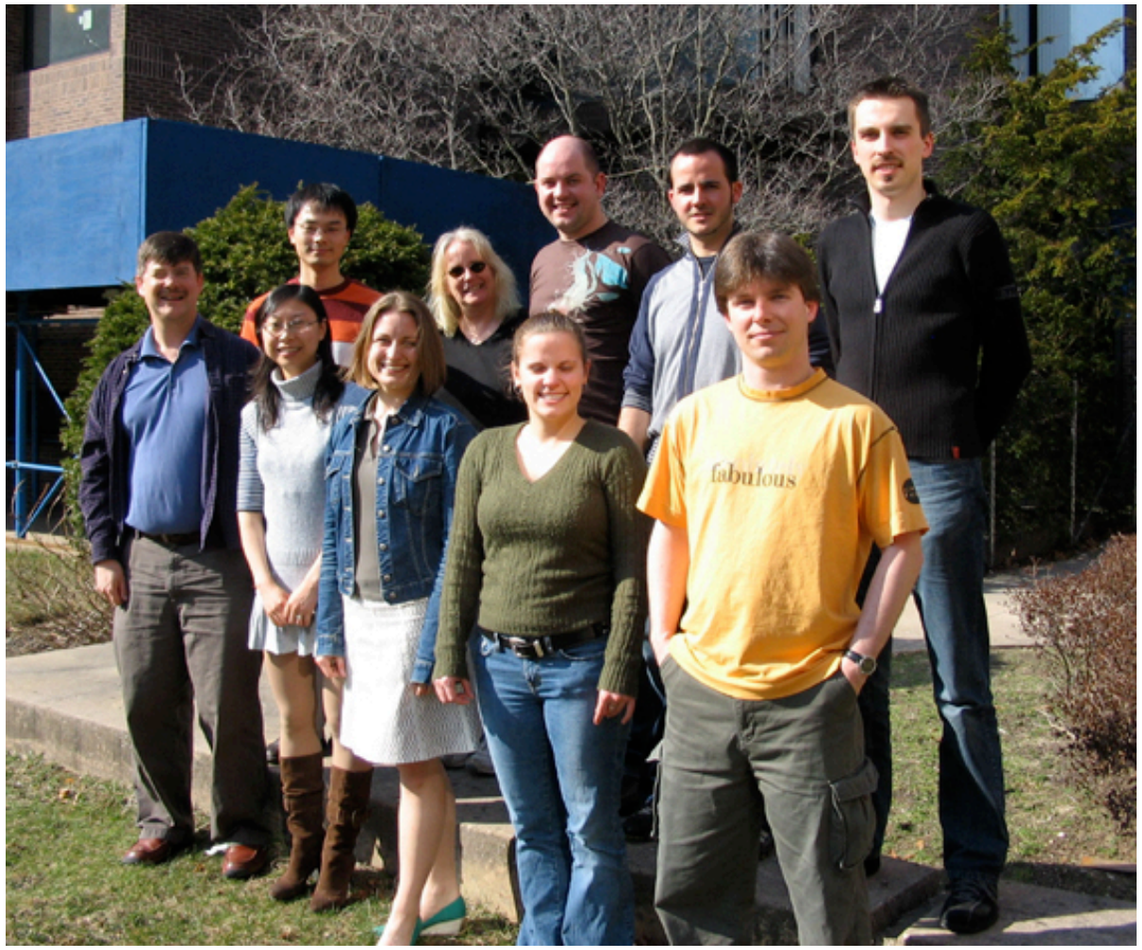
The cold, soft truth: cryo diffraction microscopy

Chris Jacobsen

Dept. Physics & Astronomy

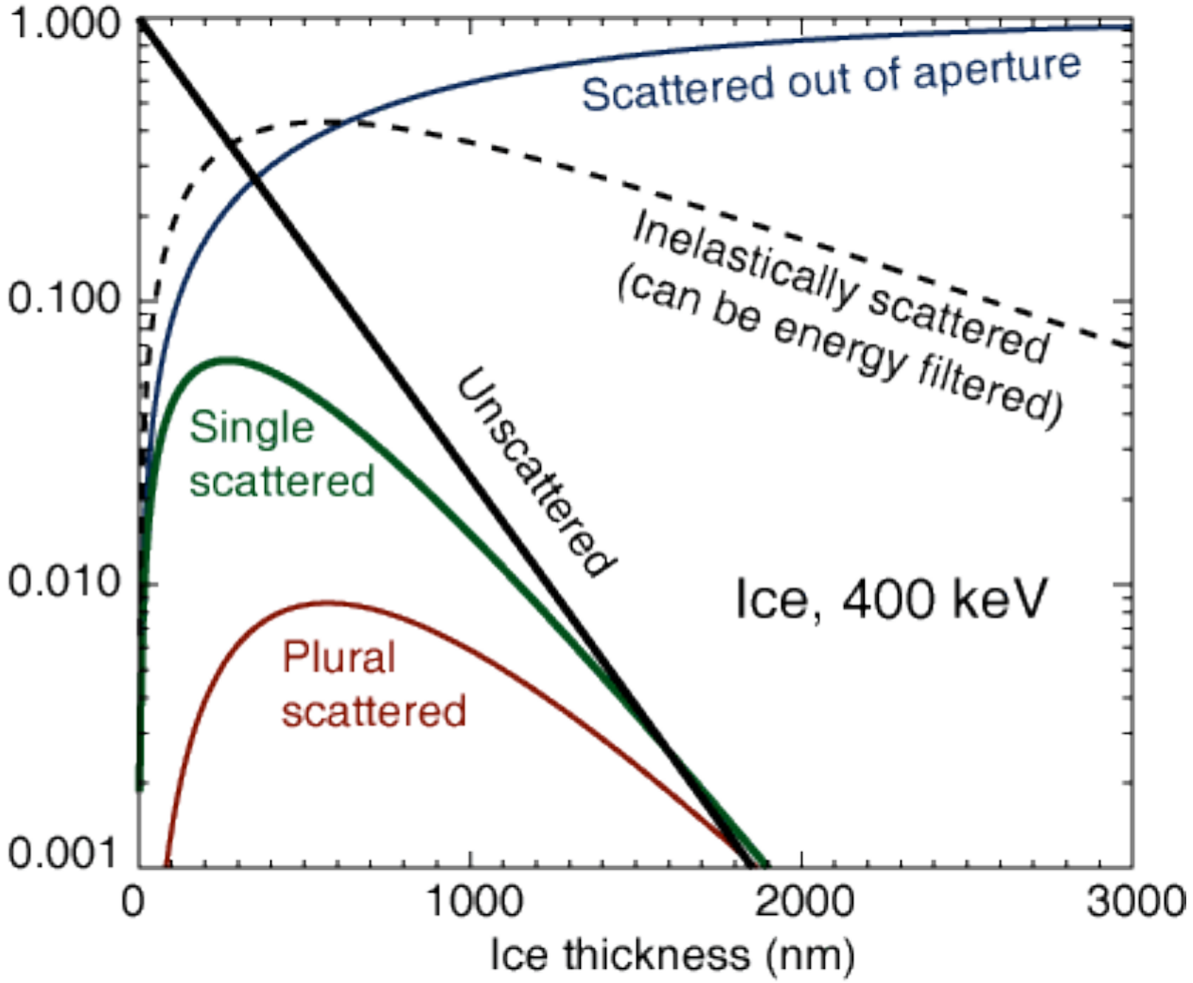
Stony Brook University

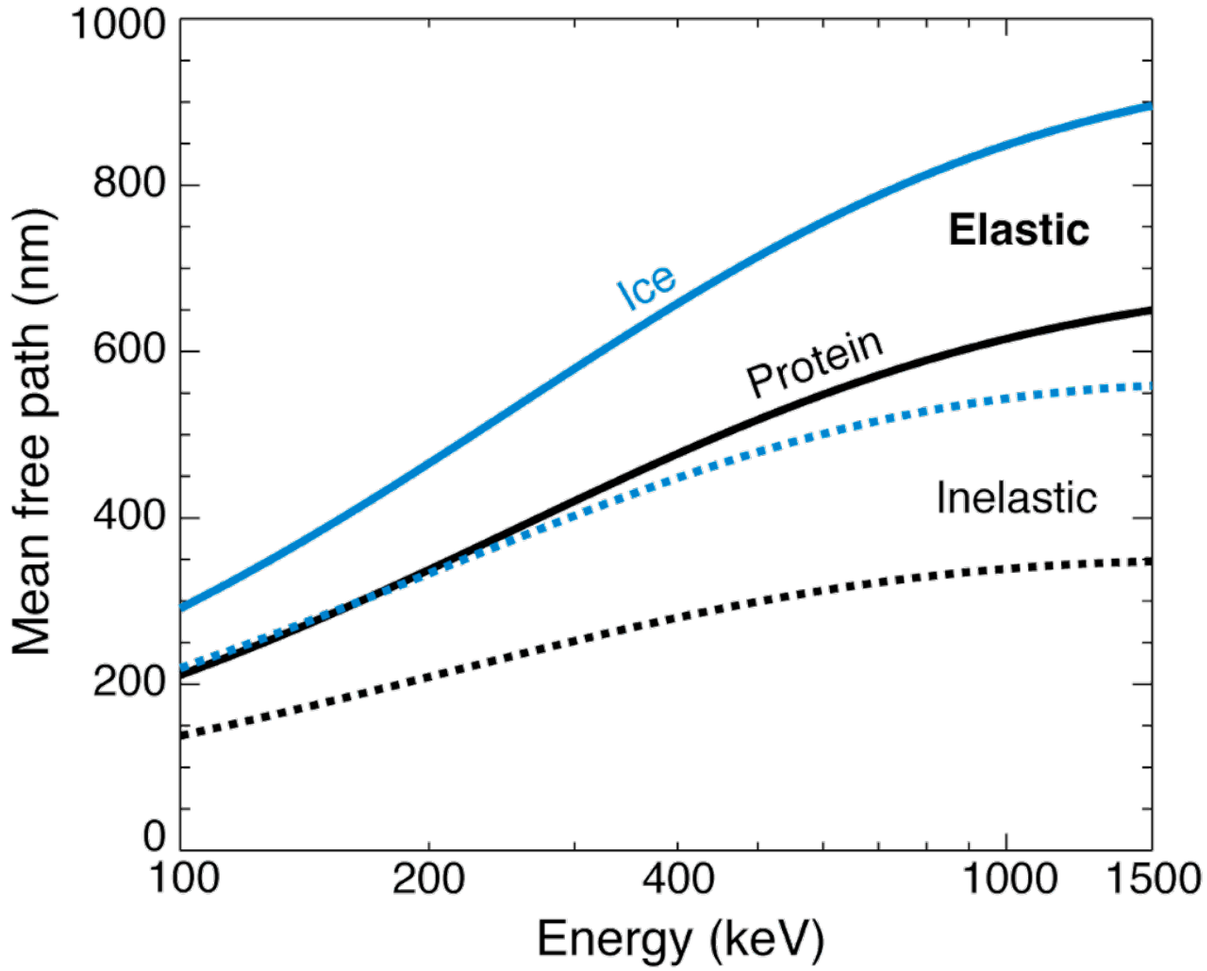


Cryo x-ray diffraction microscopy: why?

- X rays: best probe for samples thicker than ~1 μm.
- Diffraction microscopy (aka CXDI):
  - Get the most out of the exposure to the sample.
  - Freedom from depth of focus limits.
- Cryo: essential for soft and/or wet specimens.

