X-ray diffraction microscopy: What's it good for?

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What is X-ray diffraction microscopy?

- X-ray microscopy without lenses, by computational phasing of diffraction data.
- Also known as Coherent X-ray Diffraction Imaging (CXDI) but you'll get no invertible image without coherence, and it's mainly useful for microscopy.
- Proposed by David Sayre in *Imaging Processes and Coherence in Physics* (ed. Schlenker *et al.*, 1980)
- Reconstruction method: finite support constraint phase retrieval [Feinup, *Opt. Lett.* **3**, 27 (1978), and subsequent developments].

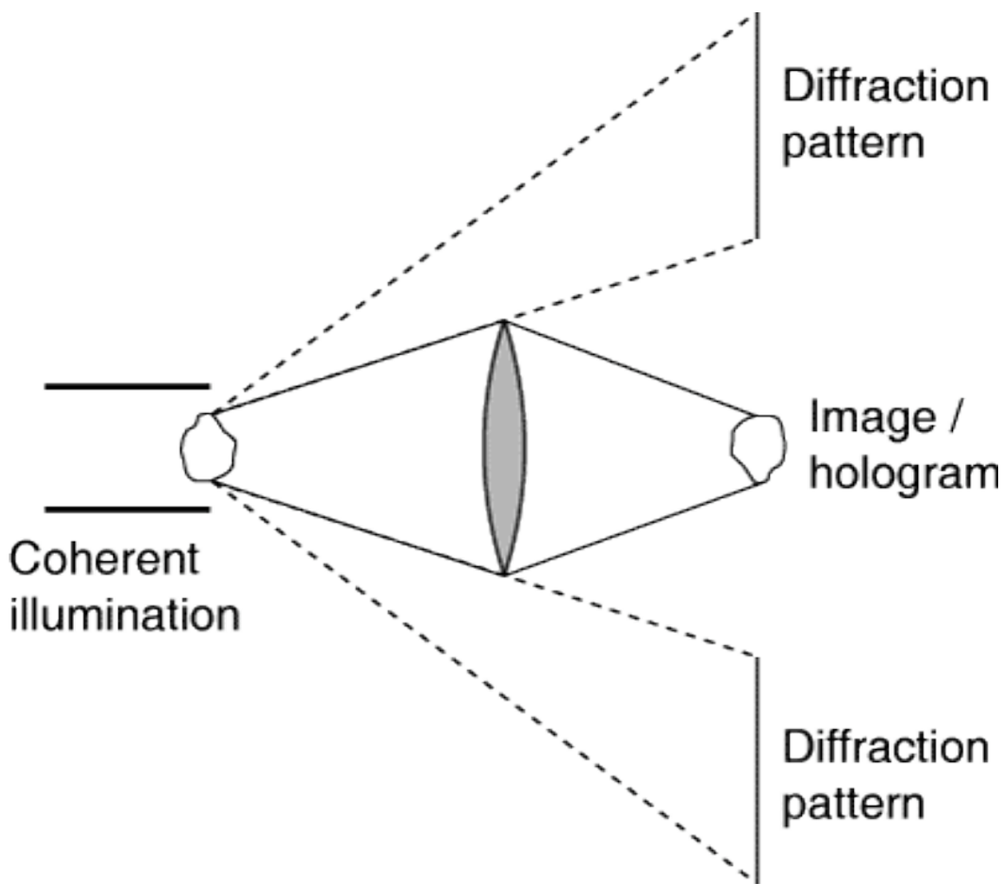
First x-ray reconstruction: Miao, Charalambous, Kirz, and Sayre, *Nature* **400**, 342 (1999).
Previous experiments: Sayre, Kirz, Yun, Chapman.

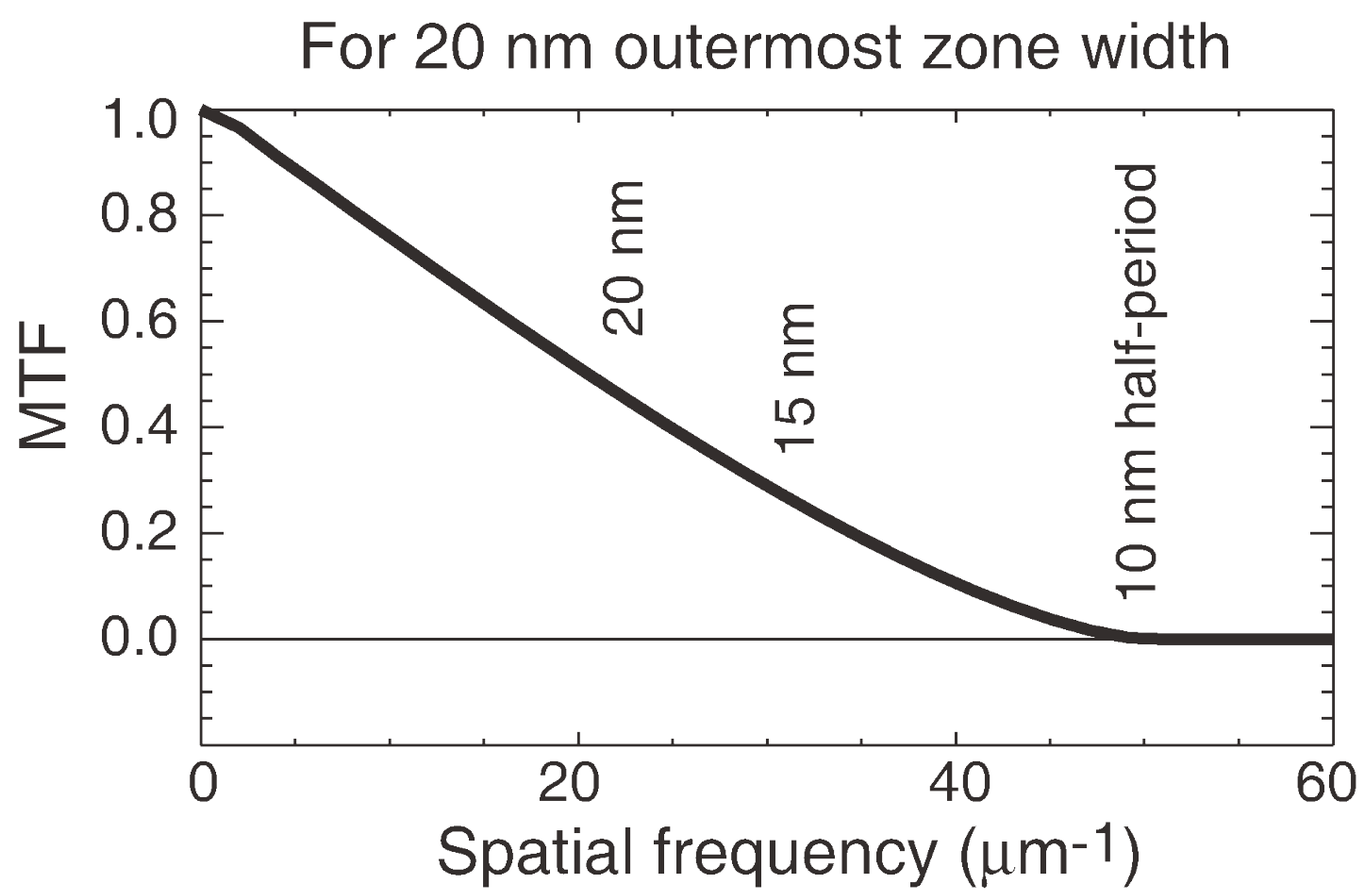
Why not X-ray lenses?

- 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:

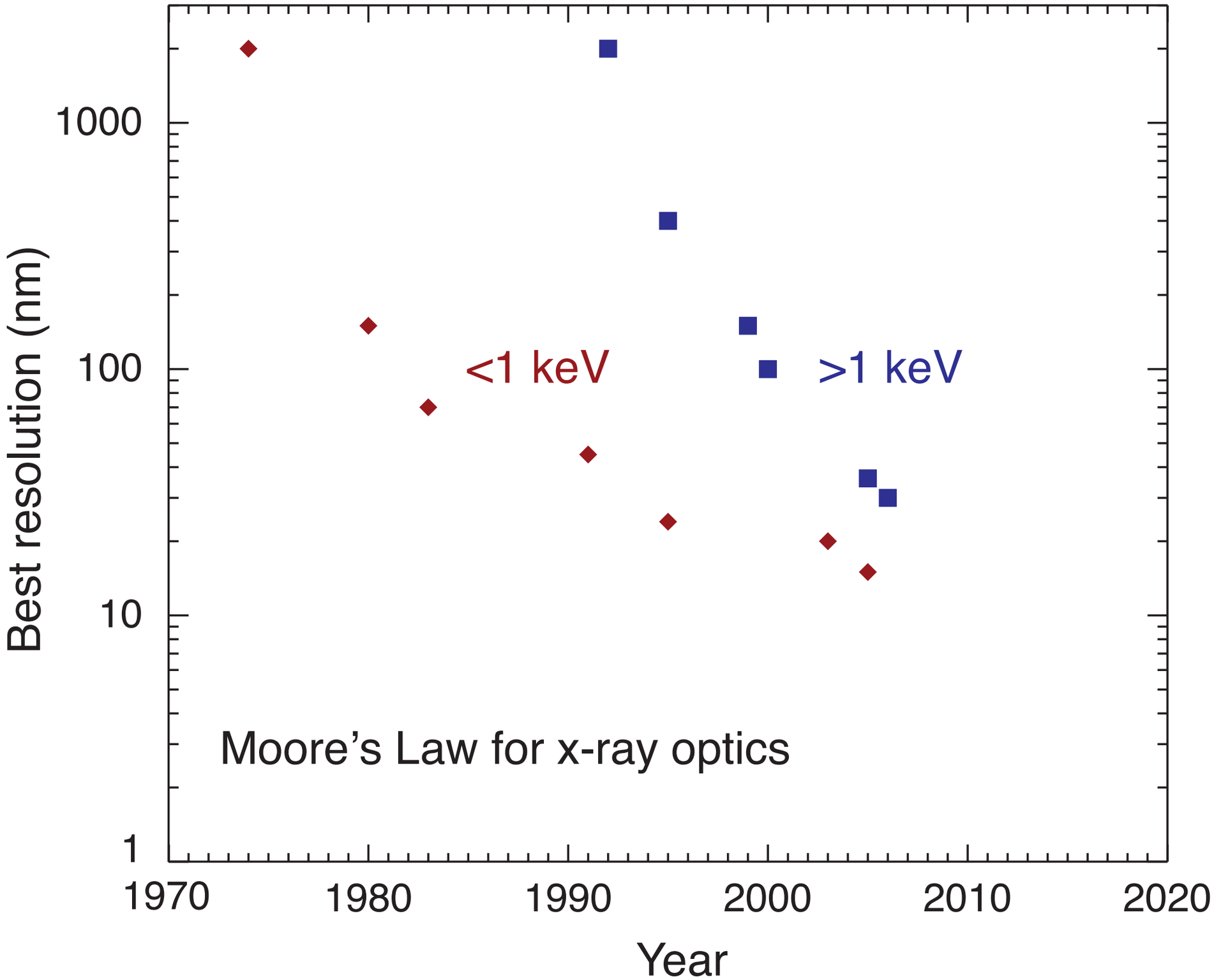
  net transfer of 1% for high spatial frequencies

- Can we avoid this ~100x signal loss, and also go beyond numerical aperture limit of available optics?



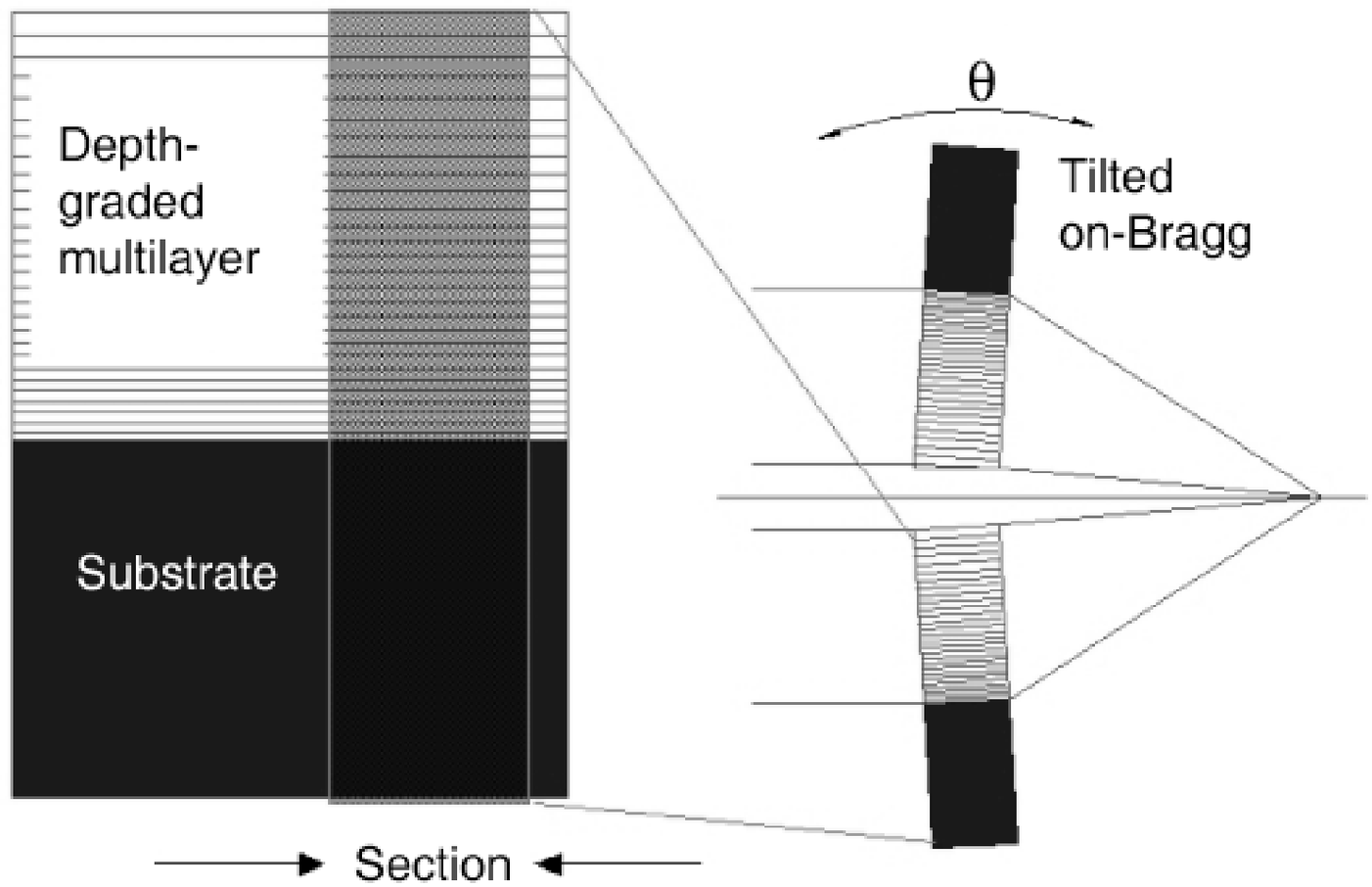


X-ray optics: best resolution



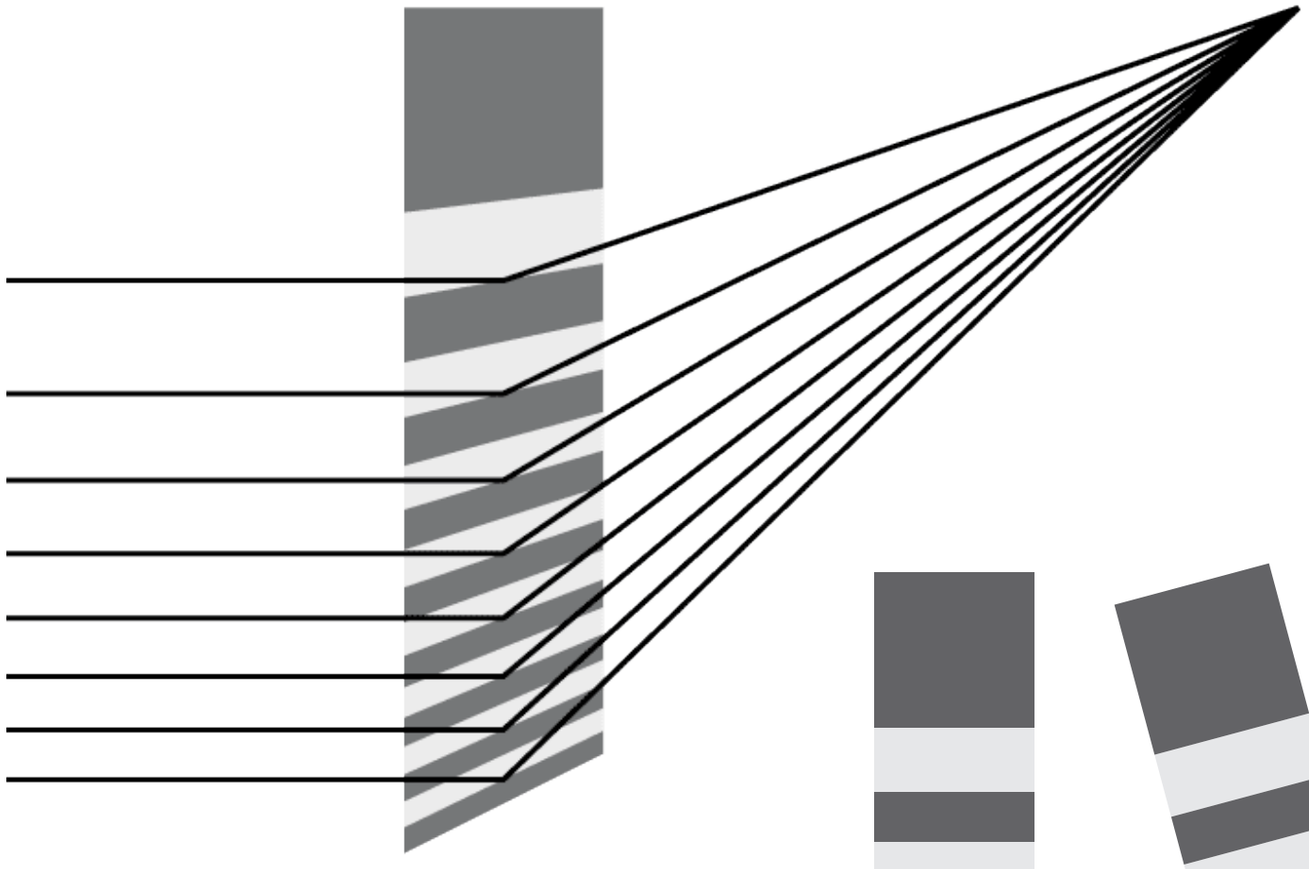
Multilayer Laue lenses

- Forget top-down circles, and go sideways! Start by depositing thinnest zones first on a flat substrate, and work your way up to thicker zones. Cross two 1D lenses for 2D focusing.
- For thick optics, you want to tilt to be on the Bragg condition anyway [Maser, PhD thesis; Maser and Schmahl, *Opt. Comm.* **89**, 355 (1992)]

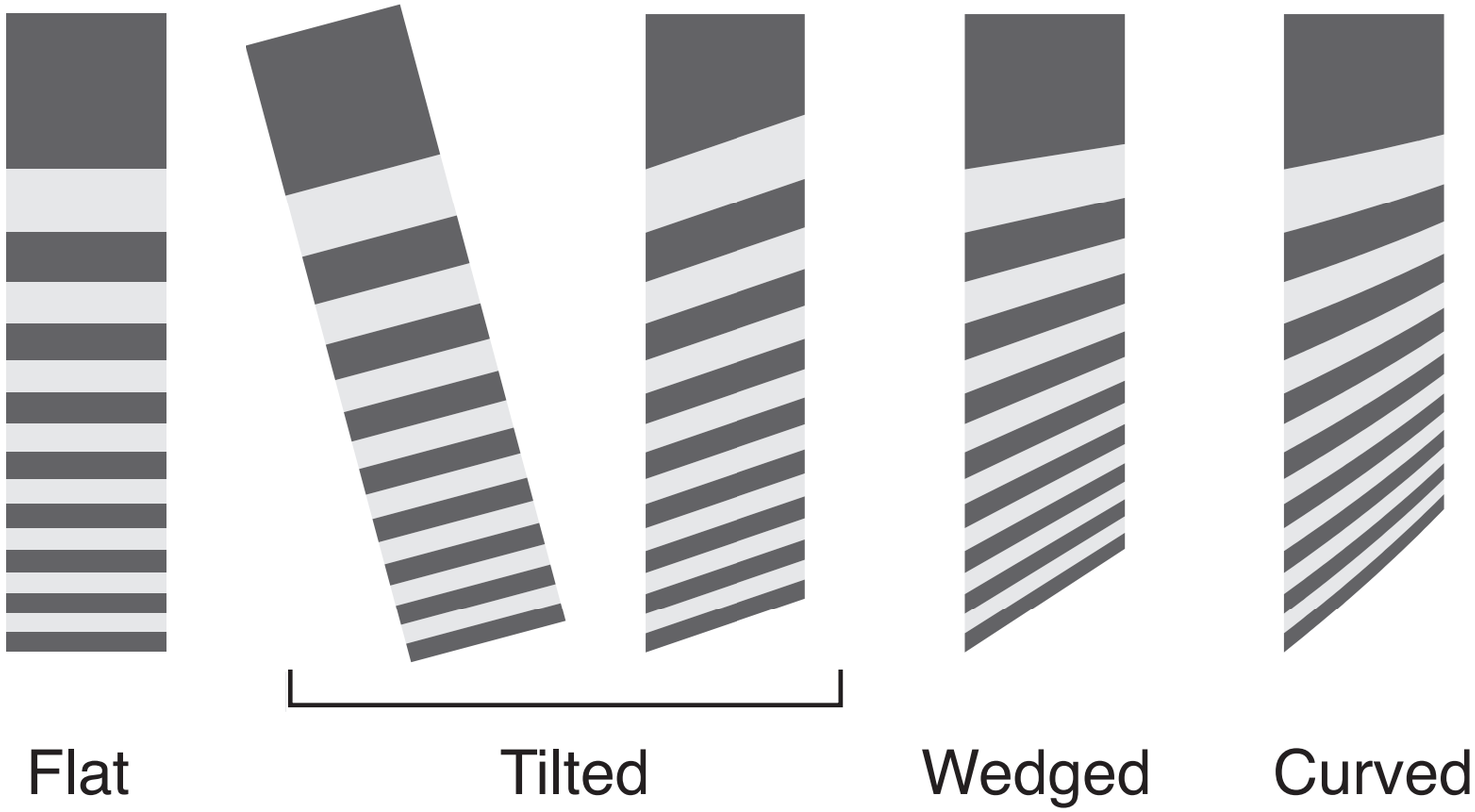


J. Maser et al., *SPIE* **5539**, 185 (2004); plus tests by Liu *et al.*, *J. Appl. Phys.* **98**, 113519 (2005); Kang *et al.*, *Appl. Phys. Lett.* **86**, 151109 (2005); Kang *et al.*, *Phys. Rev. Lett.* **96**, 127401 (2006).

MLLs are not without challenges

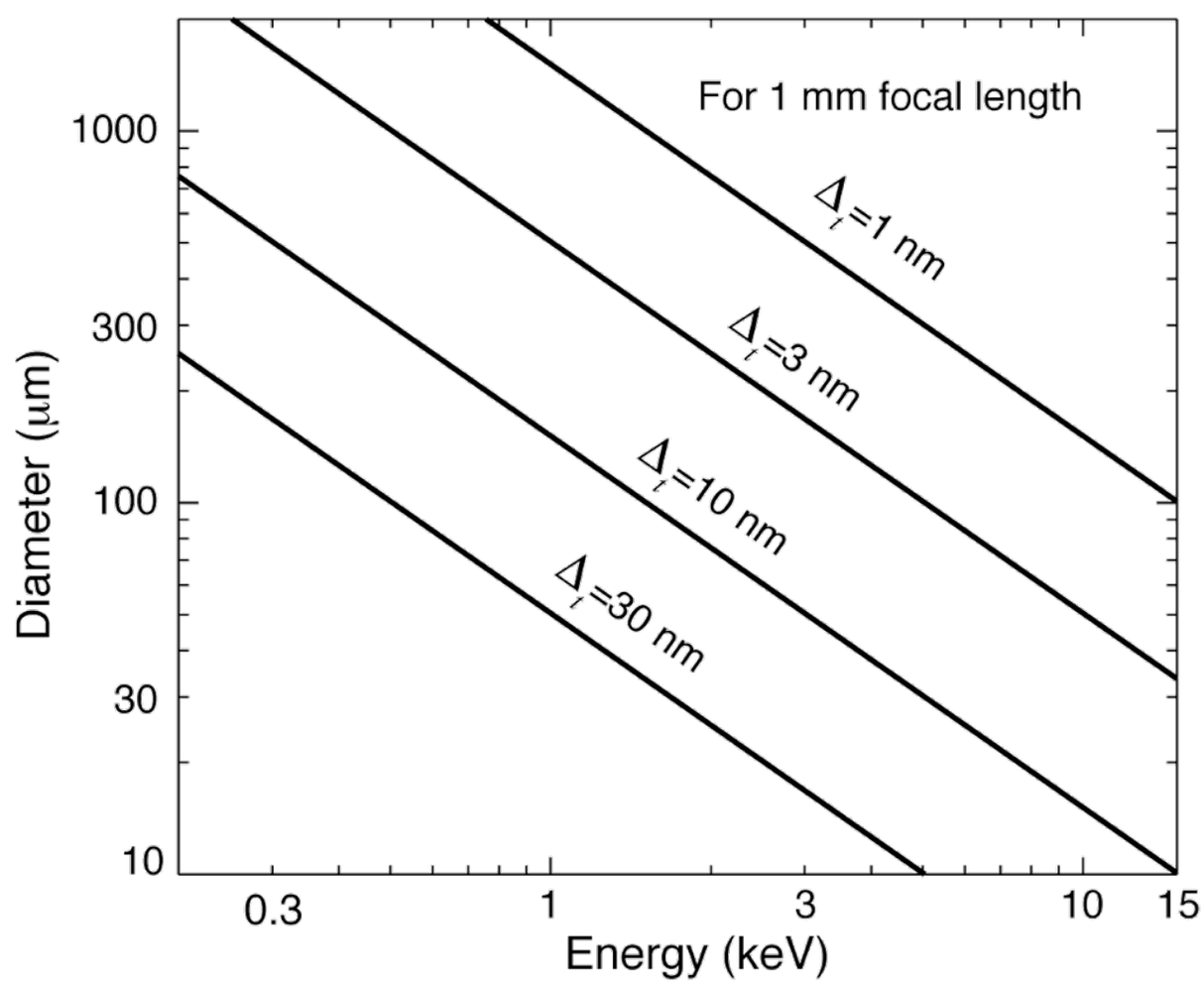


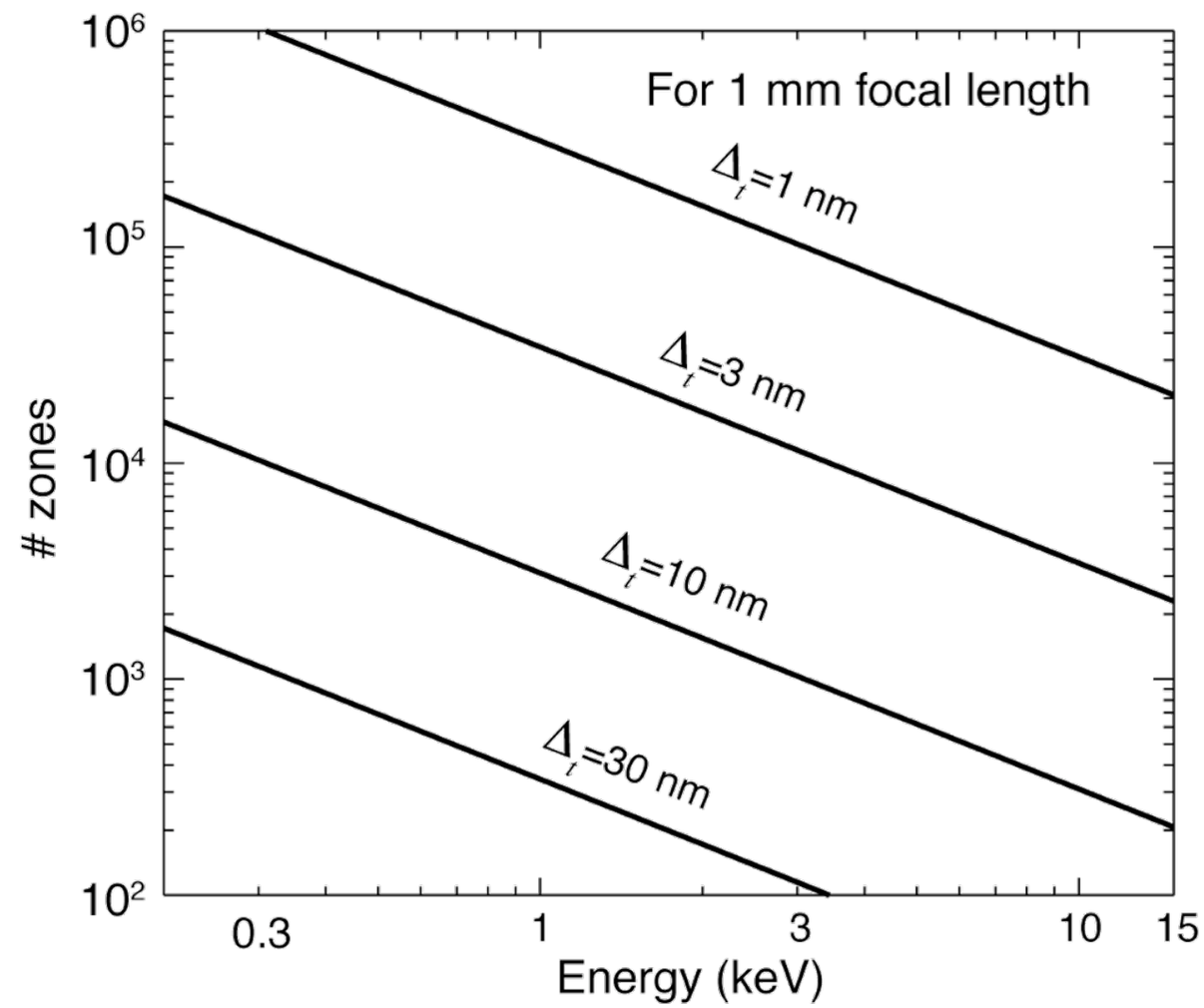
Must stay on-Bragg for good efficiency



High resolution: lots of layers!

