Milestones in X-ray microscopy: Scanning X-ray microscopy, spectromicroscopy and recent achievements

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Stony Brook University

Outline

- 1/ History – as I see it
  - Comments on X-ray microprobes
- 2/ STXM at the ALS, NSLS and elsewhere
- 3/ Variations: SPEM, SLXM, SXRF
- 4/ Conclusions
  - where different forms of x-ray microscopy fit in.

H. H. Pattee Jr.
The Scanning X-ray Microscope
JOSA 43, 61 (1953)

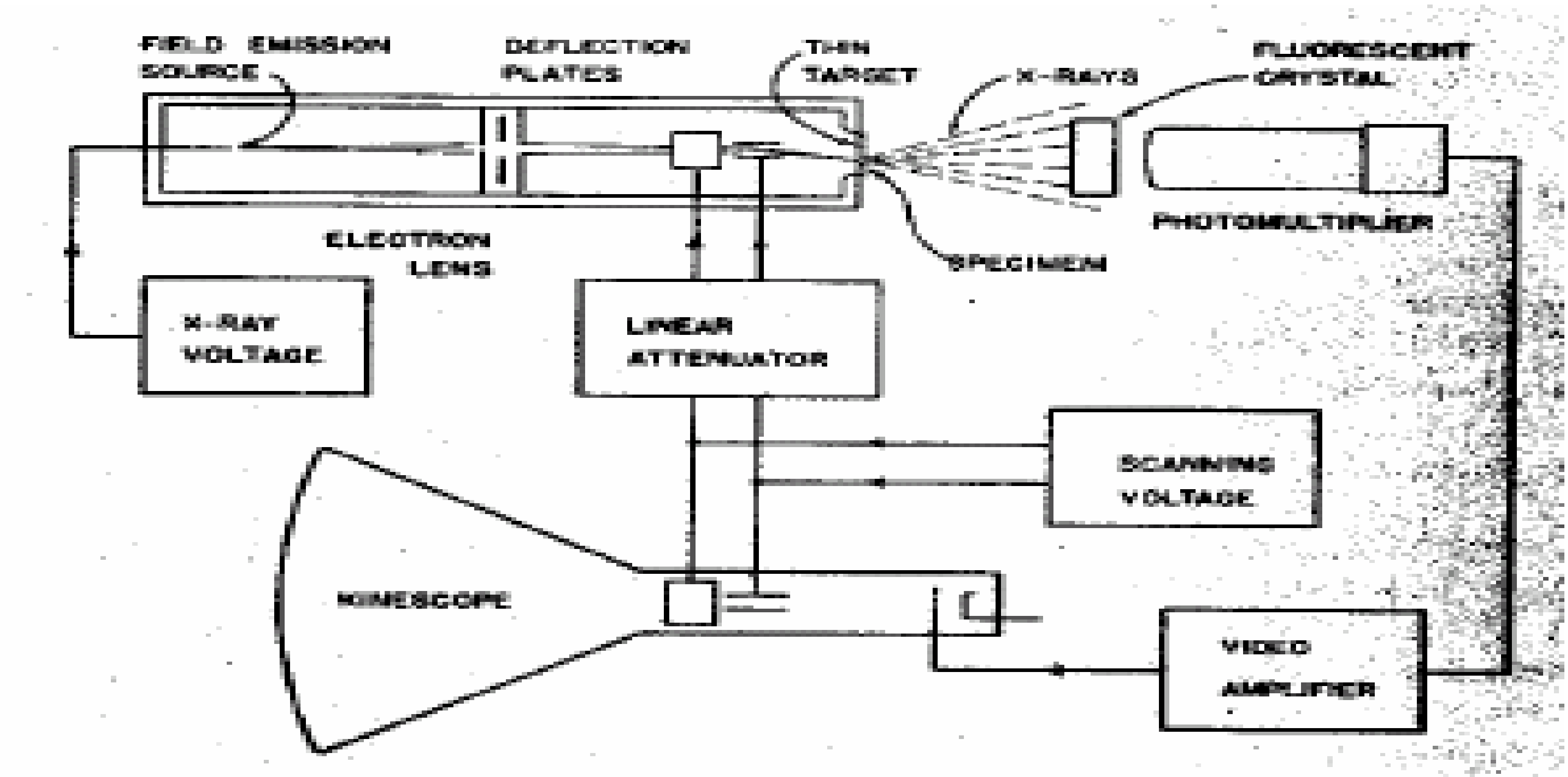
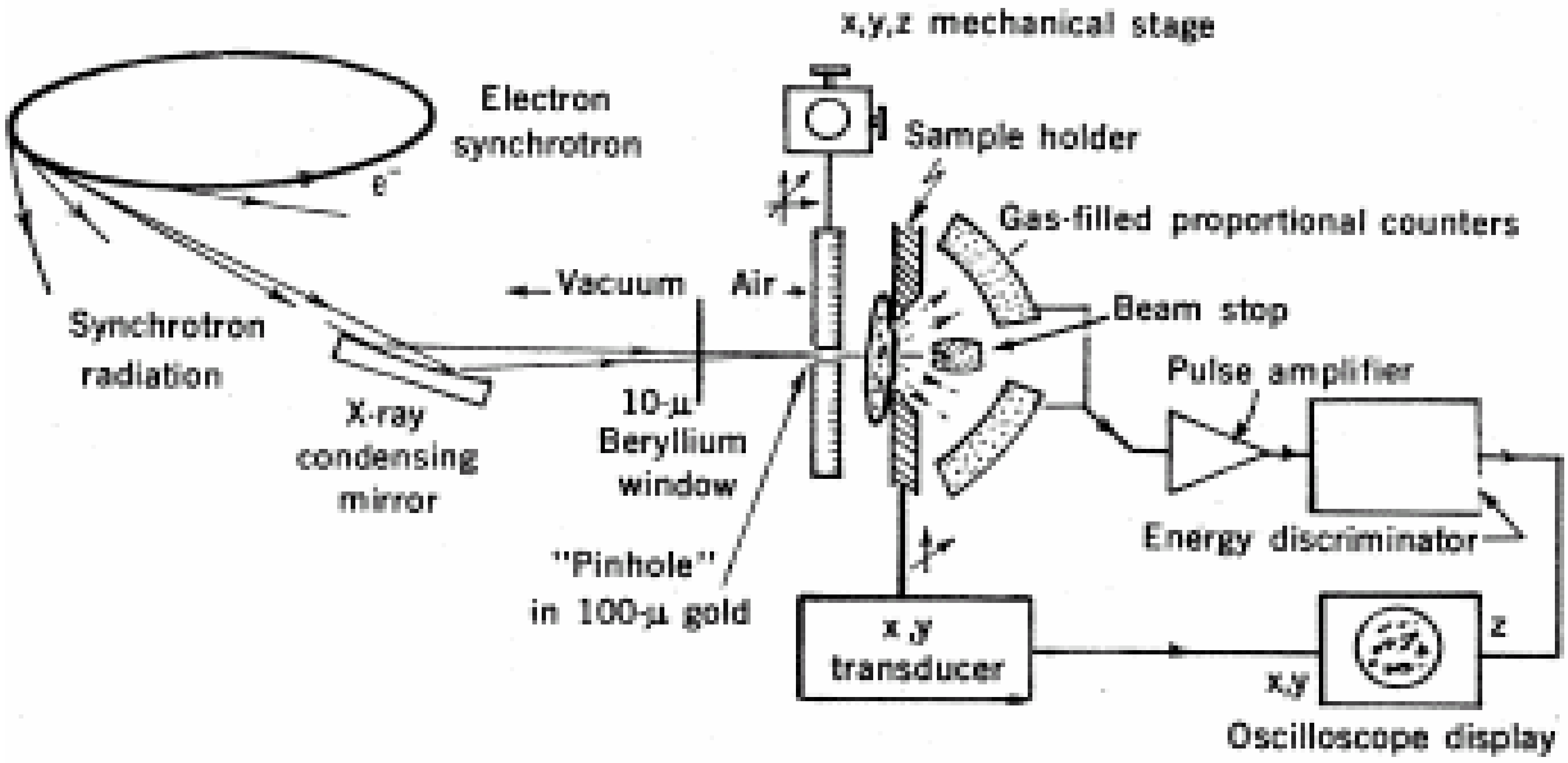


FIG. 1. Scanning x-ray microscope.

Horowitz and Howell
A Scanning X-ray Microscope using Synchrotron Radiation
Science, 178, 608 (1972)



Horowitz and Howell
1-2 micron pinhole collimator

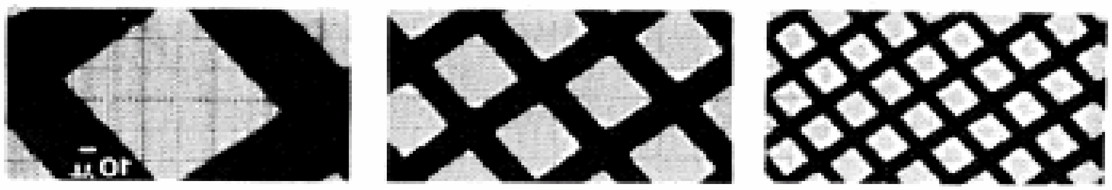


Fig. 2. Transmission micrographs of a 200 mesh per inch (80 grids per centimeter) copper grid at three different magnifications. The faint horizontal and vertical lines in these micrographs are from the oscilloscope graticule.

transmission

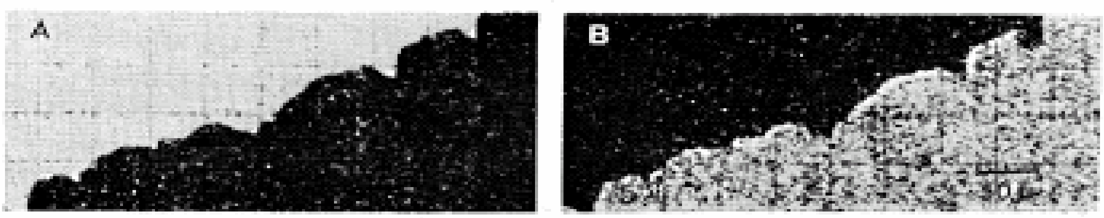


Fig. 3. An aluminum foil 10 μ thick viewed: (A) in transmission and (B) in aluminum K fluorescence.

transmission and Al fluorescence

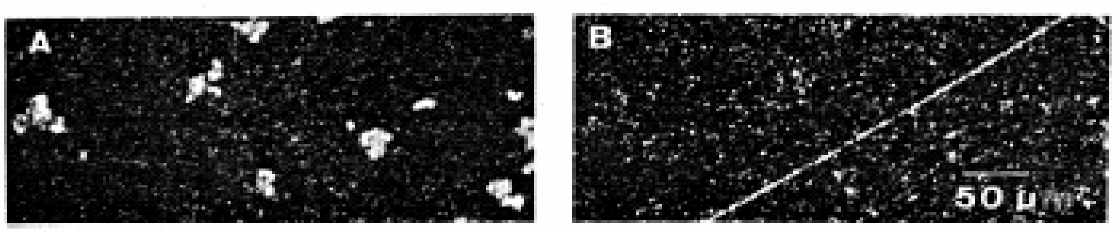


Fig. 4. A sample consisting of sulfur dust and a 2-μ silicon whisker viewed: (A) in sulfur K fluorescence and (B) in silicon K fluorescence.

S and Si fluorescence

Scanning microscopy

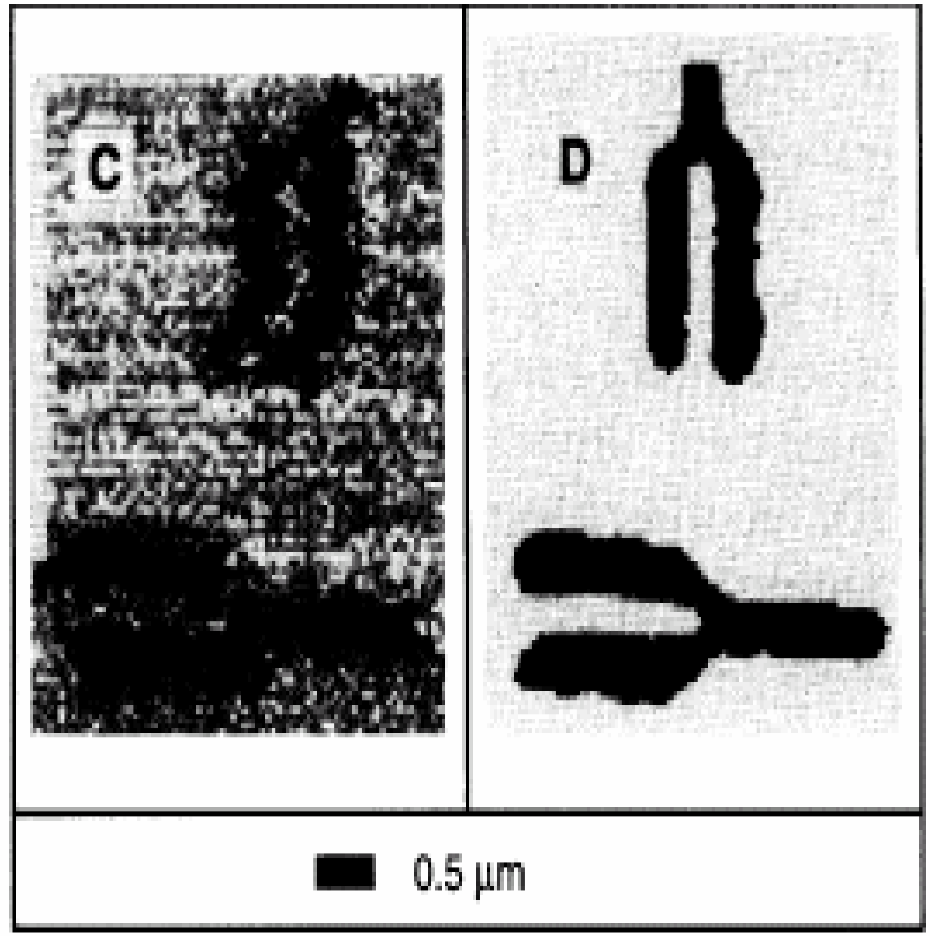
- Scan specimen (or probe) mechanically
  - Collect image pixel by pixel
- Detect
  - transmitted x-rays (STXM),
  - fluorescence (SXRF)
  - photoelectrons (SPEM)
  - visible light (SLXM)
  - diffracted X-rays
- Size of microprobe determines resolution
  - here ~ 2 μ, formed by pinhole
- Brightness limited
- Scan parameters determine object area, “magnification”

Comments on the formation of the probe

- Major types:
  - Pinholes; Tapered capillaries; Waveguides
    - Mostly for high energy X-rays
  - Mirrors
    - Normal incidence (multilayer) - <200 eV
    - Grazing incidence 3 – 30 KeV
  - Lenses
    - Compound refractive – high energy
    - Zone plates 200 eV – 20 KeV

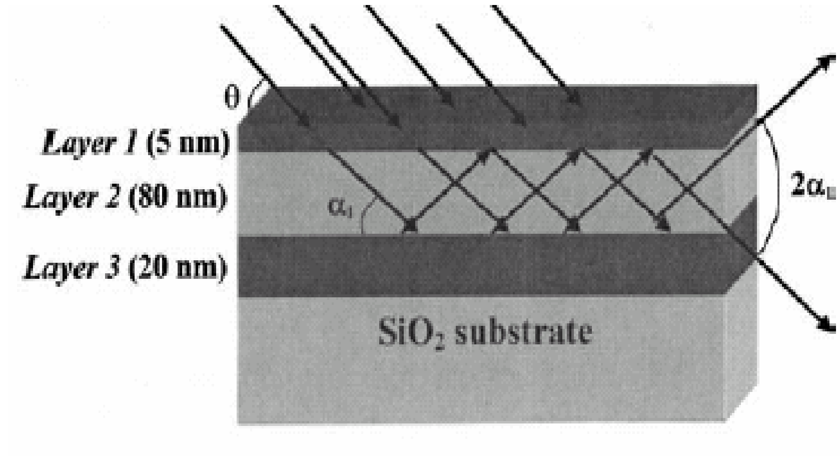
Tapered capillaries

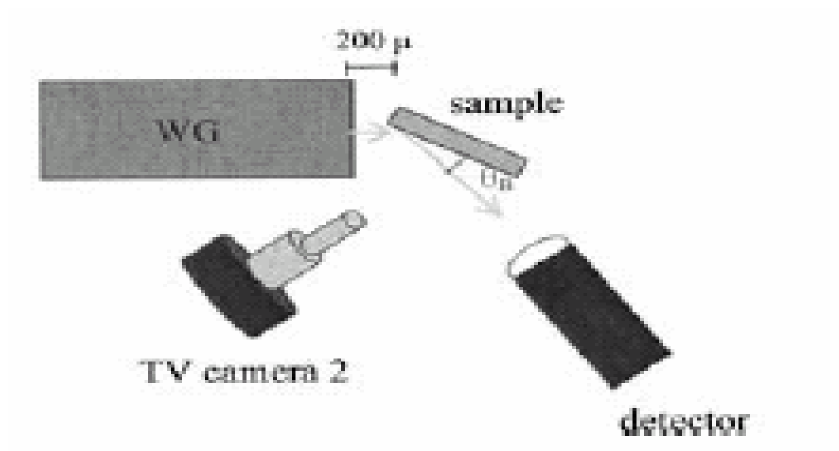
D. H. Bilderback, S. A. Hoffman, & D. J. Thiel, Science 263, 201 (1994)



Waveguides

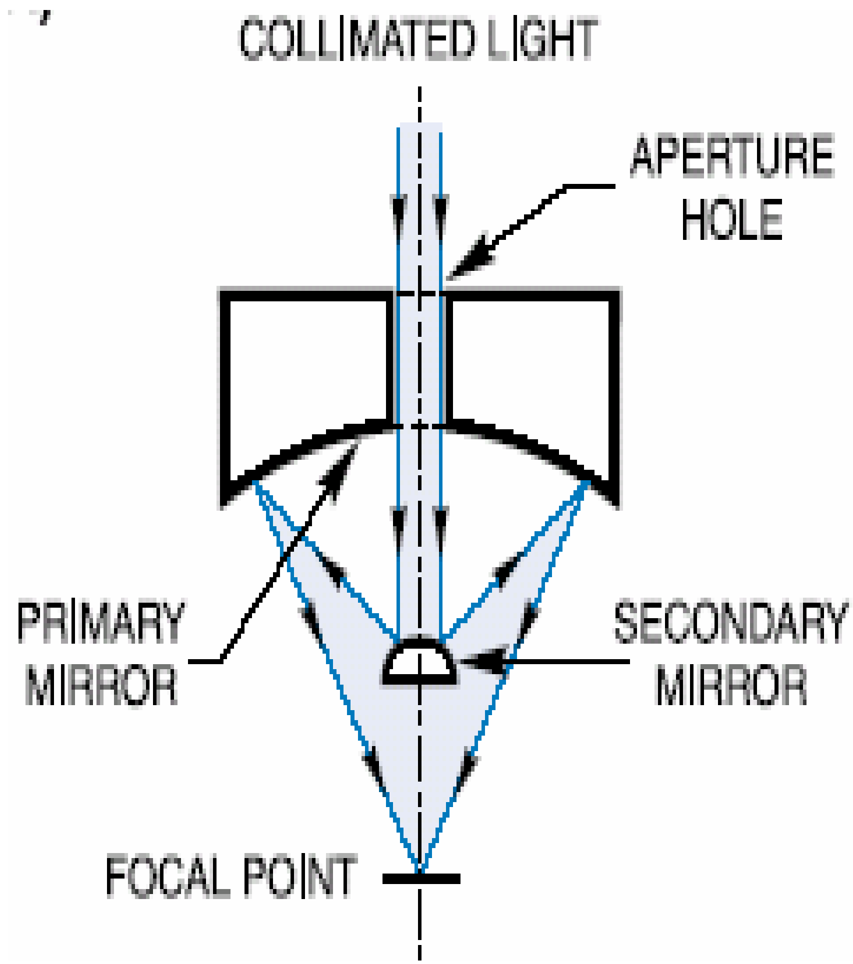
A. Cedola, S. Lagomarsino, F. Scarinci, M. Servidori, V. Stanic, J. Appl. Phys. 98, 1662 (2004)





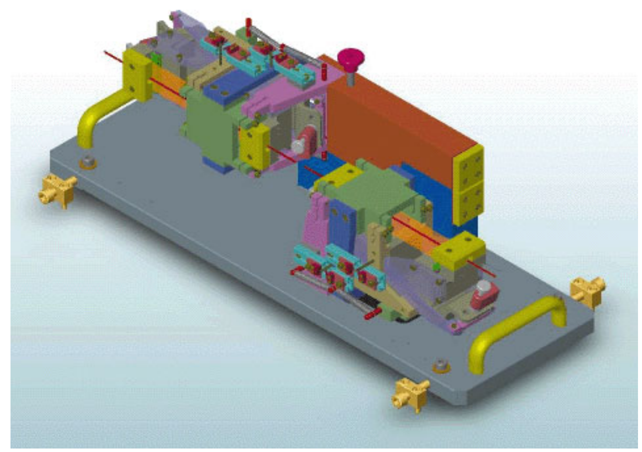
Mirrors – normal incidence

- Schwarzschild objective
- Multilayer coating:
  - Spiller
- First attempt: R.-P. Haelbich, W. Staehr, C. Kunz, HASYLAB 1980
- Hoover – NASA
- MAXIMUM
- SuperMAXIMUM



Mirrors – grazing incidence

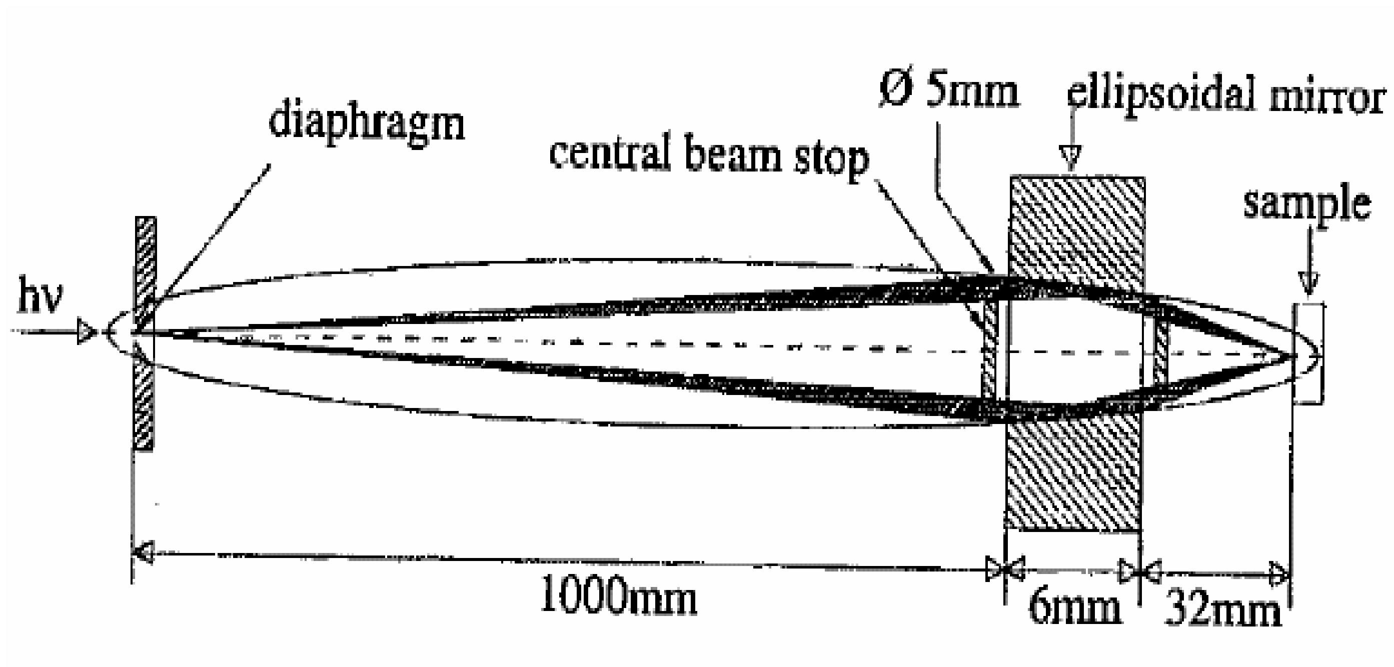
- achromatic!
- Kirkpatrick-Baez: separate x and y focusing
- Elliptical bending
- 100 nm focal spot (A. Freund et al. ESRF)



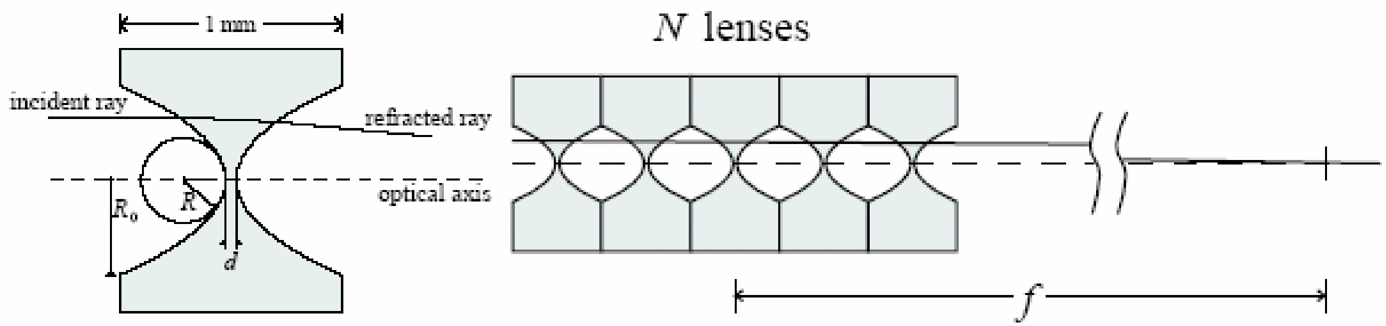
Mirrors – grazing incidence

- Ellipsoids:

  J. Voss, H. Dadras, C. Kunz, A. Moewes, G. Roy, H. Sievers, I. Storjohann, and H. Wongel, J. X-ray Sci. Technol. 3, 85 (1992).