Electron interactions

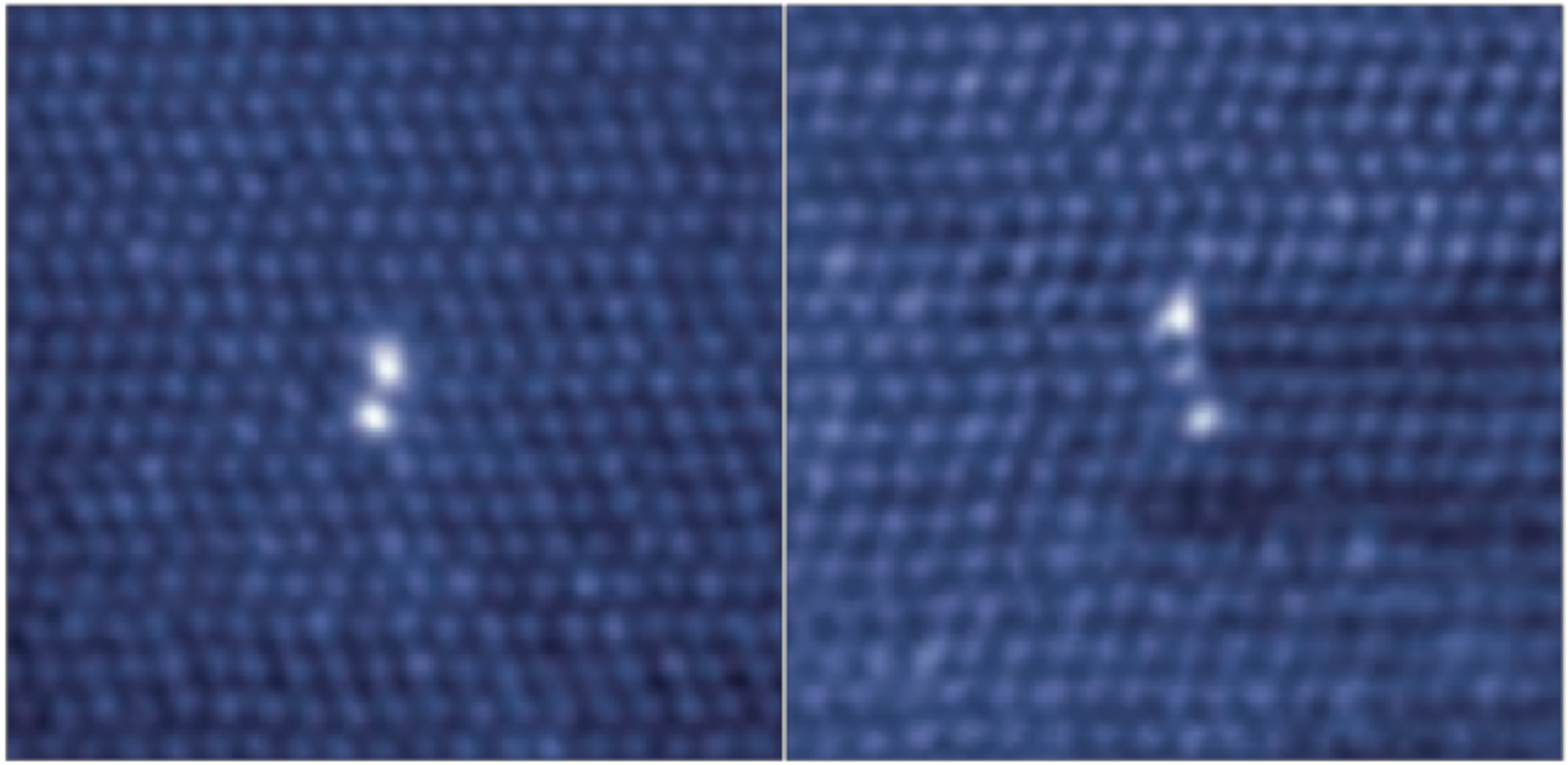




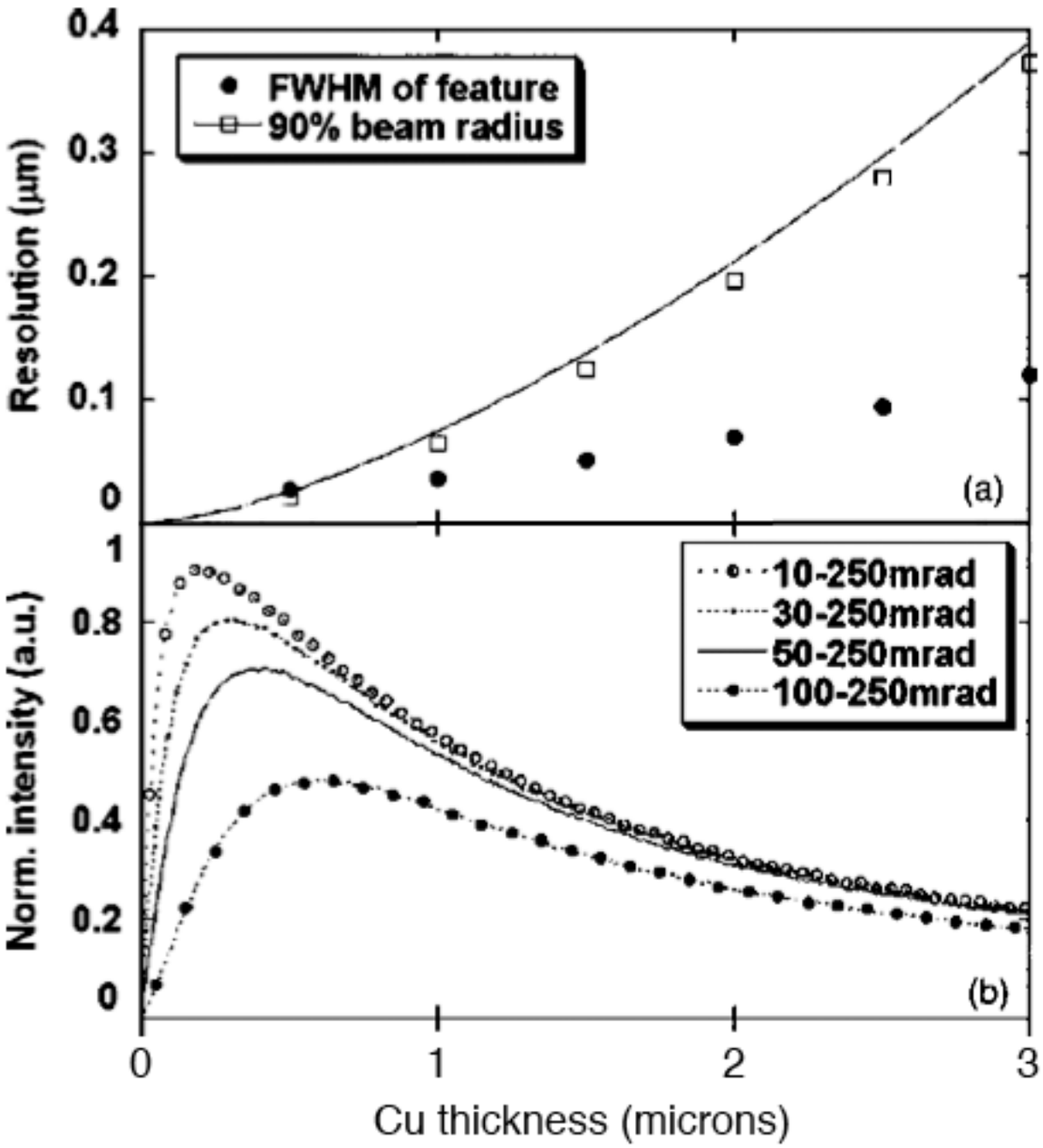
These plots: Jacobsen, Medenwaldt, and Williams, in **X-ray Microscopy & Spectromicroscopy** (Springer, 1998)

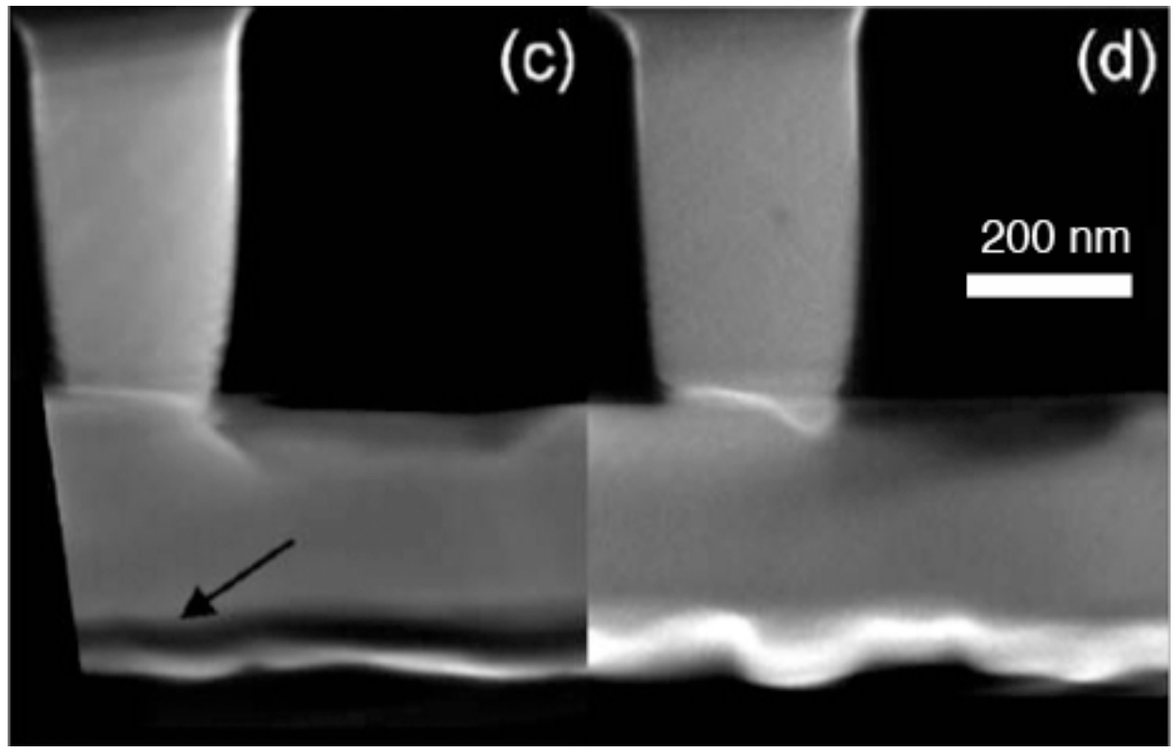
TEM of dopant atoms

Erbium atom columns (10-15 atoms each) in 8-20 nm thick silicon (001; lattice spacing 0.252 nm). Kaiser *et al.*, *Nature Materials* **1**, 102 (2002).



If dose doesn't matter, electrons can go pretty thick!

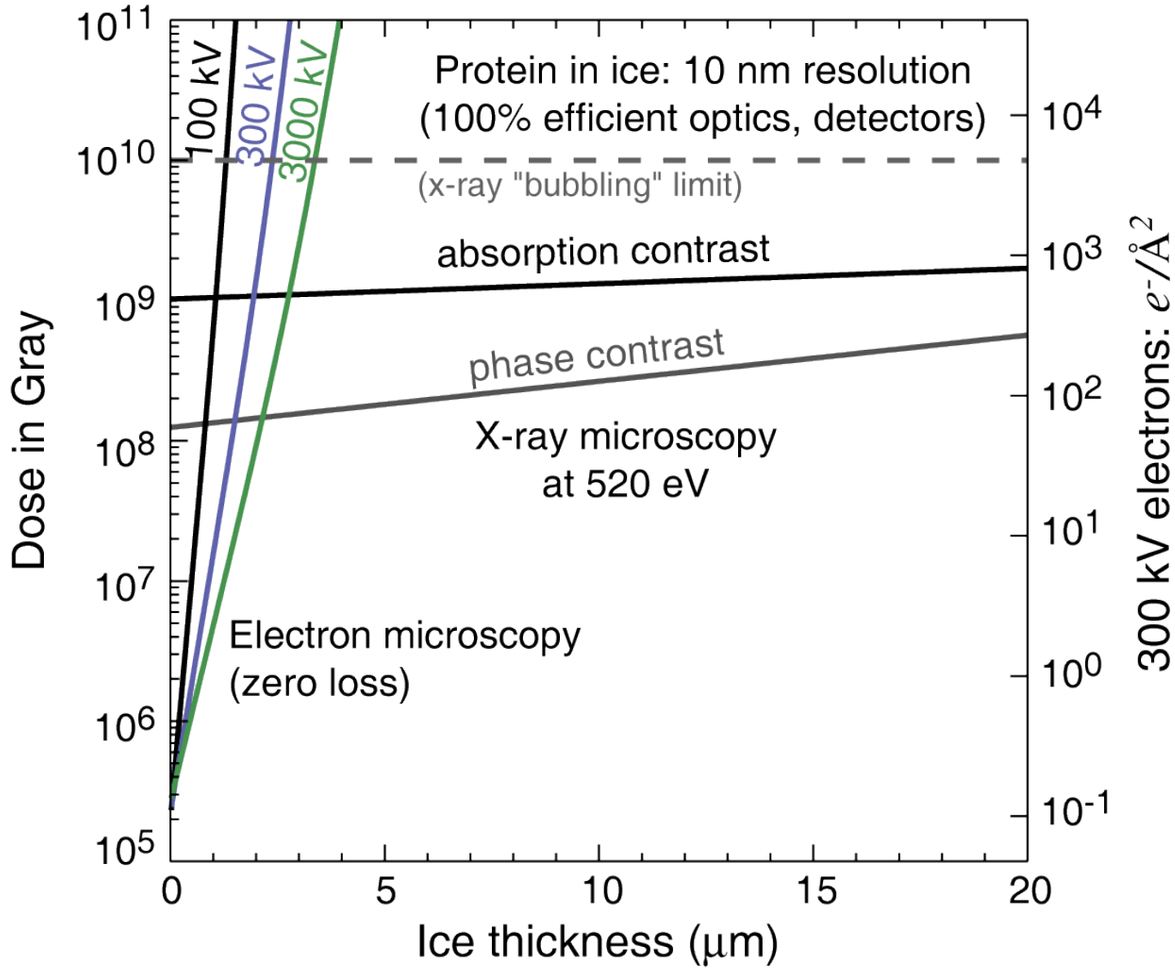


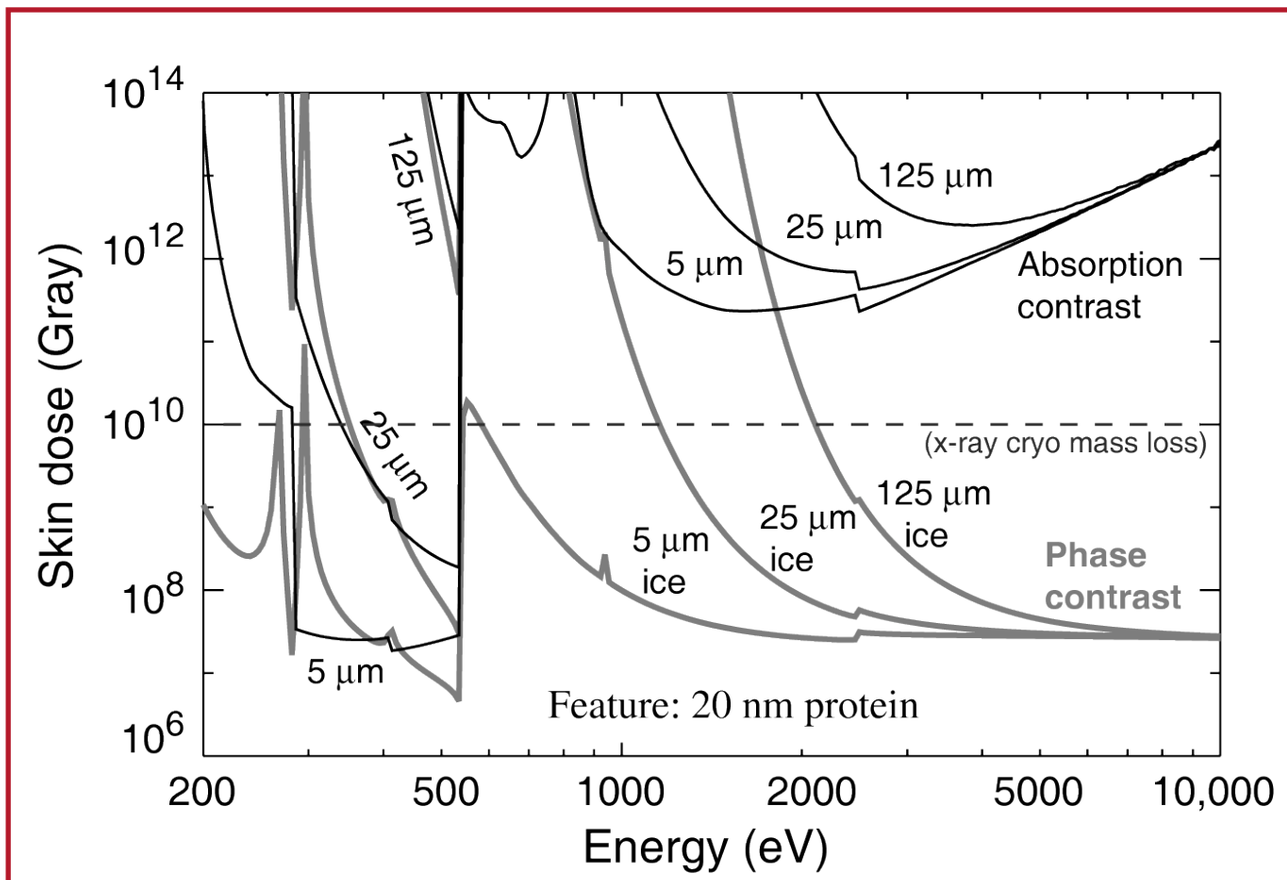


Voids in Cu interconnects: slices from a tomographic reconstruction.
Ercius *et al.*, *Appl. Phys. Lett.* **88**, 243116 (2006)

X rays and thick specimens

X-rays: better for thicker specimens. Sayre *et al.*, *Science* **196**, 1339 (1977); Schmahl & Rudolph in **X-ray Microscopy: Instrumentation and Biological Applications** (Springer, 1987)





Phase contrast is important!

