X-ray microscopy

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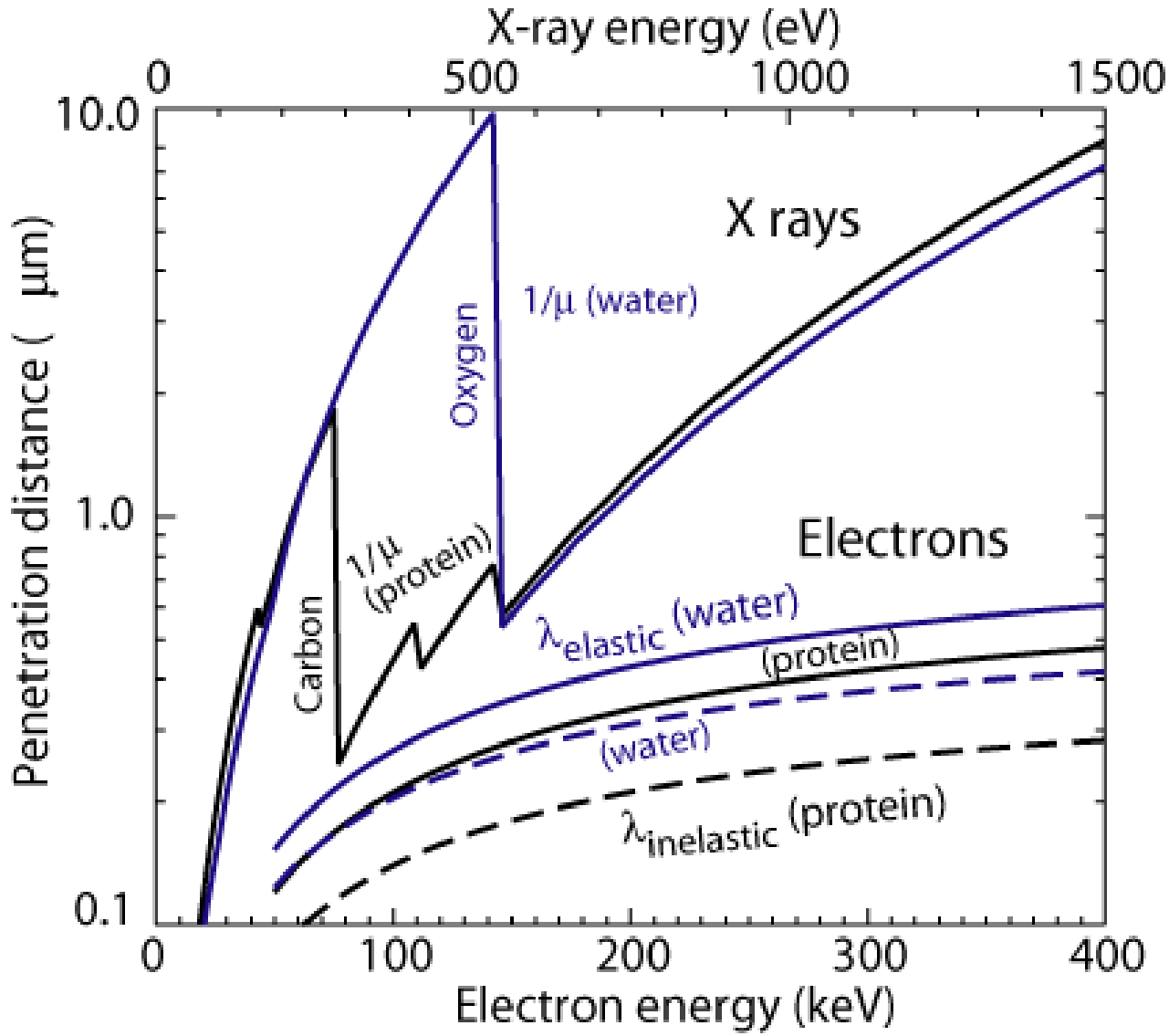
EPSCoR Program review 7/21/2009

Why do X-ray microscopy?

- Tools to visualize the nanoworld
- Resolution limited by wavelength
- Electron microscopes
  - Limited thickness
  - Limited contrast in unstained biological samples
- X-rays
  - Short wavelength,
  - Penetrating
  - Absorption edges provide contrast
    - "water window"; XANES – chemical contrast


X-ray energy (eV)
0
500
1000
1500
10.0
1.0
0.1
Penetration distance (μm)
X rays
1/μ (water)
Oxygen
Carbon
1/μ (protein)
Electrons
λelastic (water)
(protein)
(water)
λinelastic (protein)
0
100
200
300
400
Electron energy (keV)

History

- W. C. Röntgen 1895
  - lenses and mirrors don't work
- A. Einstein 1918
  - Index of refraction <1.0 :
  - Grazing incidence mirrors
- P. Kirkpatrick 1949
  - Grazing incidence mirrors
  - Kirkpatrick-Baez focusing

SCIENTIFIC AMERICAN

Established 1845

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ARTICLES

IS THE BOMB AN ABSOLUTE WEAPON? by P. M. S. Blackett
The celebrated British physicist's argument on this basic issue is summarized from his much-discussed current book, *Fear, War, and the Bomb*. Hopes for peace, he holds, depend on an answer to the question. 13

A U. S. PHYSICIST'S REPLY TO BLACKETT by Louis N. Ridenour
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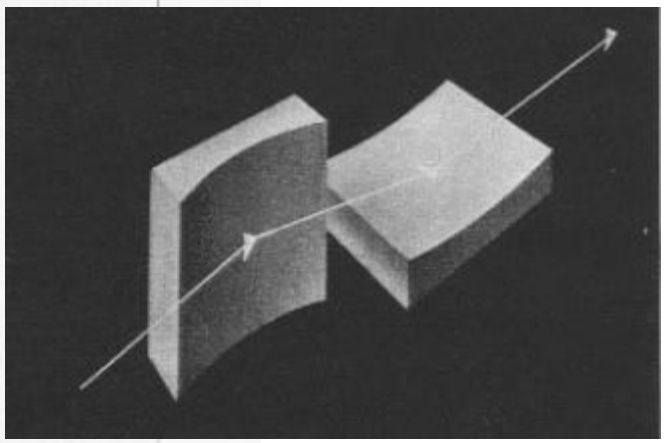
THE X-RAY MICROSCOPE by Paul Kirkpatrick
It would be a big improvement on microscopes using light or electrons, for X-rays combine short wavelengths, giving fine resolution, and penetration. The main problems standing in the way have now been solved. 44

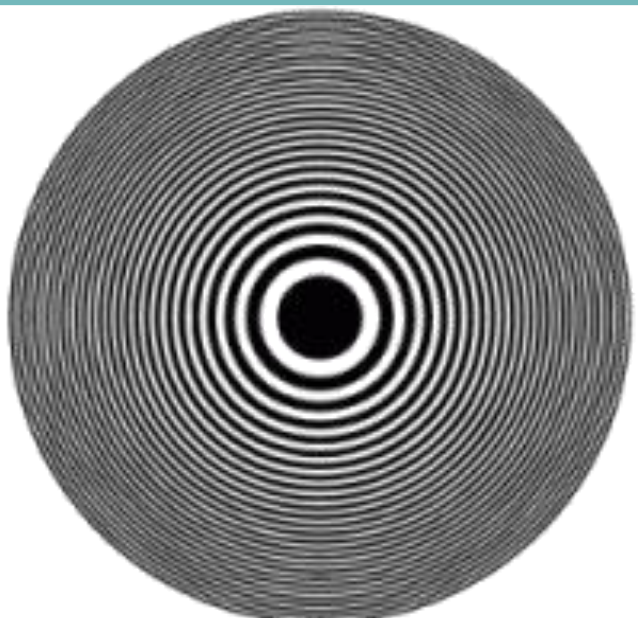
weapons with which they fight foreigners. Some even attack their own kind. A study of a recently discovered phase of plant competition. 48

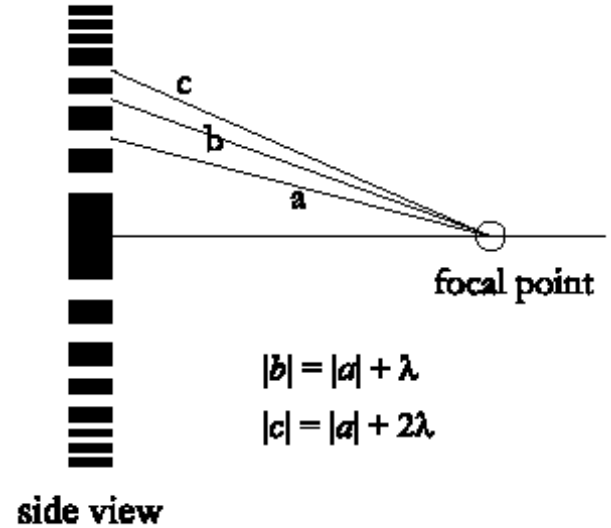


Zone plates

- Diffractive optics: radially varied grating spacing
  - Path difference through neighboring open zones increases by multiple of λ
- In first diffraction order:
  - Largest diffraction angle is given by outermost (finest) zone width δr_N as $\theta=\lambda/(2\delta r_N)$
  - Rayleigh resolution is

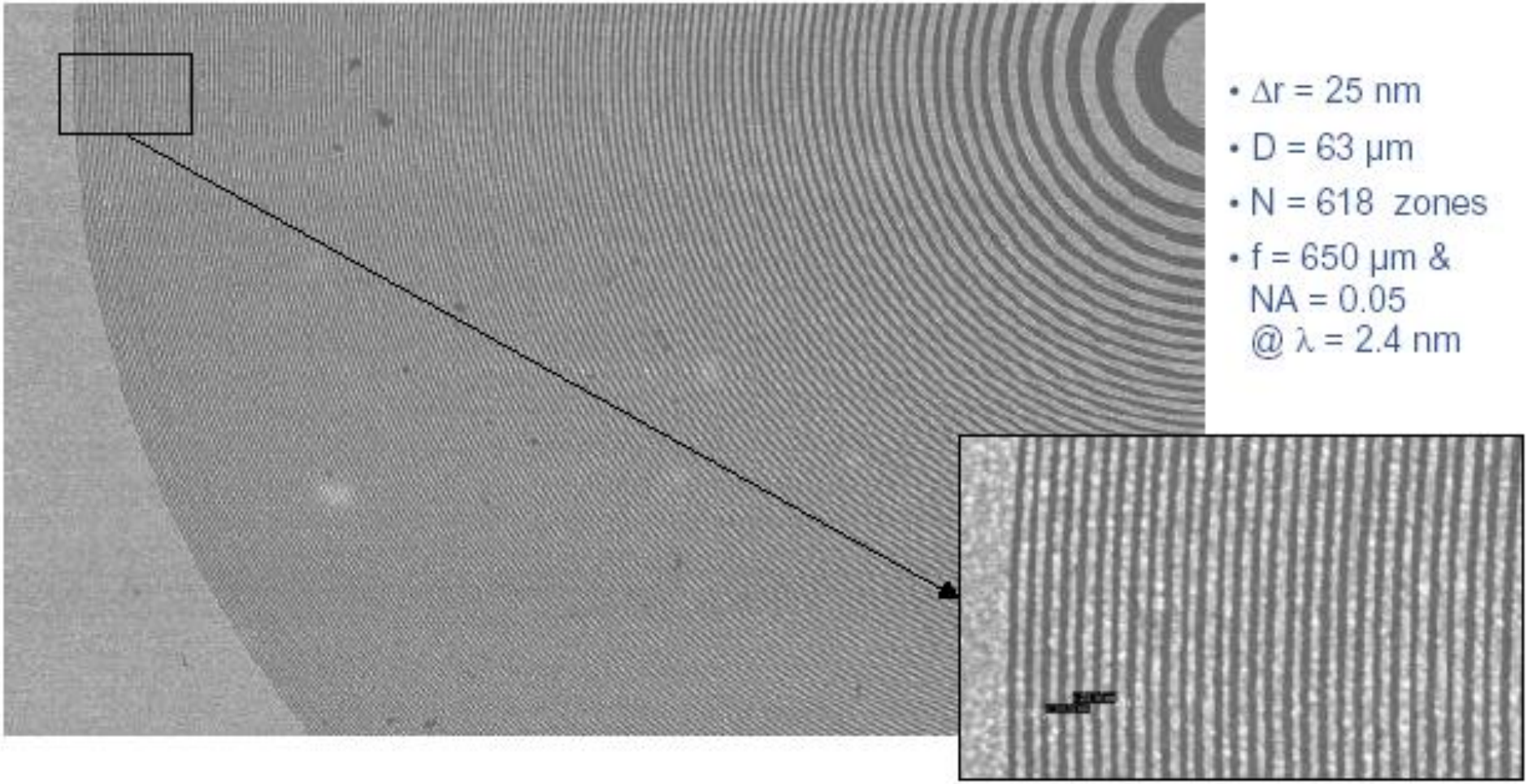
    $\delta_f=0.61\ \lambda/(\theta)=1.22\ \delta r_N$
- Zones must be positioned to ~1/3 width over diameter
  - (10 nm in 100 μm, or 1:10^4)





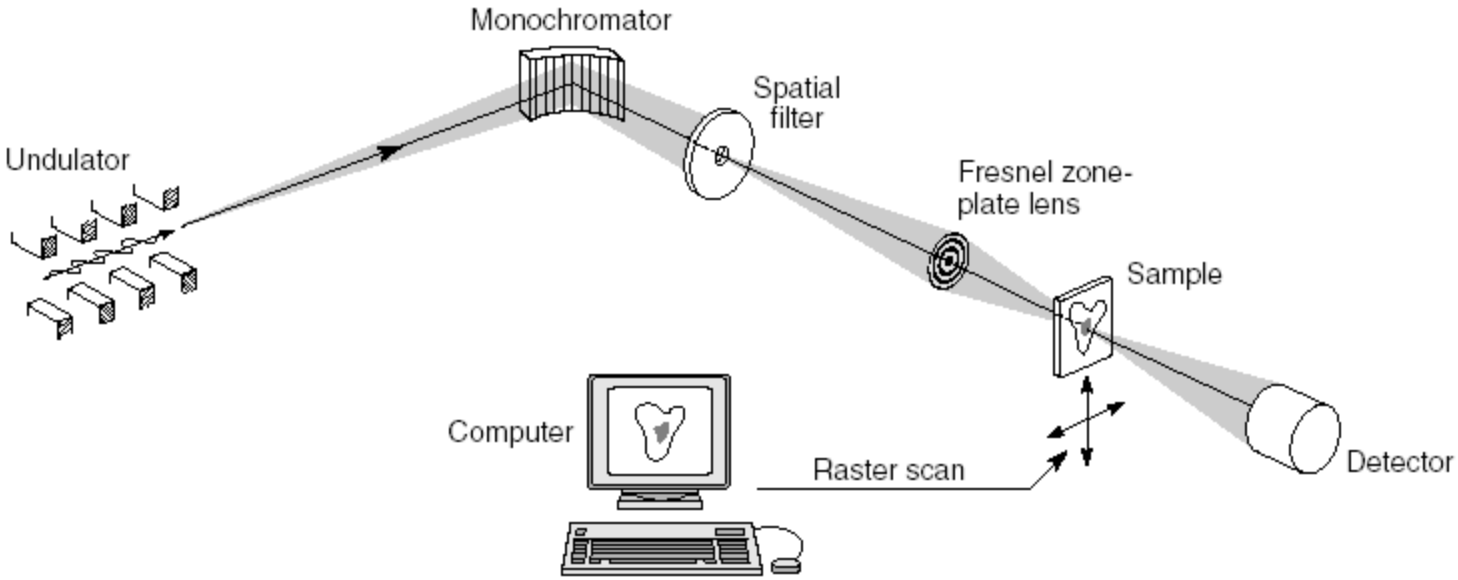
New 25 nm Zone Plate

Erik Anderson, Deirdre Olynick and Bruce Harteneck, Zone Plate Fabrication with the Nanowriter.



