

Nanoprobes: getting the most bang per photon

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Getting the most bang per photon

- Use the strongest contrast mechanism (for example, phase contrast).
- Use the most efficient optical system (scanning, or lensless).
- Make the sample as robust as possible (cryo).
- Extract your information from complex data.

X-ray refractive index

Röntgen tried refractive focusing, and failed...

We now know that the x-ray refractive index goes like

$$n = 1 - \frac{n_a r_e}{2\pi} \lambda^2 (f_1 + if_2) = 1 - \alpha \lambda^2 (f_1 + if_2)$$

Differs from $n=1$ by 10^{-3} - 10^{-6} so refractive focusing is *very* weak.

Phase velocity is faster than light in vacuum!

$$v_p = \frac{\omega}{k} \approx c(1 + \alpha f_1 \lambda^2)$$

Prisms refract x rays the opposite way from visible light!

Phase is advanced rather than retarded!

Total external reflection with critical angle

$$\theta_c \approx \sqrt{2\alpha \lambda^2 f_1}$$

Lassen sich Brechungsexponenten der Körper für Röntgenstrahlen experimentell ermitteln?

Von A. Einstein.

(Eingegangen am 21. März 1918.)

Vor einigen Tagen erhielt ich von Herrn Prof. A. KÖHLER (Wiesbaden) eine kurze Arbeit¹⁾, in welcher eine auffallende Erscheinung bei Röntgenaufnahmen geschildert ist, die sich bisher nicht hat deuten lassen. Die reproduzierten Aufnahmen — zu meist menschliche Gliedmaßen darstellend — zeigen an der Kontur einen hellen Saum von etwa 1 mm Breite, in welchem die Platte heller bestrahlt zu sein scheint als in der (nicht beschatteten) Umgebung des Röntgenbildes.

Ich möchte die Fachgenossen auf diese Erscheinung hinweisen und beifügen, daß die Erscheinung wahrscheinlich auf Totalreflexion beruht. Nach der klassischen Dispersionstheorie müssen wir erwarten, daß der Brechungsexponent n für Röntgenstrahlen nahe an 1 liegt, aber im allgemeinen doch von 1 verschieden ist. n wird kleiner bzw. größer als 1 sein, je nachdem der Einfluß derjenigen Elektronen auf die Dispersion überwiegt, deren Eigenfrequenz kleiner oder größer ist als die Frequenz der Röntgenstrahlen. Die Schwierigkeit einer Bestimmung von n liegt darin, daß $(n - 1)$ sehr klein ist (etwa 10^{-5}). Es ist aber leicht einzusehen, daß bei nahezu streifender Inzidenz der Röntgenstrahlen im Falle $n < 1$ eine nachweisbare Totalreflexion auftreten muß.

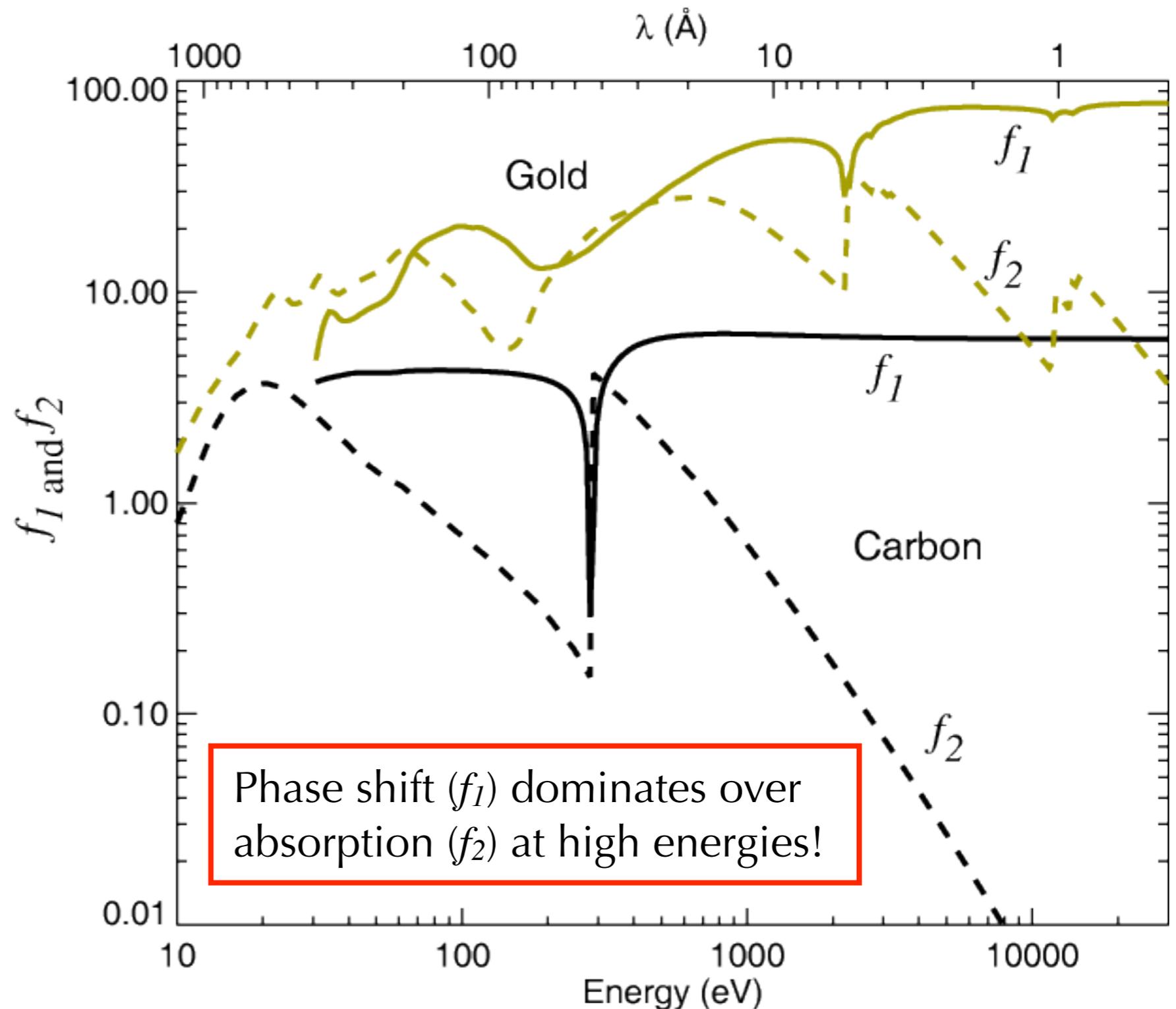
X-ray refractive index

Again, refractive index goes like

$$n = 1 - \frac{n_a r_e}{2\pi} \lambda^2 (f_1 + if_2)$$

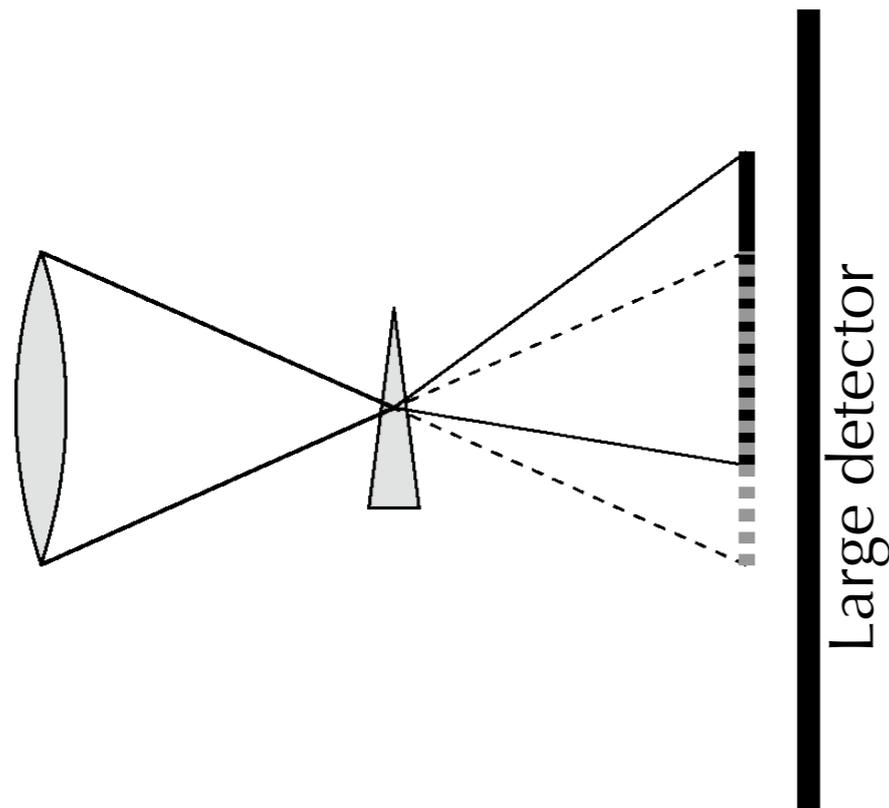
The oscillator strength ($f_1 + if_2$) has a phase shifting part f_1 and an absorption part f_2 .

Note that phase shift dominates over absorption at higher energies!

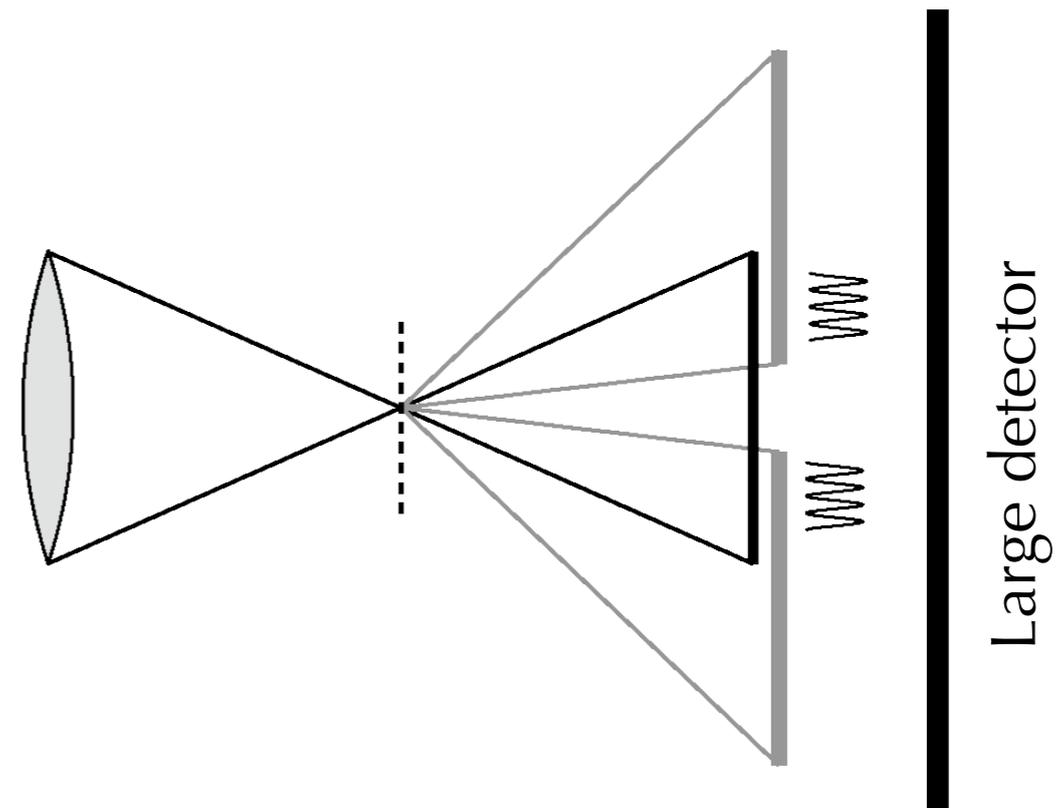


Scanning microscopes: contrast mode depends on detector

- Large area detector: sensitive only to absorption
- Point detector on-axis: coherent imaging
- Detector with restricted or segmented spatial response: some degree of phase contrast



Phase gradient (prism)

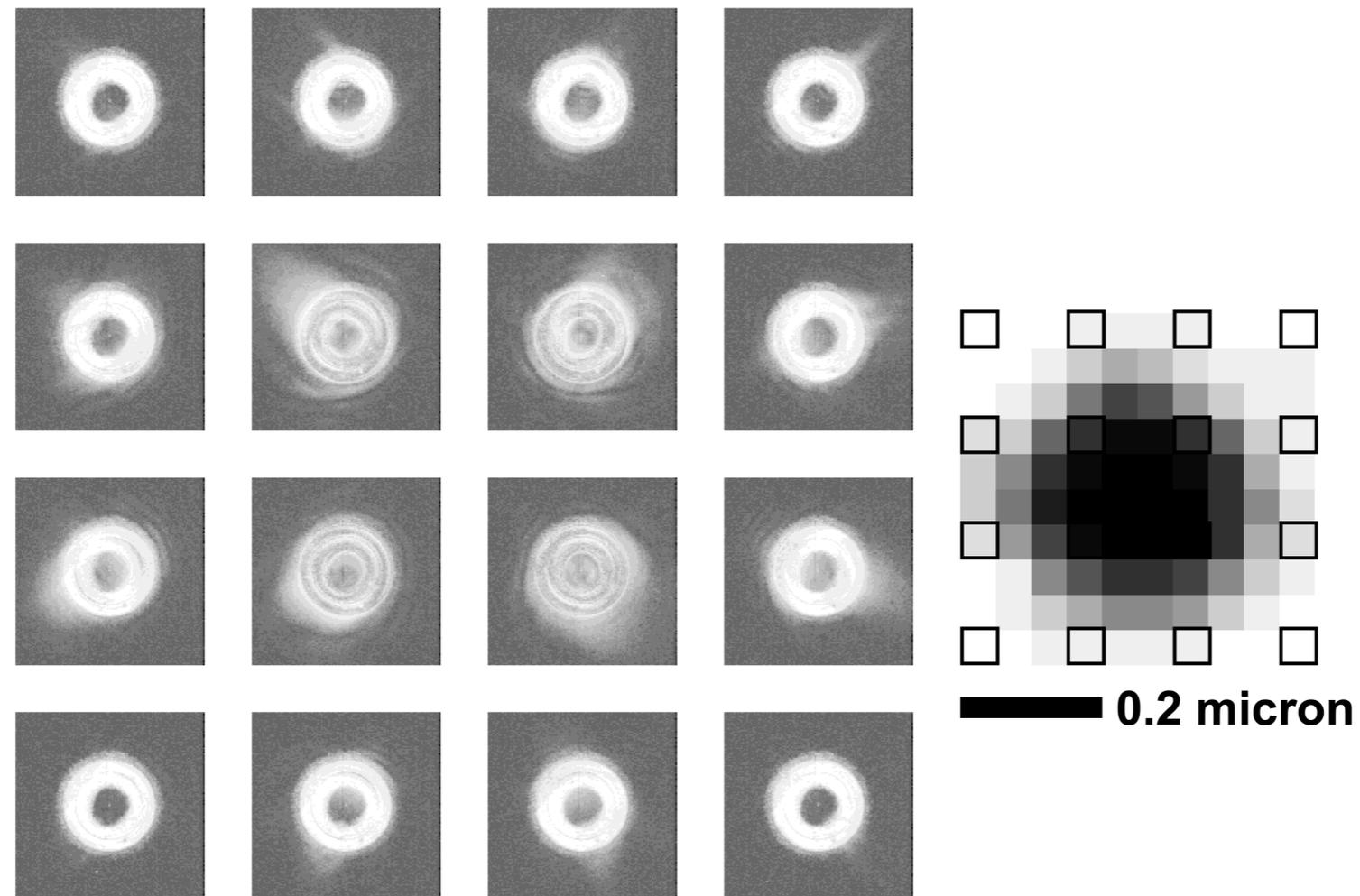


Spatial frequency in object

See e.g., Spence and Cowley, *Optik* **50**, 129 (1978); Nellist *et al.*, *Nature* **374**, 630 (1995).