These plots: based on Jacobsen, Medenwaldt, and Williams, in **X-ray Microscopy & Spectromicroscopy** (Springer, 1998)

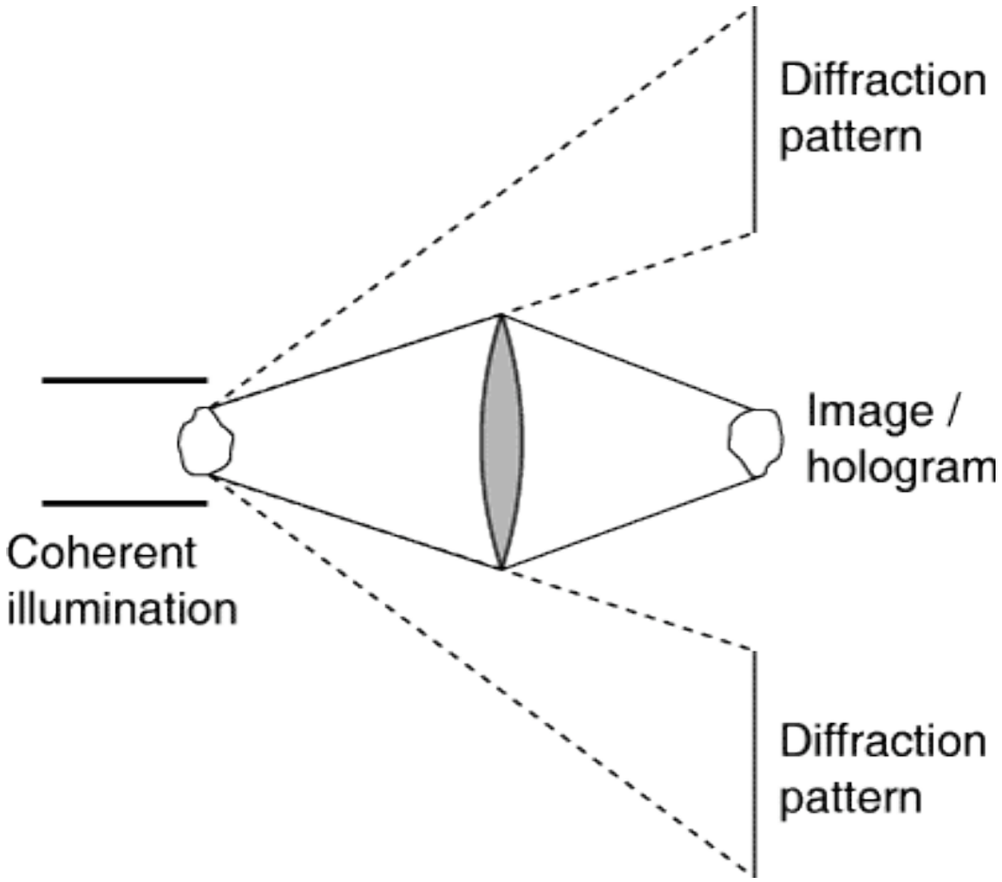
Cryo x-ray diffraction microscopy: why?

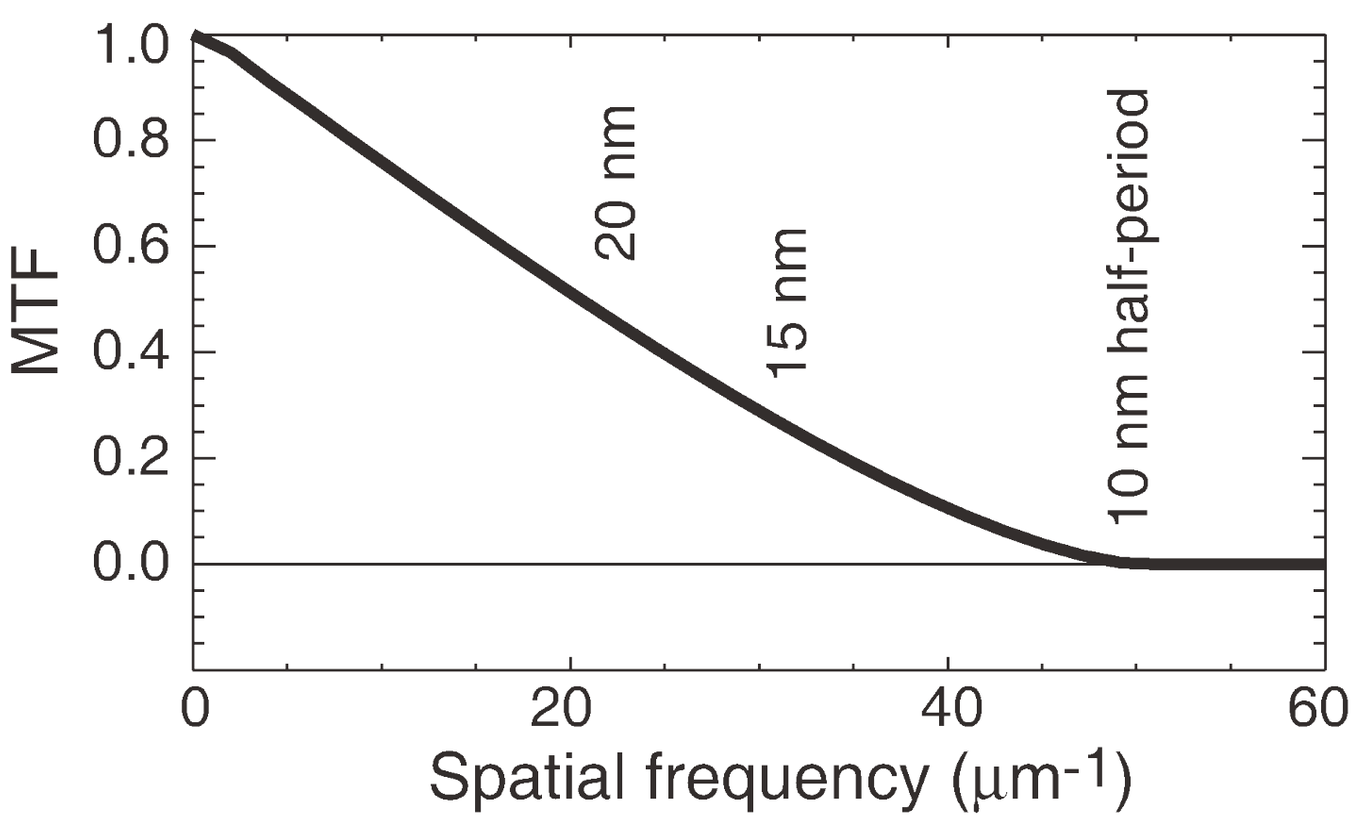
- X rays: best probe for samples thicker than ~1 μm.
- Diffraction microscopy (aka CXDI):
  - Get the most out of the exposure to the sample.
  - Freedom from depth of focus limits.
- Cryo: essential for soft and/or wet specimens.

Radiation damage sets the ultimate resolution limit

- For many specimens, radiation damage sets the ultimate limit on achievable resolution.
- Lenses phase the signal, but lose the signal. Example: 20 nm zone plate with 10% efficiency, 50% window transmission, 20% modulation transfer function (MTF) for 15 nm half-period:

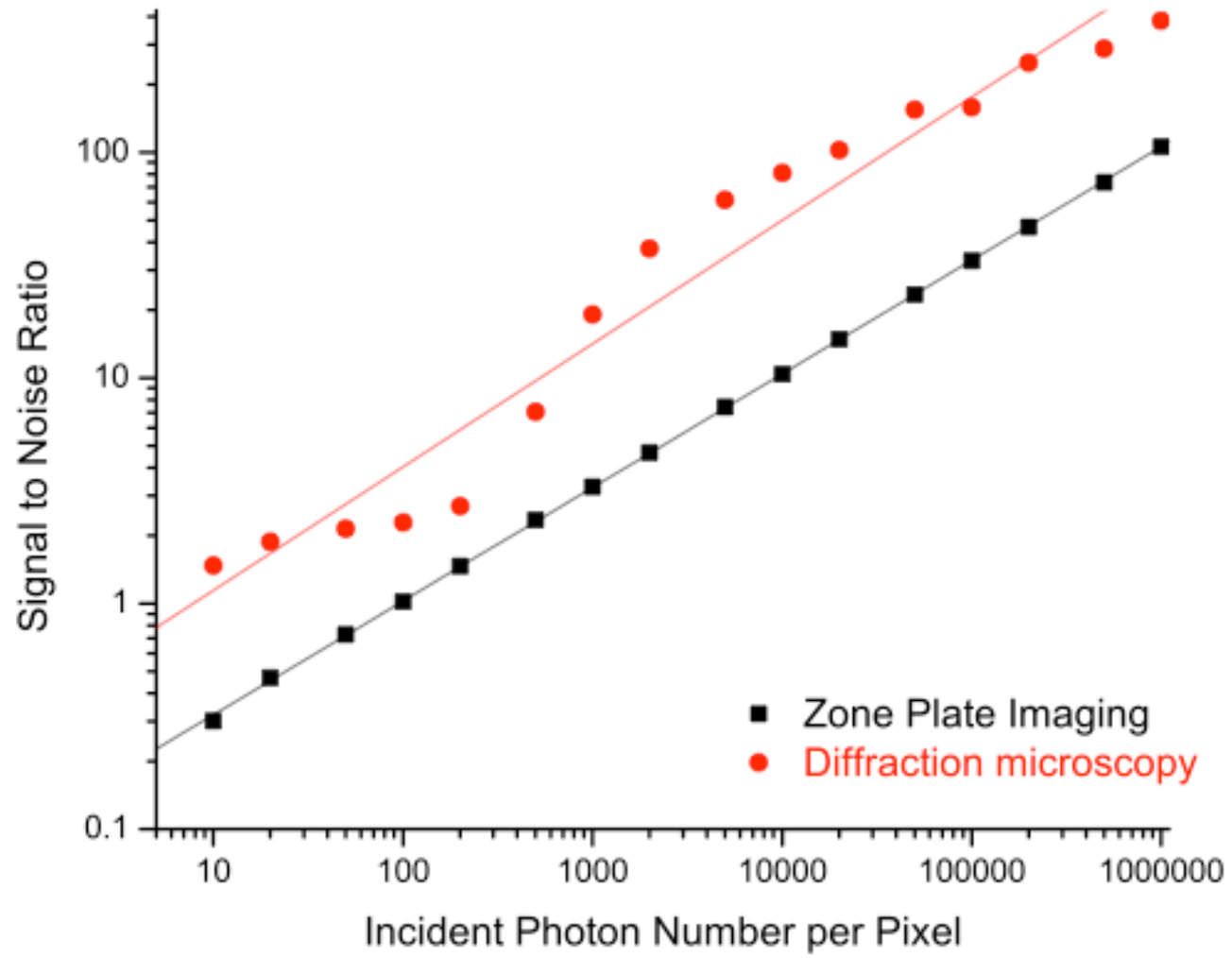
  net transfer of 1% for high spatial frequencies
- Can we avoid this ~100x signal loss, and also go beyond numerical aperture limit of available optics?





Can one recover phase from noisy data? Yes!

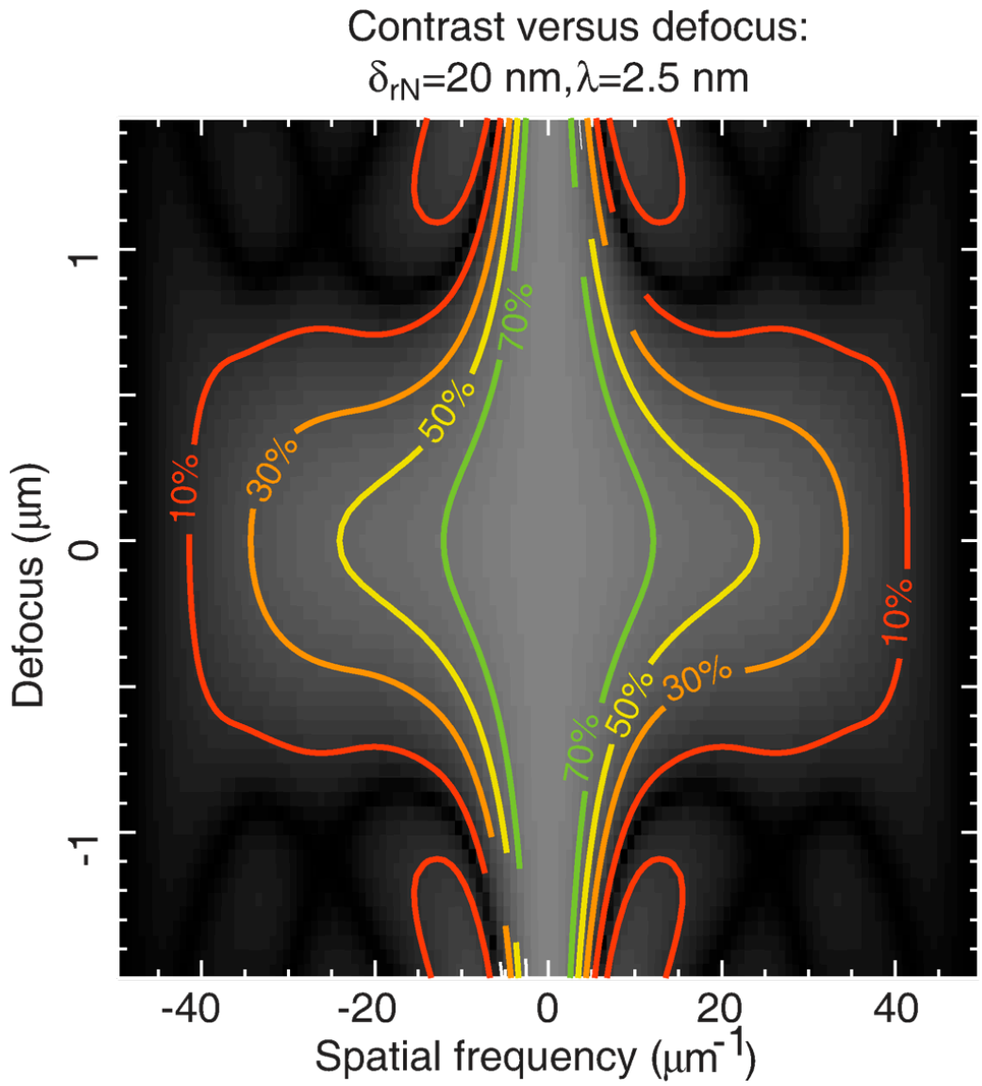
- Simulation: exit wave from thick cell (X. Huang *et al.*)
- Poisson noise on intensities
- Zone plate: 20 nm, 10% efficiency, incoherent bright field
- Diffraction: reconstruction from noisy intensity
- Direct test of low photon count builds upon earlier results by Fienup, *Optics Lett.* **3**, 27 (1978); and Williams *et al.*, *Acta Cryst. A* **63**, 36 (2007).