Parabolic refractive lenses ESRF/Aachen collaboration



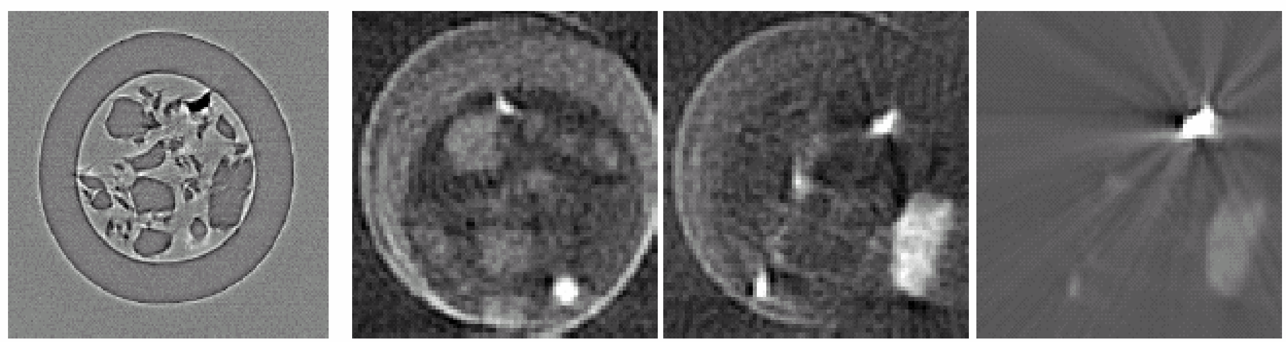
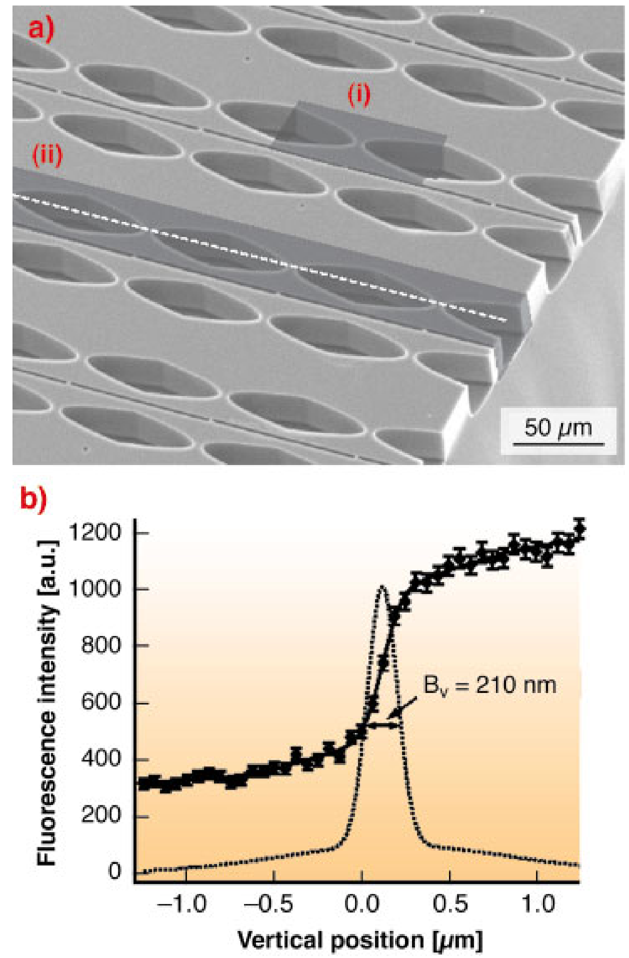


Figure 2: Results a combined transmission and fluorescence microtomography study. The sample consisted of different particles in a glass capillary (inner diameter 100 μm. The capillary wall as well as the particle outlines are clearly visible in the transmission tomogram of a slice in the sample (*far left*). The three images on the right are fluorescence tomograms of the same slice for different energies of the fluorescence radiation, namely Zr-K_α (*left center*), K-K_α (*right center*), and Fe-K_α (*far right*).

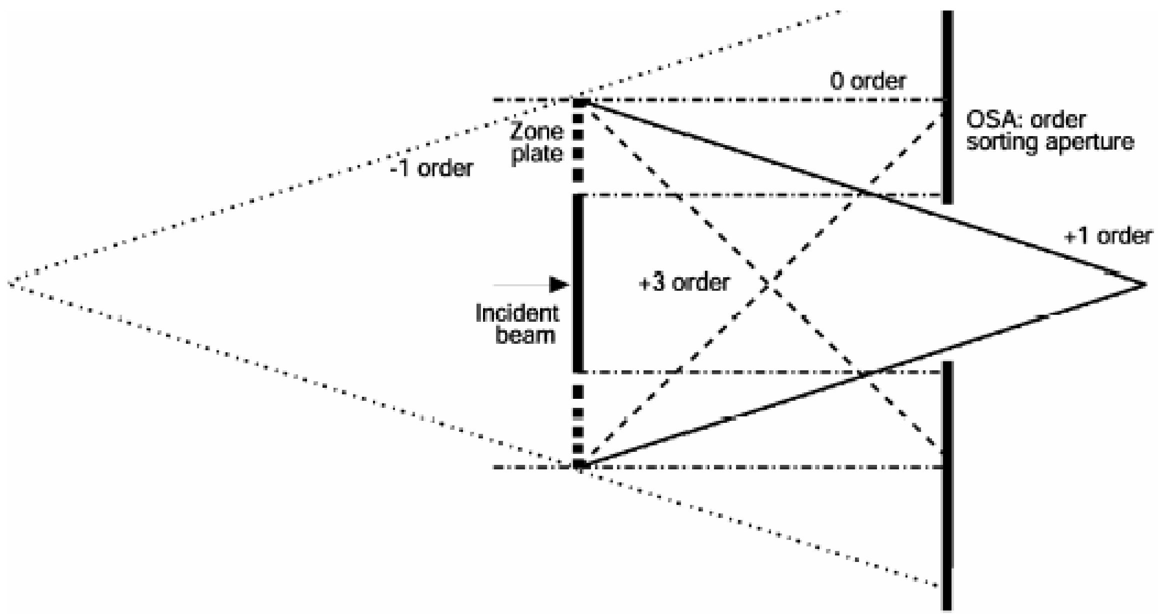
Parabolic refractive lenses

- Other efforts: cylindrical lenses by microfabrication
- Heat load not problem!
- Alignment much easier than K-B
- Chromatic
- Figure: C. G. Schroer et al., APL 82, 1485 (2003)



Zone plates

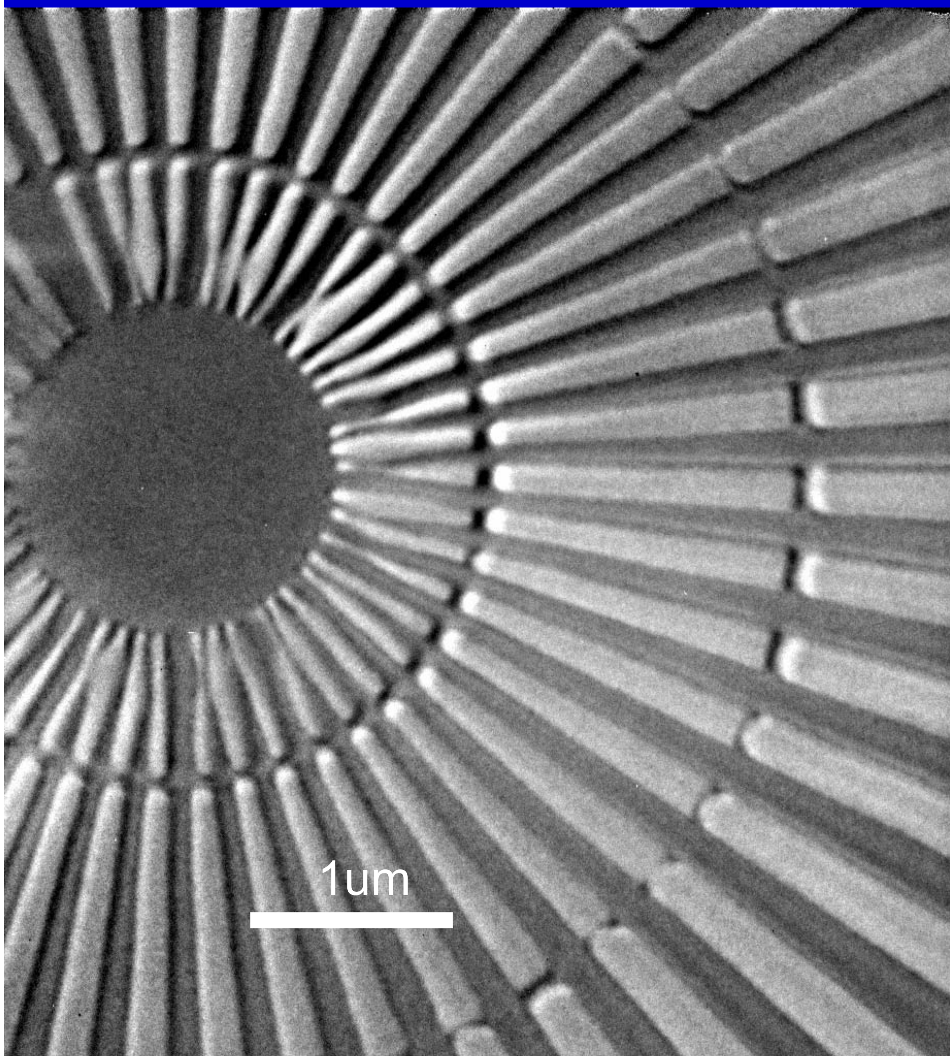
- Central stop and Order Sorting Aperture for forming microprobe and removing other orders
- No spherical aberrations!
- But chromatic!

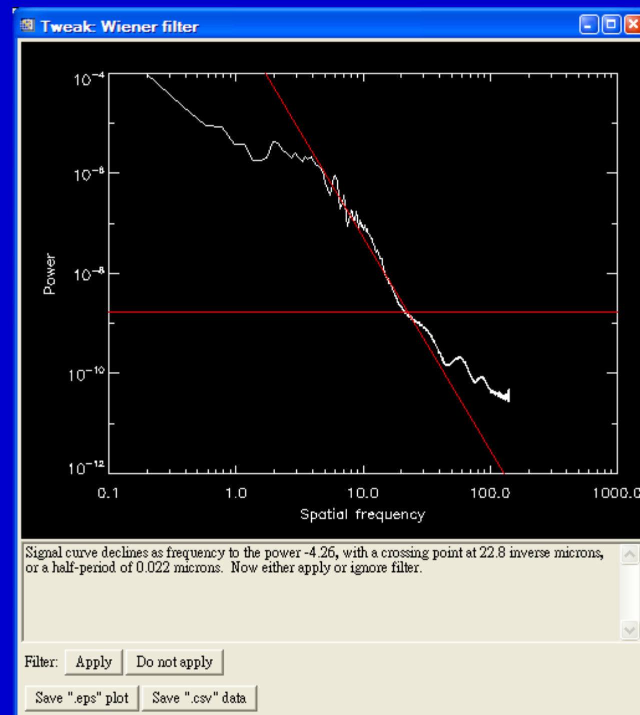


Efforts at XRADIA

- δr_n~ 100 nm, 1650 nm thick Au (for up to 24 KeV)
- δr_n~ 50 nm, <700 nm thick Au (for up to 9 KeV)
- δr_n~ 40 nm, <150 nm thick Au (for up to 1.4KeV)
- Working on achromatic X-ray lens
  - (zone plate plus non-focusing corrector)

Gold Spoke Pattern Imaged by 3rd Diffraction Order of Zone Plate





E = 8 keV

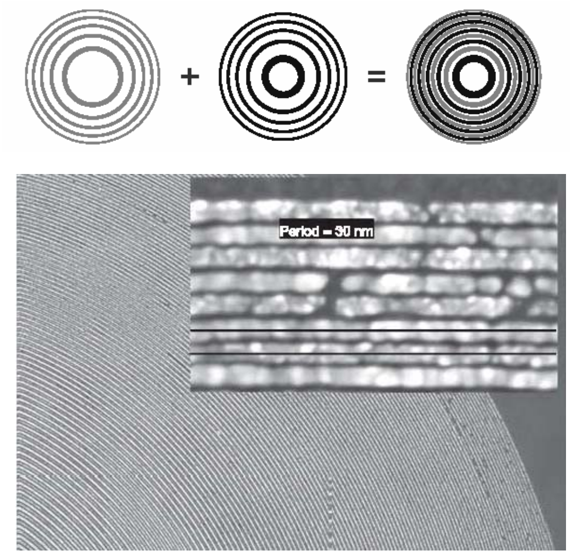
Exposure time : 10 mins

Resolution: About 25nm

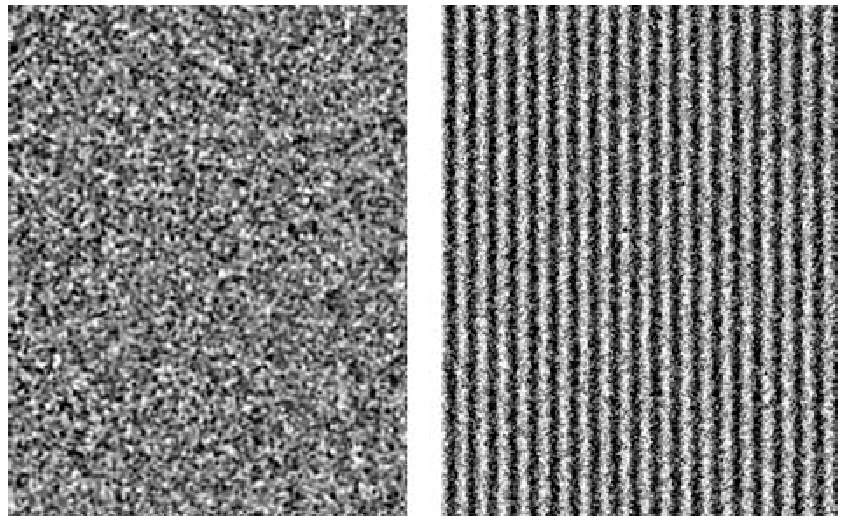
FOV : 5um x 5um

A SHARPER FOCUS FOR SOFT X-RAYS

Zone Plate Lenses with Better than 15-Nanometer Spatial Resolution



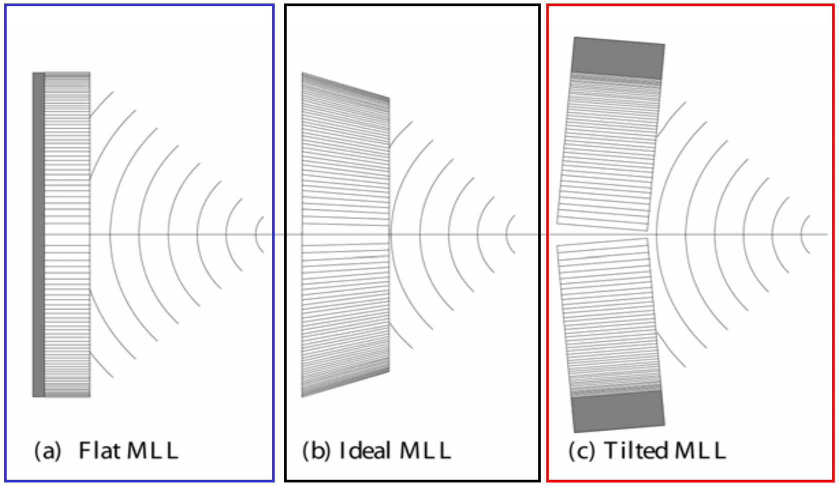
Overlay technique with separate e-beam lithography patterns for odd and even zones achieves 30-nm zone period (center-to-center) with high quality (e.g., placement accuracy of 1.7 nm).



Soft x-ray images taken with the CXRO XM-1 full-field imaging microscope at the ALS (Beamline 6.1.2). Comparison of images of a 15.1-nm test object with the previous 25-nm (left) and the new 15-nm (right) zone plates illustrates the improved spatial resolution achievable.

W.Chao and D.T. Attwood, Berkeley Lab and University of California, Berkeley) and B.D. Harteneck, J.A. Liddle, and E.H. Anderson (Berkeley Lab). [*Nature* **435**, 1210 (2005)]

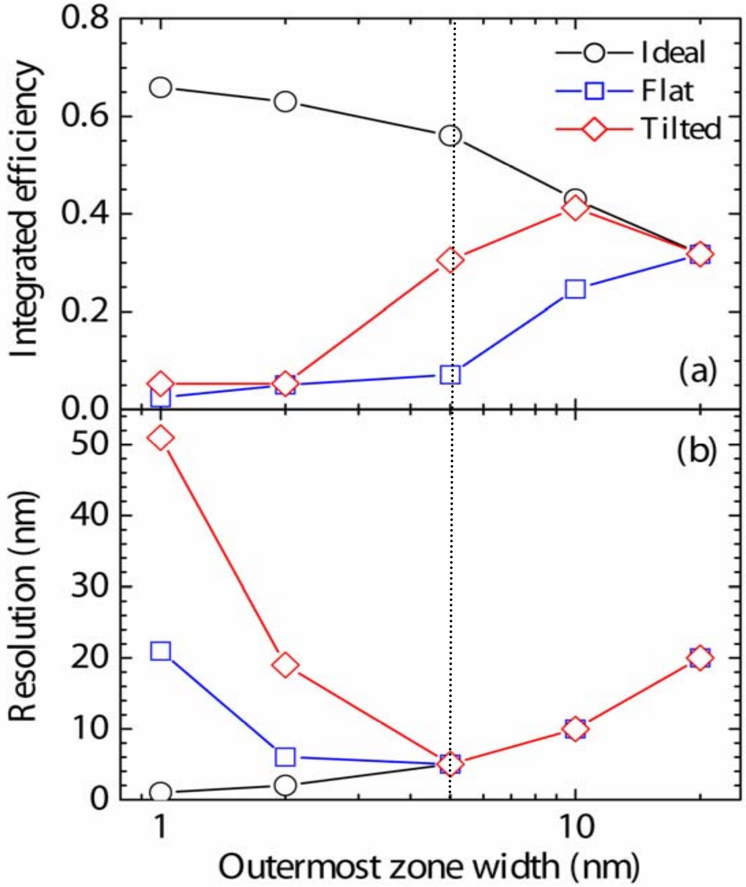
Where is the resolution limit for x-ray focusing (Diffractive Optics)?



- Ideal structure:
  - Resolution approaching 1 nm feasible,
  - Diffraction efficiency (2D) > 50%
- Tilted MLL: δ = 5 nm feasible

- Locally 1D N-wave CWT valid to ~ 1 nm
- Beyond:
  - -What is the effect of Borrman-Fan on Phase?
  - - When is curvature of zones required?

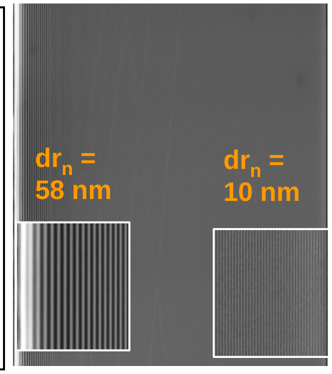
Calculations for 1D MLL



Kang et. al, PRL, 96, 127401 (2006)

Multilayer Laue Lens – Concept

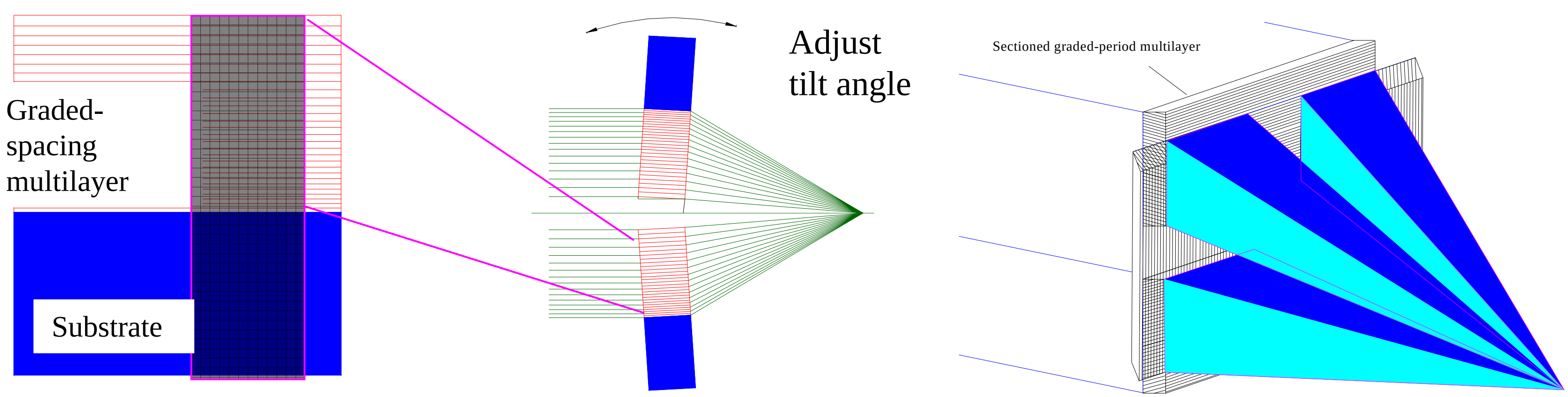
- Deposit varied depth-graded multilayer on plane substrate (thinnest structures first)
- Section to 5-20 μm depth
- Assemble into a linear MLL
- Assemble two linear MLL's into a 2D MLL.



Material: WSi_2/Si

Total deposition thickness: 12.4 um

d-spacing: 10 – 58 nm



Cross-divisional team (MSD/CNM/XFD) capitalizes on strengths of ANL science and facilities

The 43rd Anniversary R&D 100 Awards

2005 R&D 100 Award

X-ray focusing with MLL sections, dr_N = 15, dr_N = 10 nm

Sample A: dr_N = 15 nm

Sample B, C: dr_N = 10 nm

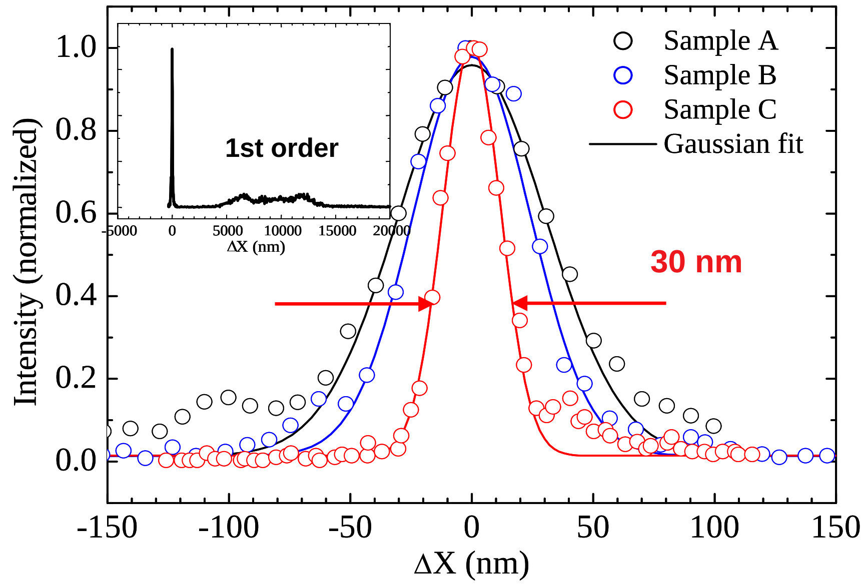
NA-limited resolution:

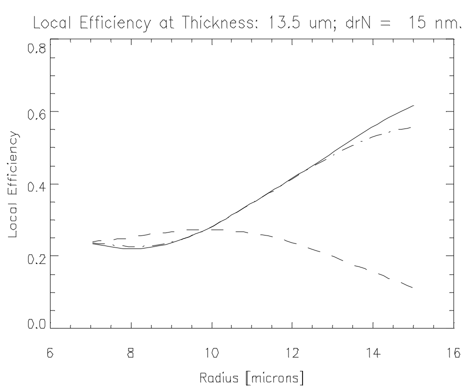
Sample A: 57 nm (27% NA)

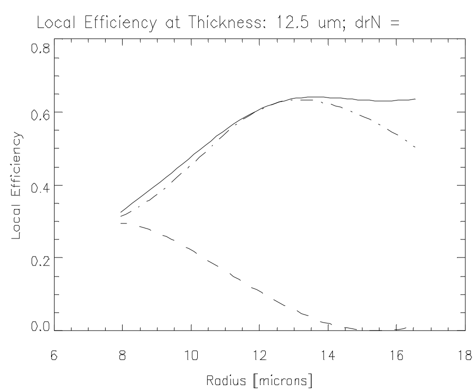
Sample B: 44 nm (23% NA)

Sample C: 24 nm (41% NA)

Resolution measurement







Photon Energy: 19.5 keV

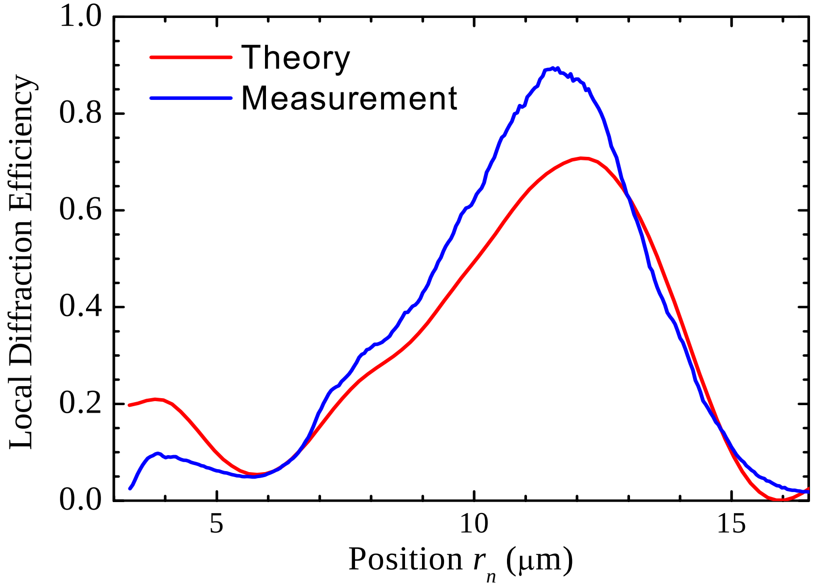
Measured Resolution: **30 nm**

Diffraction Efficiency: **44%**

Kang et. al, PRL, Apr., 2006

X-ray focusing with MLL sections, $dr_N = 5$ nm

Experiment and Theory (Coupled Wave Theory)