- Δr = 25 nm
- D = 63 μm
- N = 618 zones
- f = 650 μm & NA = 0.05 @ λ = 2.4 nm

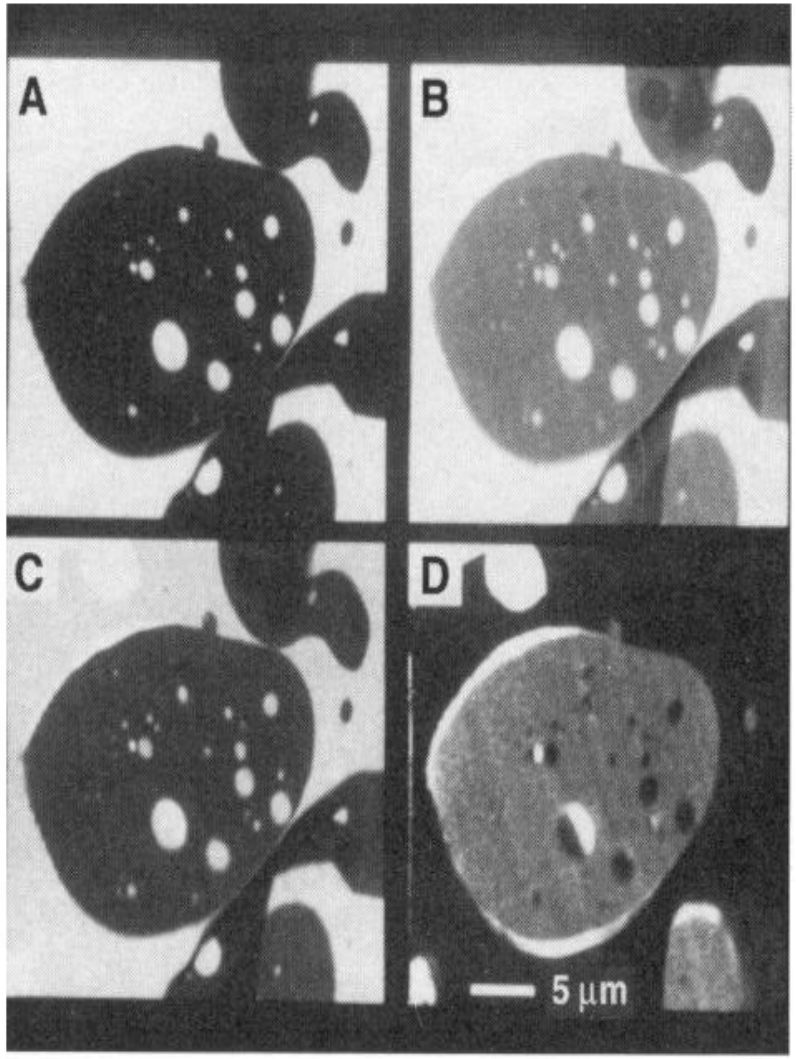
Scanning microscope schematic



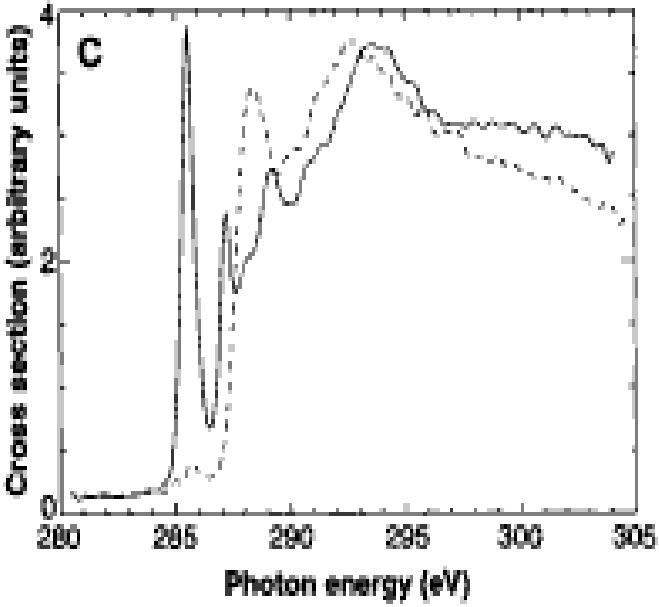
Scanning microscopy

- Scan specimen (or probe) mechanically
  - Collect image pixel by pixel
- Detect
  - transmitted x-rays (STXM),
  - fluorescence (SFM)
  - photoelectrons (SPEM)
  - visible light (SLXM)
- Size of microprobe determines resolution
- Brightness limited
- Scan parameters determine object area, "magnification"

spectromicroscopy



Polypropylene/acrylonitril blend



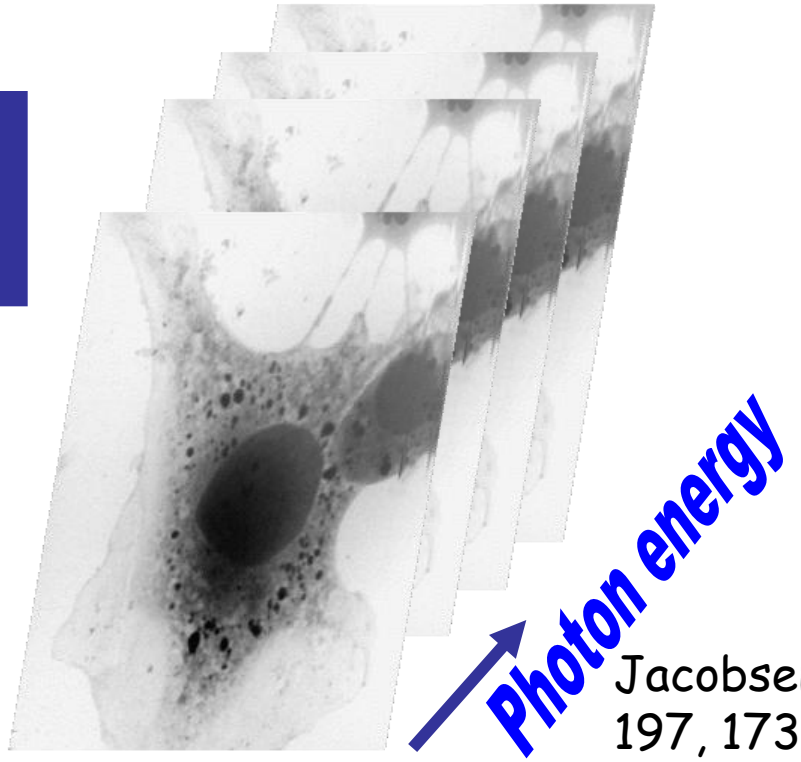
Ade et al. Science 258, 972 (1992)

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.

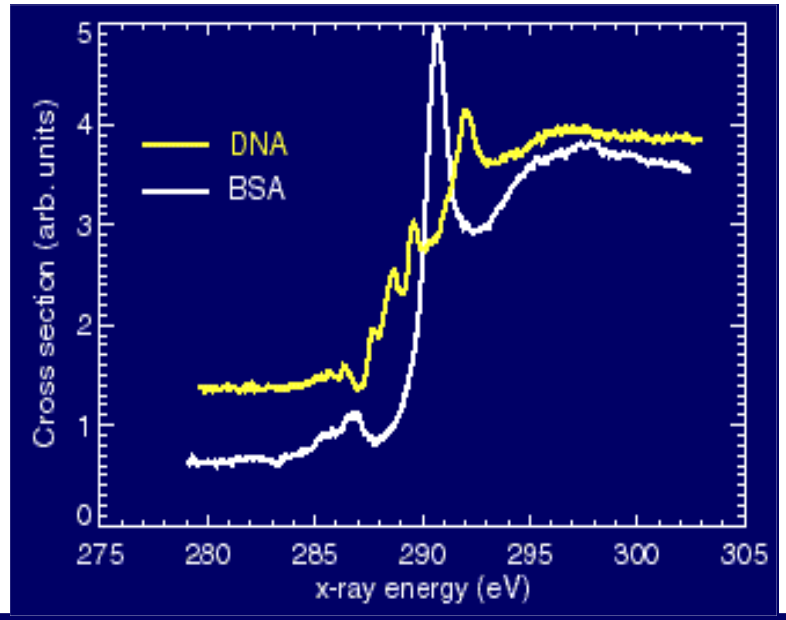
IDL-based analysis tools are made available

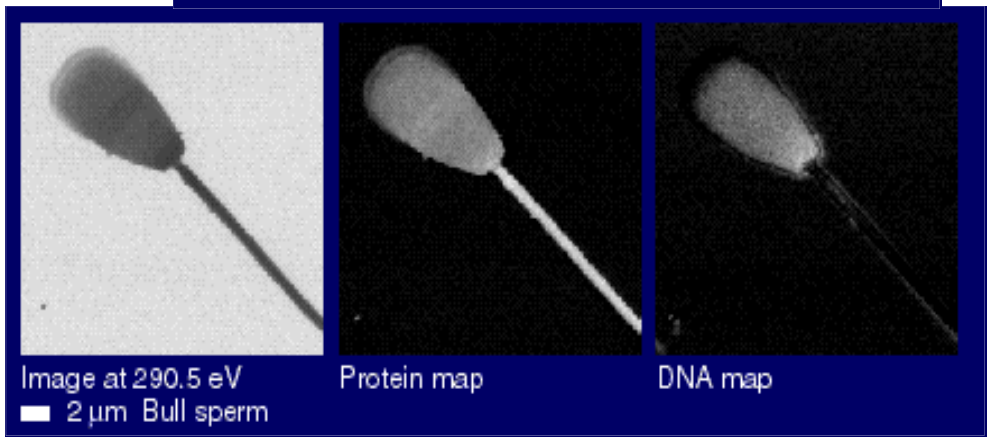


Jacobsen et al. , J. Microscopy 197, 173 (2000)

DNA packing in sperm

- X. Zhang, et al. *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

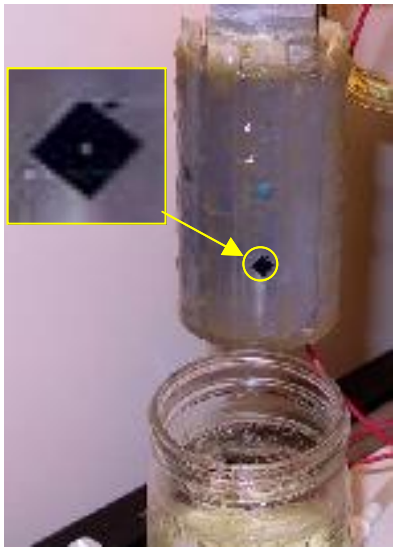
Present

- Microscopes sprouting at all SR sources
- 5 Microscopes at ALS: most oversubscribed
- Going from 2D to 3D:
  - Spectromicroscopy x,y,E
  - Tomography x,y,z
  - Time resolved microscopy x,y,t
- Radiation damage can be a problem

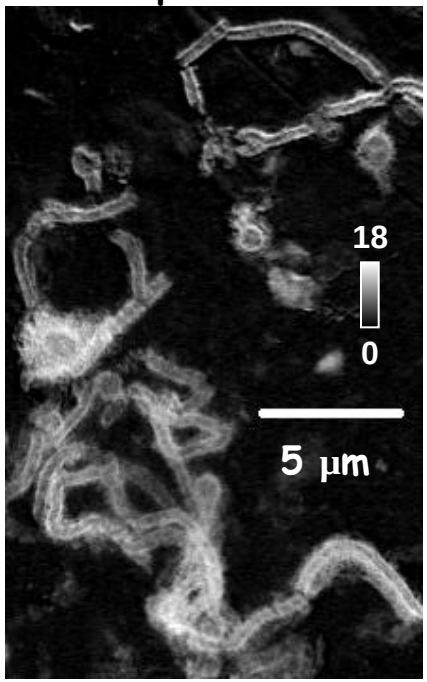
STXM 11.0.2
STXM 5.3.2

Biofilms: metal speciation & quantitative maps

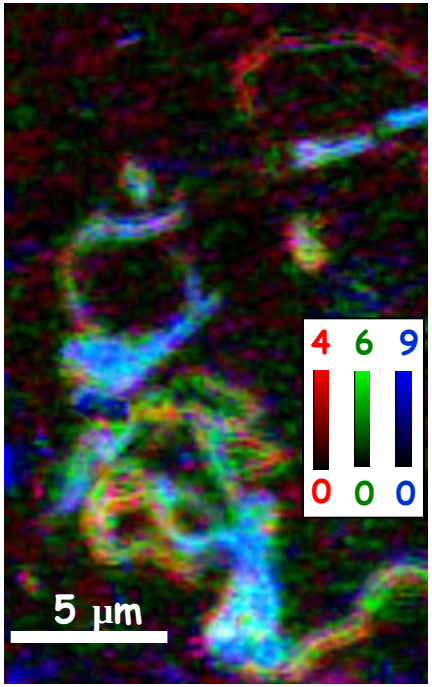
River biofilm cultured in river water; spiked with 10 ppm $NiCl_2$ for 24 hr prior to harvesting wet cell.