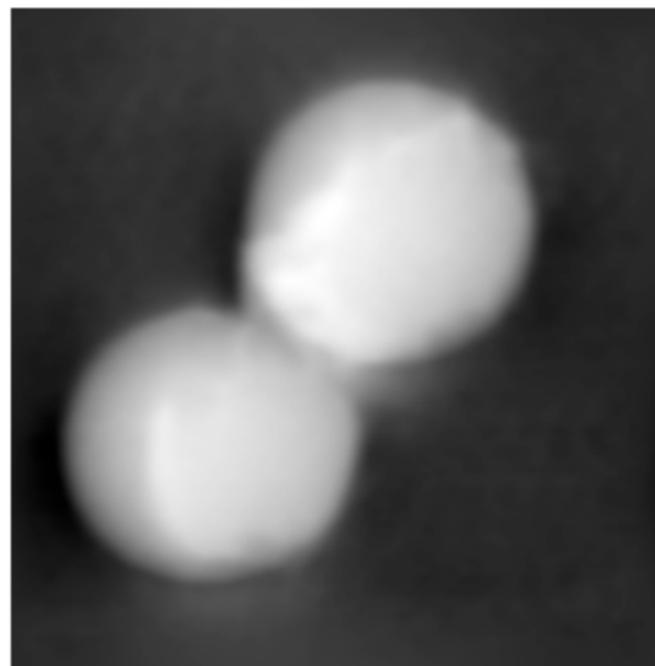
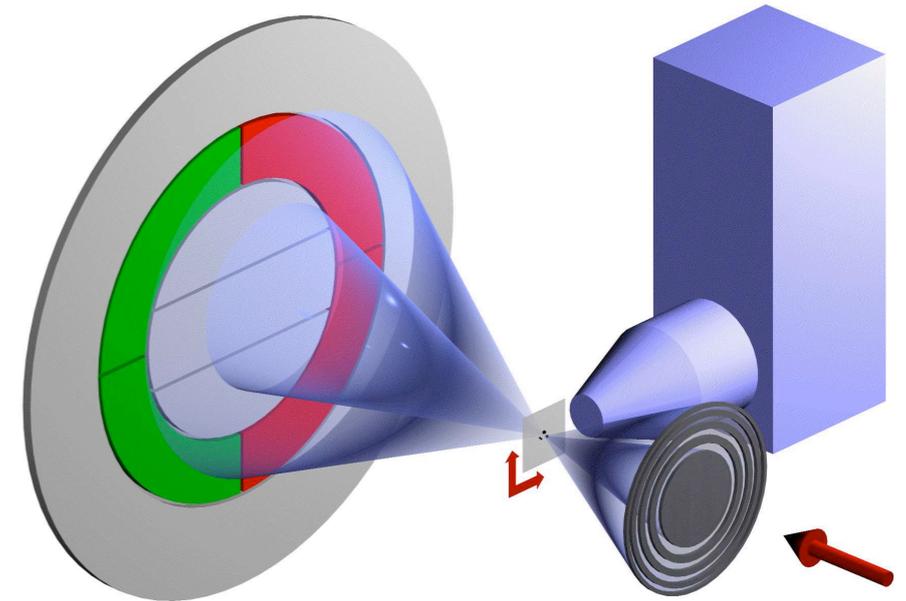
Scanning microprobes: CCD as the ultimate detector

- Record microdiffraction pattern per pixel; Wigner phase reconstruction. Chapman, *Ultramicroscopy* **66**, 153 (1996). Shown below: polystyrene sphere raw data (which was reconstructed to give amplitude and phase).

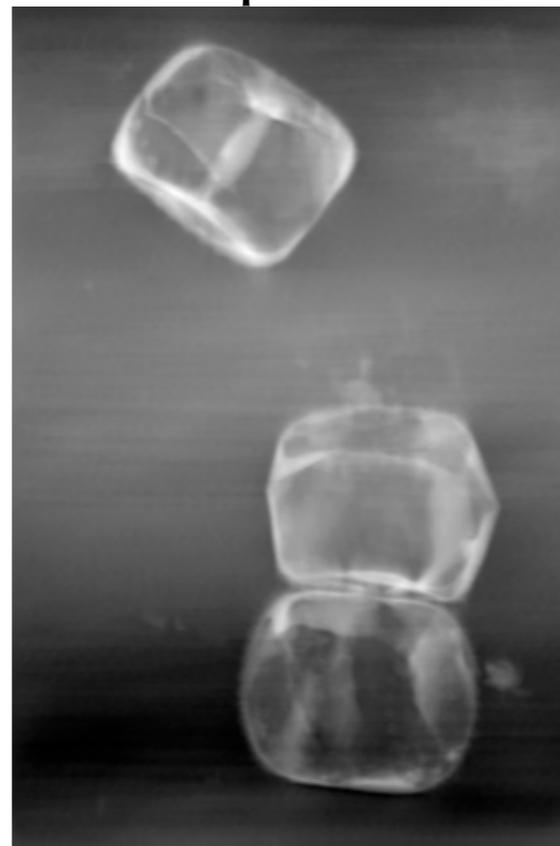


Phase contrast in microprobes

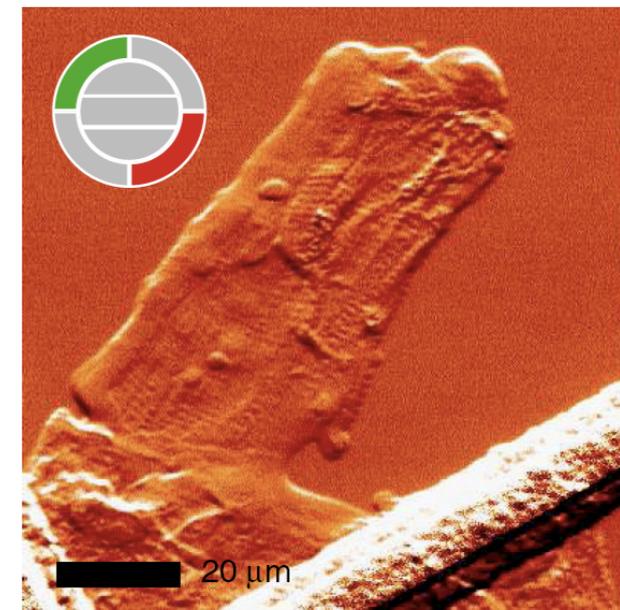
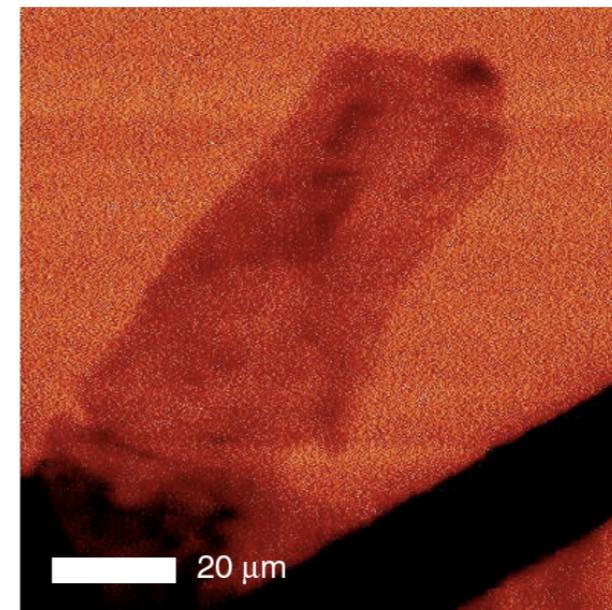
- Place elemental maps in ultrastructural context; provide quantitative concentration.
- Segmented x-ray detector (with BNL, Max Planck silicon lab)
- Fourier optics reconstruction filters, and data fusion with elemental map data



Reconstructed phase in radians of 5 μm silica sphere: CXRO data predicts 0.60, experiment gives 0.58



5 μm
Diatom: phase corresponds to max thickness of 2.8 μm

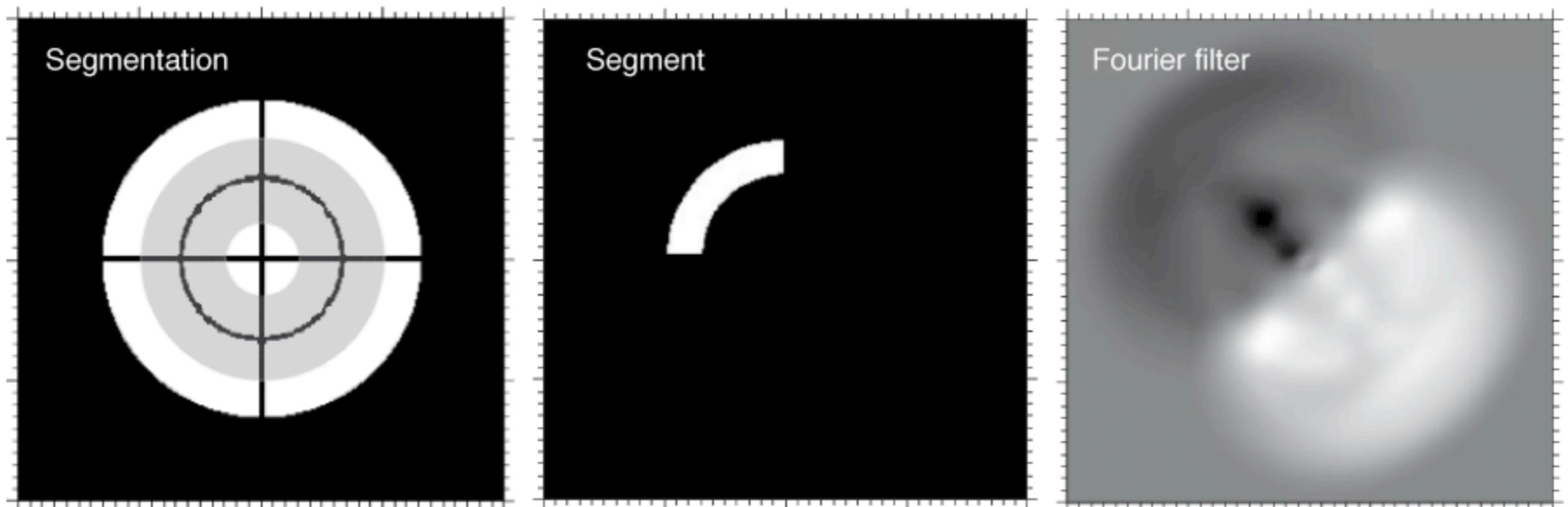


Cardiac muscle (w/Palmer, Vogt *et al.*: absorption (left) and differential phase (right) images.

Hornberger *et al.*, *Ultramic.* **107**, 644 (2007);
Feser *et al.*, *Nucl. Inst. Meth. A* **565**, 841 (2006)

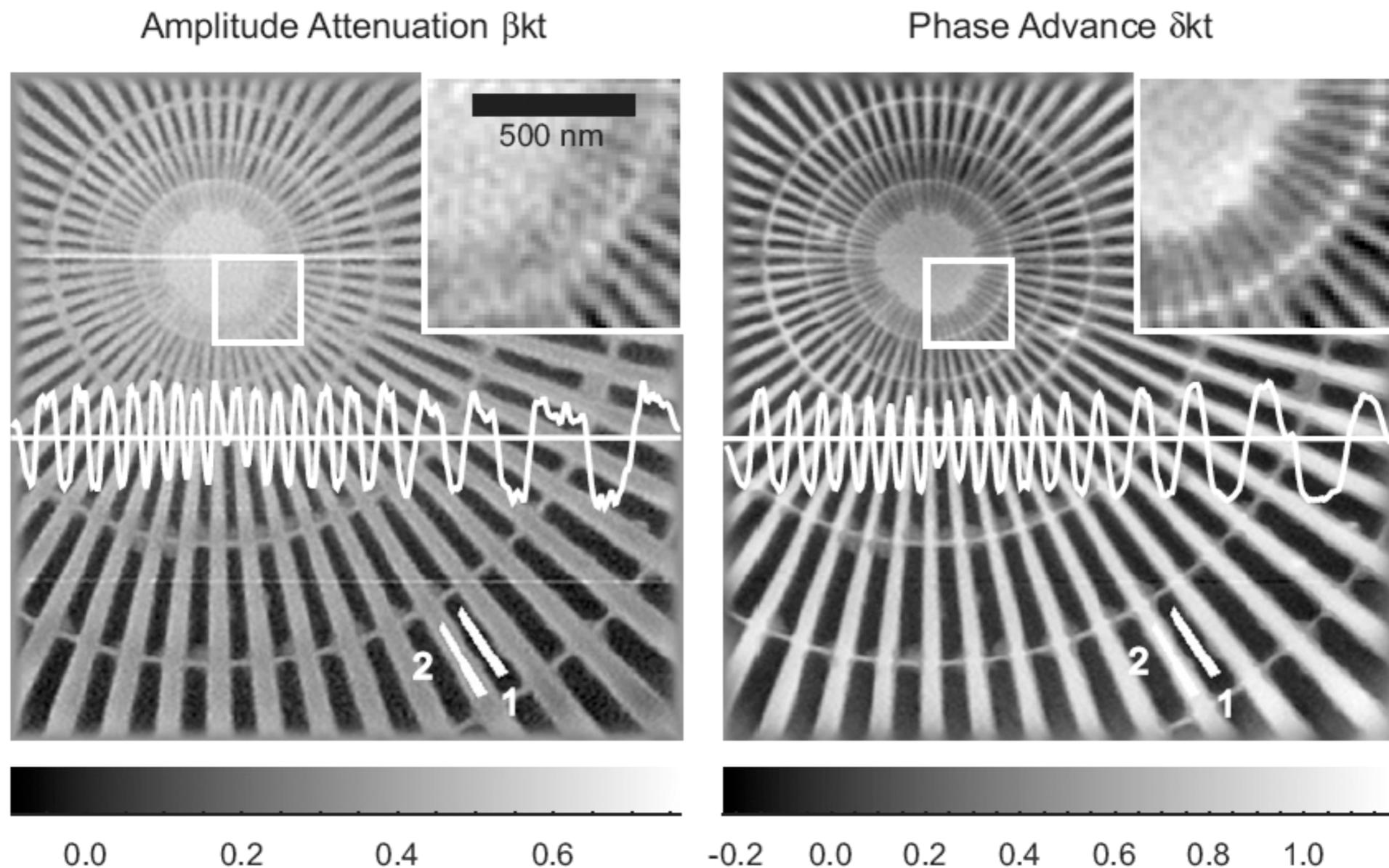
Segmented detector and Fourier filter reconstruction

- Limited number of segments means fast readout in scanning microprobe, and fast reconstruction.
- Fourier filtering approach: inspired by STEM work of McCallum, Landauer, and Rodenburg, *Optik* **103**, 131 (1996).
- Extended and implemented by Hornberger, Feser, and Jacobsen, *Ultramic.* **107**, 644 (2007).



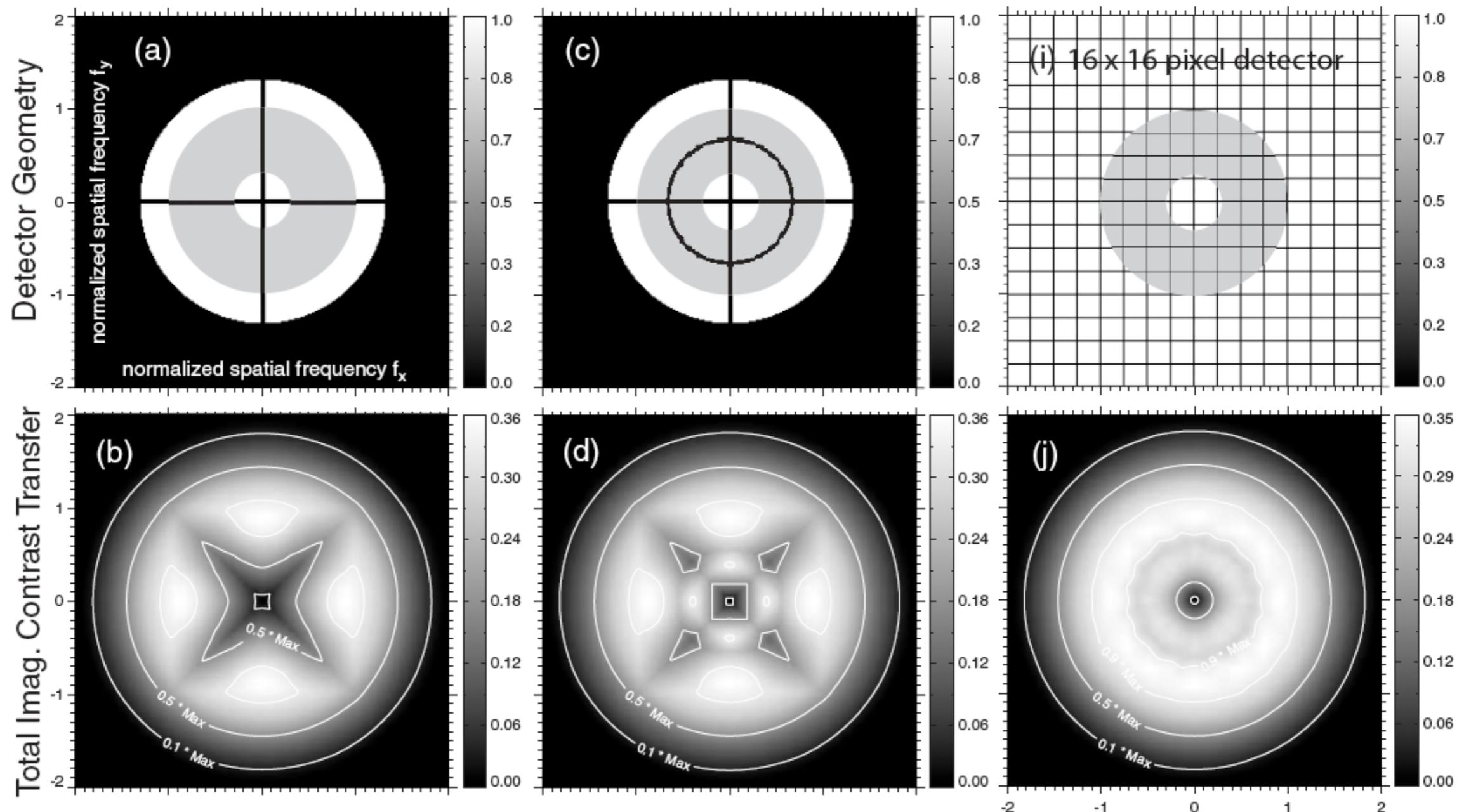
Quantitative phase reconstruction

- Hornberger, Feser, and Jacobsen, *Ultramic.* **107**, 644 (2007).
- Fourier filter applied to segmented detector data at 525 eV.
- Quantitative agreement with Henke data.