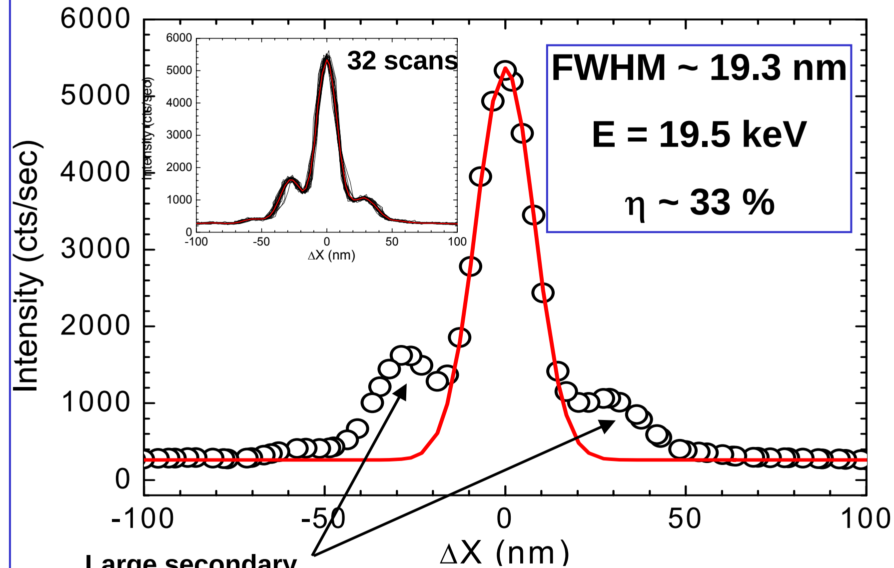
Radial change of local efficiency

Calculated integrated efficiency ~ 30 %

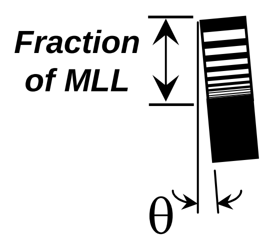
Measured Integrated efficiency ~ 33 %

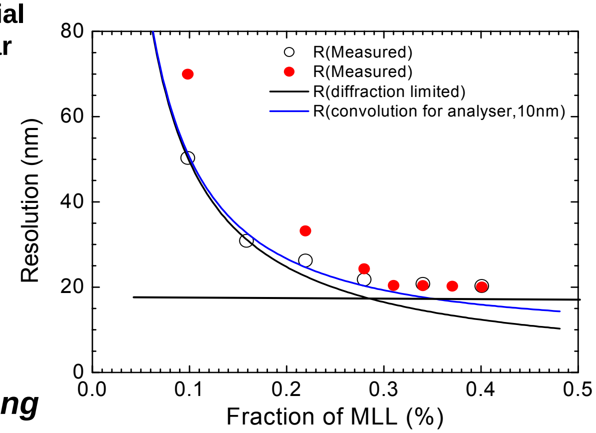
(unpublished)

Resolution measurement



Large secondary maxima due to partial structure and linear geometry



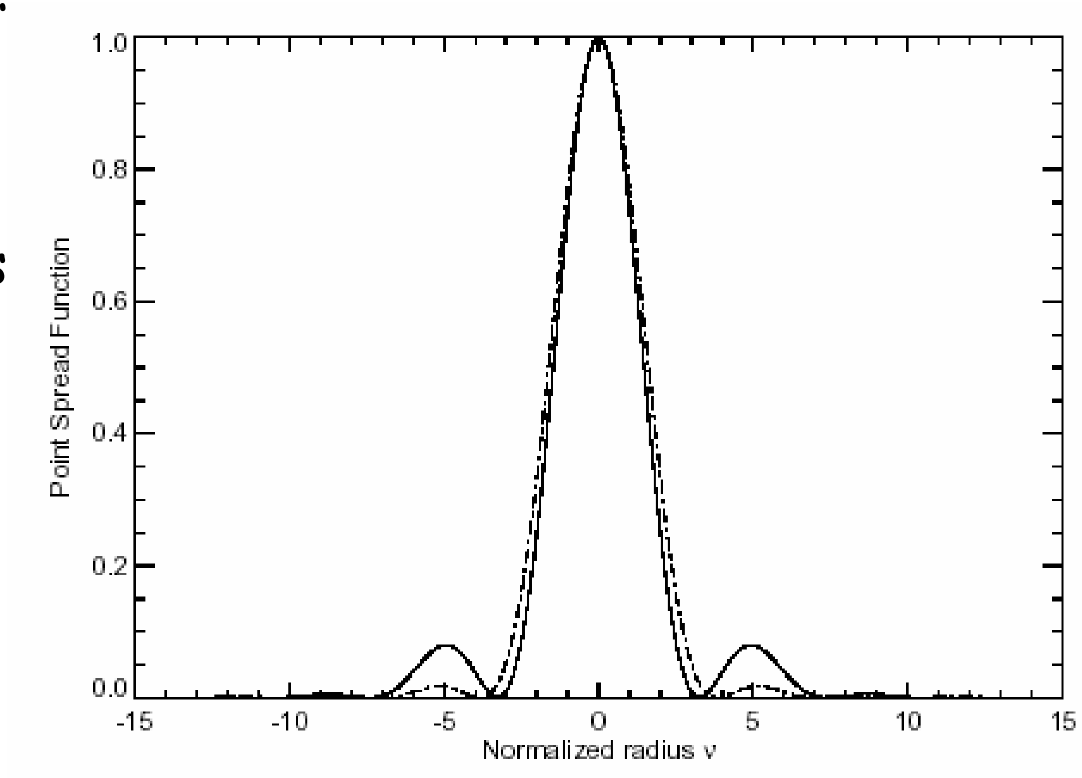


Courtesy H.C. Kang

- Mirrors and lenses demagnify small source
  - For microprobe, off-axis aberrations irrelevant!
- Diffraction-limit: focus size > λ/NA
- Strehl-ratio: What fraction of photons within focus?
- Depth of focus and working distance

Shape of focus

- Full coverage of aperture: Airy pattern
- Partial coverage (central stop, ring, segment): increased secondary rings
- Apodization

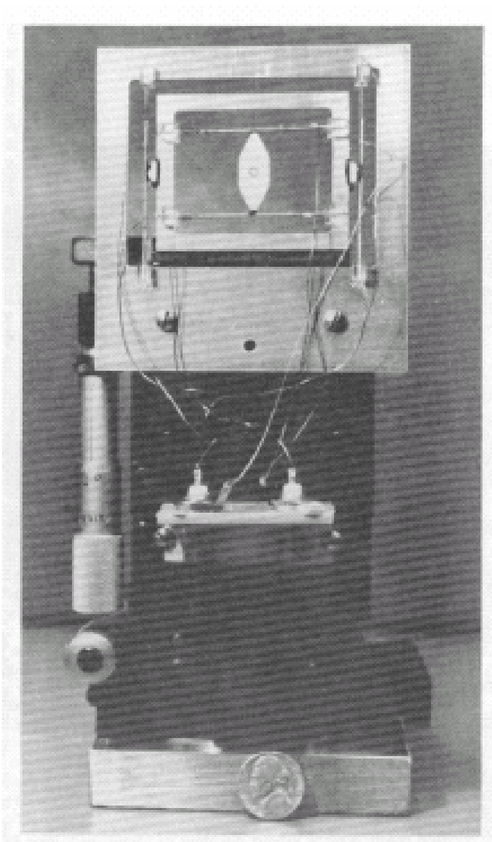


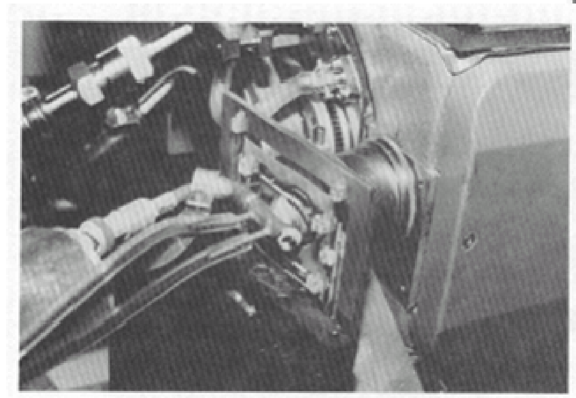
1979

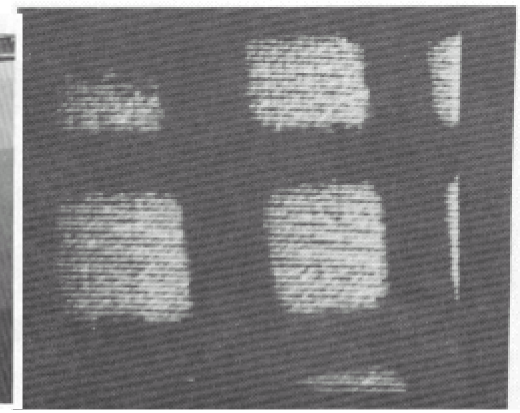
Plans for a scanning transmission X-ray microscope

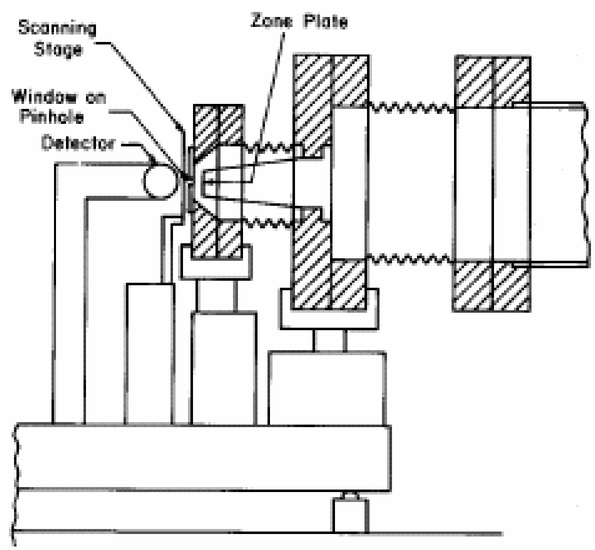
J. Kirz, R. Burg and H. Rarback

Ann. NY Acad. Sci. 342, 135 (1980)

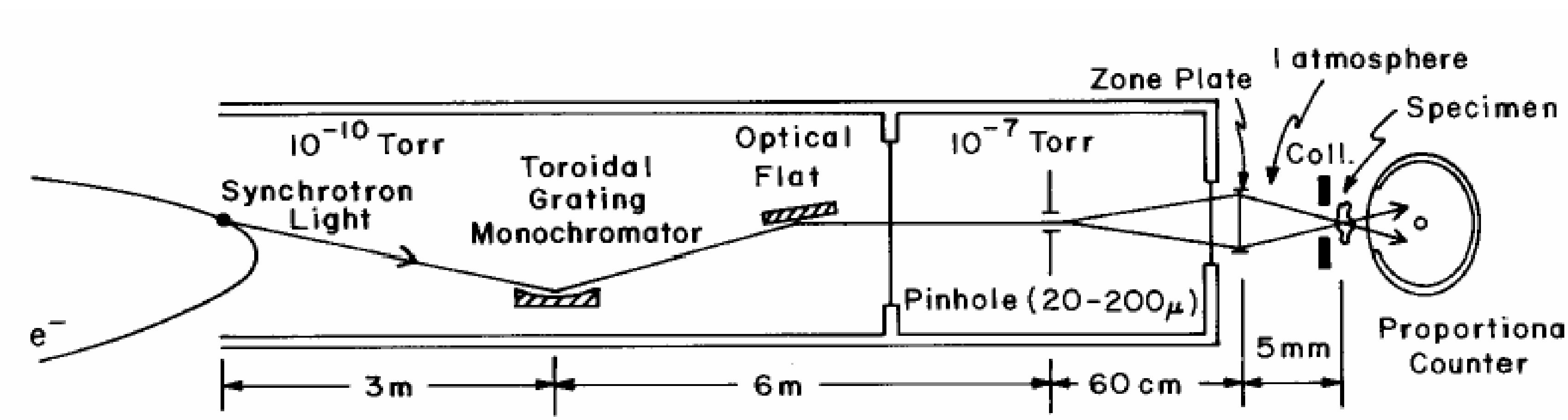








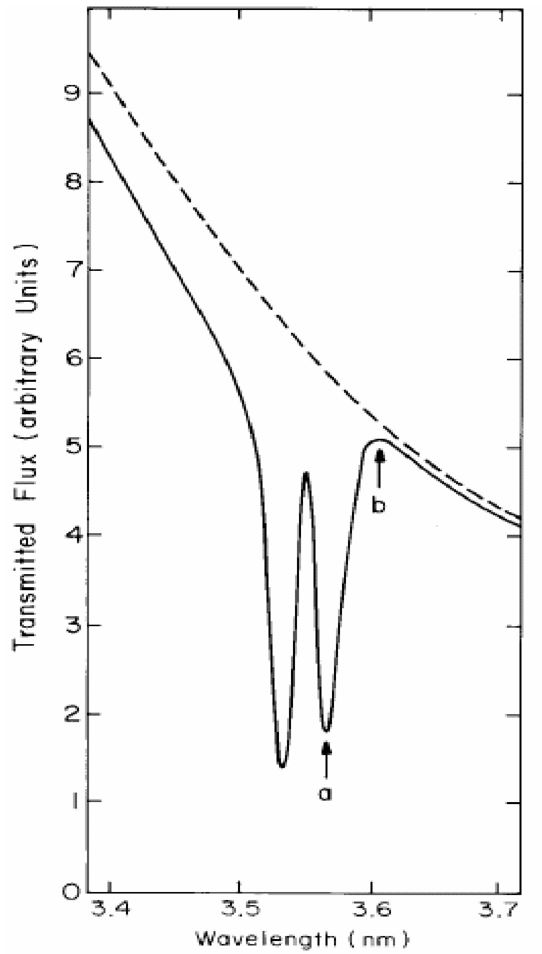
Scanning microscopy (STXM) 1983

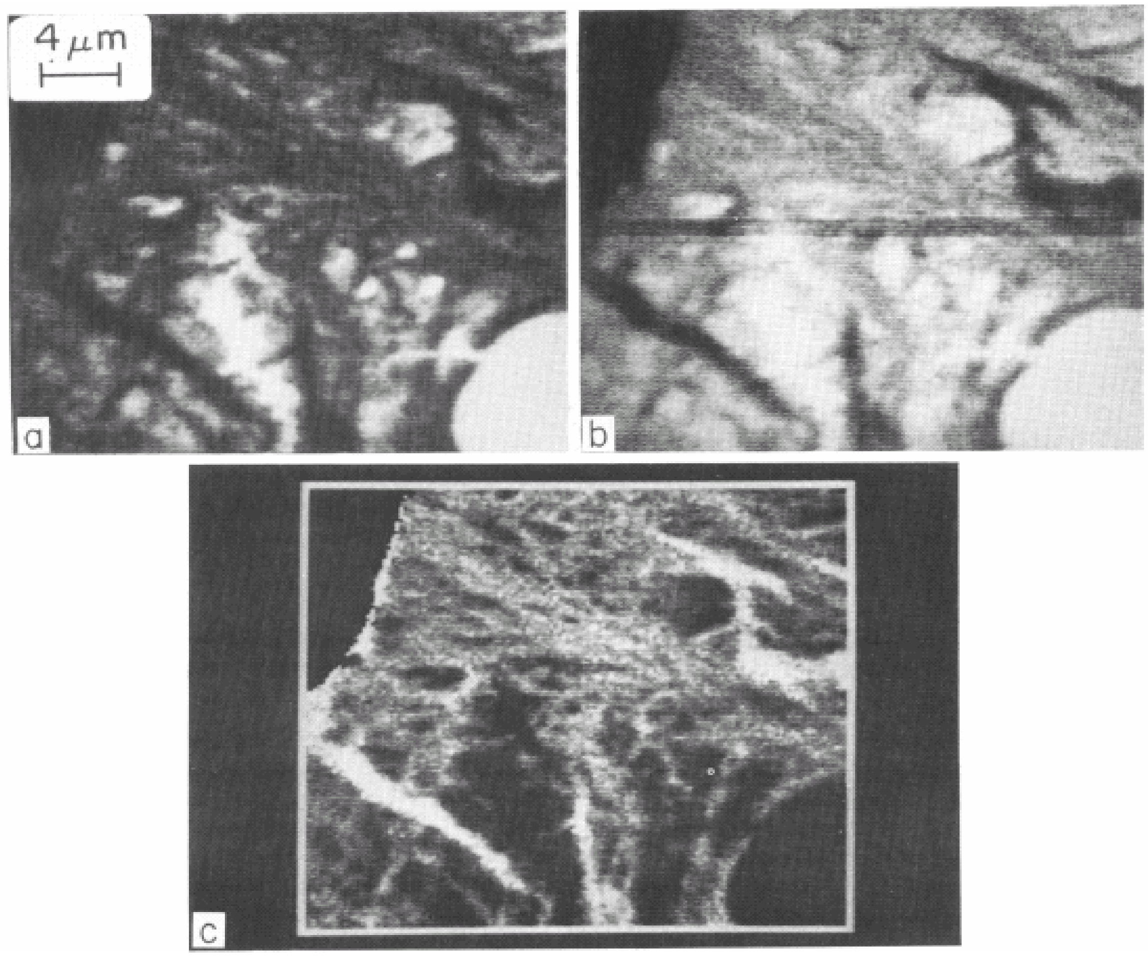


- Silicon nitride window
- Zone plate
- Proportion counter
- Scanning stage

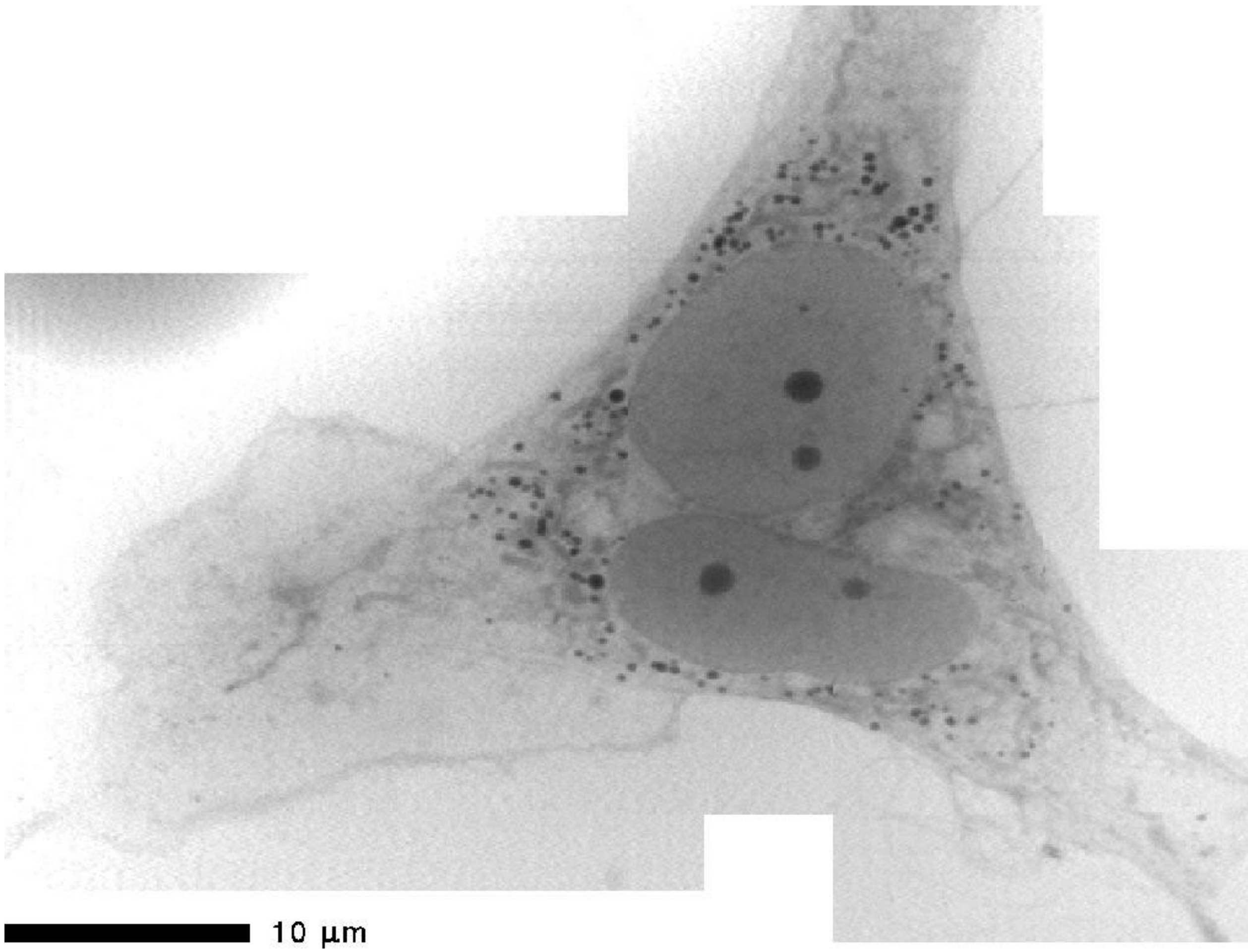
Kenney J. Microsc.1984

Absorption microanalysis with a scanning X-ray microscope: mapping the distribution of calcium in bone
J. Kenney et al, J. Microsc. 138, 321 (1985)



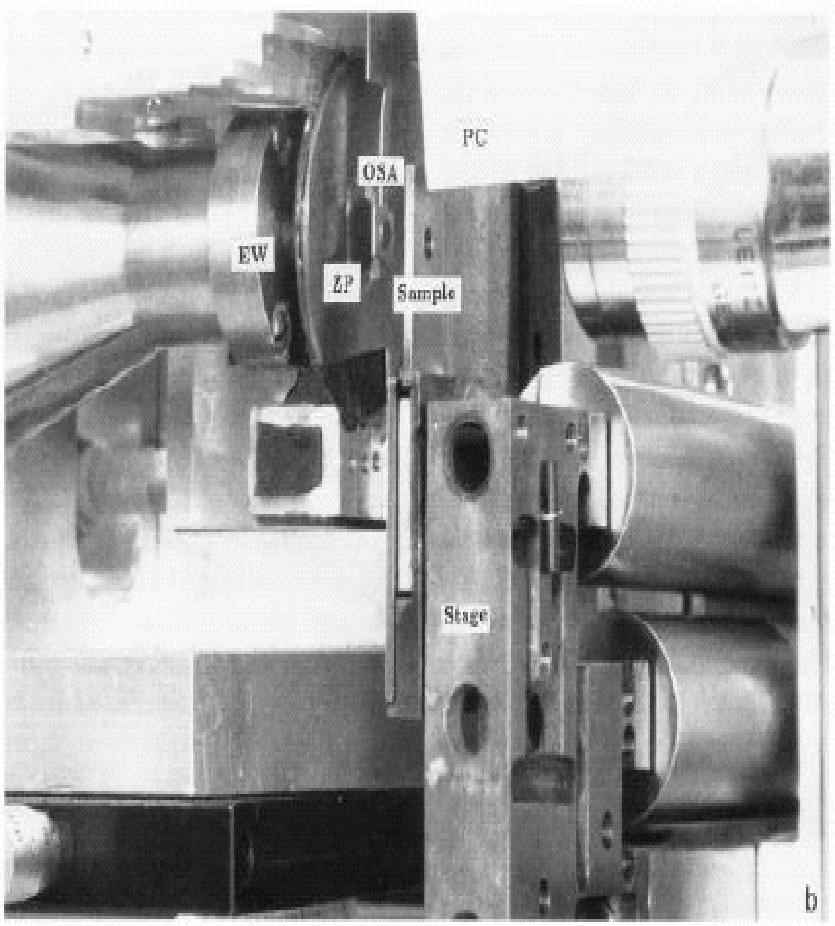


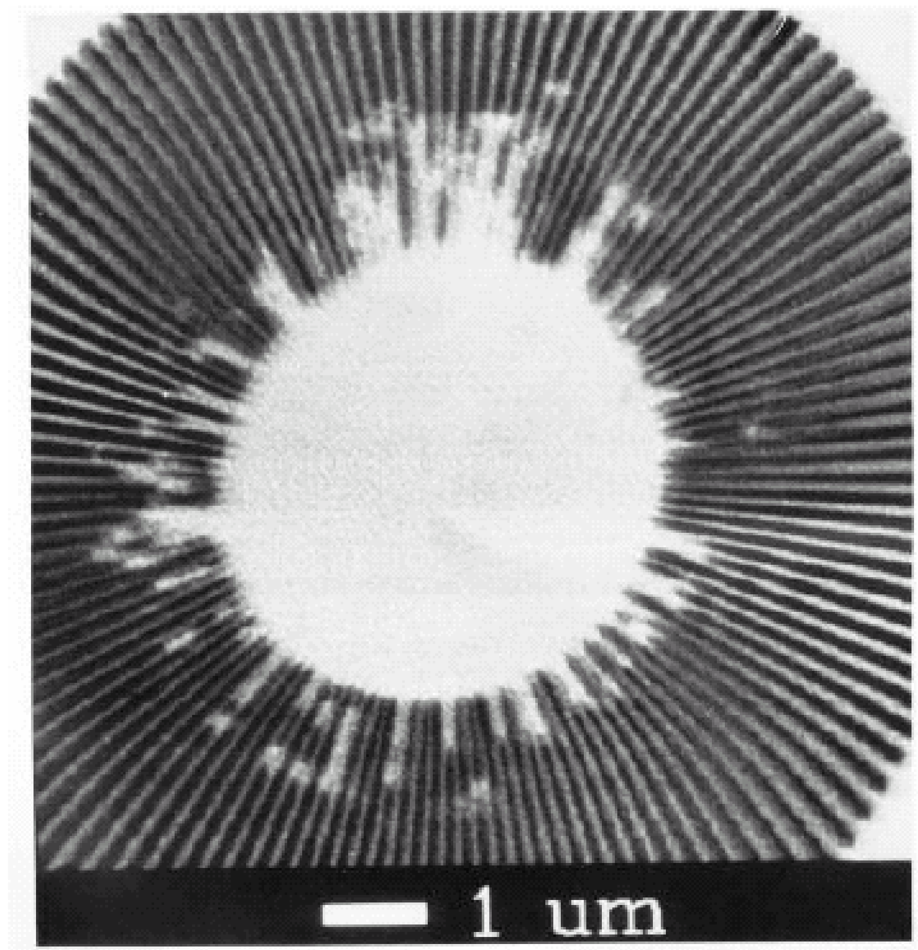
STXM – wet specimens 1986



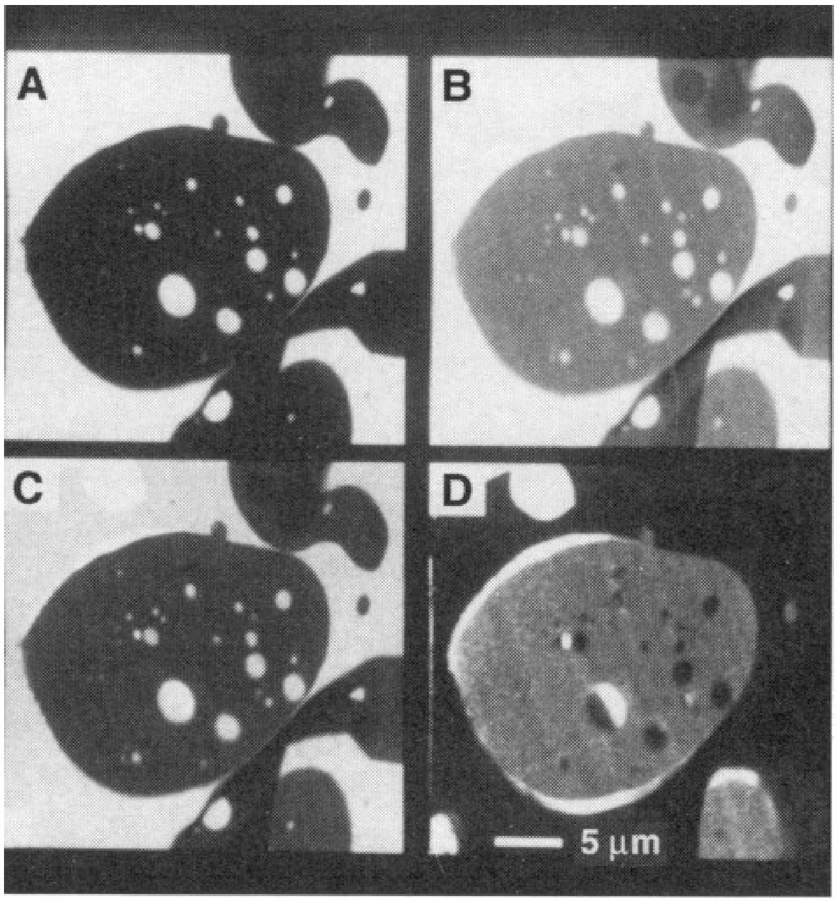
Gilbert thesis, '92

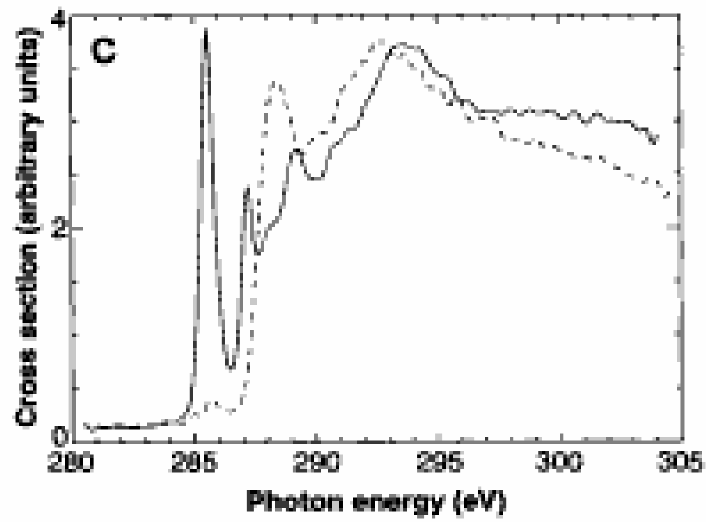
C. Jacobsen et al., Opt. Comm. 86, 351 (1991)
45 nm zone plate made at IBM/CXRO
X1 undulator, 340 eV