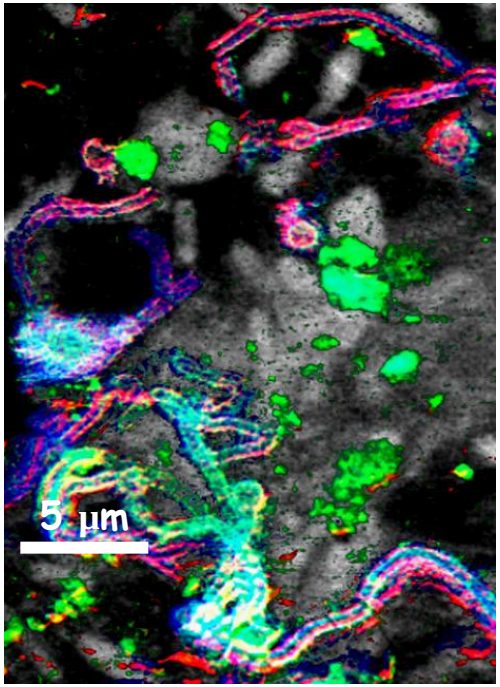
Ni map

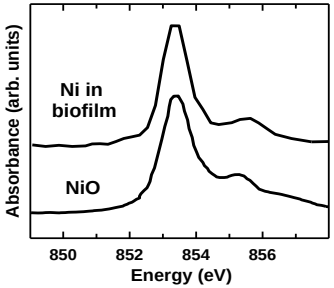


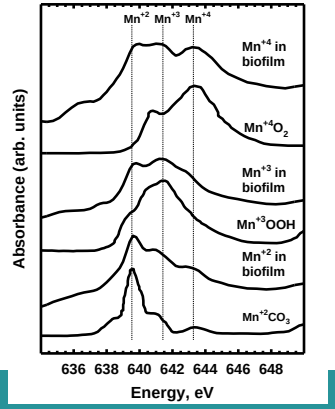
Mn^{+2} Mn^{+3} Mn^{+4}

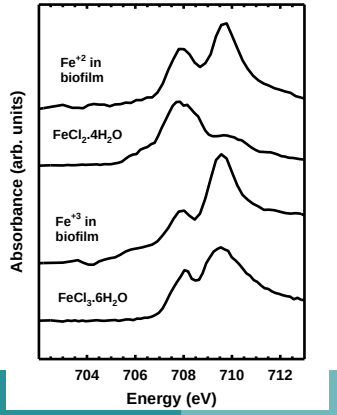


Mn Fe Ni





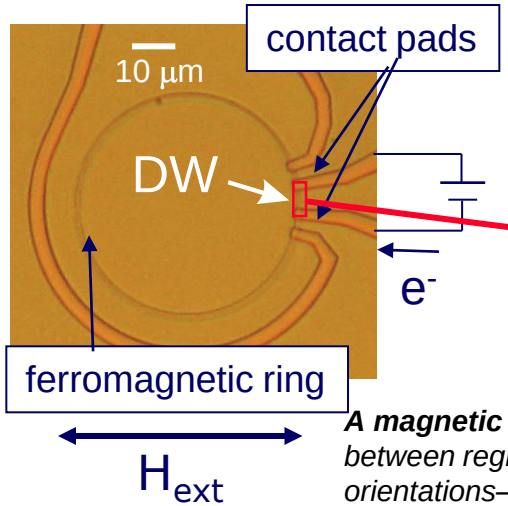




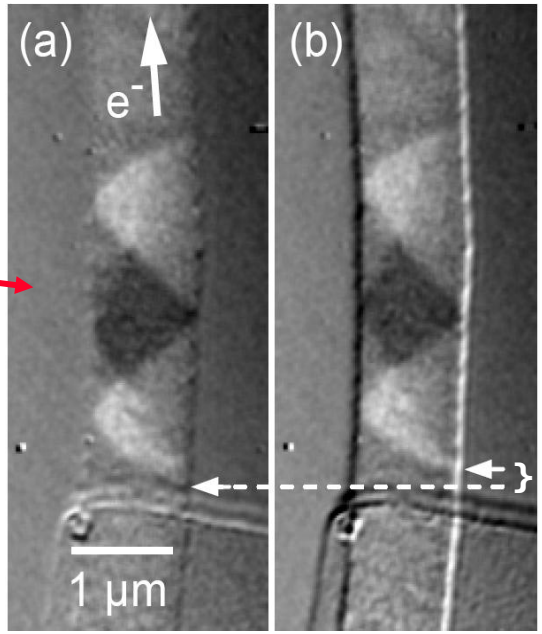
J. Dynes et al., **Geobiology** (2009) in press

Current-Driven Domain Wall Motion in Nanowires

A Step Toward Magnetic Data Storage without Rotating Disks



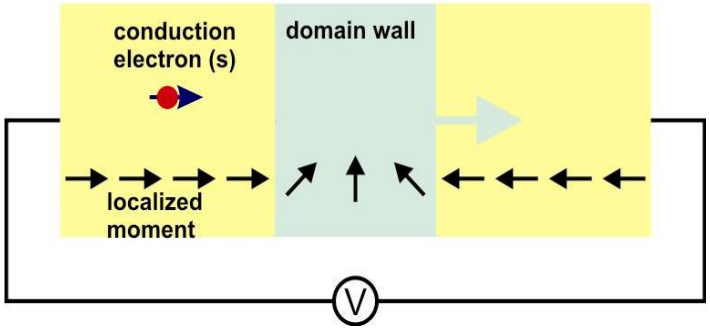
A magnetic domain wall (*DW)—the boundary between regions of different magnetic orientations—is created in a 20nm x 1000nm ferromagnetic permalloy ($Ni_{80}Fe_{20}$) ring through the application of a magnetic field (H_{ext}) between two contact pads. One nanosecond short pulses are injected into the ring to move the DW.*



Distance moved by domain wall

before ***after***

Magnetic soft x-ray microscopy *using the CXRO XM-1 x-ray microscope allows the imaging of domain wall motion with a spatial resolution as high as 15 nm. Images captured before (a) and after (b) the current pulse injection are used the measure the speed of the motion, which was 110m/s, in agreement with theoretical estimates.*

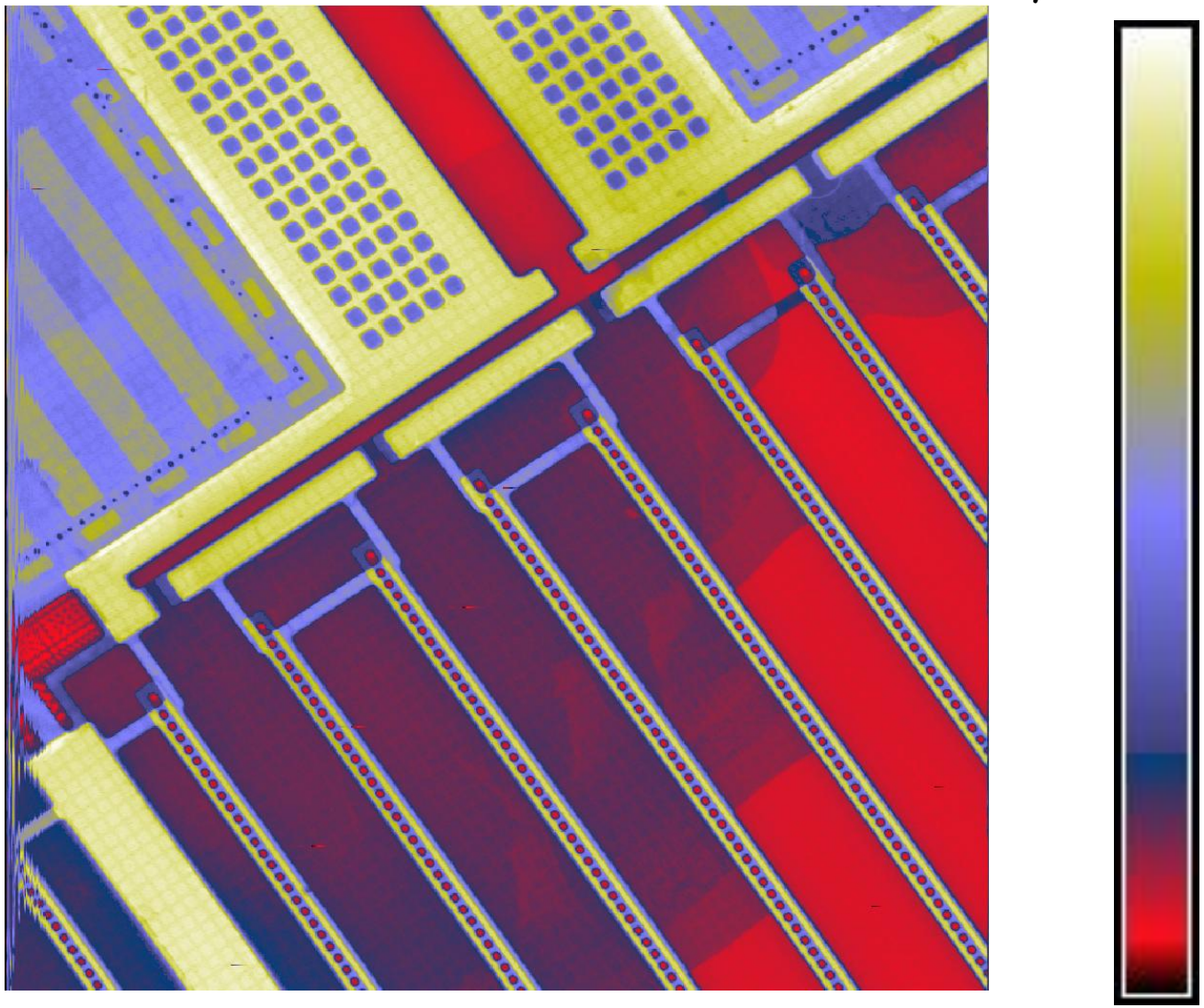


A "spin torque" *exerted by the current pulse pushes the domain wall along the wire.*

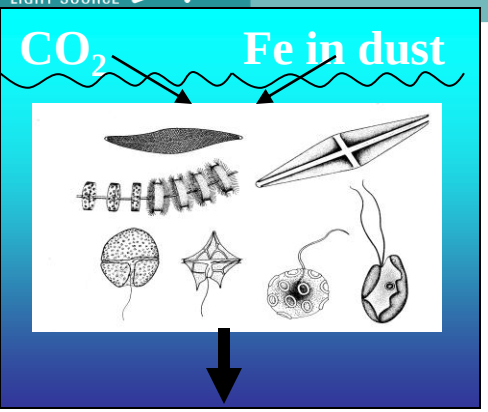
Ref: G. Meier, M. Bolte, R. Eiselt, B. Krüger, D.-H. Kim., P. Fischer, Physical Review Letters ***98****, 187202 (2007)*

STXM at APS

I. McNulty et al.

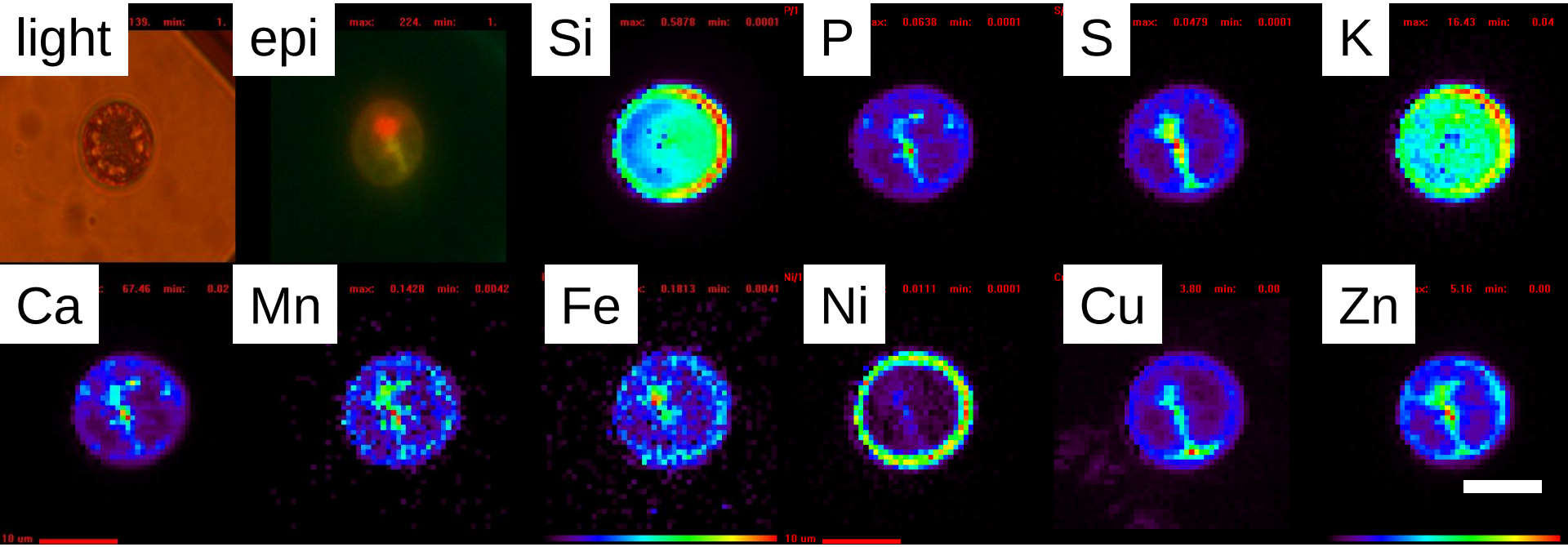


Trace metals in plankton and global carbon balance



- 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



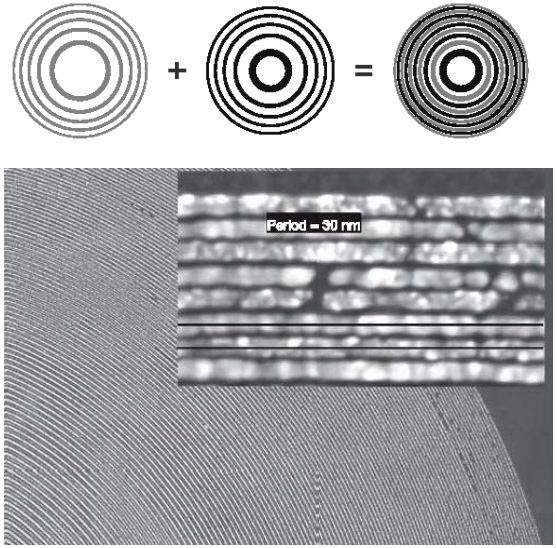
B. Twining, *et al.*, Analytical Chemistry 75, 3806-3816 (2003).