Fewer segments=fast readout

Fourier plane coverage of various detector schemes
(B. Hornberger PhD dissertation, 2007)

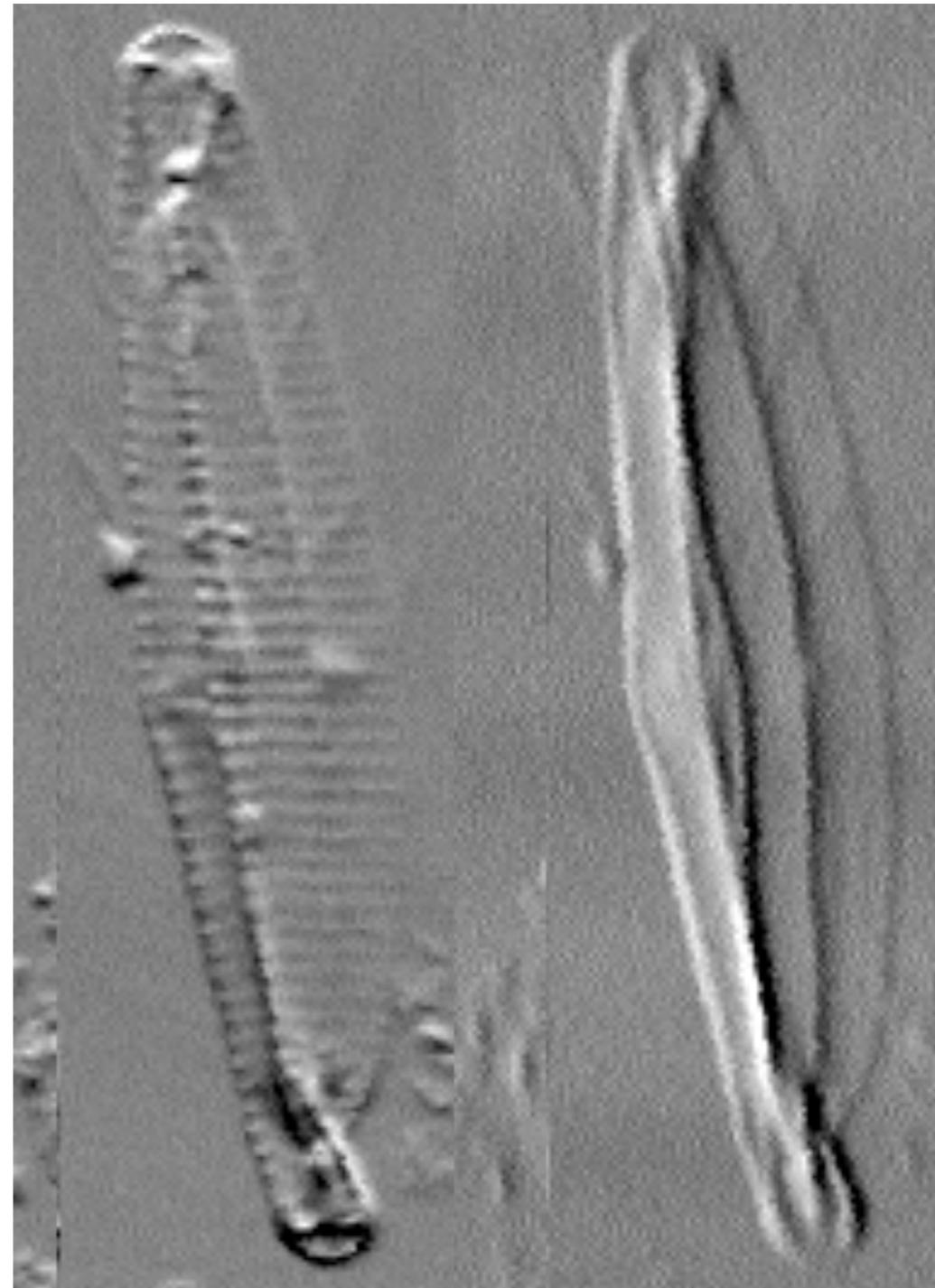


Phase contrast tomography

- Experiment: C. Holzner (Stony Brook), M. de Jonge, S. Vogt (Argonne), and others.
- Diatom study: S. Baines (Stony Brook) *et al.*
- Use differential phase contrast to align low-count, noisy fluorescence projections.
- $\pm 60^\circ$ tilt, 50 nm zone plate, 2.8 keV

Vertical
differential

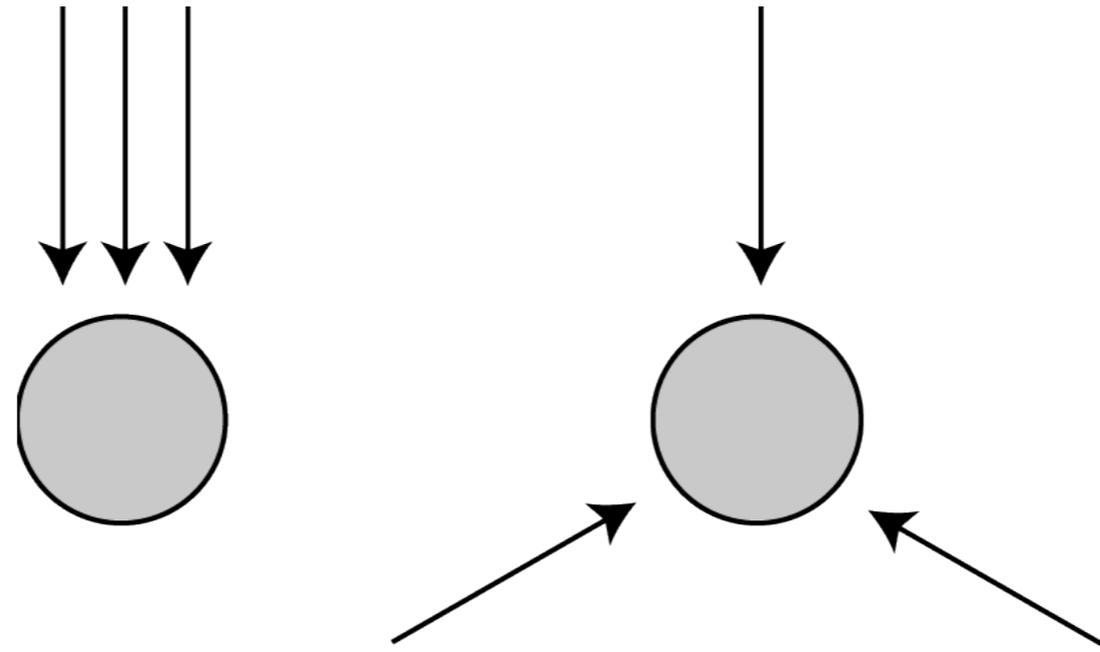
Horizontal
differential



2 μm

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).



Getting the most bang per photon

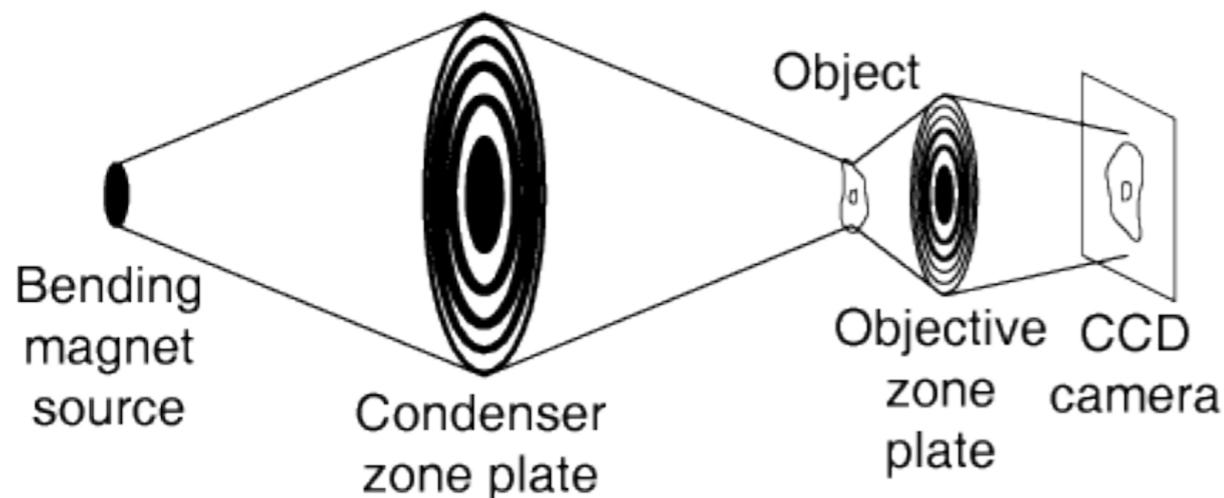
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- Make the sample as robust as possible (cryo).
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Optic-based microscopes

TXM

- Incoherent illumination; works well with a bending magnet, with fast imaging
- More pixels (e.g., 2048^2)
- Moderate spectral resolution in most cases

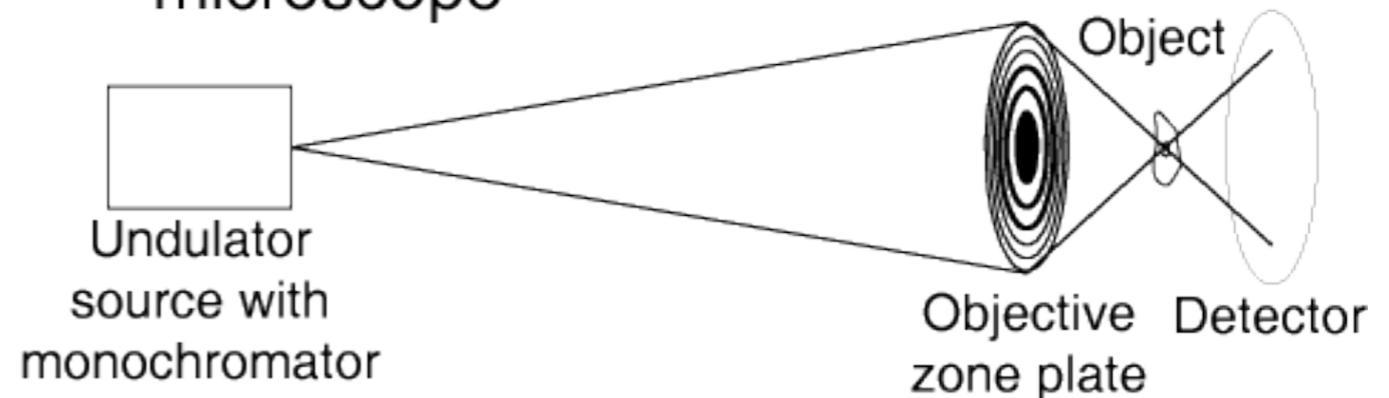
TXM: transmission x-ray microscope



STXM

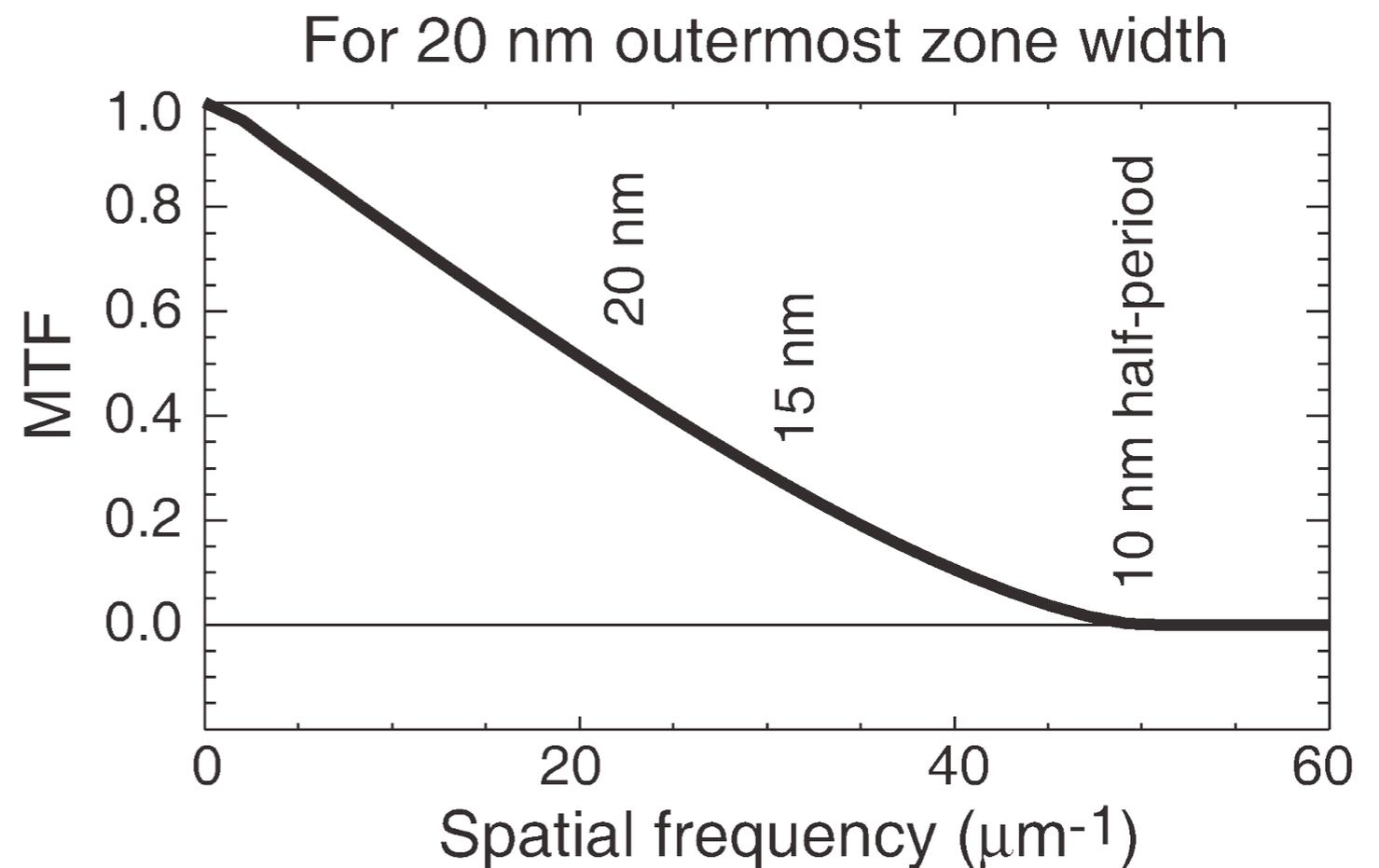
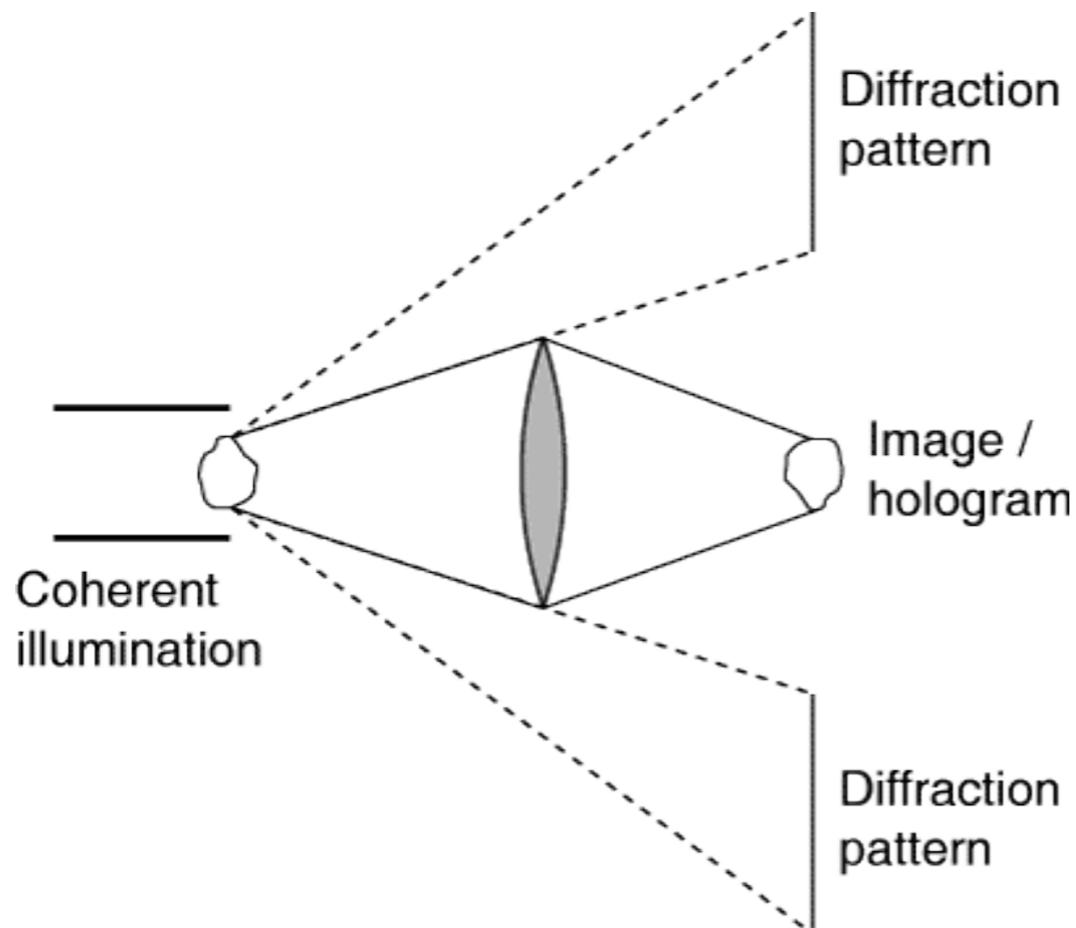
- Coherent illumination; works best with an undulator
- Less dose to sample (**optics are typically ~10% efficient**)
- Better suited to conventional grating monochromator [high $E/(\Delta E)$]
- Microprobes: fluorescence etc.