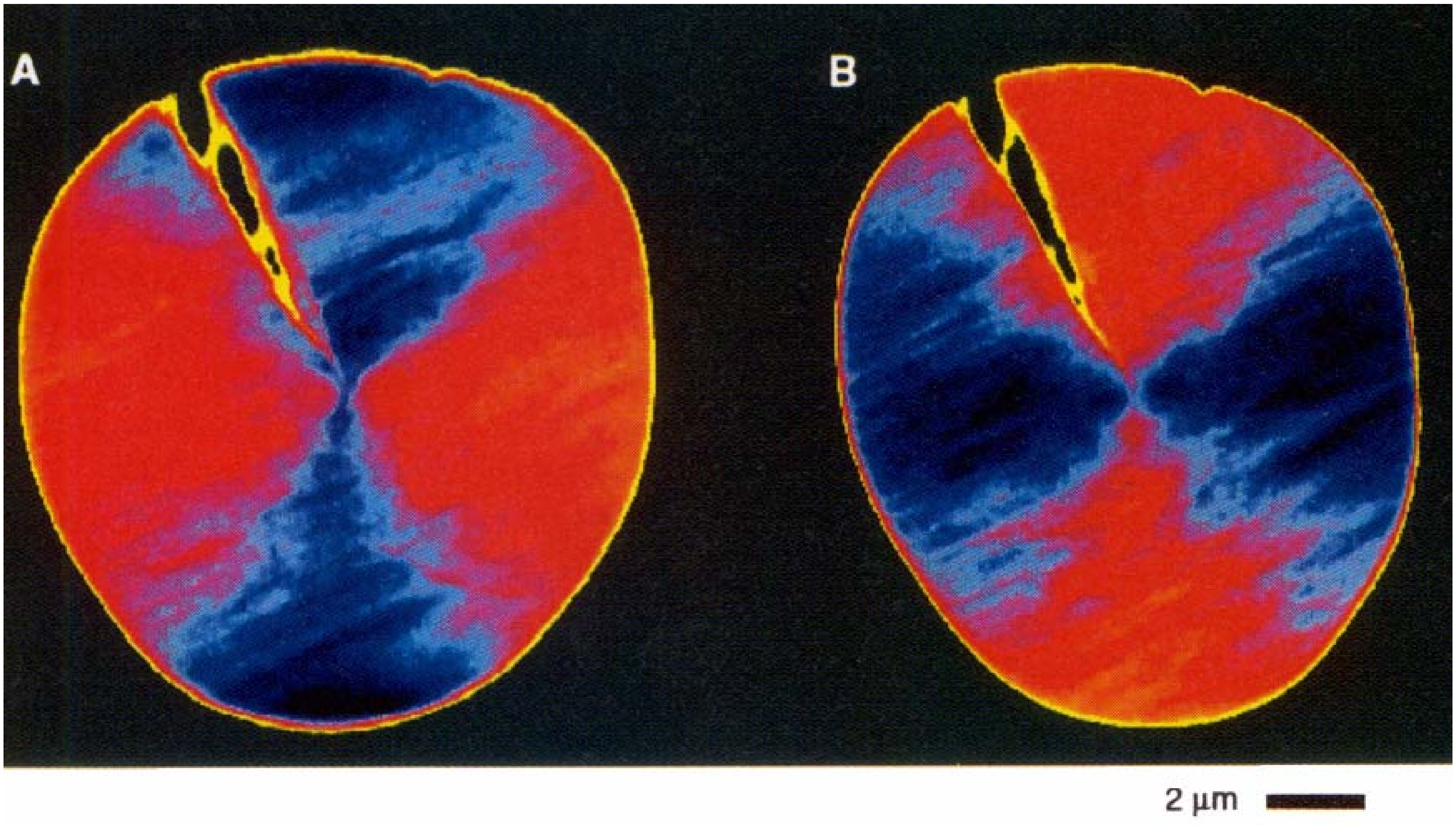
STXM 1992
spectromicroscopy





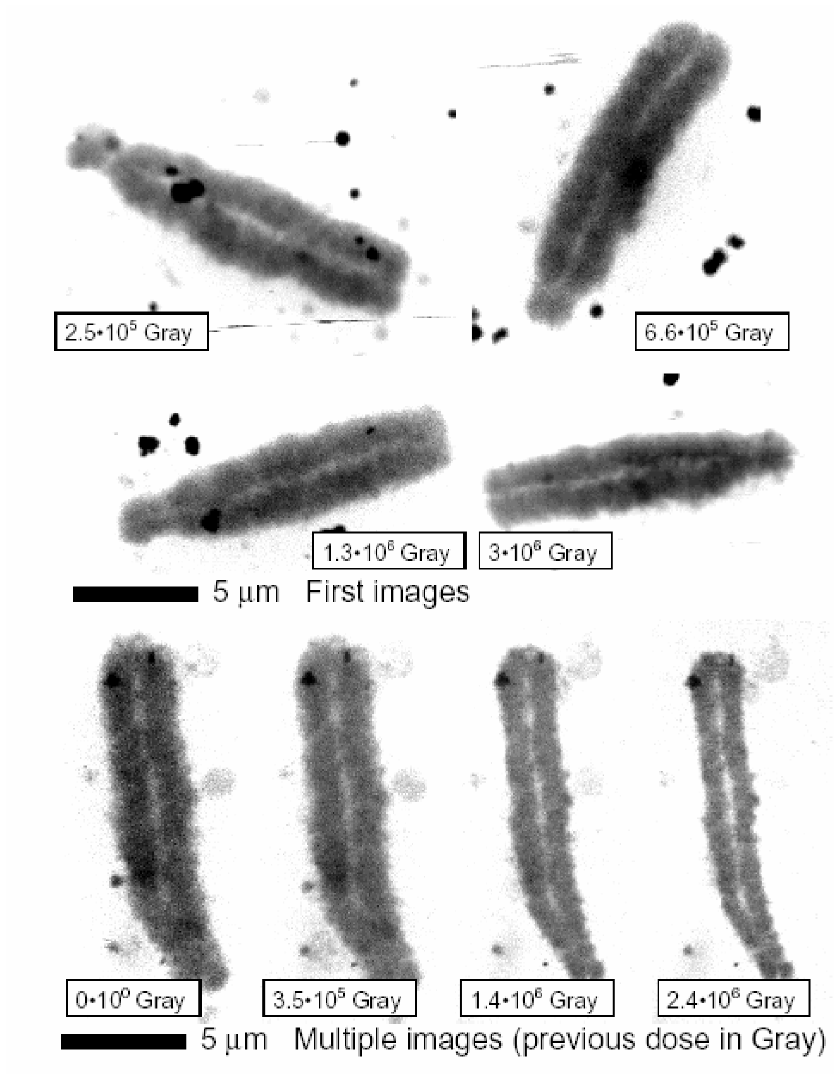
Ade et al. Science

Linear dichroism microscopy Ade & Hsiao, NSLS 1993



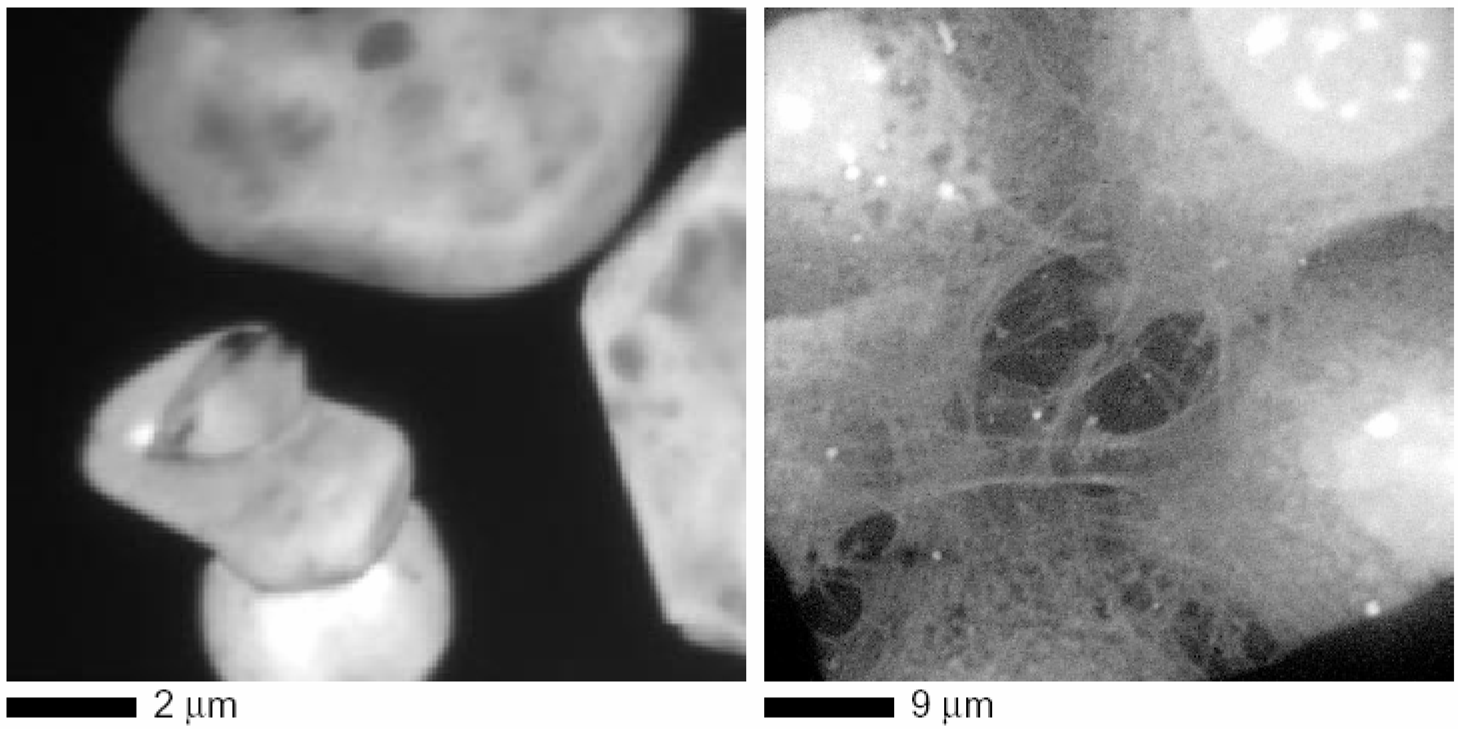
Ade & Hsiao, Science 1993

Radiation damage studies, 1993



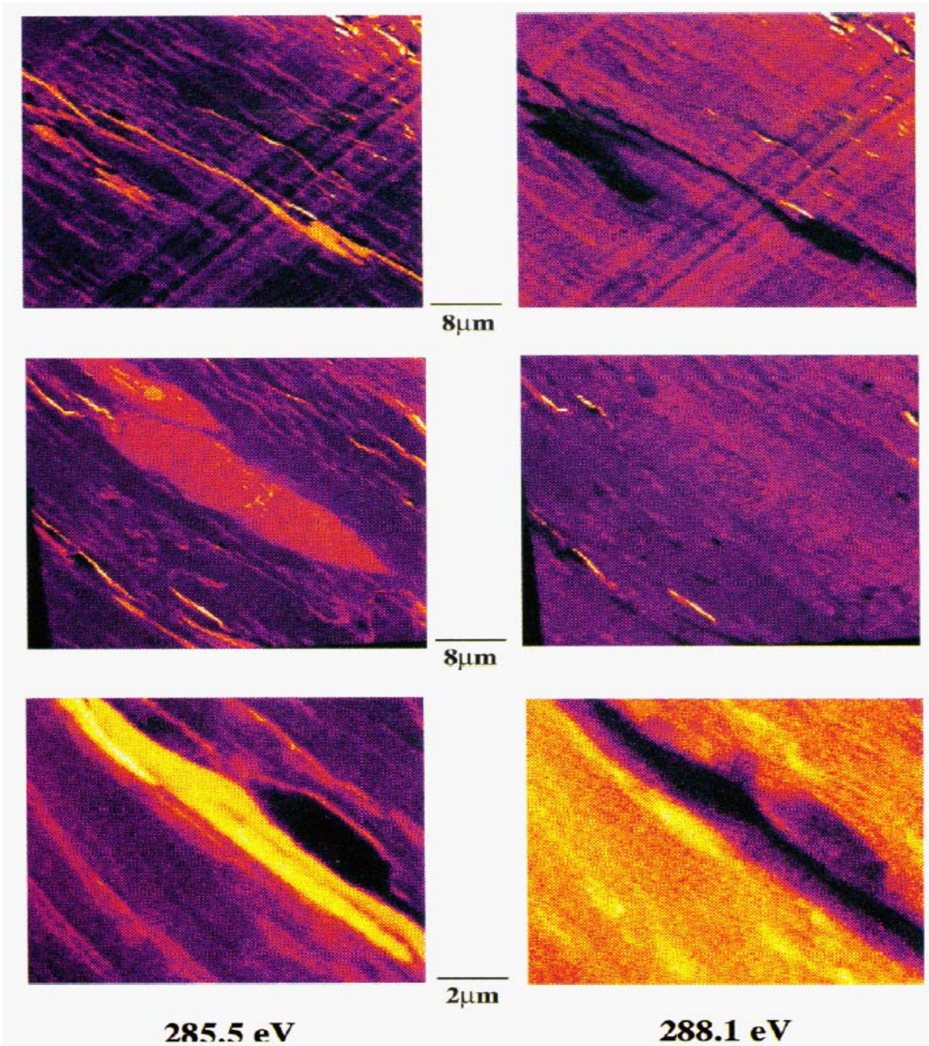
Williams et al. J. Microsc

Visible light detection:SLXM
phosphor grains 1993   actin filaments 1994



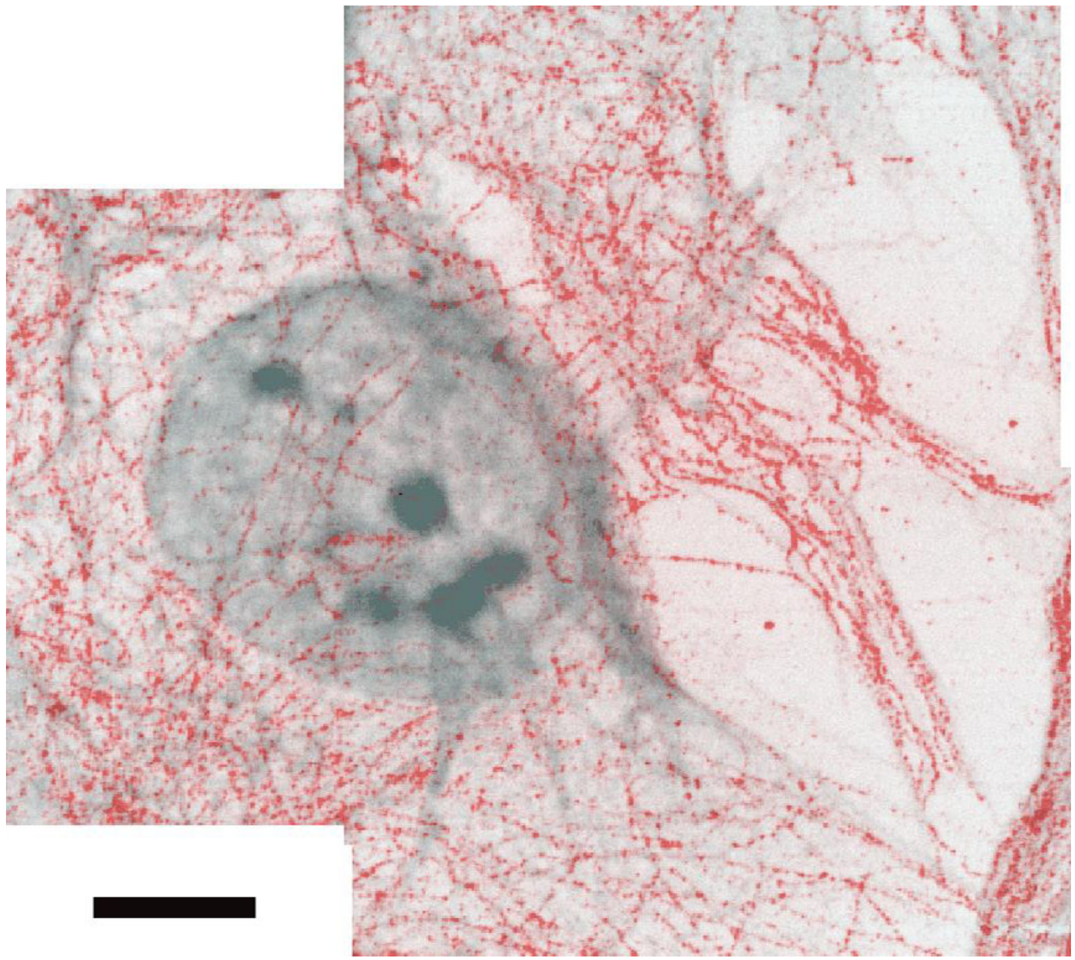
Jacobsen
Morrone

Environmental/Earth Science 1994
diagenesis of coal



Botto, Cody,... Energy&Fuels

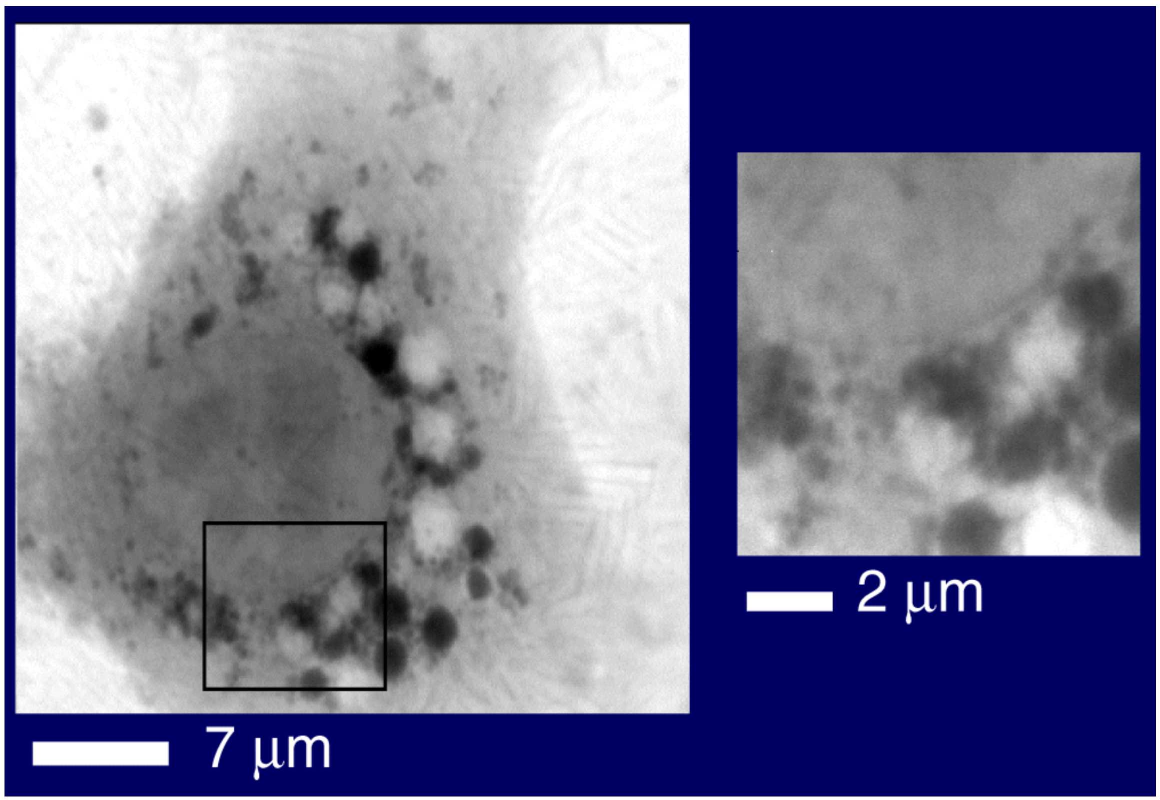
Gold labeled specimens 1996



Chapman et al. J. Micr. Soc. Am
scale bar 7 microns

Cryo-STXM: Frozen-hydrated Fibroblasts 1999

Grids with live cells are
- Taken from culture medium and blotted
- Plunged into liquid ethane (cooled by liquid nitrogen)
- Loaded into cryo holder

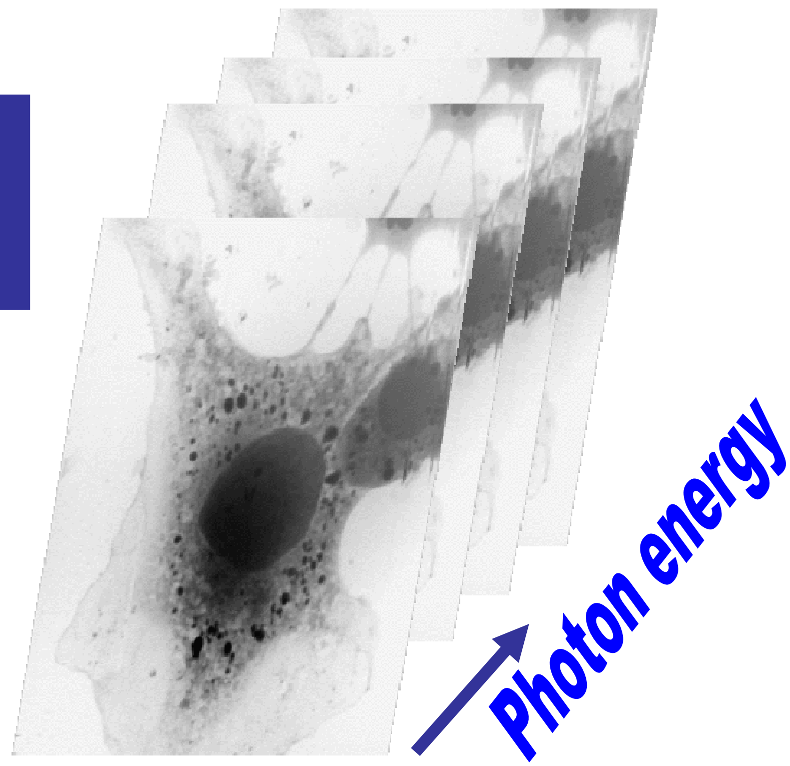


Spectromicroscopy by image stacks 1999

- Acquire sequence of images over XANES spectral region; automatically align using Fourier cross-correlations; extract spectra.

Images at N=150 energies are common.