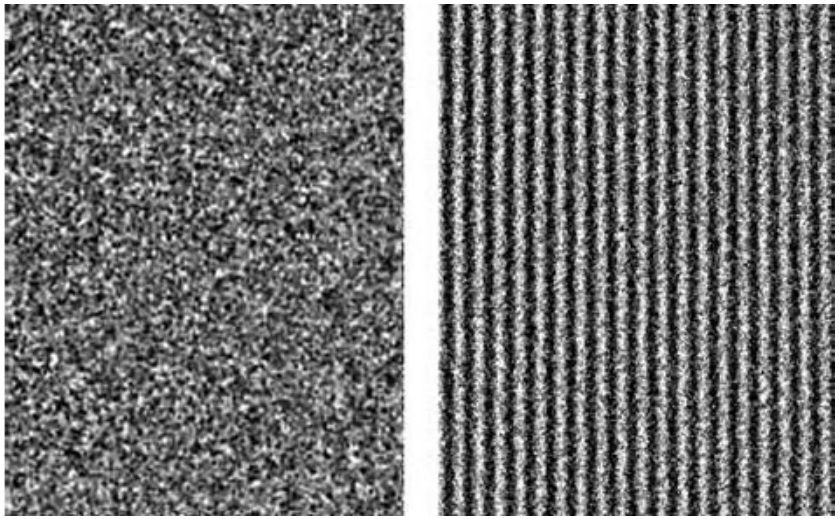
•Fe is concentrated in/near the chloroplasts (epi)

A SHARPER FOCUS FOR SOFT X-RAYS

Zone Plate Lenses with Better than 15-Nanometer Spatial Resolution

Center for X-ray Optics, LBNL





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).

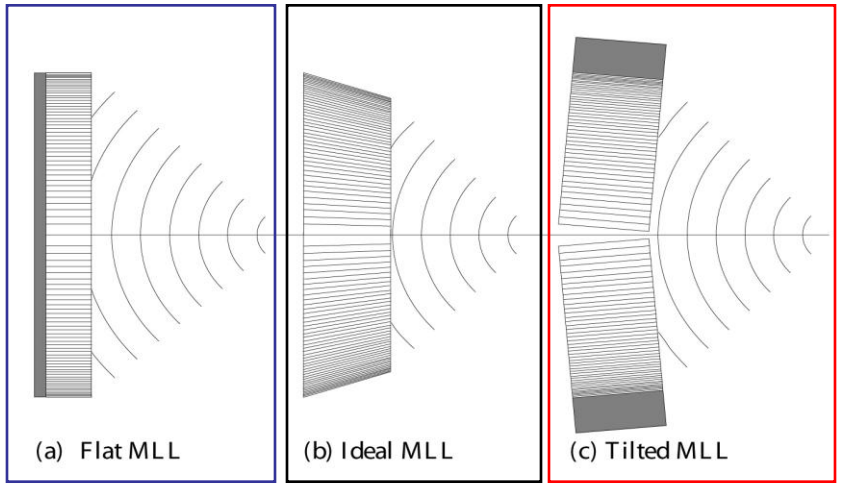
Soft x-ray images taken with the CXRO XM-1 full-field imaging microscope at the ALS (Beamline 6.1.2). Comparison of images of a 15.1-nm test object with the previous 25-nm (left) and the new 15-nm (right) zone plates illustrates the improved spatial resolution achievable.

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)]

How far can you go?

- NSLS II specification: 1 nm resolution
- (What is the scientific motivation?)
- Can you get there?

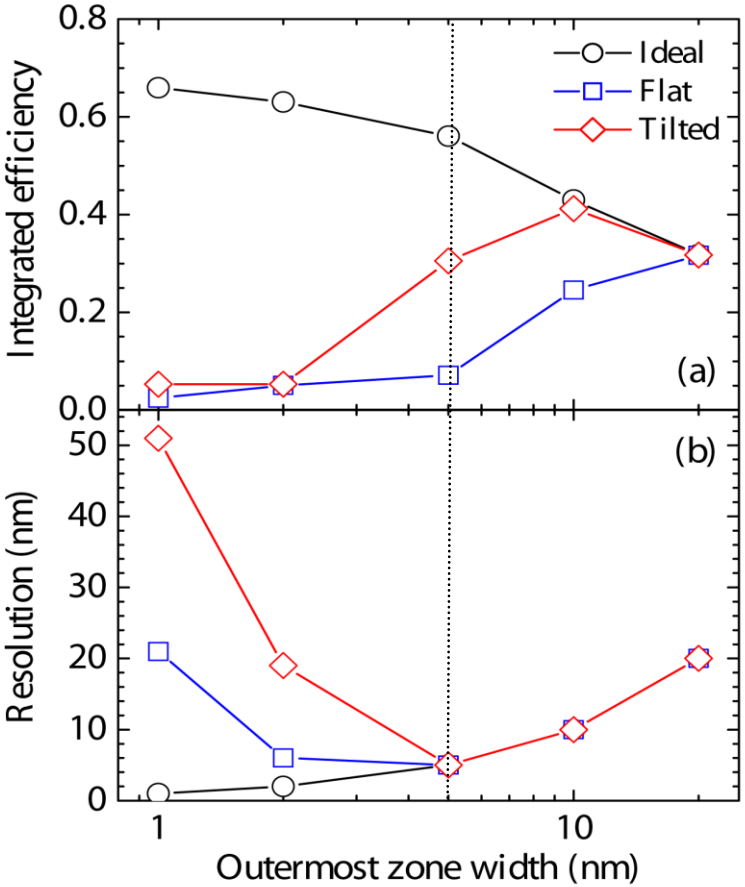
Where is the resolution limit for x-ray focusing?



- 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?

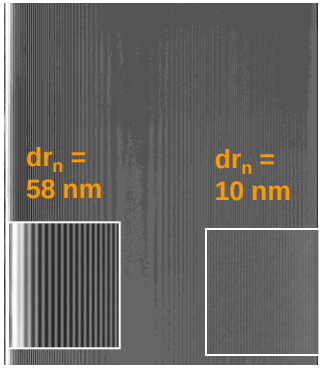
Calculations for 1D MLL



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

Multilayer Laue Lens – Concept

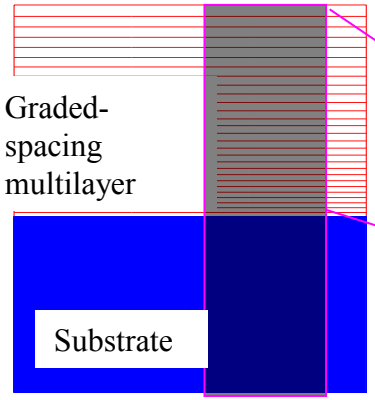
- Deposit varied depth-graded multilayer on plane substrate (thinnest structures first)
- Section to 5-20 μm depth
- Assemble into a linear MLL
- Assemble two linear MLL's into a 2D MLL.



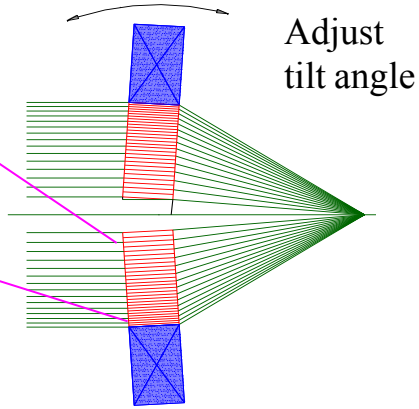
Material: WSi_2/Si

Total deposition thickness: 12.4 um

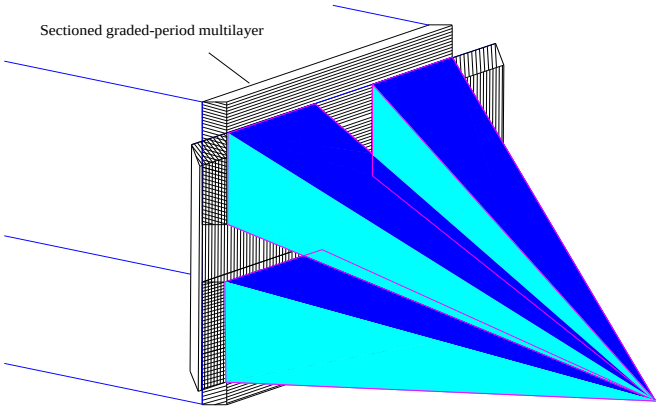
d-spacing: 10 – 58 nm



Deposited multilayer



1D MLL

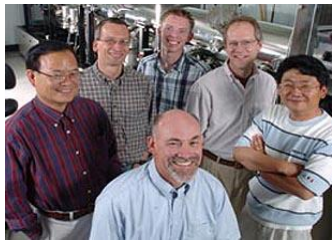


2D MLL

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

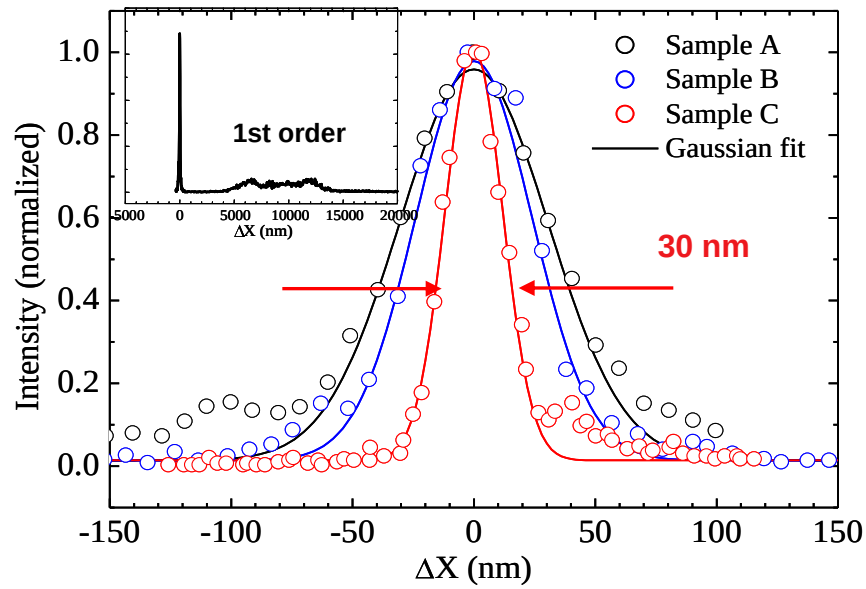
NA-limited resolution:

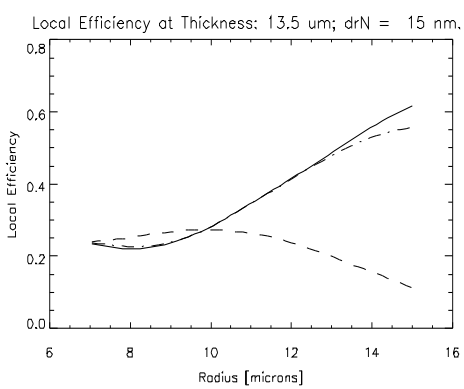
Sample A: 57 nm (27% NA)

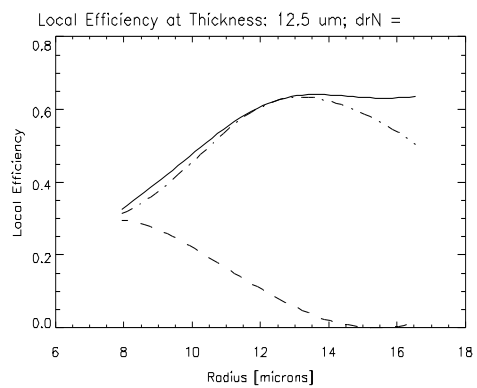
Sample B: 44 nm (23% NA)

Sample C: 24 nm (41% NA)