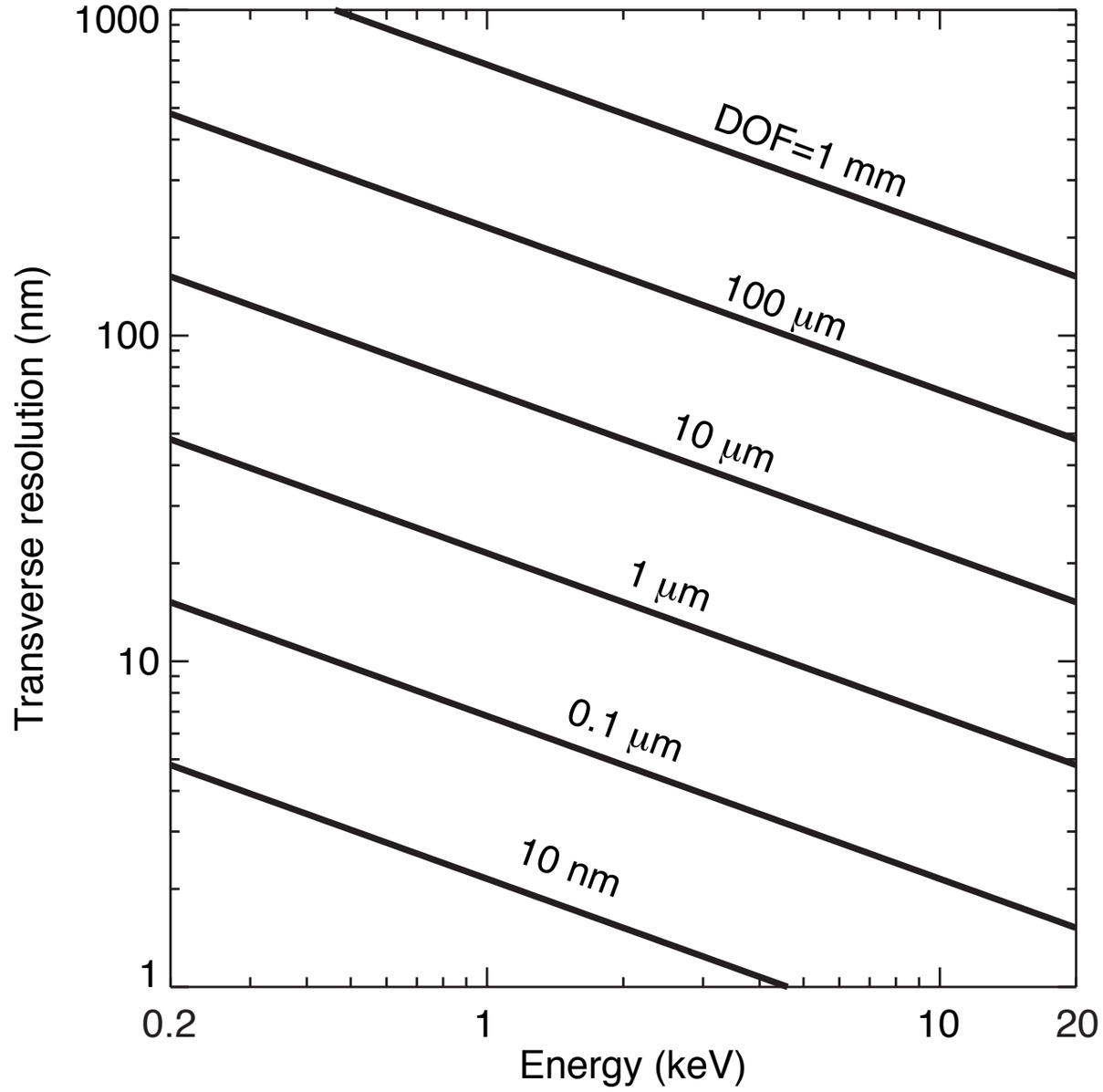
3D imaging with lenses

Transverse: $$\Delta_t \Rightarrow \frac{\lambda}{4\theta} = \frac{\Delta_{rN}}{2}$$

Longitudinal: $$\Delta_\ell \Rightarrow \frac{\lambda}{\theta^2} = 4\Delta_{rN}\,\frac{\Delta_{rN}}{\lambda}$$



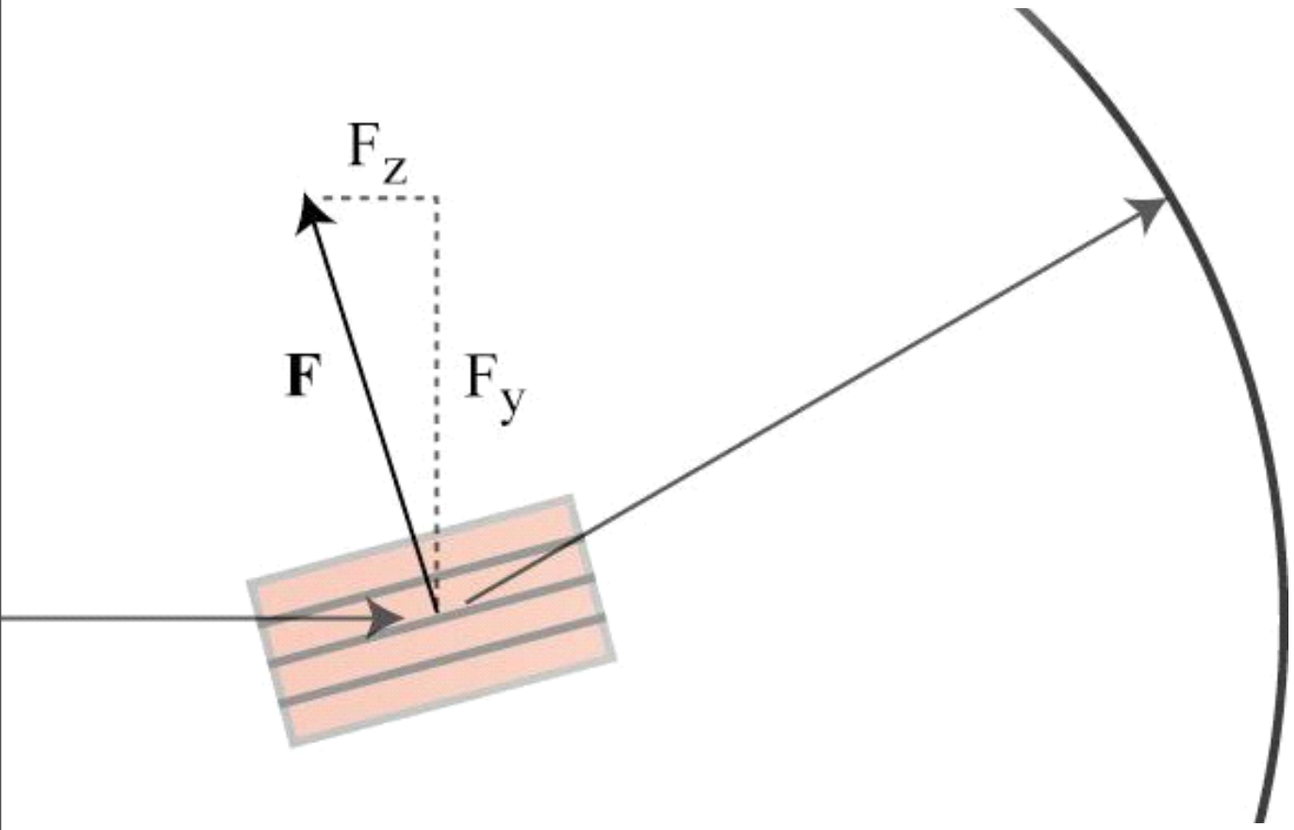
20 nm resolution at 520 eV: depth of field ~1 μm



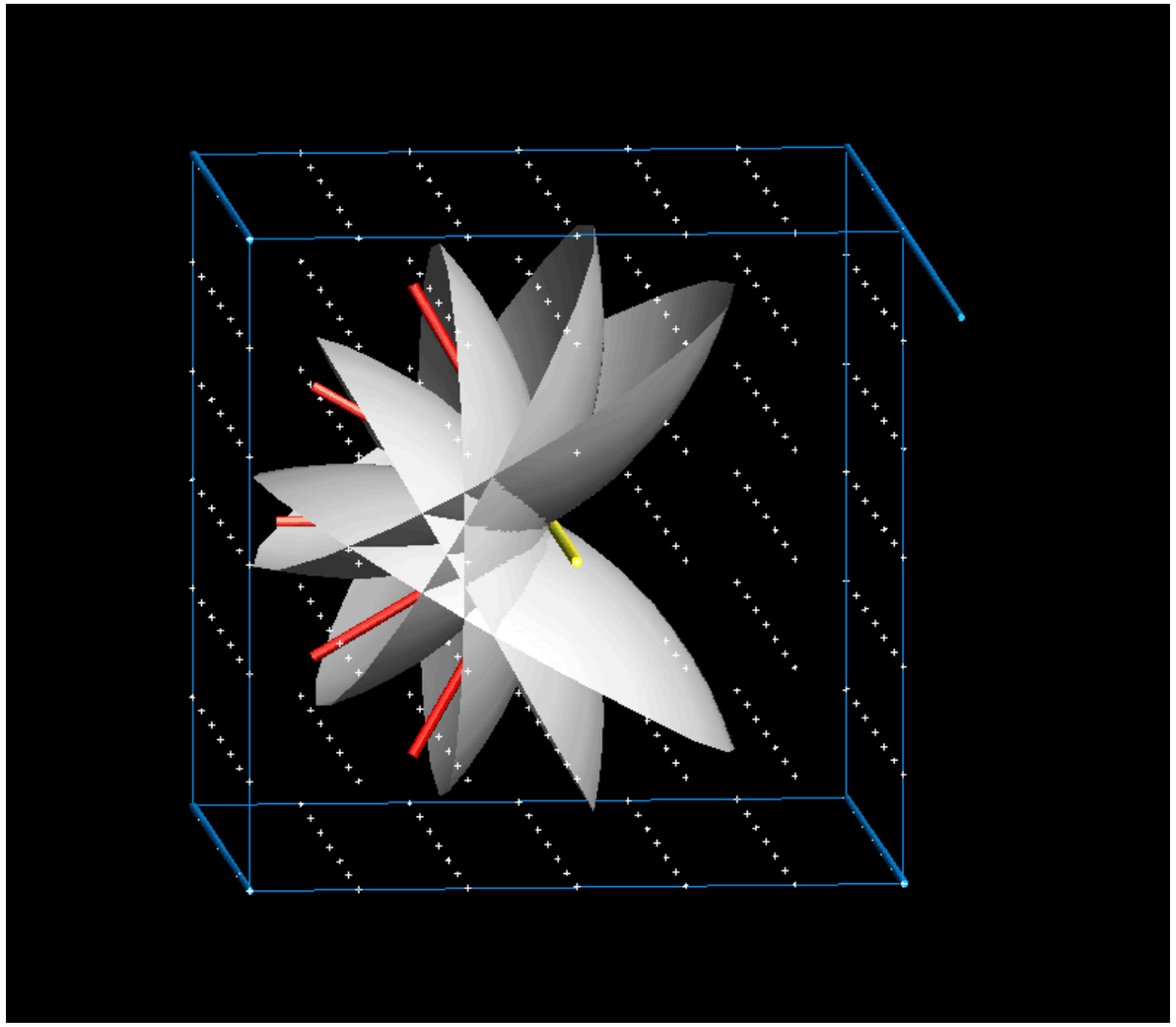
Through-focus deconvolution with lenses:

- Confocal: fully incoherent (fluorescence)
- EM: phase only, coherent
- TXM: partially coherent, equal absorption and phase contrast, need for experimental CTF

Diffraction microscopy in 3D



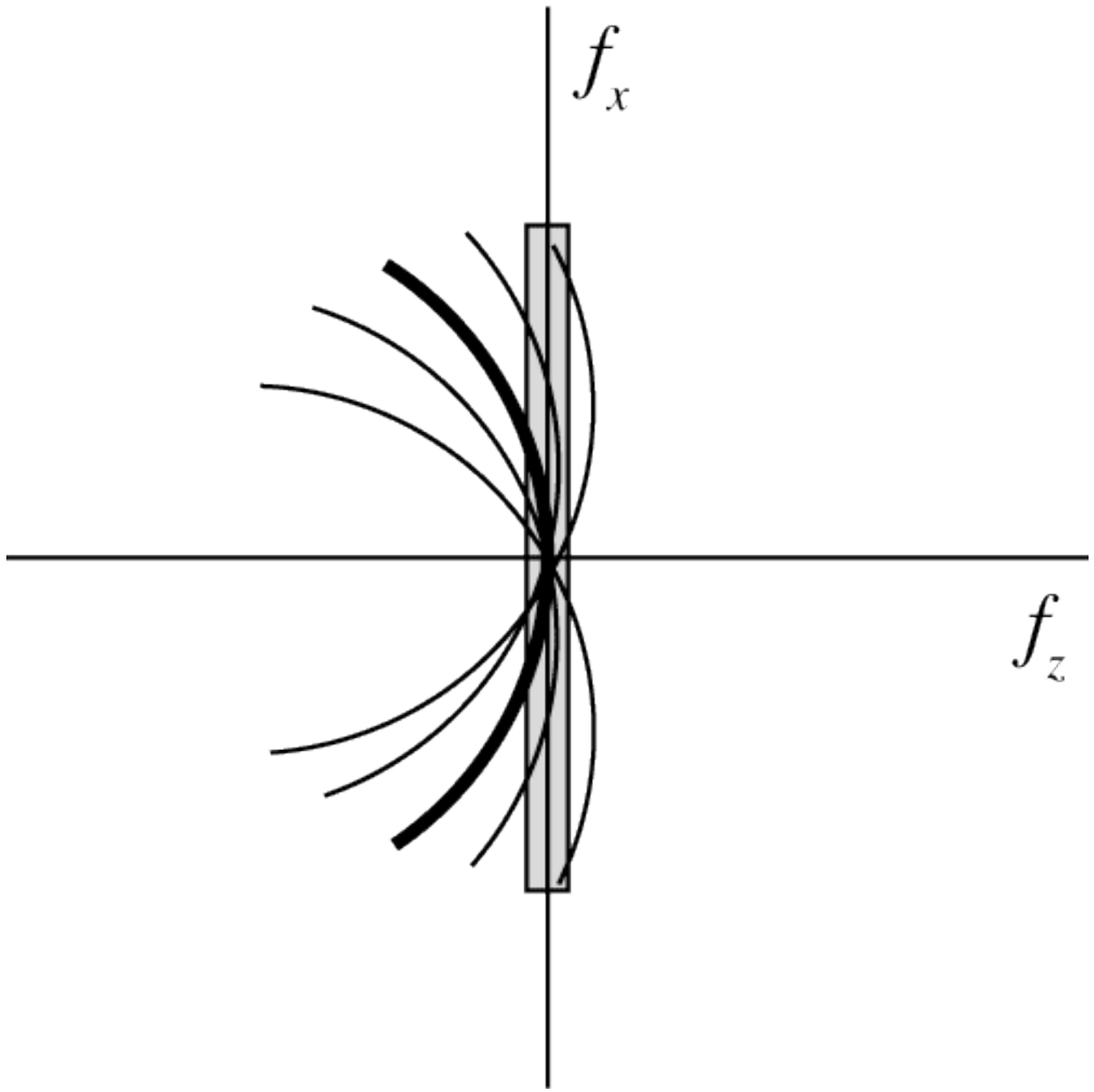
Bragg gratings that diffract to a certain angle represent a specific transverse and longitudinal periodicity (Ewald sphere)