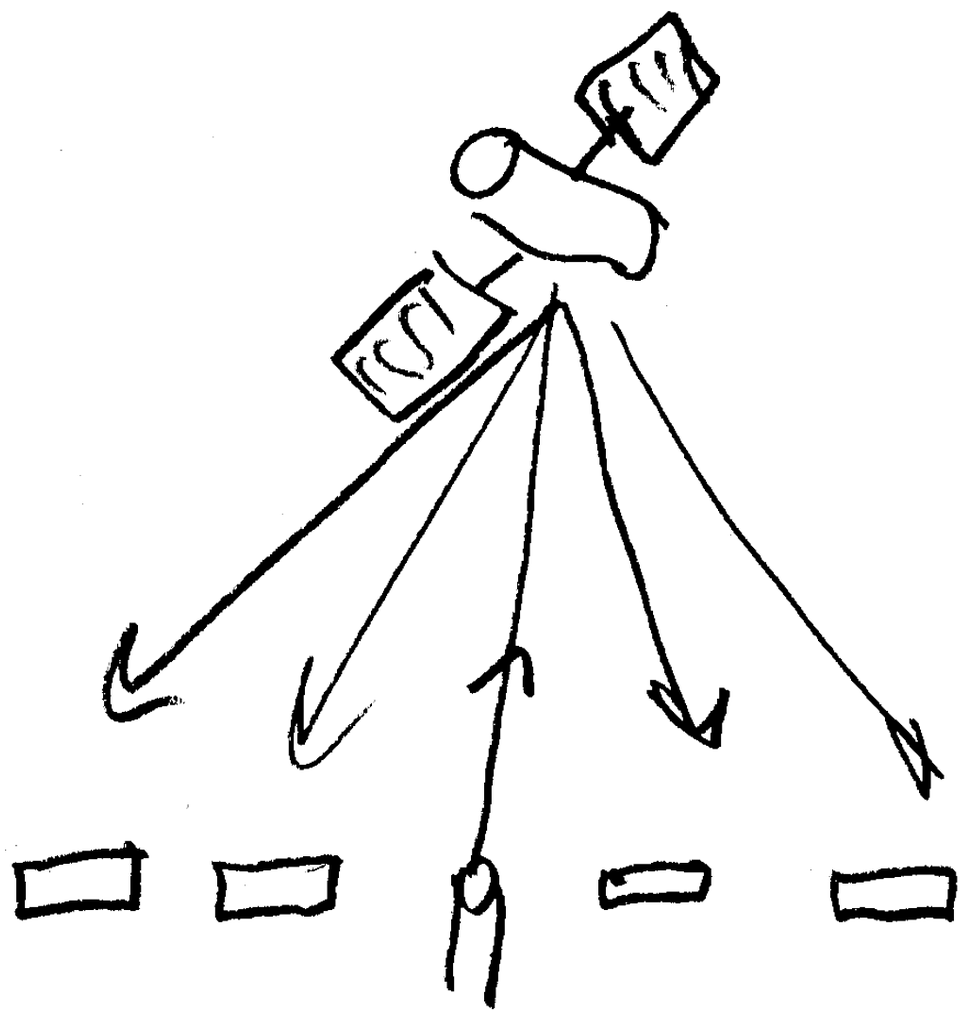
- Transverse resolution $\Delta_t = 1.22\Delta_{rN}$, where Δ_{rN}=outermost zone width
- Diameter $d = 1.22\ \lambda f/\Delta_t$, # zones $N = 1.22^2\ \lambda f/\Delta_t^2$





What can you do without lenses?

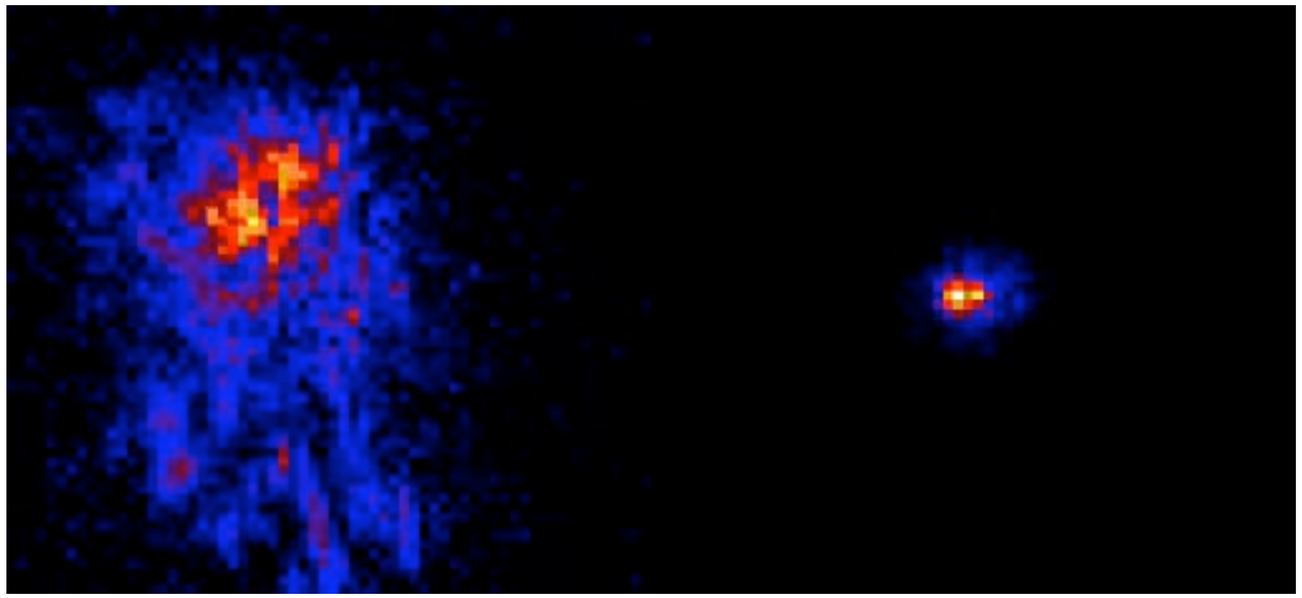
Example: phase of light in the far-field lost due to atmospheric turbulence.

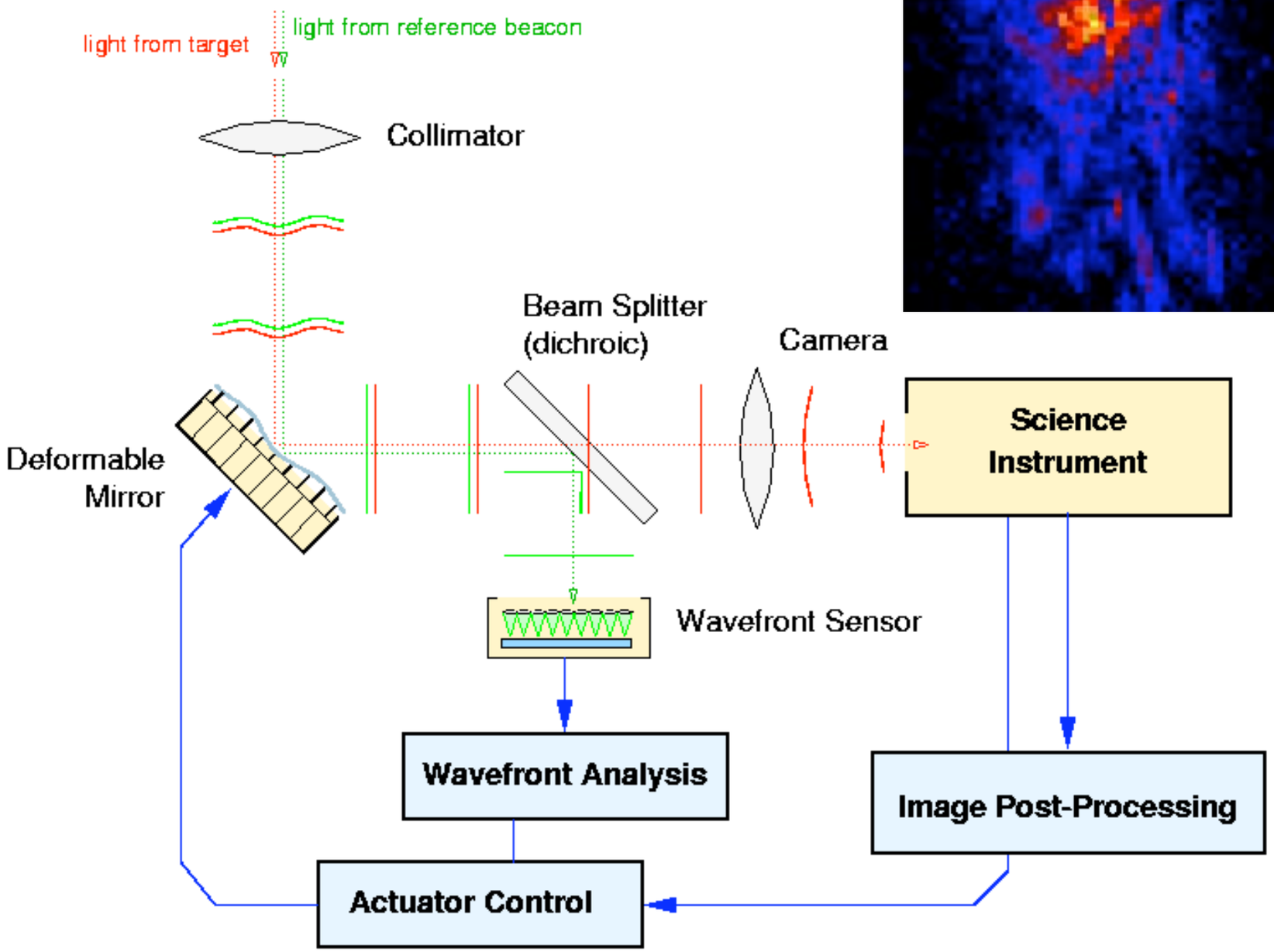


Cartoon based on presentations by Fienup from ERIM (Environmental Research Institute of Michigan). Fienup is now at U. Rochester

Losing phase

- Atmosphere: random rearrangement of optical phase due to temperature fluctuations
- Rubber mirrors (adaptive optics) can fix this - but only for nearly point-sized objects!





Center for Adaptive Optics, UC Santa Cruz

Phase matters

Image→ Fourier transform→ zero magnitude or phase→ inverse Fourier transform

Malcolm Howells at La Clusaz

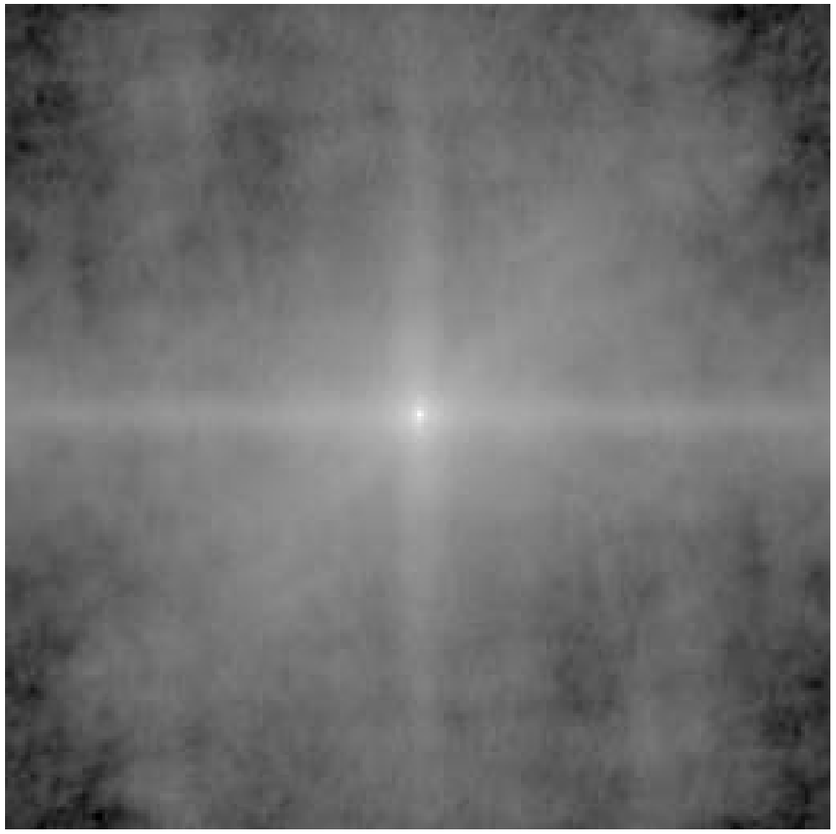


Image using only Fourier magnitudes

Image using only Fourier phases

How can we get phases when we measure only intensities?

Gerchberg & Saxton, *Optik* **34**, 275 (1971); **35**, 237 (1972).

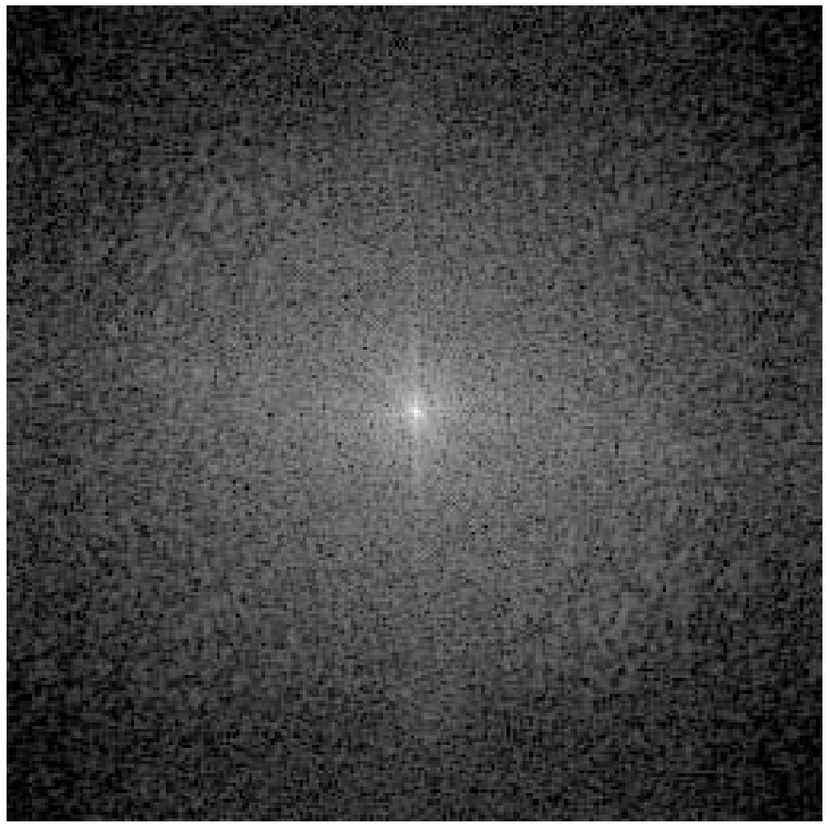
Recovery of phase information in electron microscopy, where you can tune the objective lens to record a diffraction pattern.

Image plane

⇐Fourier⇒

transform

(insert measured magnitudes at each plane while leaving phase untouched)



Diffraction plane

Helped by complete mixing of information between real and Fourier space:

$$G(f) = \int g(x) e^{i2\pi x f}\, dx$$

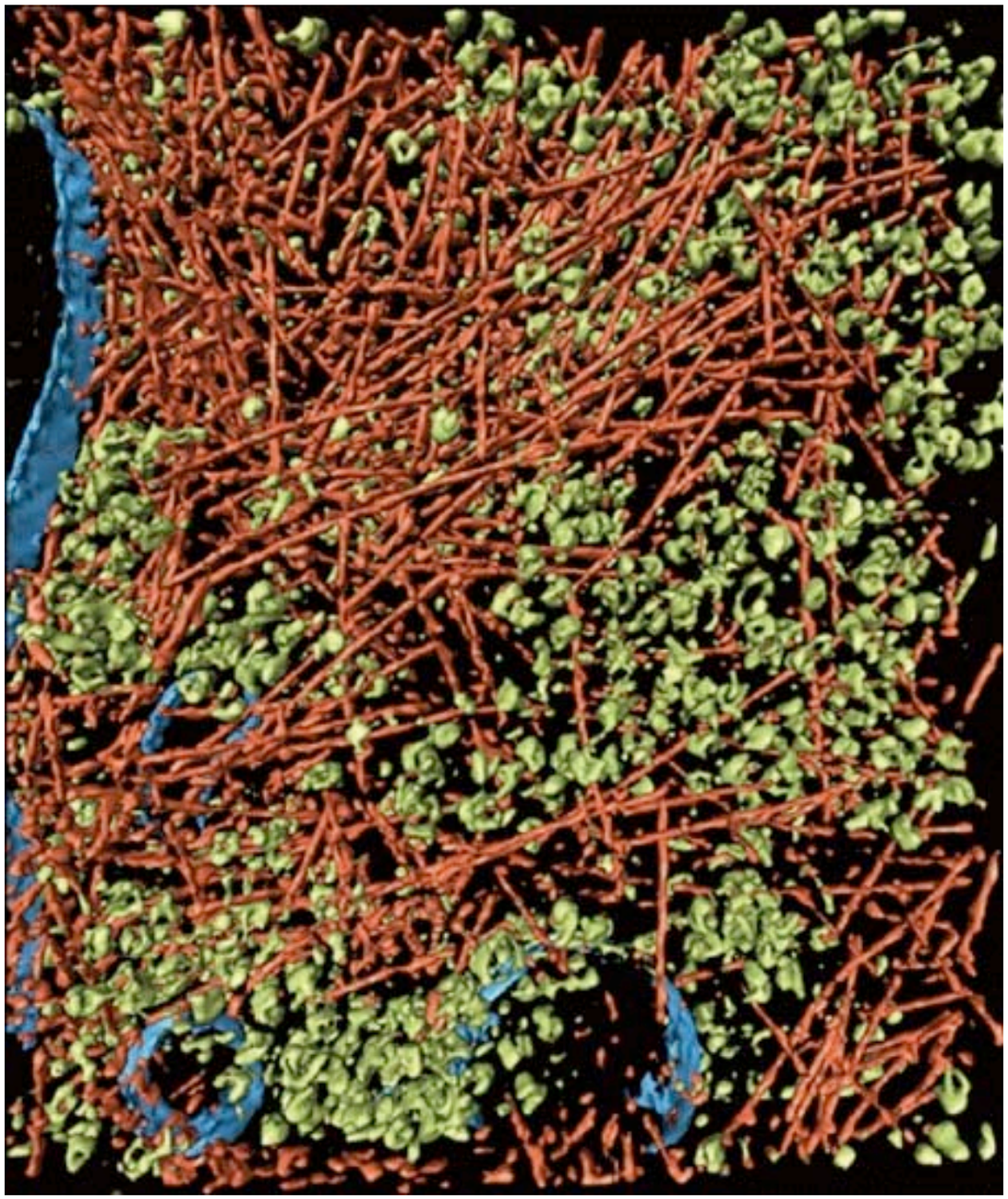
$$g(x) = \int G(f) e^{-i2\pi f x}\, df$$

What's it good for?

- When are X rays the right probe? We have to know about the competition.
- When is x-ray diffraction microscopy the right way to get images? We have to understand its limitations.
- What are some of the outstanding challenges?

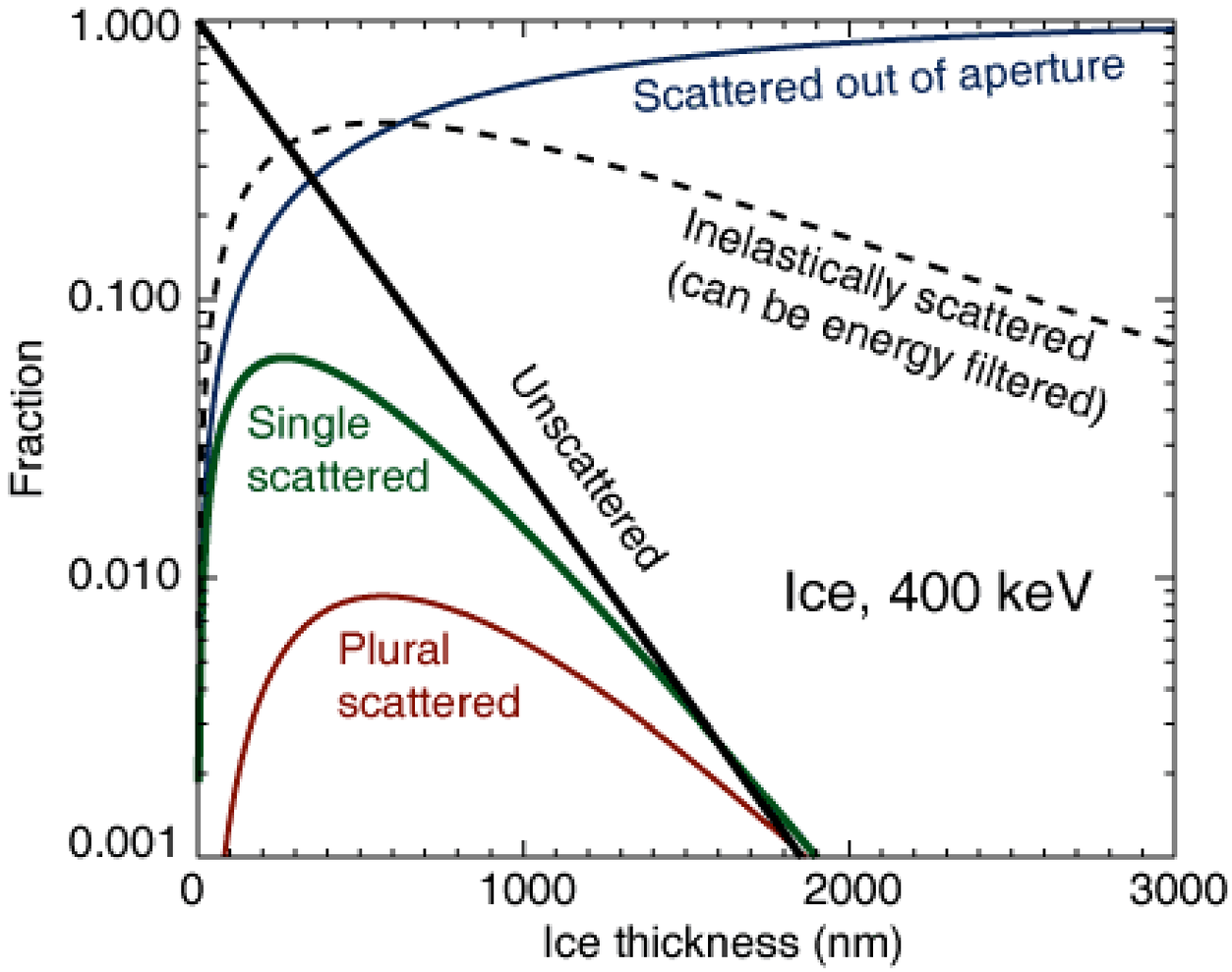
Cryo-electron tomography

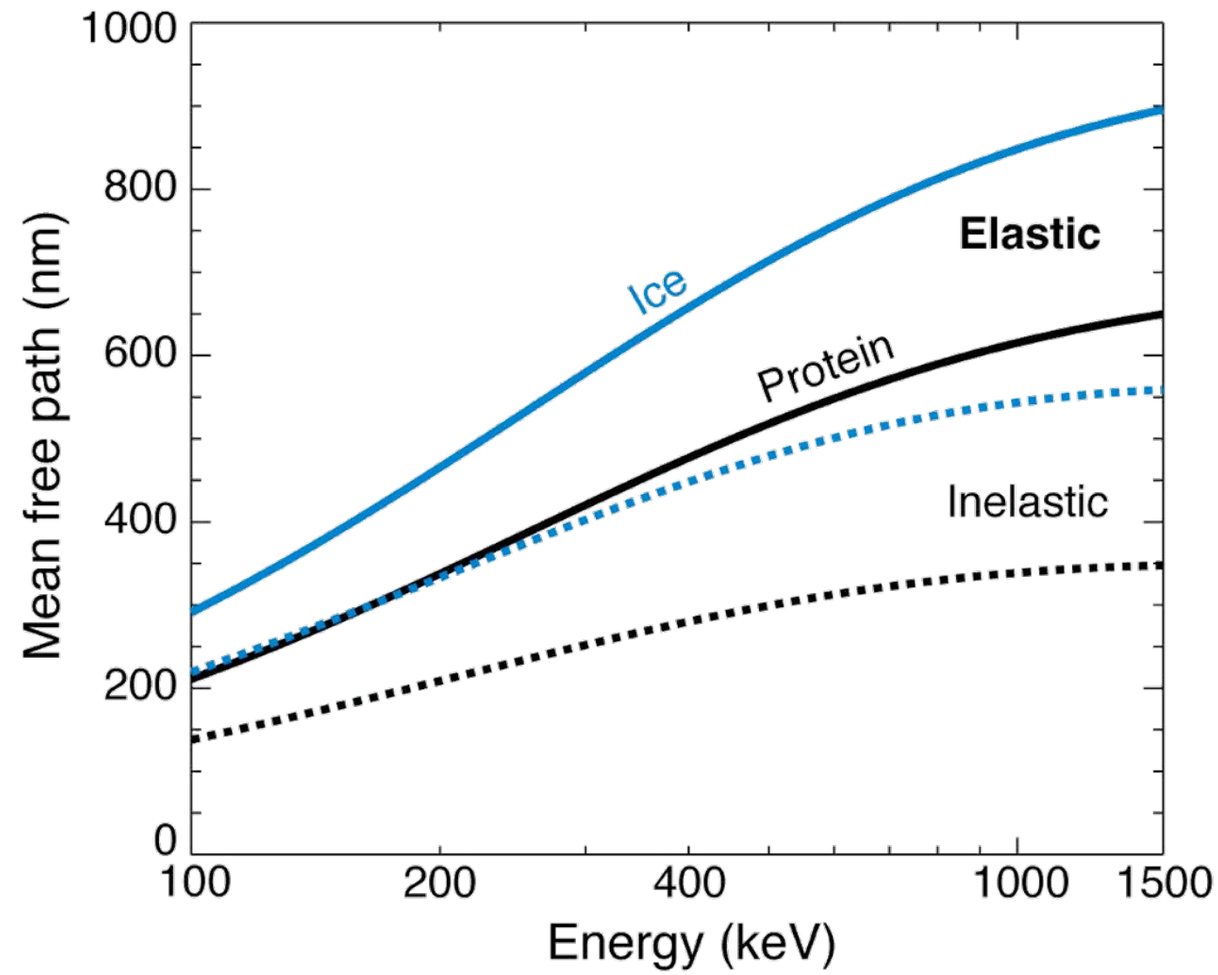
- Frozen-hydrated *Dictyostelium discoideum* viewed at LN2 temperature
- Tomography dataset with 300 keV field-emission TEM equipped with energy filter
- 5-6 nm resolution in 300-600 nm sample thickness
- Medalia *et al.*, *Science* **298**, 1209 (2002).



Actin, membranes, ribosomes and other macromolecular complexes.

How thick can electrons go?