IDL-based analysis tools are made available



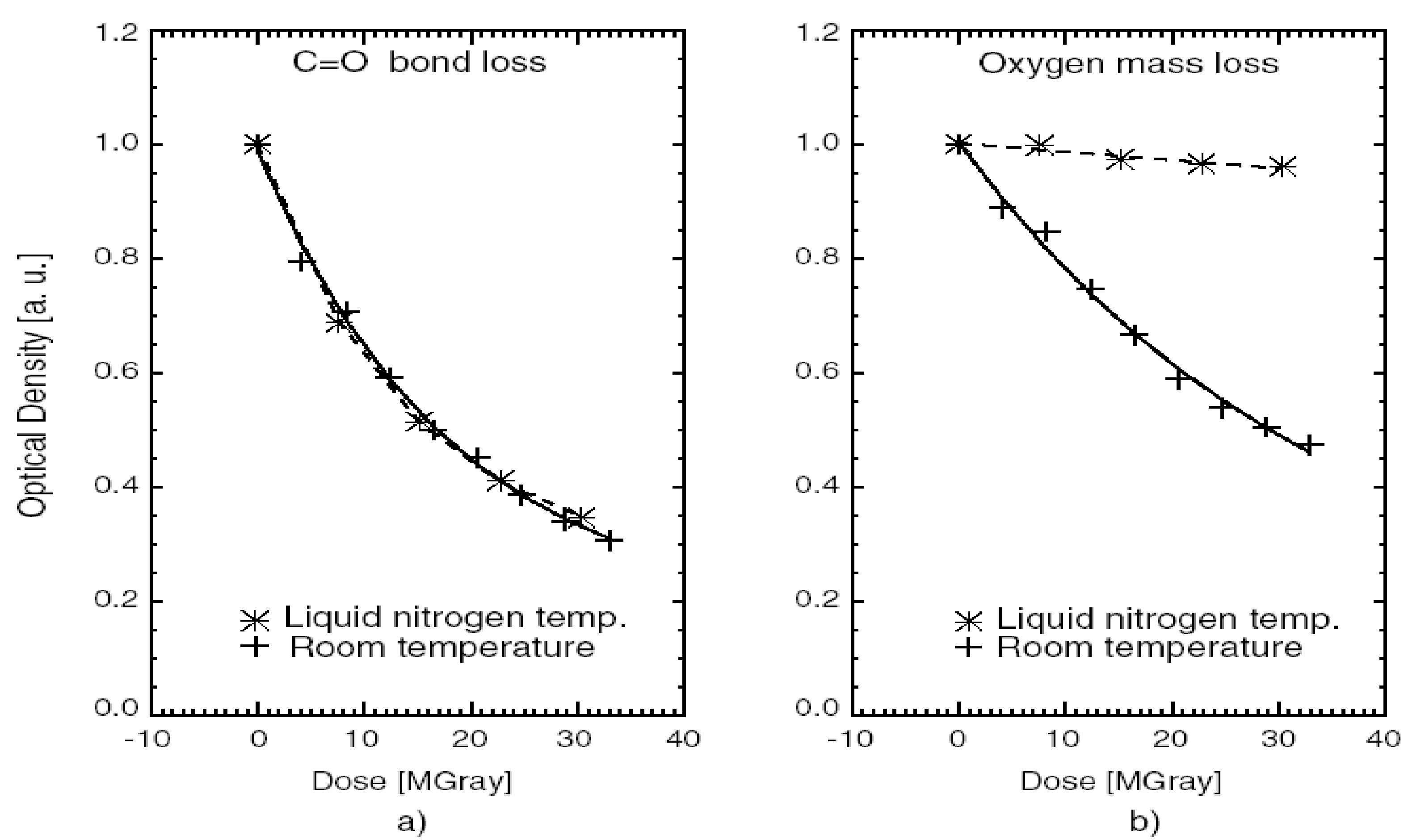
Jacobsen et al., J. Microsc. 2000

Analysis of stacks

C. Jacobsen and students

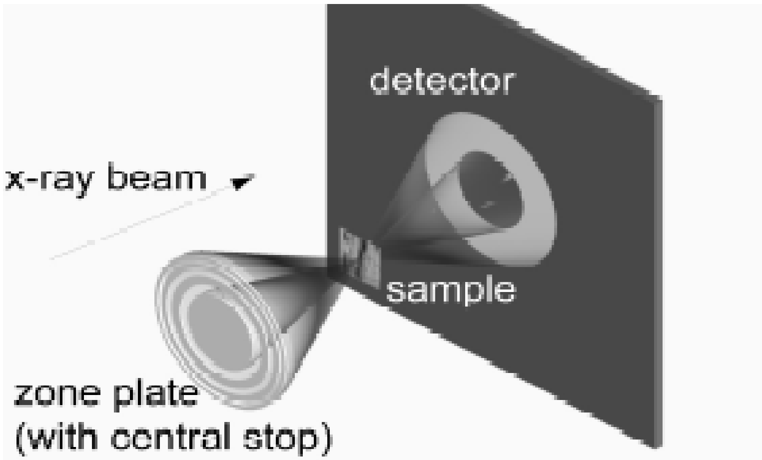
- Singular Value Decomposition
  - (components and model spectra known)
- Principal Component Analysis
  - (components unknown)
- Cluster analysis

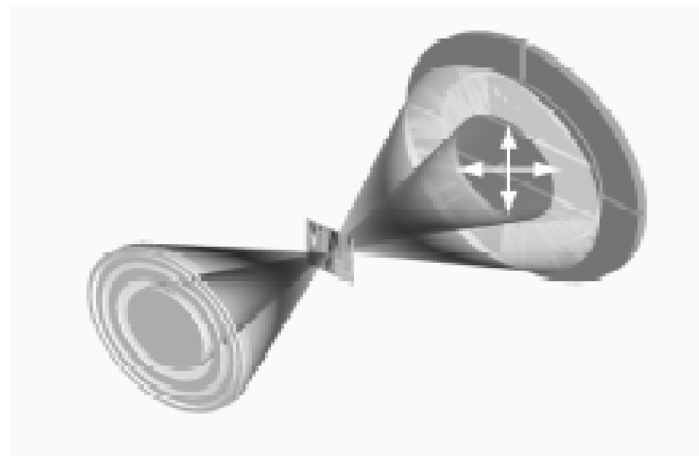
Cryo protects PMMA against mass loss, but not against chemical change!

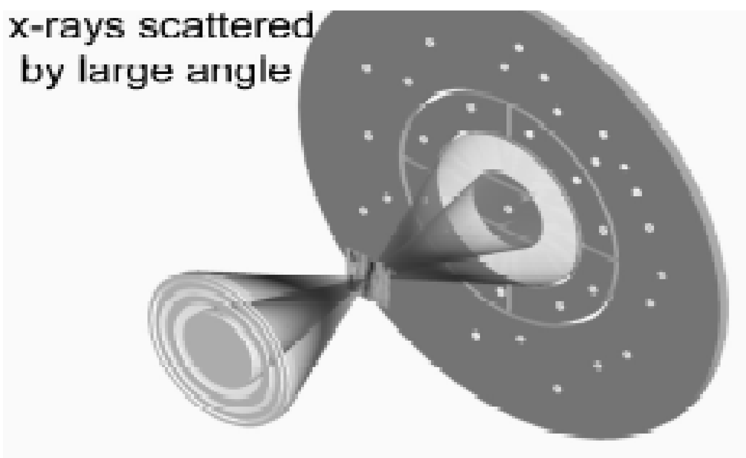


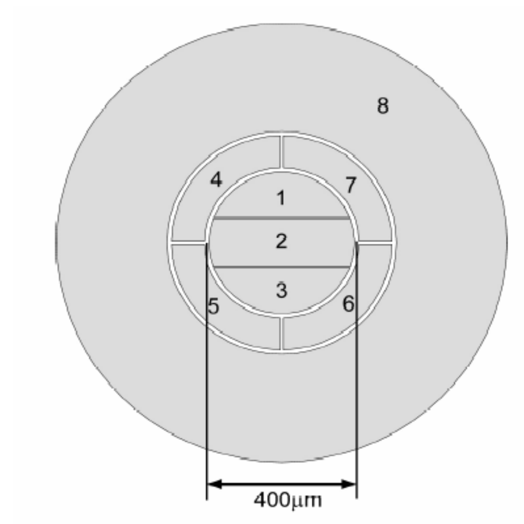
Beetz & Jacobsen, J. Synch. Rad. 2003

segmented detector 2001
(Stony Brook, BNL Instrumentation)



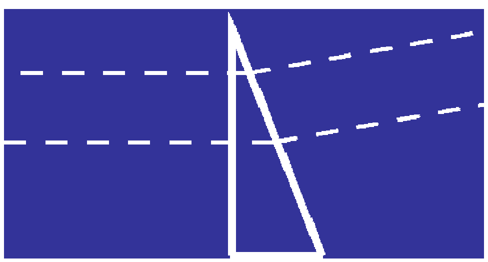




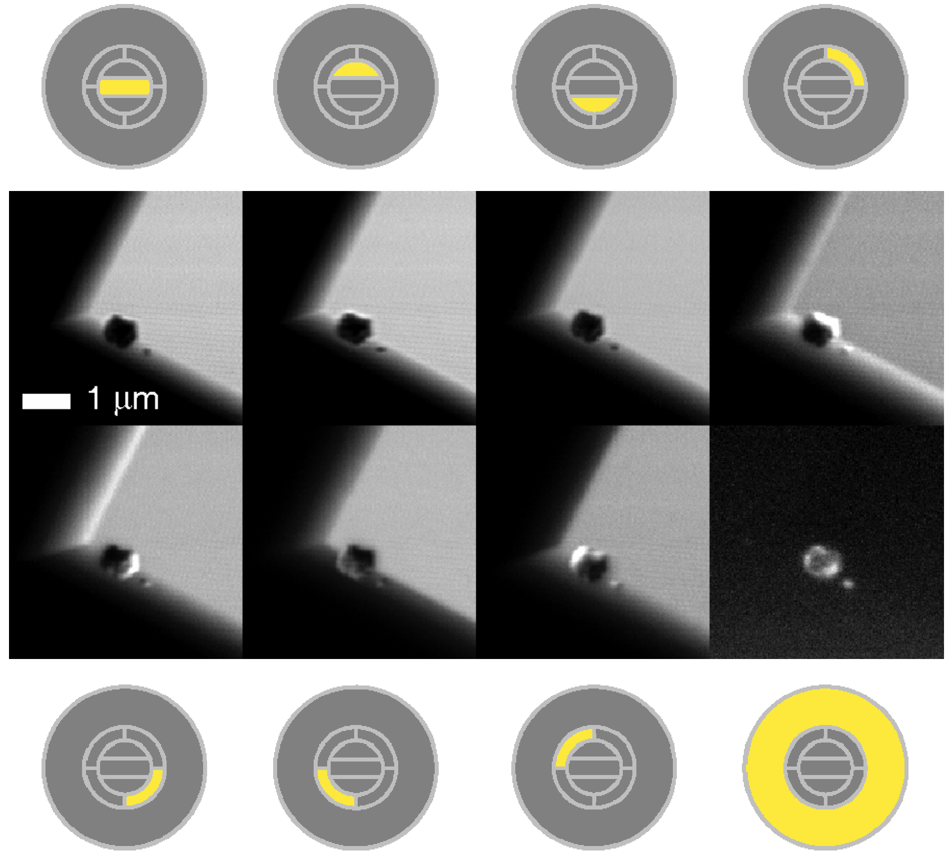


Segmented silicon drift detector

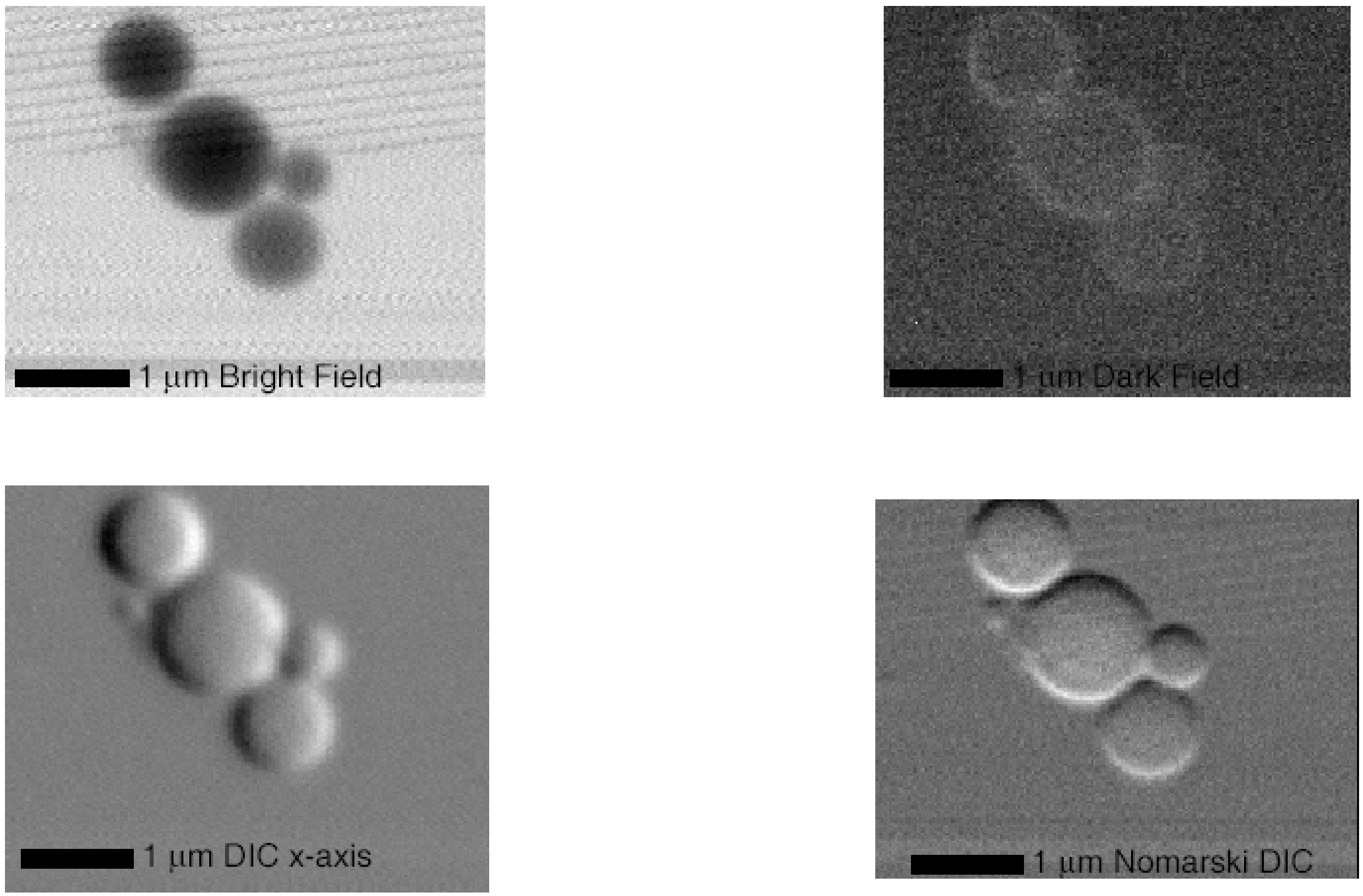
- Corner of silicon nitride window: silicon at ~54° wall slope forms a prism
- Refraction of x-ray beam in *opposite* direction from visible light prisms



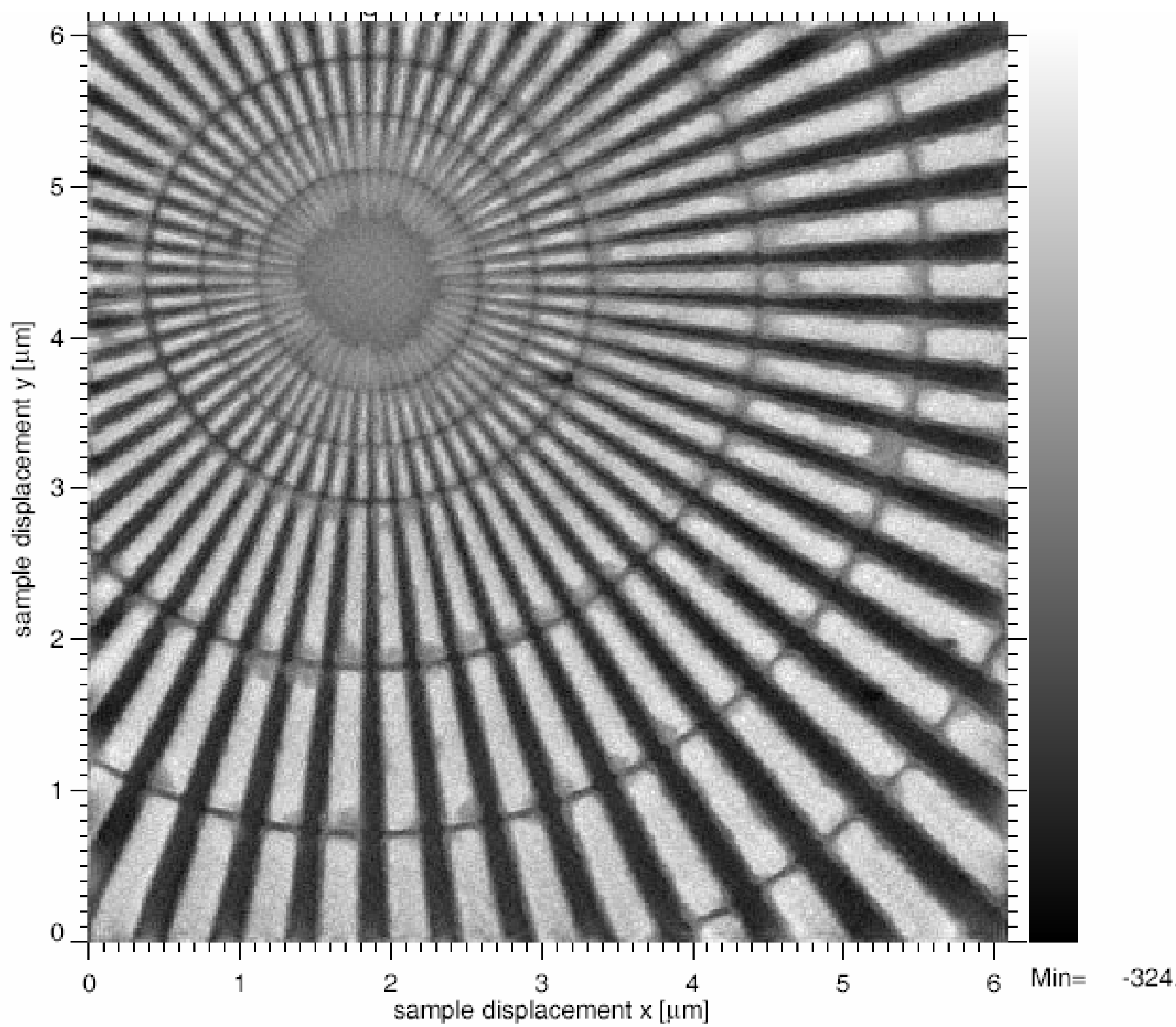
X-ray refractive index:
$n=1-\delta-i\beta$



All channels acquired simultaneously



Feser thesis



Feser thesis

- Bright field: easy to make quantitative
- Phase contrast: edge enhancement, good for high energy
- Dark field: emphasizes strong scatterers
- Luminescence: locates special labels
- Fluorescence: trace element sensitivity (high energy)

STXM developments
Stony Brook / NSLS

- Zone plates 1983-'85 IBM
  1987-'94: IBM/CXRO
  1995- Bell Labs/SB
- Many students, postdocs, over the years:
  - Ade, Buckley, Chapman, Feser, Jacobsen, Kaznacheyev, Kenney, Ko, Lerotic, Lindaas, Maser, McNulty, Miao, Neuhäusler, Osanna, Rarback, Schäfer, Spector, Vogt, Wang, Winn, Yang, Yun, Zhang…

Stony Brook group today:

- Faculty: Chris Jacobsen, Janos Kirz
- Students: Holger Fleckenstein, Benjamin Hornberger, Bjorg Larsen, Enju Lima, Ming Lu, David Shapiro,
- Guest scientist: David Sayre
- Beamline scientist: Sue Wirick

Agere Inc.: Don Tennant

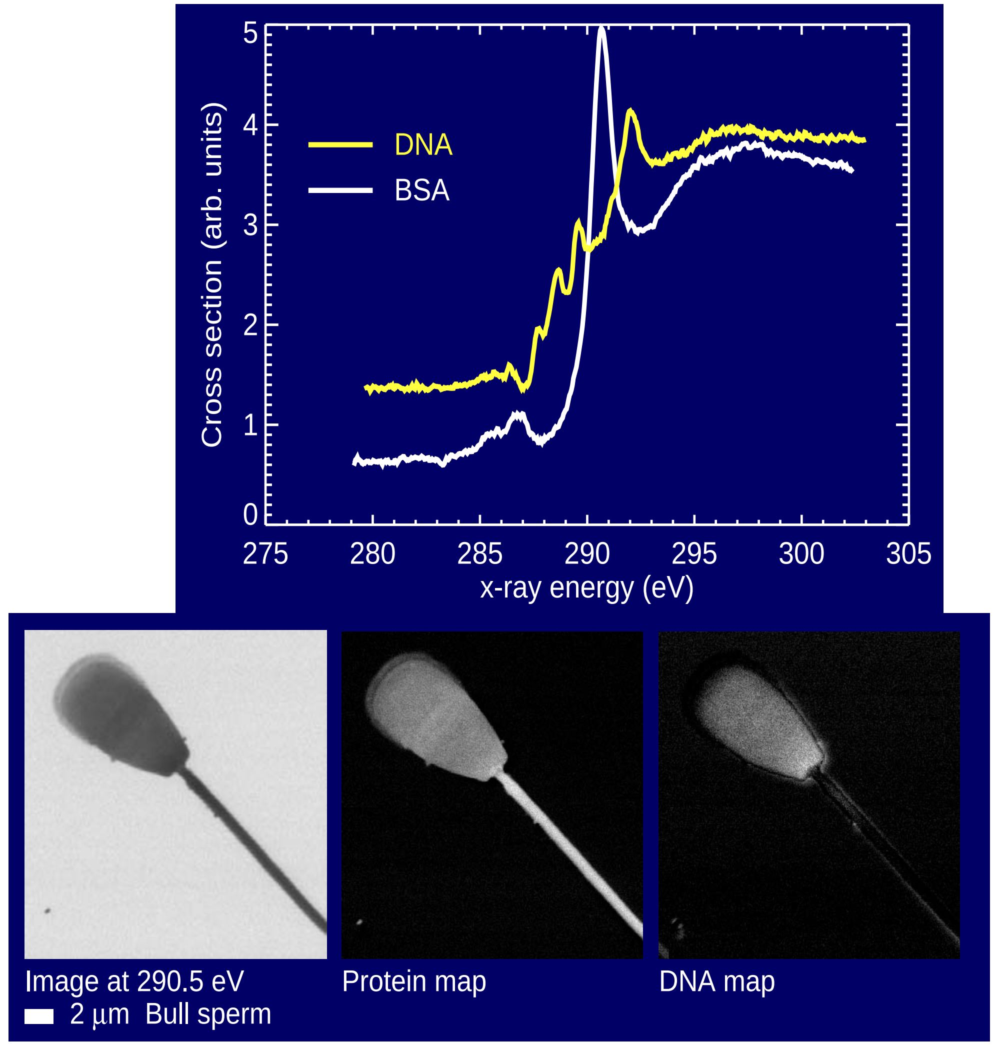
Many collaborators…

Applications @ NSLS X1A

• Sperm morphology / infertility	Jacobsen, USB
• Interplanetary dust, meteoritics	Flynn, SUNY/Plattsb.
• Organic geochemistry / wood, coal	Cody, Carnegie Inst.
• Nuclear waste transport	Schaefer, Karlsruhe
• Marine organic matter	Brandes, U. Texas
• Bacteria and uranium chemistry	Gillow, BNL
• Humic acid aggregates	Rothe, Karsruhe
• Humic and fulvic acids	Scheinost, Zurich
• Biofilms	Thieme, Goettingen
• Emulsion stability	Urquhart, Saskatchewan
• PMMA: damage as fn of temp.	Jacobsen, USB
• ……	

DNA packing in sperm

- X. Zhang, R. Balhorn, J. Mazrimas, and J. Kirz, *J. Structural Biology* **116**, 335 (1996)
- DNA packing in sperm mediated by protamine I and protamine II; fraction of protamine II can vary from 0% to 67% among several species
- Bulk measurements: compromised by immature or arrested spermatids
- Conclusion: protamine II replaces protamine I, rather than binding to protamine I complex



Air-dried bull sperm

Sperm morphology and infertility

- Study to shed light on correlations of morphology, and biochemistry, in male infertility
- Image of wet, unfixed human sperm

STXM / Spectromicroscopy today

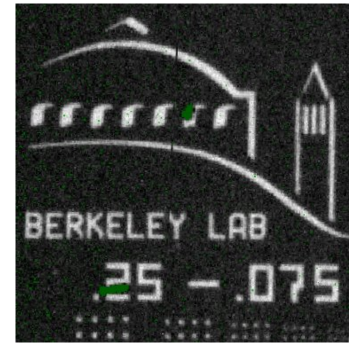
- ALS:

  Polymer STXM - 5.3.2

  - Interferometric stage control (NCSU/ALS/McMaster team 2001)

  MES STXM – 11.0.2
- NSLS: STXM IV, CRYO, (STXM V)
- Elsewhere: ESRF, APS, Hsinchu
  BESSY II, (CLS), (SLS),…

Advanced Light Source



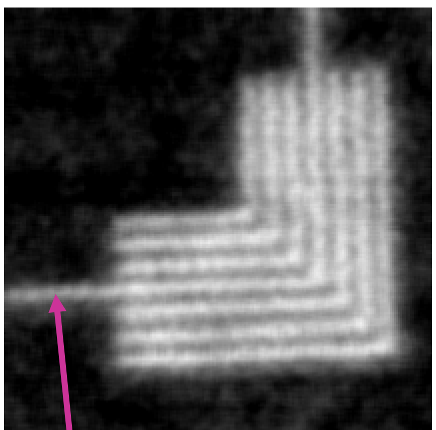
Polymer STXM image of test pattern

5.3.2 Polymer STXM: First Tests

A.L.D. Kilcoyne, T. Tylisczak, M. Kritcher, Peter Hitchcock, S. Fakra, K. Frank, C. Zimba, M. Rafailovich, J. Sokolov, G. Cody, E. Rightor, G. Mitchell, I. Koprinarov, E. Anderson, B. Harteneck, Adam Hitchcock, T. Warwick, H. Ade