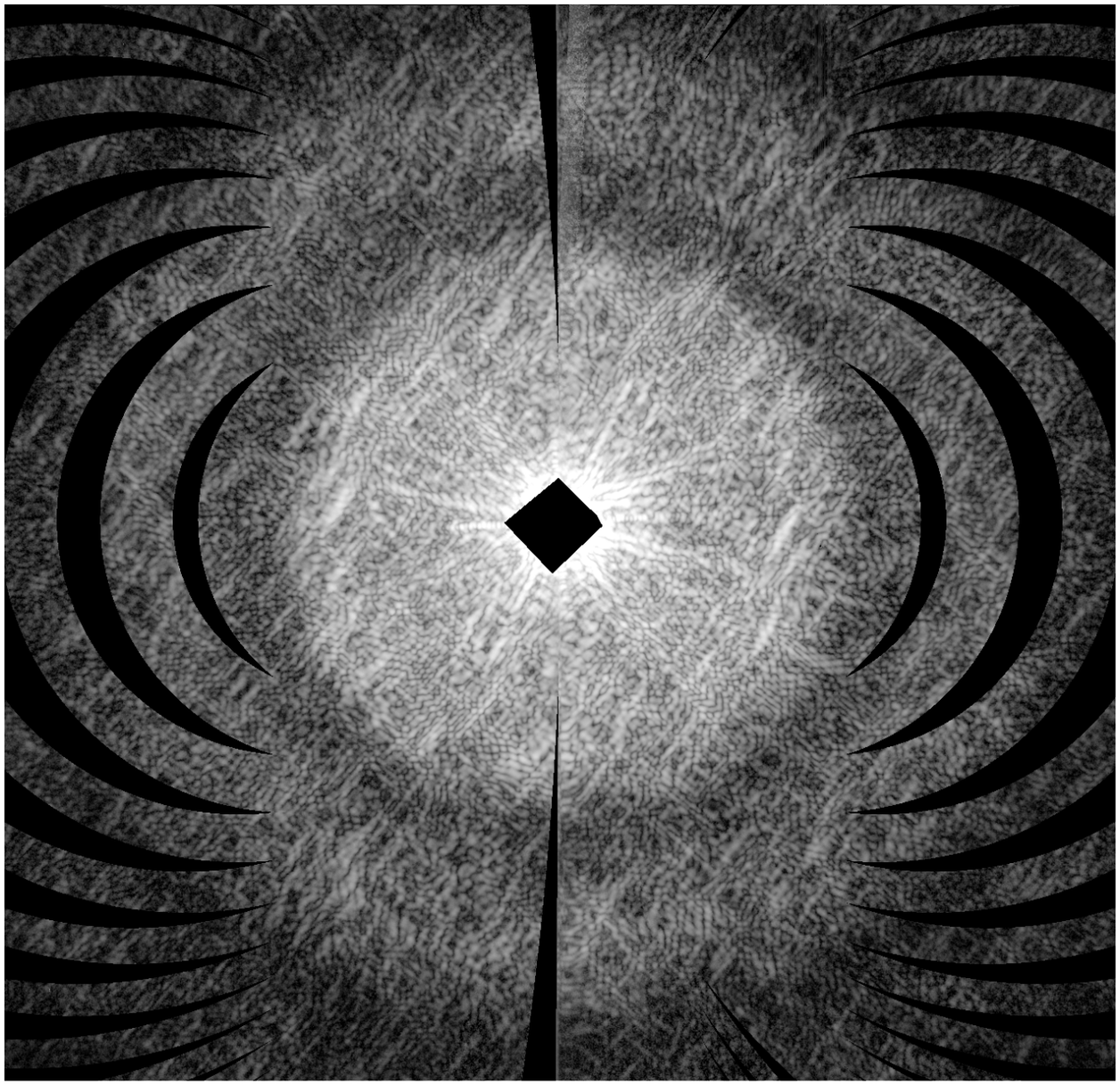
Data collection over a series of rotations about an axis fills in 3D Fourier space for phasing

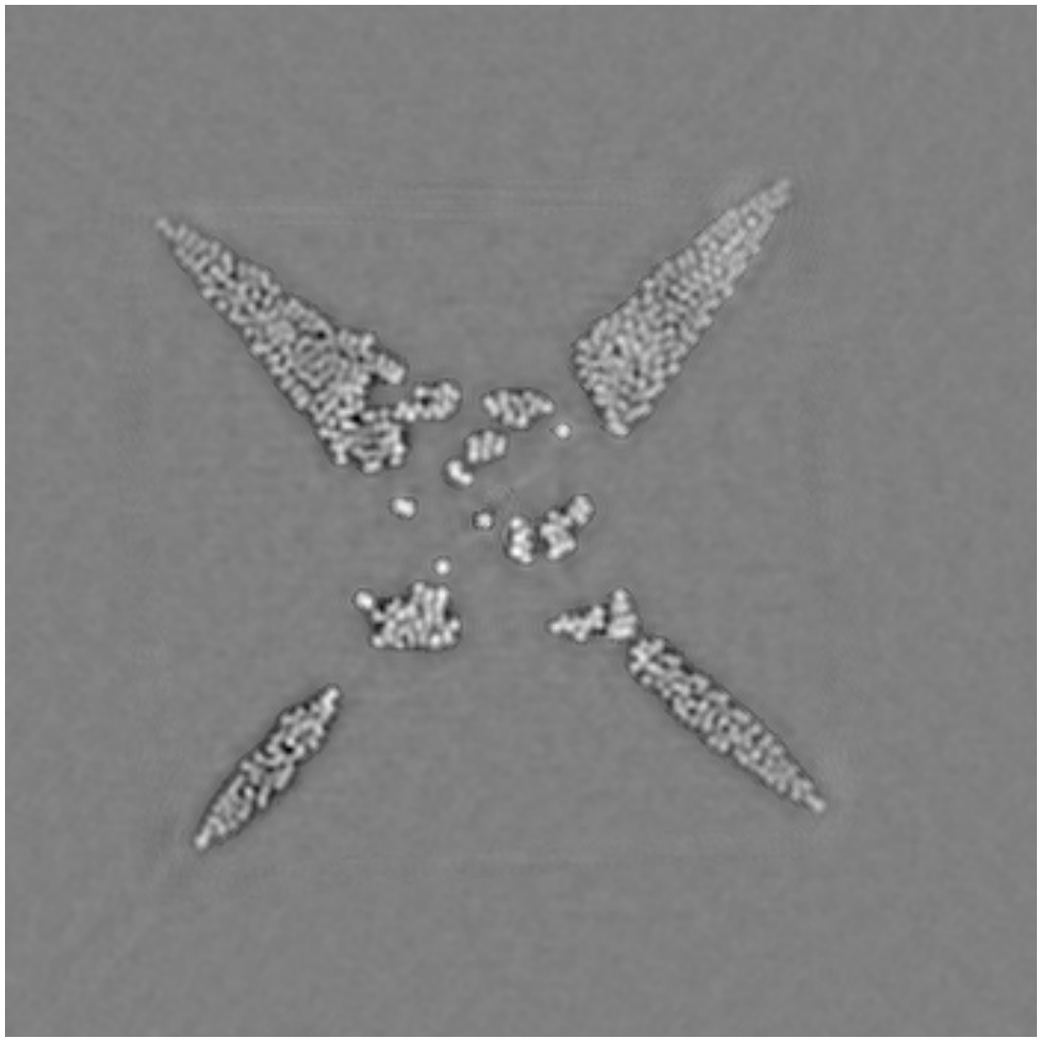
Pure projections from phased 3D data

Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)

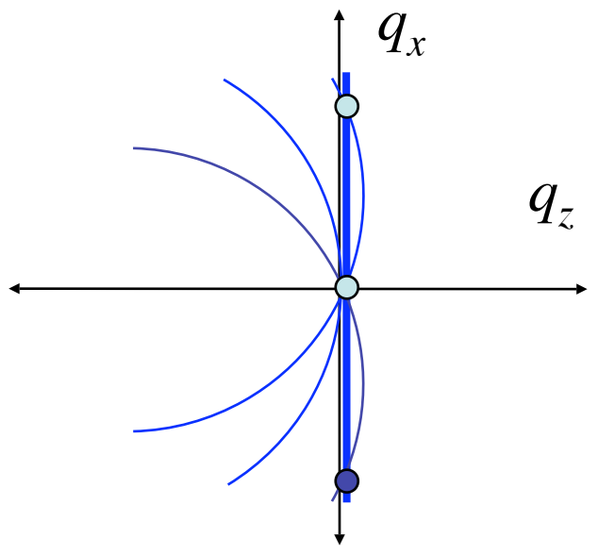


Experimental realization





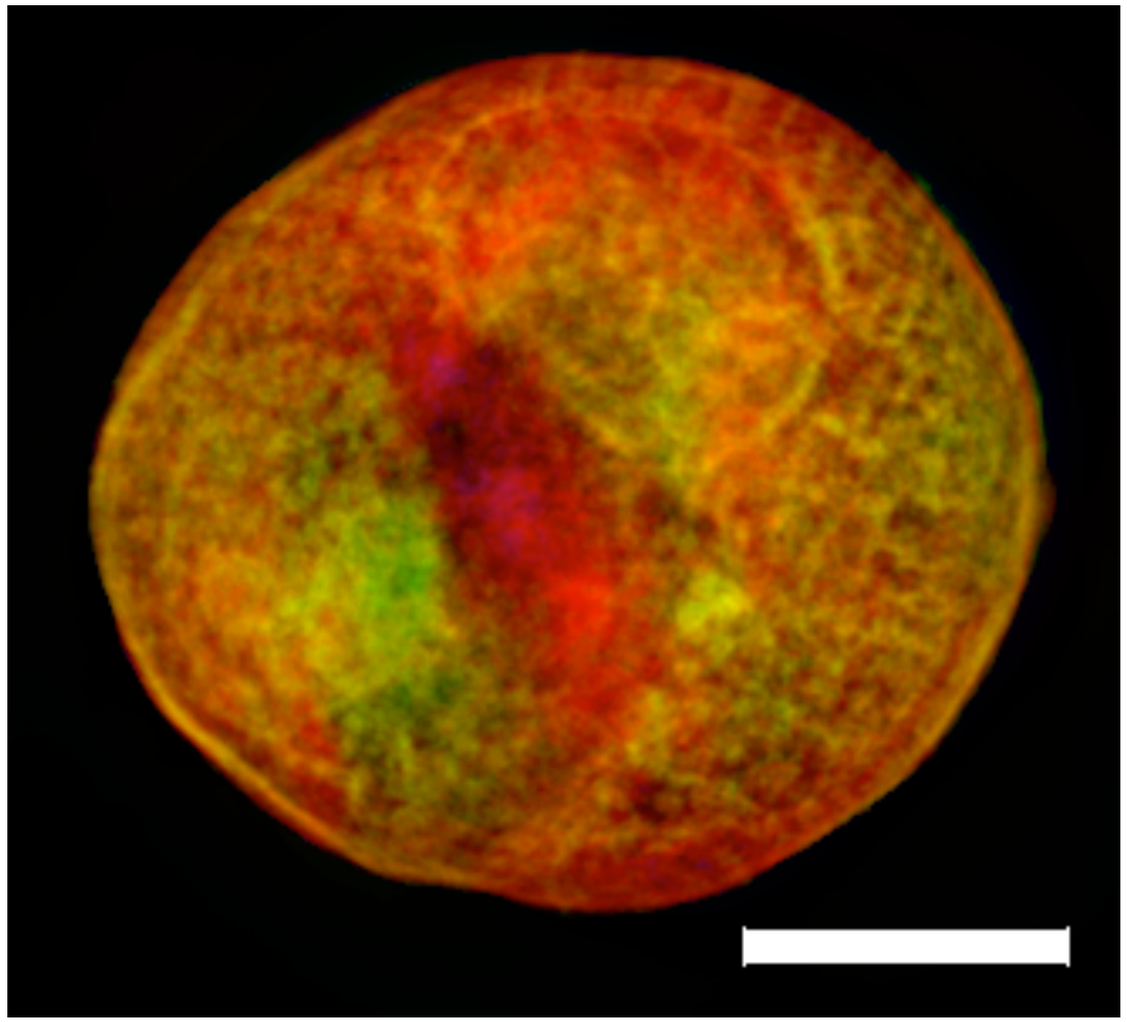
Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)



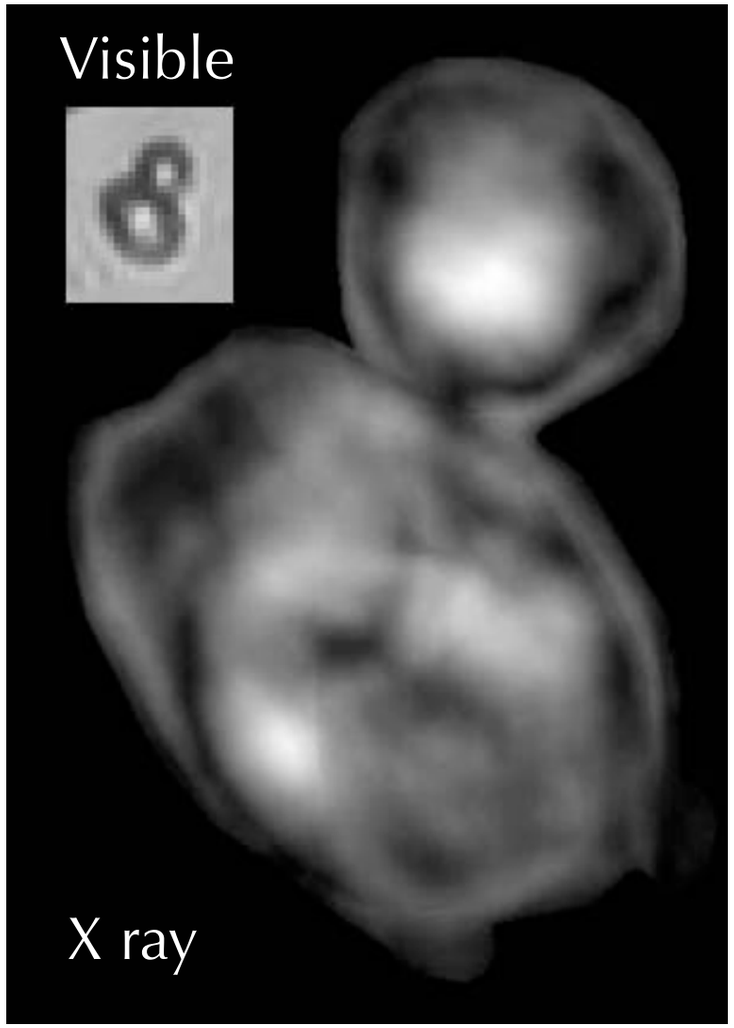
Cryo x-ray diffraction microscopy: why?

- X rays: best probe for samples thicker than ~1 μm.
- Diffraction microscopy (aka CXDI):
  - Get the most out of the exposure to the sample.
  - Freedom from depth of focus limits.
- Cryo: essential for soft and/or wet specimens.