STXM: scanning transmission x-ray microscope



Radiation damage sets the ultimate resolution limit

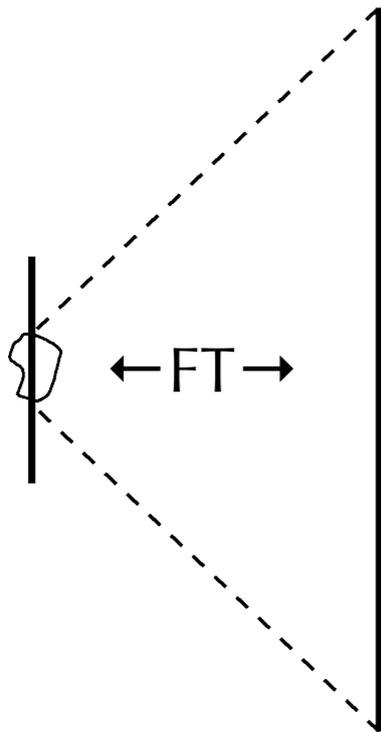
- For many specimens, radiation damage sets the ultimate limit on achievable resolution.
- Lenses phase the signal, but lose the signal. Example: 20 nm zone plate with 10% efficiency, 50% window transmission, 20% modulation transfer function (MTF) for 15 nm half-period:
net transfer of 1% for high spatial frequencies
- Can we avoid this ~100x signal loss, and also go beyond numerical aperture limit of available optics?



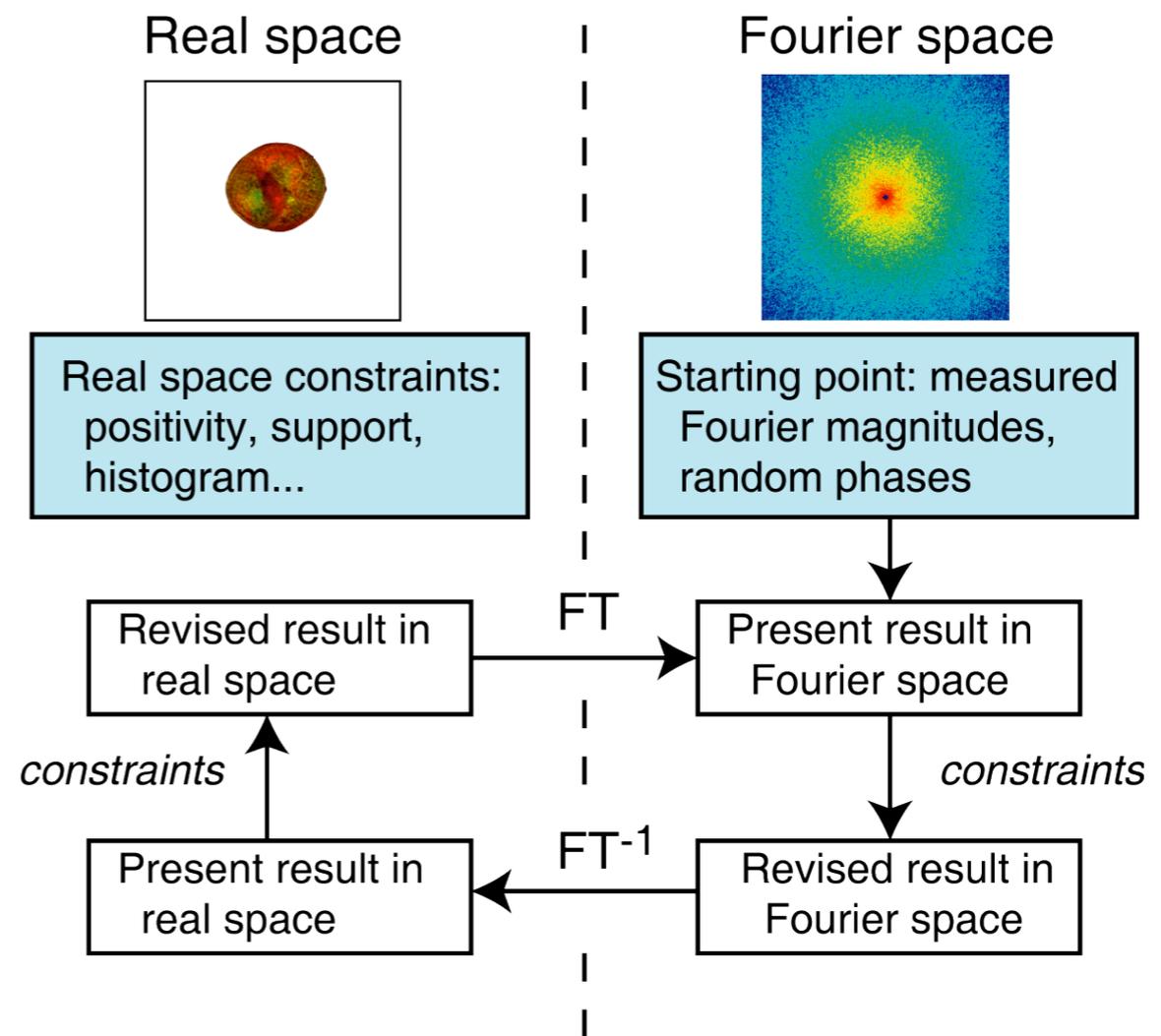
Imaging without lenses

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

Real space: finite support
(or other constraints)



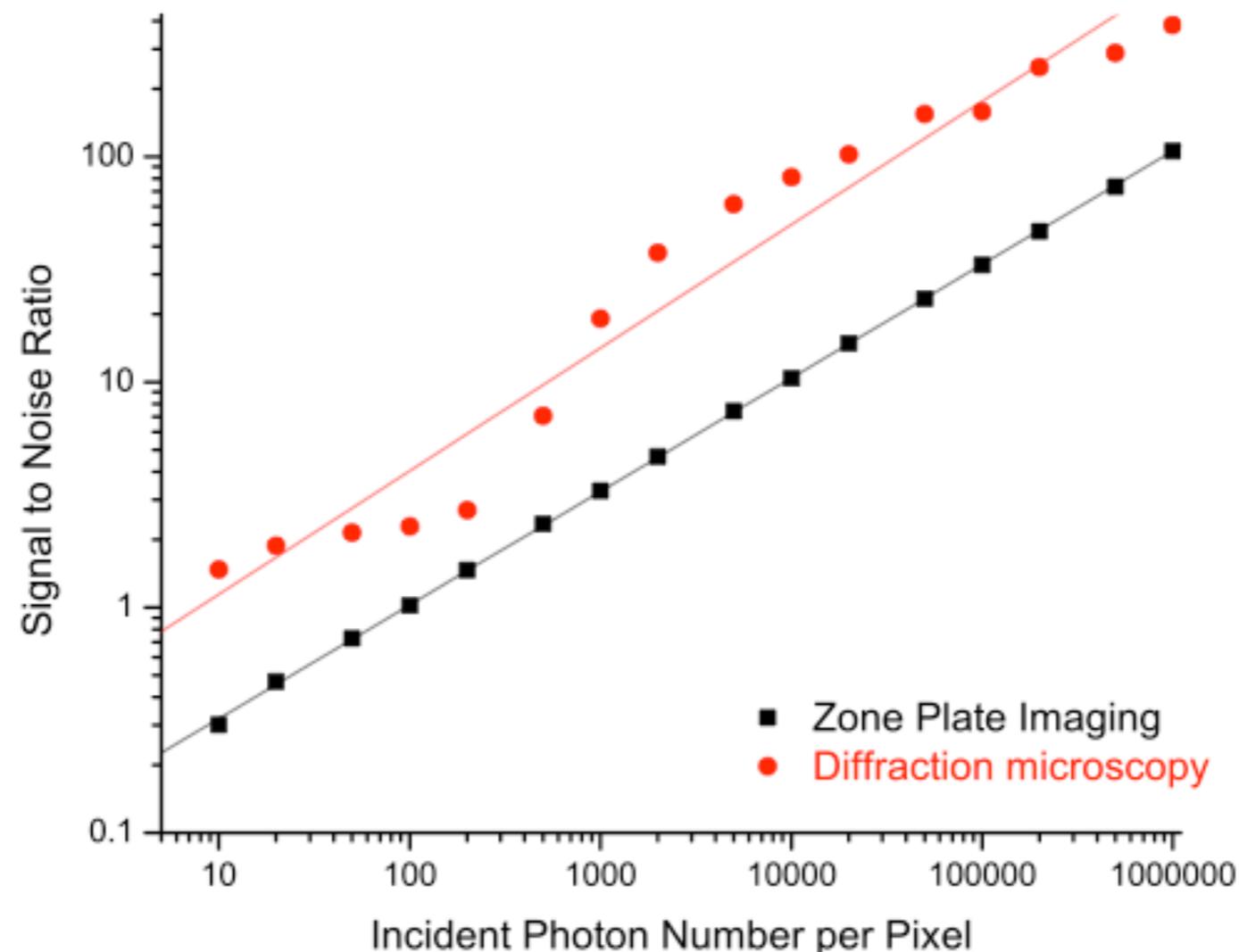
Fourier space: magnitudes
known, but phases are
not



Phasing algorithms: Feinup, *Opt. Lett.* **3**, 27 (1978); Elser, *JOSA A* **20**, 40 (2003); and others.

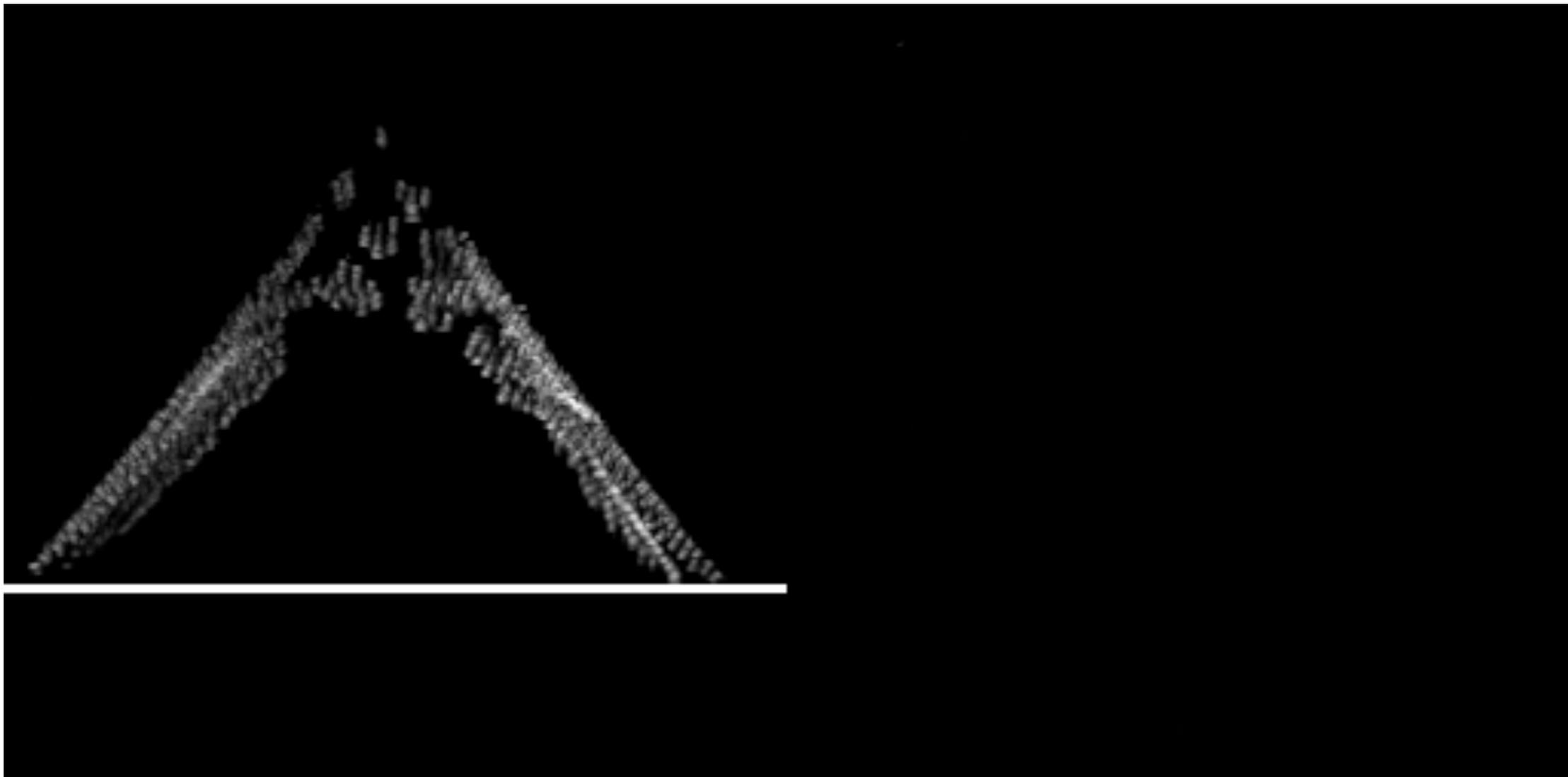
Can one recover phase from noisy data? Yes!

