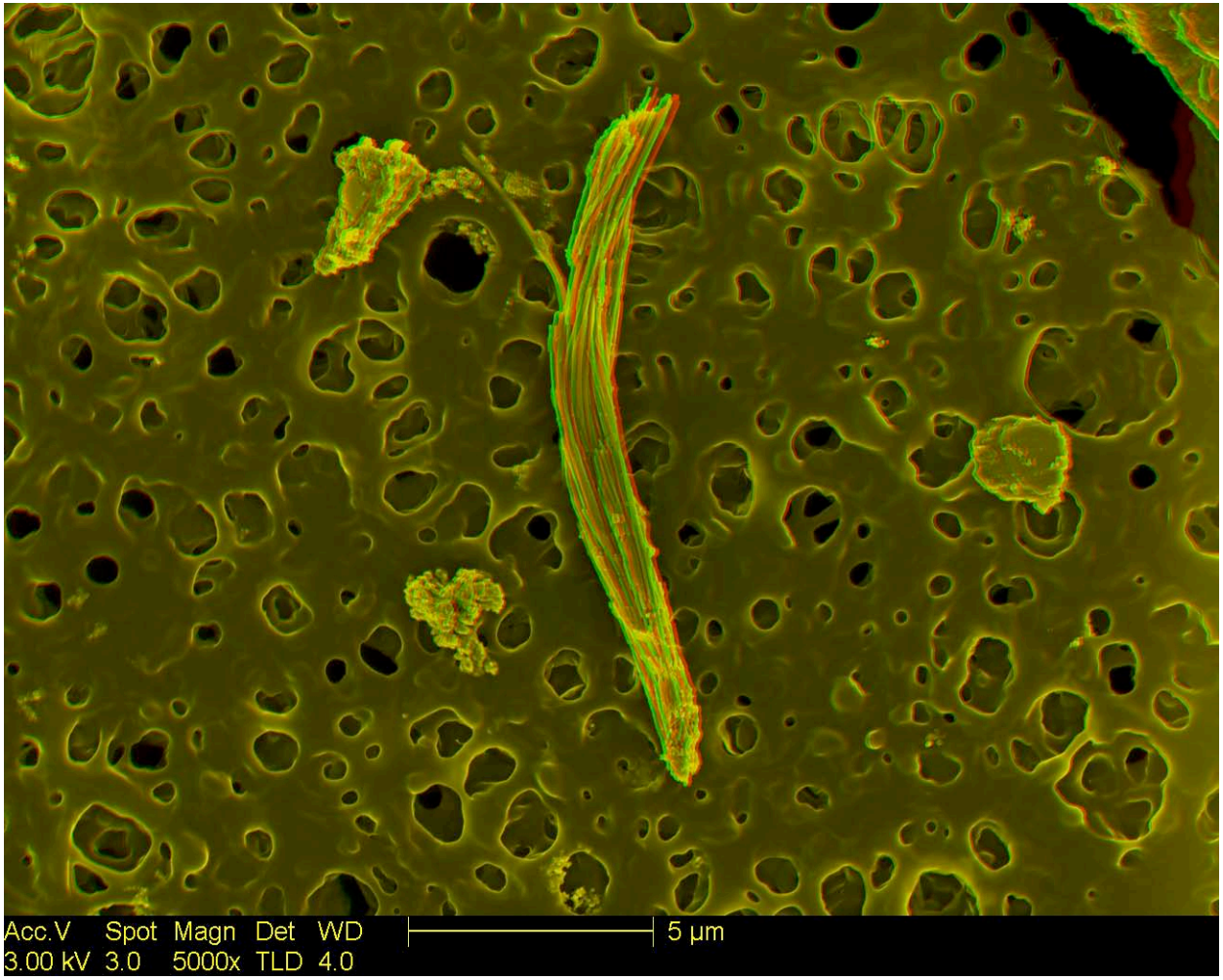


Particle End



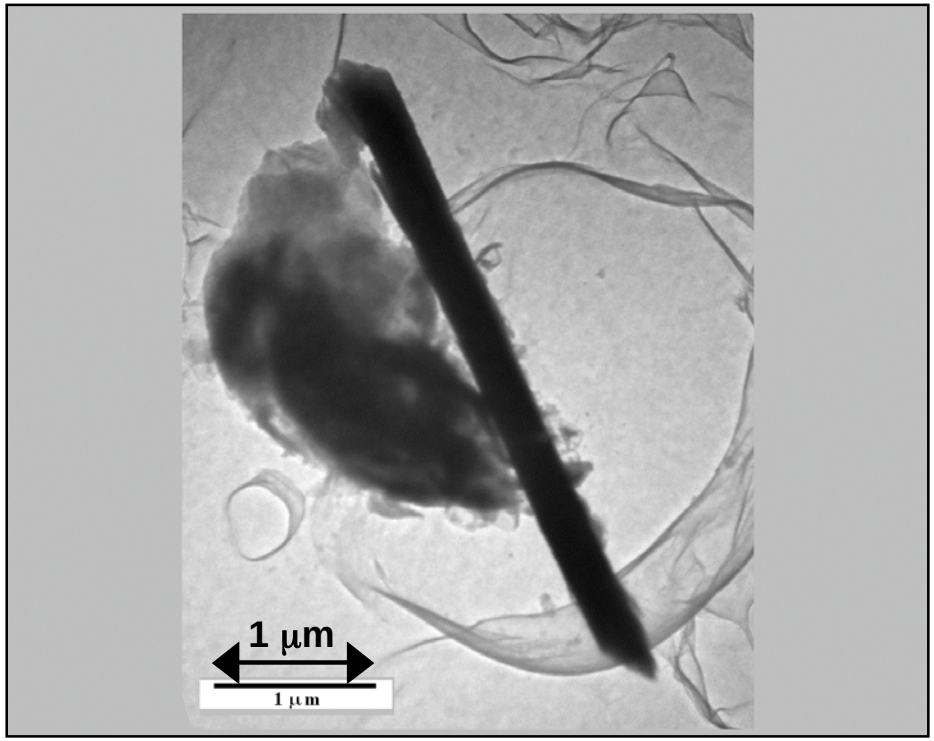
FESEM images for the surface and one end of a chrysotile asbestos bundle from a air sample.

Stereo Pair Imaging Possible with FESEM

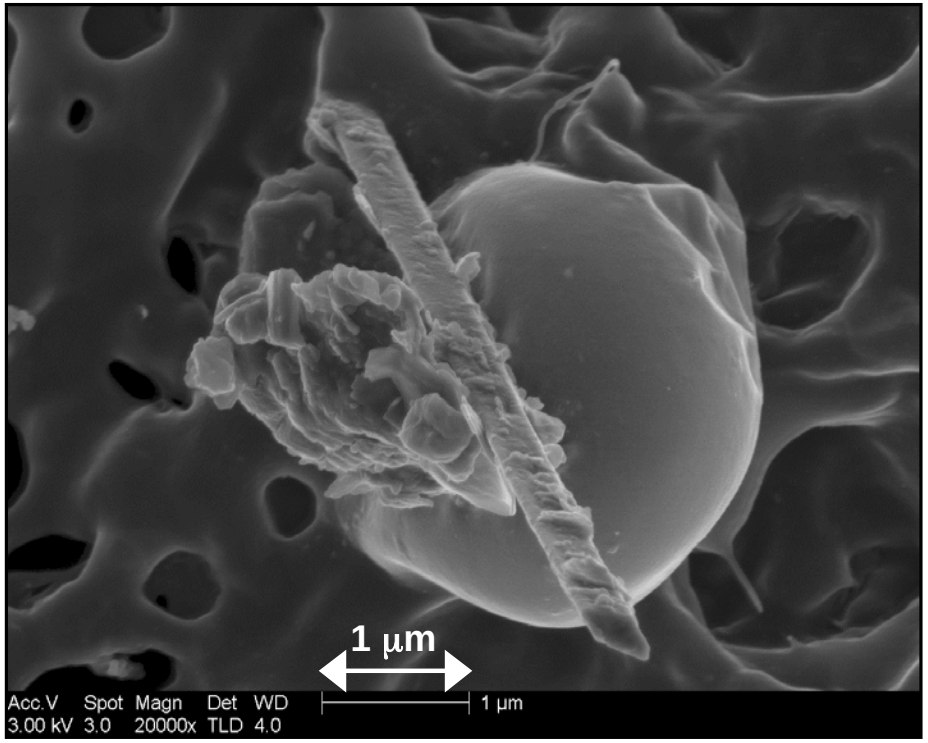


Complementary TEM and FESEM Characterization

TEM Image



FE-SEM Image



What appears to be a long, thin fiber in the TEM image is revealed in the FESEM image to be a prismatic particle coming out of the plane of the projection screen.

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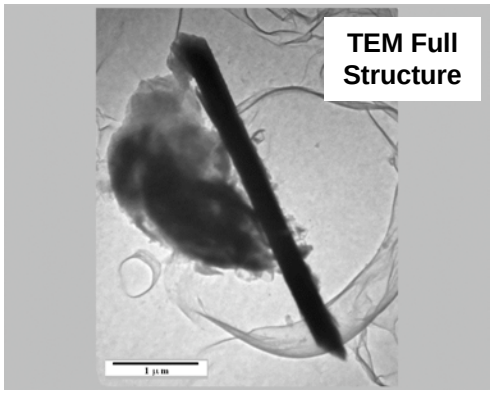
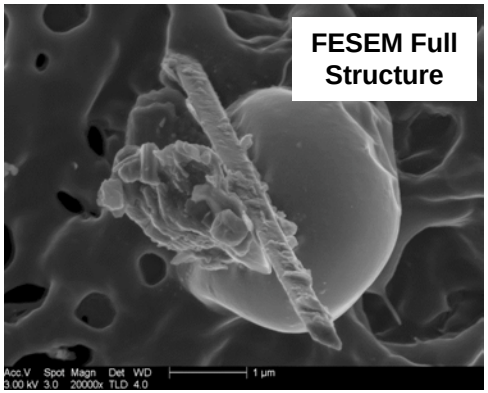
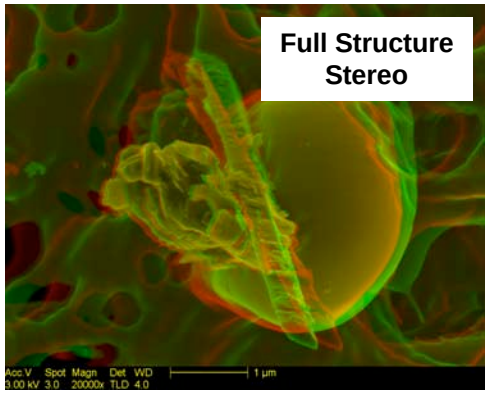
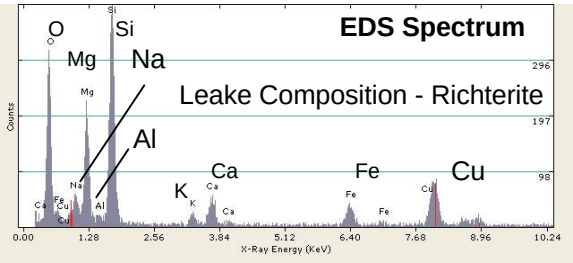
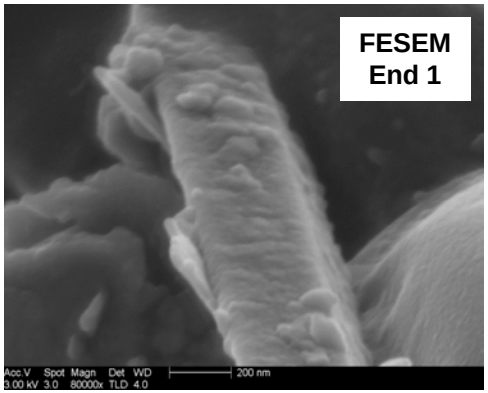
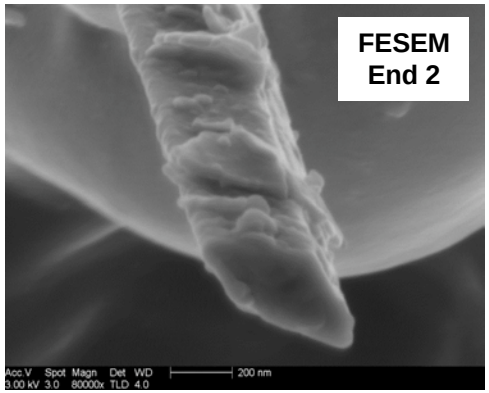