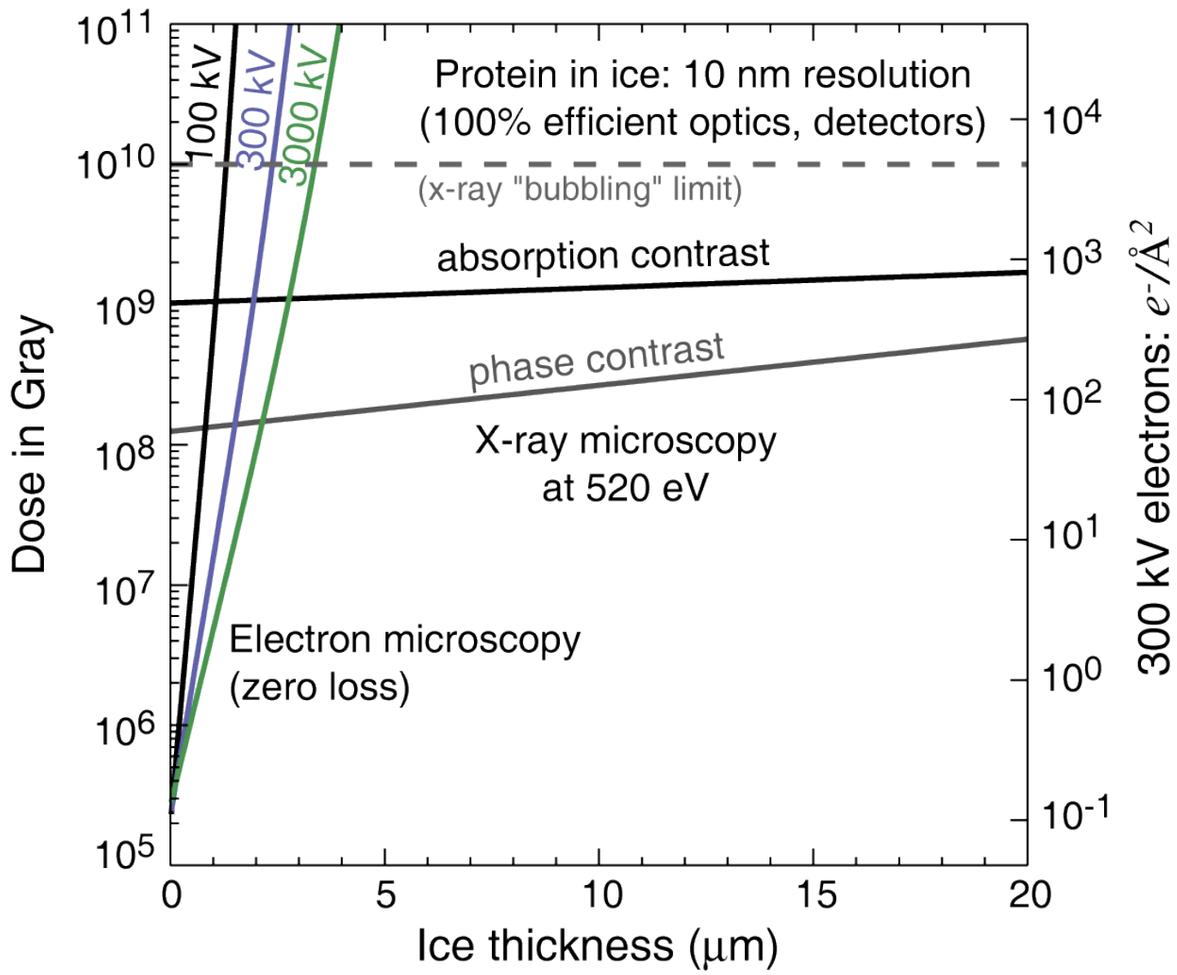


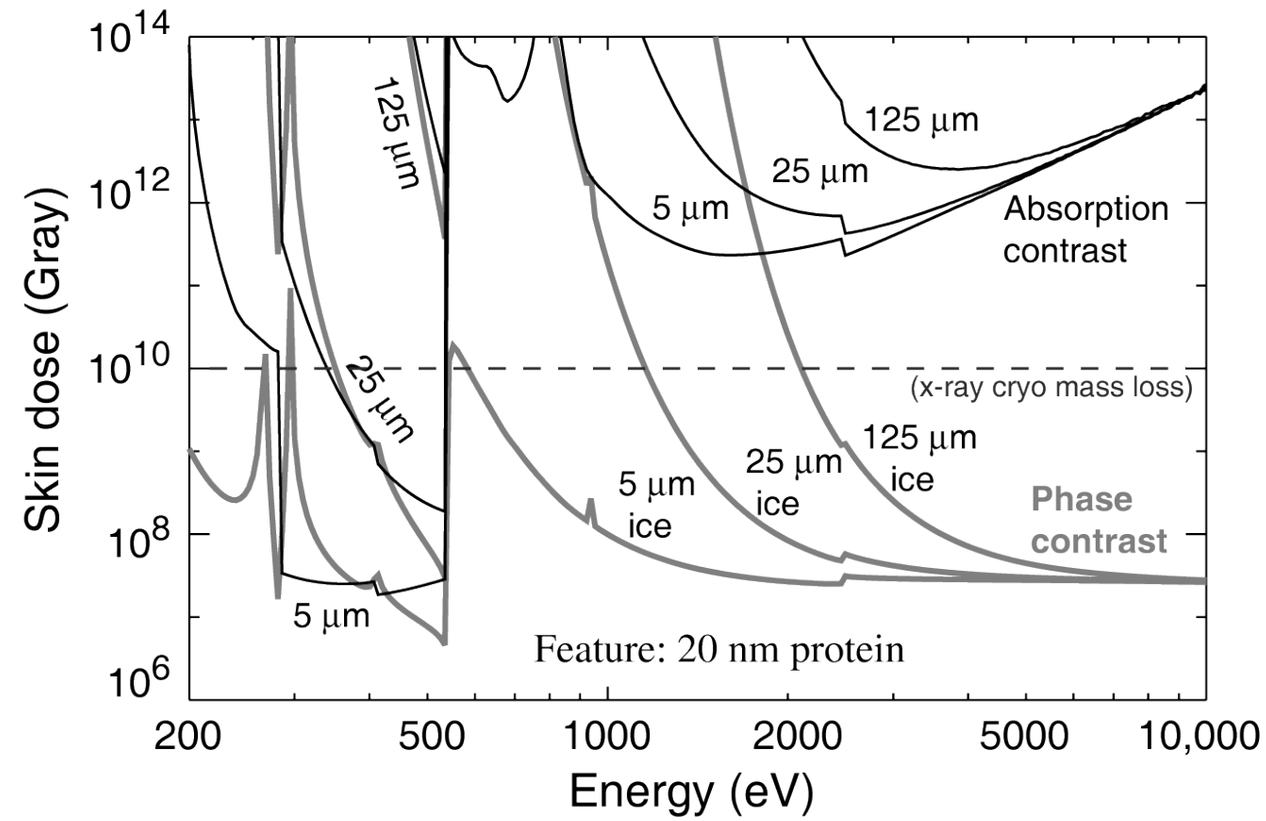


These plots: Jacobsen, Medenwaldt, and Williams, in **X-ray Microscopy & Spectromicroscopy** (Springer, 1998)

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)



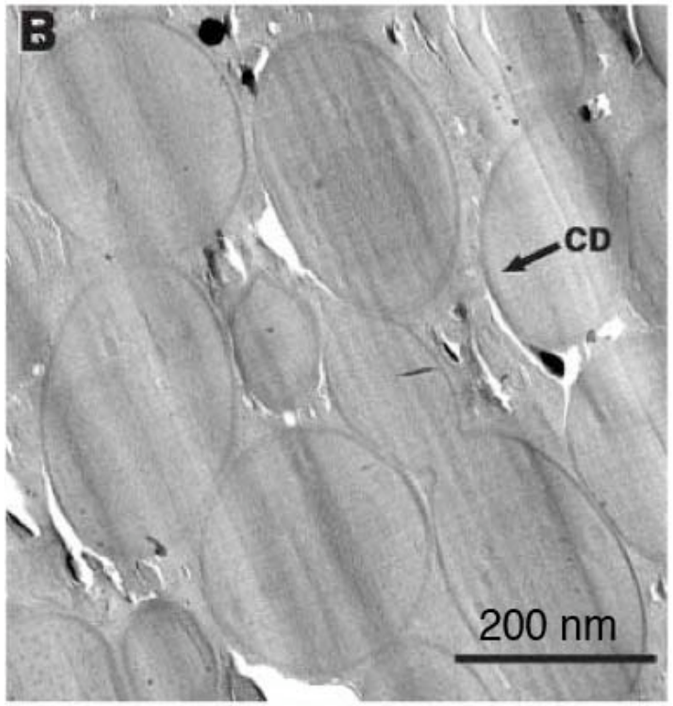


Phase contrast is important!

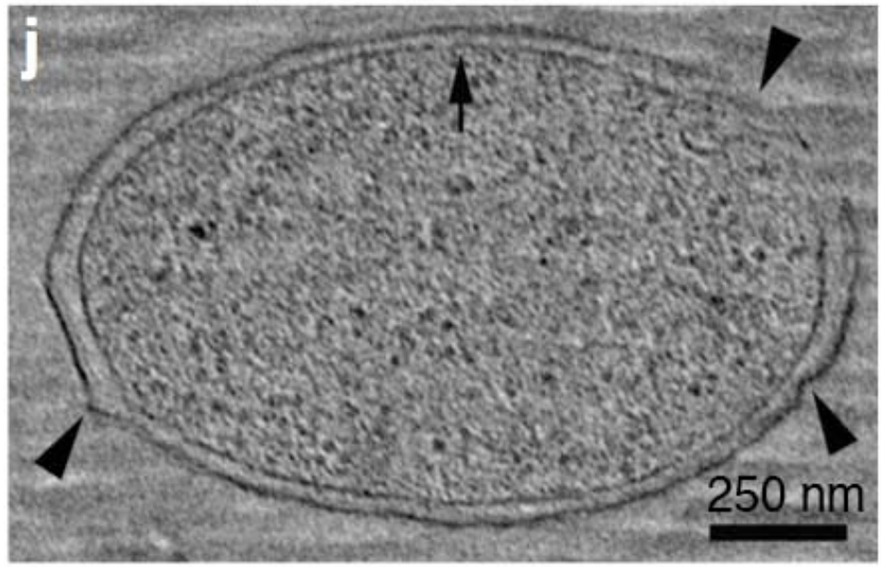
These plots: based on Jacobsen, Medenwaldt, and Williams, in **X-ray Microscopy & Spectromicroscopy** (Springer, 1998)

Making the specimen thinner

Serial sectioning: difficult but not impossible! Hoenger talk, Wednesday morning.

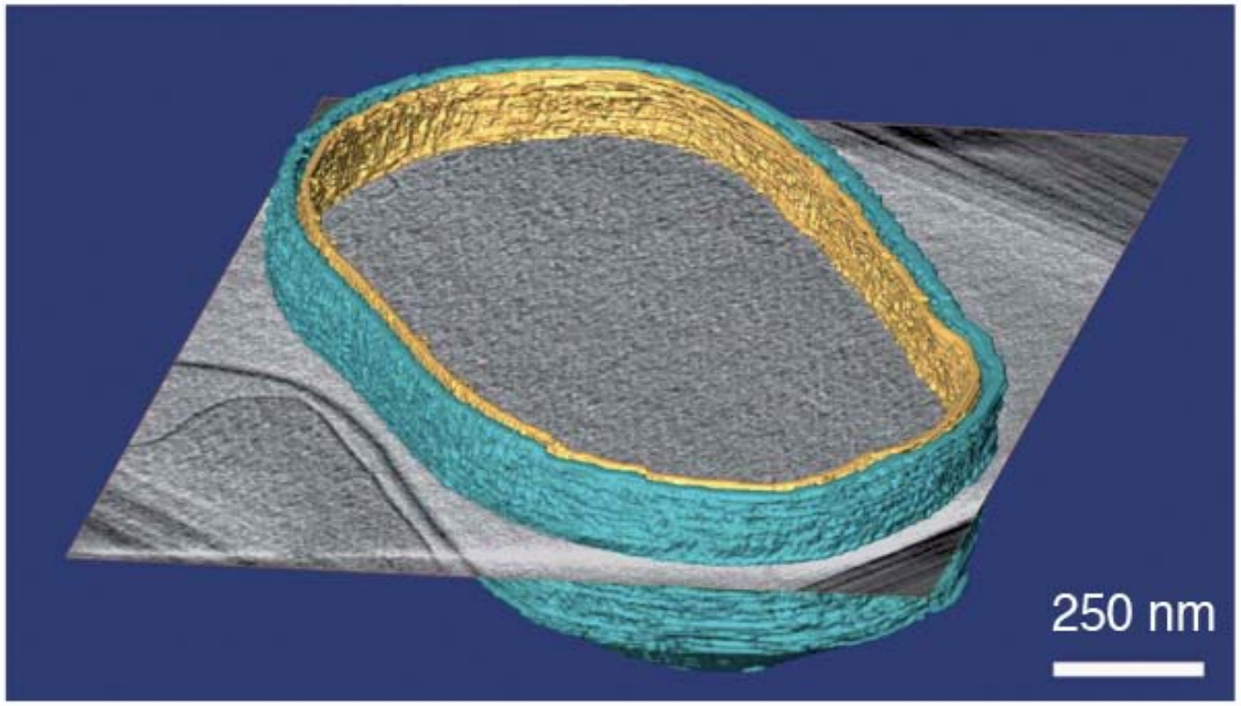


Ladinsky *et al.*, *J. Micros.* **224**, 129 (2006)



Marko *et al.*, *Nature Methods* **4**, 215 (2007)

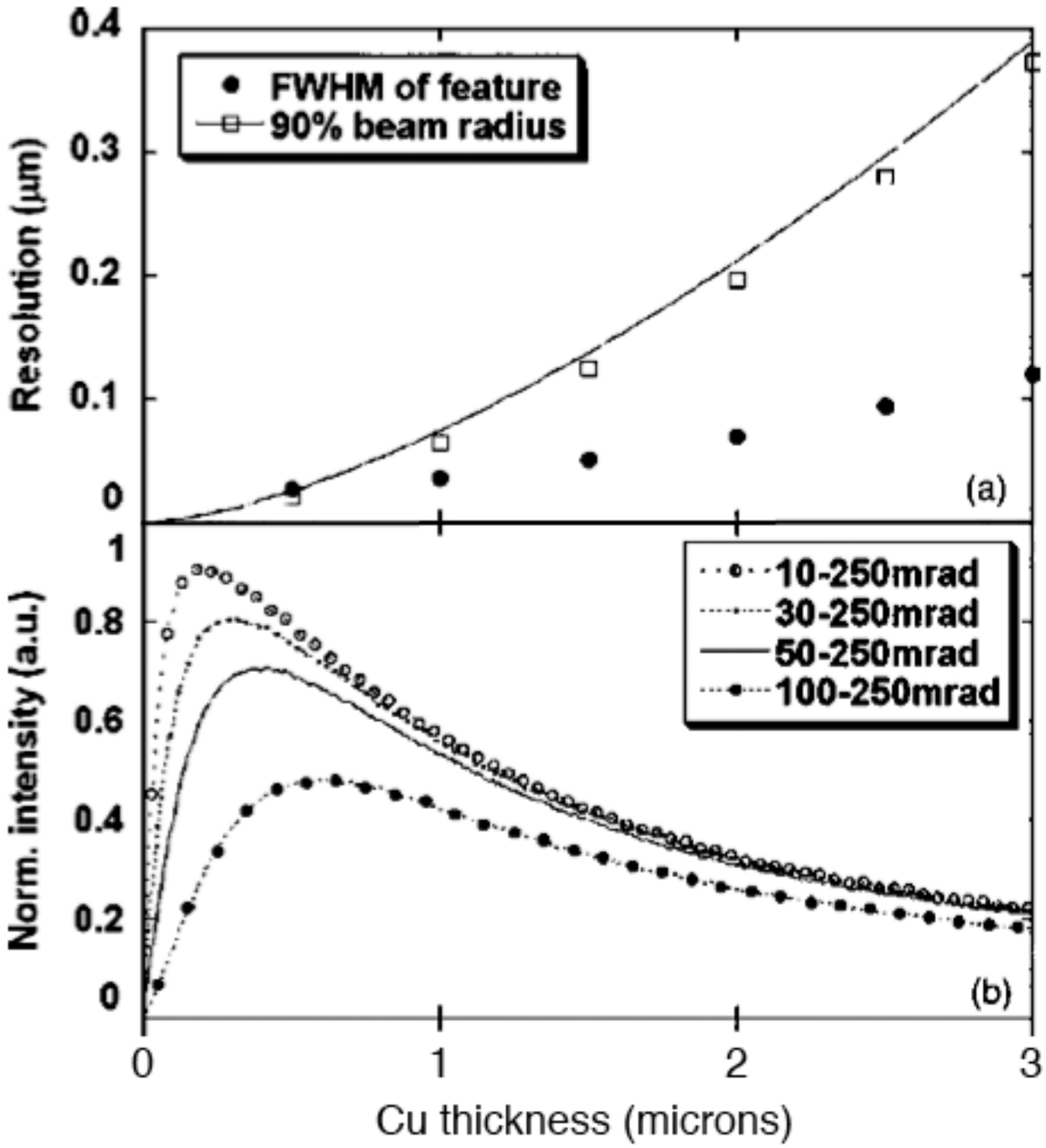
Challenges with sectioning: knife chatter, distortion

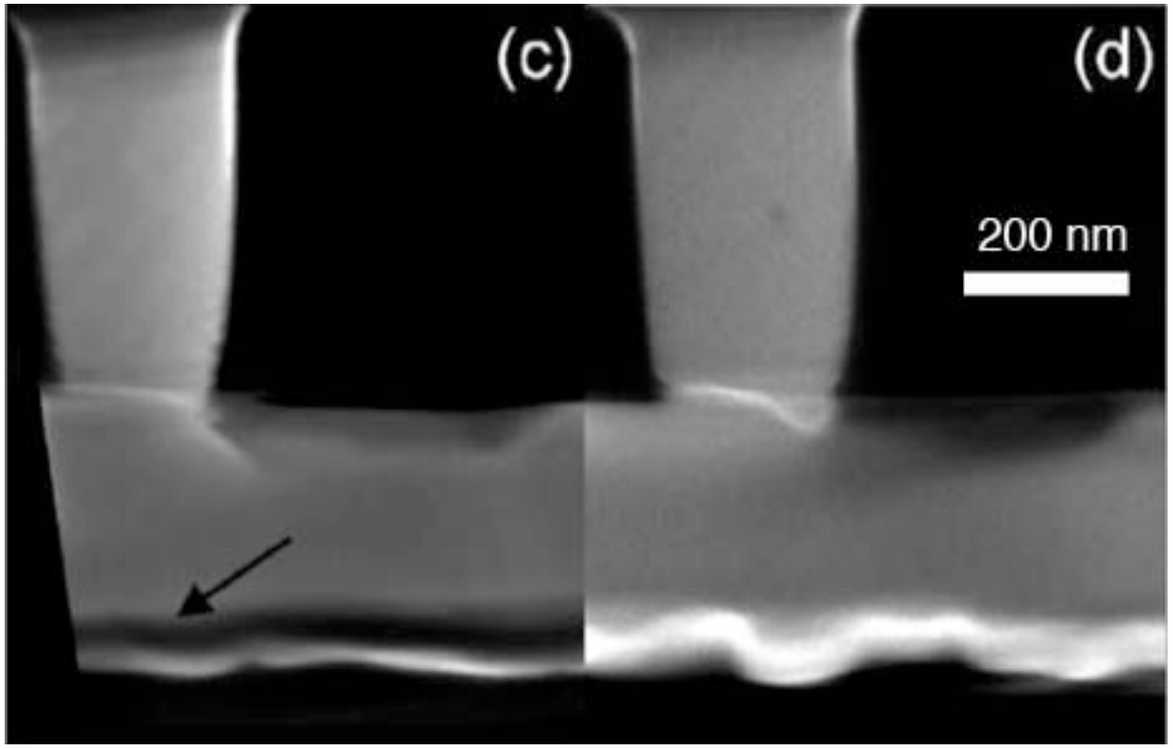


Marko *et al.*, *Nature Methods* **4**, 215 (2007)

Cryo FIB sectioning: Marko *et al.*, Wadsworth Center

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





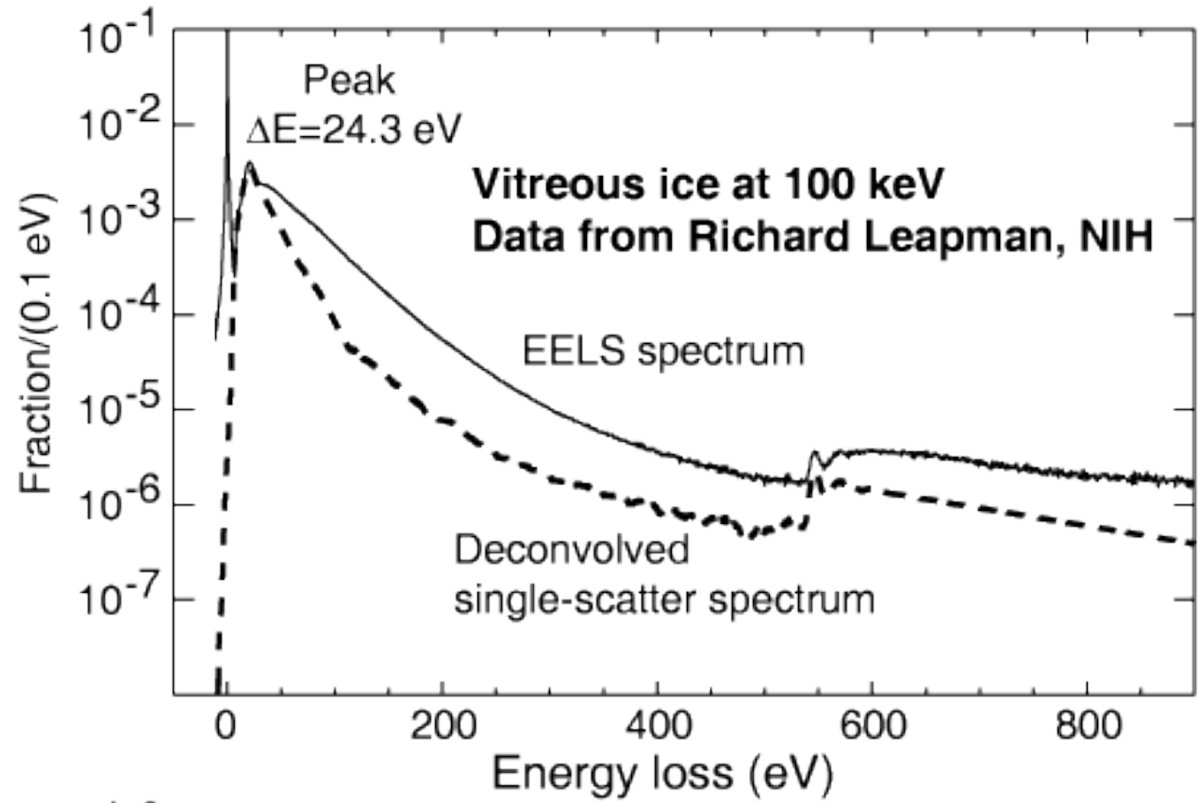
Voids in Cu interconnects: slices from a tomographic reconstruction.
Ercius *et al.*, *Appl. Phys. Lett.* **88**, 243116 (2006)

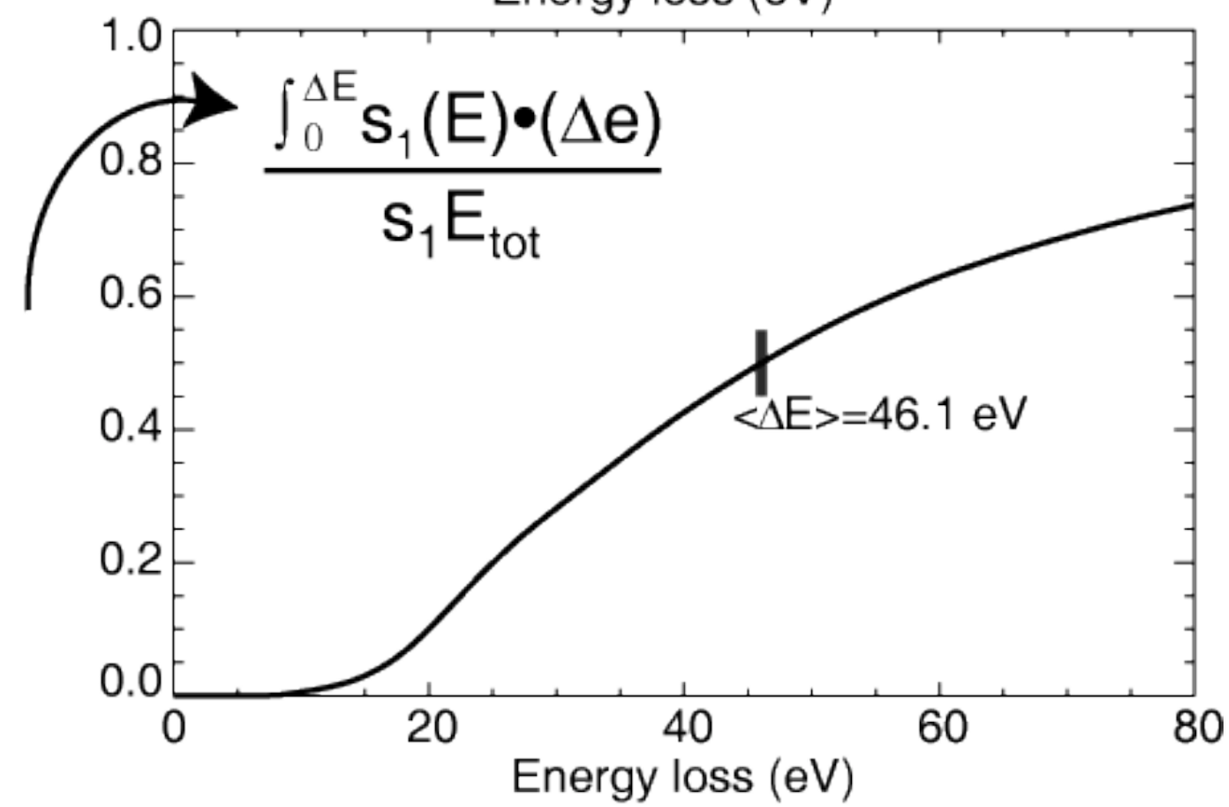
Electrons: inelastic energy deposition

Inelastic scattering: energy transfer of 35-45 eV

At 100 keV, 1 $e^-/nm^2 \Rightarrow 3.2 \times 10^4$ Gray

(1 Gray=1 J/kg)





Atomic resolution imaging: electrons or photons?

10 keV photons

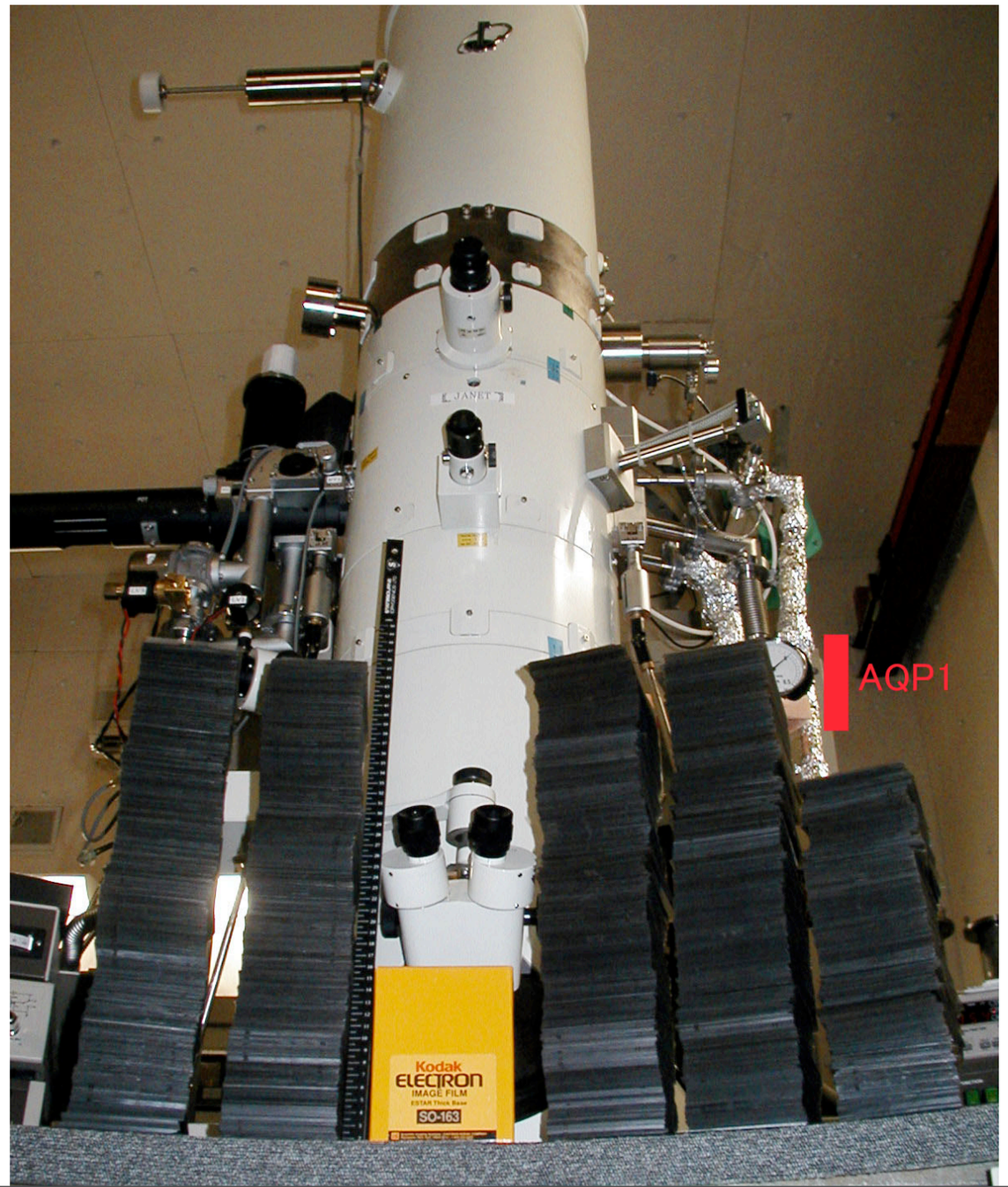
- About 100 absorption events per elastic scatter
- About 10 keV deposited per absorption
- Therefore about 10^6 eV deposited per elastic scatter
- A thousand scattered photons: $10^3 \bullet 10^6$ eV into $(2\ \text{Å})^3$, or 2×10^{13} Gray

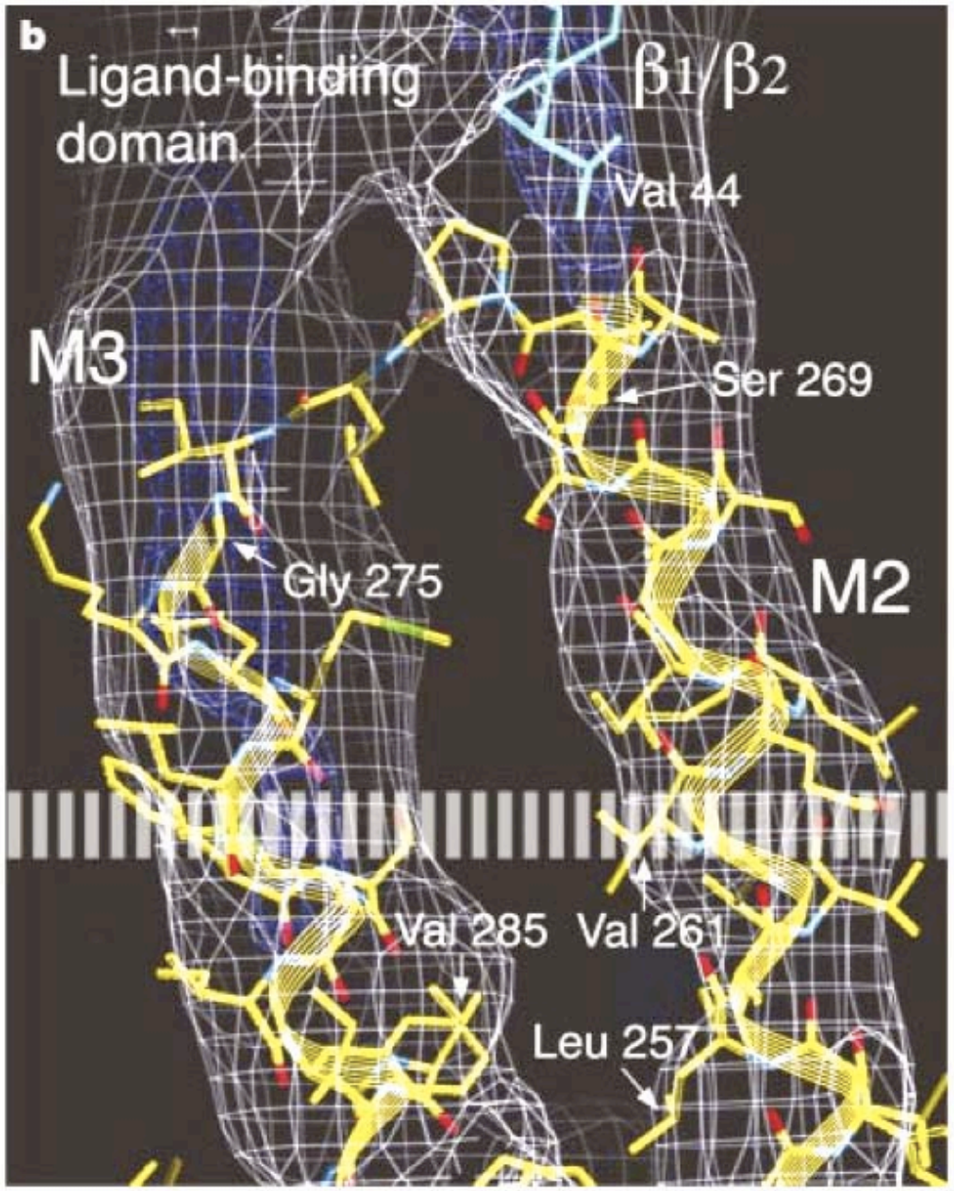
100 keV electrons

- About 2.5 inelastic scatters per elastic scatter
- About 45 eV deposited per inelastic scatter
- Therefore about 10^2 eV deposited per elastic scatter
- A thousand scattered electrons: $10^3 \bullet 10^2$ eV into $(2\ \text{Å})^3$, or 2×10^9 Gray