Resolution measurement







Photon Energy: 19.5 keV

Measured Resolution: **30 nm**

Diffraction Efficiency: **44%**

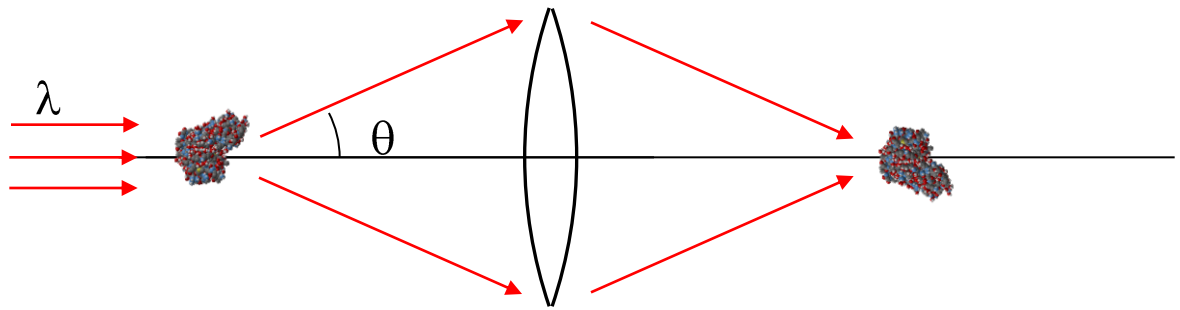
Kang et. al, PRL, Apr., 2006

Completely different approach

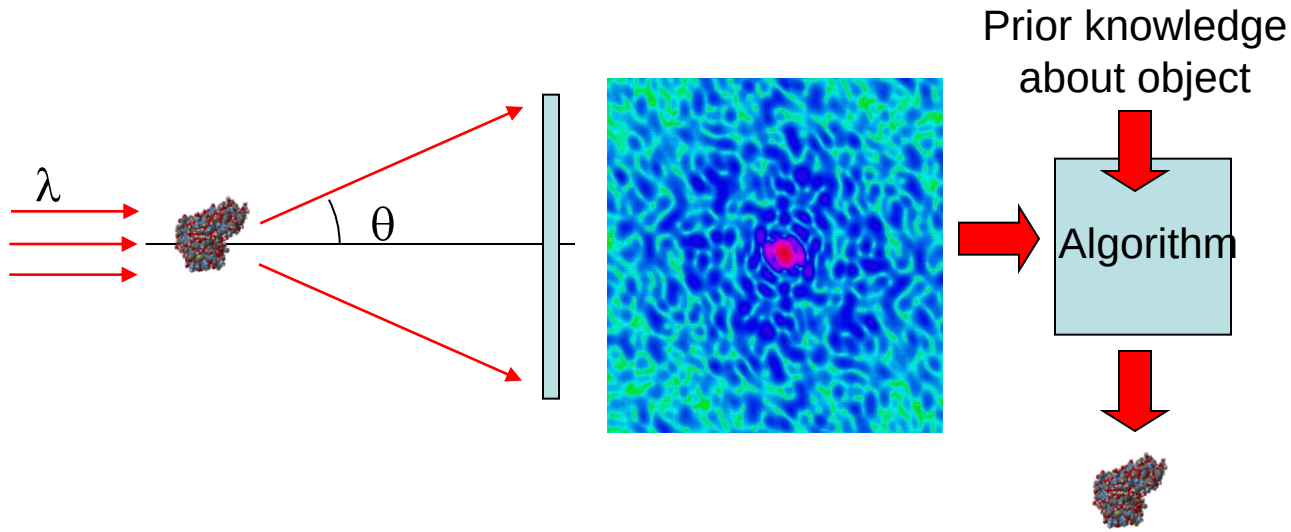
Get rid of optics!

Diffraction microscopy is lensless

Use a computer to phase the scattered light, rather than a lens



A lens recombines the scattered rays with correct phases to give the image



An algorithm finds the phases that are consistent with measurements and prior knowledge

Resolution: $\delta = \lambda / \sin\theta$

Idea of David Sayre

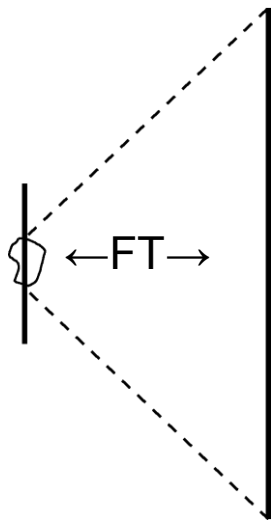
Image reconstruction from the diffraction pattern

- Lenses do it, mirrors do it
  - but they use the full complex amplitude!
- Recording the diffraction *intensity* leads to the "phase problem"!
- Holographers do it – but they mix in a reference wave, need very high resolution detector or similar precision apparatus
- Crystallographers do it – but they use MAD, isomorphous replacement, or other tricks (plus the amplification of many repeats)

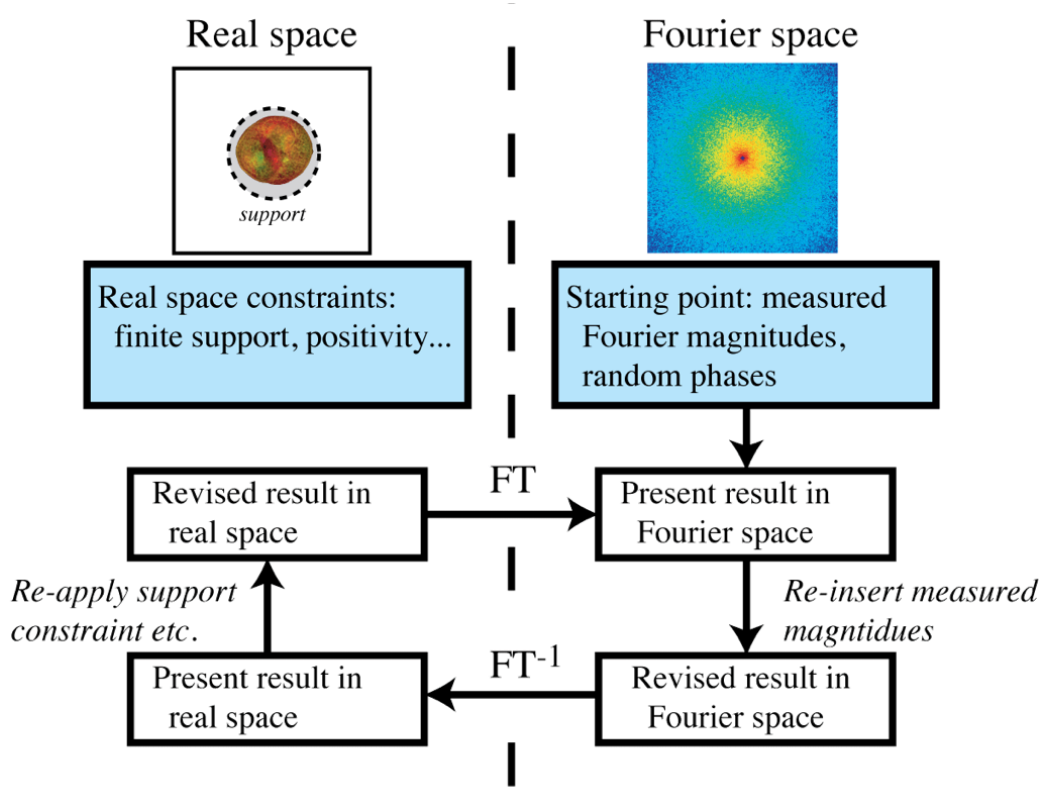
Imaging without lenses

- Avoid losses of lens efficiency and transfer function
- Must phase the diffraction intensities

Real space: finite support
(or other constraints)



Fourier space: magnitudes known, but phases are not

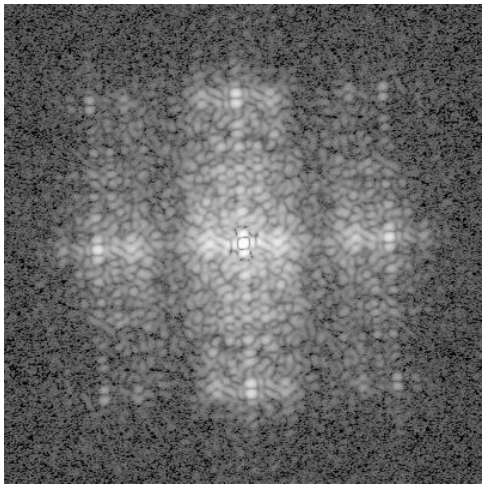


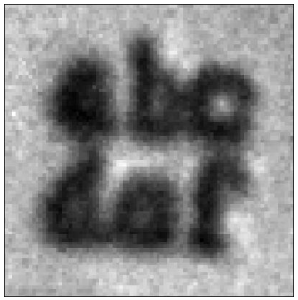
Phasing algorithms: Feinup, *Opt. Lett.* **3**, 27 (1978); Elser, *JOSA A* **20**, 40 (2003); and others. First x-ray demonstration: Miao *et al.*, *Nature* **400**, 342 (1999).

First experimental demonstration

Miao, et al. *Nature* **400**, 342 (1999).

λ=1.8 nm soft x-ray diffraction pattern





Low angle data From optical micrograph

Scanning electron micrograph of object

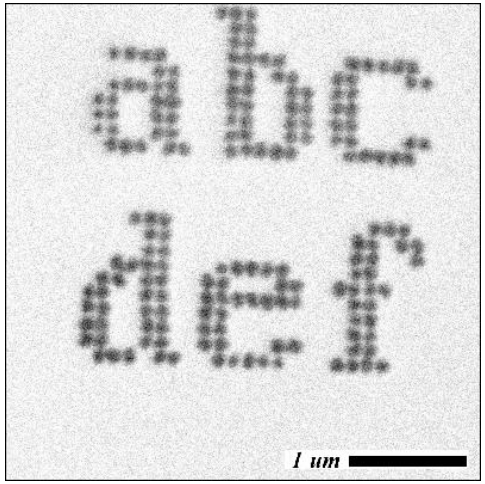
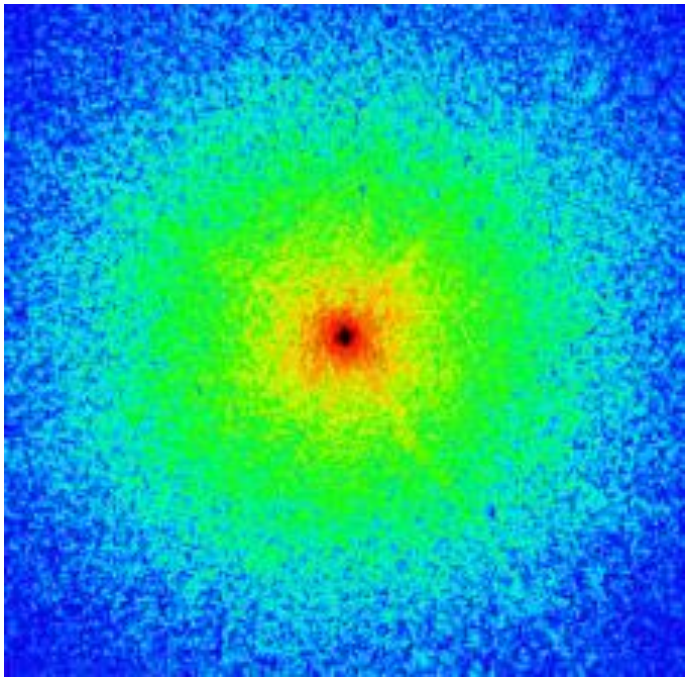


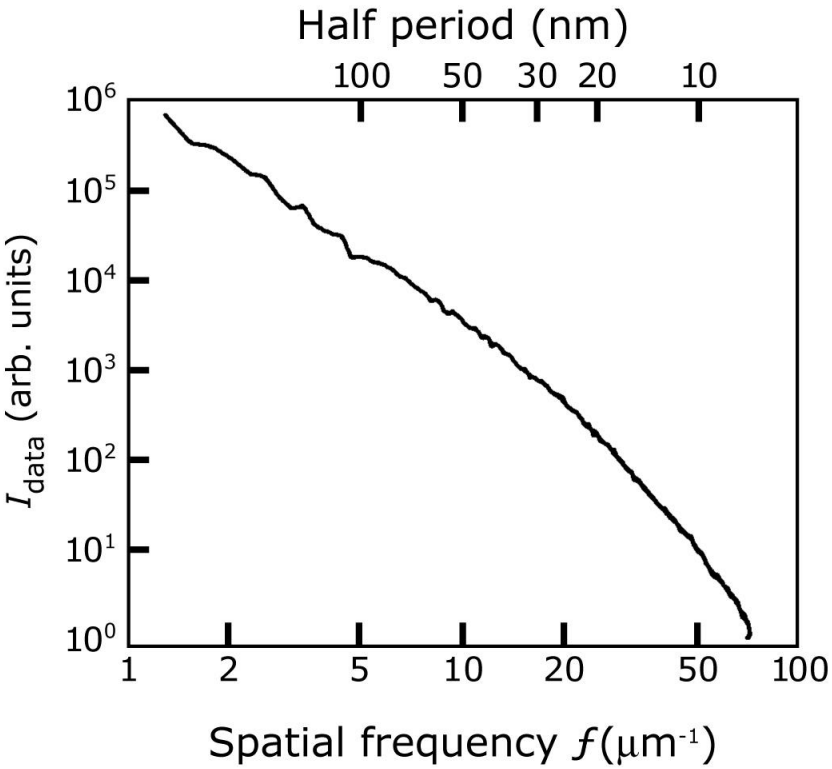
Image reconstructed from diffraction pattern (θ_{max} corresponds to 80 nm). Assumed positivity

Diffraction data of freeze-dried yeast cell

Yeast cell: 2.5 micron thick, unstained freeze-dried, at 750 eV
Total dose ~ 10^8 Gray (room temperature)