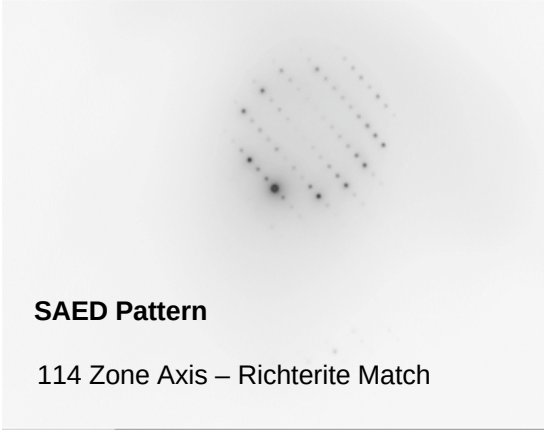


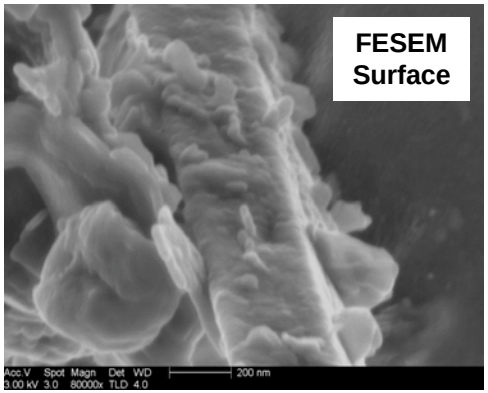


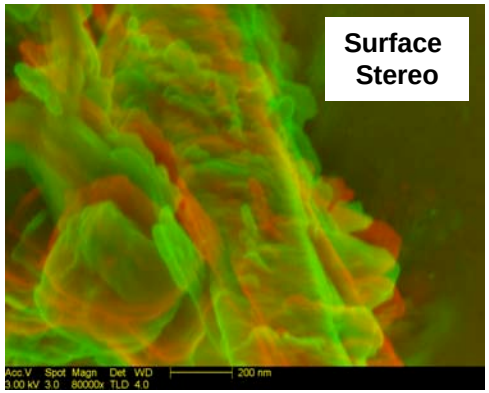












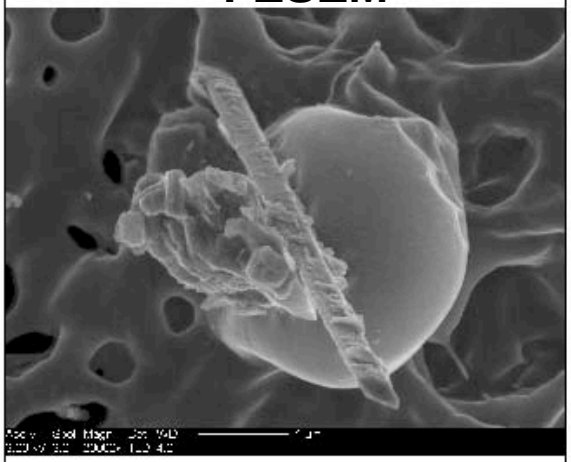
Full Characterization of Particles by TEM/FESEM

Particle Classification Database

Side-by-side EPA Air -- EPA #1R00349 or IR00352 or IR00353 -- Screening Plant; Styrofoam Planting Boxes; Personal

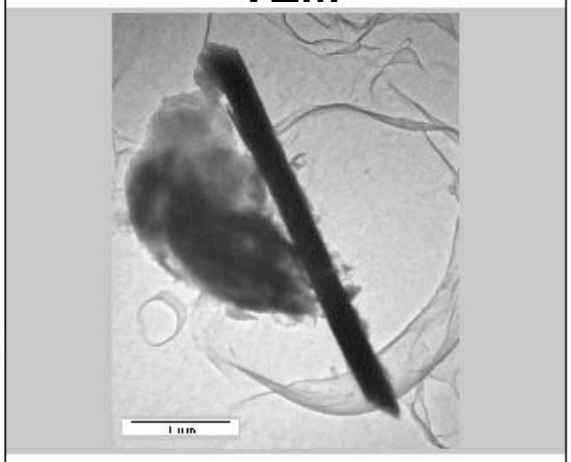
SEM Note:
TEM Note:

FESEM



Set First Fiber

TEM



Shapes	☐ Acicular ☒ Prismatic ☐ Bladed ☐ Columnar ☐ Layered ☐ Fiber ☐ Curvature ☐ Bundle ☐ Irregular ☐ NA
Surface	☐ Smooth ☐ Textured ☒ Rough ☐ Mottled ☐ Steps ☐ Striations ☐ Embedded ☐ Coated ☐ Superstructure ☐ NA
Sides	☒ Parallel ☐ NonParallel ☐ Tapered ☐ Irregular ☐ Stepped ☒ Rough ☐ NA
Ends	☐ Blunt ☒ Perpendicular ☐ Angled ☐ Irregular ☐ Stepped ☐ Needle-like ☐ Tapered ☐ Splayed ☐ NA
Association	☐ Anchored ☒ Coherent ☒ Contact ☐ Free Standing ☐ NA
Part.Relation to Filter	☐ Flat ☐ Upright ☒ Inclined ☐ Suspended ☒ Supported ☐ NA

L = 4 W = .25 AR = 16:1

☐ X-Ray map ☐ Indeterminate

Final Call: ☐ Asbestos ☒ Non-Asbestos

Damaged: ☐

Comment: Non-asbestiform richterite particle

RJLG # 3045299.HT Field 28, Fiber 6

Summary of the Database Classification Terminology used for Describing the Morphology and Other Physical Characteristics of Mineral Particles by FESEM

Particle Characteristic	**Classification Terminology Used for Each Particle Characteristic**								
Shape:	Fiber	Acicular	Prismatic	Bladed	Bundle	Columnar	Layered	Irregular	Curvature
Surface:	Smooth	Textured	Rough	Mottled	Steps	Striations	Embedded	Coated	Super-structure
Sides:	Parallel	Non-Parallel	Tapered	Irregular	Stepped	Rough	-	-	-
Ends:	Blunt	Perpendicular	Angled	Irregular	Stepped	Needle-Like	Tapered	Splayed	-
Association with Other Nearby Particles:	Anchored	Coherent	Contact	Free Standing	-	-	-	-	-
Orientation Relative to Sample Grid/Replica:	Flat	Upright	Inclined	Suspended	Supported	-	-	-	-

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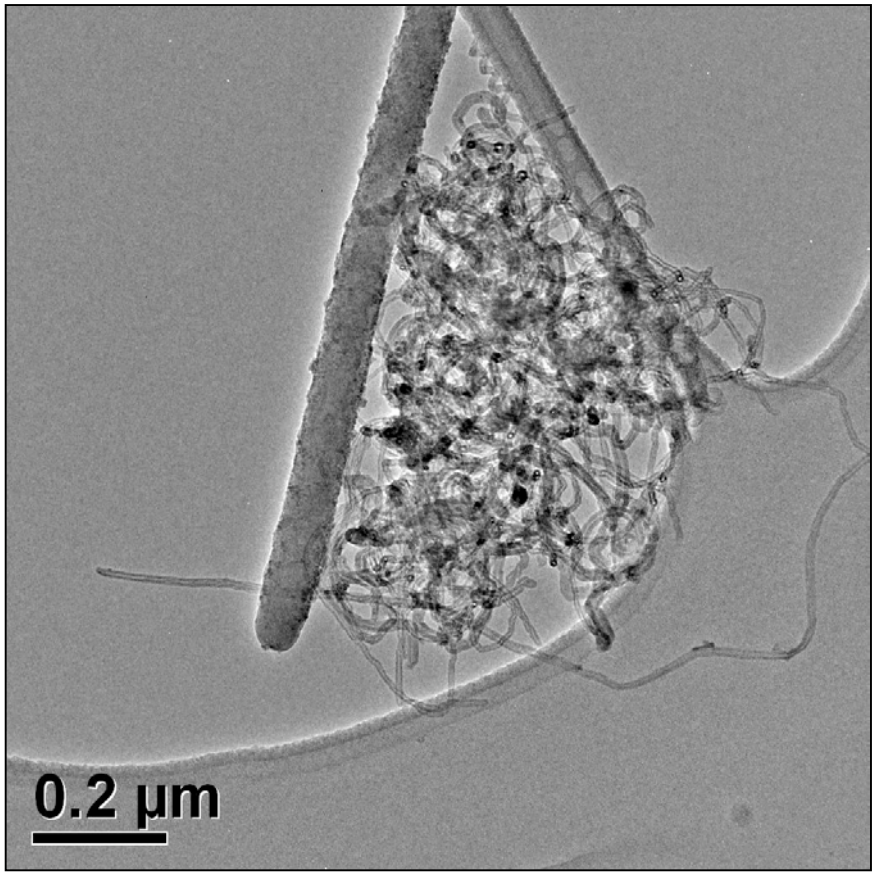
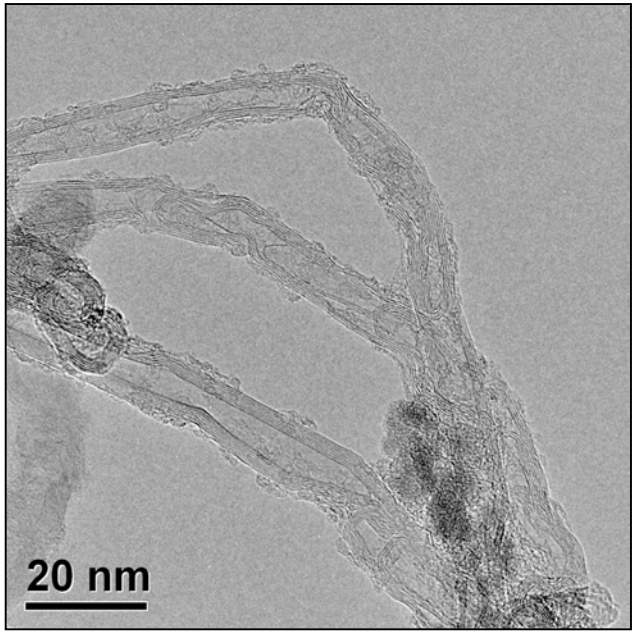
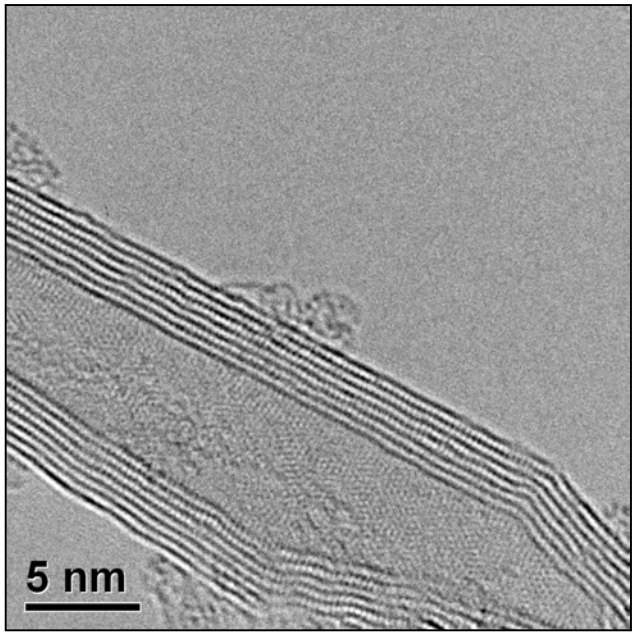
"What you should do in order for us to make more rapid progress is to make the electron microscope 100 times better."