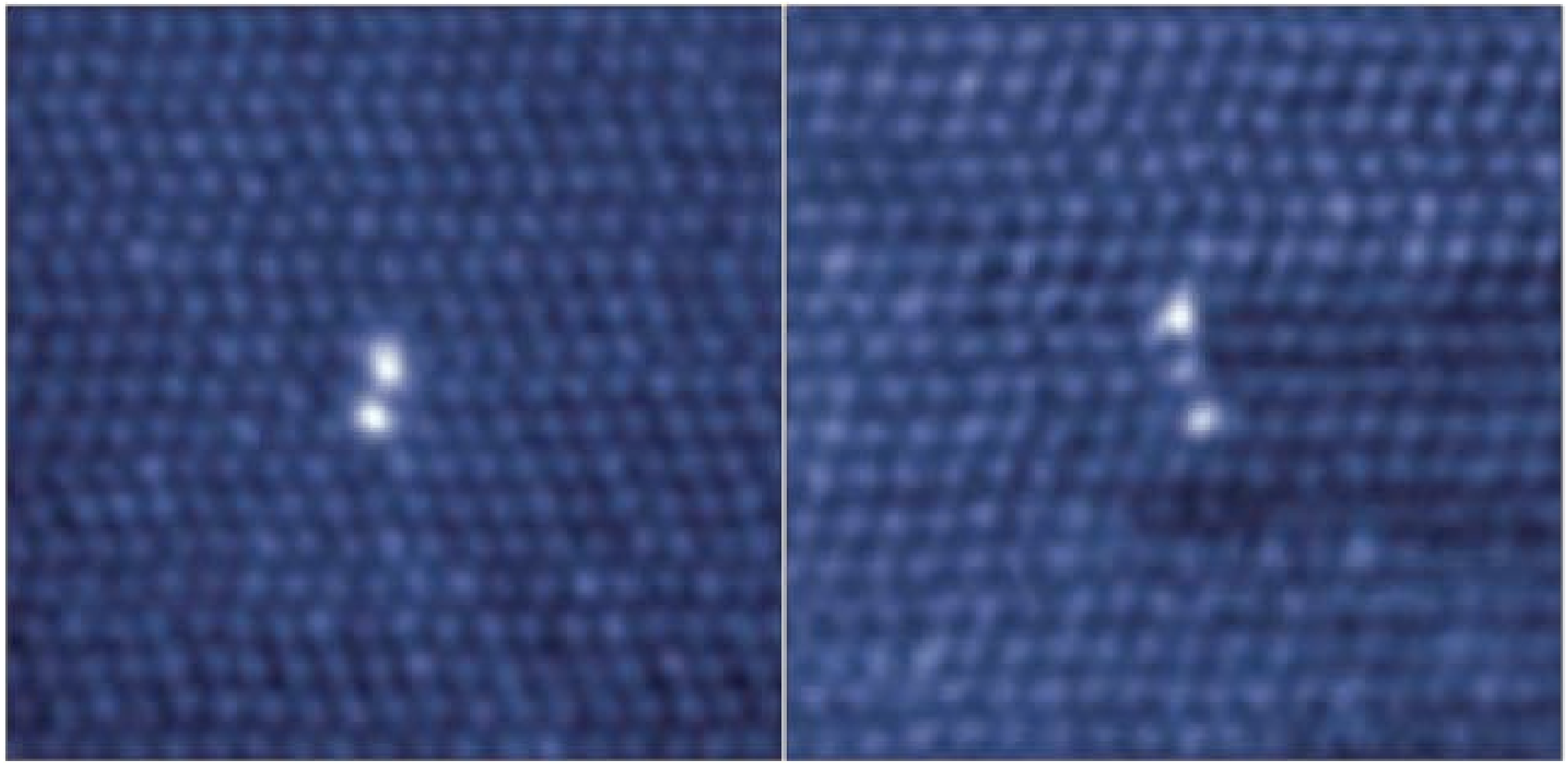
- Electrons are better than photons for atomic resolution imaging: J. Breedlove and G. Trammel, *Science* **170**, 1310 (1970); R. Henderson, *Q. Rev. Biophys*. **28**, 171 (1995).
- Crystallography's answer: spread the dose out over many identical unit cells
- XFEL answer: get the information before it's damaged

Acetylcholine receptor at 4 Å. Miyazawa, Fujiyoshi, and Unwin, *Nature* **423**, 949 (2003). AQP1: aquaporin-1, Murata, Mitsuoka, Hirai, Walz, Agre, Heymann, Engel, and Fujiyoshi, *Nature* **407**, 599 (2000). (Agre: 2003 Nobel Prize in Chemistry)





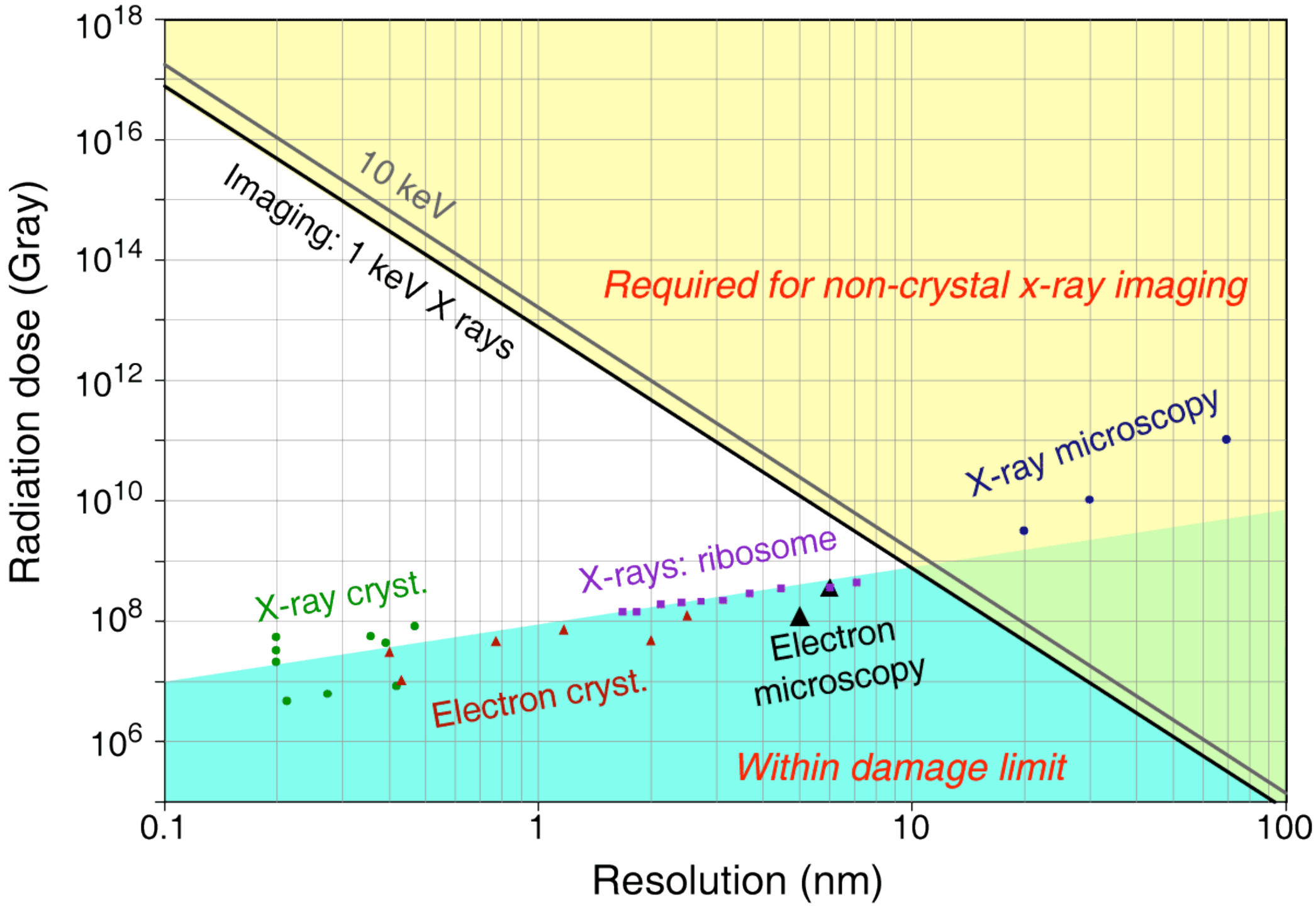
Erbium atom columns (10-15 atoms each) in 8-20 nm thick silicon (001; lattice spacing 0.252 nm). Kaiser *et al.*, *Nature Materials* **1**, 102 (2002).



What's x-ray diffraction microscopy good for?

- High resolution imaging of samples too thick for electrons.
  - Alternative route to the challenges of lenses
  - Implies need for 3D imaging.
- Contrast mechanisms which are unique to X rays, or which X rays do better. But these include 10-100 image spectromicroscopy data, trace elements via fluorescence...
- Beating damage limits (and pulsed electron beam space-charge limits) by going to femtosecond pulses.

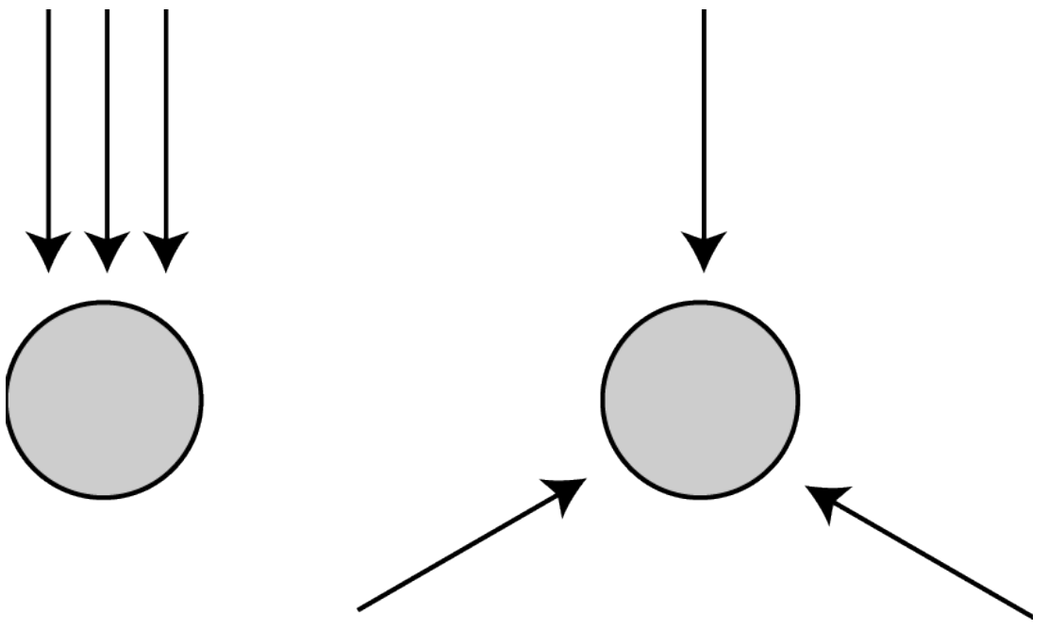
What's the limit for cells?



Howells et al., *JESRP* (submitted).
See also Shen et al., *J. Sync. Rad.* **11**, 432 (2004)

Dose fractionation

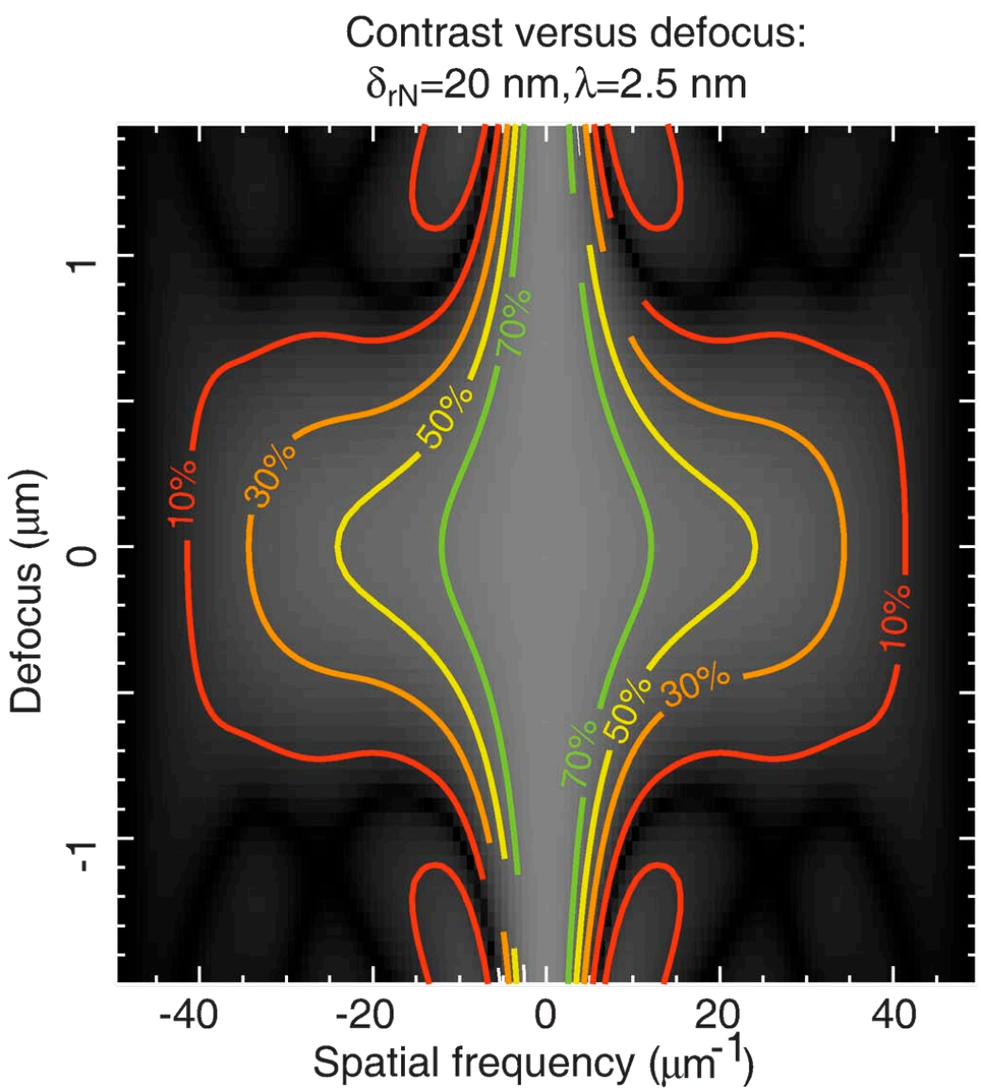
- You can divide the number of photons needed for a good 2D view into 3D views.
- Hegerl and Hoppe, *Z. Naturforschung* **31a**, 1717 (1976); McEwen *et al.*, *Ultramic.* **60**, 357 (1995).



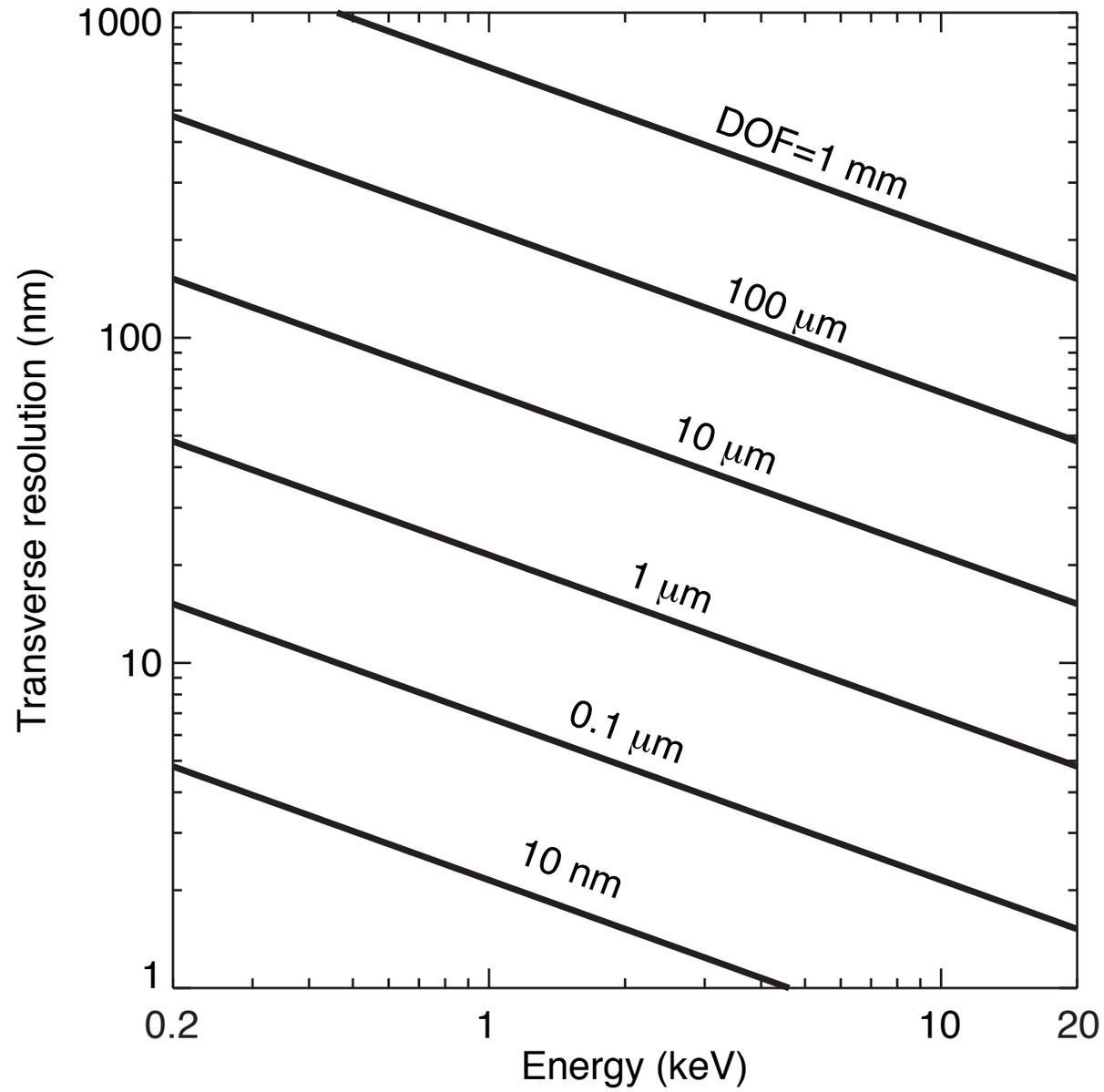
3D imaging with lenses

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

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



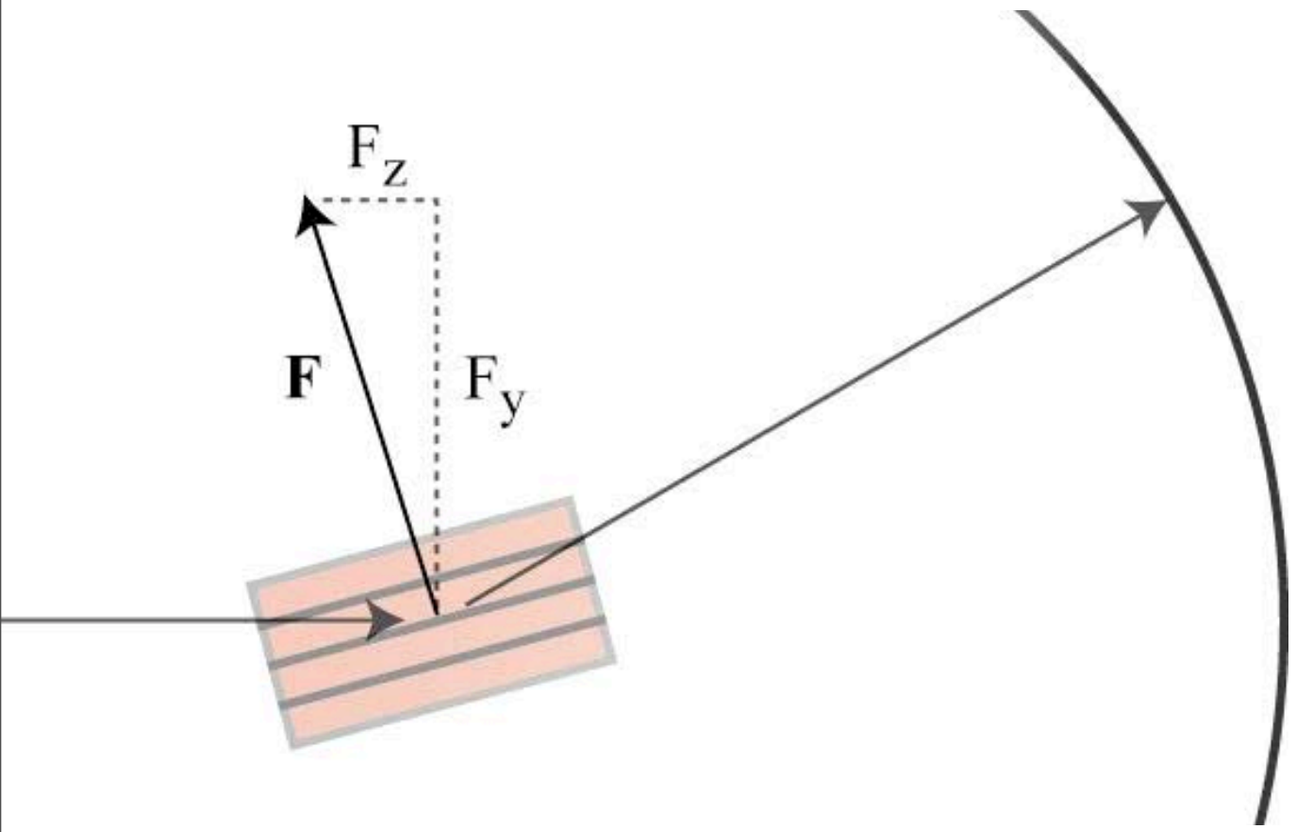
20 nm resolution at 520 eV: depth of field ~1 μm



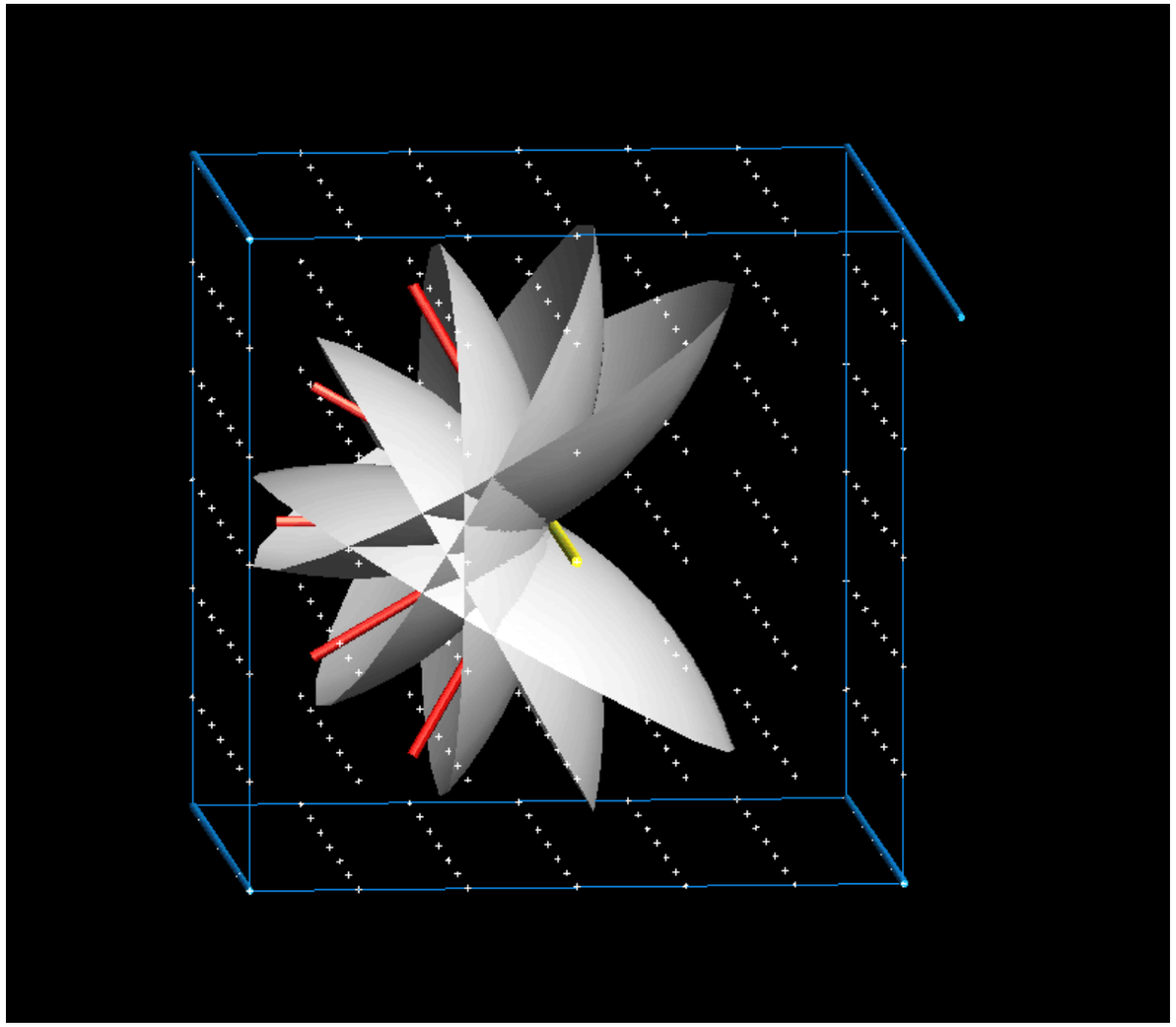
Through-focus deconvolution with lenses:

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

Diffraction microscopy in 3D



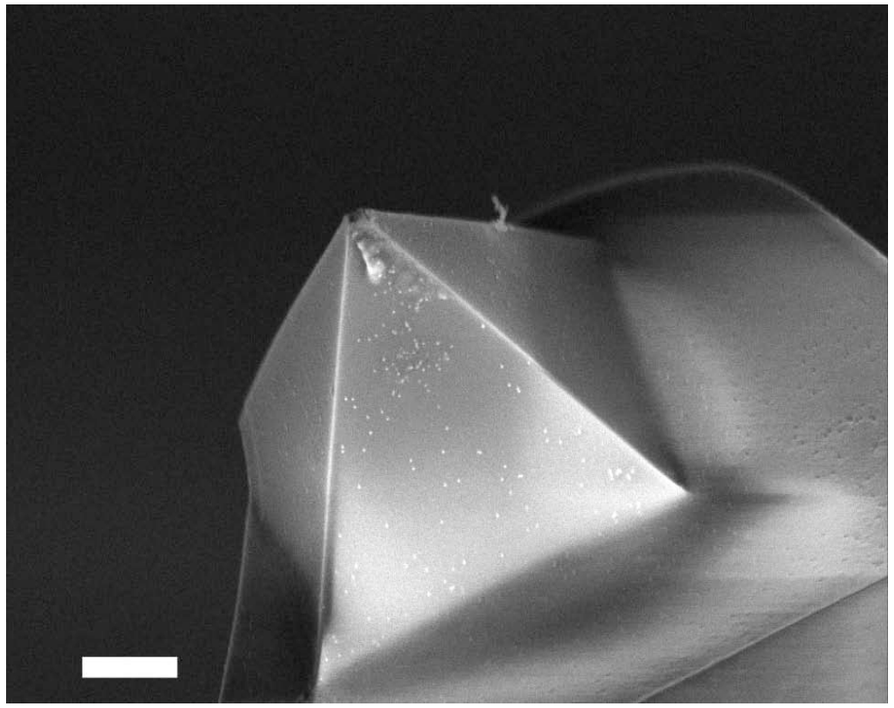
Bragg gratings that diffract to a certain angle represent a specific transverse and longitudinal periodicity (Ewald sphere)