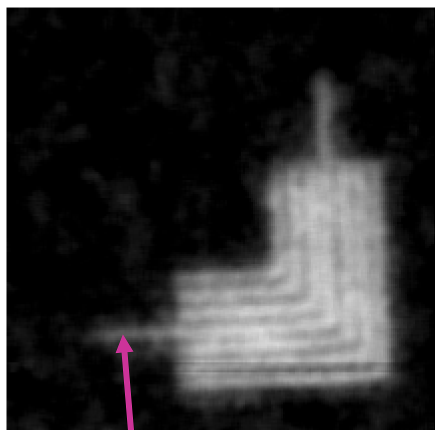
CXRO test-pattern imaged at 390 eV

Resolved 40 nm 1:1 features



Isolated 40 nm line

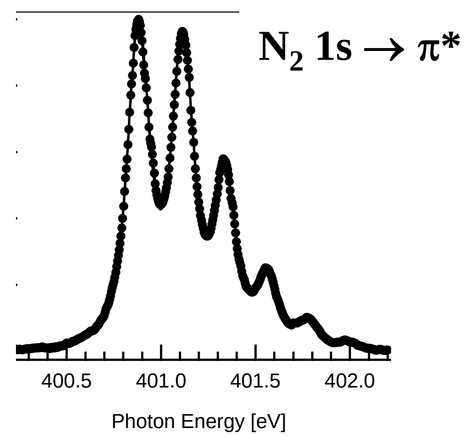
Resolved 30 nm 1:1 features



Isolated 30 nm line

Experiments: T. Tyliszczak. H. Ade, D. Kilcoyne

Excellent, stable flux: > 1 MHz
Excellent energy resolution: <60 meV

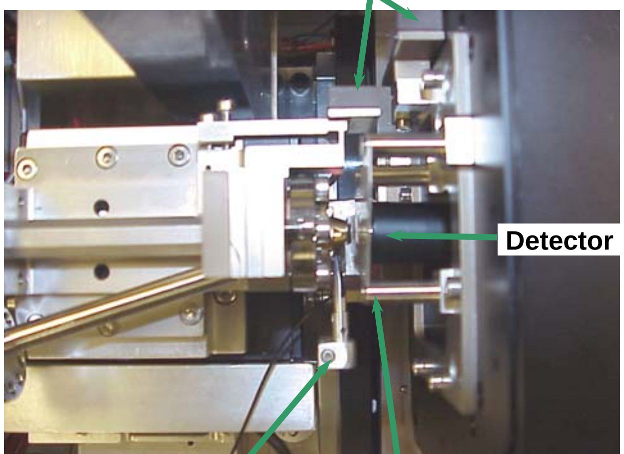


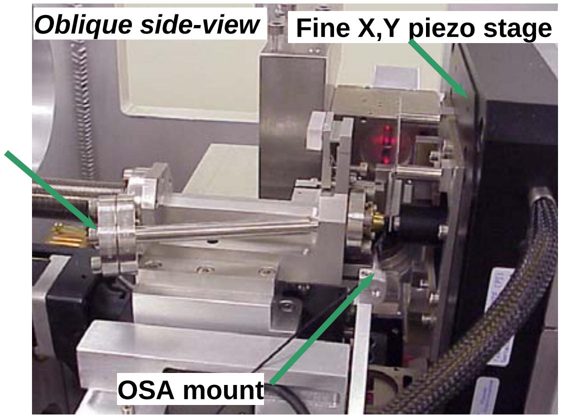
Some improvements still ahead

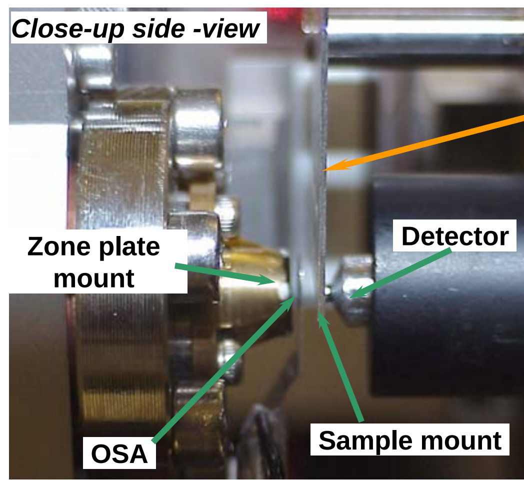
Supported by NSF DMR-9975694, DOE DE-FG02-98-ER45737 Dow Chemical and NSERC

First 5.3.2 STXM Results

Some close-up views of 5.3.2 hardware





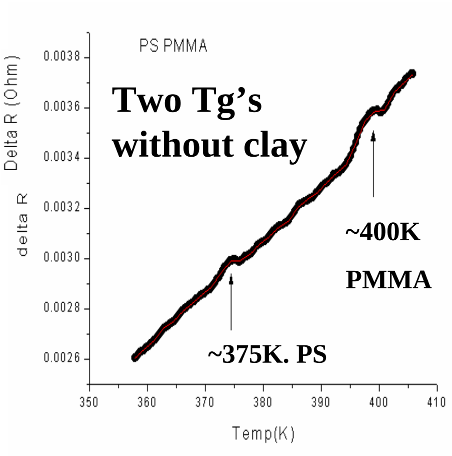


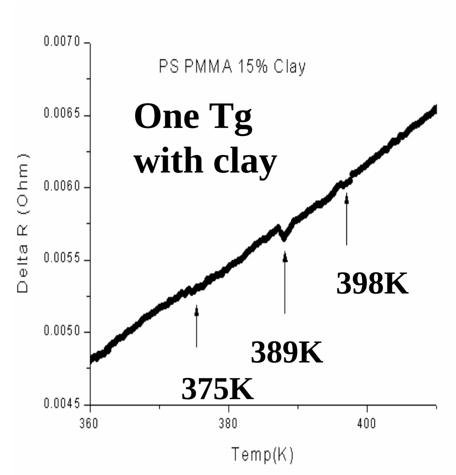
~20 micron reproducible mounting. - VLM pre-indexing with absolute coordinate system

A.L.D. Kilcoyne et al. *J Synchrotron Rad.* **102**, 125-136 (2003)

Polymer Clay Composites: PS/PMMA

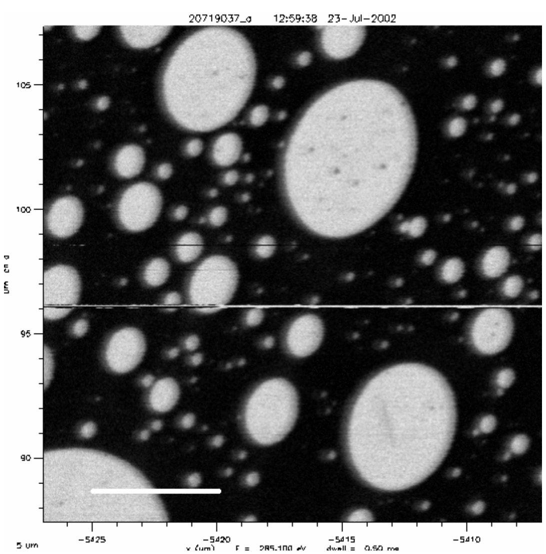
- Clay works better than copolymer to “compatibilize” the blend
- Observe the same in bi-layer geometry



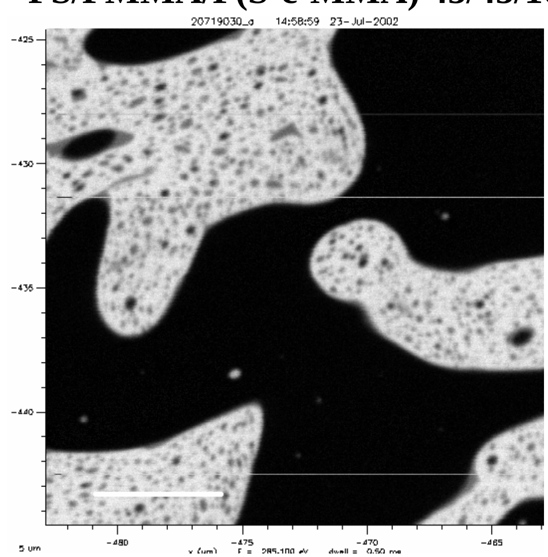


DSC suggest formation of “single” phase in the presence of clay

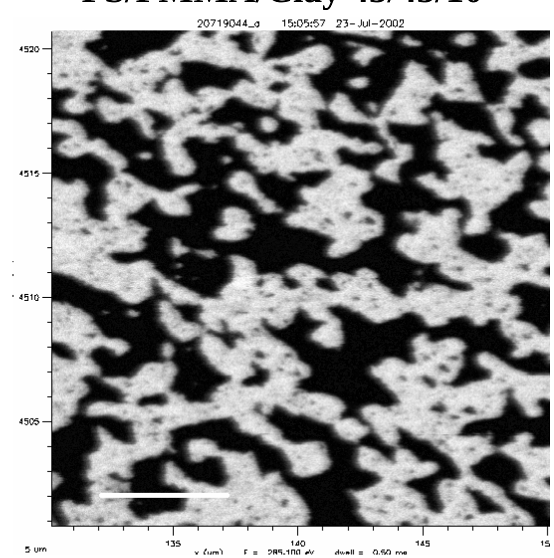
PS/PMMA 70/30



PS/PMMA/P(S-c-MMA) 45/45/10



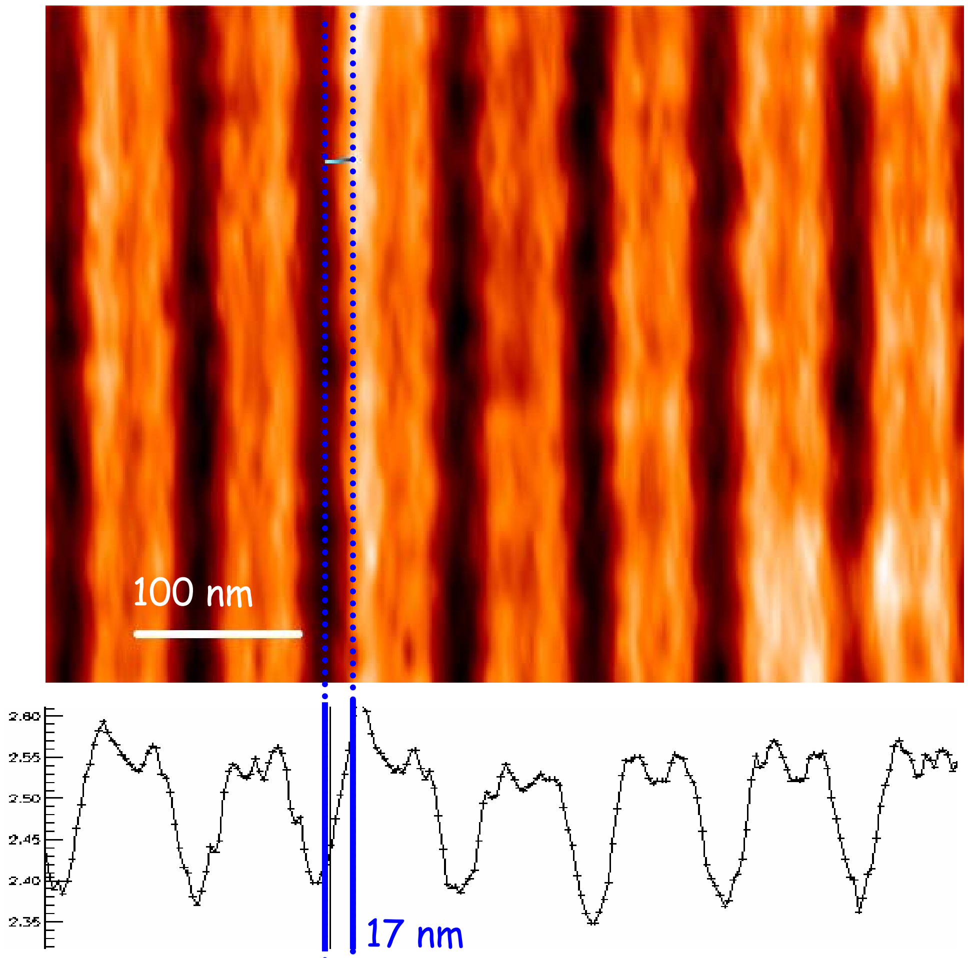
PS/PMMA/Clay 45/45/10



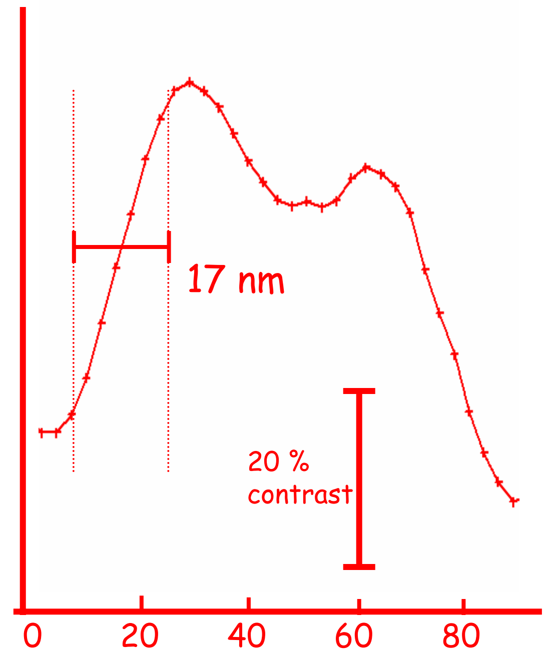
H. Ade, A.L.D. Kilcoyne, A. Winesett, O. Dhez, W. Zhang, X. Hu, M. Lin, M. Rubinstein, M. Rafailovich, J. Sokolov

Data: 5.3.2. STXM

Very good Resolution with 25 nm zone plate

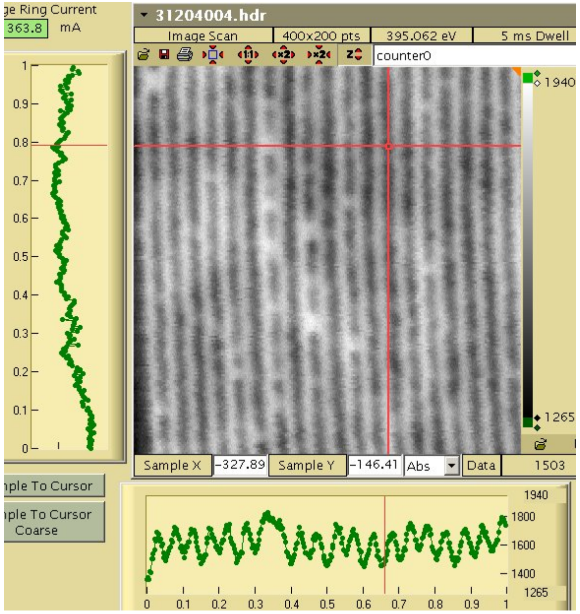


Scanning transmission x-ray microscope image of a 25 nm test pattern (1:2 spacing)

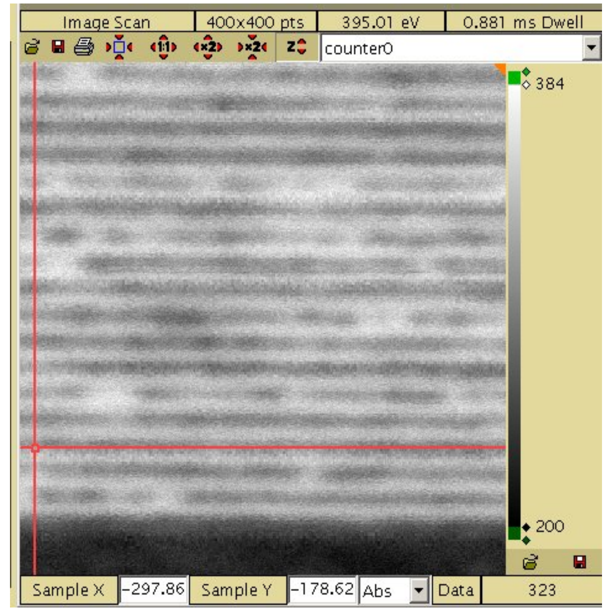


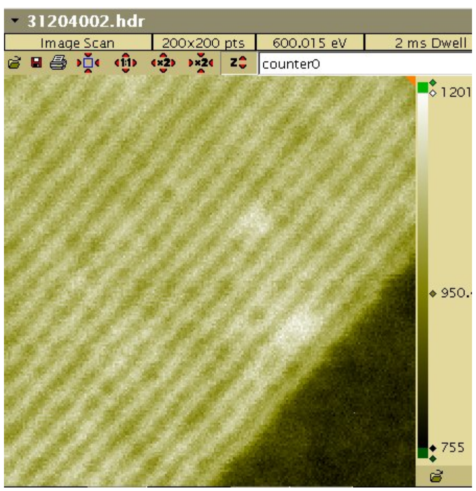
Images of 25 nm test pattern

ALS-MES 11.0.2

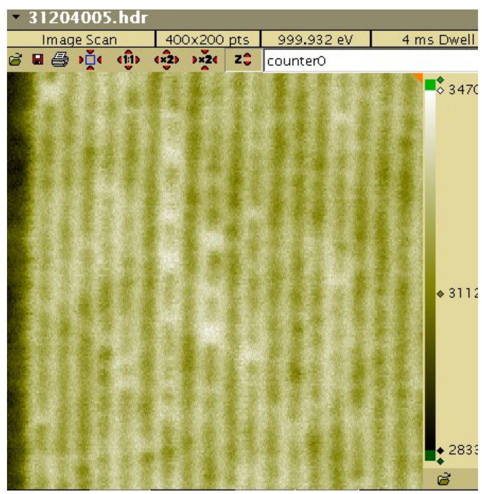


395eV

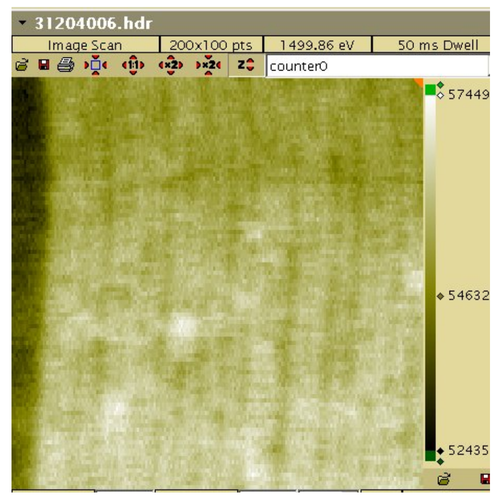




600eV



1000eV



1500eV

Current ALS-MES STXM Status

- Energy range: 80 eV – 2100 eV – energy resolution > 7500
- Spot size: 30 nm (theoretical)
- Can resolve smaller structures (15 nm)
- Photon flux: up to 10^9 ph/s with full spatial resolution and energy resolving power > 3000
- Resolution limited by the zone plate
- 4 basic zone plates: 25 nm and 35 nm , 40 nm and 45
- Maximum scanning rate: 12 Hz
- Scanning range: 4000x2000 pixels – up to 20 x 4 mm
- Minimum step size 2.5 nm
- Precision of staying at the same spot for spectra acquisition:
  - — < 50 nm (laser interferometry)
- EPU – polarization dependence, circular dichroism + electromagnet
- Possibility to scan sample at 30 deg to the beam – out of plane polarization, in plane magnetization
- Single photon timing capabilities (100 ps)

Scanning Transmission X-ray Microscopy Study of Microbial Calcification

ALS-MES 11.0.2

Microbes are often associated with calcium-containing minerals in nature, but it is difficult to determine if these organisms are involved in mineral nucleation: search for biosignatures in fossilized microbial cells.

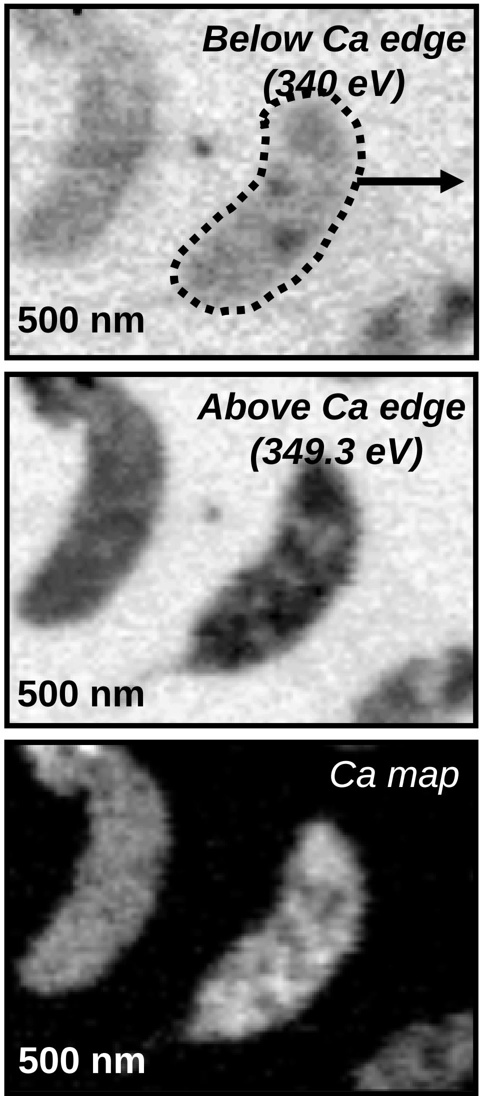
Experiment:

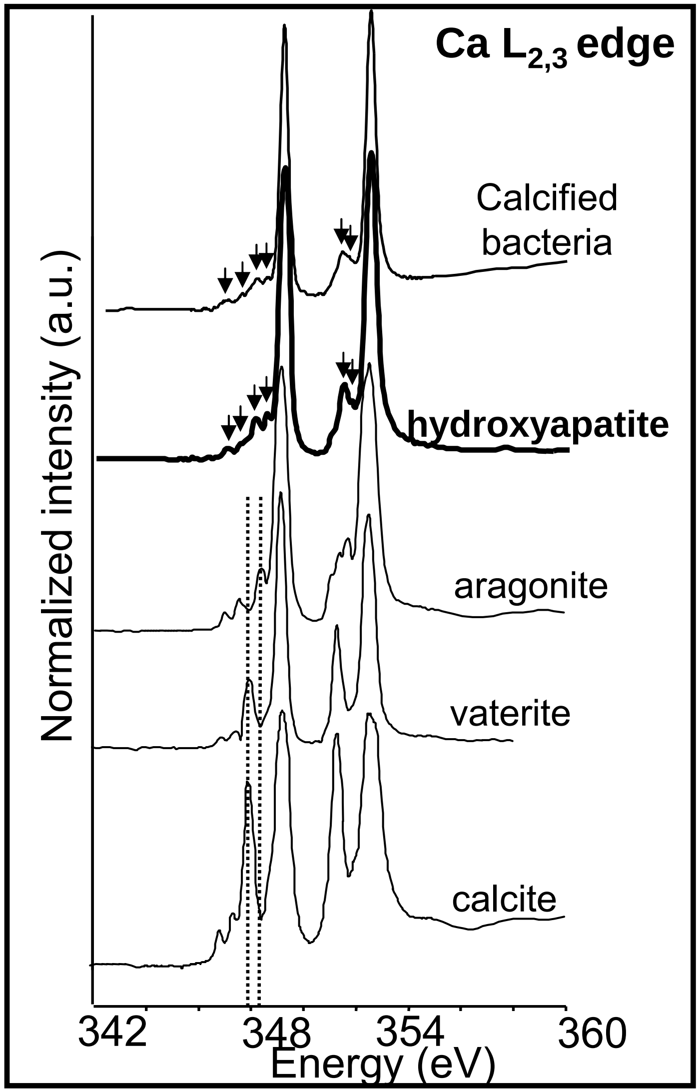
STXM used to obtain NEXAFS spectra at the C K-edge and the Ca L2,3-edge on both the minerals and associated organics during biomineralization by *Caulobacter crescentus* cells under laboratory conditions.

Results:

Ca $L_{2,3}$-edges for hydroxyapatite, calcite, vaterite, and aragonite are unique and can be used as probes to detect these different mineral phases.