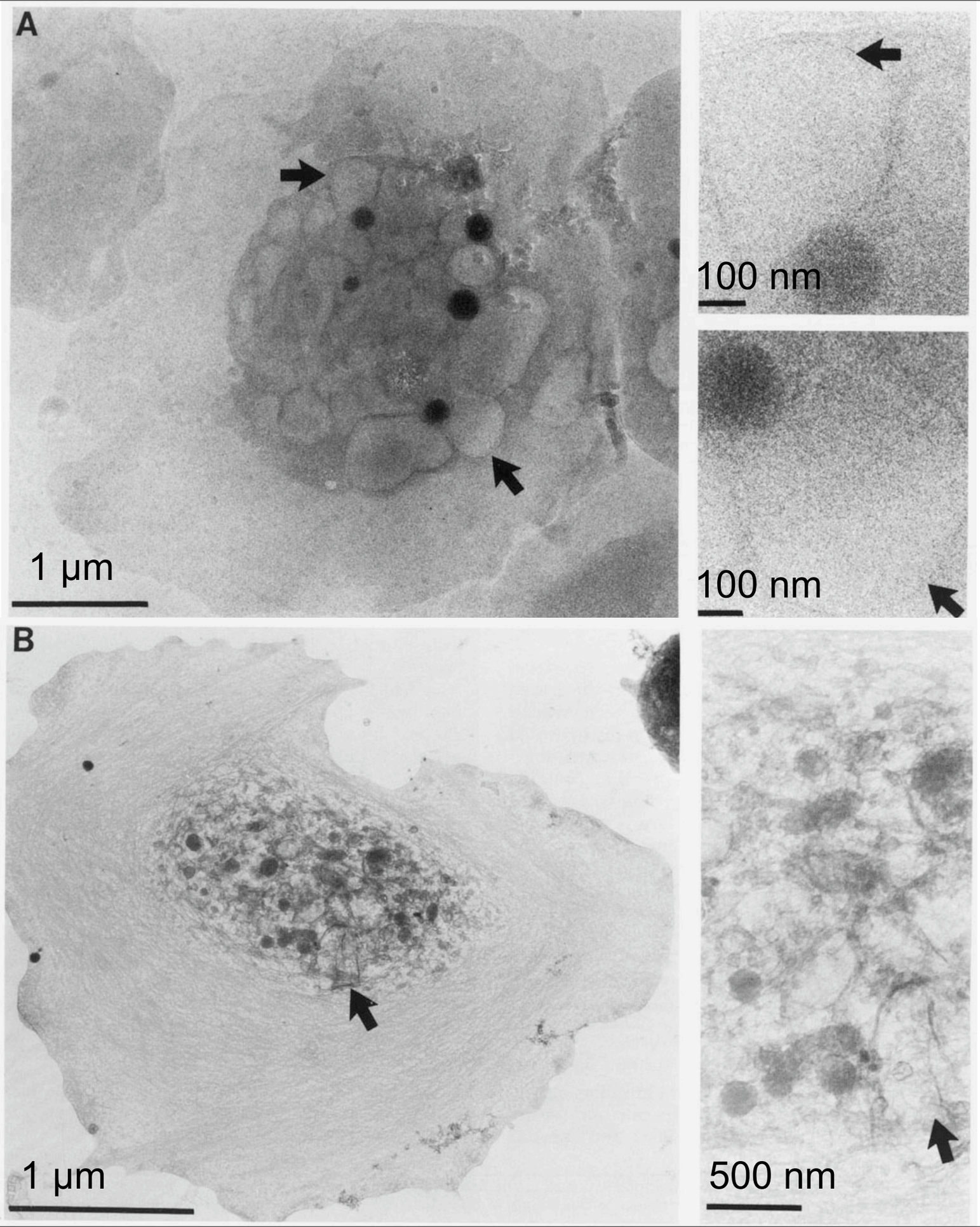
Dried yeast: Stony Brook/ALS



D. Shapiro *et al.*, *Proc. Nat. Acad. Sci.* **102**, 15343 (2005).



J. Nelson, X. Huang, J. Steinbrener *et al.*

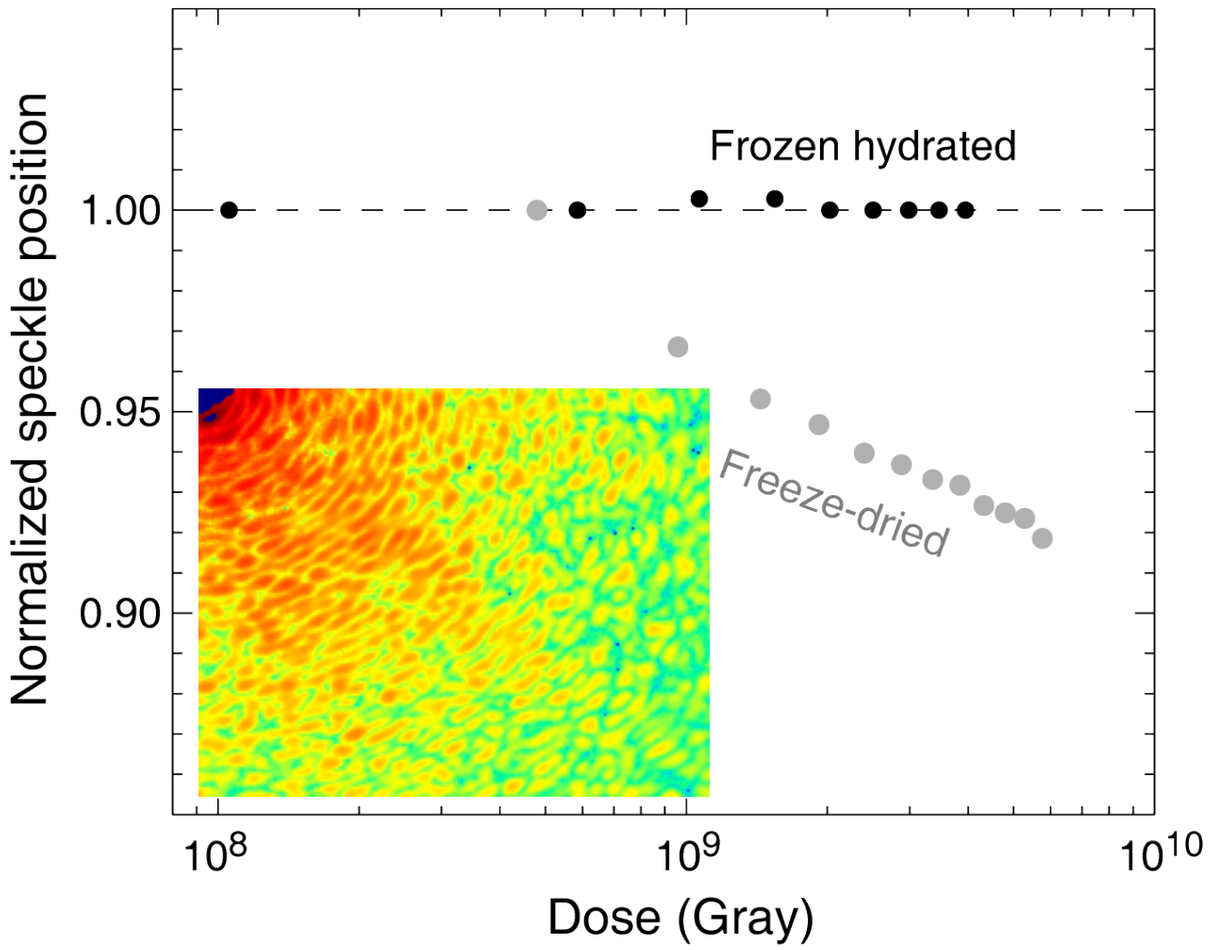


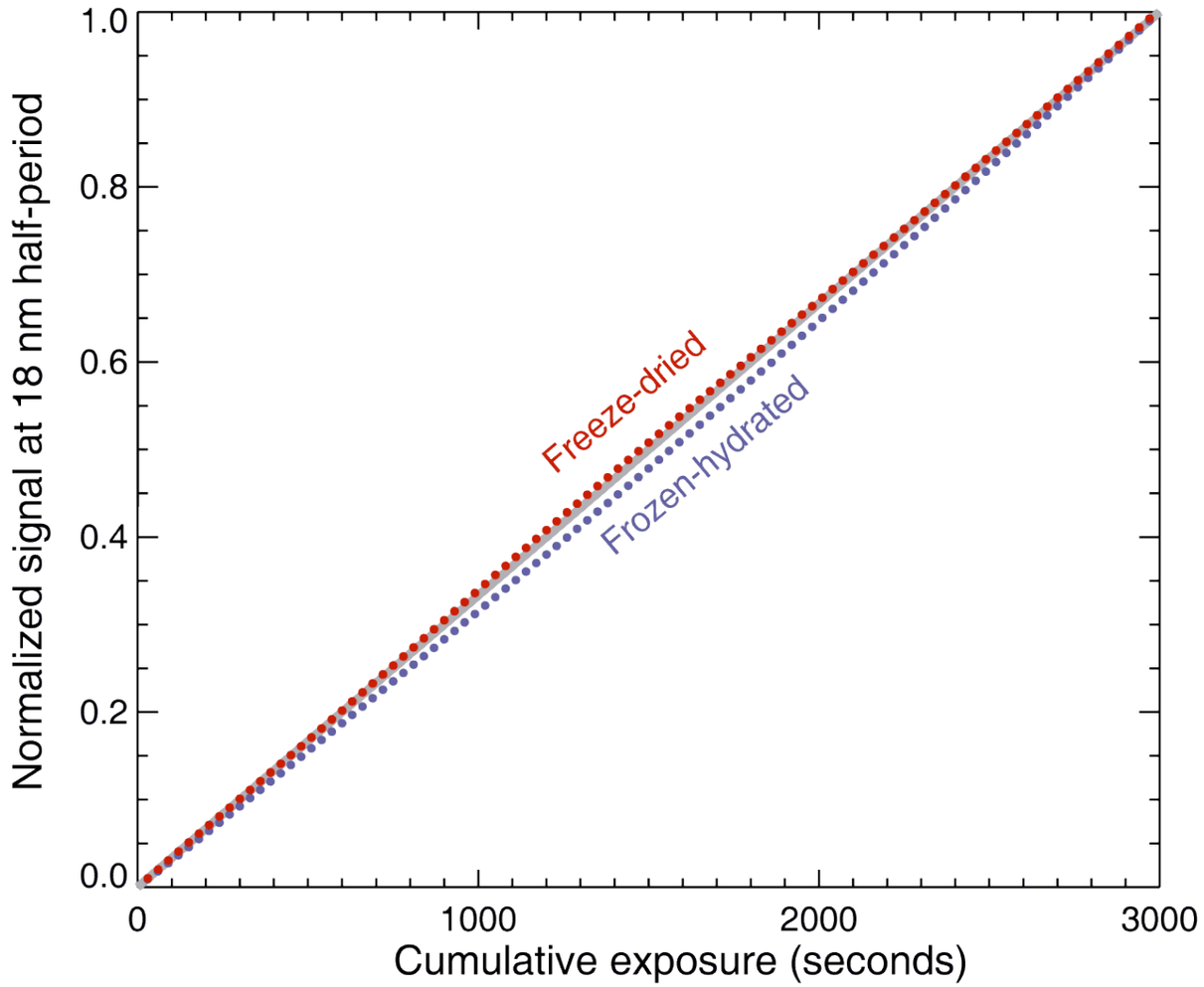
Frozen hydrated

- Human blood platelets
- 1 MeV transmission electron microscope (JEOL-1000)
- O'Toole, Wray, Kremer, and McIntosh, *J. Struct. Bio.* **110**, 55 (1993)

2% glutaraldehyde fix
1% OsO_4 postfix
critical-point dry

Frozen hydrated: stable specimens!





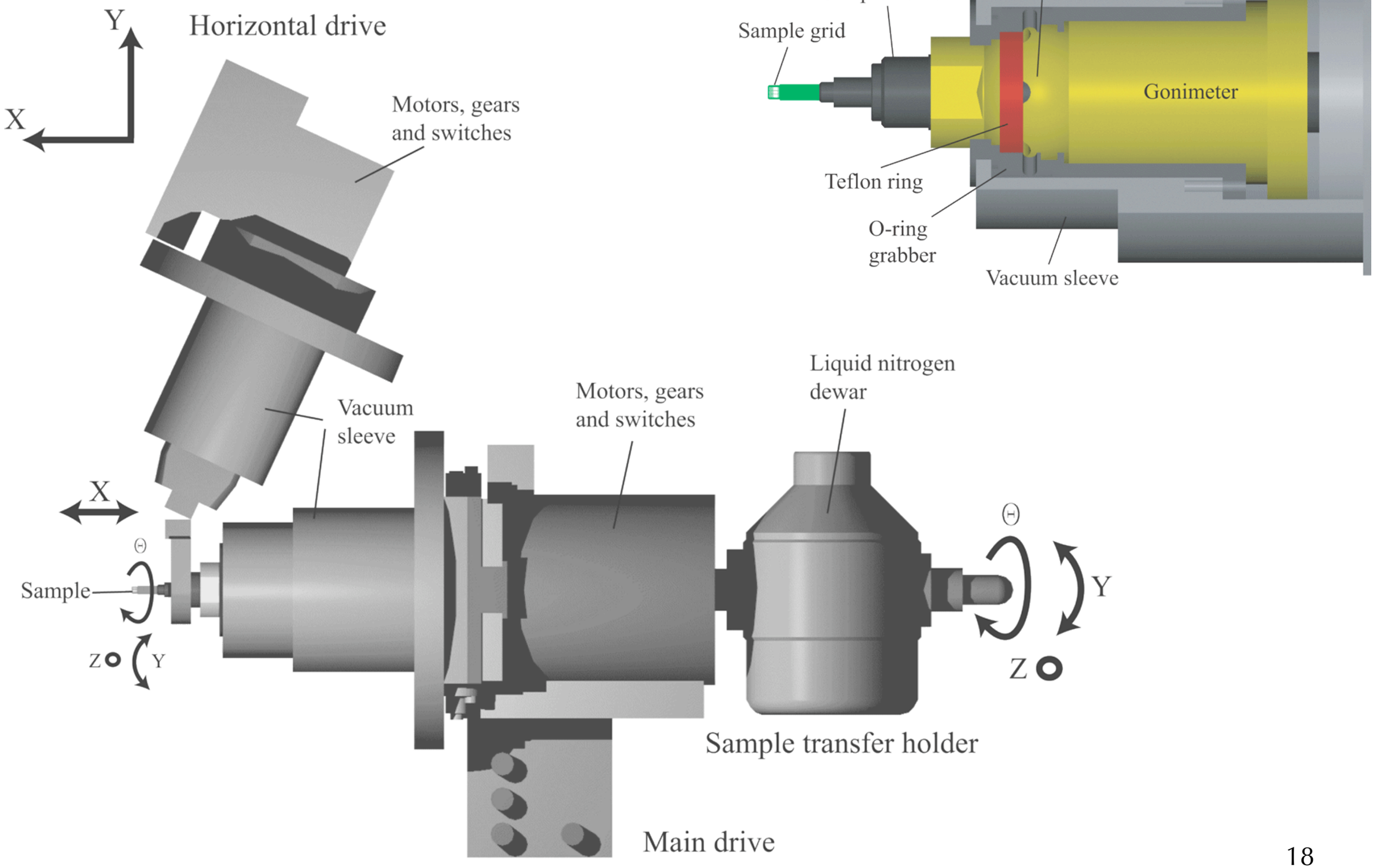
Frozen hydrated specimens don't shrink in the beam (freeze-dried specimens do)

Scattering power is linear with dose thus far in both cases

David Shapiro, PhD dissertation, Stony Brook, 2004

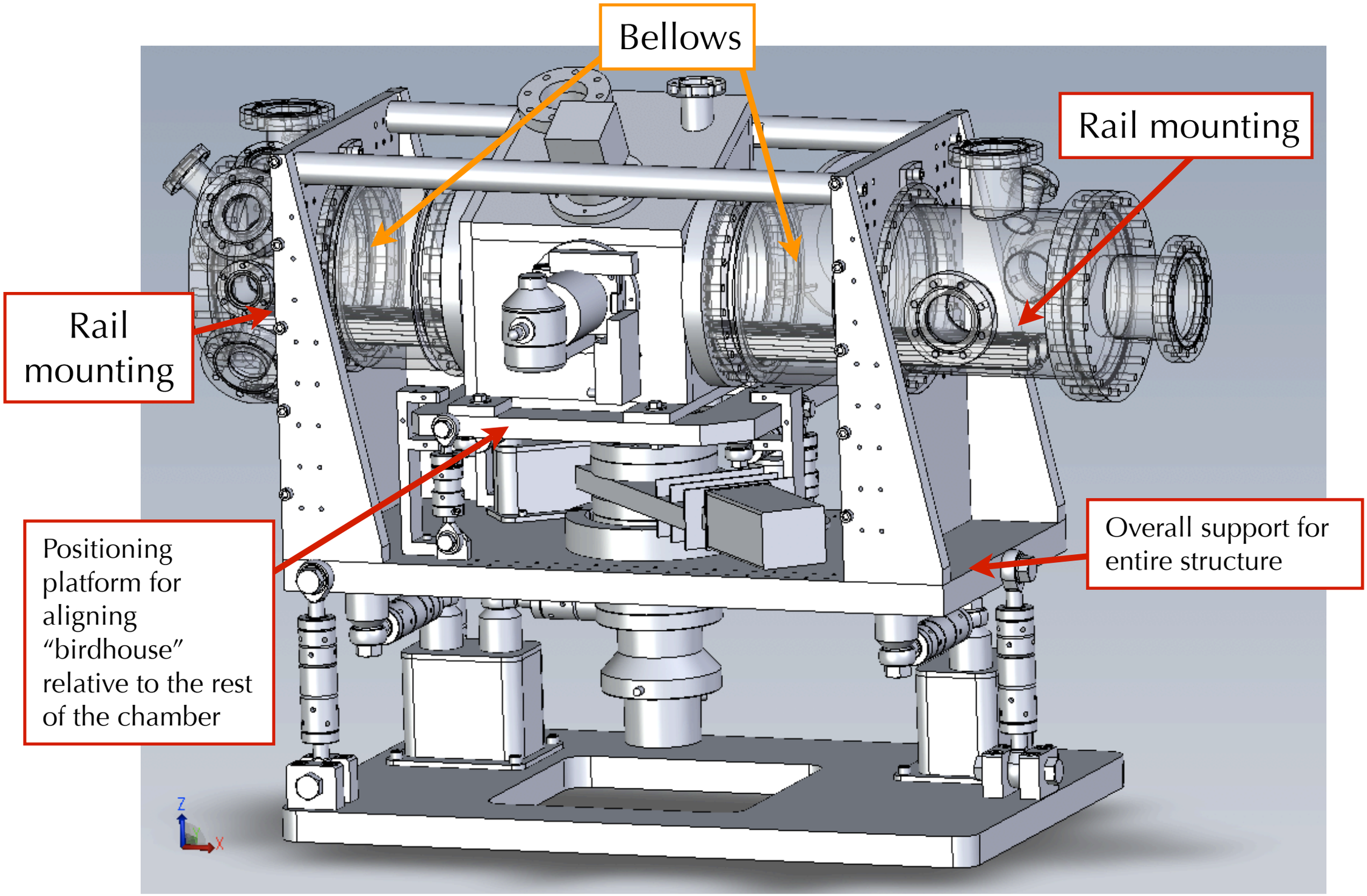
Stony Brook cryo chamber at ALS 9.0.1

Incorporates JEOL goniometer, Gatan cryo holder.
Beetz *et al.*, *Nucl. Inst. Meth. A* **545**, 459 (2005).