- Simulation (Huang *et al.*, Stony Brook): exit wave from thick cell, with 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)



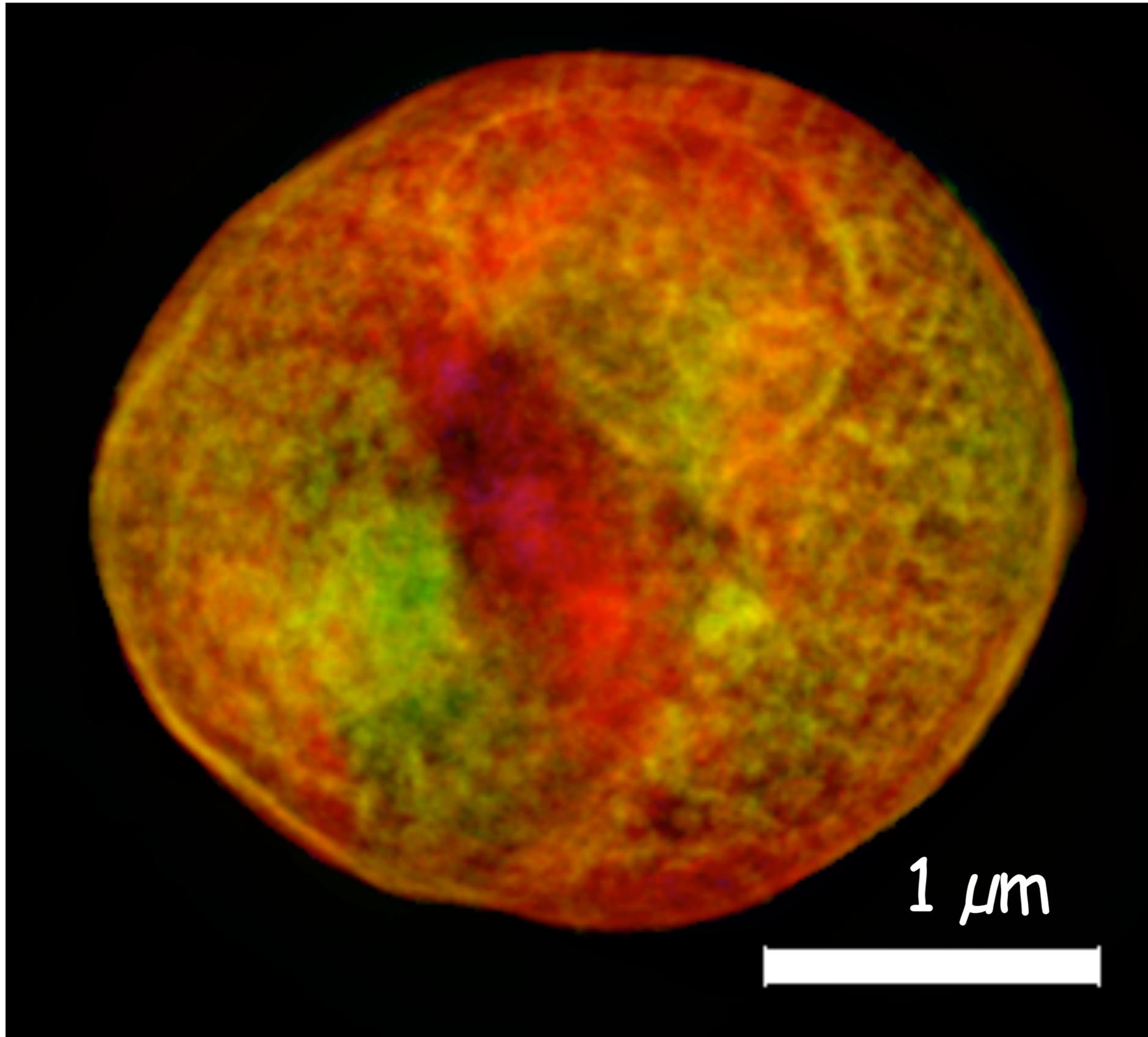
Slices through reconstruction

- Chapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)
- Resolution $\sim 10 \times 10 \times 50$ nm



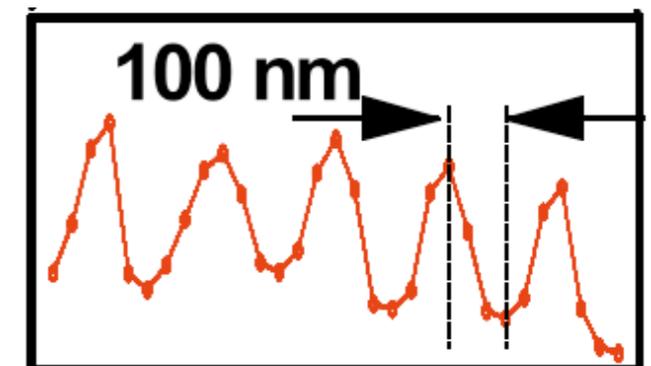
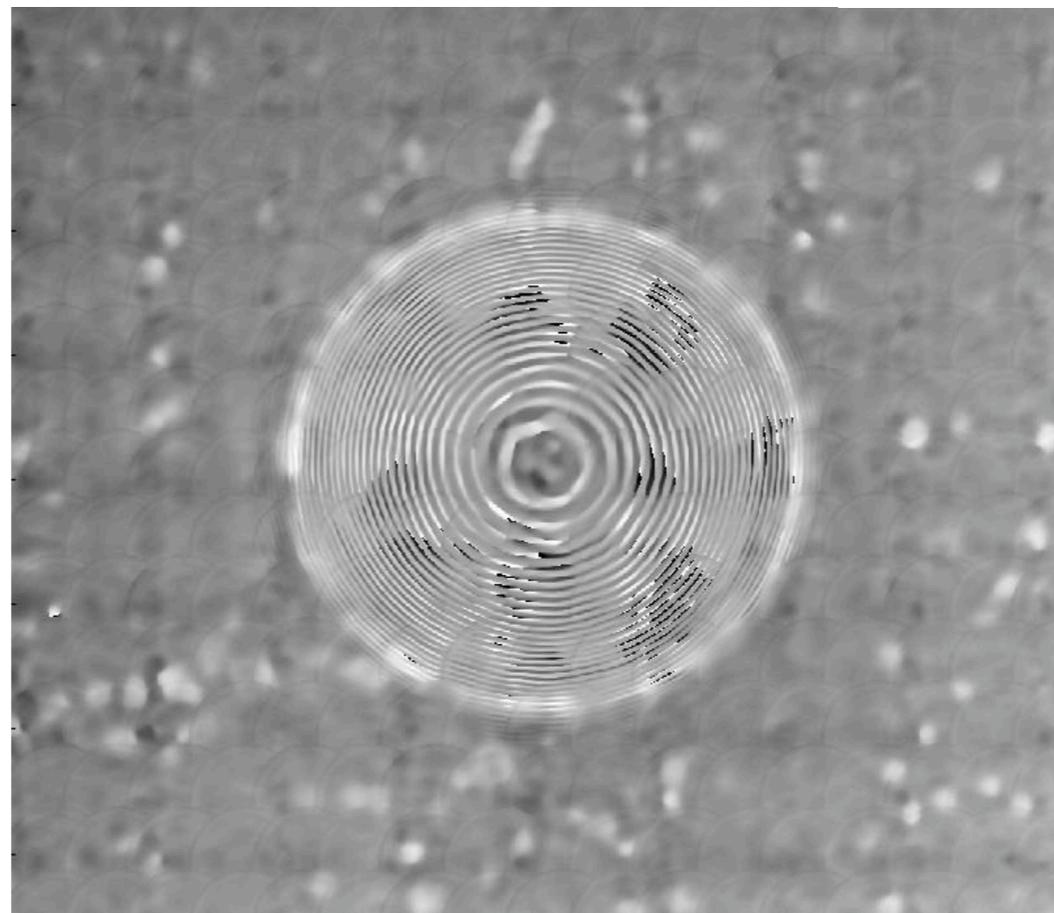
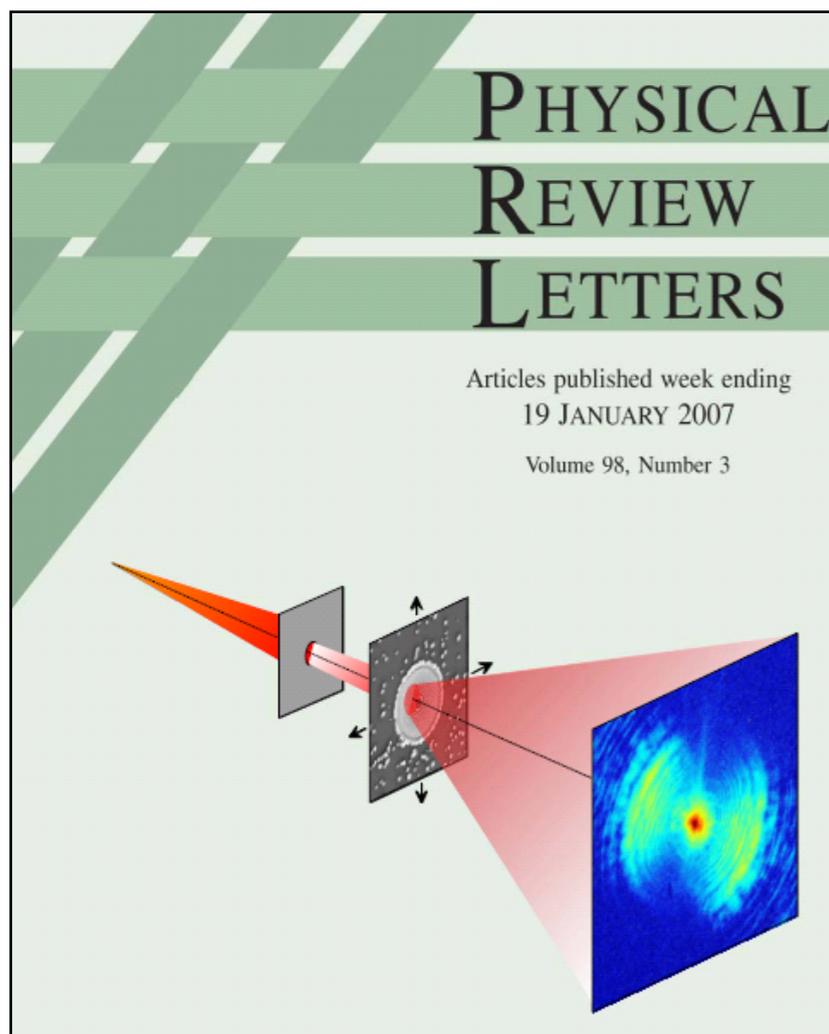
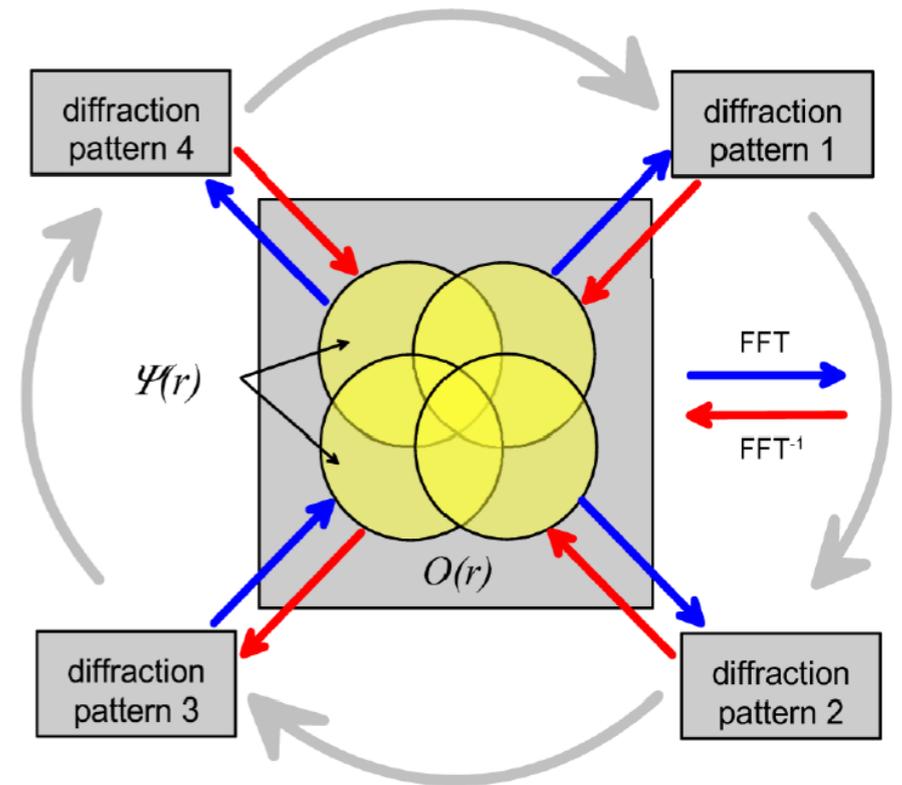
Reconstructed image

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



Alternative approach: ptychography

- R. Hegerl, W. Hoppe, *Ber. Bunsen-Ges. Phys. Chemie* **74**, 1148 (1970).
- H. M. L. Faulkner and J. M. Rodenburg, *Phys. Rev. Lett.* **93**, 023903 (2004).
- J.M. Rodenburg, A.C. Hurst, A.G. Cullis, B.R. Dobson, F. Pfeiffer, O. Bunk, C. David, K. Jefimovs, and I. Johnson, *Phys. Rev. Lett.* **98**, 034801 (2007).



What does diffraction microscopy need that's different?

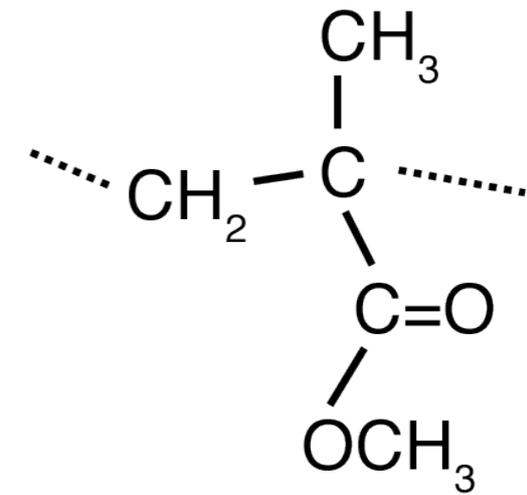
- Coherent diffraction:
 - All of the coherent photons in a $\sim 10\ \mu\text{m}$ field, where the sample sits. Monochromator exit slit? Low demagnification optic?
 - Area detector with *lots* of pixels (10^7), and *lots* of dynamic range (10^7).
 - Guard slits and beamstops.
- Nanoprobe:
 - All of the coherent photons in a $\sim 100\ \mu\text{m}$ field, where the optic sits.
 - Transmission, fluorescence, mass spec(?), electrons(?), ...