--- Richard P. Feynman – 1959
"There's Plenty of Room at the Bottom"

HRTEM







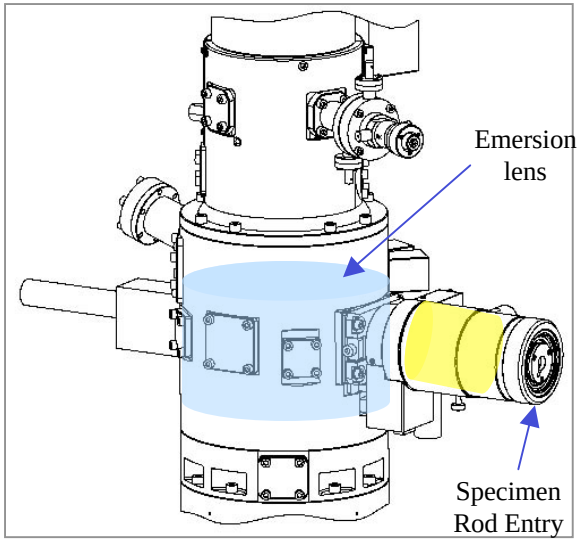
Hitachi S-5500 Ultra-high Resolution FESEM/STEM

- World's highest resolution SEM imaging – 0.4 nm at 30 kV; Maximum magnification – 2,000,000x
- Secondary electron (SE) and backscattered electron (BSE) SEM imaging
- Bright field (BF) and dark field (DF) STEM imaging
- Energy dispersive X-ray spectroscopy (EDS)
- Switching between all operation modes without changing the specimen position
- Potential for automation (CCSEM)

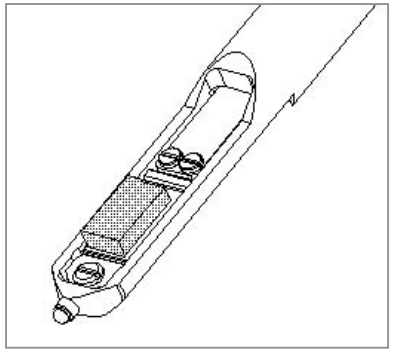
In-lens Technology: EMERSION LENS

- Specimen positioned within high excitation lens *achieves less than 1nm spot size by FE-SEM*
- No need to change stage height when specimen is in the lens field
- Holders are designed to maximize X/Y traverse and tilt angles
- Simple mounting procedure makes sample exchange routine.
- Imaging is immediate-seconds from mounting to live image.