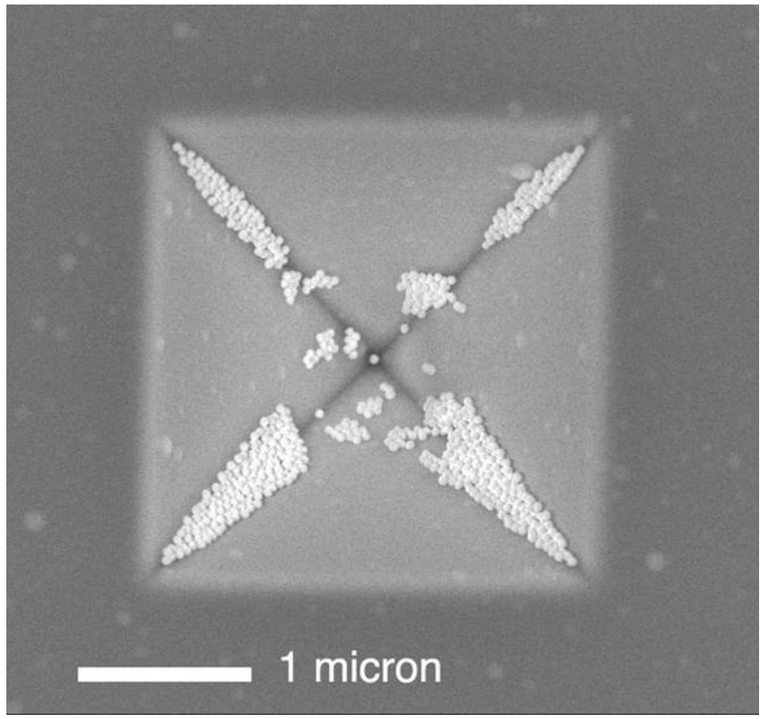


Data collection over a series of rotations about an axis fills in 3D Fourier space for phasing

3D diffraction microscopy of materials science specimens

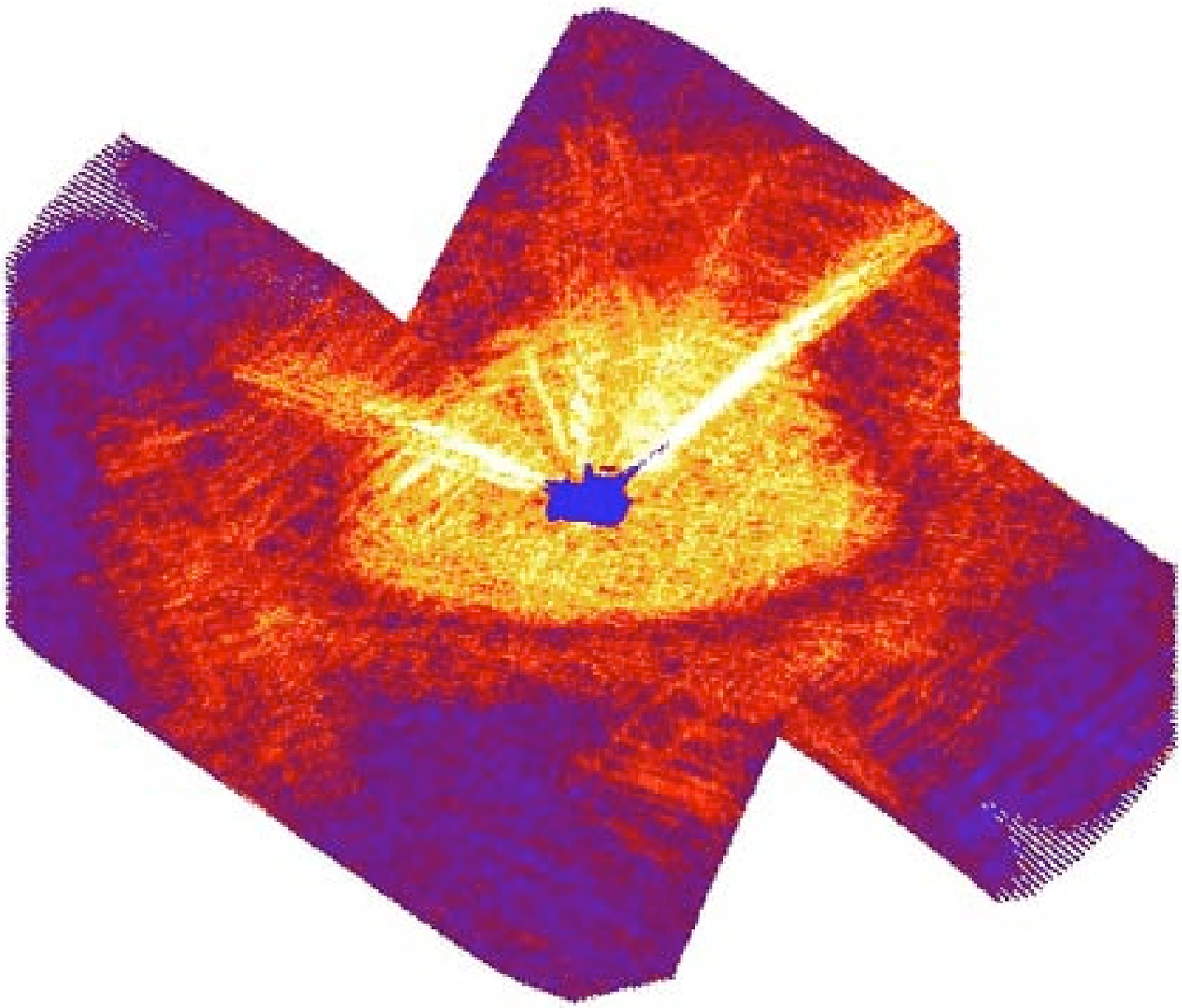
- Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)
- 50 nm gold spheres placed on hollowed AFM tip "pyramid"
- Data taken using Stony Brook apparatus at ALS beamline 9.0.1





3D data cube

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

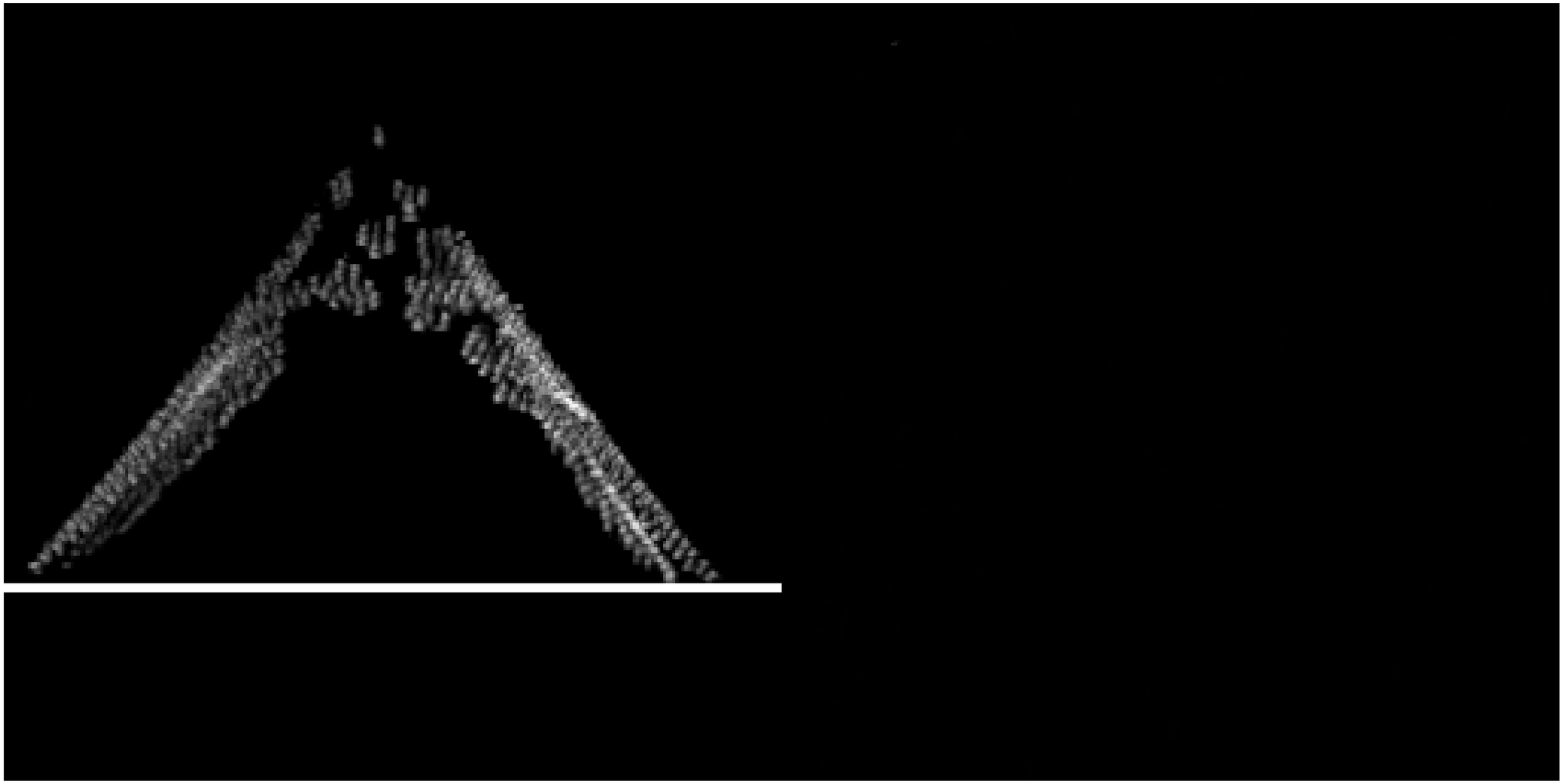


Reconstruction

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

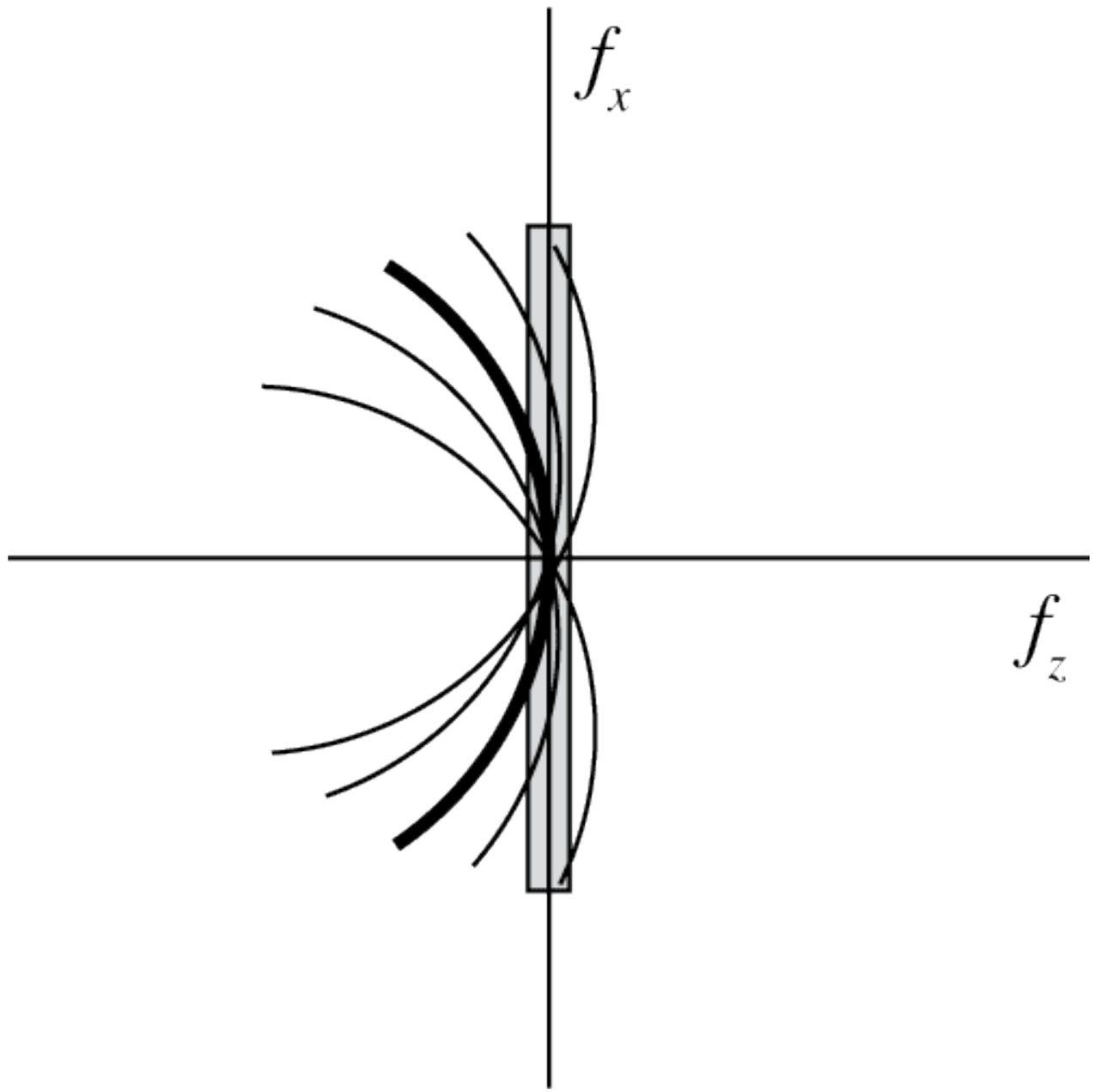
Slices through reconstruction

- Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)
- Resolution ~10x10x50 nm

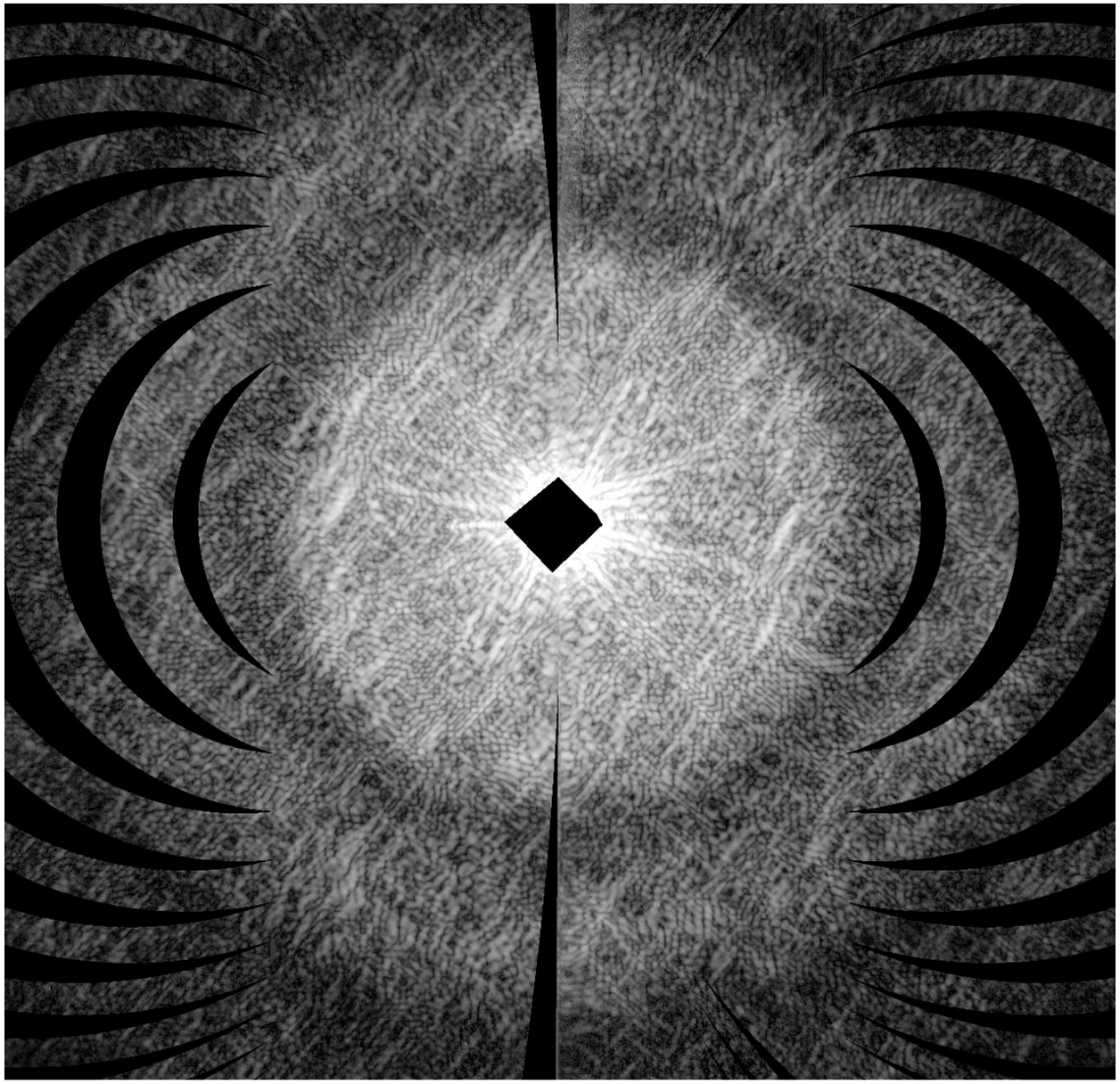


Pure projections from phased 3D data

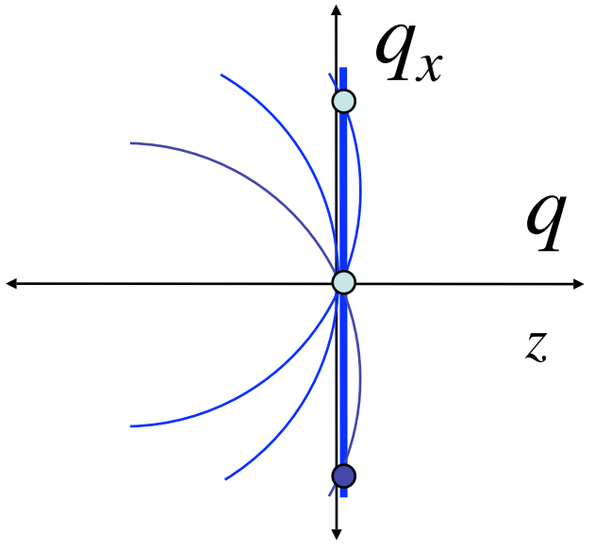
Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)



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)



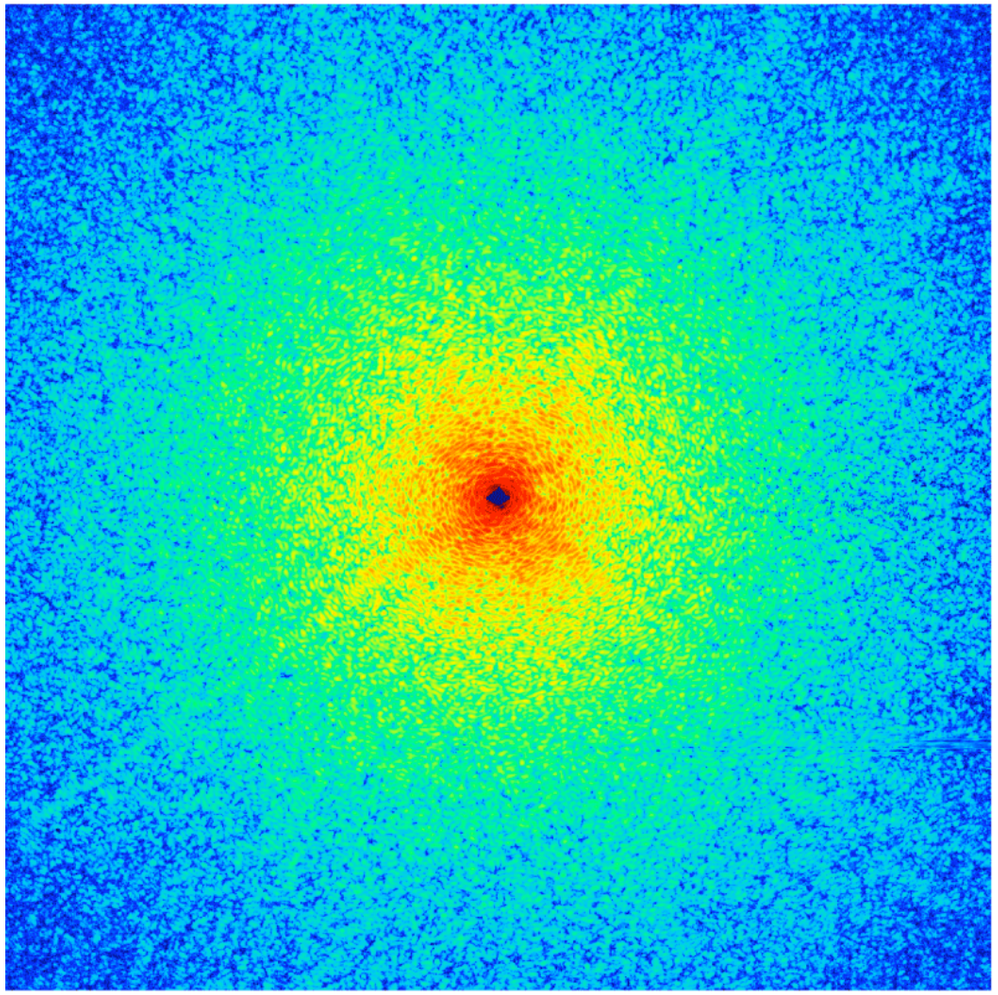
Great Barrier Reef, Australia, August 2006

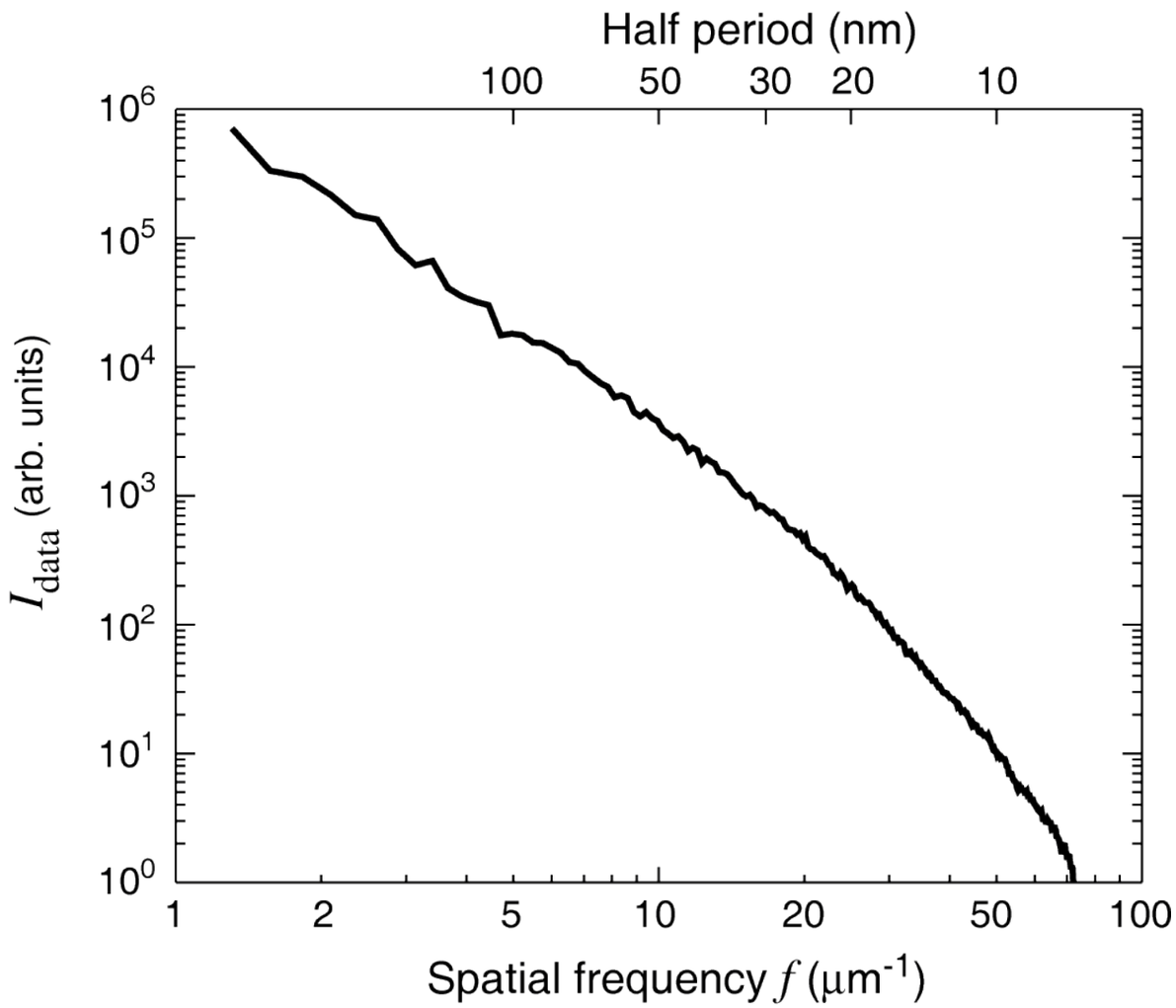
Challenges and lurking dangers

- When your lens is an iterative computer algorithm, how can you trust the “lens”?
- Obeying the law of information: empty specimen? True knowledge of curved wavefield or pinhole?
- Experimental realities: how to handle cryo specimens? How to place pinholes near cryo specimens?
- Bragg spots? Beware!

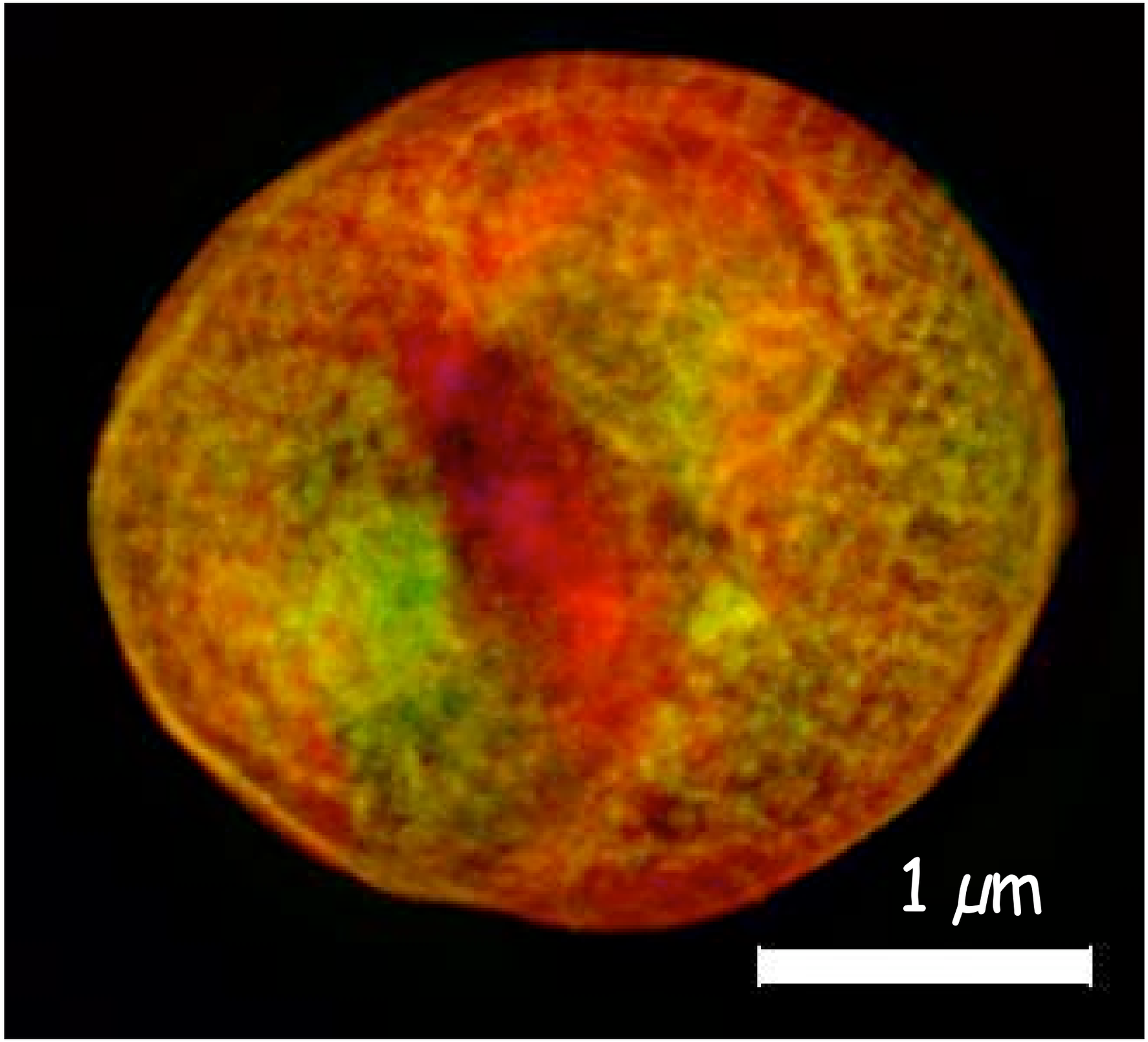
Diffraction from a yeast cell

- Shapiro, Thibault, Beetz, Elser, Howells, Jacobsen, Kirz, Lima, H. Miao, Nieman, Sayre, *Proc. Nat. Acad. Sci.* **102**, 15343 (2005). ALS beamline 9.0.1 operated at 750 eV
- Total dose to freeze-dried, room temperature, unstained cell around 10^8 Gray