Getting the most bang per photon

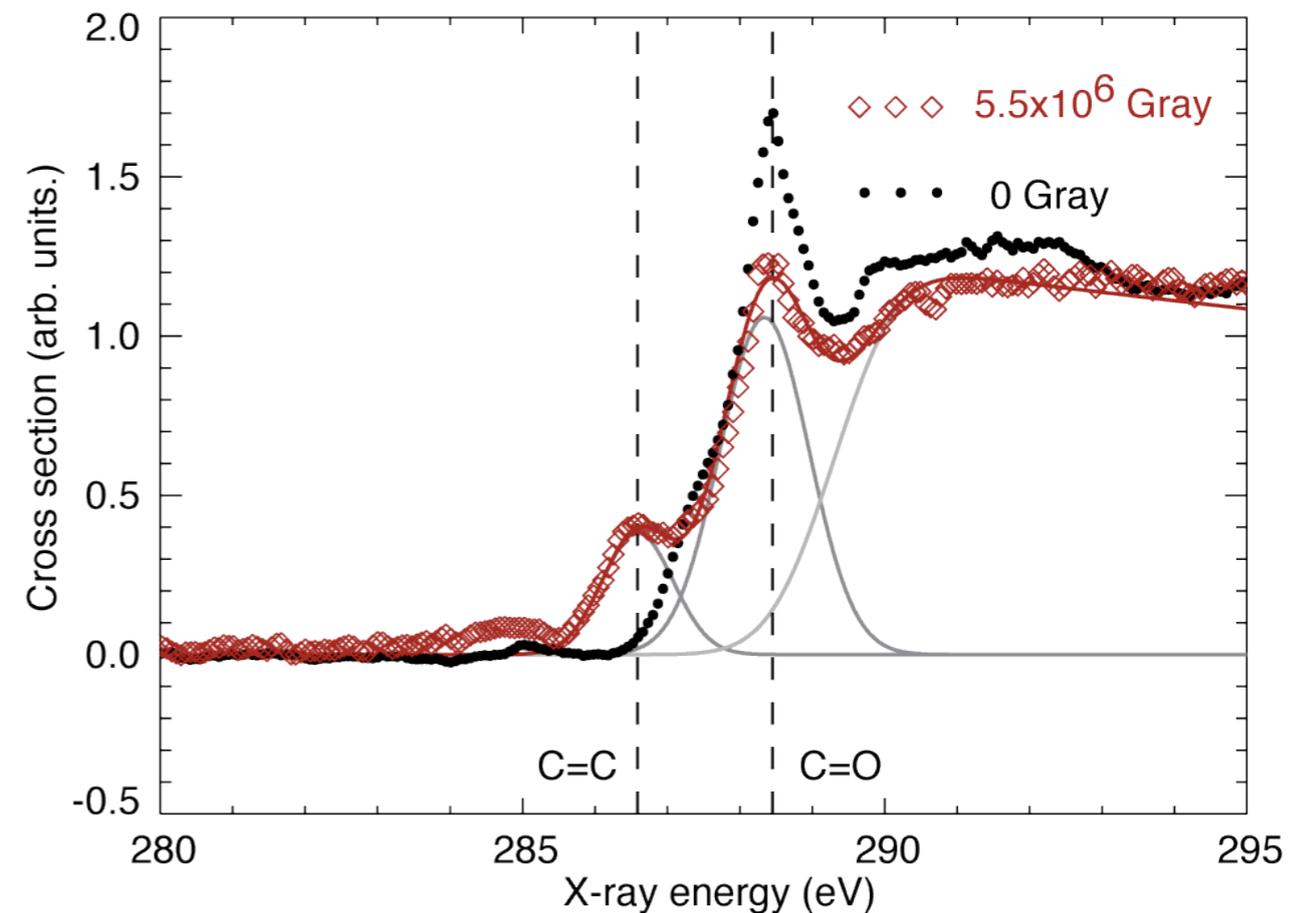
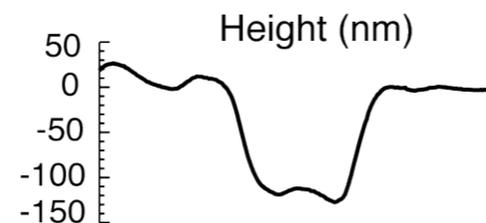
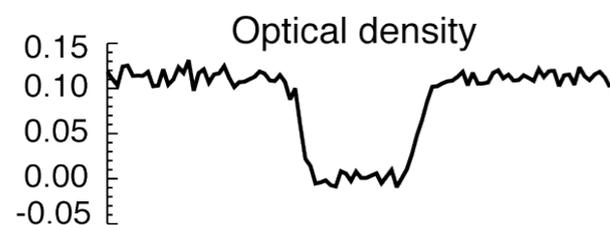
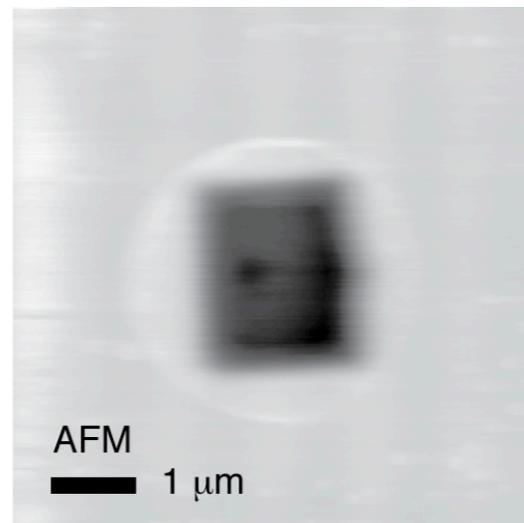
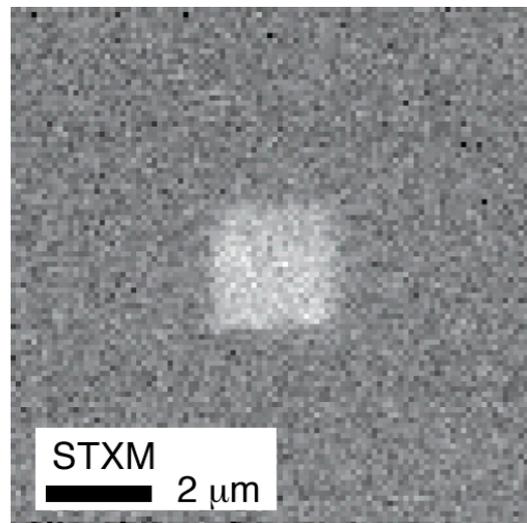
- Use the strongest contrast mechanism (for example, phase contrast).
- Use the most efficient optical system (scanning, or lensless).
- **Make the sample as robust as possible (cryo).**
- Extract your information from complex data.

X-ray irradiation: poly(methyl methacrylate) (PMMA)

- PMMA: poly methyl methacrylate (plexiglass!) which is especially radiation sensitive – it's used as a resist for electron beam lithography
- X. Zhang et al., J. Vac. Sci. Tech. B **13**, 1477 (1995)
- Fine step size, high flux image for dose
- Slightly defocused beam for low dose image off XANES peaks
- At end, AFM for thickness



- Defocused beam for spectrum
- Gaussian fit to measure peak strengths at XANES resonances

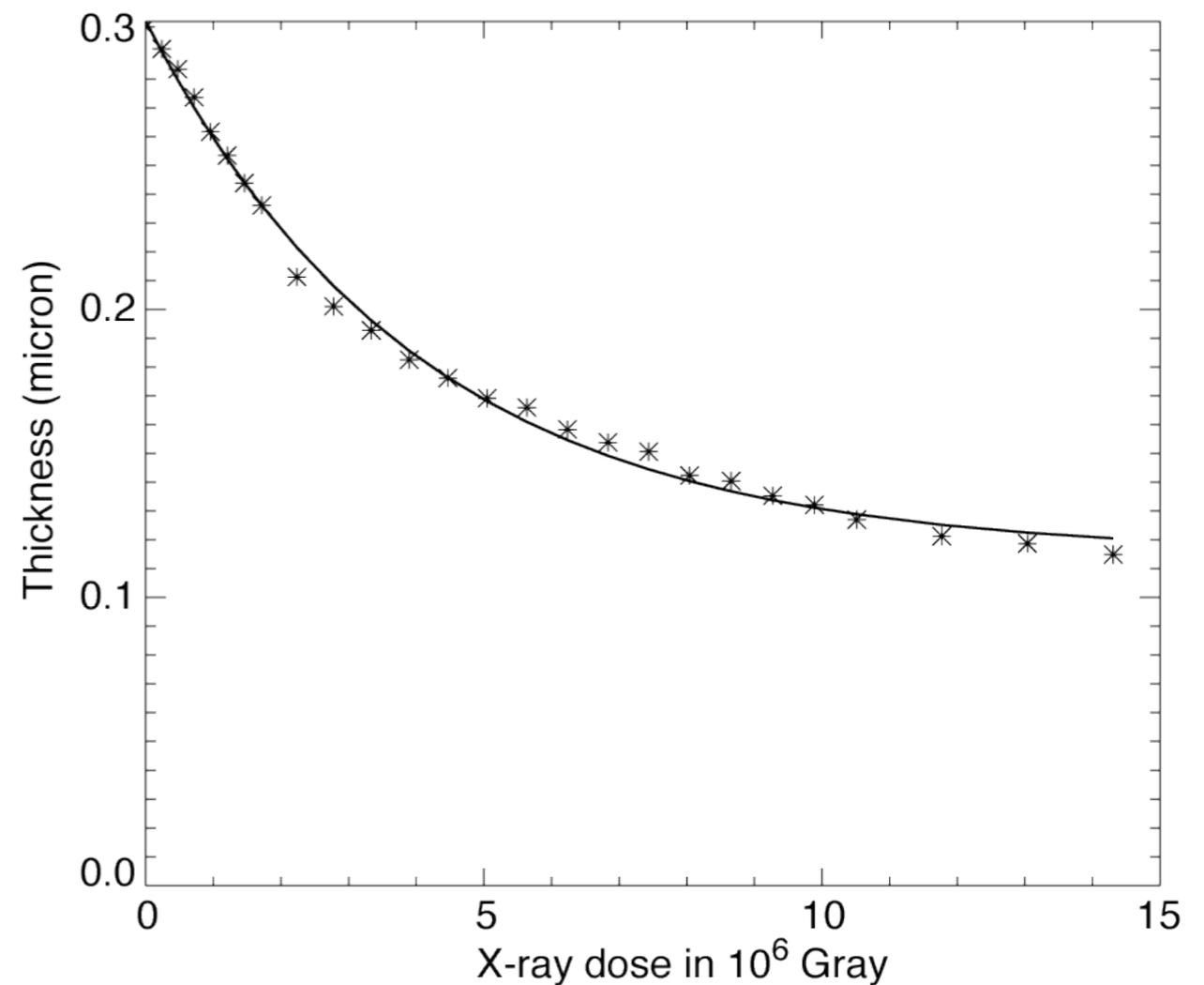
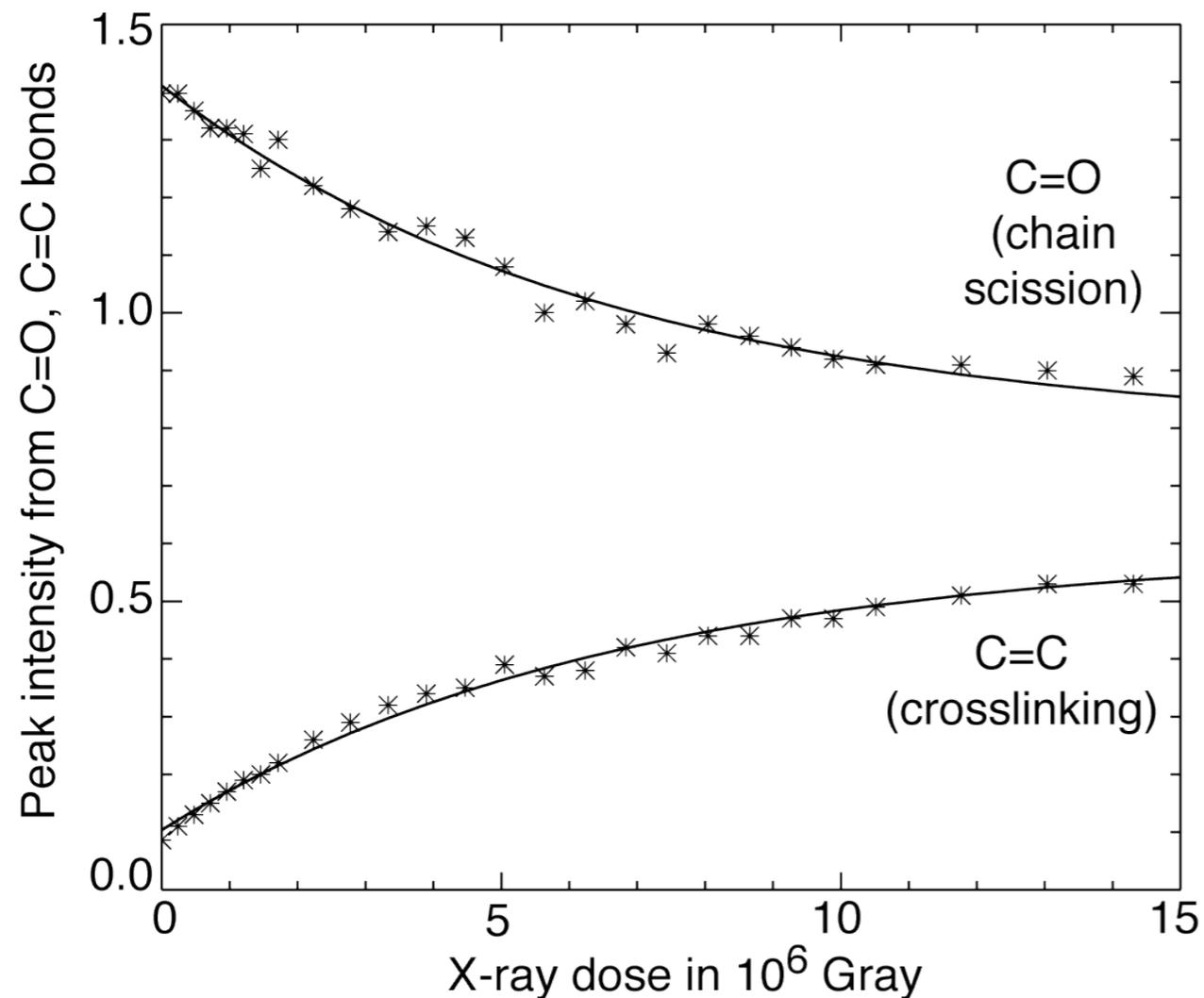


Mass loss: small pieces fly away

X. Zhang, C. Jacobsen, S. Lindaas, S. Williams, *J. Vac. Sci. Tech. B* **13**, 1477 (1995)

- Chain scission: C=O peak decrease
- Crosslinking: C=C peak increase

- Mass loss: optical density, AFM verification



Mass spectroscopy of fragments: see Tinone *et al.*, *J. Vac. Sci. Tech. A* **13**, 1885 (1995)

Atomic resolution imaging: electrons or photons?

10 keV photons

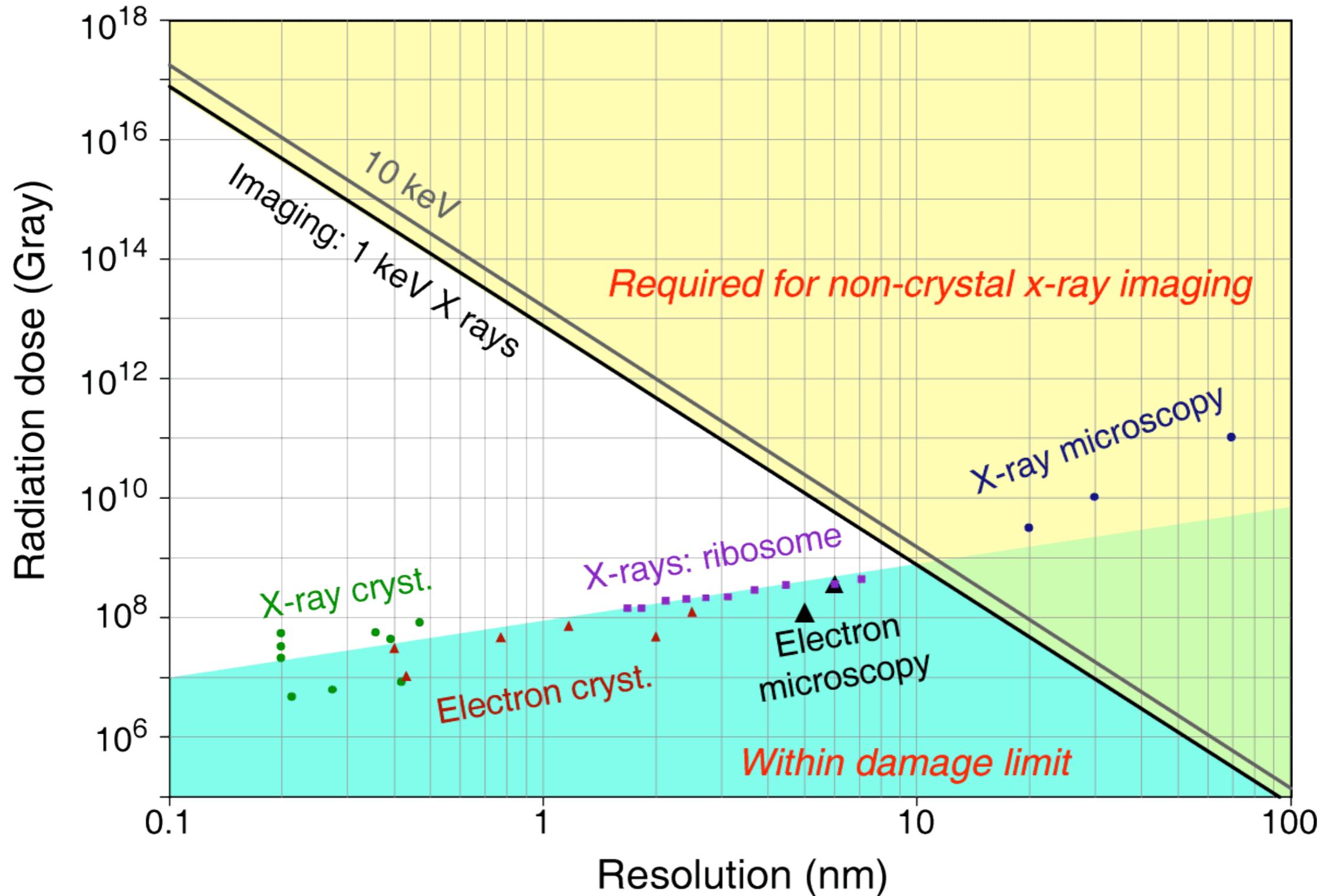
- About 100 absorption events per elastic scatter
- About 10 keV deposited per absorption
- Therefore about 10^6 eV deposited per elastic scatter
- A thousand scattered photons:
 $10^3 \cdot 10^6$ eV into $(2 \text{ \AA})^3$, or 2×10^{13} Gray

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:
 $10^3 \cdot 10^2$ eV into $(2 \text{ \AA})^3$, or 2×10^9 Gray

- Electrons are better than photons for atomic resolution imaging: J. Breedlove and G. Trammell, *Science* **170**, 1310 (1970); R. Henderson, *Q. Rev. Biophys.* **28**, 171 (1995).
- X-ray crystallography's answer: spread the dose out over many identical unit cells
- X-ray Free Electron Lasers: get image in <100 fsec, before damage

What's the limit for cells?