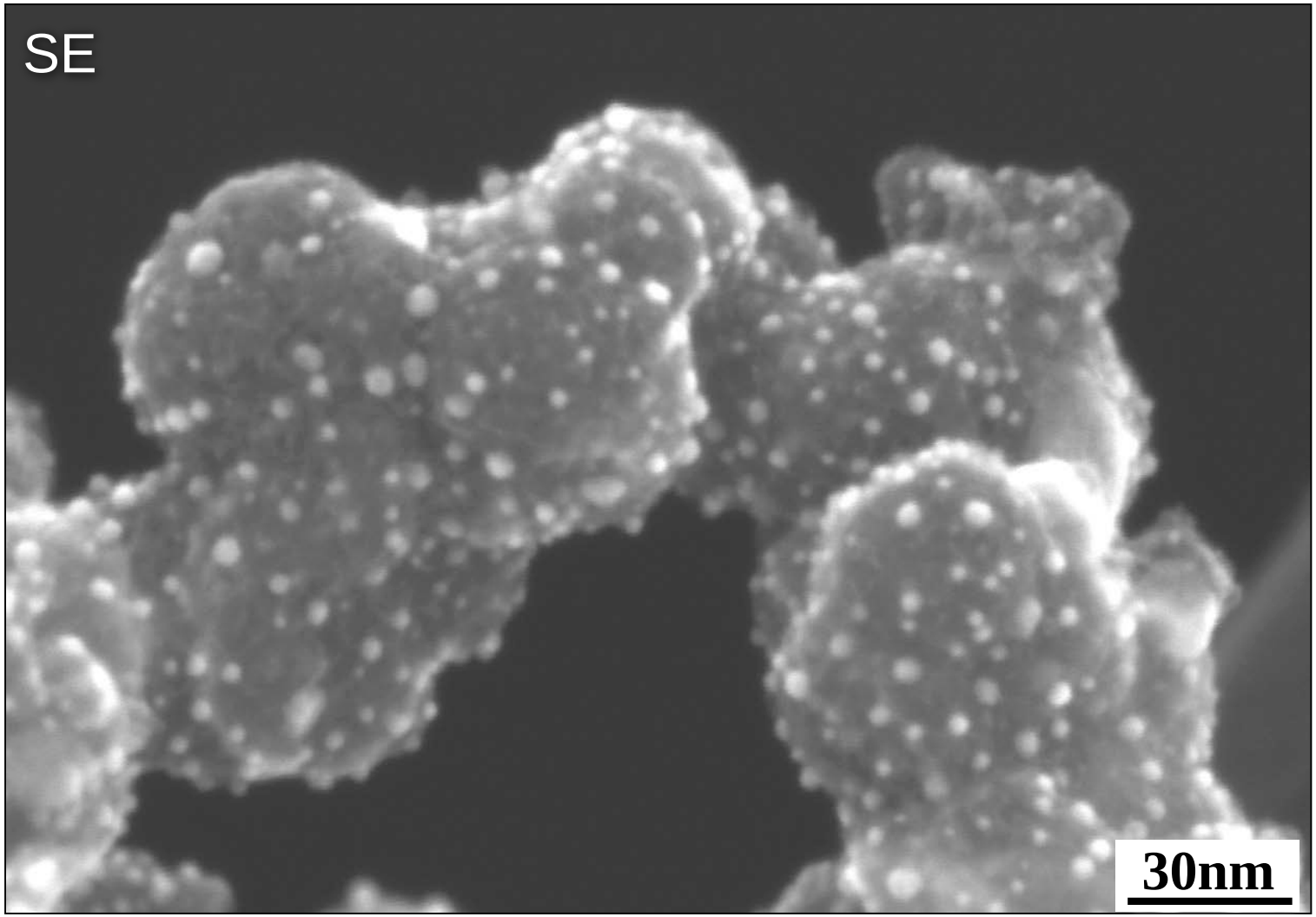
In-lens Column



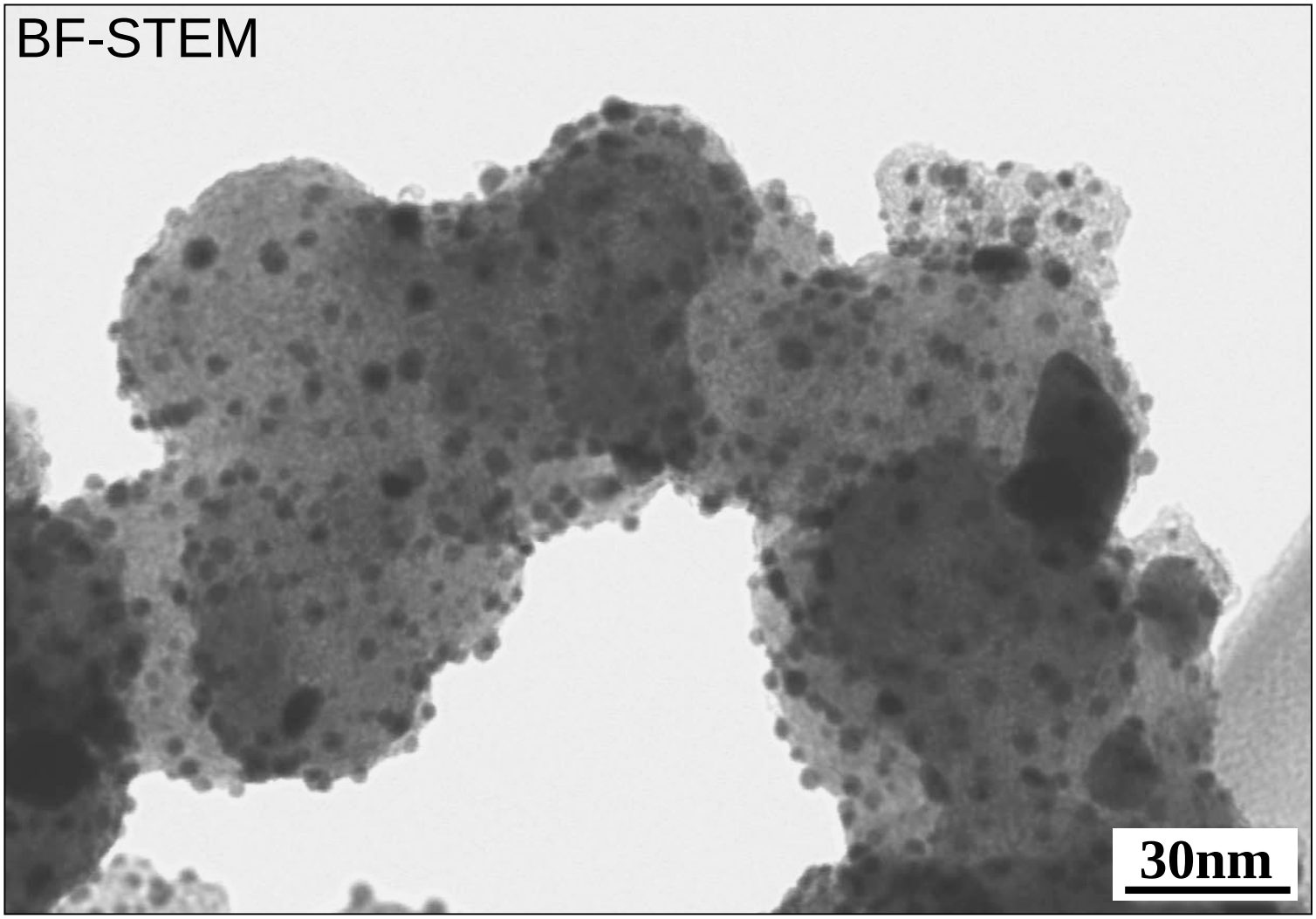
Standard Holder



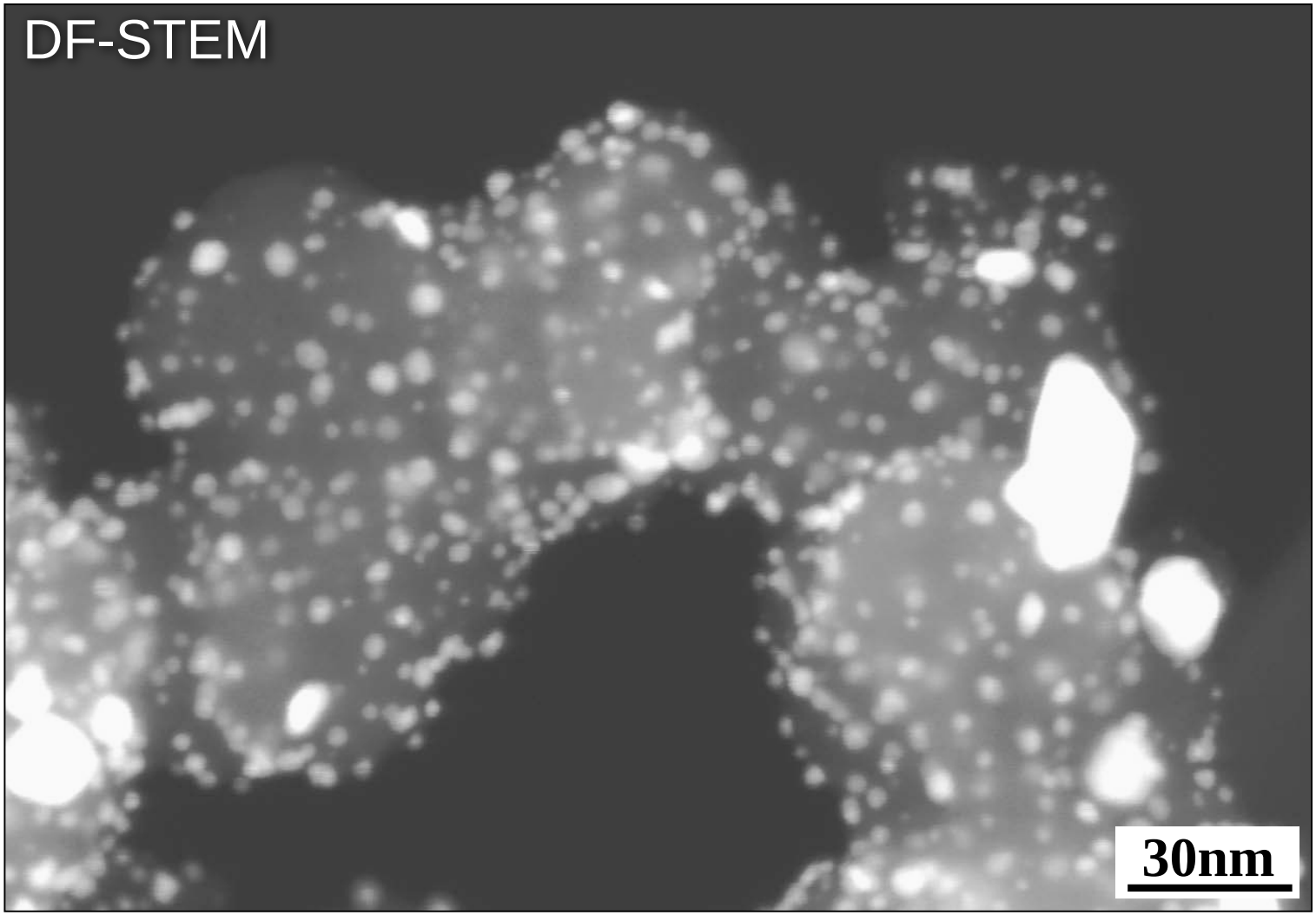
X	±3.5 mm
Y	±2.0 mm
Z	±0.3 mm
T	±40°



Pt/Ru Catalyst HV : 30kV Mag : 500kX

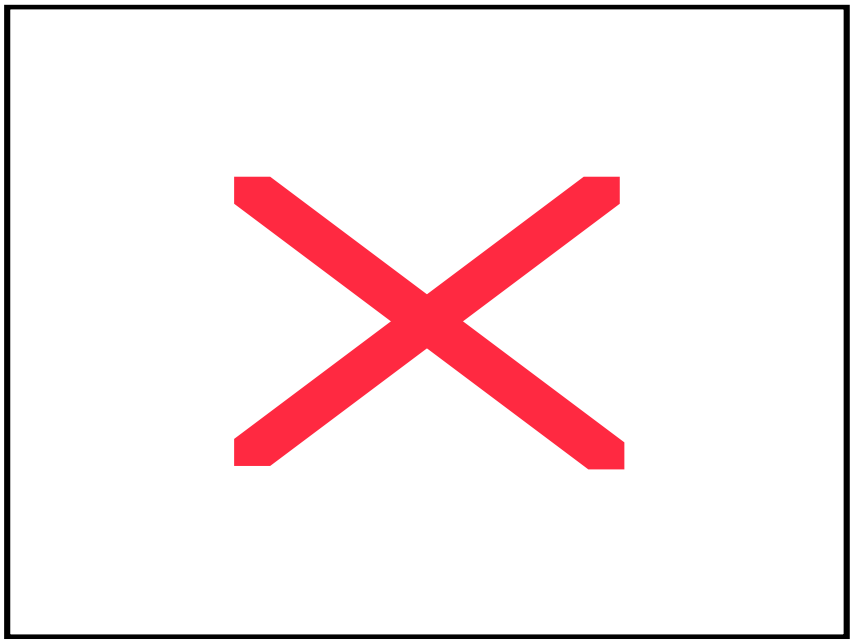


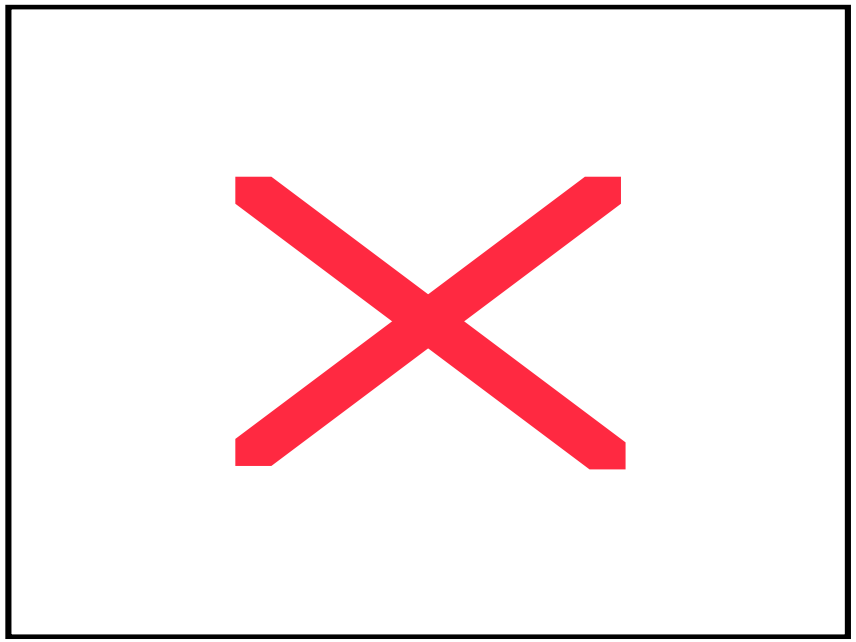
Pt/Ru Catalyst HV : 30kV Mag : 500kX



Pt/Ru Catalyst HV : 30kV Mag : 500kX

South African Crocidolite

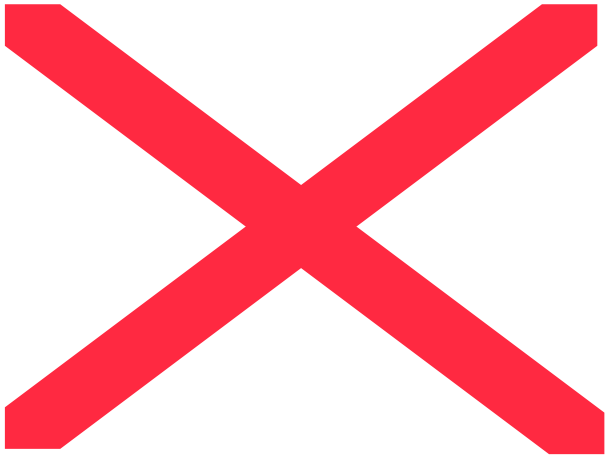




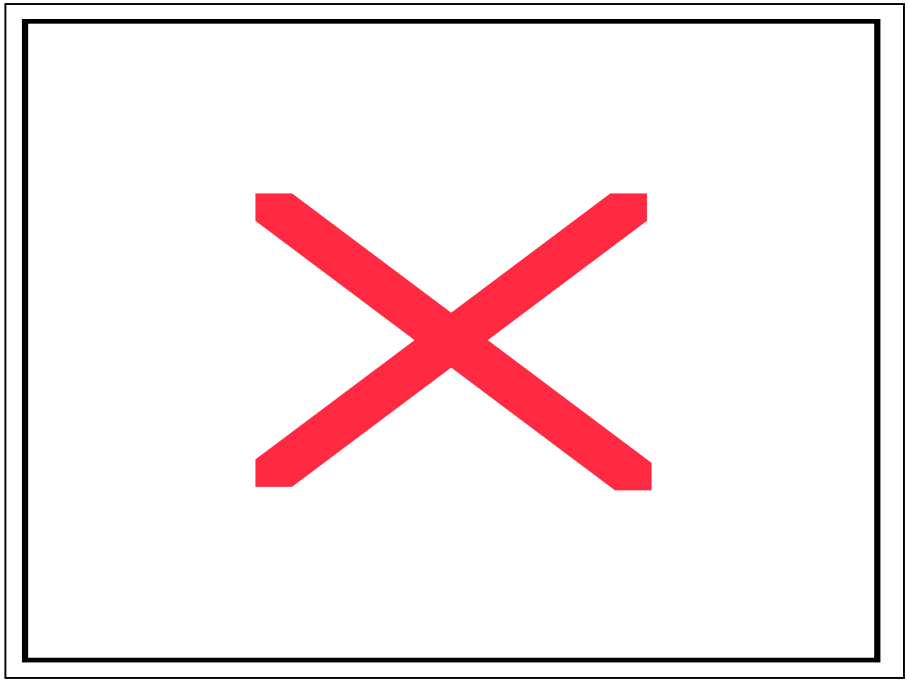
- 30.0 kV
- Secondary Electron (SE) mode
- 35 kx – Fiber body (left); 110 kx – Fiber end (right)