Upgrade at ALS 9.0.1

Better stability, higher resolution positioning (curved beam, ptychography), better visible light positioning, better anticontamination… With T. Warwick *et al.*, ALS.

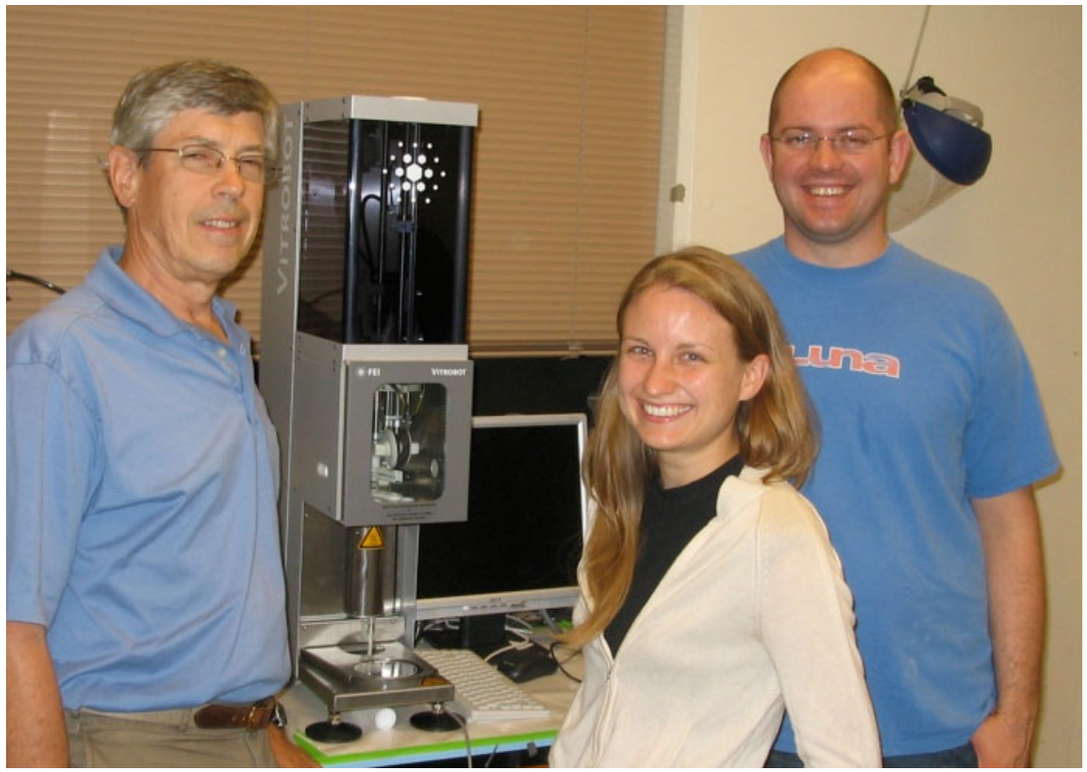


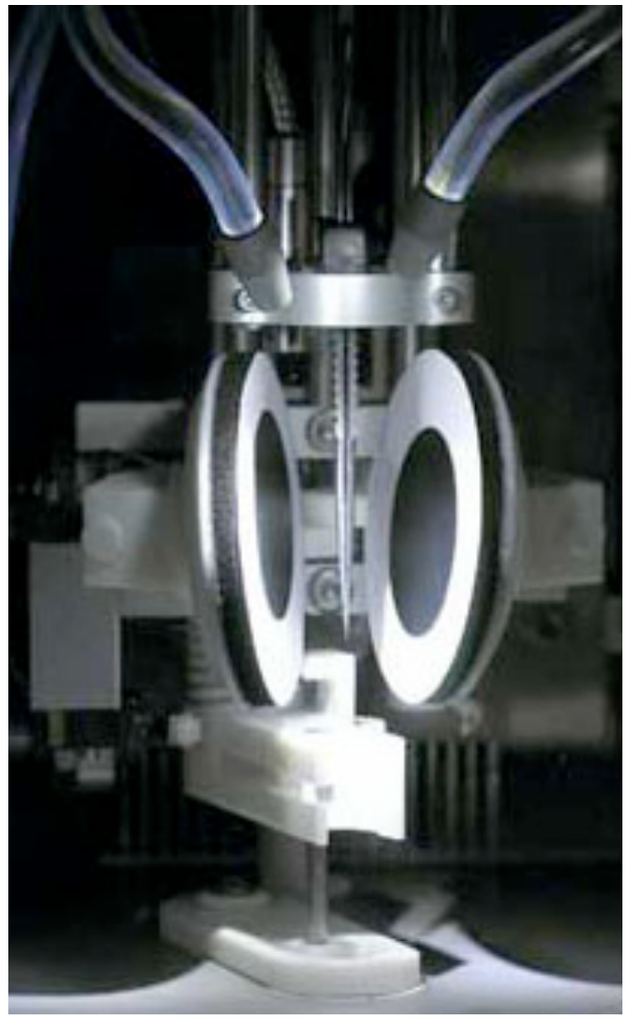
Cryo specimen preparation

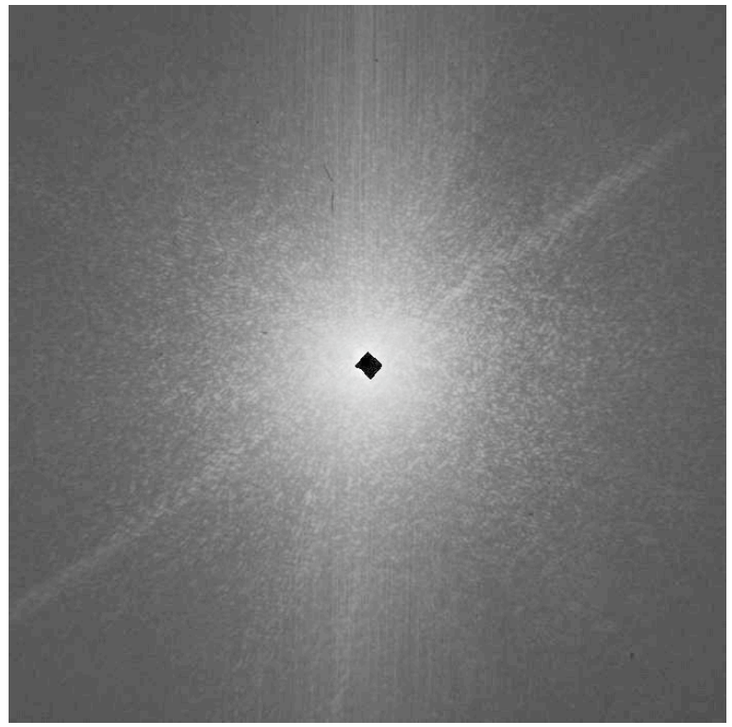
- Cryo prep lab should include cryo plunger, high pressure freezer, cryo ultramicrotome, and LN_2 storage vessels.
- One approach: mount delicate sample in a cartridge/ crystal pin mount once, and move cartridge from technique to technique.
- Evaluation of specimen quality: cryo light microscopy (gives new science opportunities!), lab x-ray source for checking for ice crystallization diffraction rings.
- Specimen preselection: indexing between cryo light microscope, x-ray diffraction microscopy, and x-ray/IR microscopes and nanoprobes.

Sample freezing

Plunge freezing: FEI Vitrobot (hopping between Donner Lab and ALS)







Ken Downing, Bjorg Larson, and Andrew Stewart. Thanks also to Eva Nogales and her lab!

Journal of Microscopy, Vol. 227, Pt 2 2007, pp. 98–109
Received 3 April 2007; revised 19 April 2007; accepted 24 April 2007

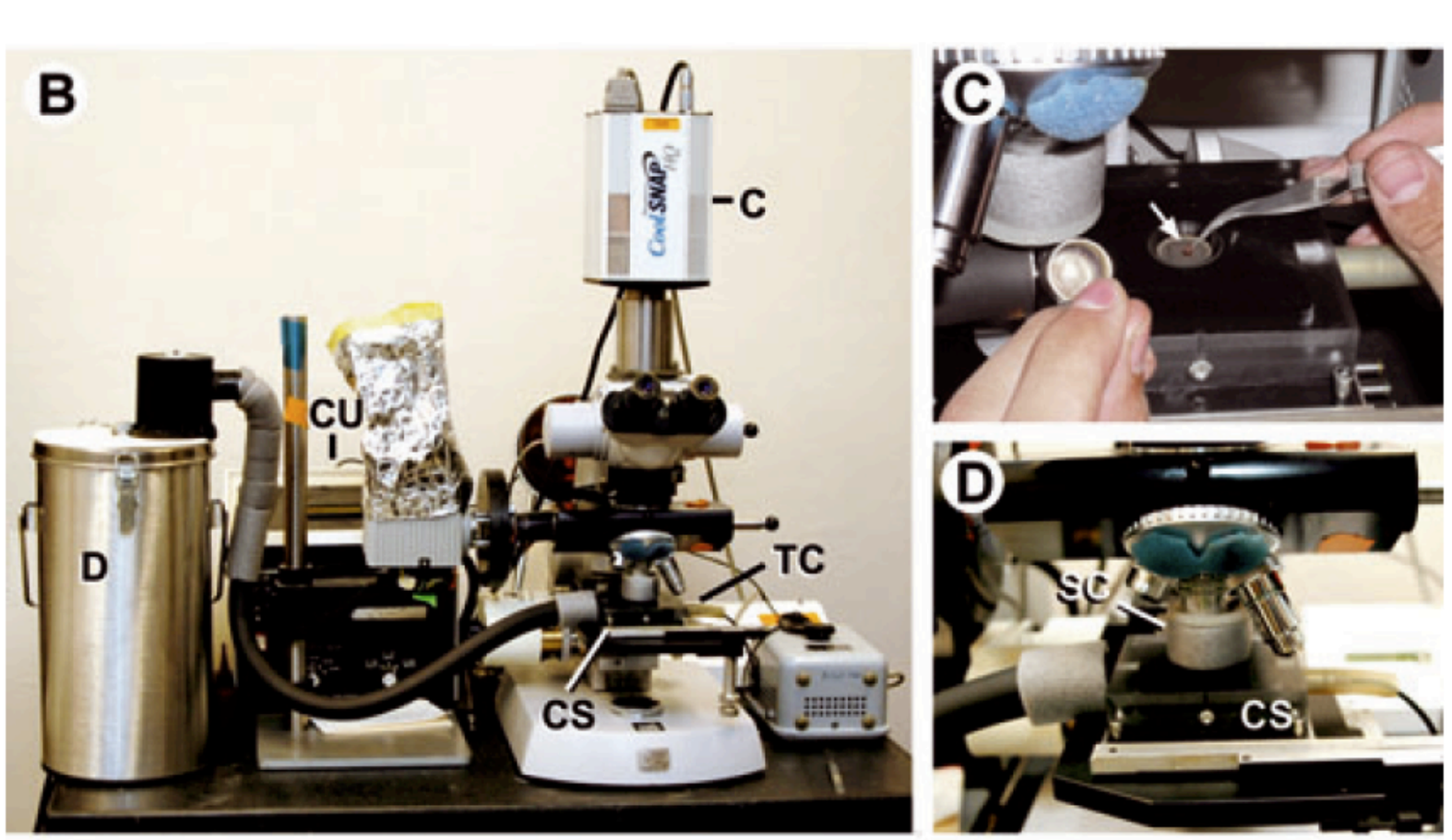
Cryo-fluorescence microscopy facilitates correlations between light and cryo-electron microscopy and reduces the rate of photobleaching

CINDI L. SCHWARTZ*, VASILY I. SARBASH†, FAZOIL I. ATAULLAKHANOV†, J. RICHARD MCINTOSH* & DANIELA NICASTRO‡

* *Boulder Laboratory for 3D Electron Microscopy of Cells, University of Colorado, Department of Molecular, Cellular, and Developmental Biology, Boulder, CO, USA*

†*National Research Center for Hematology, Moscow, Russian Federation*

‡*Brandeis University, Rosenstiel Basic Medical Sciences Research Center/Biology Department, Waltham, MA, USA*