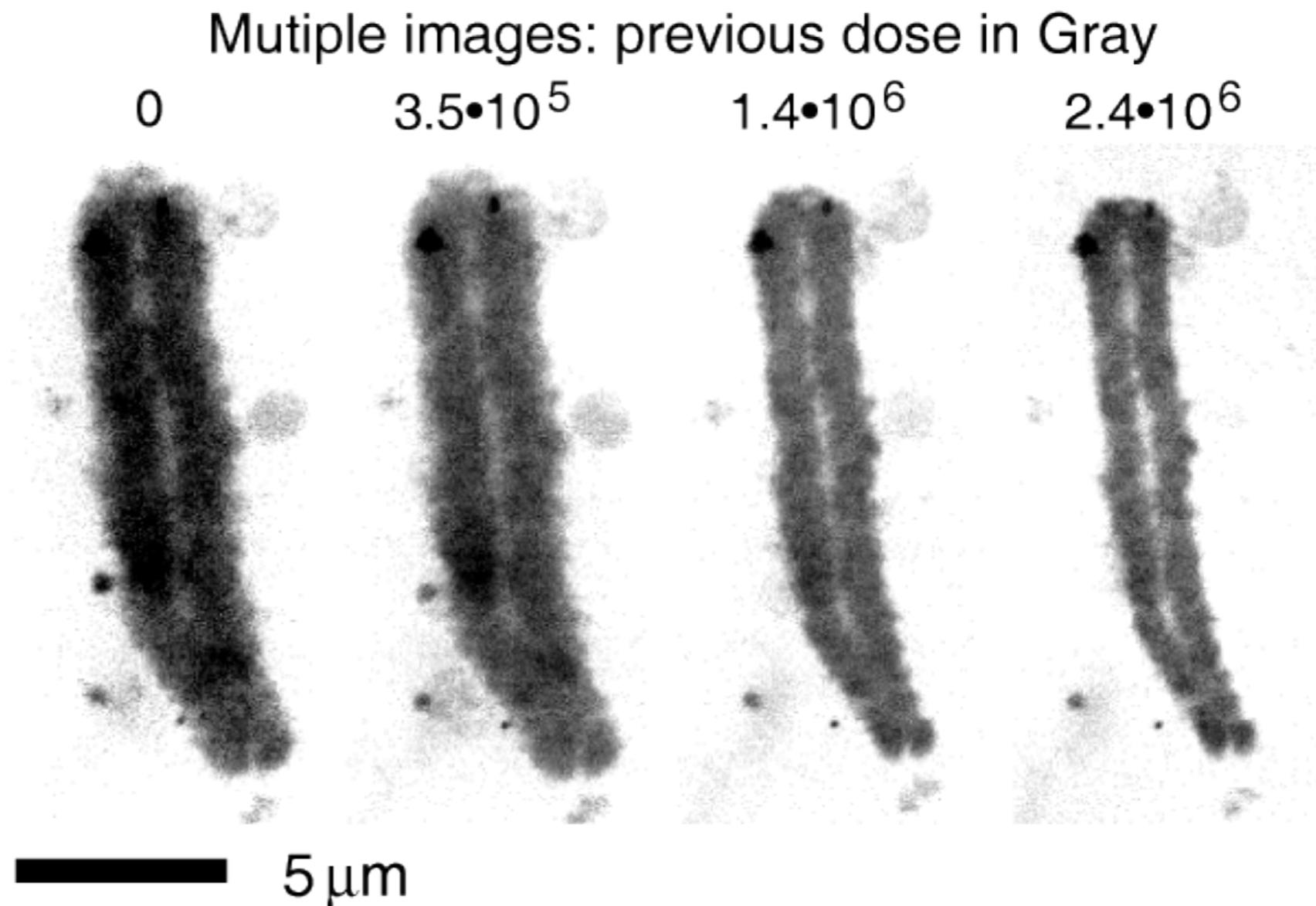


Howells et al., *JESRP* (submitted).

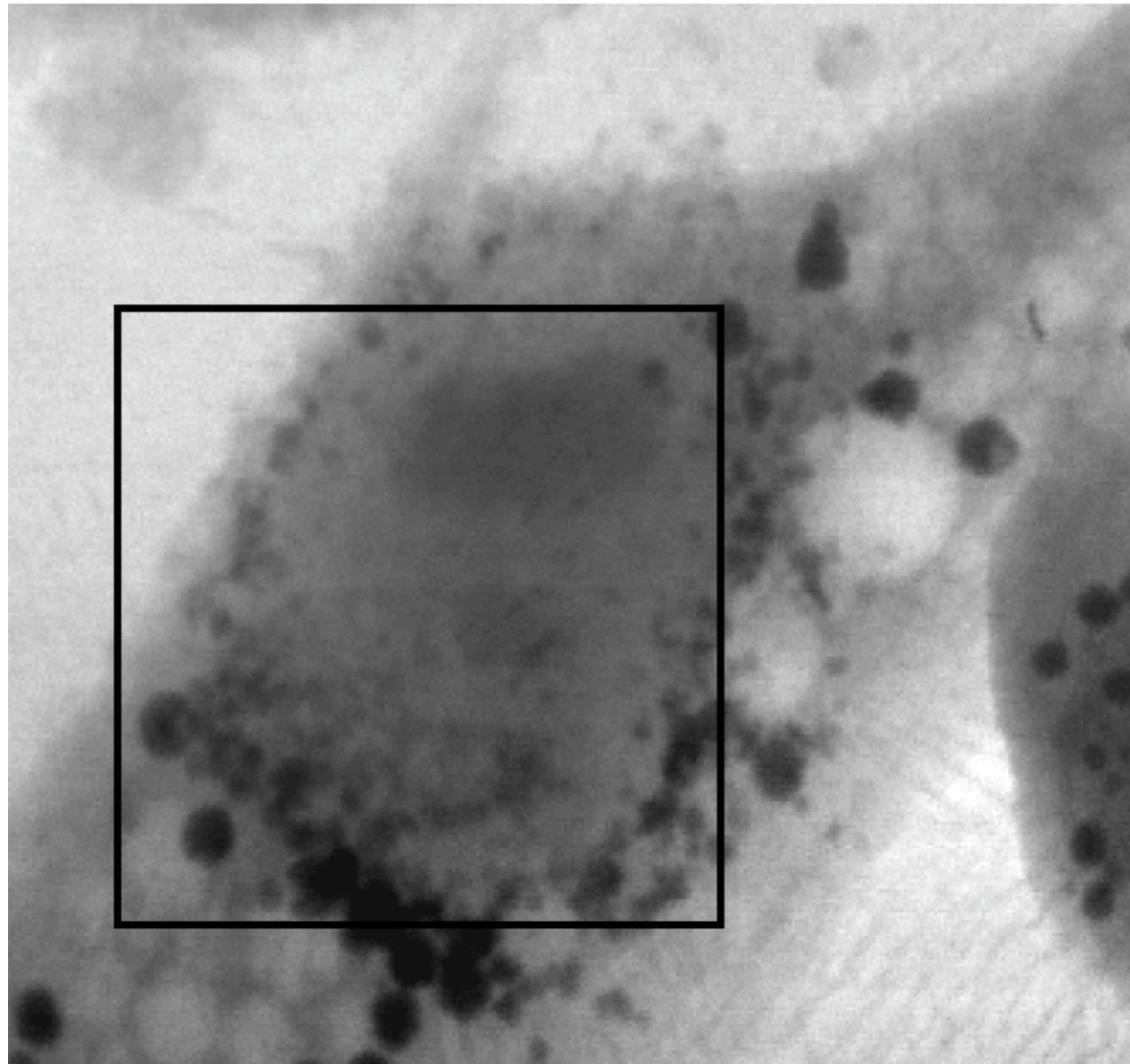
See also Shen et al., *J. Sync. Rad.* **11**, 432 (2004)

Wet, fixed samples: one image is OK

- Chromosomes are among the most sensitive specimens.
- *V. faba* chromosomes fixed in 2% glutaraldehyde. S. Williams et al., J. Microscopy **170**, 155 (1993)
- Repeated imaging of one chromosome shows mass loss, shrinkage



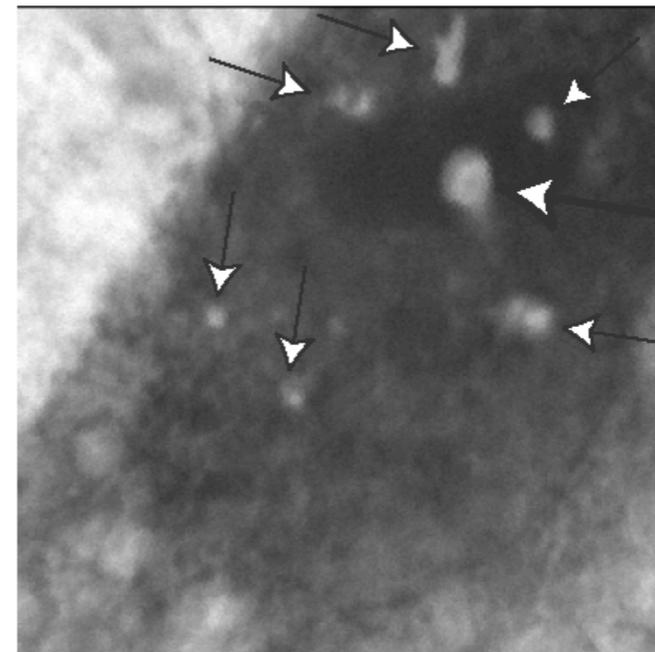
Radiation damage resistance of wet specimens at liquid nitrogen temperature



Frozen hydrated image **after** exposing several regions to $\sim 10^{10}$ Gray

Maser et al., *J. Micros.* **197**, 68 (2000)

After warmup in microscope (eventually freeze-dried): holes indicate irradiated regions!



— 7 μm

Cryo specimen preparation

- Ice crystals create a “Swiss cheese” effect above -135°C . For hydrated samples: if you’re born cold, stay cold!
- Possible future approach: mount delicate sample in a cartridge 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 and x-ray/IR microscopes and nanoprobes.

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

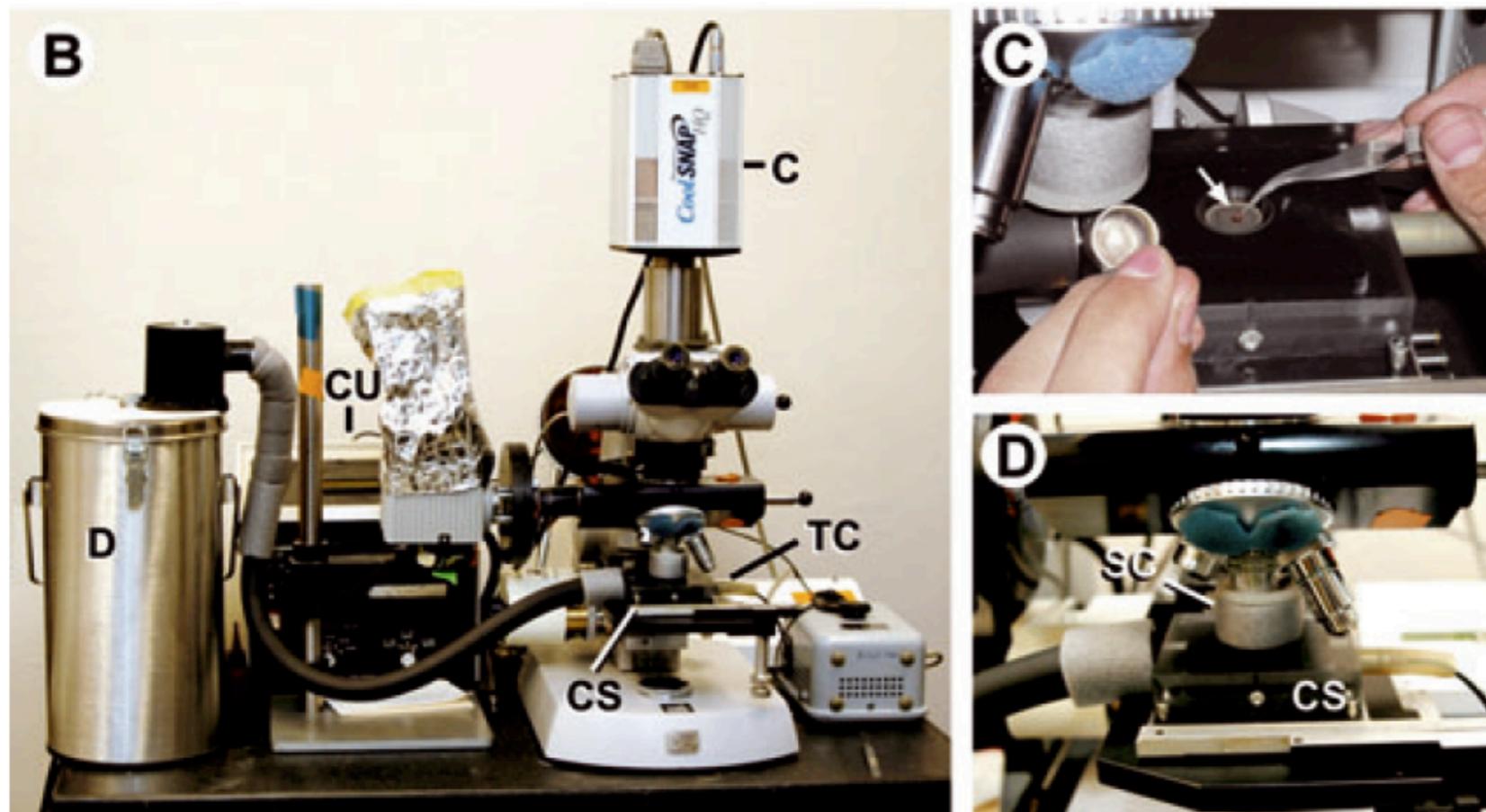
CINDI L. SCHWARTZ^{*}, VASILY I. SARBASH[†],
FAZOIL I. ATAULLAKHANOV[†], J. RICHARD MCINTOSH^{*}
& DANIELA NICASTRO[‡]