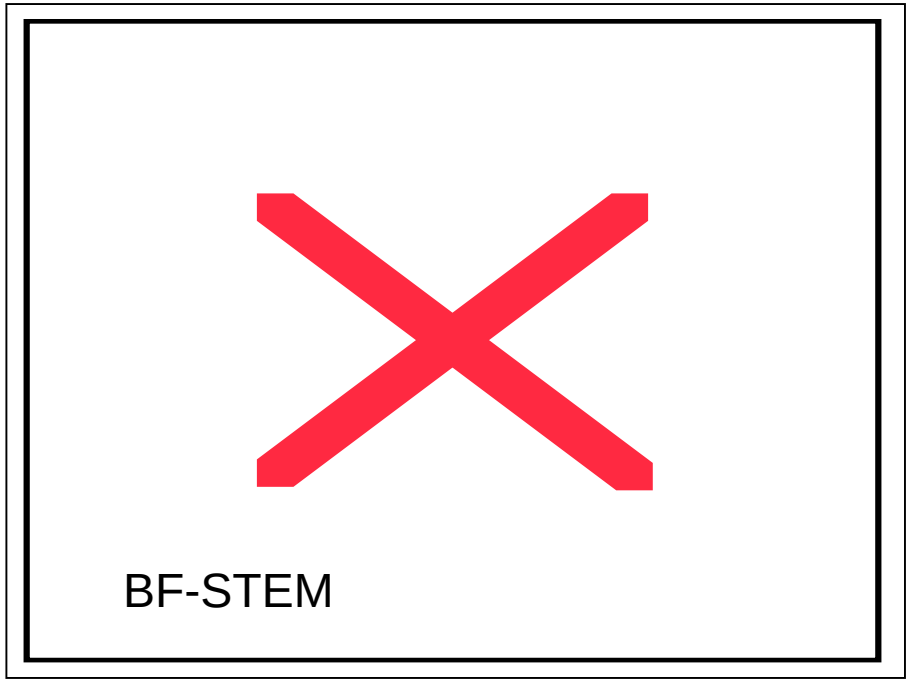
South African Crocidolite

BF-STEM



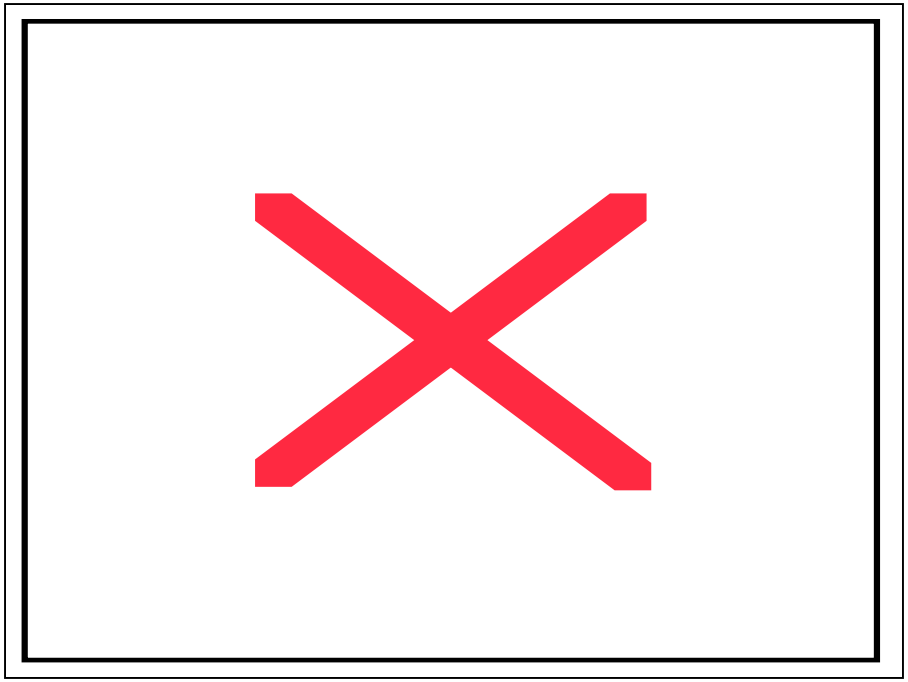
Carbon Black

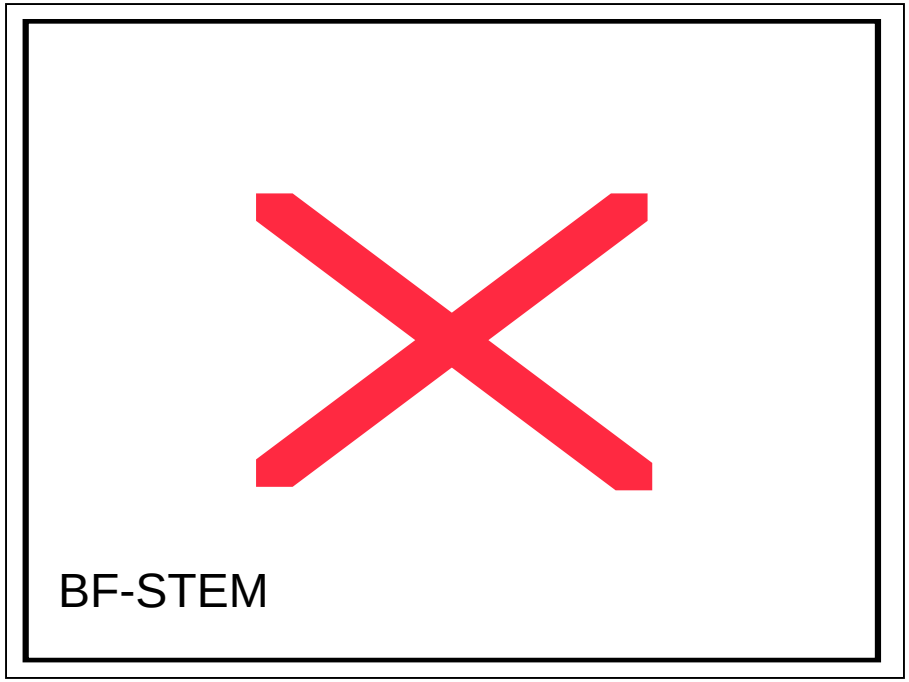




SE and BF-STEM image of Carbon Black at 200kX

Carbon Black

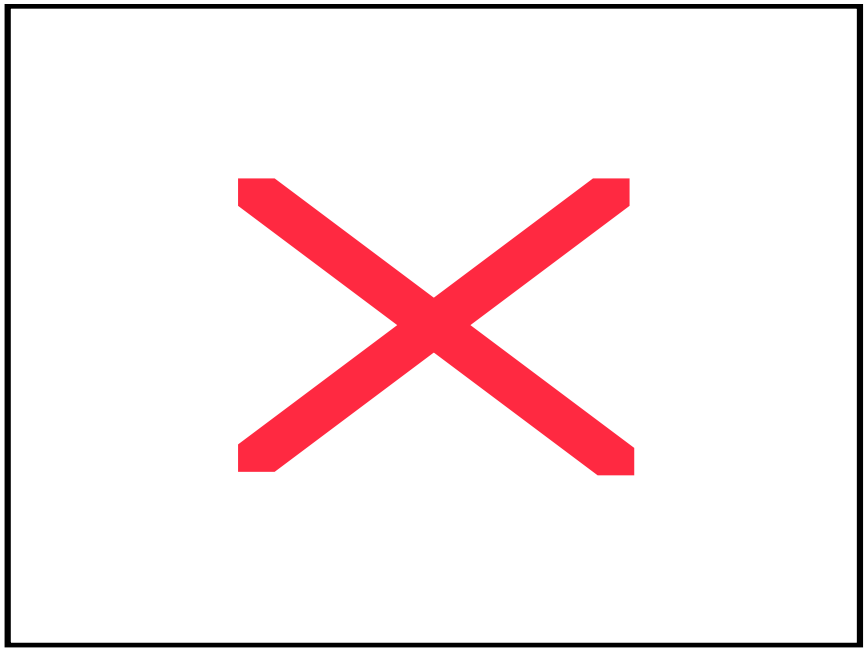


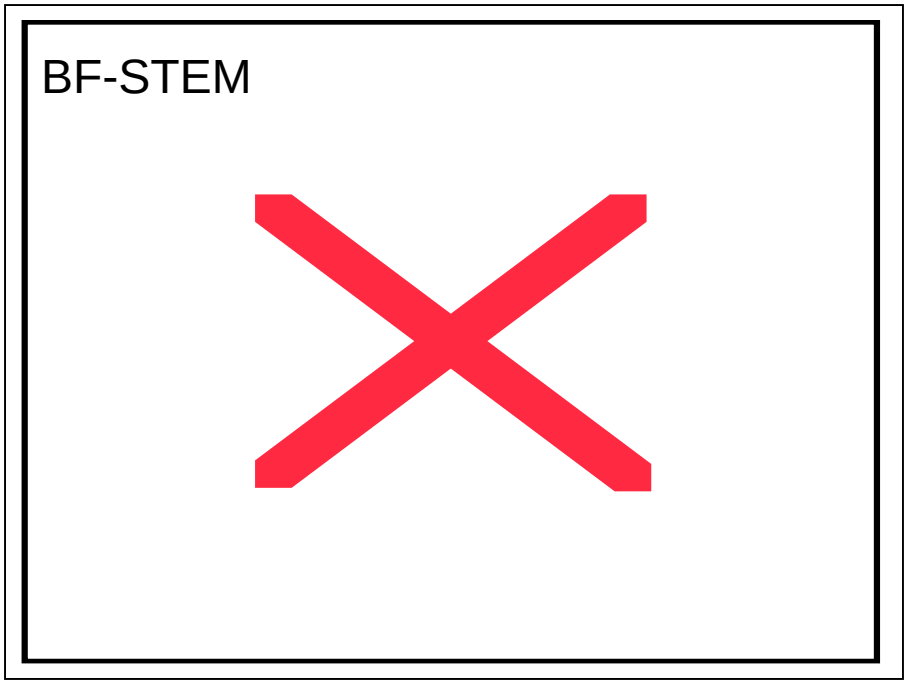


SE and BF-STEM image of Carbon Black at 1500kX

- SE image shows surface features
- BF-STEM shows internal structure

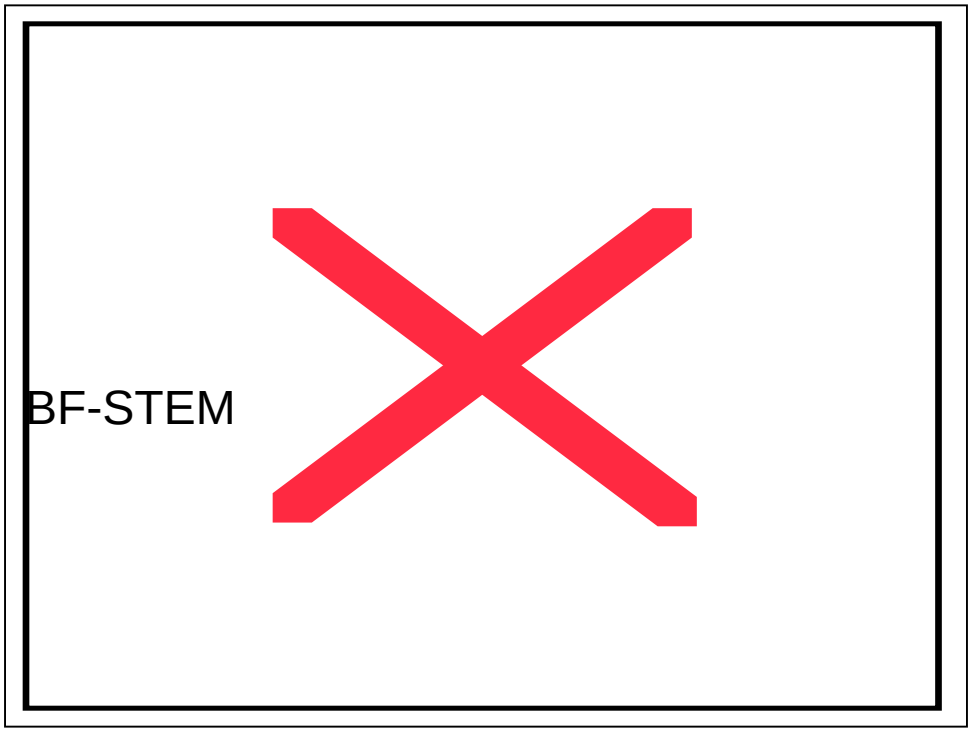
Hexane Soot – 30 kV





- Secondary Electron (SE) mode at 30 kV; right
- Bright Field (BF) mode at 30 kV
- SE image acquired at 500kX and BF image acquired at 400kx –
- Chain of particles over hole in carbon film

Hexane Soot – 30 kV



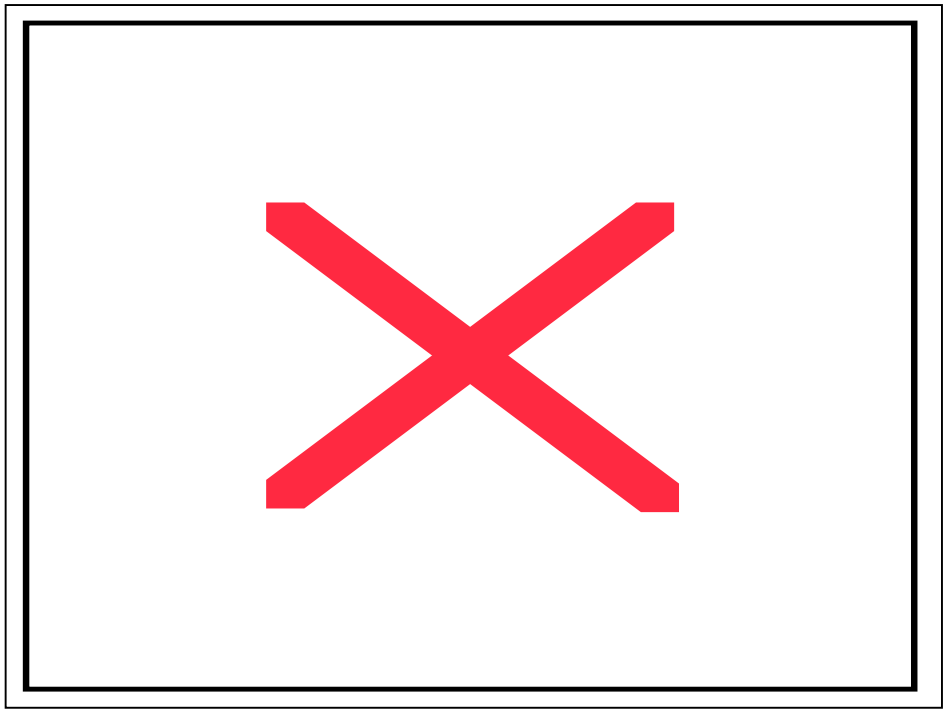
- Bright Field (BF) mode at 30 kV
- Image acquired at 1000kX!
- Internal structure of soot particles visible in BF image