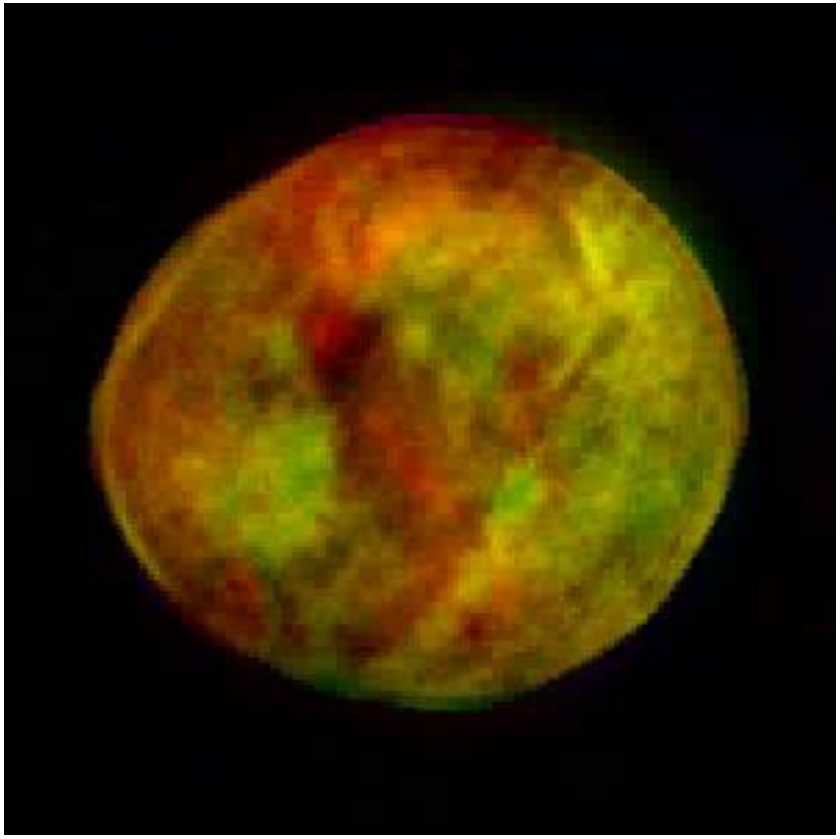
Oversampling is about 5 in each dimension





David Shapiro, Stony Brook, now at ALS

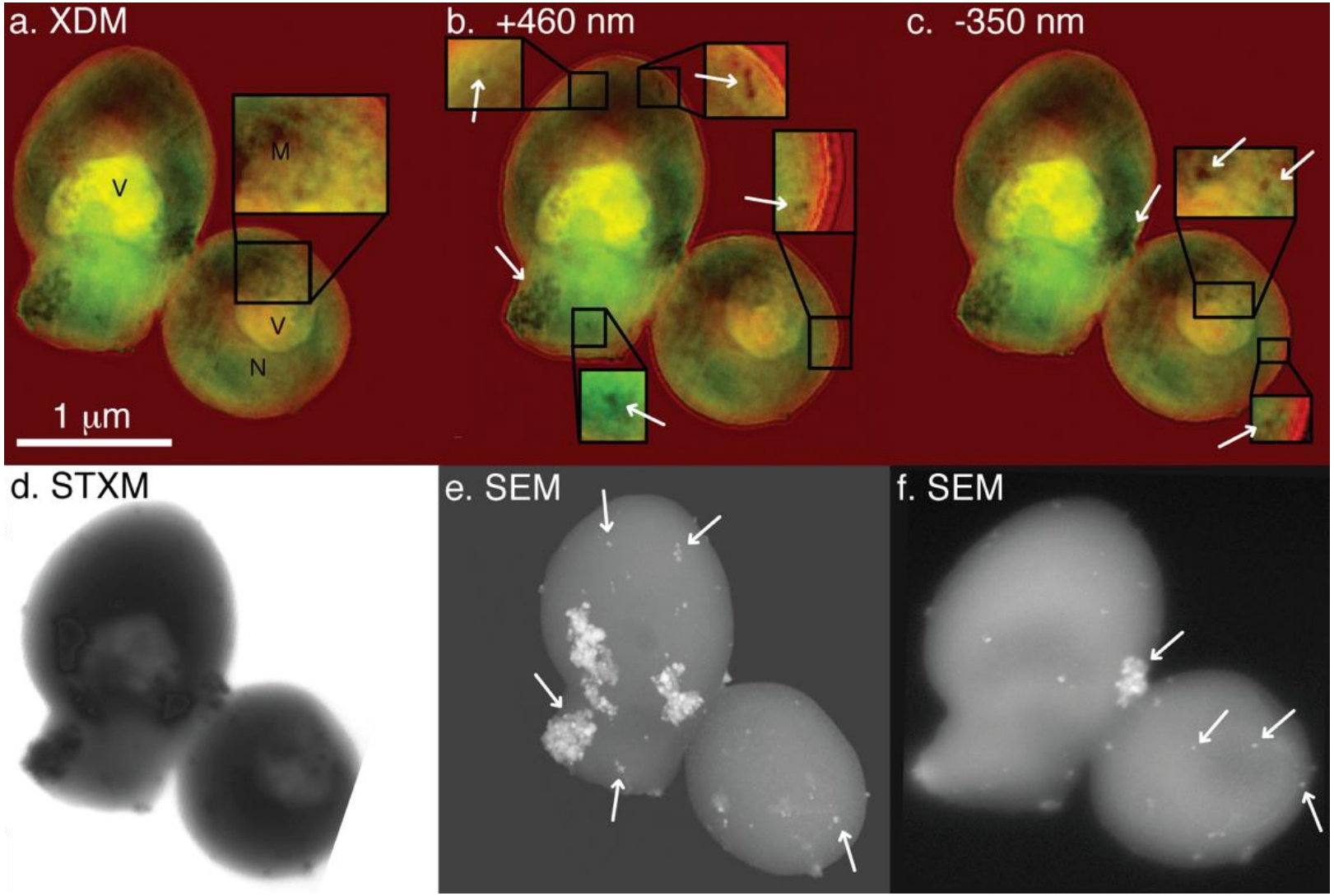
Movie: tilt from -3 to +5 degrees in 1 degree steps



Pierre Thibault

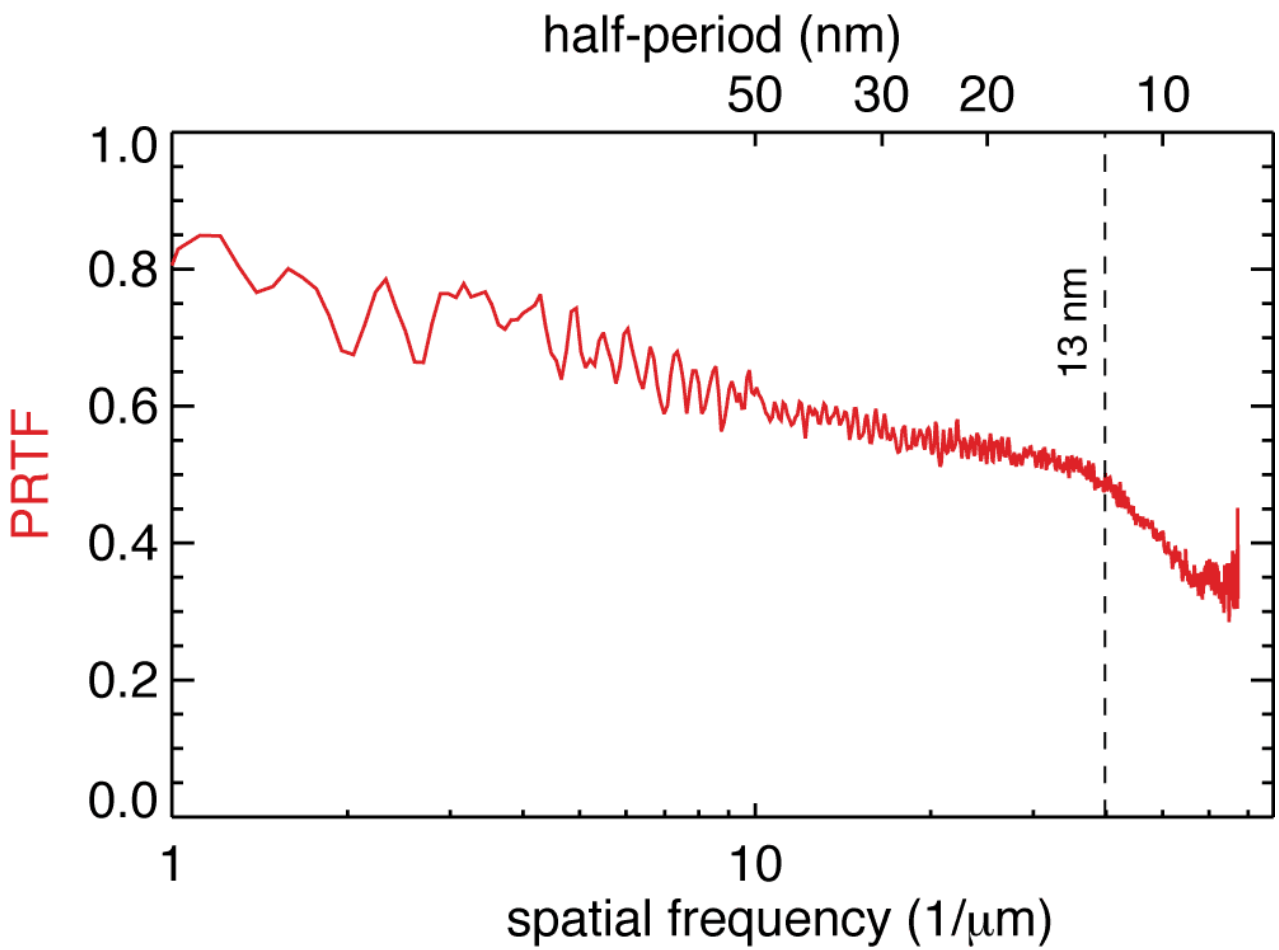
Gold-labeled yeast

1.8 nm gold, silver-enhanced, freeze-dried: Johanna Nelson, Stony Brook.
Propagation of complex reconstructed wave to “focus” on different planes.



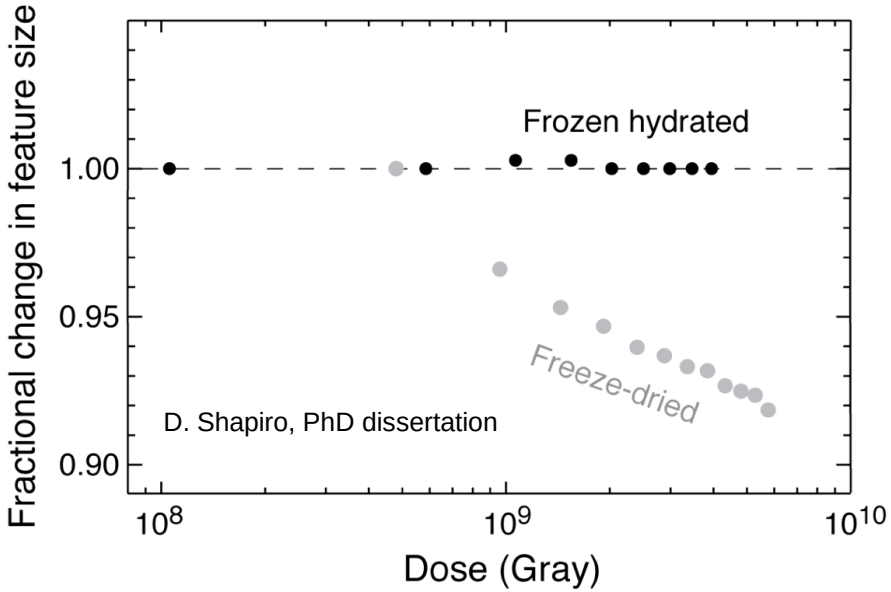
Gold-labeled yeast resolution: ~13 nm

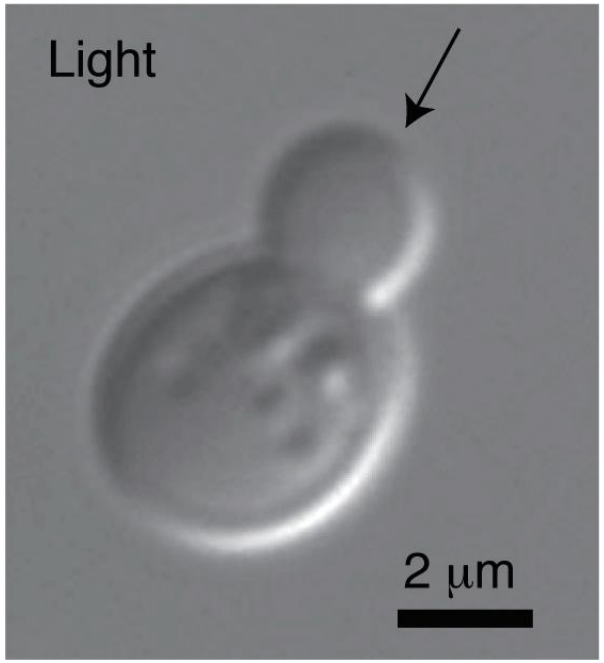
Phase retrieval transfer function (PRTF) measures reproducibility of phasing versus length scale; can compare with modulation transfer function (MTF)

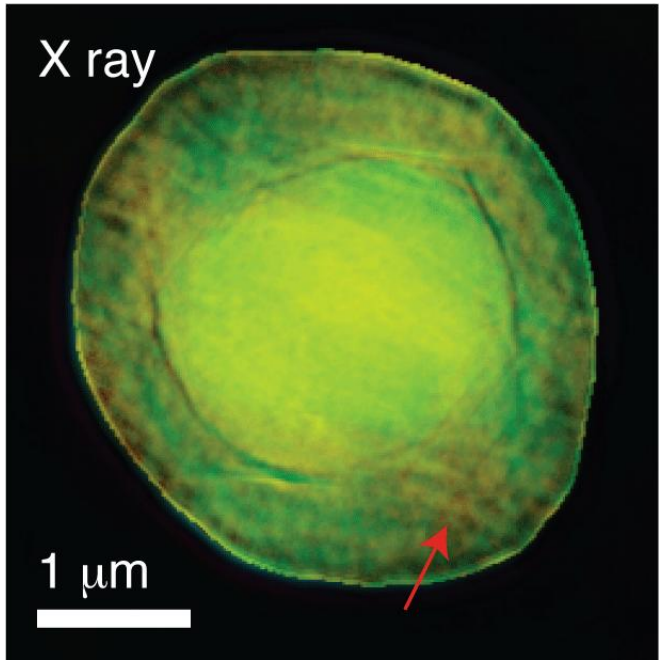


Frozen hydrated yeast

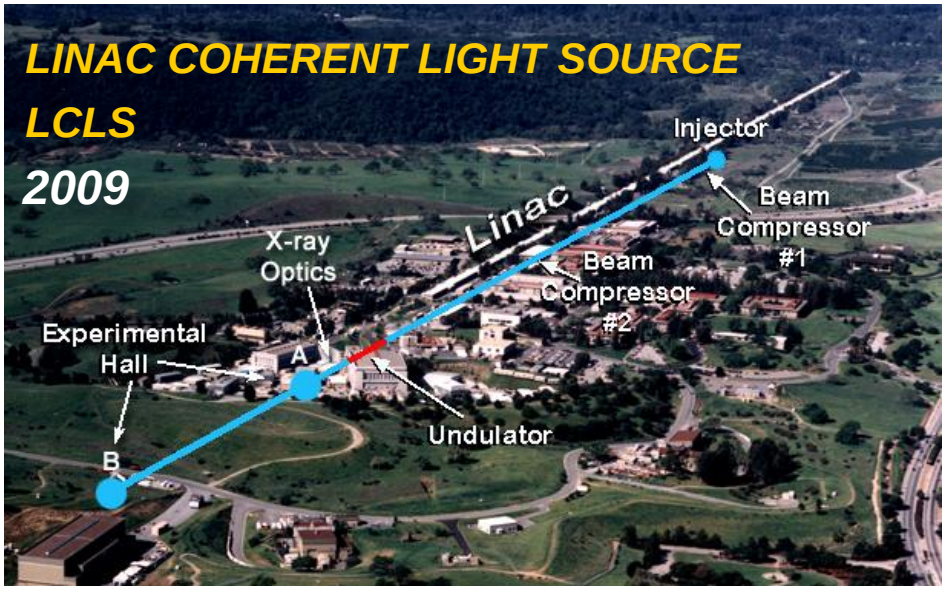
- X. Huang *et al.*, Stony Brook/ALS
- See also E. Lima *et al.* images of *Deinococcus radiodurans* bacteria at ESRF.