C. crescentus cells, when cultured in the presence of high calcium concentration, precipitate carbonate hydroxyapatite.

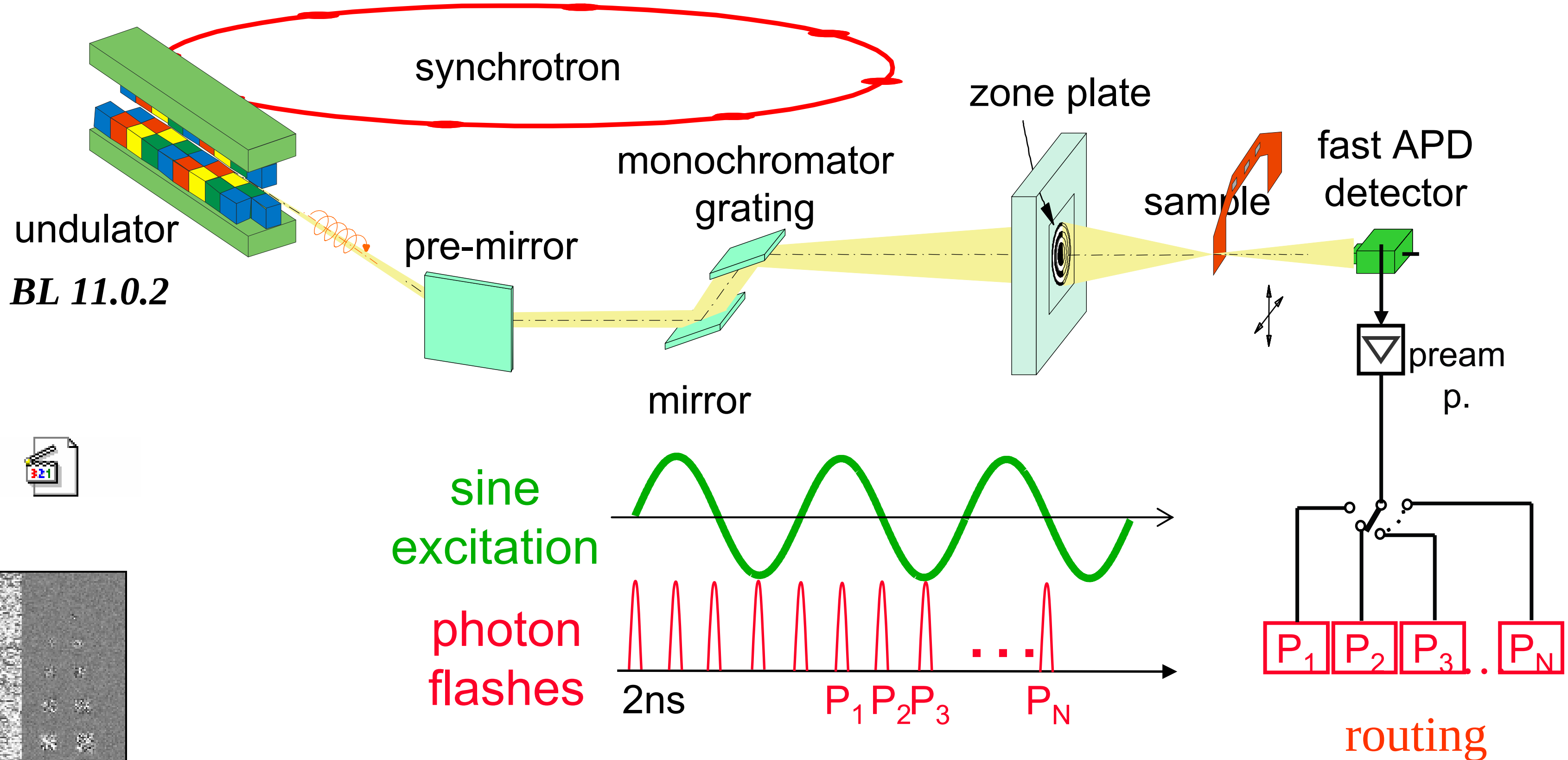


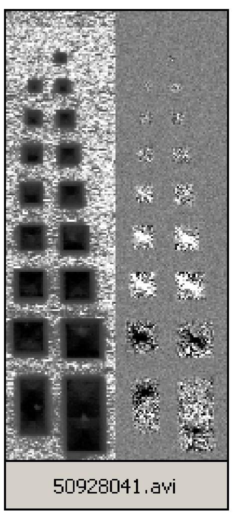


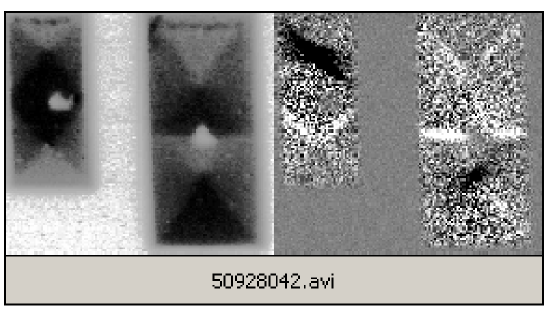
K. Benzerara, T.H. Yoon, T. Tyliszczak, B. Constantz, A.M. Spormann, and G. E. Brown, Jr. Geobiology

Time-Resolved STXM Magnetic Measurements in multi-bunch mode

ALS-MES 11.0.2



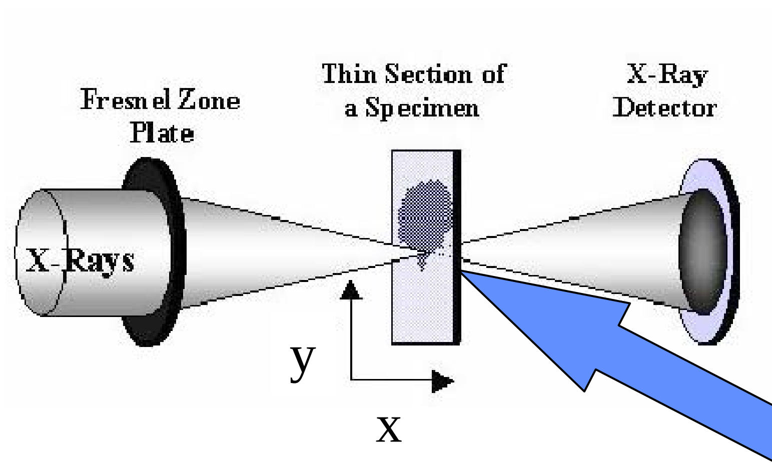




A. Puzic, B. Van Waeyenberge, K.W. Chou, P. Fischer, H. Stoll, G. Schütz, T. Tyliszczak, K. Rott, H. Brückl, G. Reiss, I. Neudecker, T. Haug, M. Buess, C.H. Back, „Spatially Resolved Ferromagnetic Resonance: Imaging of ferromagnetic eigenmodes“ J. Appl. Phys. 97 10E704 (2005). LBNL-56923

Cu Oxidation/Reduction at 760 Torr in-situ Scanning Transmission x-ray Spectroscopy

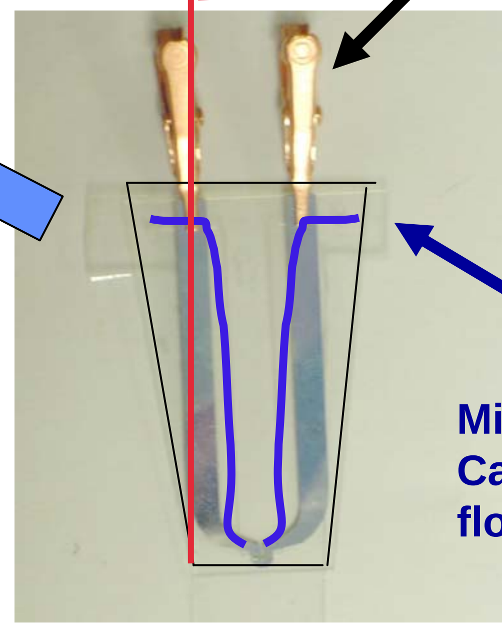
ALS-MES 11.0.2



Data Acquisition
- **Flow Switching**
- **Pressure**
- **Temperature Control**

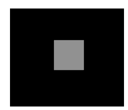
Thermocouple

Resistive heater



Micro machined Capillary flow tubes

Sandwich two silicon nitride windows around this holder to create gas cell



Sample is placed on one of the windows

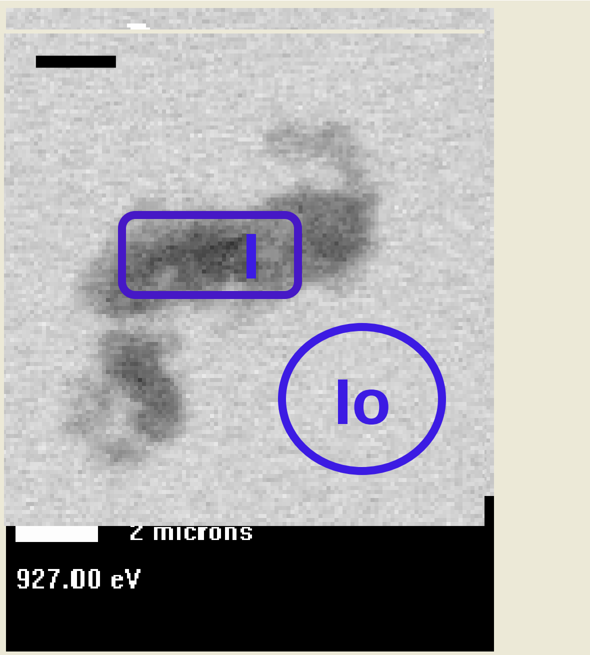
Applications

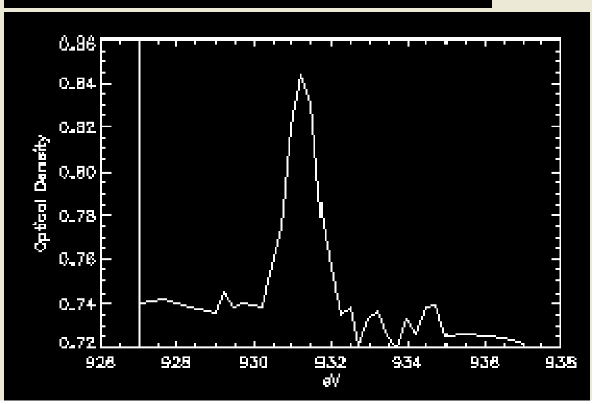
Soot oxidation and subsequent particle growth
Catalysis
Water uptake on patterned photo resists

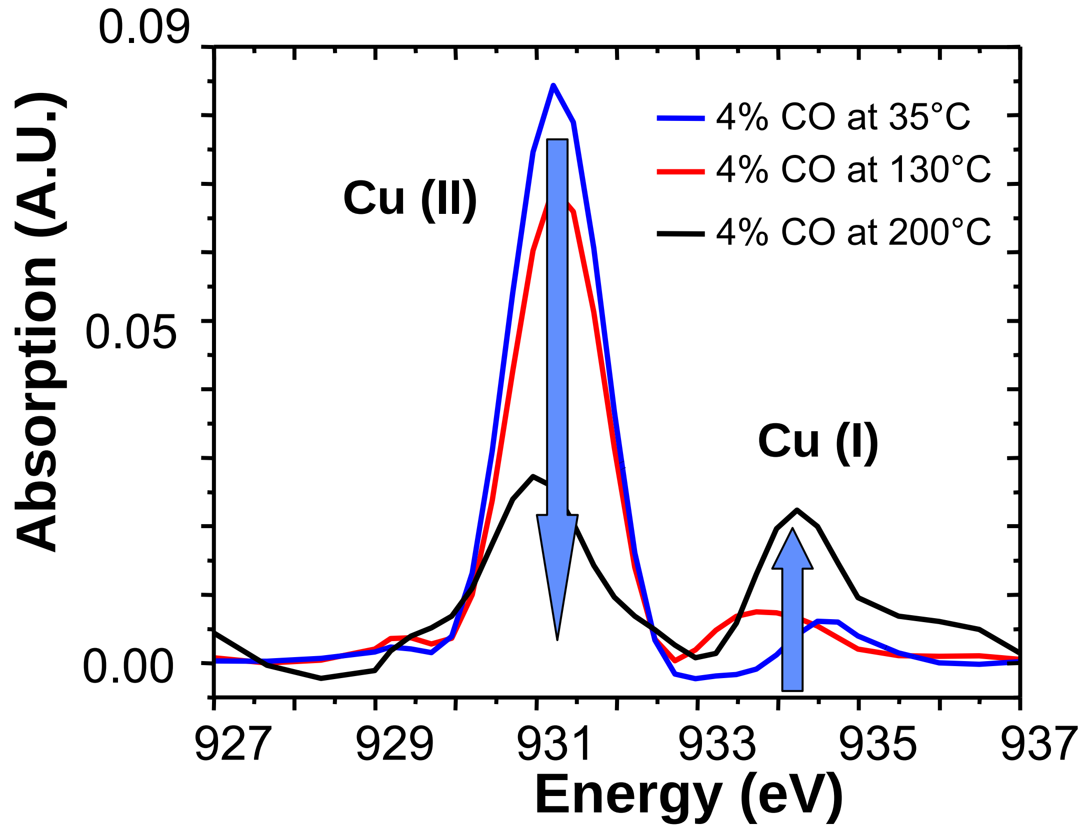
I. Drake, T.C. N. Liu, M. K. Gilles, T. Tyliszczak, A.L.D. Kilcoyne, D.K. Shuh, R.A. Mathies, and A.T. Bell "Micro reactor for in-situ Transmission Soft X-ray Absorption Studies of Supported Catalysts and Environmental Materials." Rev. Sci. Inst. 75(10) 3242-3247 (2004).

In situ cell

ALS-MES 11.0.2



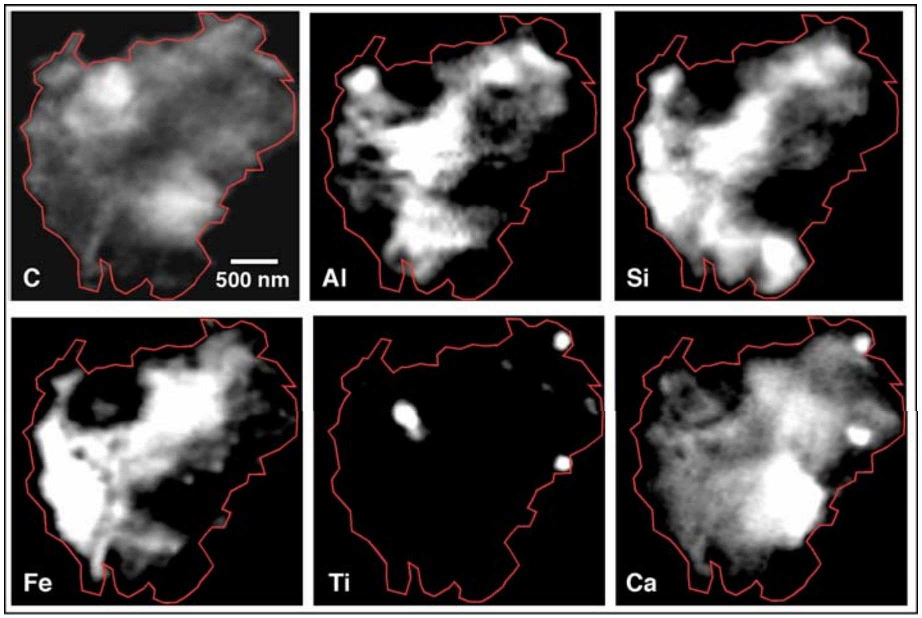


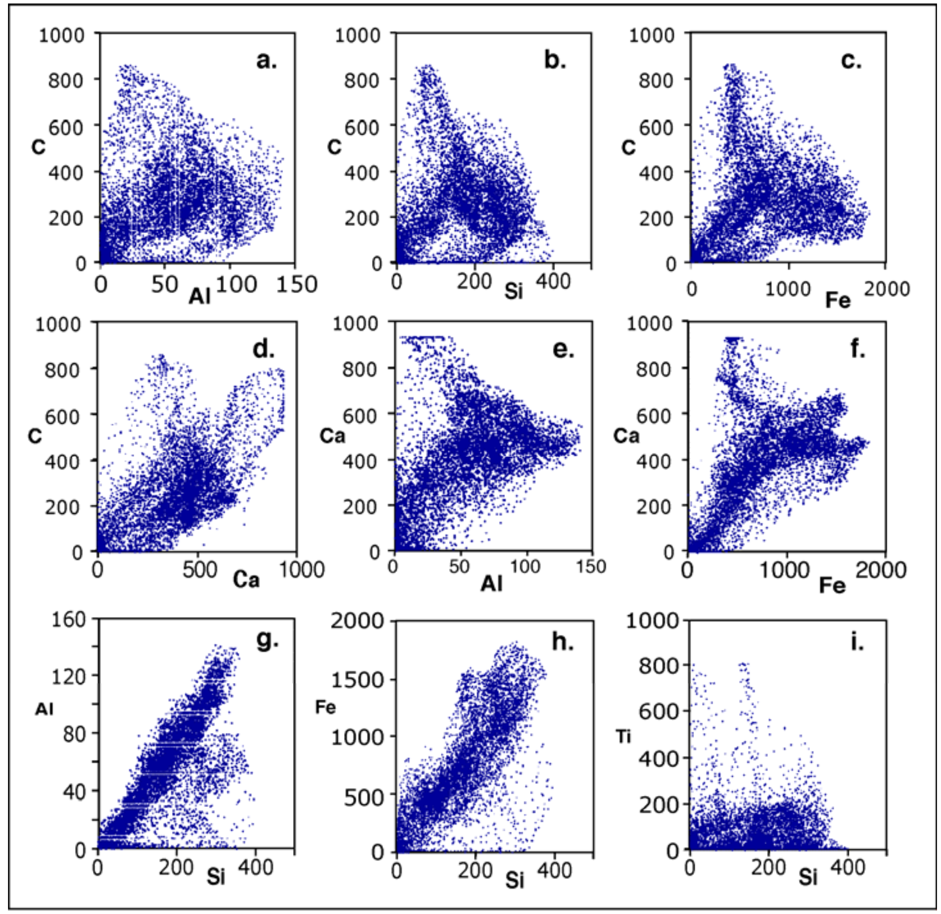


Monitor changes on a single particle during oxidation and reduction

Elemental correlation maps in soil

ALS-MES 11.0.2



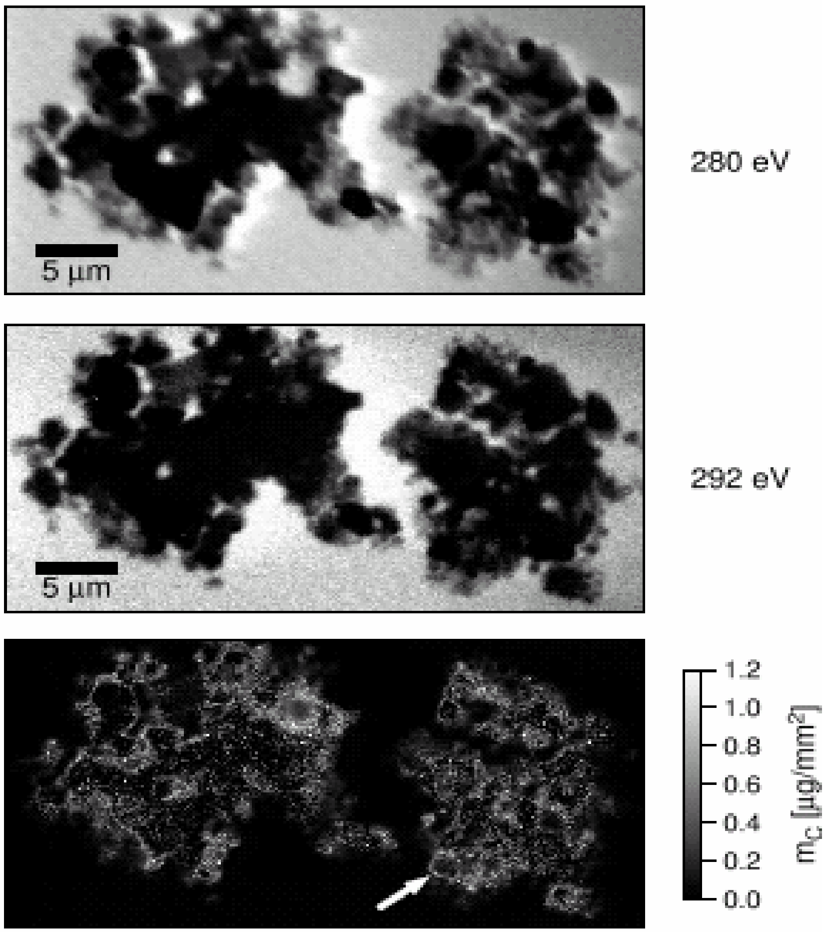


C – “organic” carbon (map at 288.3 eV)

Correlation plots of net counts (arbitrary units) obtained from STXM maps Both maps and plots are based on 30 nm pixels over a 9 μm^2 area (10^4 pixels).

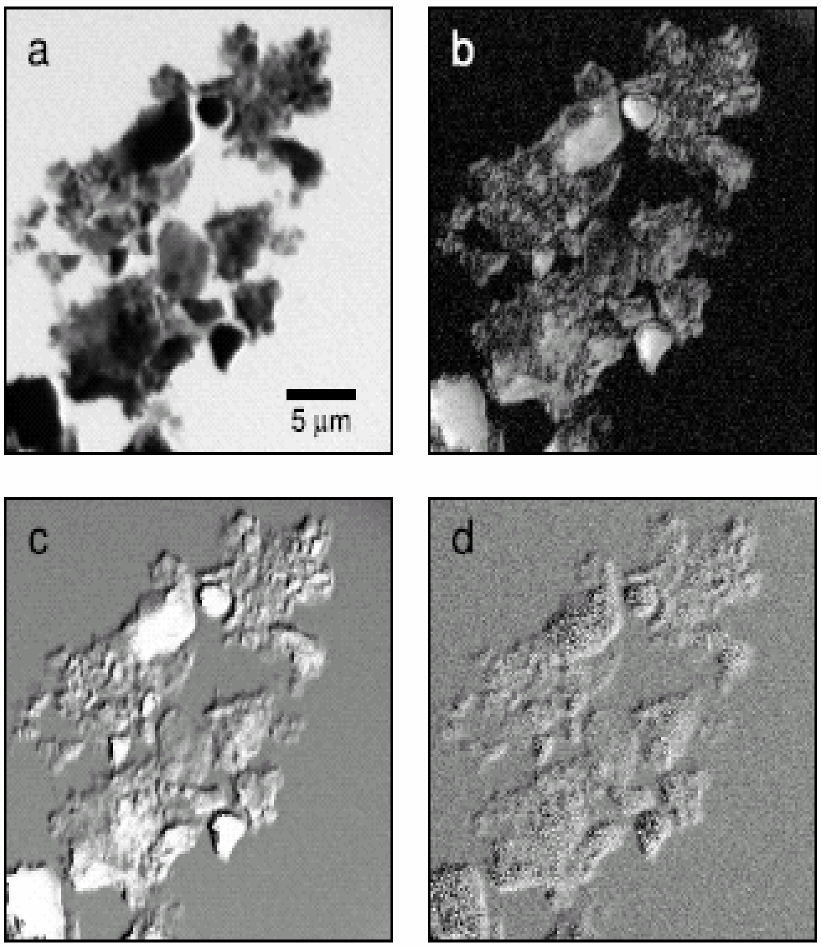
J. Wan, T. Takunaga, T. Tyliszczak, LBNL

STXM @ BESSY II
Urs Wiesemann thesis, 2003



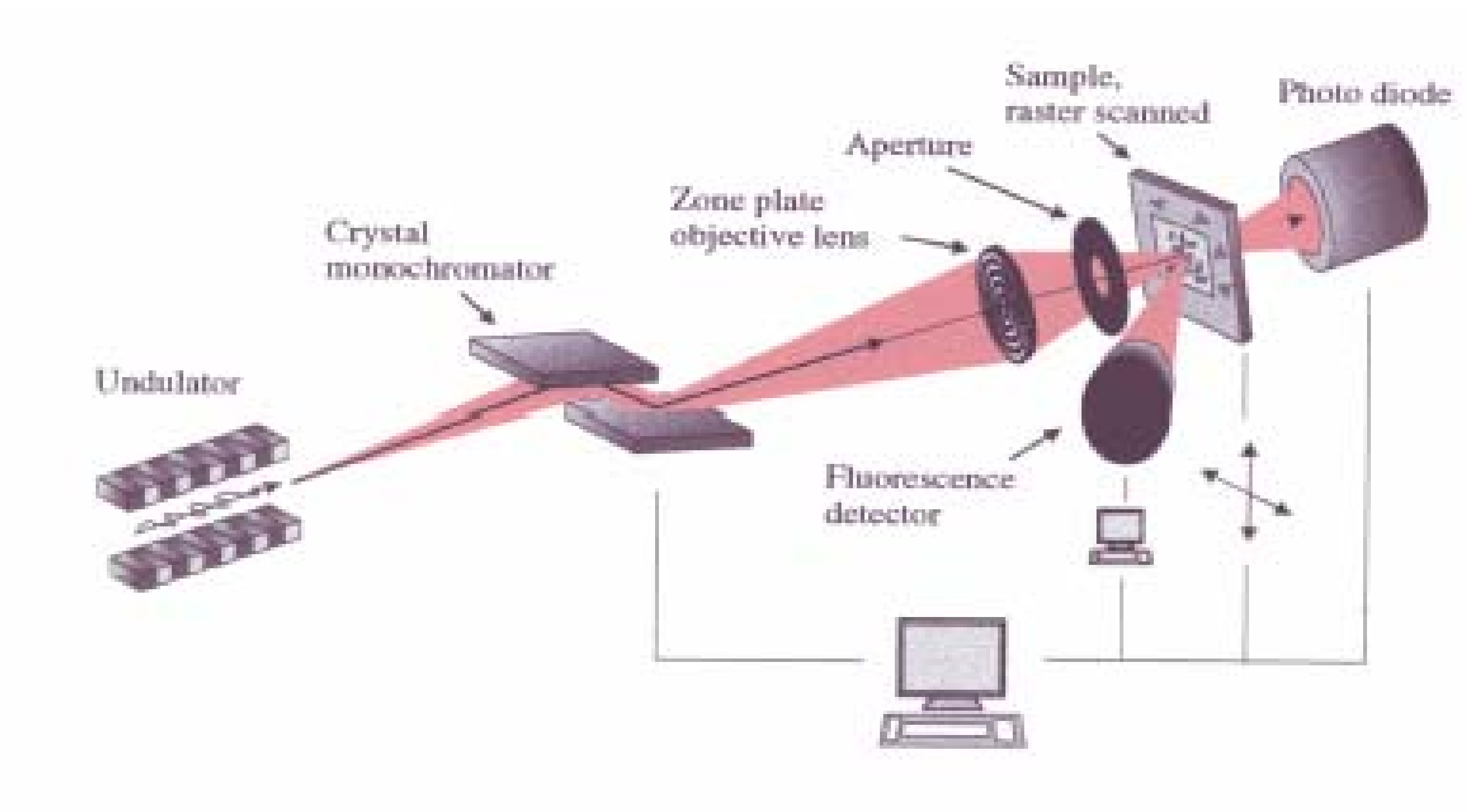
Elemental imaging at the carbon absorption edge of two flocks of Colloidal particles from a chernozem soil in dry state on a silicon membrane. Shown are the absorption contrast images below the absorption edge at 280 eV (I11, top), above the absorption edge at 292 eV (I12, center), and the carbon mass density mC calculated using Eq. (6.1) (bottom). The arrow in the mass density image indicates an organically coated particle. The images have 400 × 200 pixels of 100 × 100 µm2 size; the pixel dwell time is 6ms.