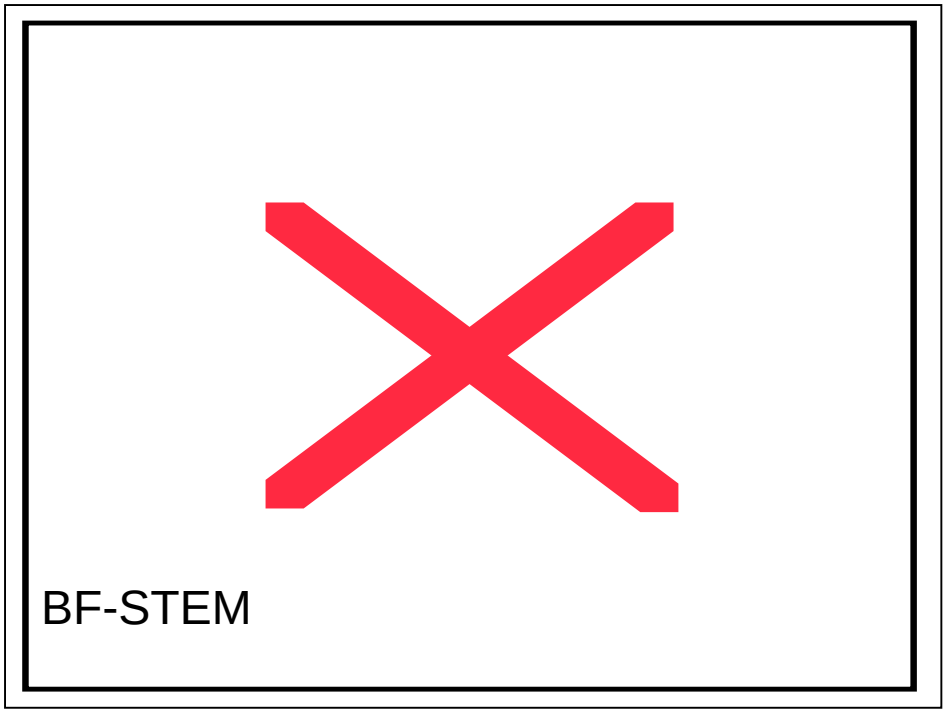
SWCNT Solid Sample

BF-STEM

- 30.0 kV
- Secondary Electron (SE) mode and Bright-Field (BF) STEM-mode
- 100kx

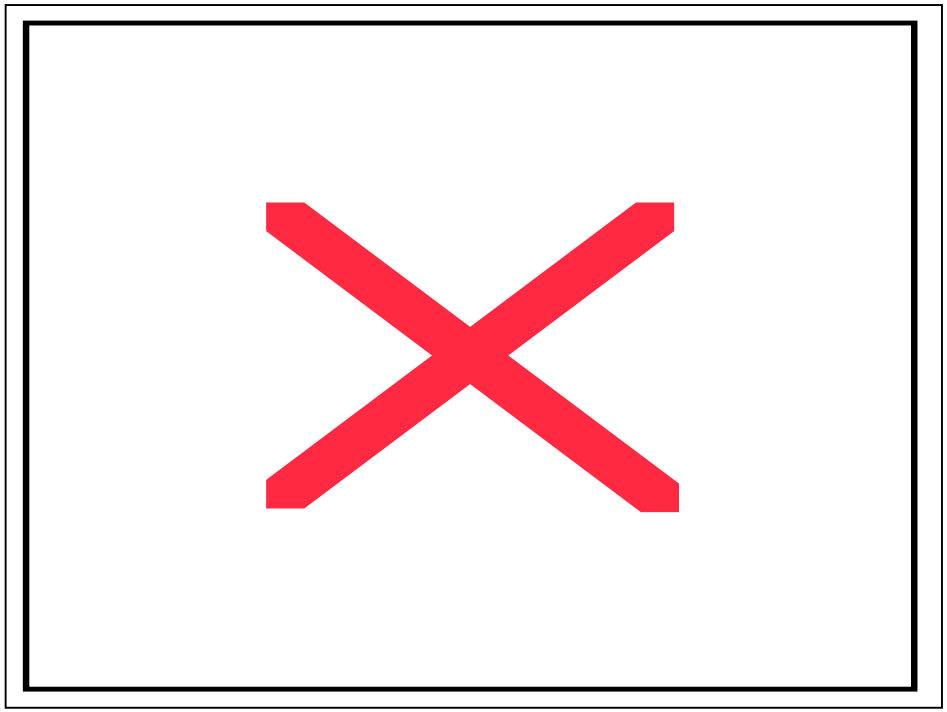
SWCNT Suspension Sample

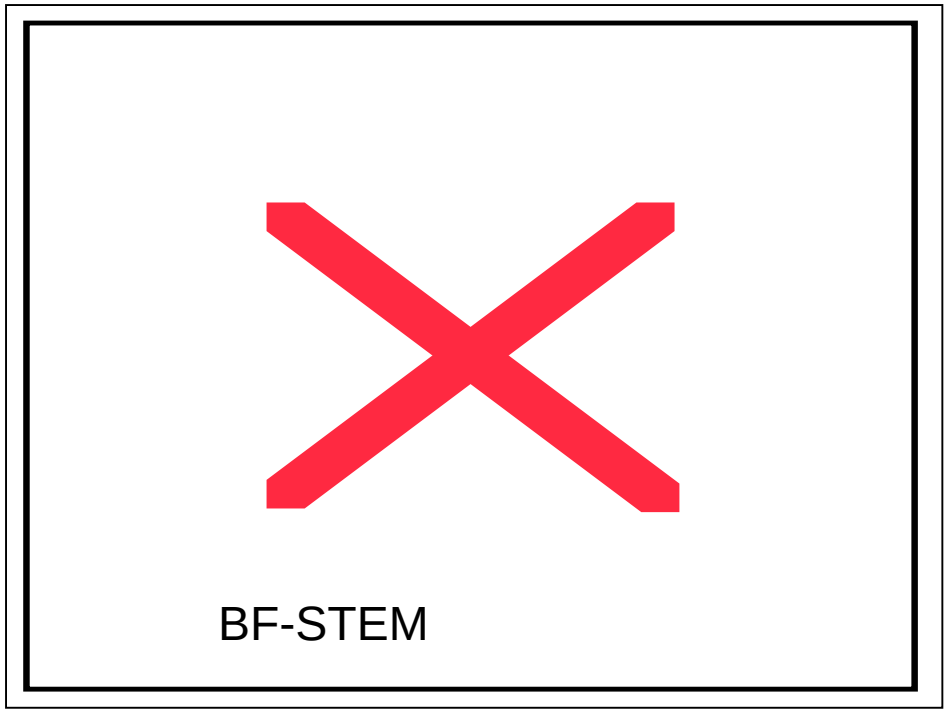




- 30.0 kV
- Secondary Electron (SE) mode and Bright-Field (BF) STEM-mode
- 200kx

SWCNT Solid Sample





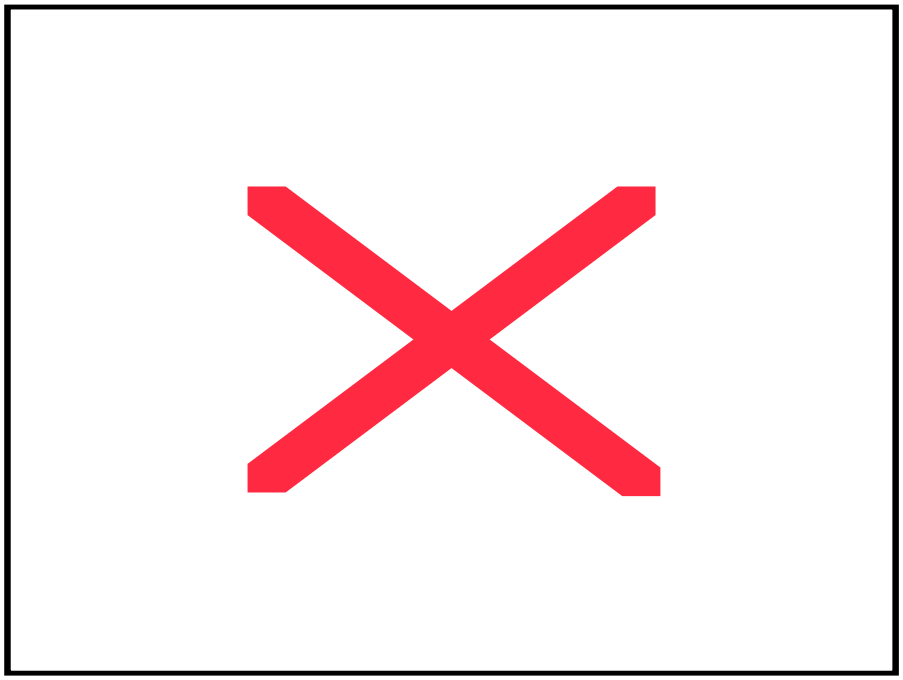
- 30.0 kV
- Secondary Electron (SE) mode and Bright-Field (BF) STEM-mode
- 400kx

AF-SWCNT Solid Sample

BF-STEM

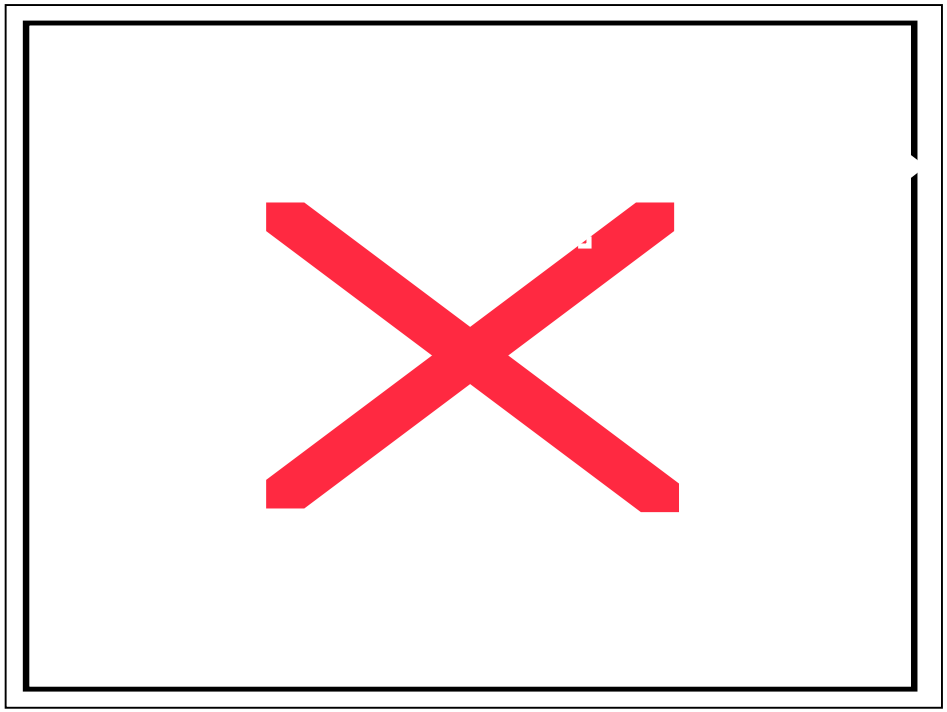
- 30.0 kV
- Secondary Electron (SE) mode and Bright-Field (BF) STEM-mode
- 11.0kx