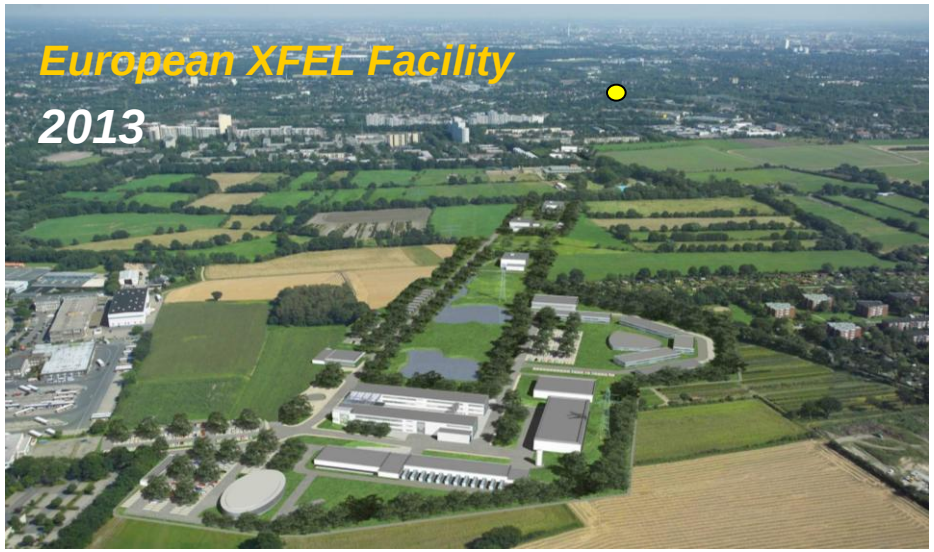


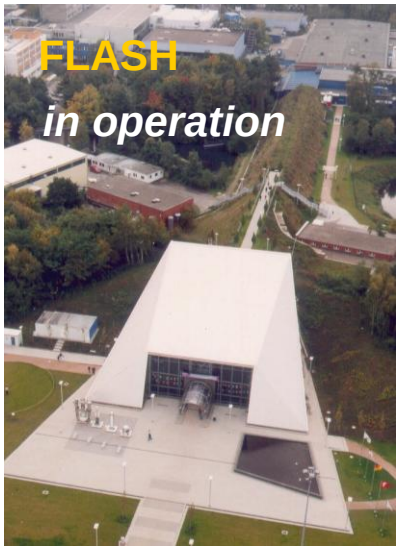




Hard X-ray SASE Free Electron Lasers







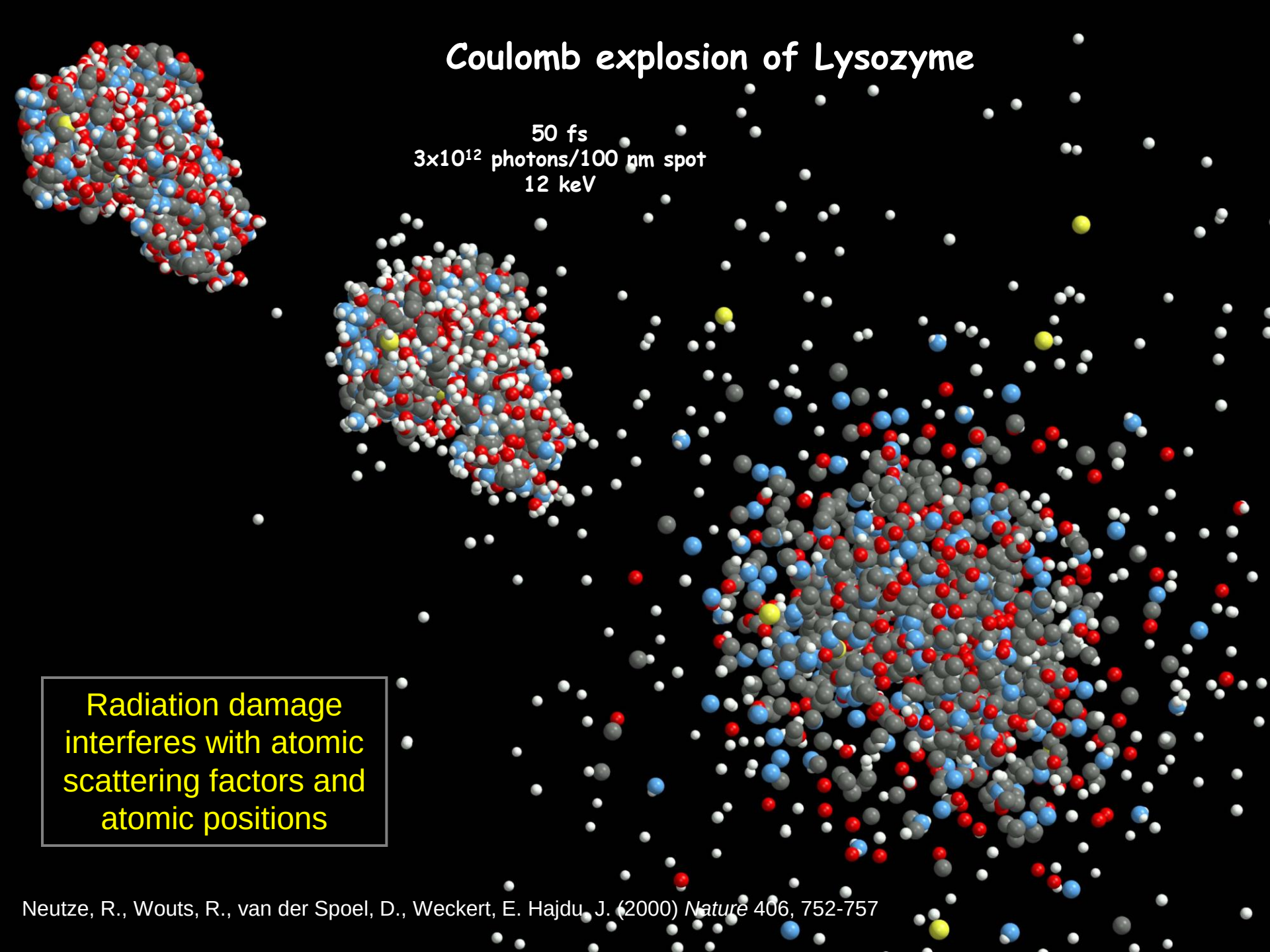
What's really going on…

- Given n electrons radiating (~10^9)
- Given N magnet periods (~10^2)
- Bend magnet output ~ n
- Wiggler output ~ nN
- Undulator output ~ nN^2
- FEL output ~ n^2N^2

What can FEL's do for X-ray microscopy?

- Fully coherent, very high peak power
- Structural biology using crystals
- The challenge of membrane proteins
- Single shot turns molecule into plasma
- Capture structure fast!
- Many identical specimens

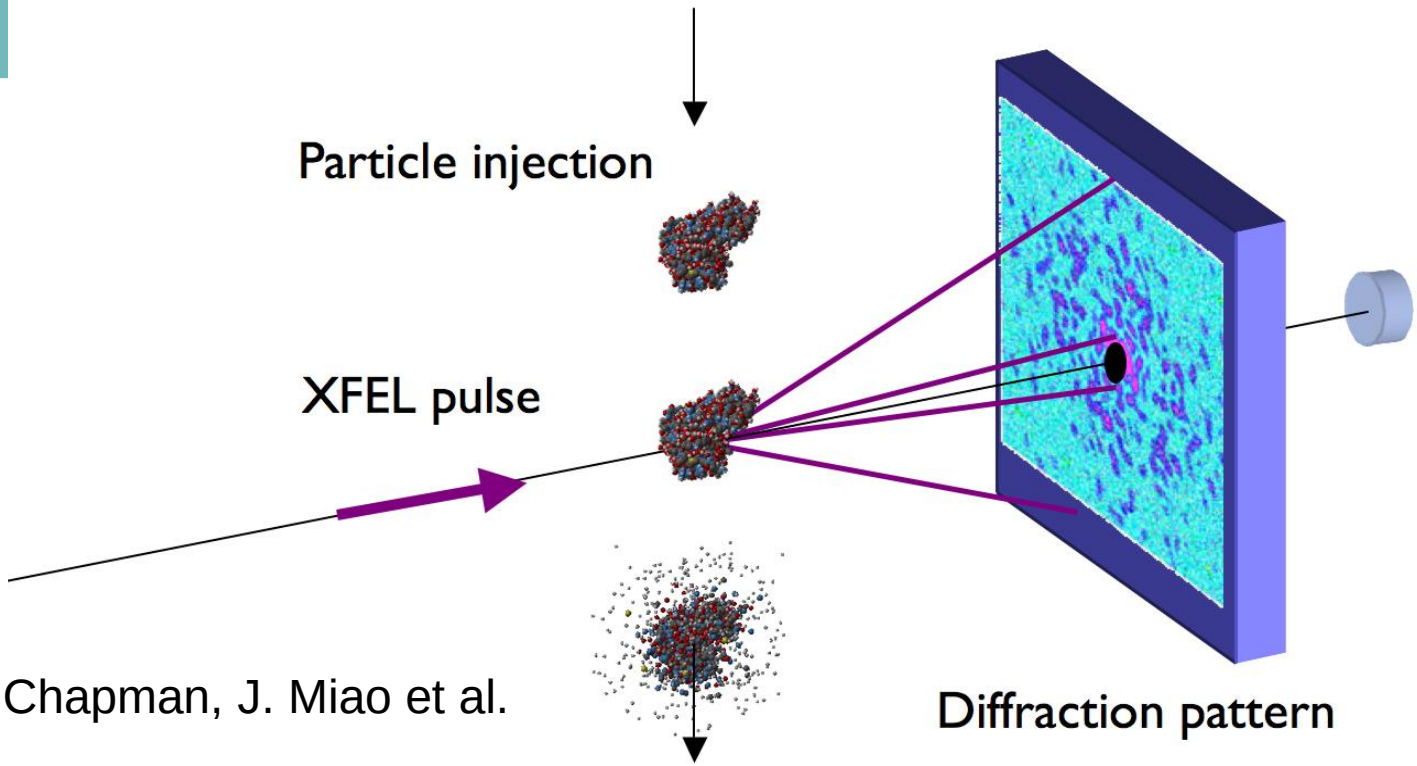
Coulomb explosion of Lysozyme



Radiation damage interferes with atomic scattering factors and atomic positions

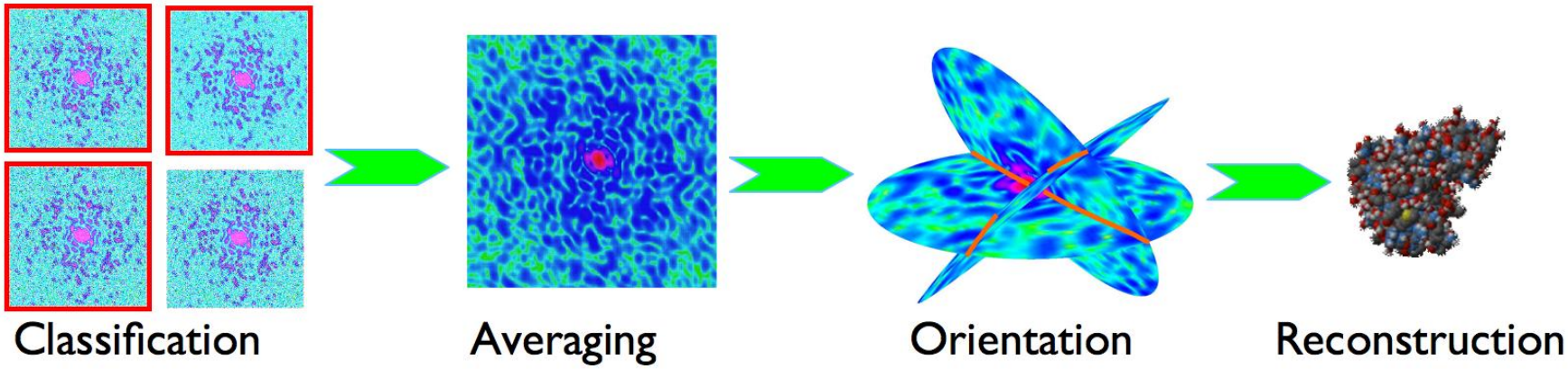
Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) *Nature* 406, 752-757