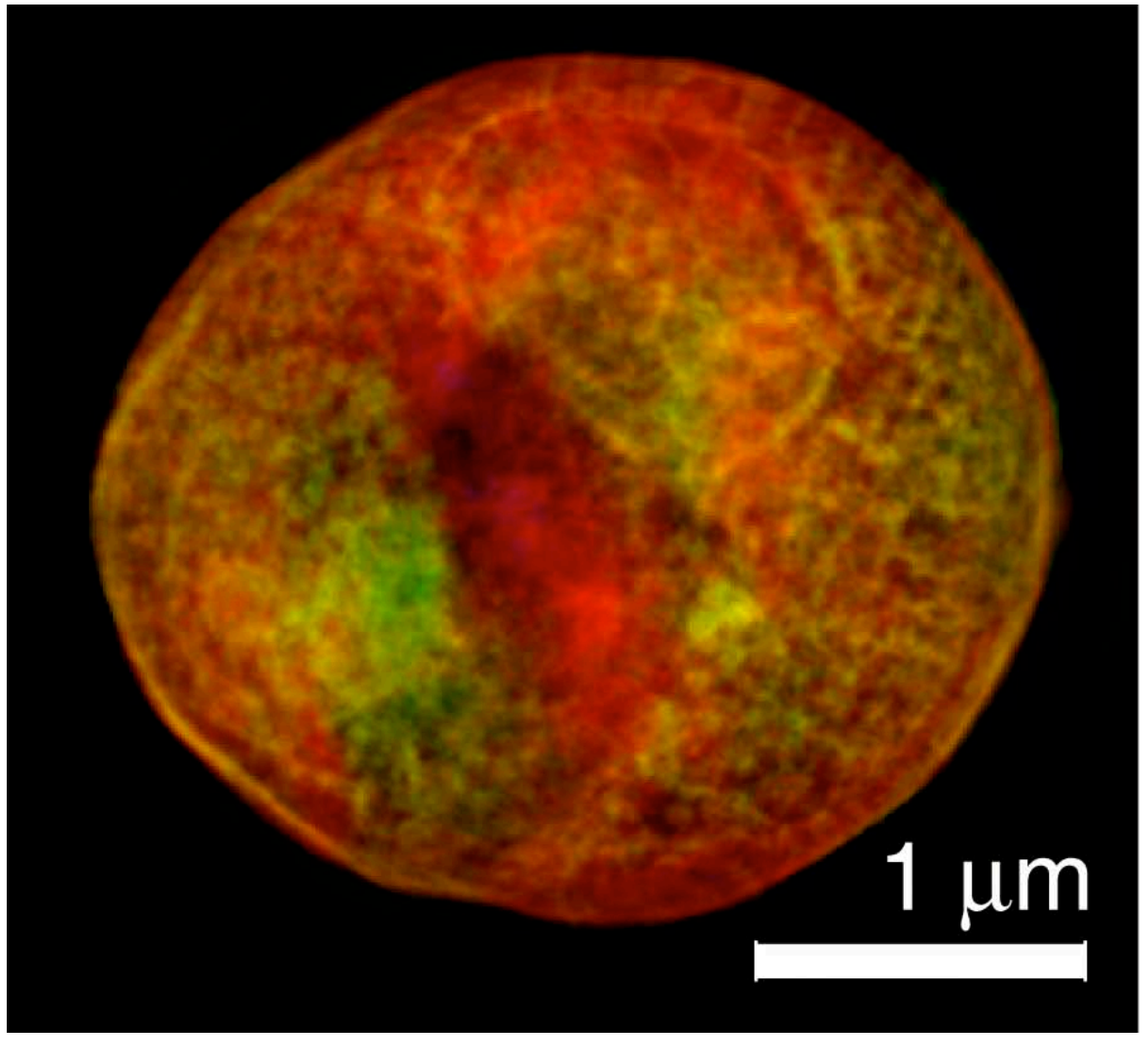


Reconstructed image

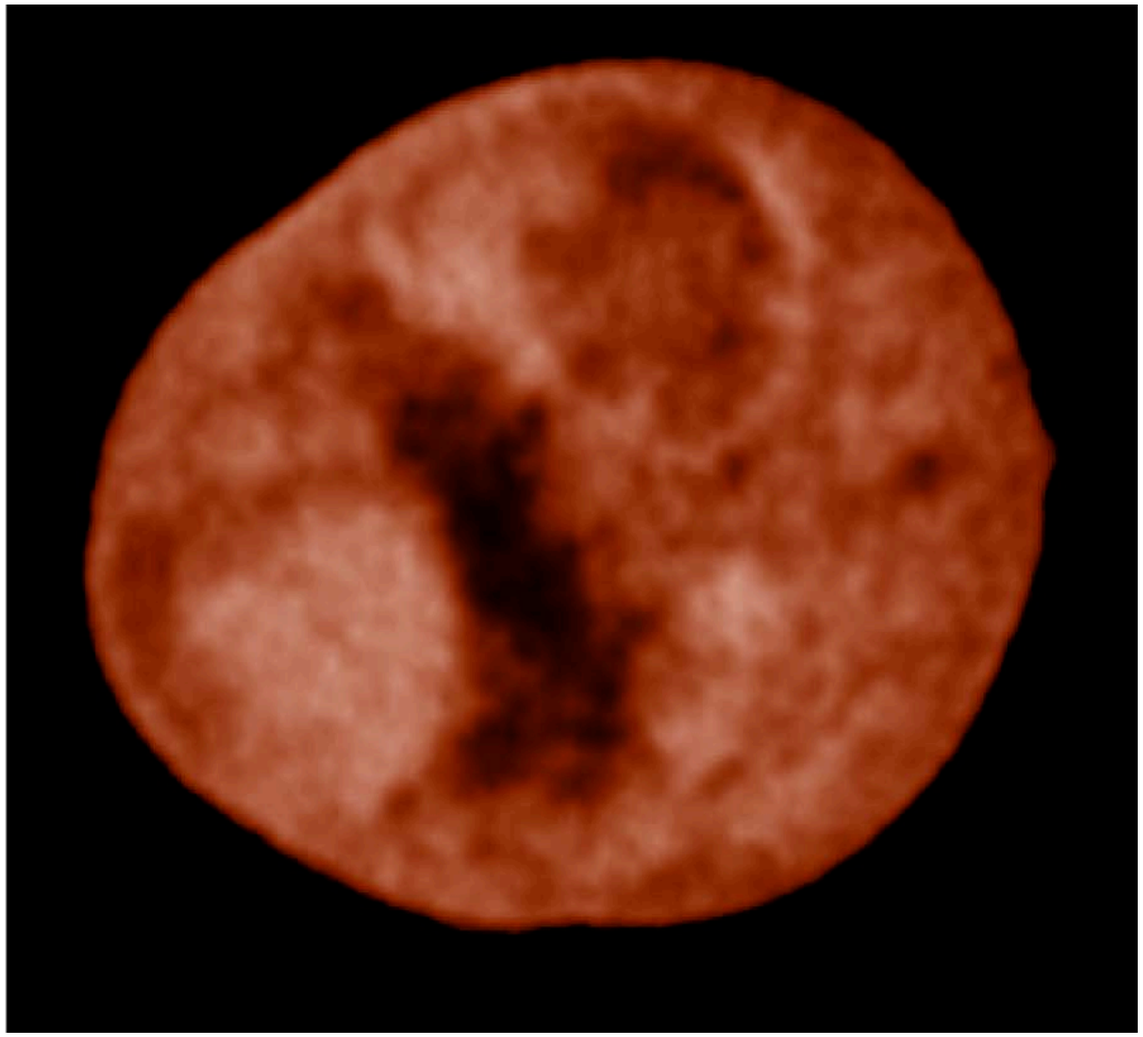


Shapiro *et al.*, *Proc. Nat. Acad. Sci.* **102**, 15343 (2005).

Comparison with a microscope

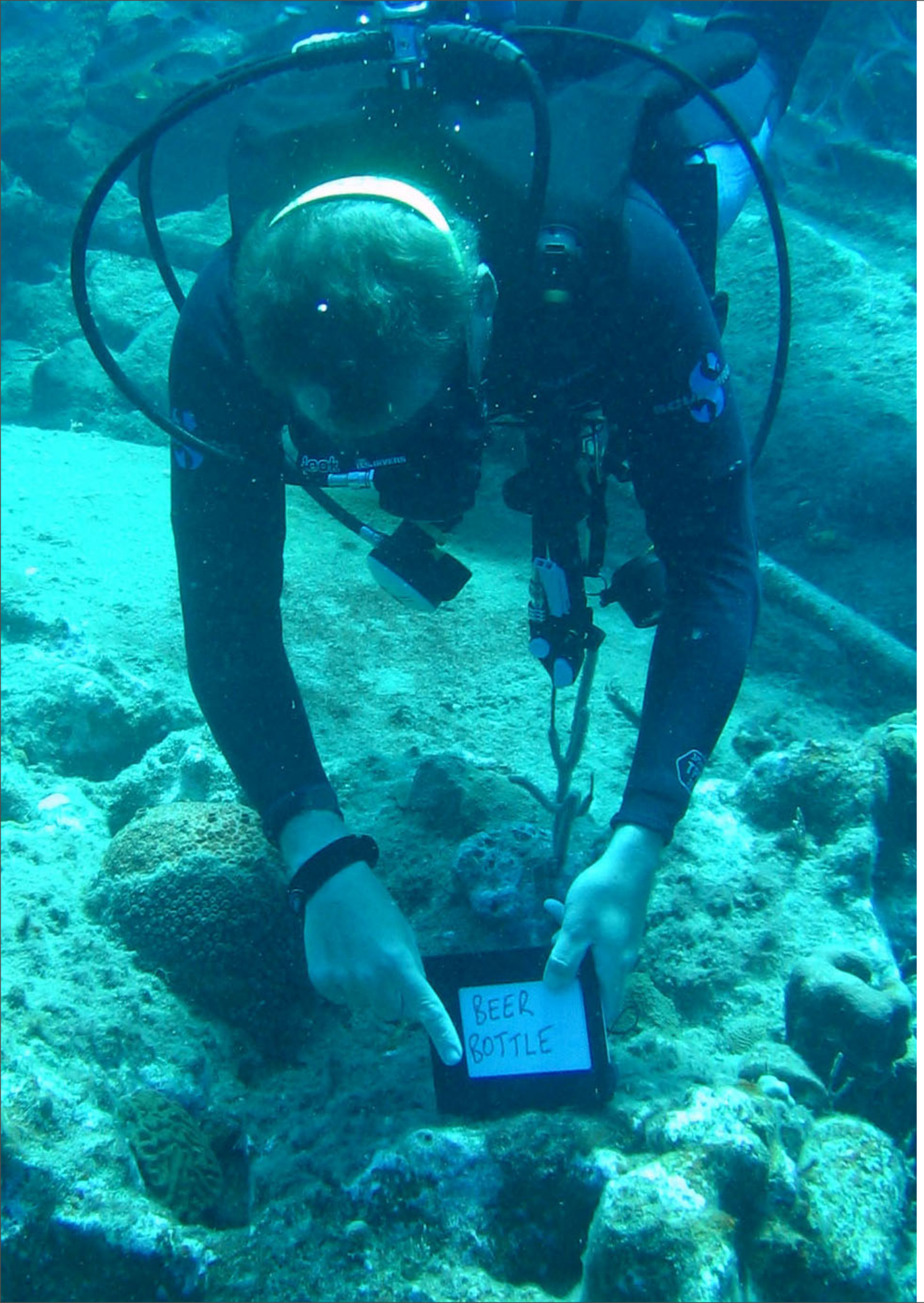


Diffraction reconstruction (data taken at 750 eV; absorption as brightness, phase as hue).

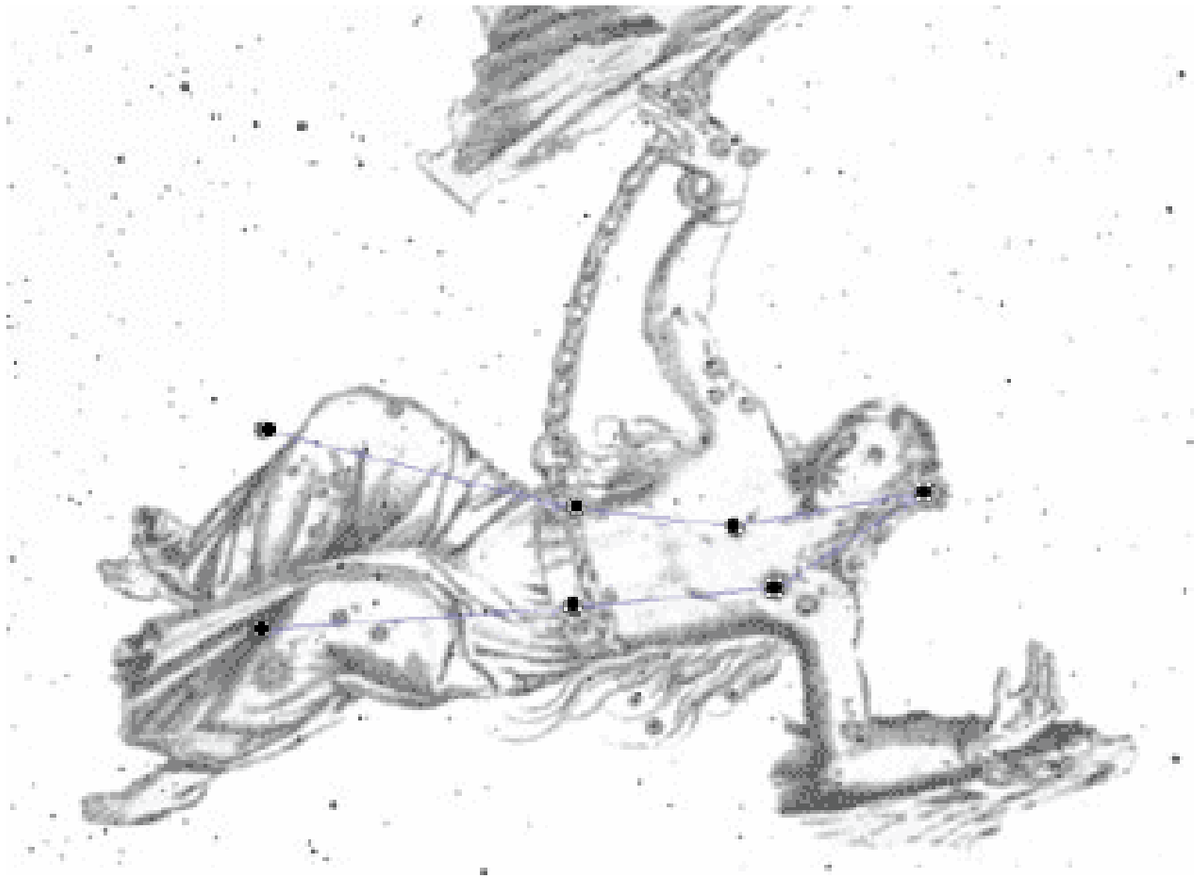


Stony Brook/NSLS STXM image with 45 nm Rayleigh resolution zone plate at 520 eV (absorption as brightness)

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

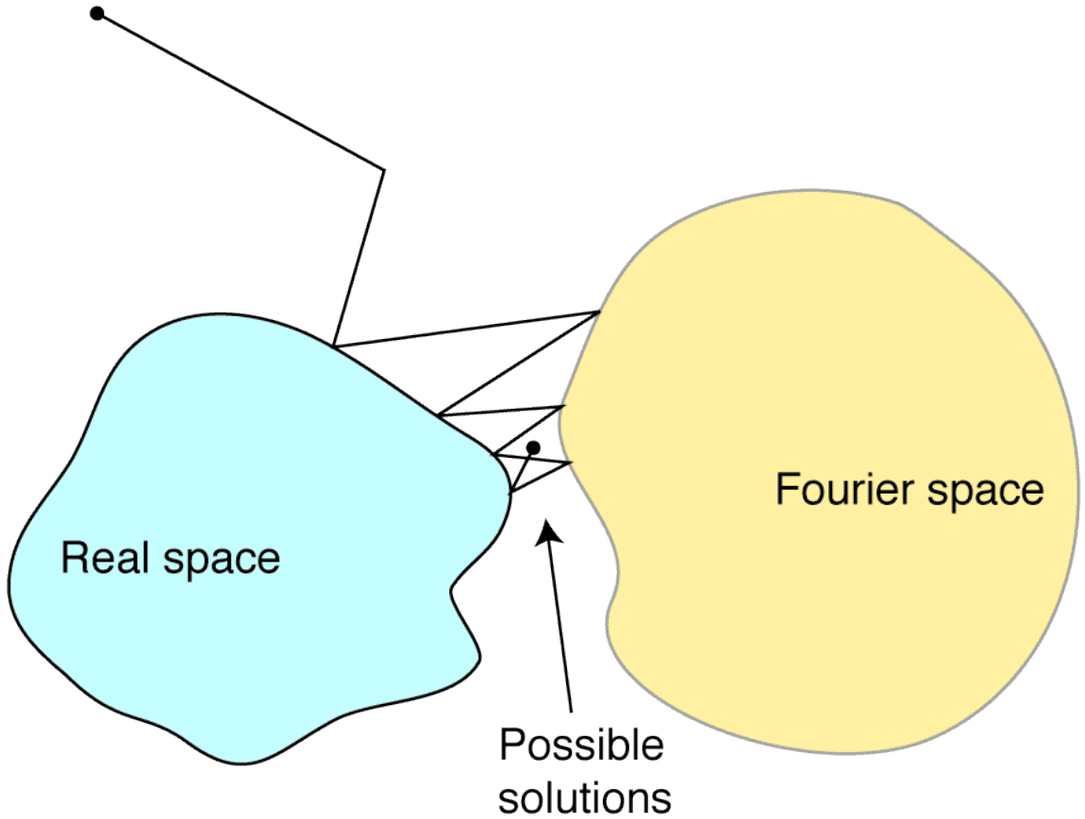


Should we see what we believe?

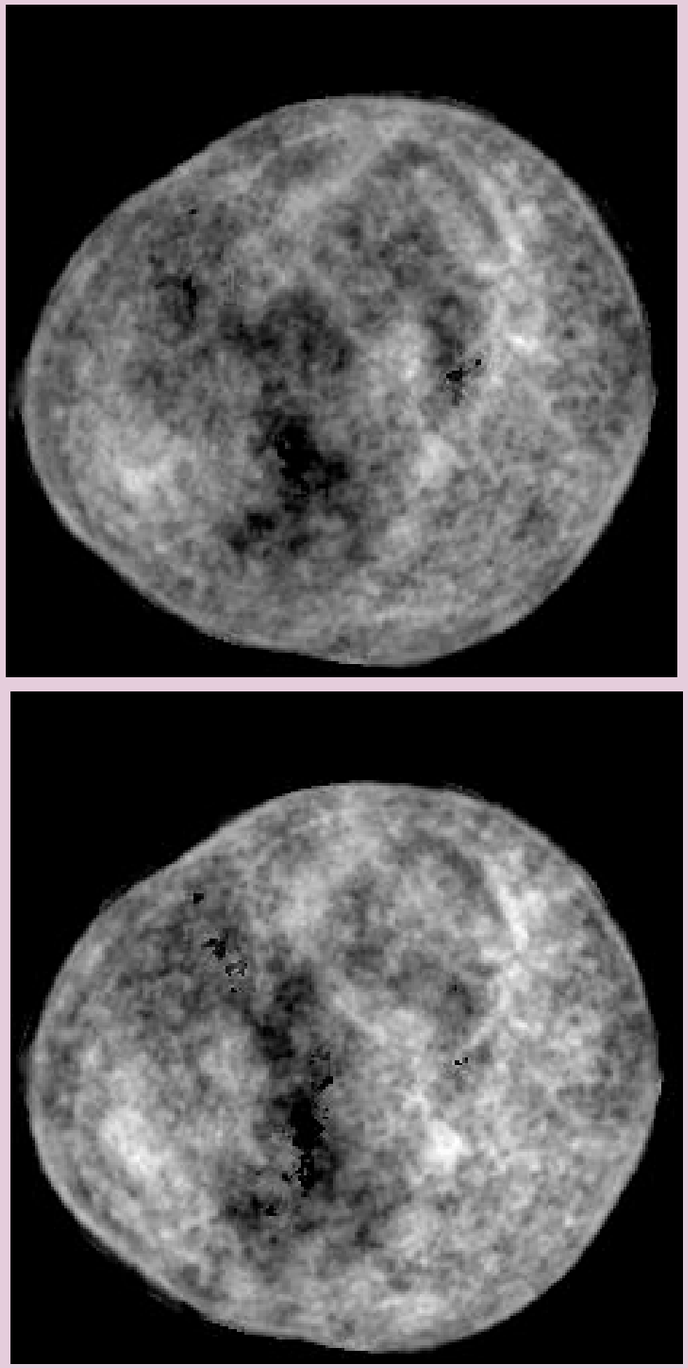


The constellation
Andromeda

Fluctuating solutions



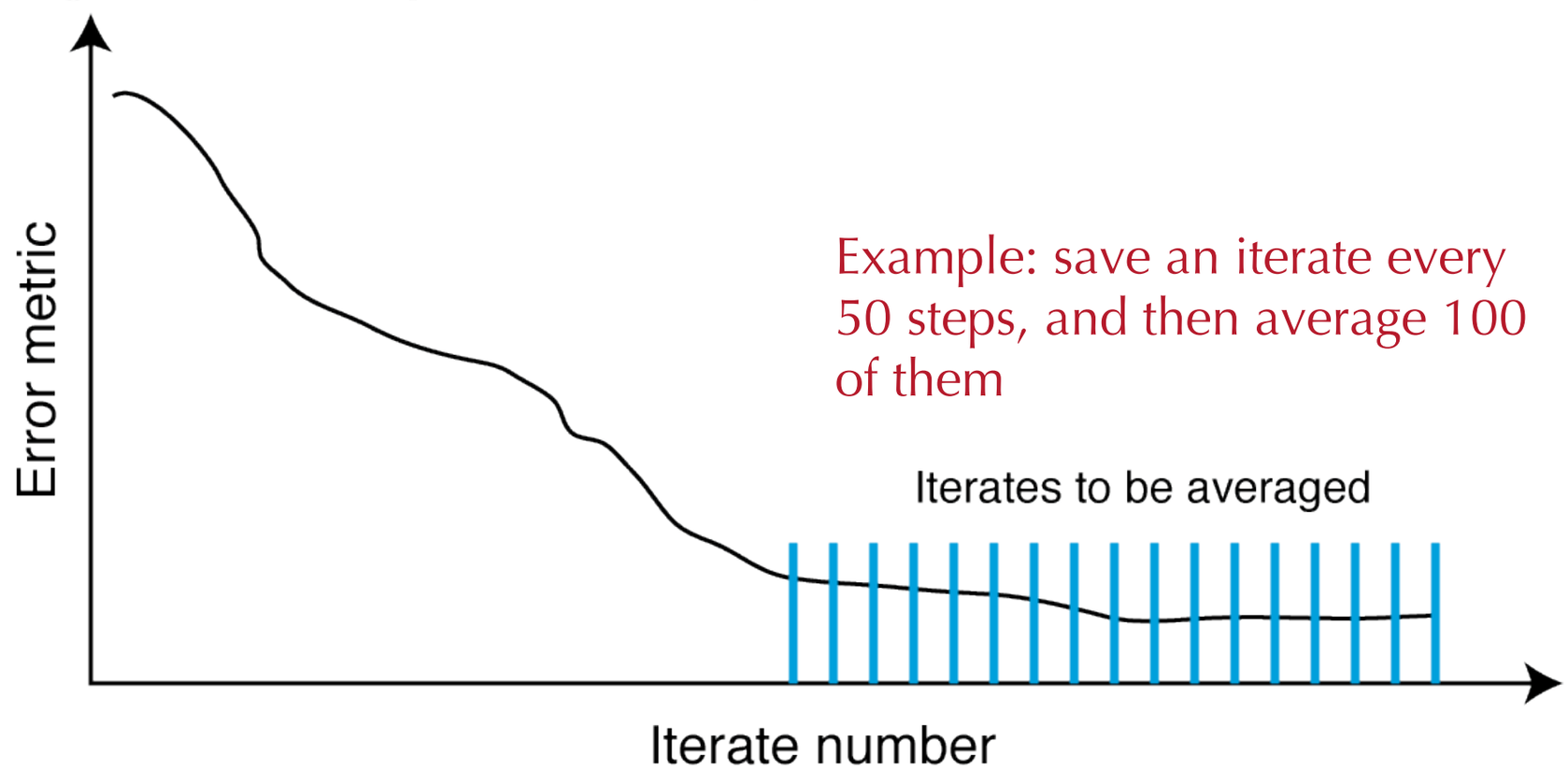
- Ideally, there would be a single point of best agreement between various constraints.
- Noise and incomplete constraints only provide a neighborhood of acceptable solutions.
- Choosing one iterate from one random starting phase as “the” solution is unwarranted!



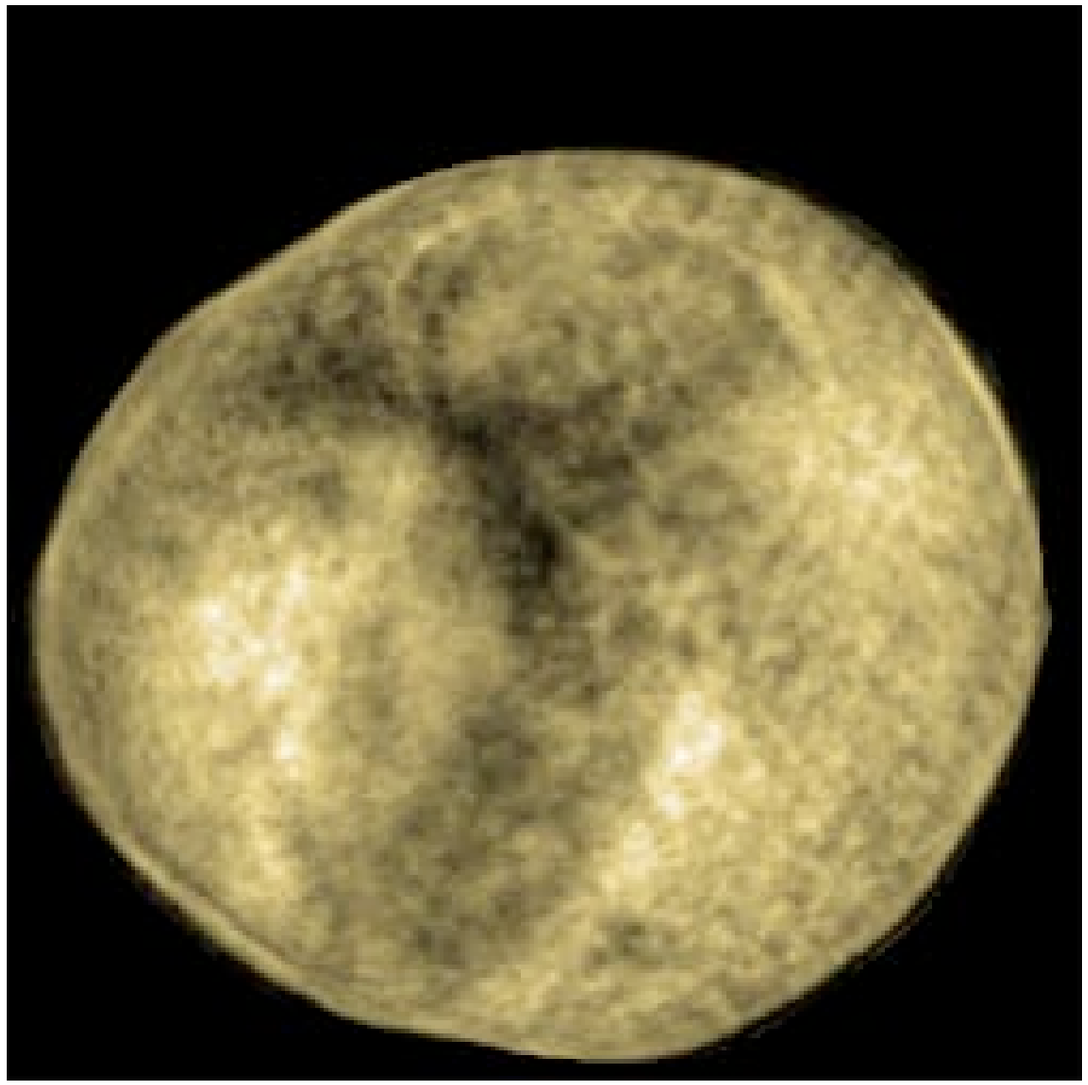
Two individual iterates

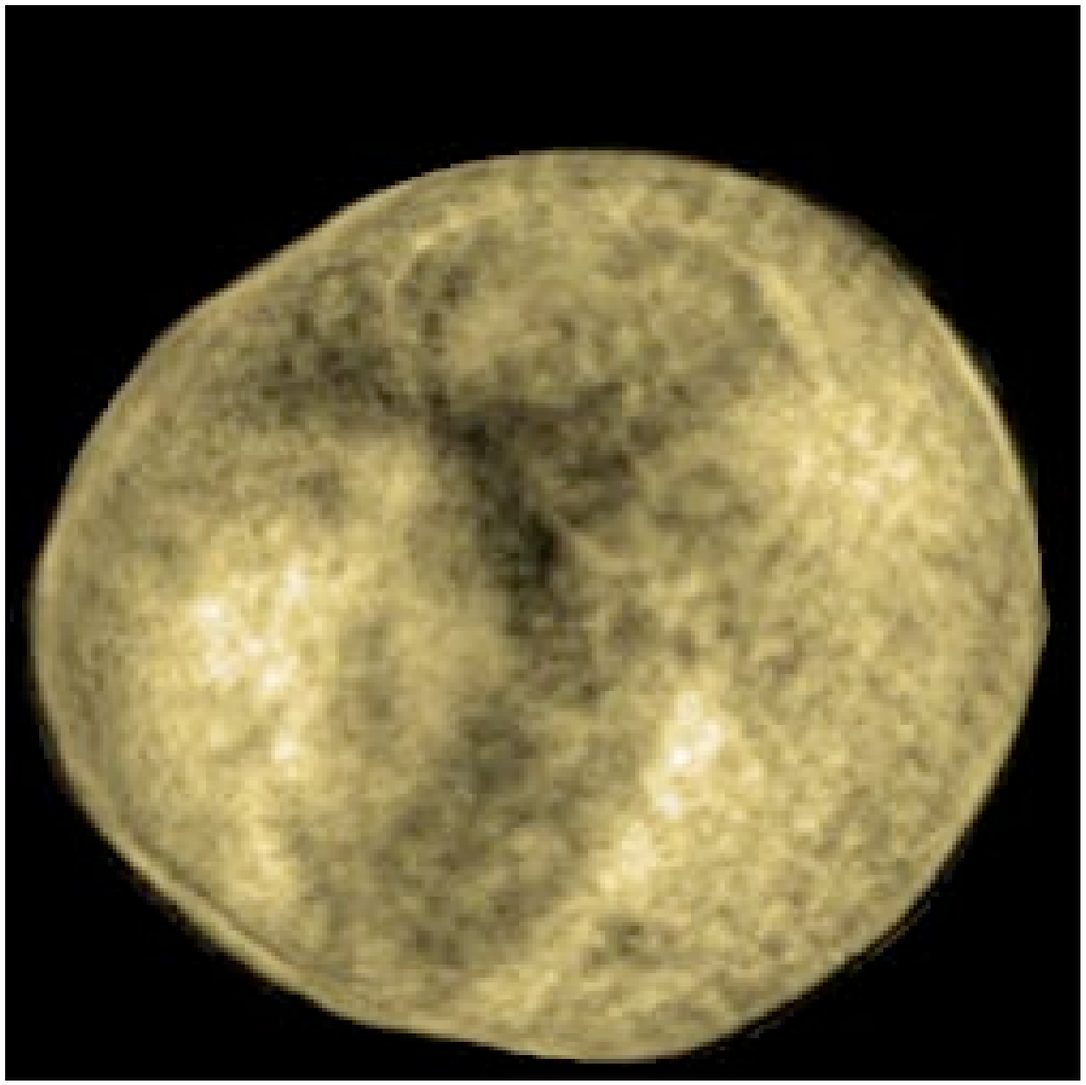
Iterate averaging

- If the solution fluctuates, let's take many samples and average them!
- Non-reproducible phases get washed out; reproducible phases get reinforced
- Thibault, Elser, Jacobsen, Shapiro, and Sayre, *Acta Crystallographica A* **62**, 248 (2006)
- Other approaches: compare results from several different starting random phases (*e.g.*, Miao, Robinson)