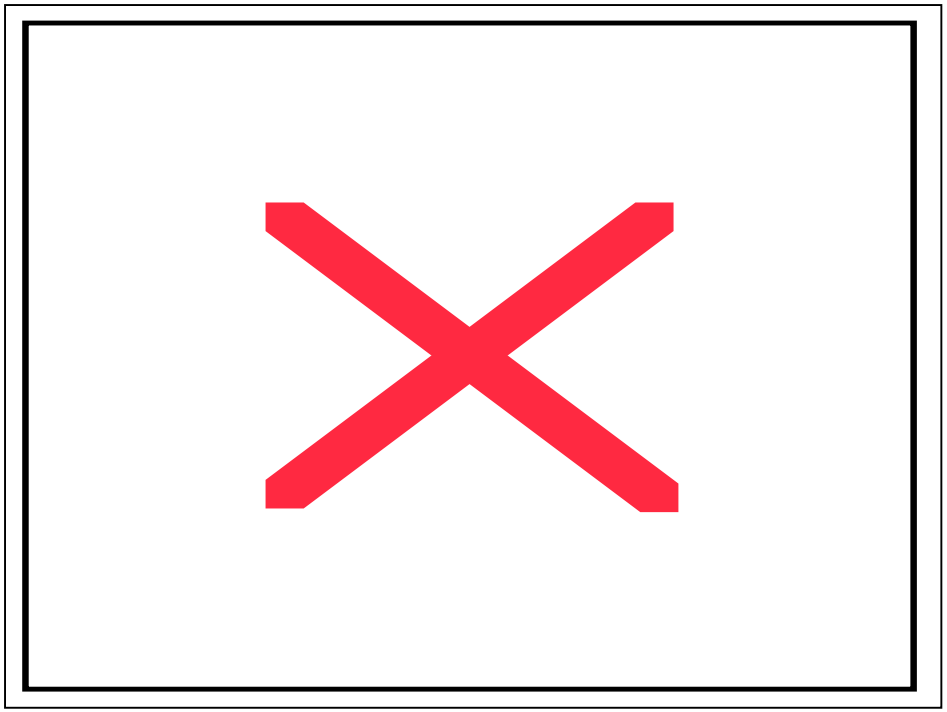
AF-SWCNT Solid Sample



- 30.0 kV
- Secondary Electron (SE) mode and Bright-Field (BF) STEM-mode
- 60.0kx

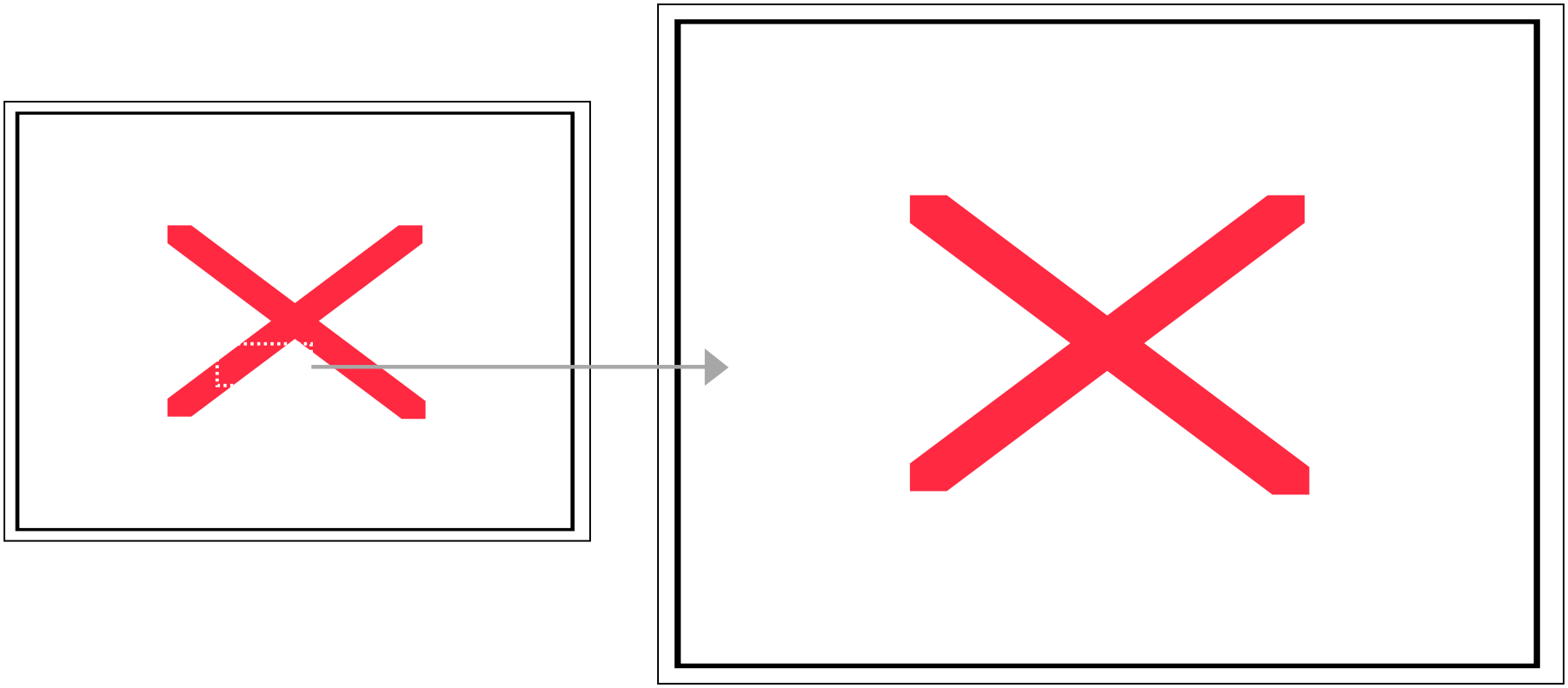
SWCNT Suspension Sample





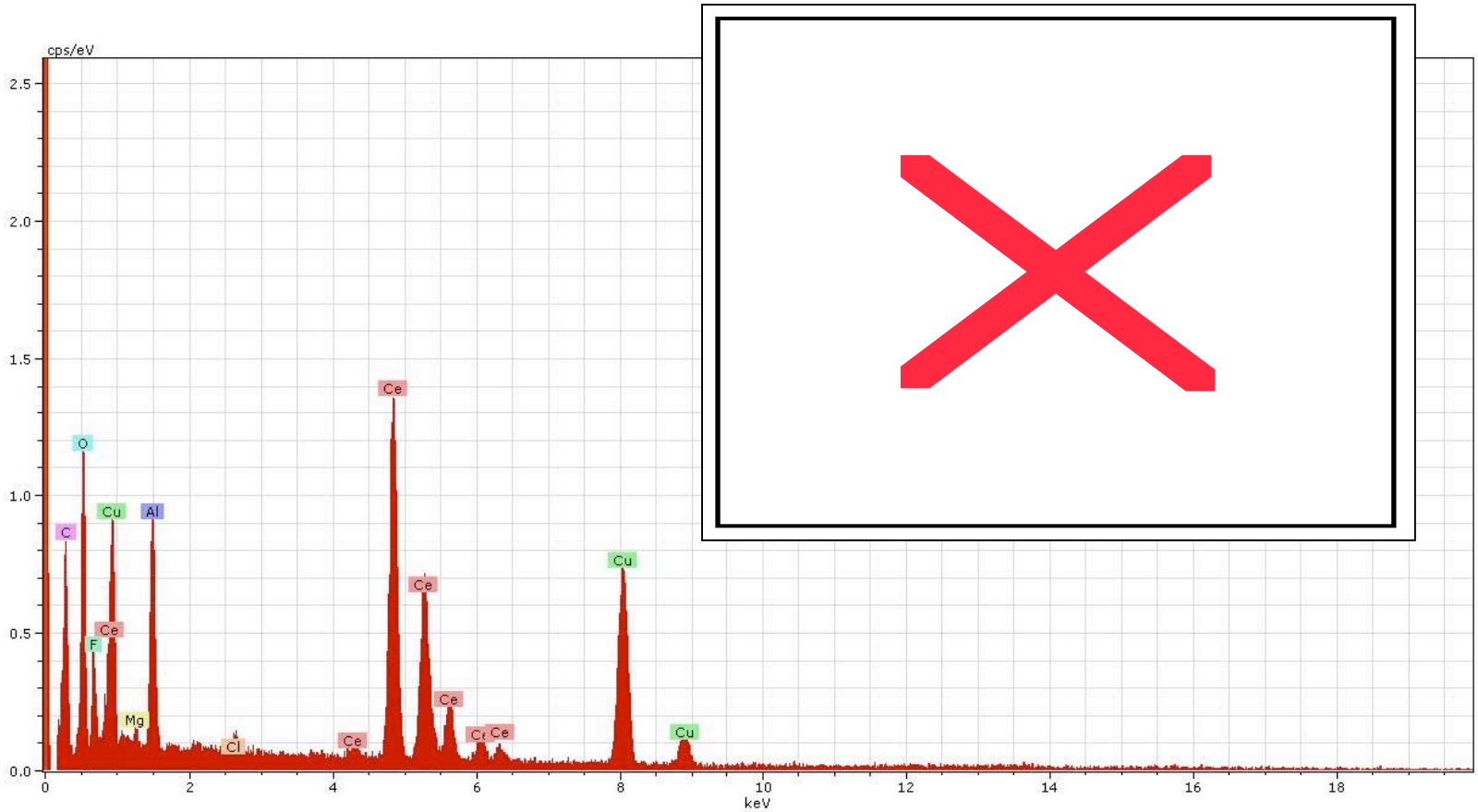
- 30.0 kV
- Secondary Electron (SE) mode
- 22.0kx

5nm TiO_2 Nanoparticles



- 2.0 kV
- Secondary Electron (SE) mode
- 200.0kx

7nm CeO_2 Nanoparticles

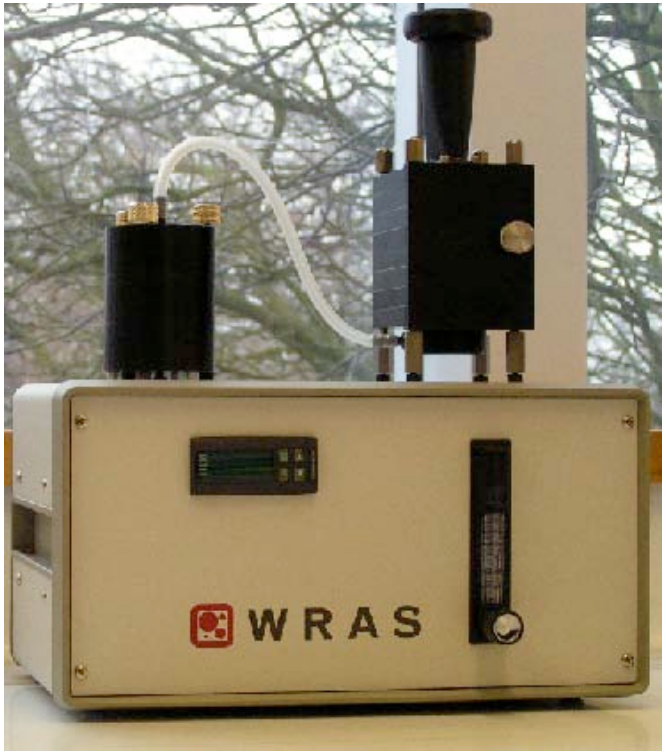


How do we take advantage of these analytical tools?