Urs Wiesemann thesis



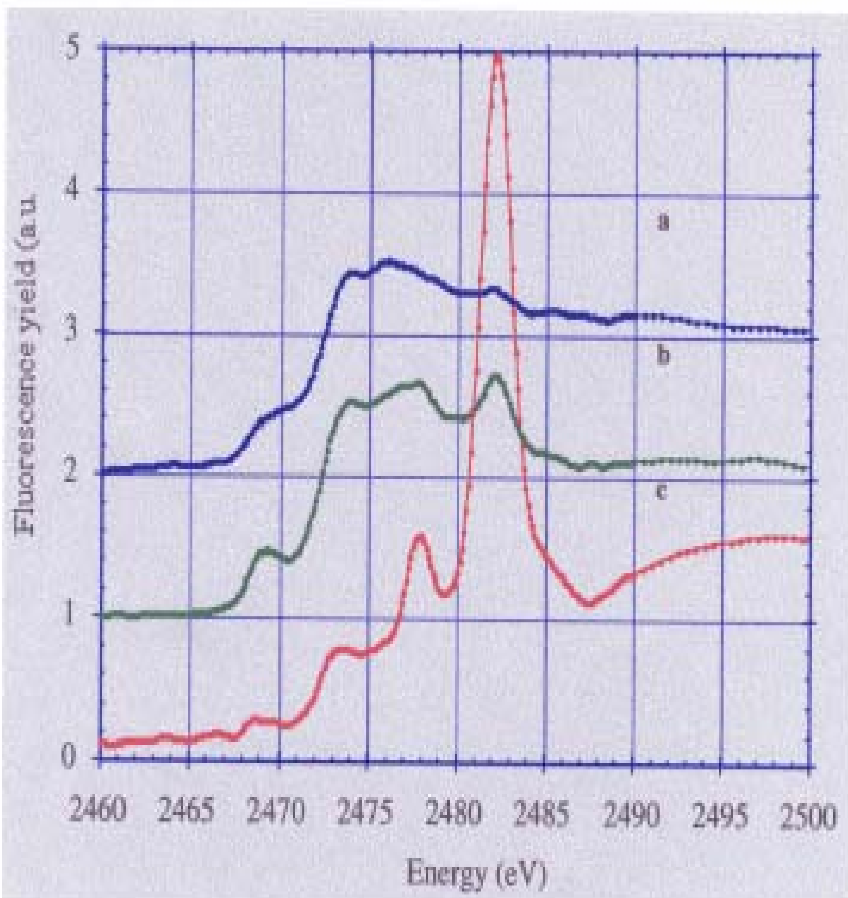
Colloidal particles from a chernozem soil in dry state on silicon membrane imaged in incoherent bright field and differential phase contrast. (a)Incoherent bright field contrast; (c) differential phase contrast in horizontal direction; (d) differential phase contrast in vertical direction; (b) square root of the quadratic sum of the horizontal and vertical phase contrast images (bottom). All images are generated from a single scan of 400 × 400 pixels with a step size is 75nm and 6ms dwell time; the photon energy is 408 eV.

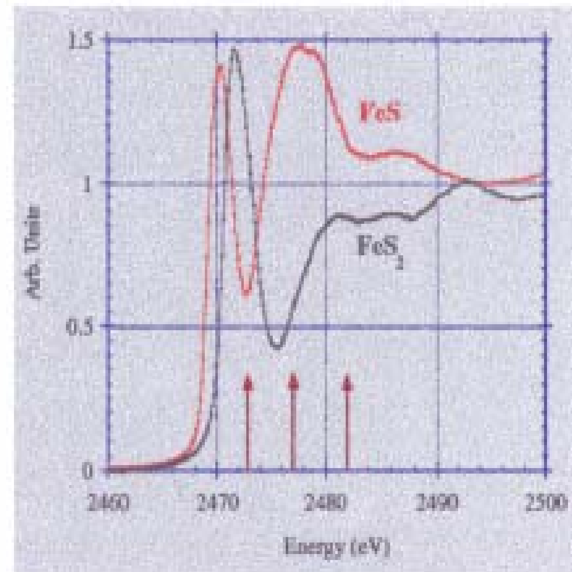
ESRF



Sample,
raster scanned
Photo diode
Aperture
Zone plate
objective lens
Crystal
monochromator
Undulator
Fluorescence
detector

Small glassy inclusions in volcanic olivine grains, with 900 – 1700 ppm Sulphur

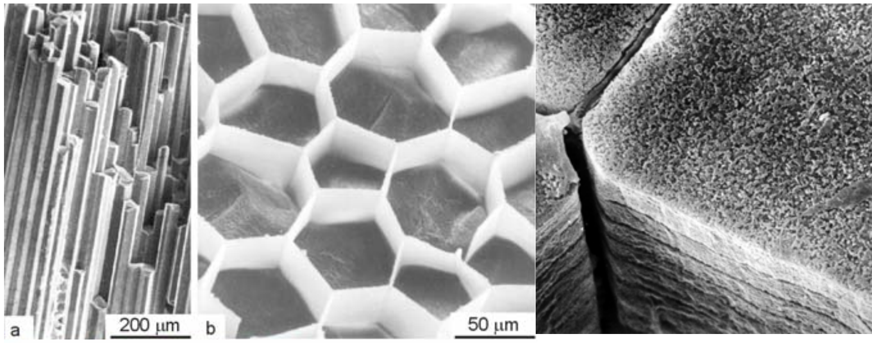


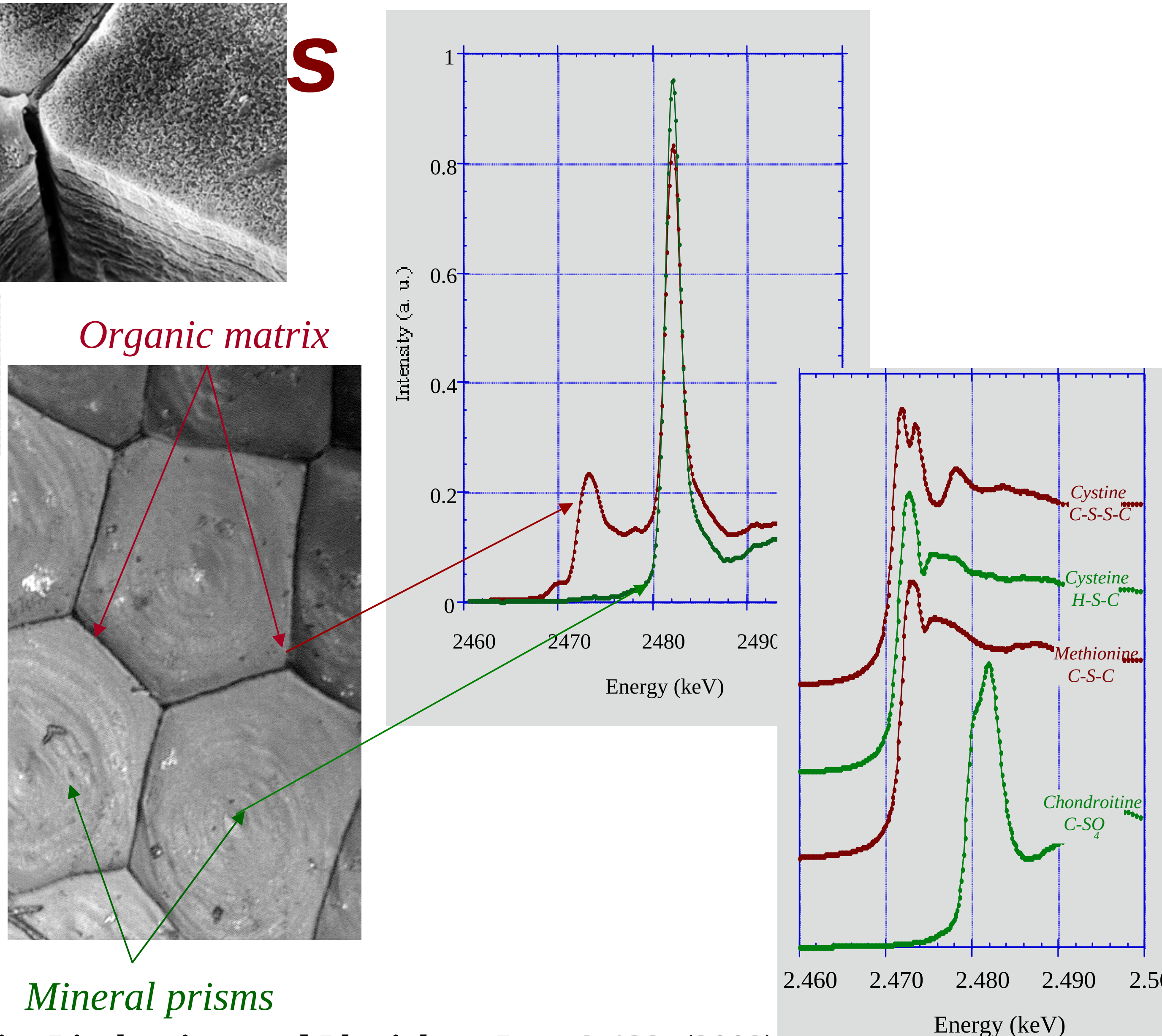


Susini et al., Surf. Rev. 2002

Sulfur K-edge in Pinna

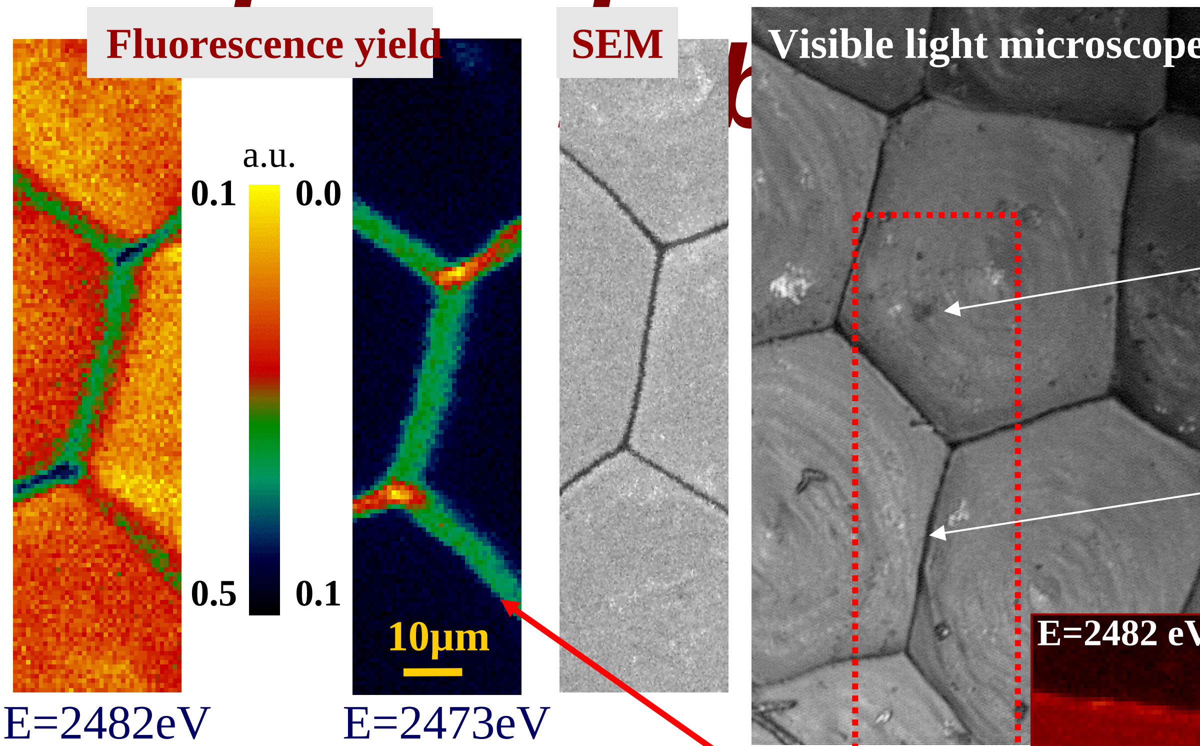
s

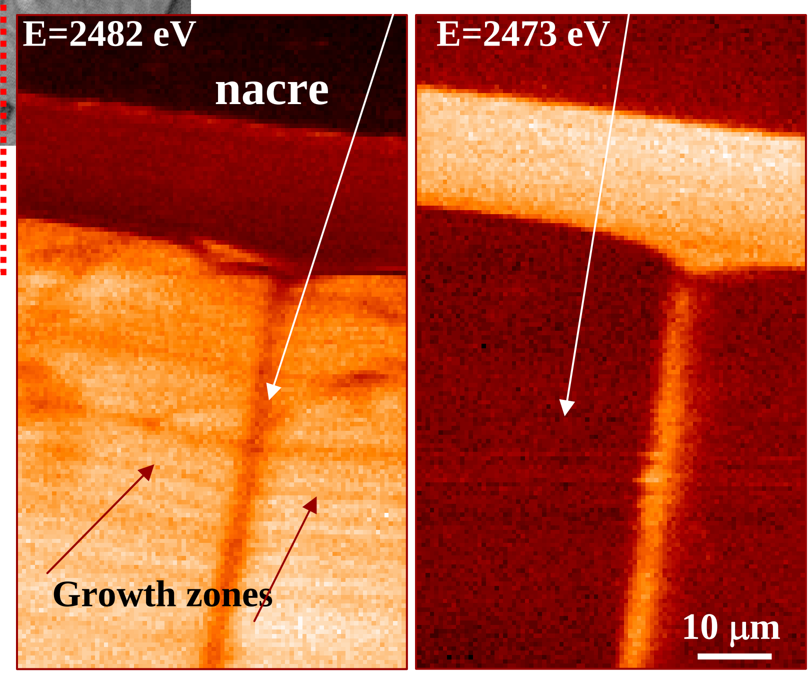




Y. Dauphin et al., ***Comparative Biochemistry and Physiology*****, Part A** **<u>132</u>, (2002)**

Sulphur species in Pinna





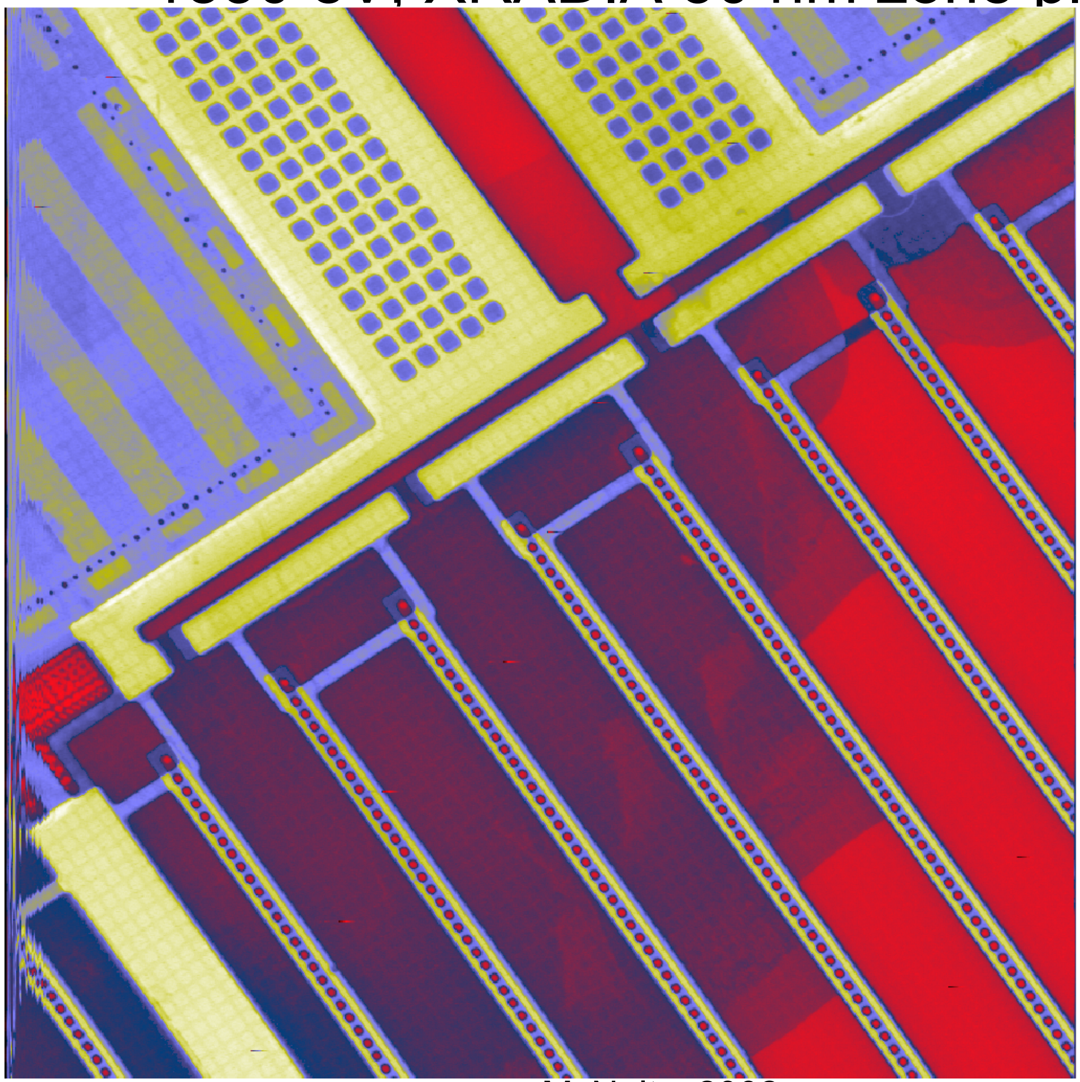
- probe: 0.25x0.30 μm^2
- dwell time: 1 sec/pixel

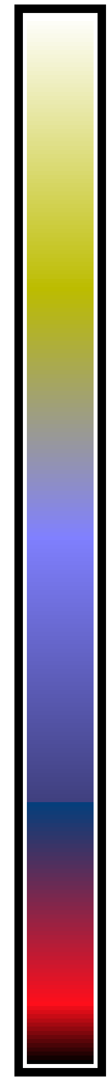
Y. Dauphin et al., *Journal of Marine Biology*, <u>142</u>, 299, (2003)

Y. Dauphin et al. *J. Structural Biology*, <u>142</u>, 272, (2003)

STXM at APS

1830 eV; XRADIA 50 nm zone plate

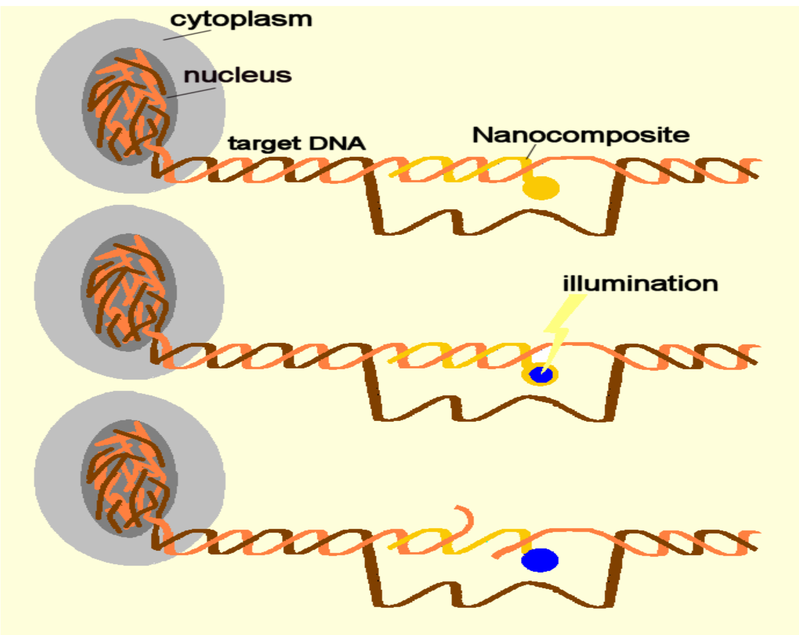




McNulty, 2002

TiO_2-DNA nanocomposites as intracellular probes

- Cell is transfected with TiO_2-DNA nanocomposites (4.3 nm Ø)
- DNA is used to target nanocomposite to specific chromosomal region
- TiO_2 allows photocleavage of targeted DNA strand upon illumination
- potential to be used to for gene therapy

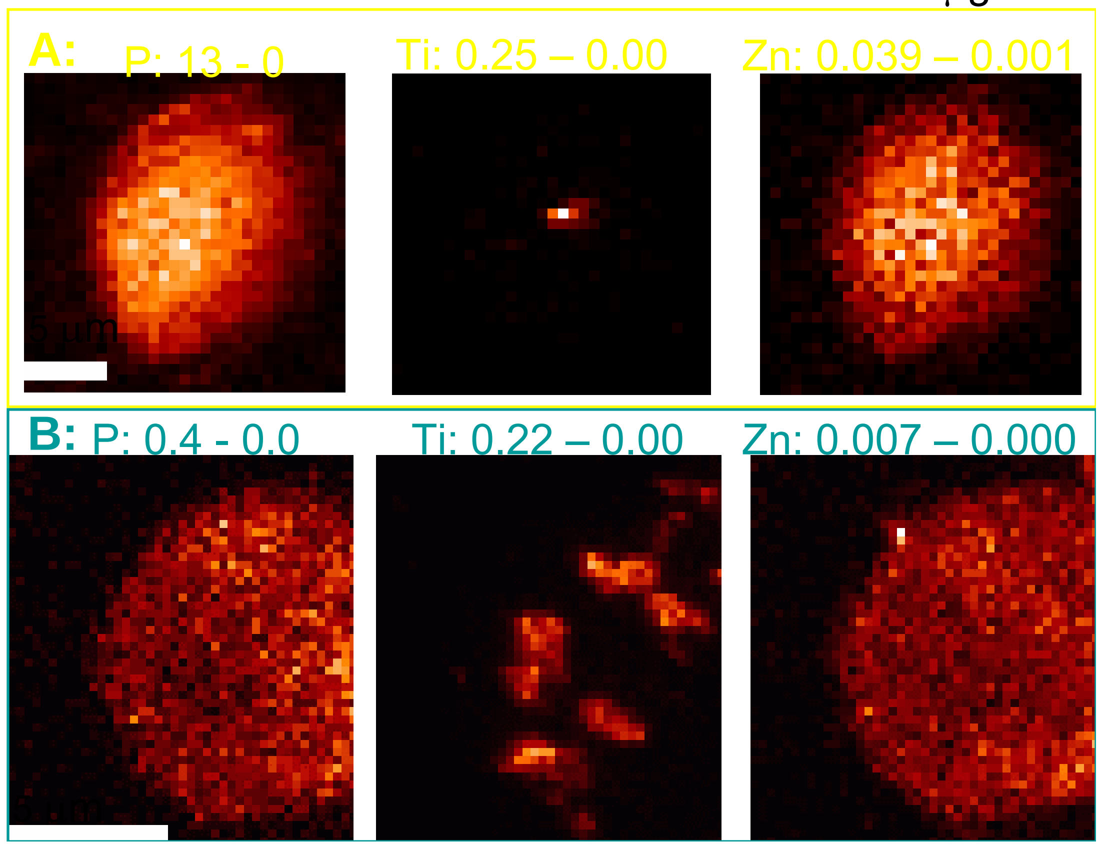


5/8/2006

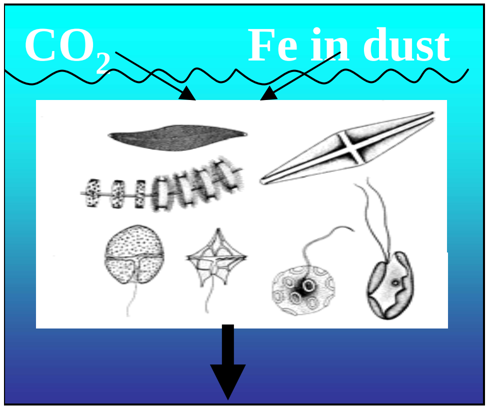
T. Paunesku *et al.*, Nature Materials 2, 343-346 (01. May 2003)

- map Ti distribution using X-ray induced K_α fluorescence, to quantify the succesrate of TiO_2-DNA transfection, and visualize target
- A: scan of a MCF7 cell transfected with nanocomposites targeted to nucleolus
- B: scan of a PC12 cell transfected with nanocomposites targeted to mitochondria

Units: $\mu g/cm^2$

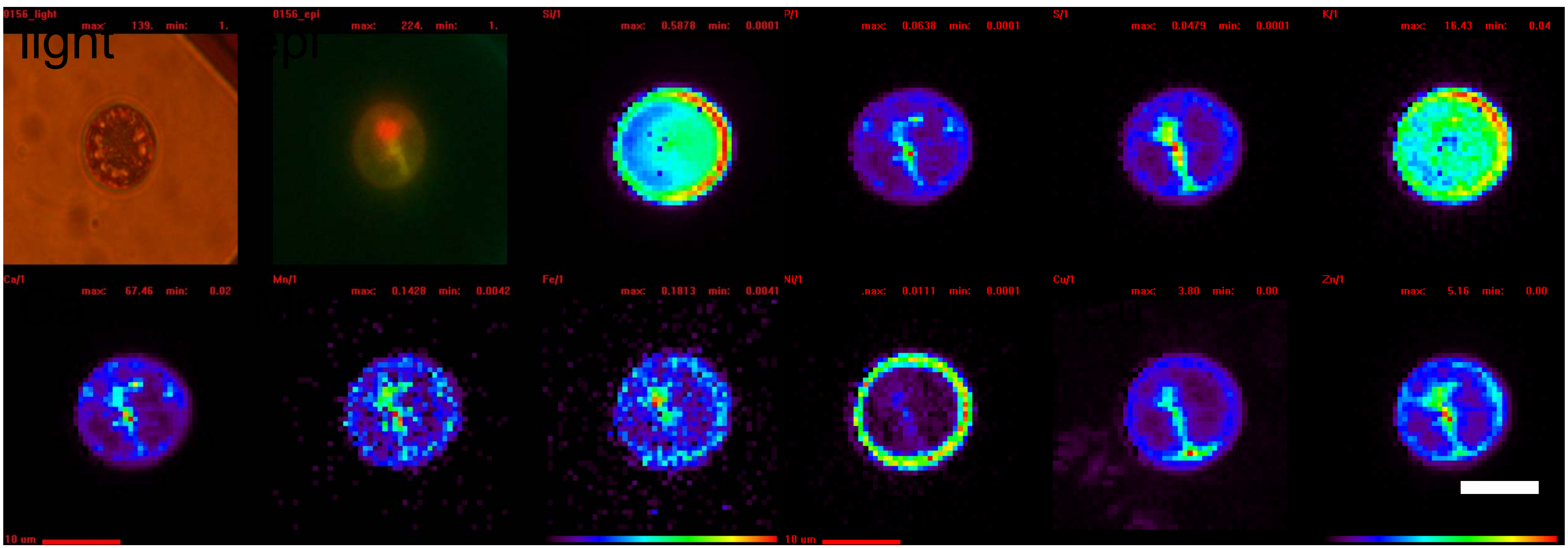


Trace metals in plancton and global carbon balance



- CO_2 sequestration in the ocean seems to be limited by the availability of Fe (necessary for Chlorophyll production).

CO_2 sequestration by Fe seeding



B. Twining, *et al.*,
Analytical Chemistry 75, 3806-3816 (2003).

Why I like scanning?

- XANES spectroscopy
- Multi-channel detector
- Low dose, large area overview scans
  - Convenient correlation to visible light microscopy
- Wet, dry, or cryo specimens
- Minimizes radiation dose
- Quantitative

- Relatively slow: pixel by pixel