Different starting random phases

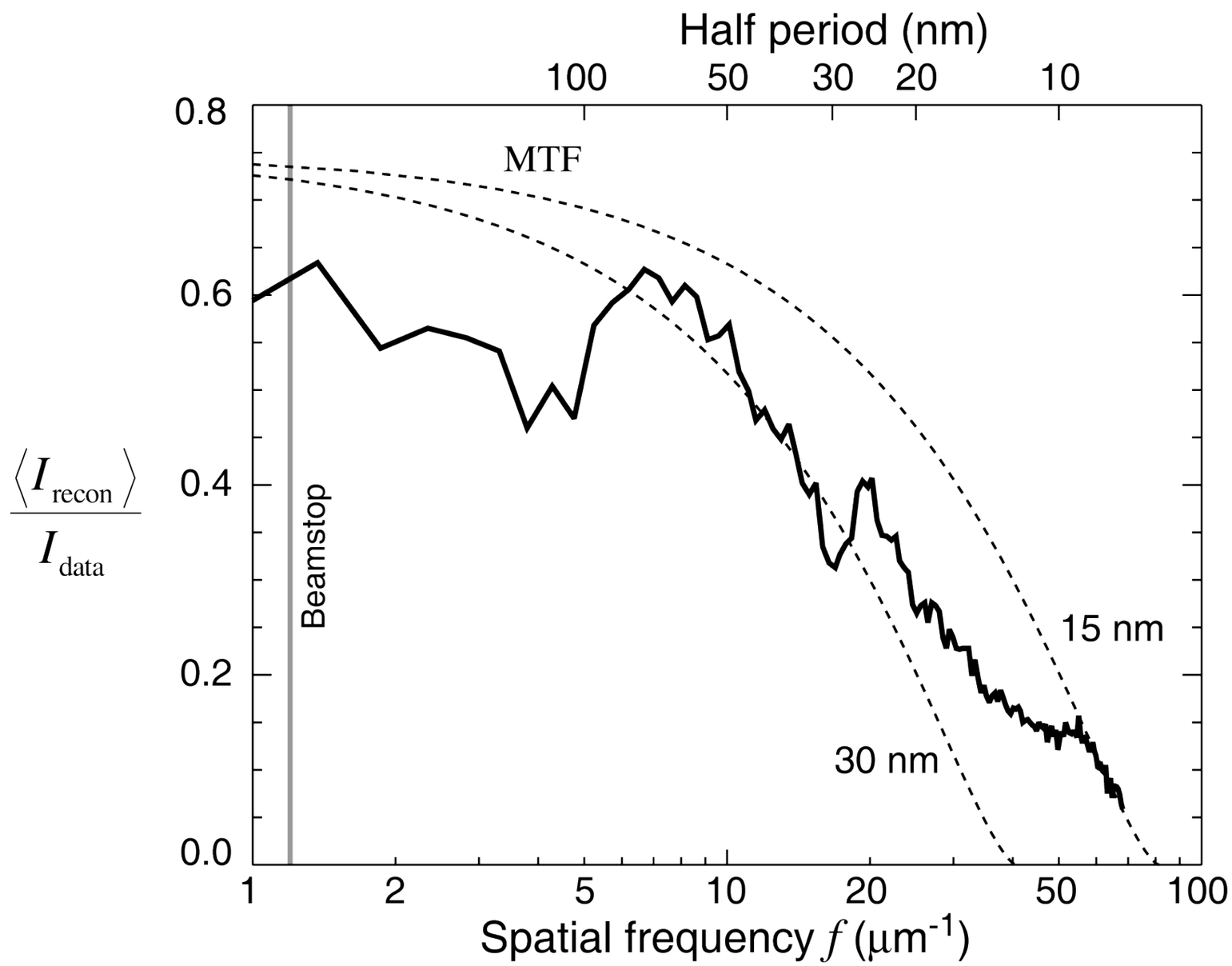




Two separate runs of algorithm with different random starting phases. In both cases, 125 iterates spaced 40 iterations apart were averaged (E. Lima).

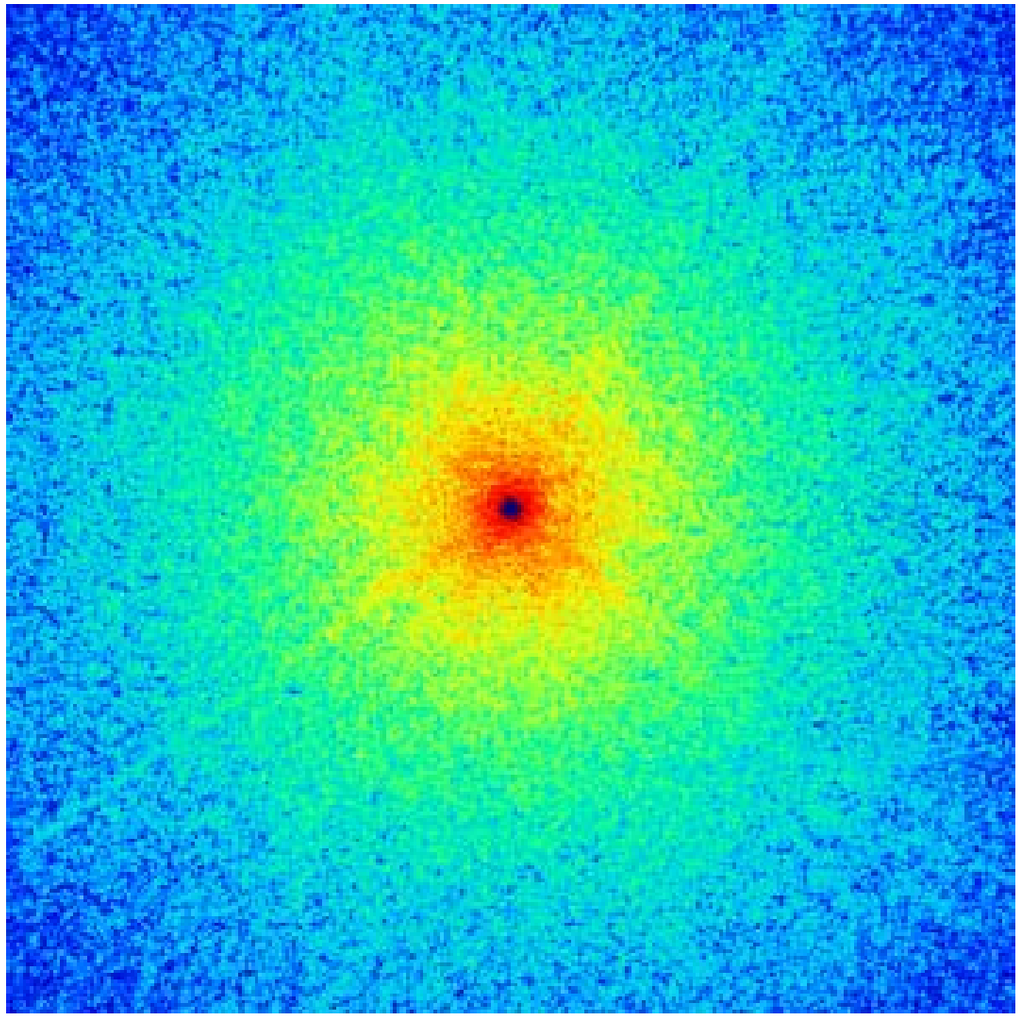
What is the resolution?

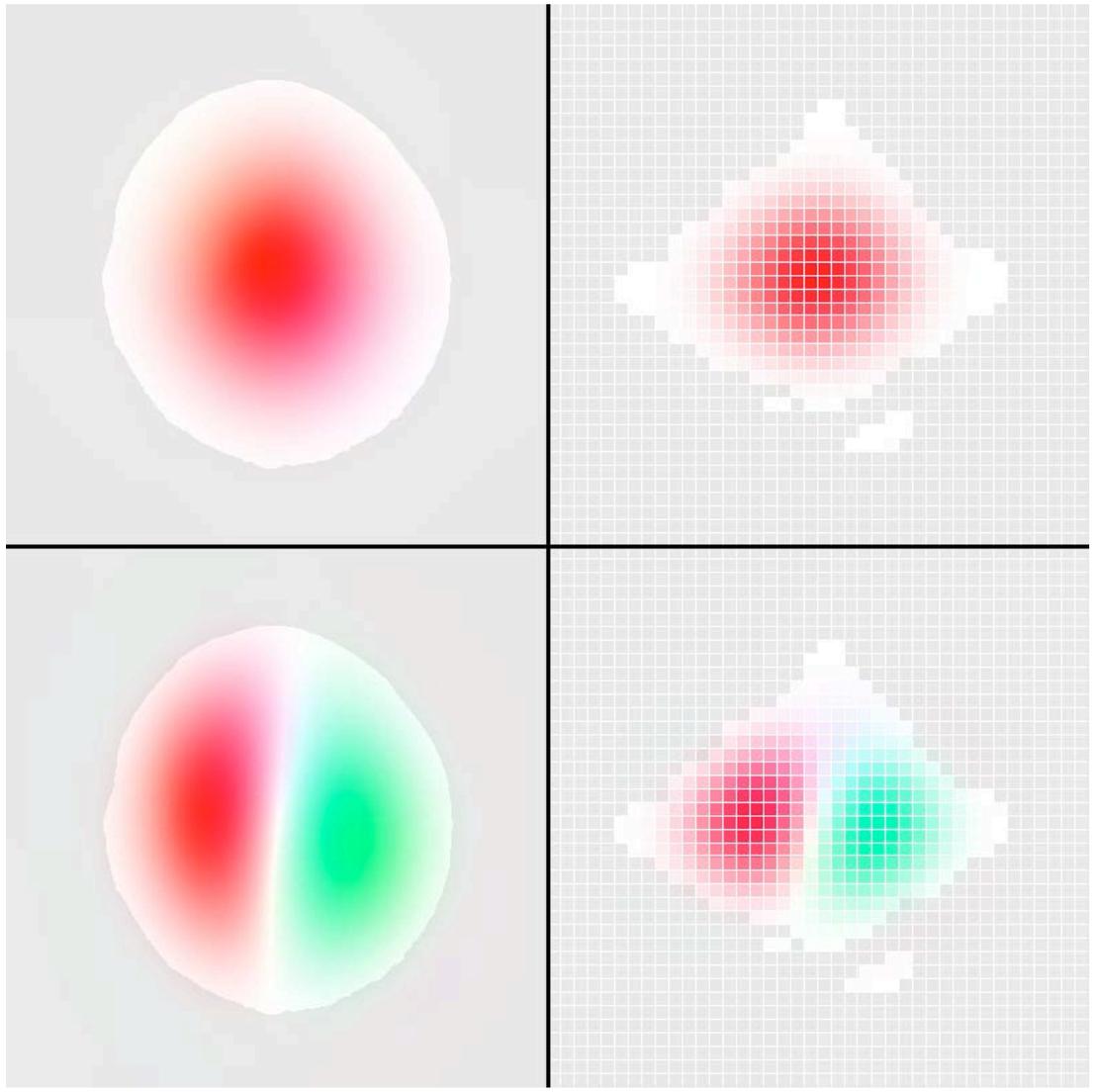
- We do *not* claim maximum angle of speckles as the resolution of the reconstructed image.
- Instead, one can look at how much iterate preserves Fourier intensities at various spatial frequencies – like an MTF.
- Equivalent alternative: phase error versus spatial frequency (Chapman *et al.*)



We miss those low frequencies…

Unconstrained modes in the reconstruction: Thibault, Elser, Jacobsen, Shapiro, and Sayre, *Acta Crystallographica A* **62**, 248 (2006)

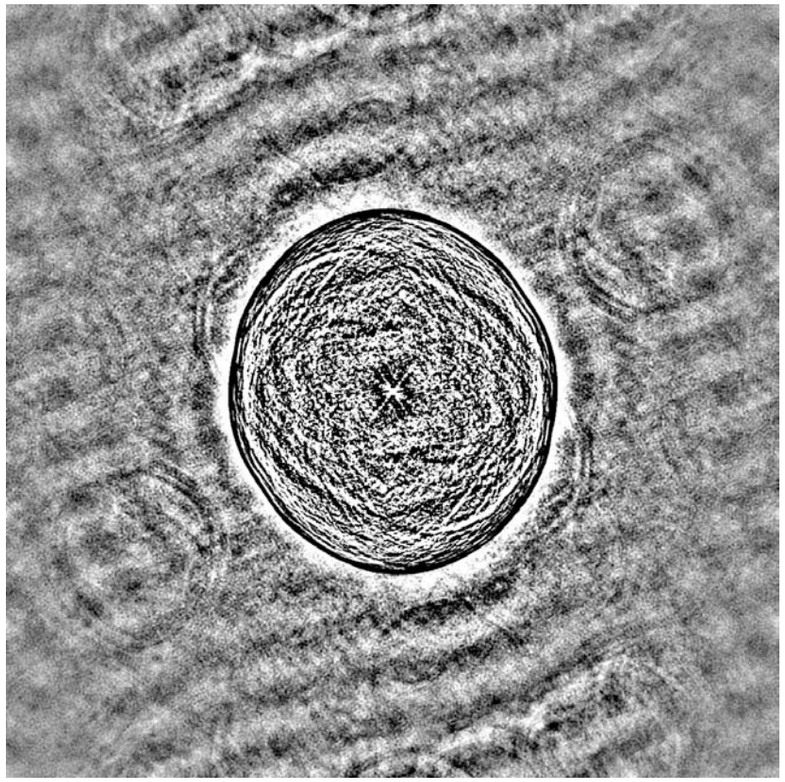




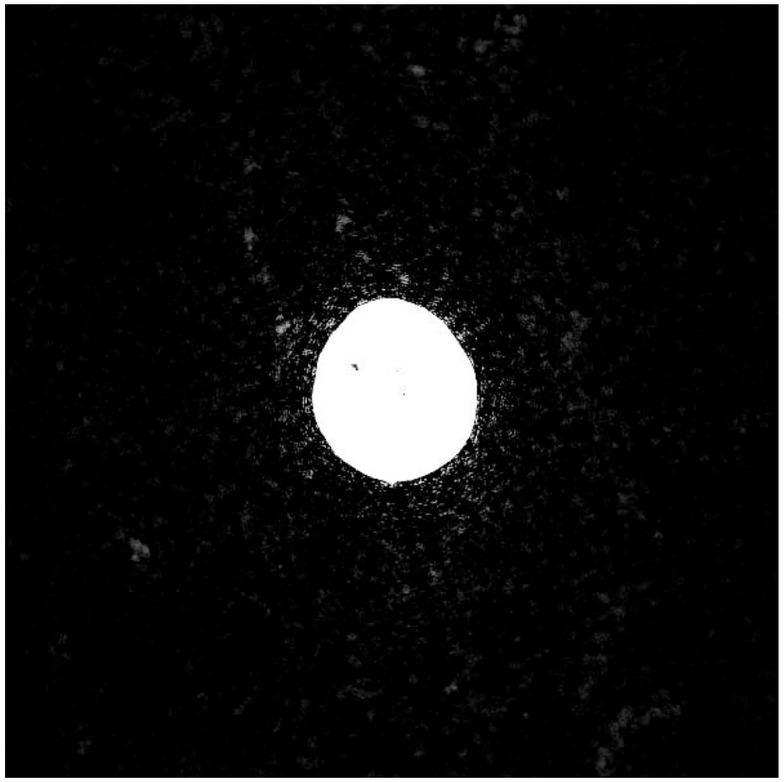
Real space Fourier space

Is it really empty outside the support?

Thibault, Elser, Jacobsen, Shapiro, and Sayre, *Acta Crystallographica A* **62**, 248 (2006)



Real part of the (Laplacian filtered) autocorrelation of the yeast cell data showing evidence for the presence of dirt around the cell.

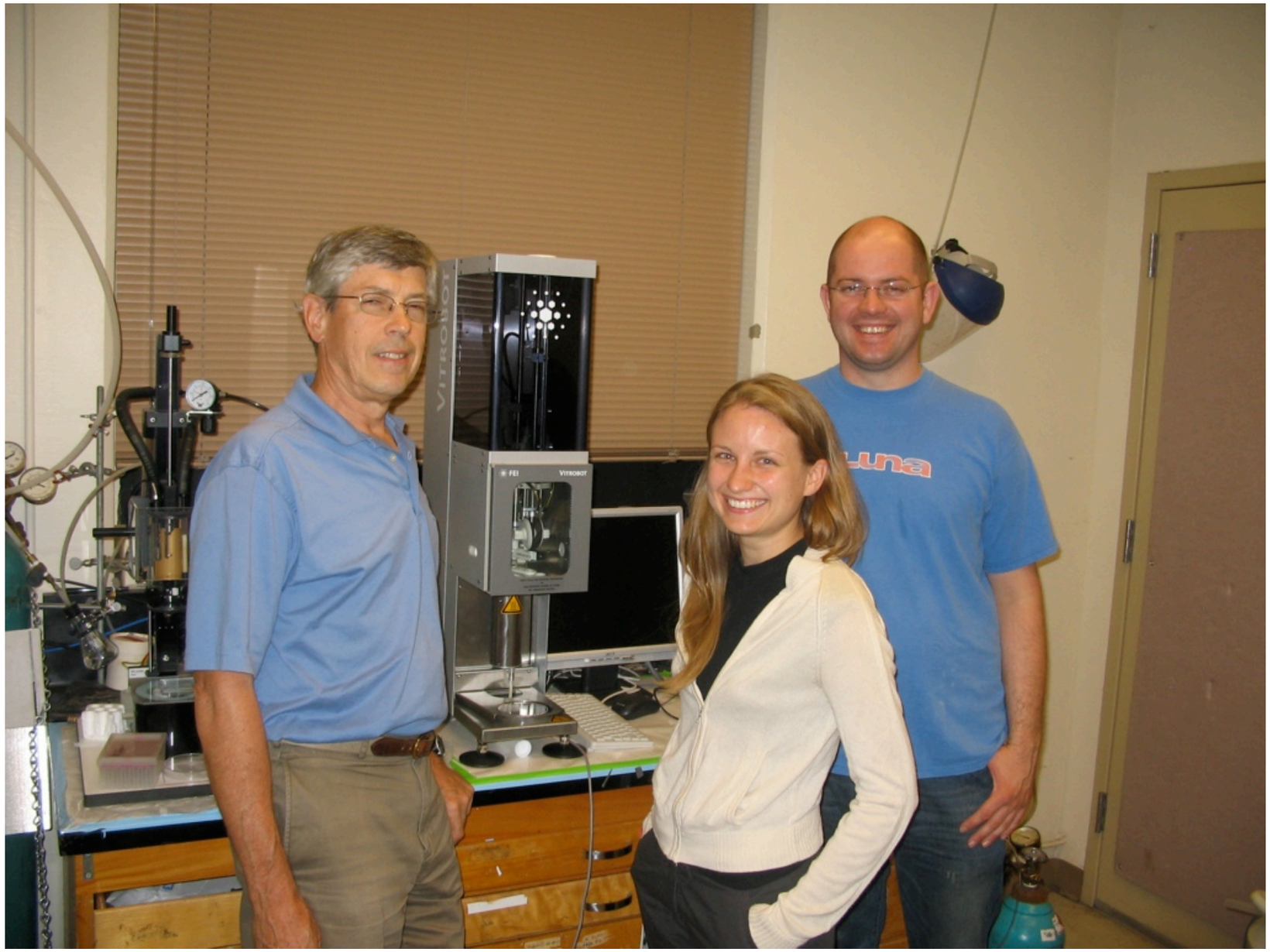


"Dirt" recovered around the yeast cell. A threshold-based support has been added to the fixed support of the cell.

Alternative: curved beams or dancing pinholes

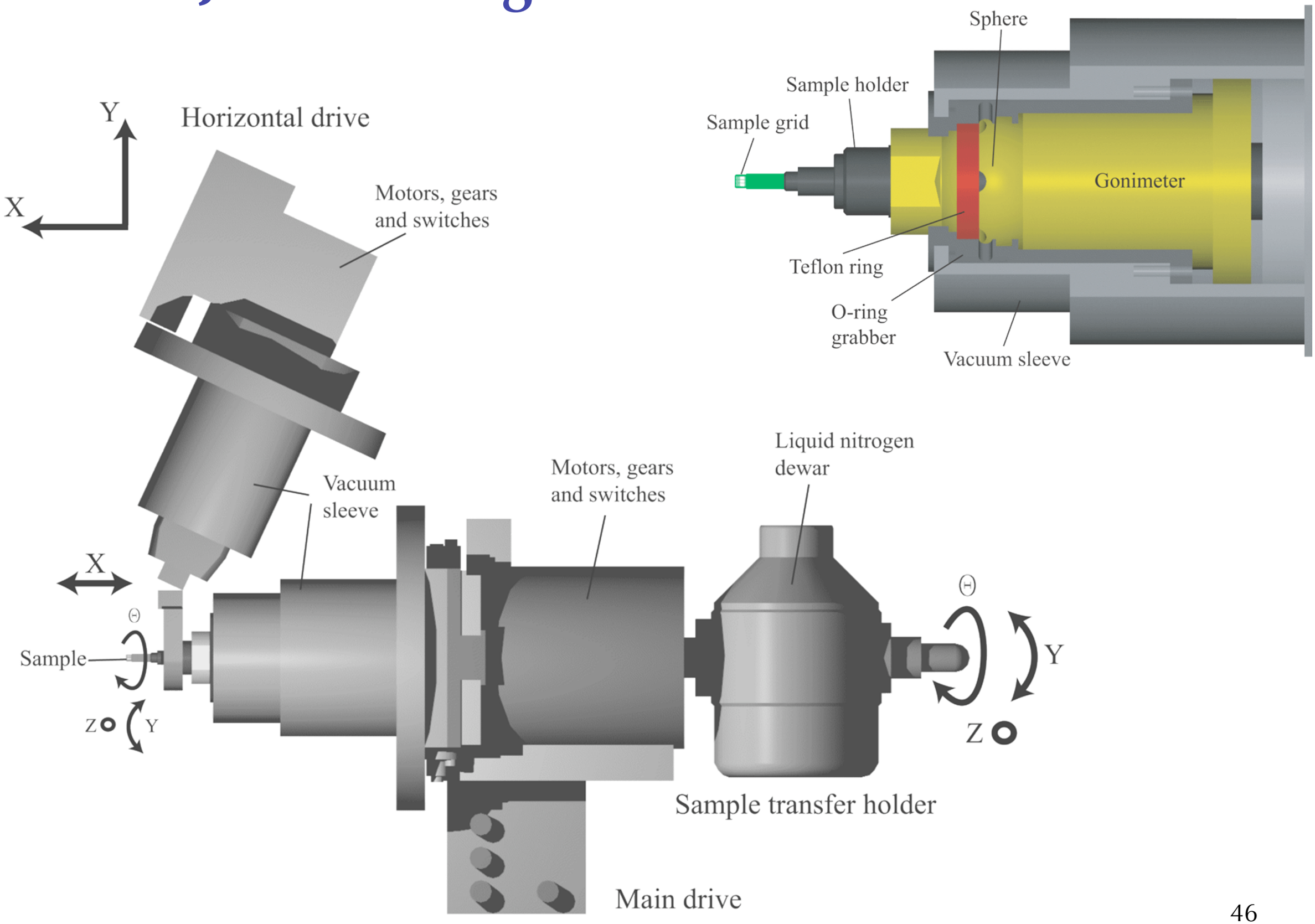
Frozen hydrated!

Specimen preparation has been challenging… Now using Vitrobot (hopping between Donner Lab and ALS)

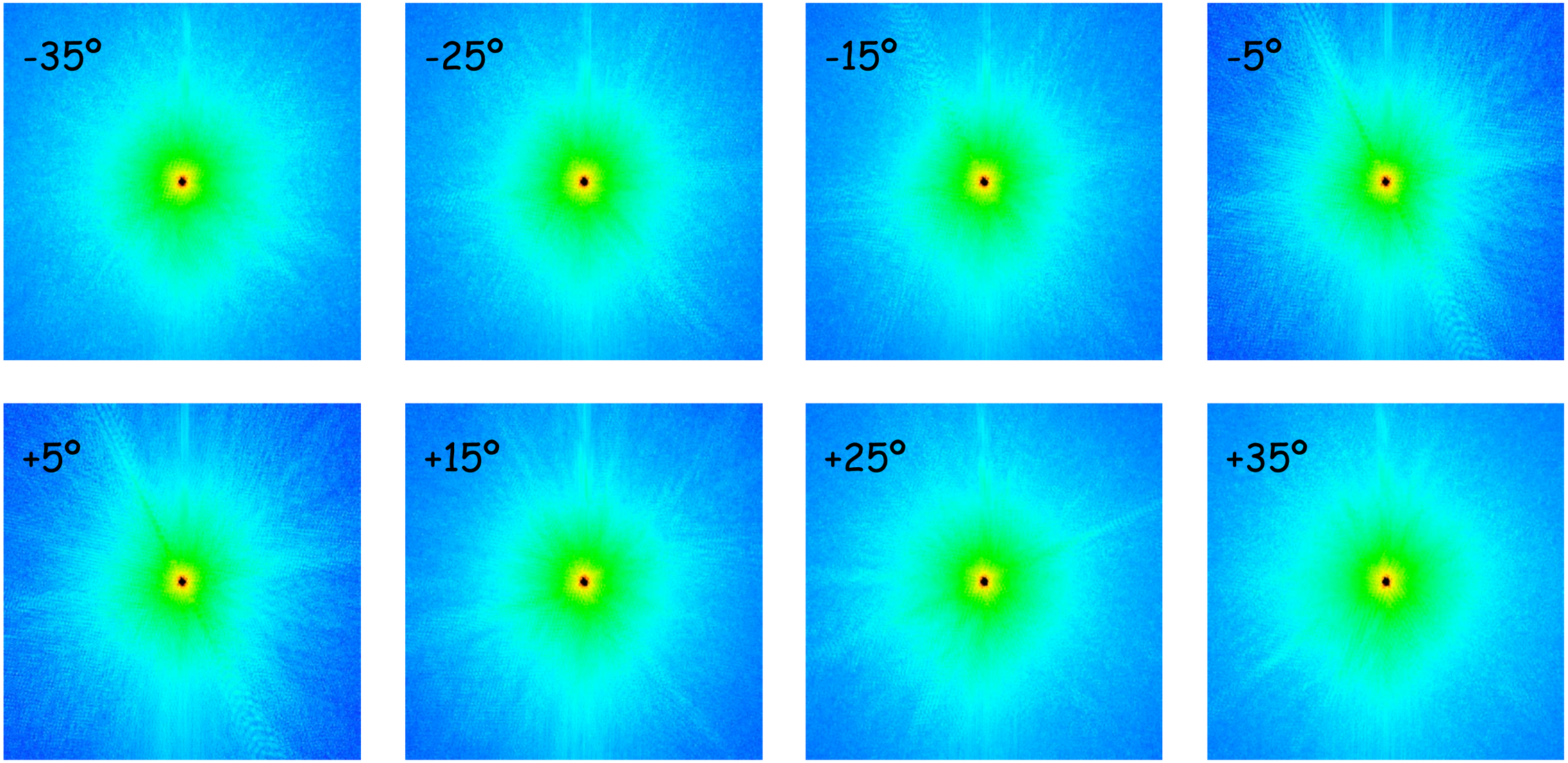


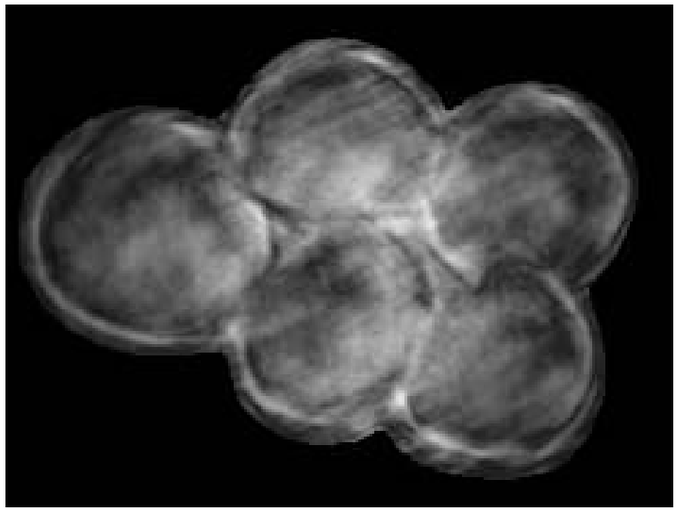
Ken Downing, Bjorg Larson, and Andrew Stewart.
Thanks also to Eva Nogales and her lab!