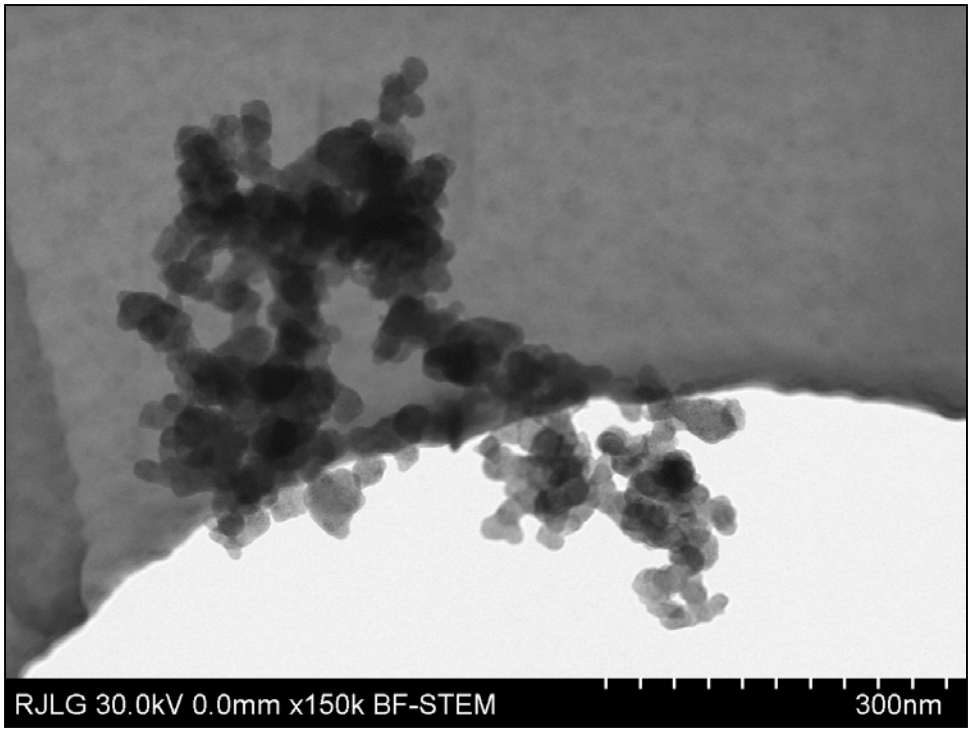
- Develop sampling technology capable of collecting nanoparticle samples directly on suitable substrate
  - TEM grids
  - Polycarbonate filters
- Integrate these samplers with real time measurements
  - Trigger collection of sample with “spikes” in concentration

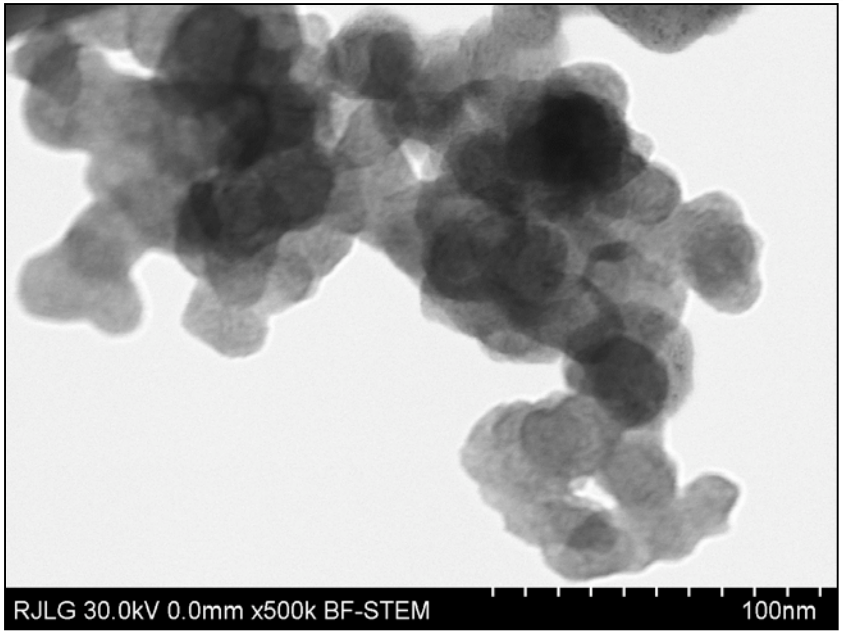
Naneum WRAS – Wide Range Aerosol Sampler

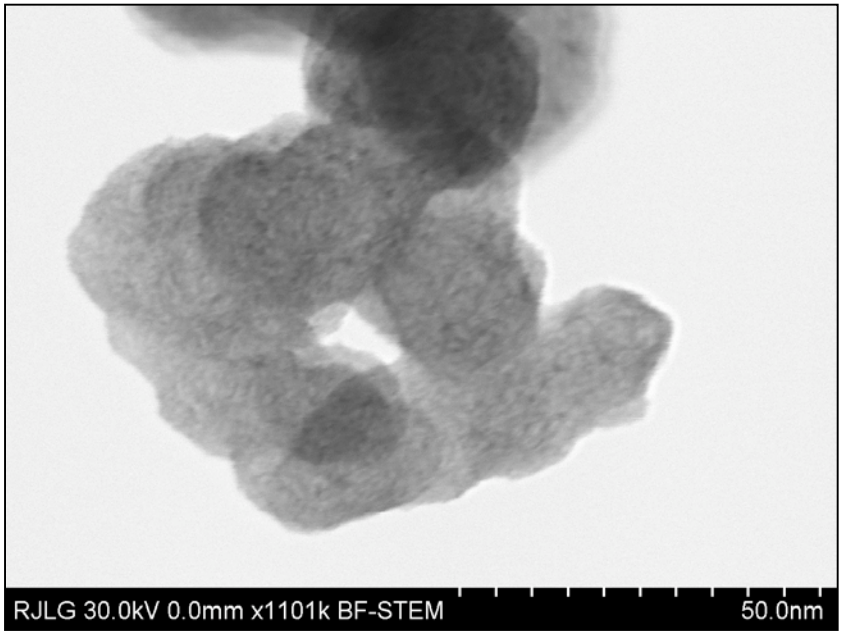
- Monitoring for wide range of particles
  - Micron, sub micron, nano-scale
  - 2 nm to 30 μm
- Samples can be analyzed chemically and by electron microscopy



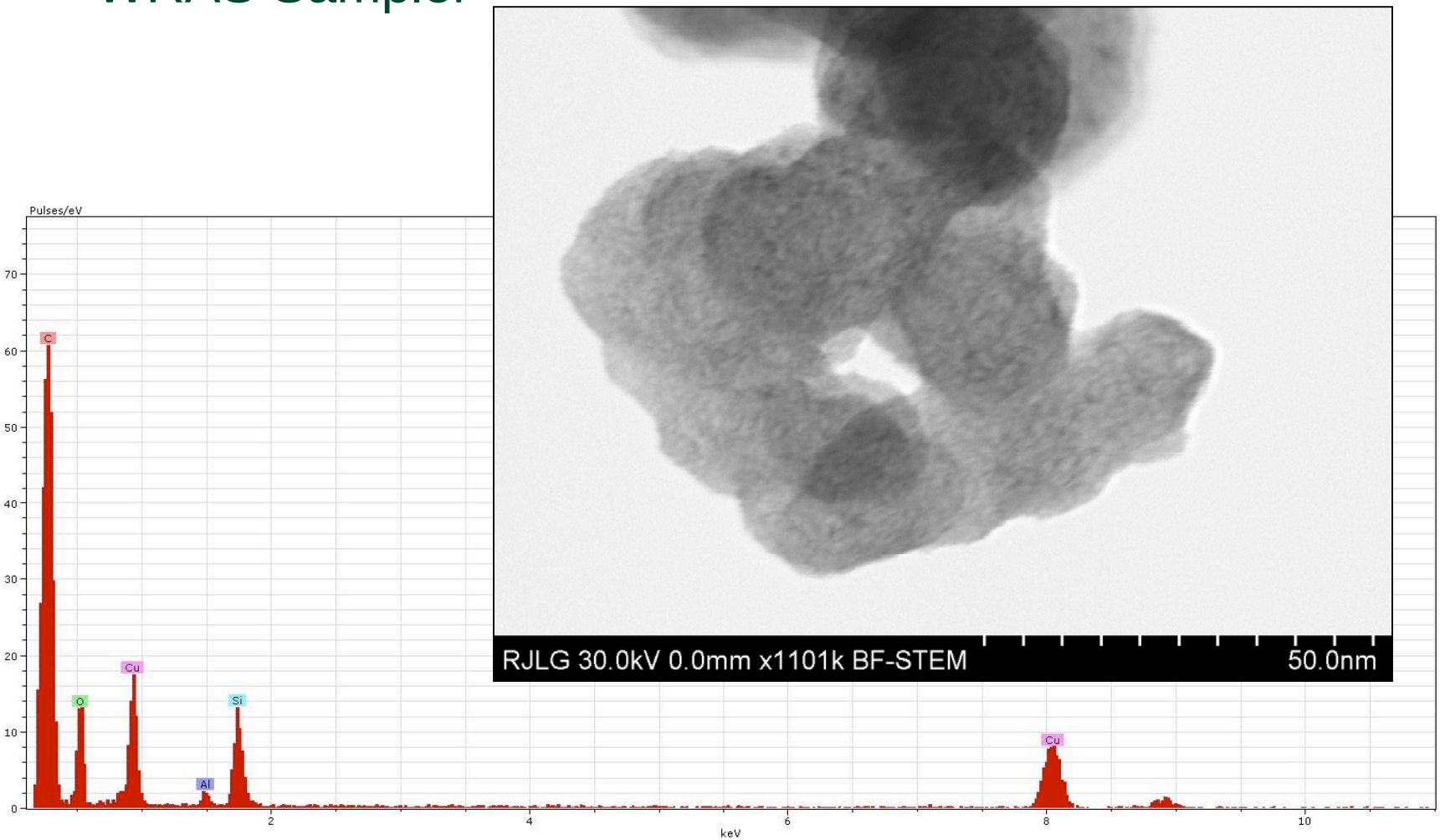
WRAS Sample


RJLG 30.0kV 0.0mm x150k BF-STEM
300nm


RJLG 30.0kV 0.0mm x500k BF-STEM
100nm


RJLG 30.0kV 0.0mm x1101k BF-STEM
50.0nm

WRAS Sampler



Summary

- Advanced analytical tools are available for the characterization of nanoparticles
  - How do we use these tools in a cost-effective manner?
- Learn from previous experiences
- Sample in a “smart” manner
- Automate the process where possible
- Incorporate information in databases
- Sampling and analysis protocols are evolving and will continue to evolve as more information becomes available

Determining Concentration of Airborne Single-Crystal Ceramic Whiskers (SCCW) in the Workplace Environment

- ASTM Standard:
  - D6056: Transmission Electron Microscopy
  - D6057: Phase Contrast Microscopy
  - D6059: Scanning Electron Microscopy
  - D6058: Standard Practice
- TEM is considered to be the reference technique for the analysis of airborne SCCW
- PCM or SEM are considered to be the primary screening methods for the analysis of airborne SCCW

ASTM D6058: Standard Practice

- It describes sampling and analytical techniques used to assess the airborne concentration and size distribution of SCCW, which may occur in and around the workplace where these materials are manufactured, processed, transported, or used.
- Can be used as a guide in the analysis of nanoparticles.

ASTM D6058: Standard Practice

- The practice is based on a three-tier approach to the quantitative assessment of airborne SCCW levels. It includes detailed procedures to analyze standard air sampling cassettes by PCM, SEM, and TEM.
- The choice of a particular analytical method is based on the visibility limitation of each instrument and an understanding of the actual size distribution of the fibers being analyzed.