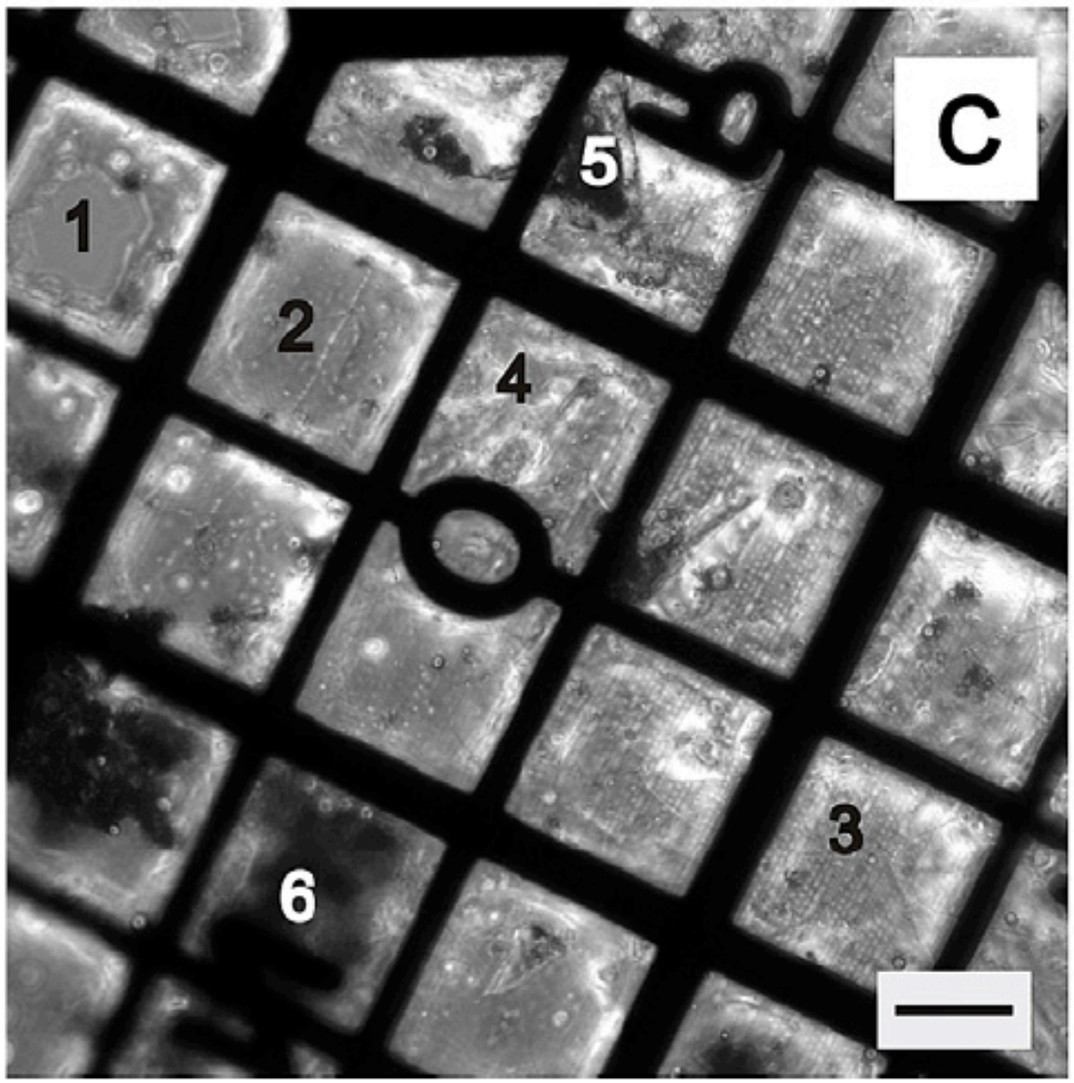
Journal of Structural Biology 160 (2007) 135–145

Correlative microscopy: Bridging the gap between fluorescence light microscopy and cryo-electron tomography

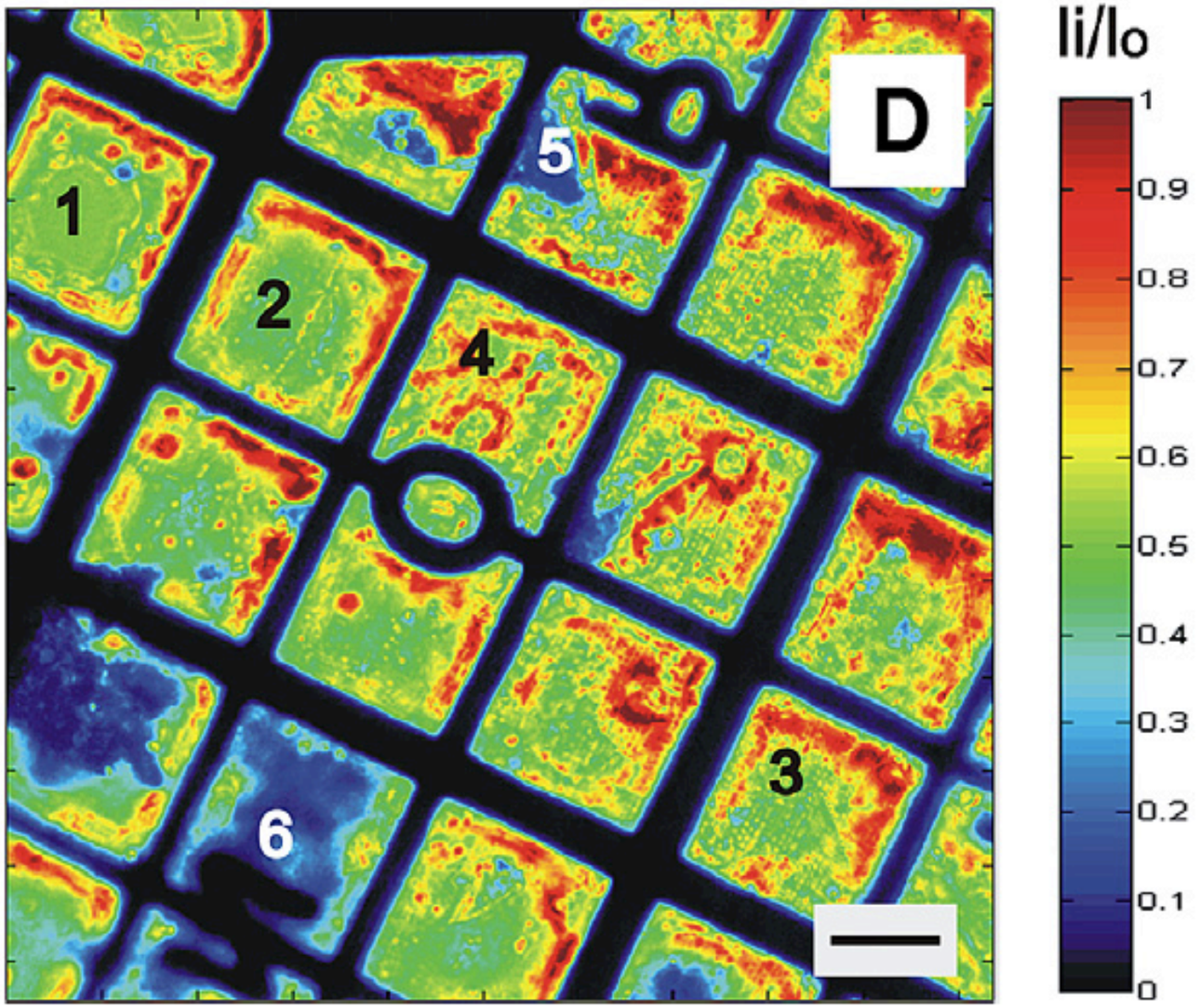
Anna Sartori *, Rudolf Gatz, Florian Beck, Alexander Rigort, Wolfgang Baumeister, Juergen M. Plitzko

Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Am Klopferspitz 18, 82152 Martinsried, Germany

Received 14 May 2007; received in revised form 19 July 2007; accepted 25 July 2007
Available online 16 August 2007



Visible light phase contrast



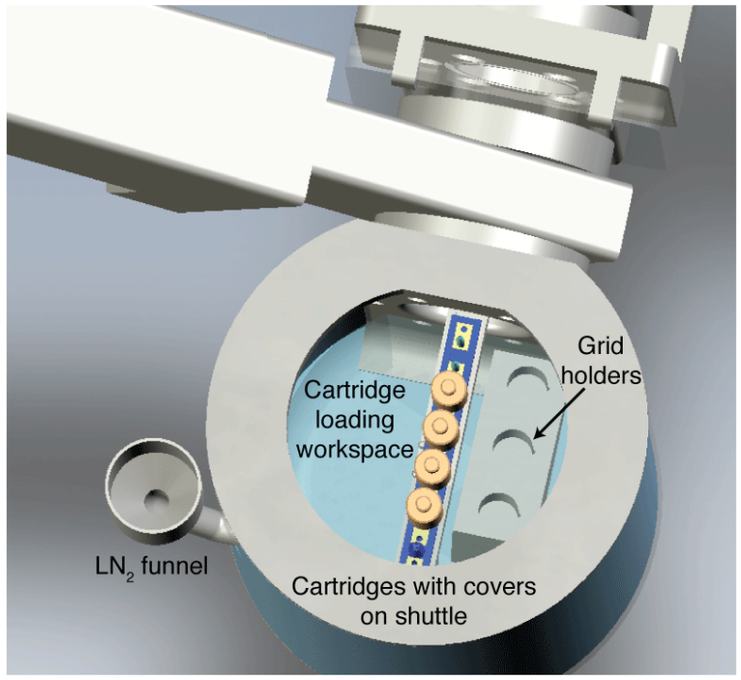
Estimating ice thickness

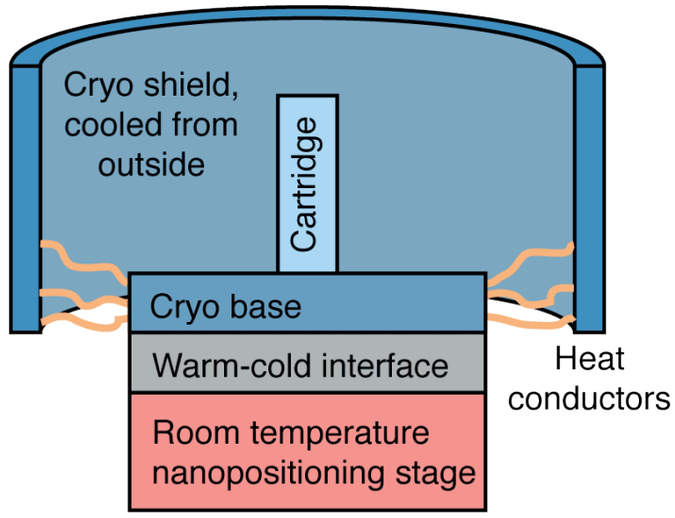
FEI Titan Krios TEM

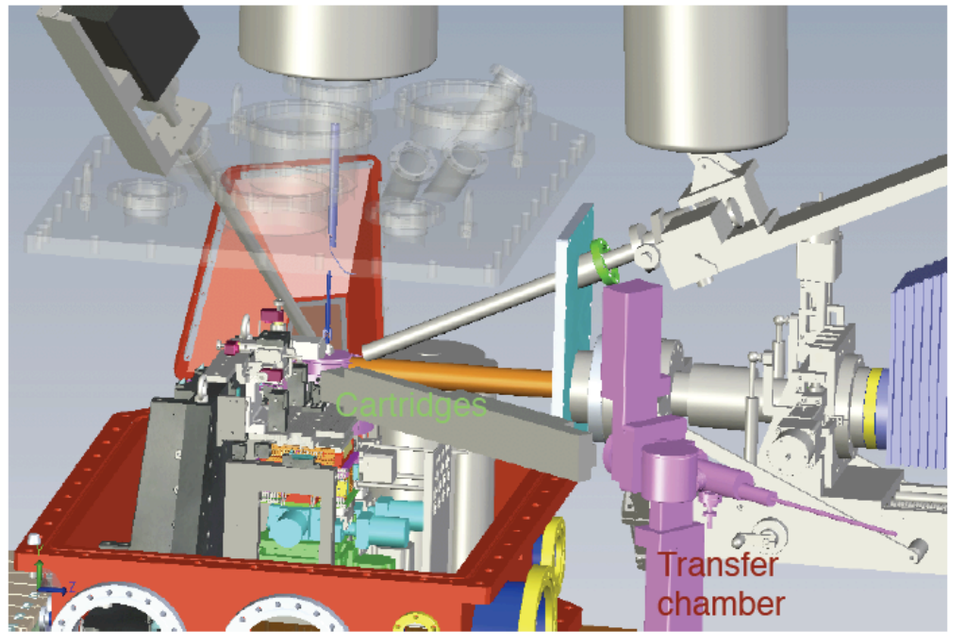
- Being introduced at MPI Martinsried on April 15.
- 300 keV, field emission gun, energy filter.
- **"Maximize Sample Integrity** - achieve contamination-free sample transfer and imaging"
- **"Increase Productivity** - The AutoLoader™ allows for fully automated and contamination-free loading and analysis of up to 12 samples contained in specially designed AutoGrid™ sample holder"

FEI COMPANY™
TOOLS FOR NANOTECH

Cryo system: Xradia example

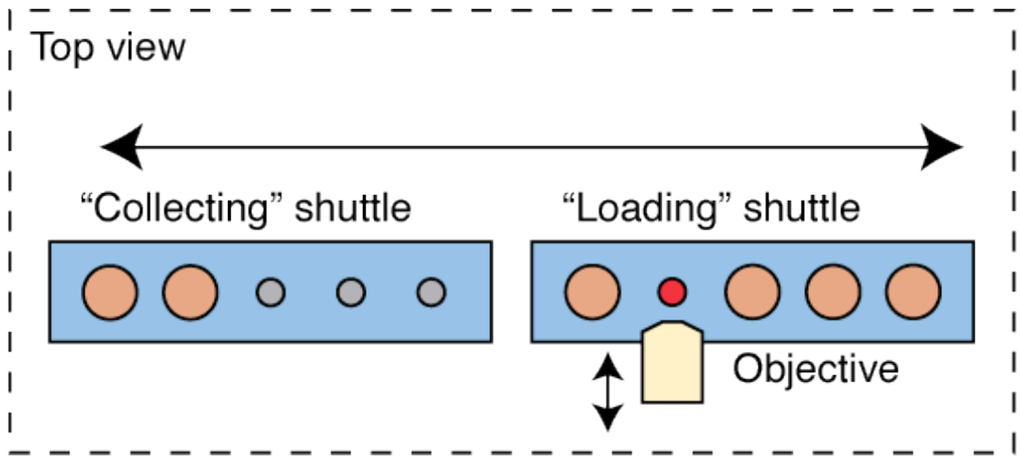


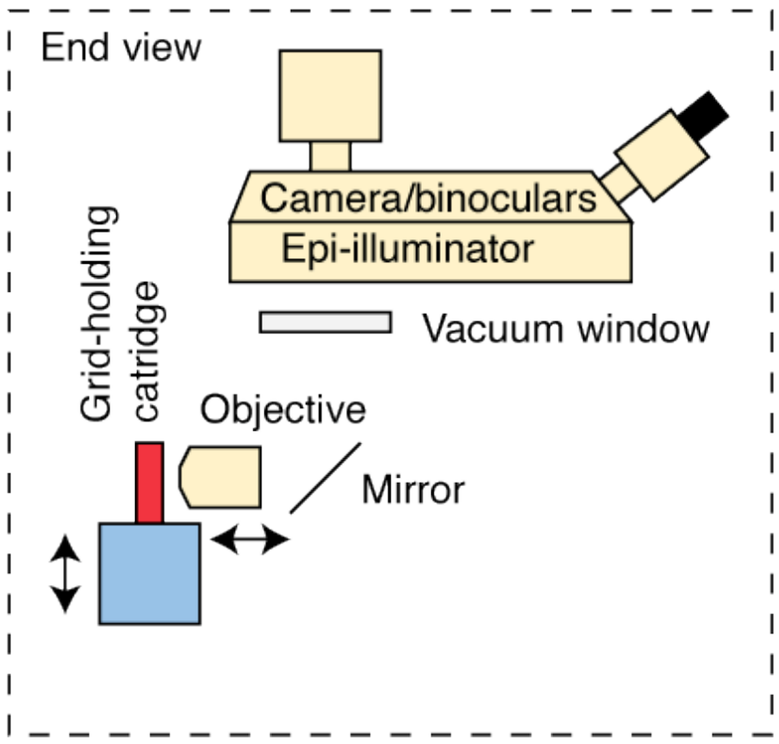




- Mount fragile grid in cartridge once; grids, capillaries, ...
- Transfer cartridge between visible light and various X-ray microscopes (including scanning, tomography).
- Robotic sample insertion in microscope.

Xradia cryo team:
C. Jacobsen, D. Trapp, *et al*.





Cryo x-ray diffraction microscopy: why?

- X rays: best probe for samples thicker than ~1 μm.
- Diffraction microscopy (aka CXDI):
  - Get the most out of the exposure to the sample.
  - Freedom from depth of focus limits.
- Cryo: essential for soft and/or wet specimens.

NSLS II should include cryo for soft and/or wet materials!