JEOL 2000 goniometer schematic



Frozen hydrated data



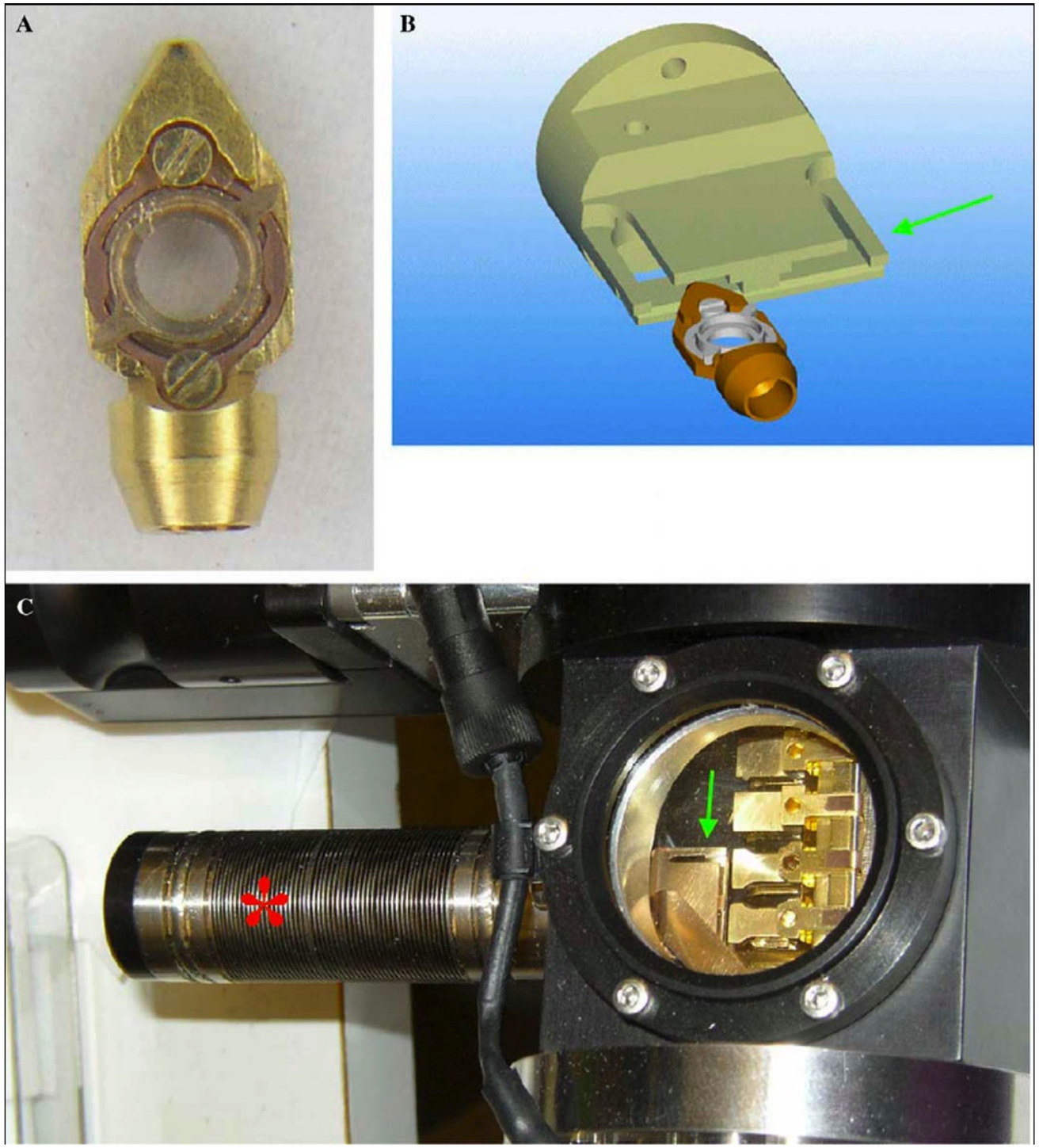


Enju Lima PhD thesis

- Too much scattering from “empty” area
- Too large a specimen was selected (insufficient oversampling)
- Too large a beam stop (low spatial frequencies)

Dreams for the future

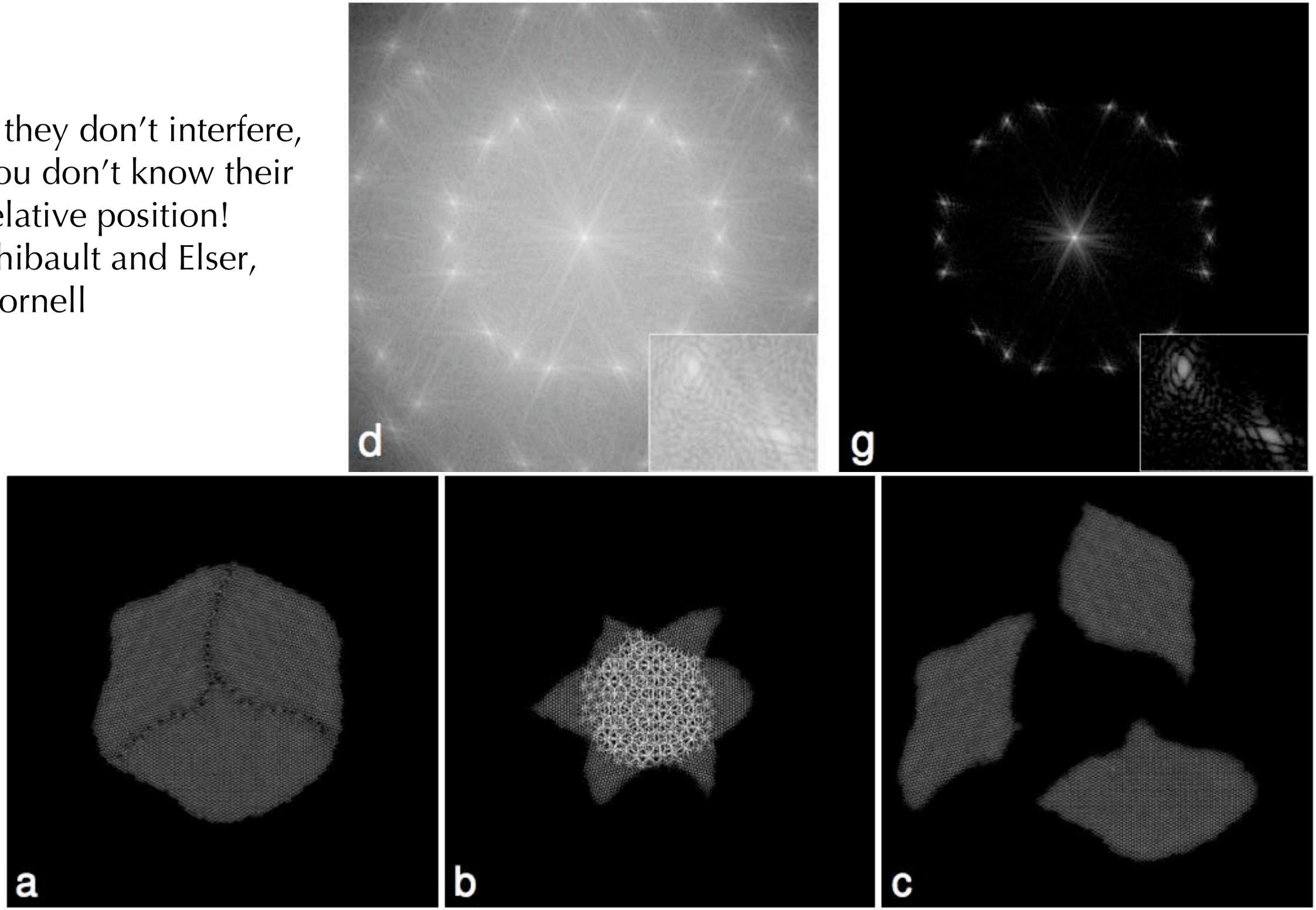
- "Cartridge" cryo system like FEI Polara?
- Automatic acquisition of tilt series to reduce dose and time (frosting)
- Cold sample in vacuum? Cold gas stream?
- Compatibility with scanning (STXM, nanoprobe) would be nice!



From Iancu *et al.*, *J. Struct. Biol.* **151**, 288 (2005).

Bragg spots? Beware!

If they don't interfere, you don't know their relative position! Thibault and Elser, Cornell

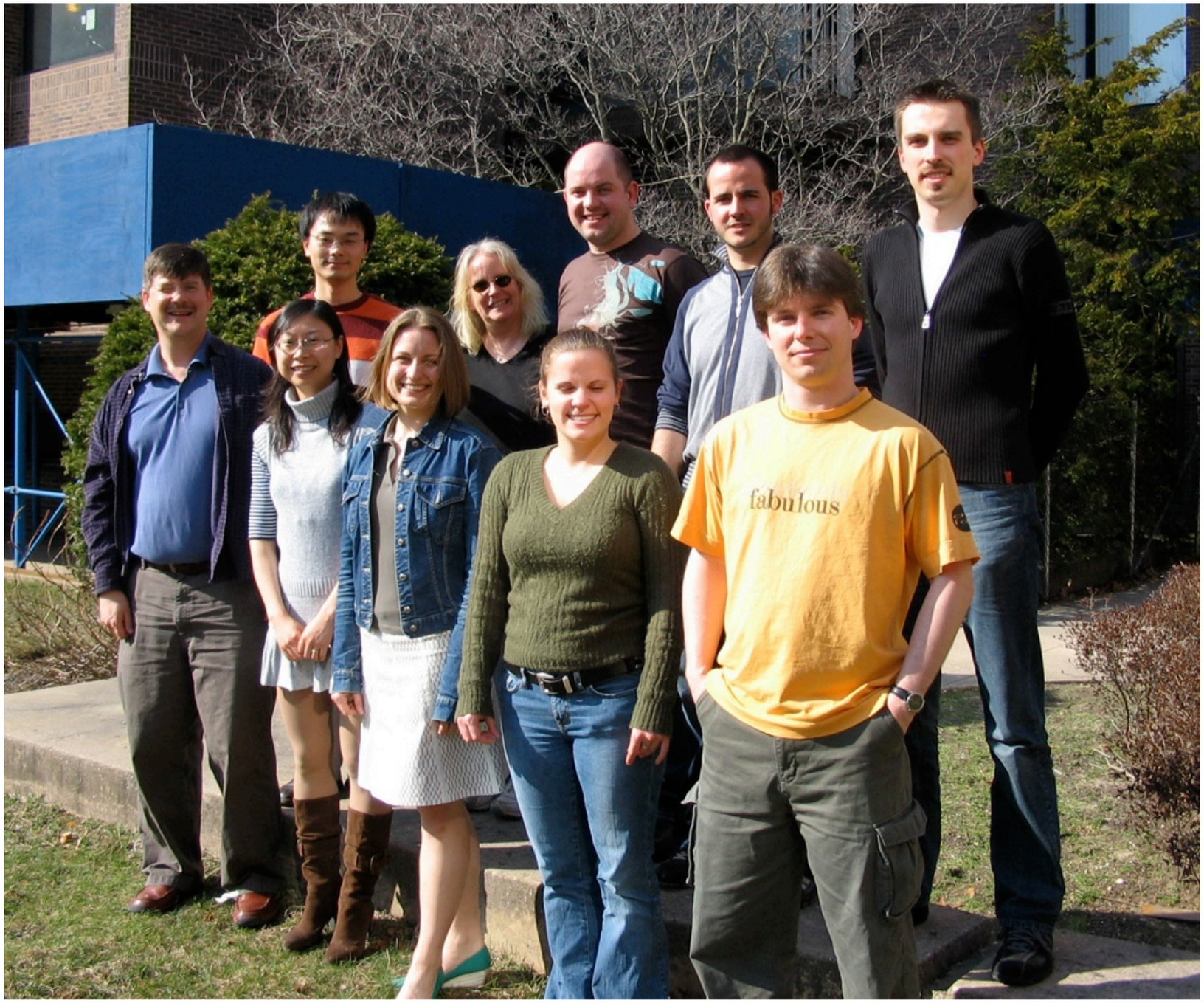


"All generalities are false, including this one"

- Stay clear of thin samples; can't compete with electrons.
  - Exceptions, exceptions... Including XFEL exploding molecules.
- If you have a lens, use it.
  - Exceptions, exceptions... Minimum dose, resolution limits, depth of focus.
- Must do 3D!
  - Requires stable specimen, which often means cryo.
  - Requires bright beam - not just for impatient experimenters, but for minimal sample frosting and contamination.
- Exploit commonalities.
  - Cryo engineering for tilts but also for scanning.
  - Software: learn from CCP4 to have standard data formats and a variety of interoperable software tools.

X-ray optics group at Stony Brook

Front: Huijie Miao, Bjorg Larson, Johanna Nelson, Holger Fleckenstein
Back: me, Xiaojing Huang, Sue Wirick, Andrew Stewart, Jan Steinbrener, Christian Holzner
Not shown: Benjamin Hornberger, Janos Kirz (Research Professor, Berkeley), David Sayre (retired)
Many alumni!



Funding: DoE (method development), NIH (application to cells)

Credit and blame

- All good data and calculations from team efforts, as indicated
- All bad interpretations and extrapolation are from me.

"It's hard to make predictions - especially about the future"

- Robert Storm Petersen (aka Storm P, Denmark, 1882-1949)?
- Yogi Berra (NY Yankees catcher, 1947-1963)? "I really didn't say everything I said"

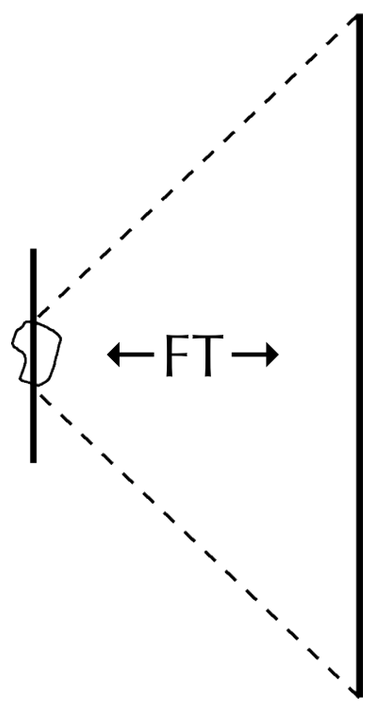
"The best way to predict the future is to invent it"

- Alan Kay (Xerox Palo Alto, 1971)

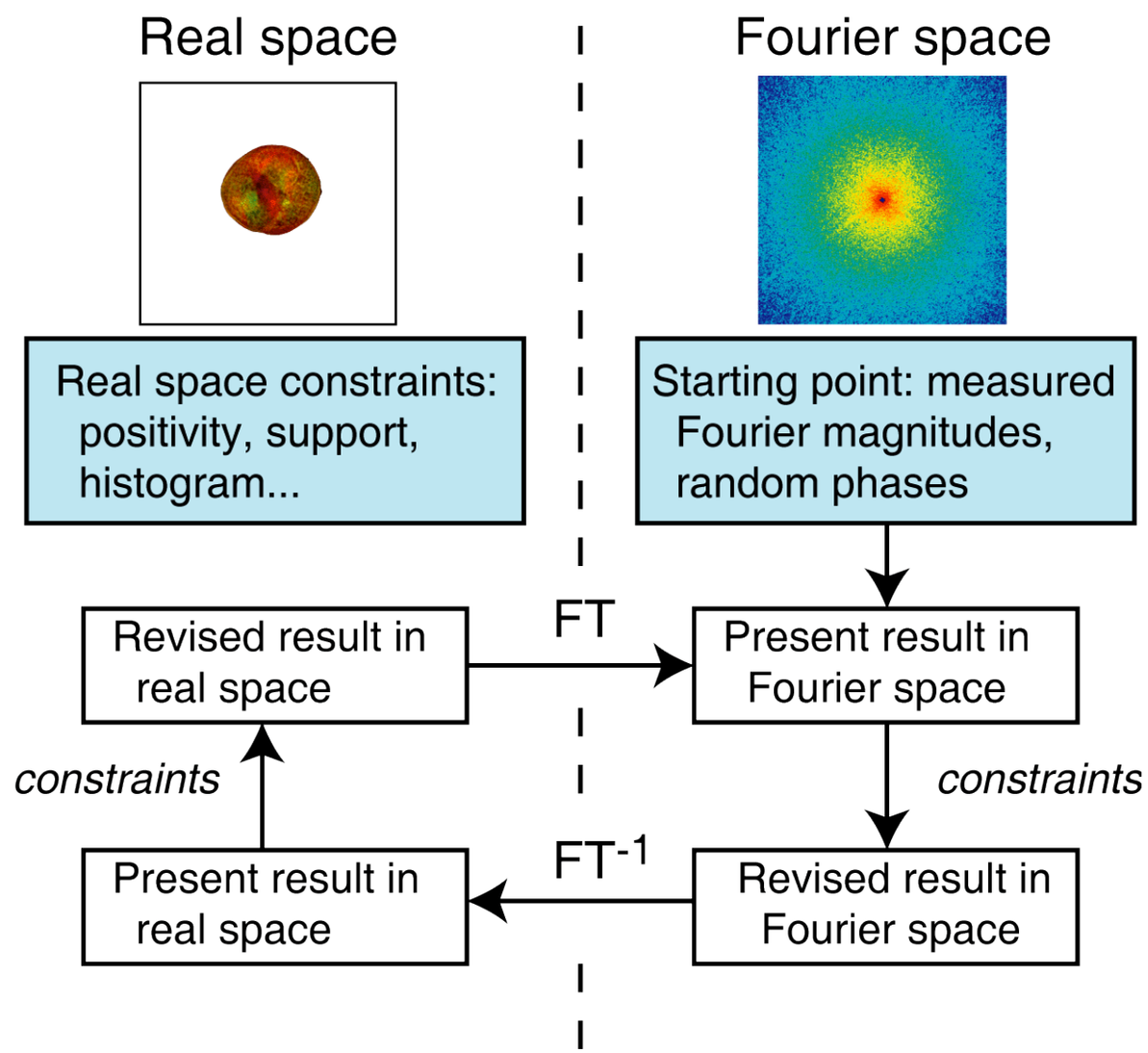
What if no real-space image?

Use *a priori* information in real space!

Real space: finite support
(or other constraints)



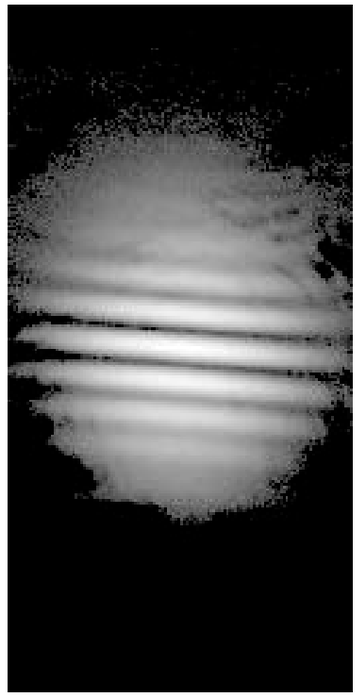
Fourier space: magnitudes known, but phases are not



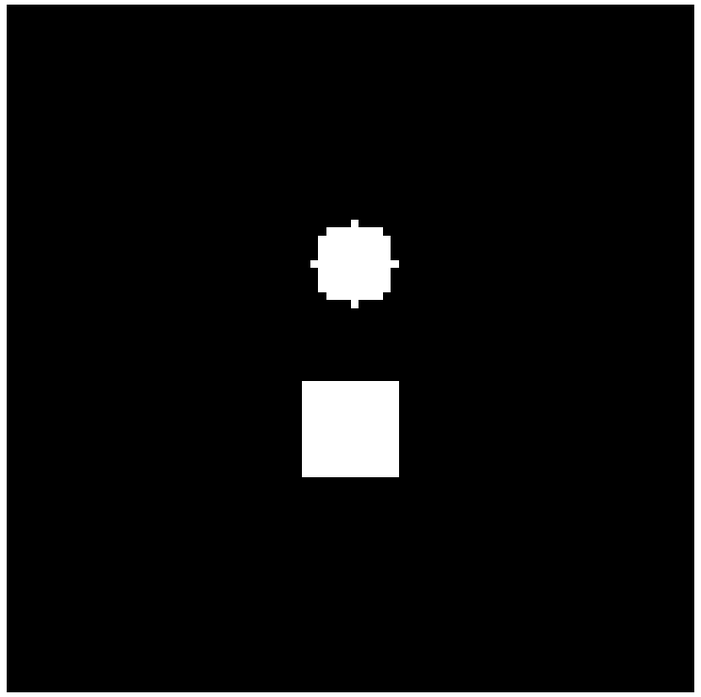
Phasing algorithms: Feinup, *Opt. Lett.* **3**, 27 (1978); many subsequent developments.

Iterative phasing: simple example

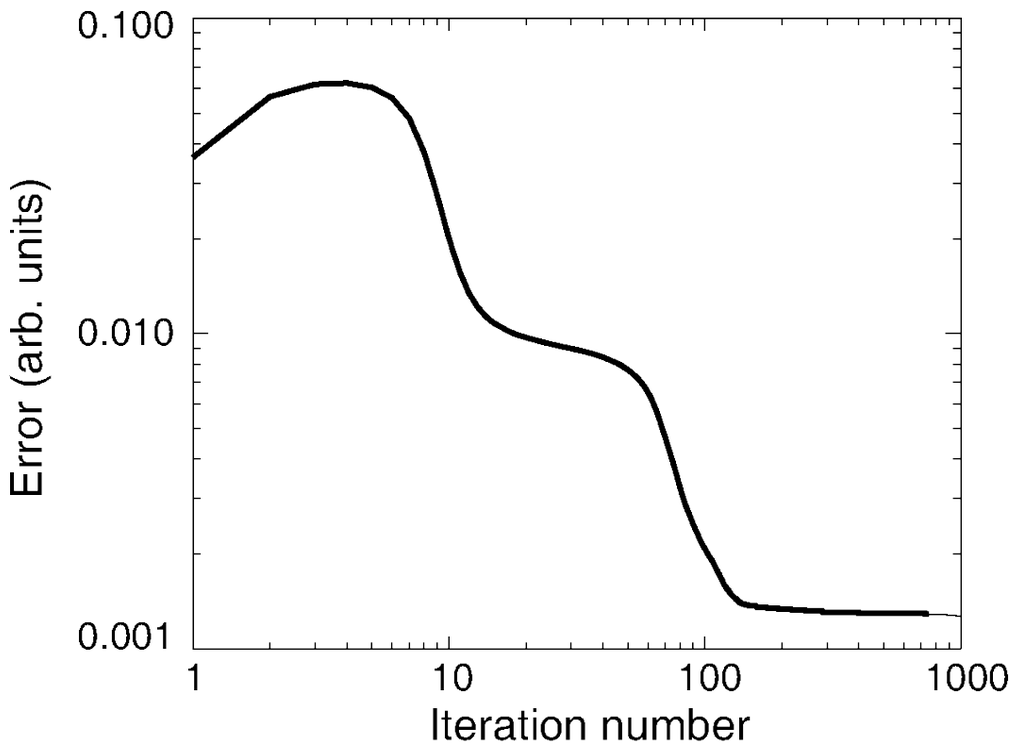
- High harmonic generation of XUV radiation from femtosecond lasers, illuminating two pinholes.
  - R. Bartels, A. Paul, H. Green, H.C. Kapteyn, M.M. Murnane, S. Backus, I.P. Christov, Y. Liu, D. Attwood, C. Jacobsen, *Science* **297**, 376 (2002)



Data

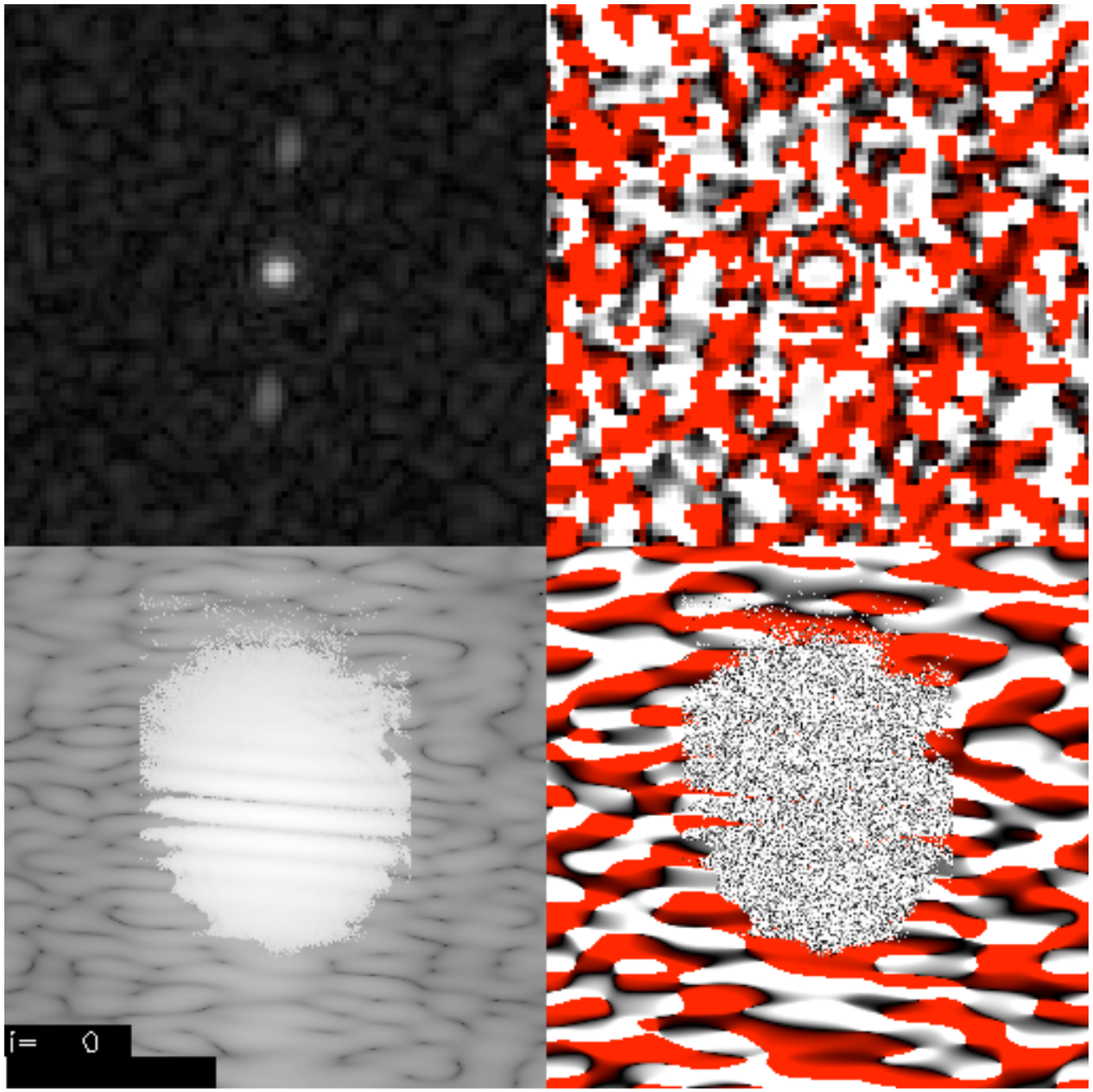


Support constraint
(very loose)

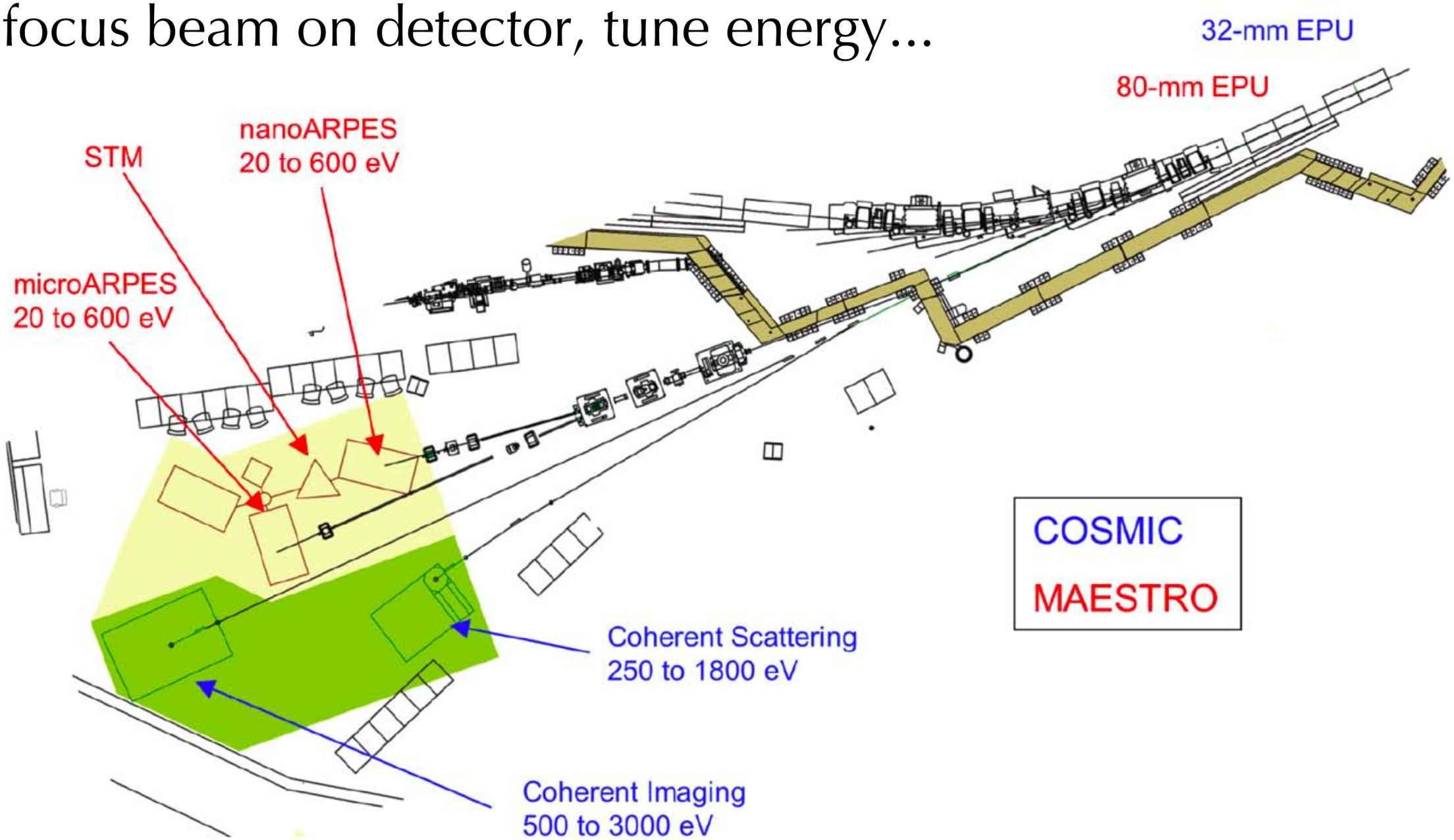


Reconstruction error

The reconstruction



Real space Magnitude	Real space Phase
Far field Magnitude	Far field Phase

To reach those goals...

COSMIC@ALS: optimized undulator (with EPU!) with ~500x more coherent flux on sample than ALS 9.0.1, focus beam on detector, tune energy...