One pulse, one measurement



J. Hajdu, H. Chapman, J. Miao et al.

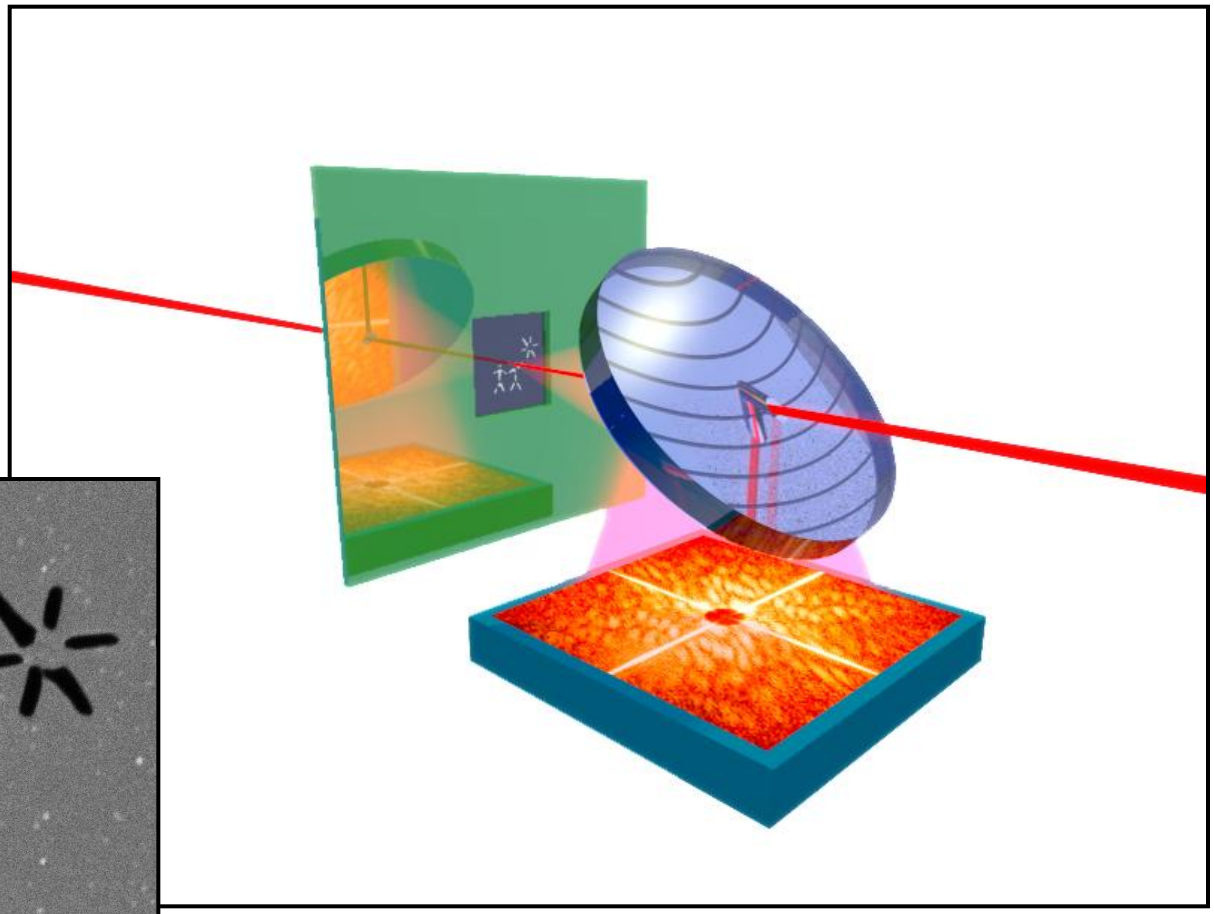
Combine 10^5 – 10^7 measurements

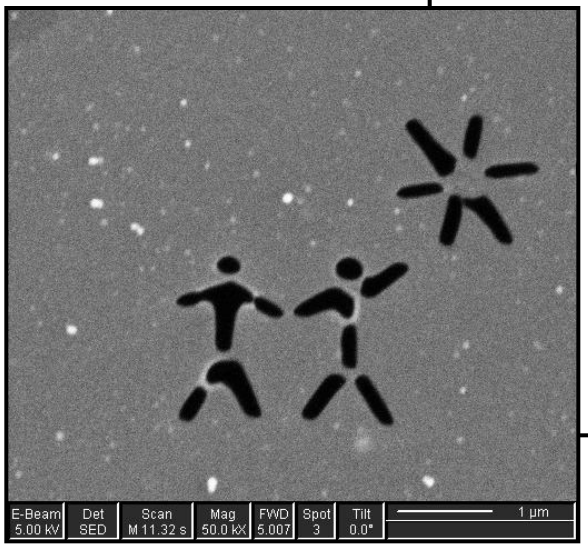


Toward a proof of principle

Ultrafast coherent diffraction at 32 nm

Incident FEL pulse:
30 fs, 32 nm,
3 x 10^{13} W cm^{-2}

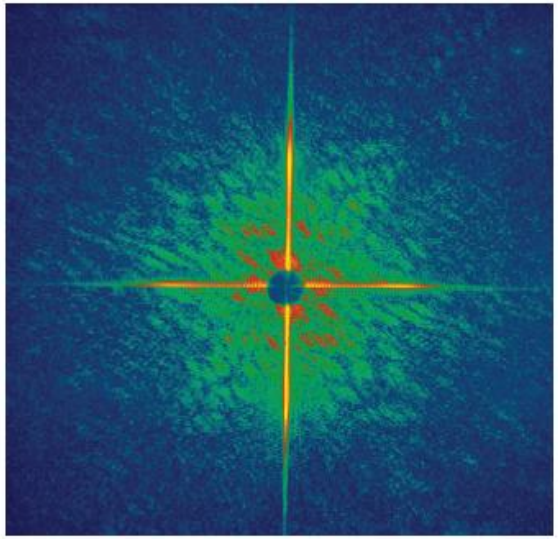


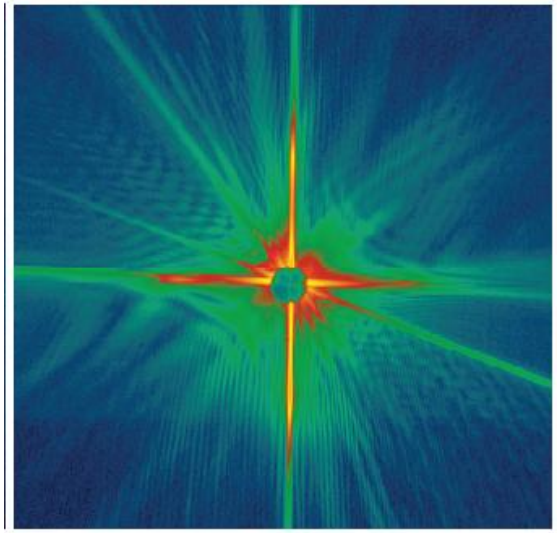


H. Chapman, J. Hajdu et al.

Ultrafast coherent diffraction

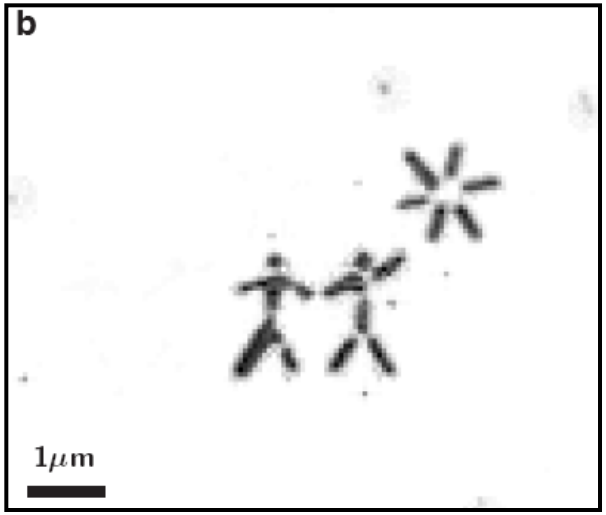
diffraction pattern from first pulse

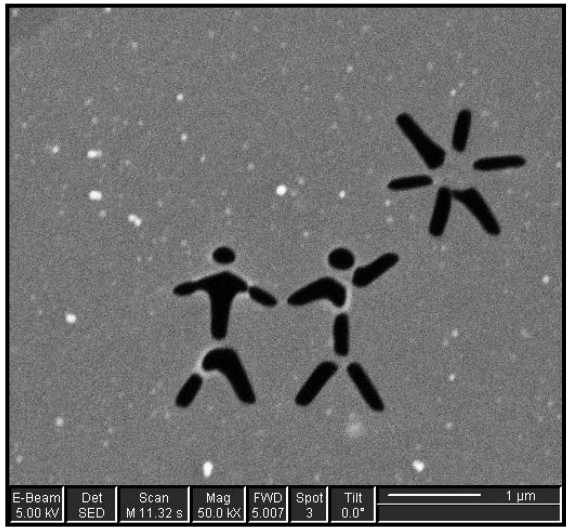




diffraction pattern from second pulse

reconstructed picture





TEM picture from original structure

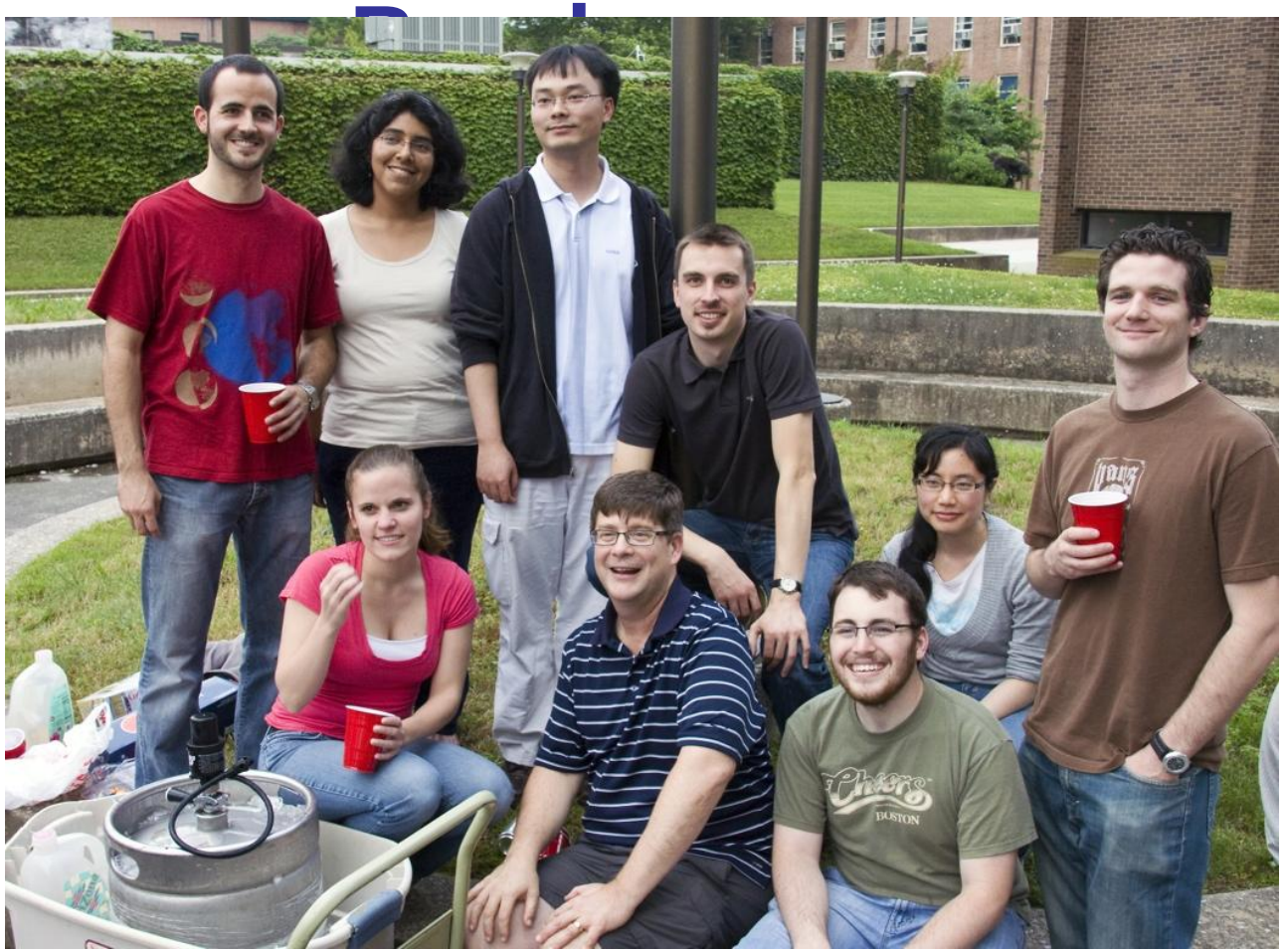
H. Chapman, J. Hajdu et al.

Conclusions

- X-ray microscopes complement other forms of microscopy
- Rapidly evolving field with many applications, and much potential

Acknowledgment I:
X-ray microscopy group at Stony Brook

Jan Steinbrener, Lisseth Gavilan, Xiaojing Huang, Christian Holzner, Rachel Mak, Josh Turner, Johanna Nelson, Chris Jacobsen, Robert Towers.
Not shown: Sue Wirick, Chris Peltzer.
Many alumni and collaborators!



Funding: DoE (method development), NIH (application to cells, phase contrast), NSF (environmental science), NYSTAR (coherent optics and applications)

Acknowledgment II

- ALS collaborators: Malcolm Howells, David Shapiro, Stefano Marchesini
- former Stony Brook colleagues, students and postdocs: David Sayre, John Miao, Enju Lima, Henry Chapman, Wenbing Yun, Harald Ade, Ian McNulty
- Other Colleagues at ALS and elsewhere
- Adam Hitchcock, McMaster U.
- Dave Attwood & Peter Fischer, CXRO
- DOE/BES; NIH