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

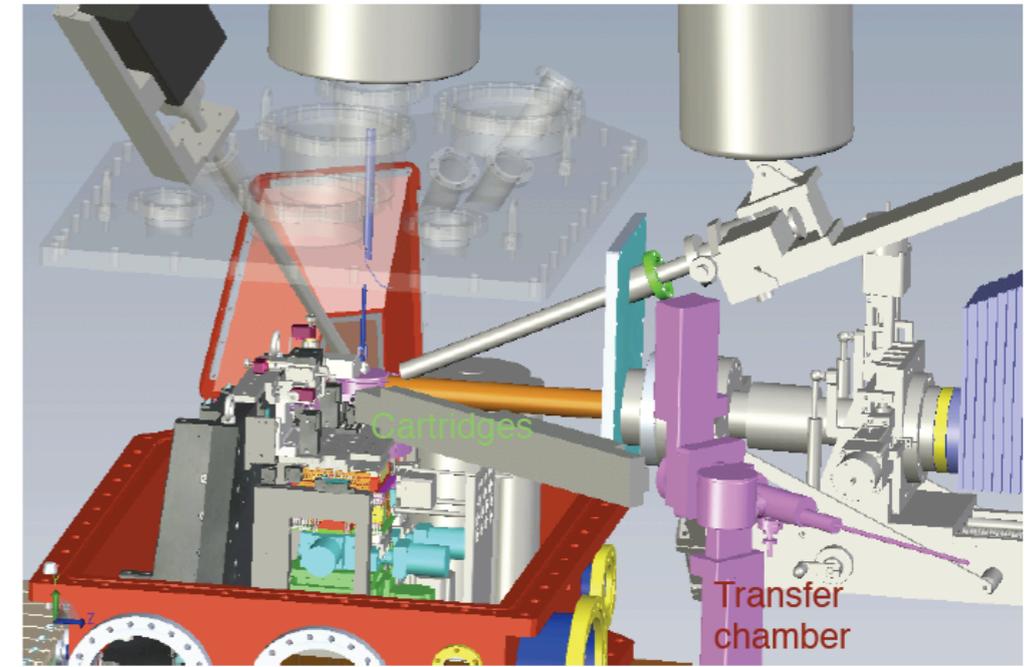
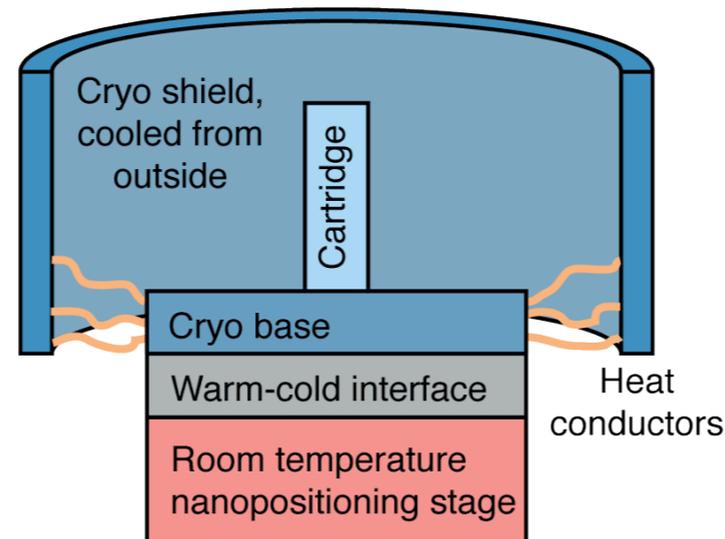
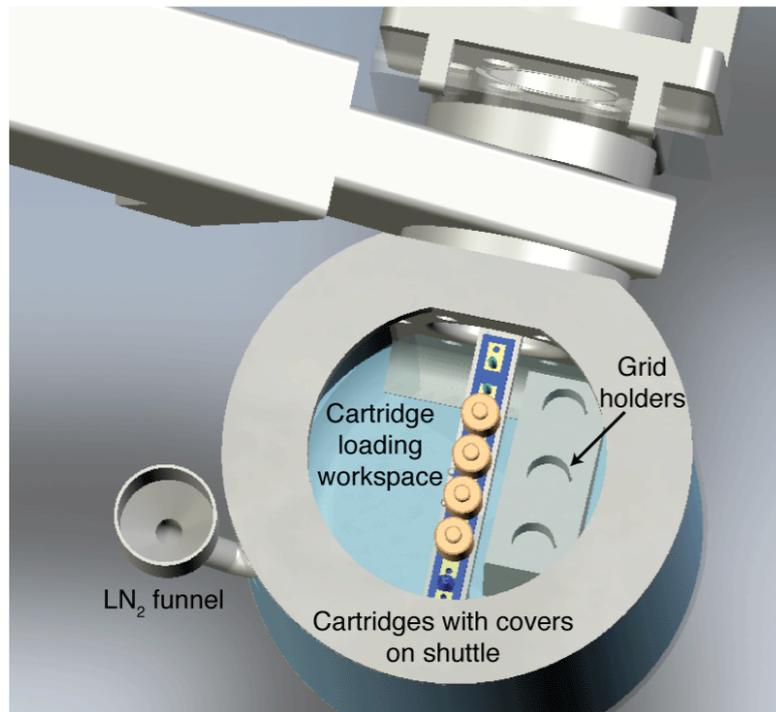
[†]*National Research Center for Hematology, Moscow, Russian Federation*

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

See also Sartori *et al.*, *J. Struct. Bio.* **160**, 135 (2007).

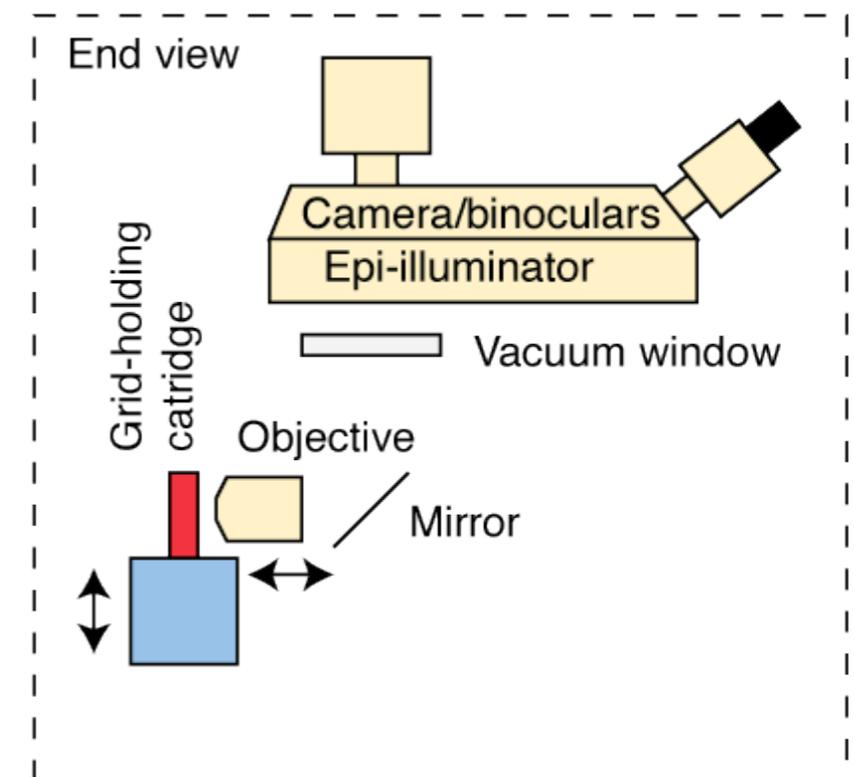
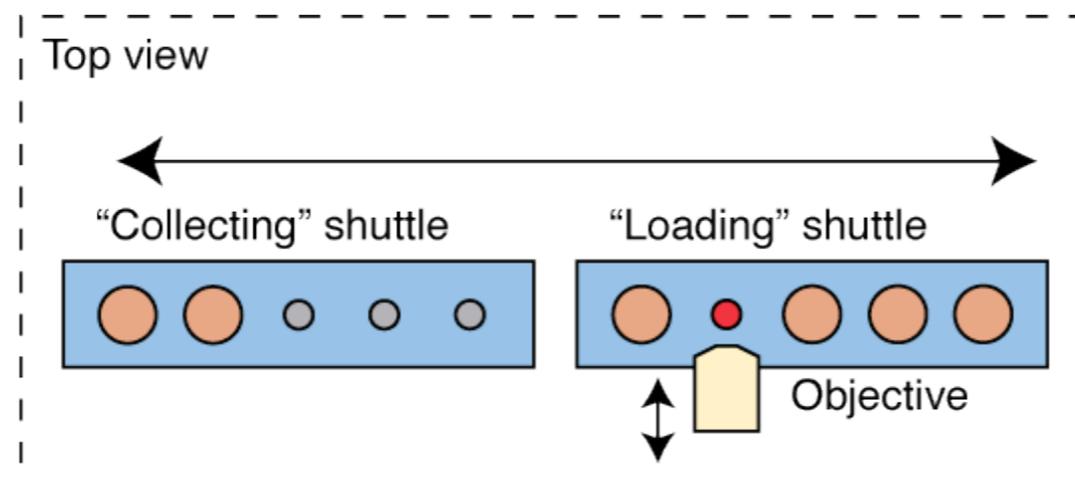


Cryo system: Xradia example



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

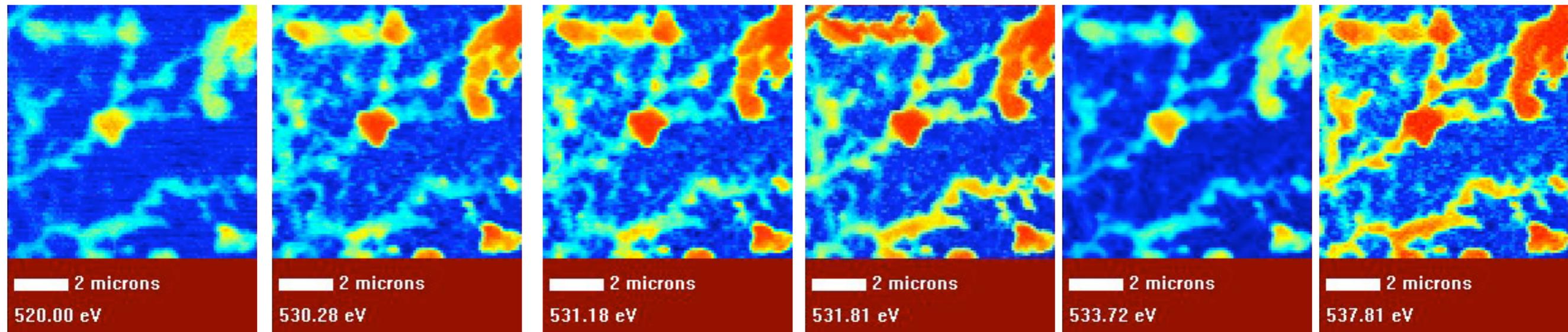
Xradia cryo team:
C. Jacobsen, D.
Trapp, H. Singh, M.
Howells, C. Cork



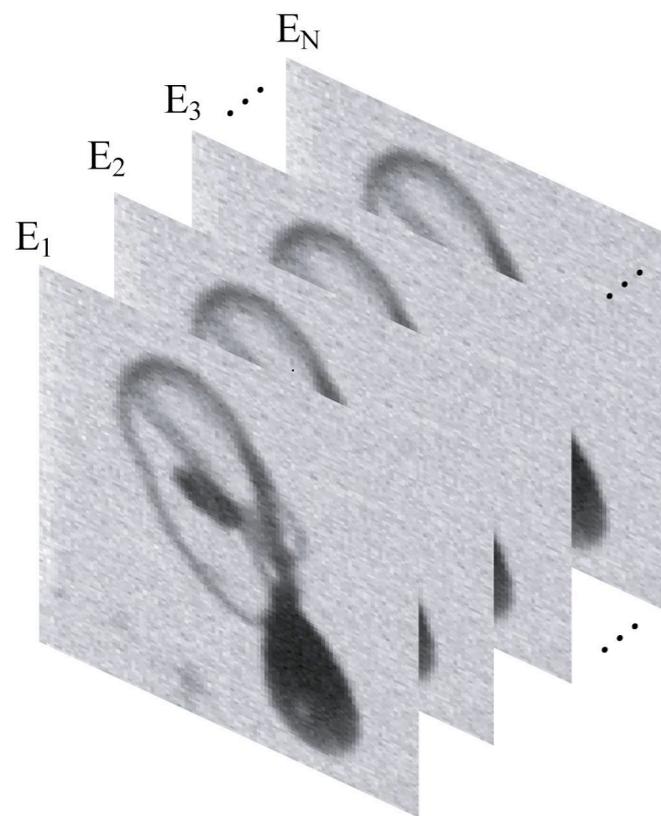
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Spectromicroscopy from image sequences



Lu in hematite (T. Schäfer)

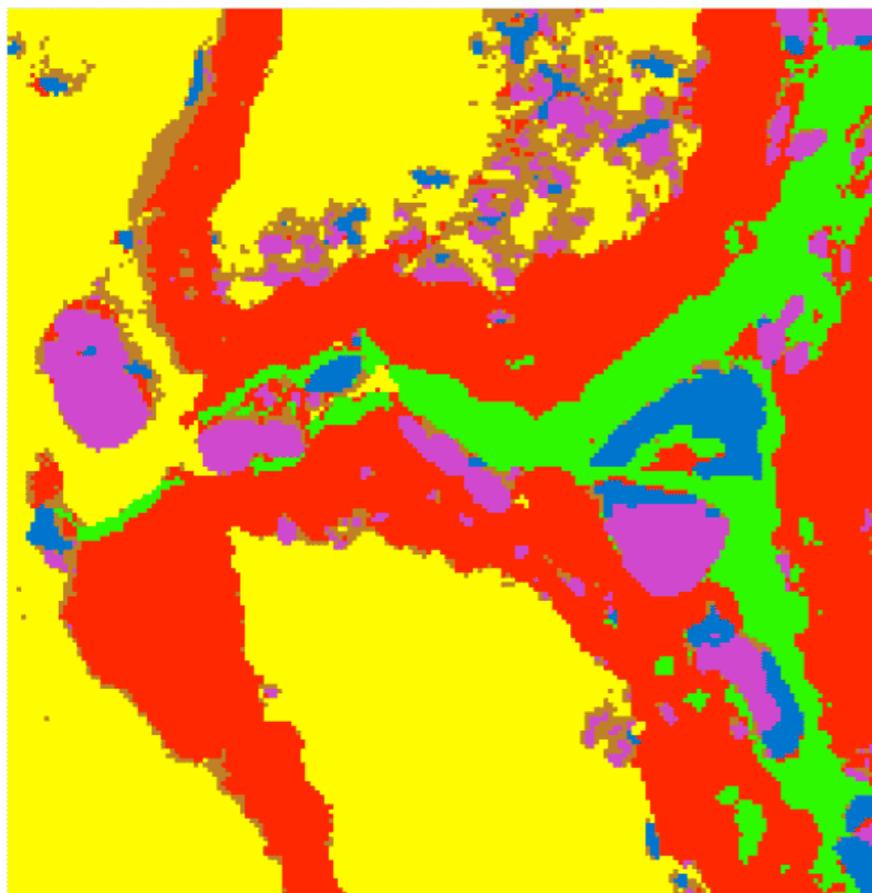


10^4 - 10^5 spectra! Too many to analyze “by hand.” Complex mixtures *etc.*; life is not made up of uniform thin films. How to deal with the complexity? Pattern recognition algorithms. Lerotic *et al.*, *Ultramicroscopy* **100**, 35 (2004).

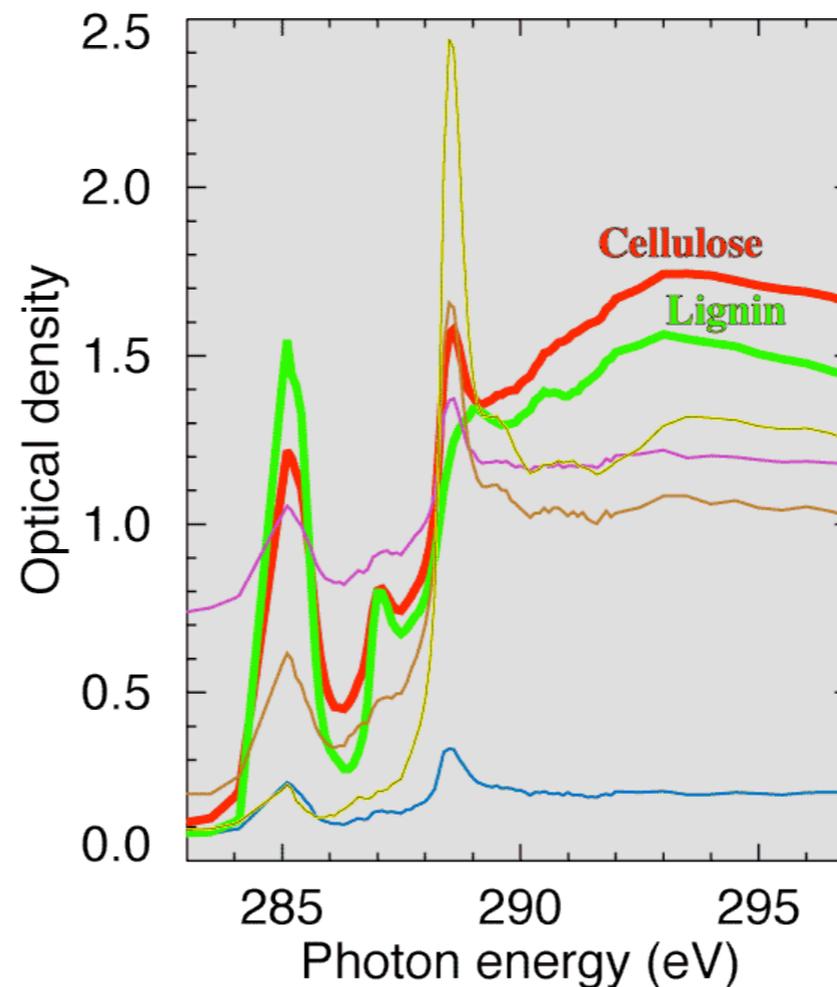
Aligned spectral image sequences:
Jacobsen *et al.*, *J. Microscopy* **197**,
173 (2000)

X-ray nanoprobes and energy?

- Ethanol from lignocellulose materials is promising: large fraction of total biomass, easier cultivation.
- But there are great challenges in economically separating cellulose from lignin!
- Soft x-ray spectromicroscopy can map cellulose and lignin so that one can see the effects of various enzymes.
- DoE proposal with David Wilson (Cornell microbiology), George Cody (Carnegie)



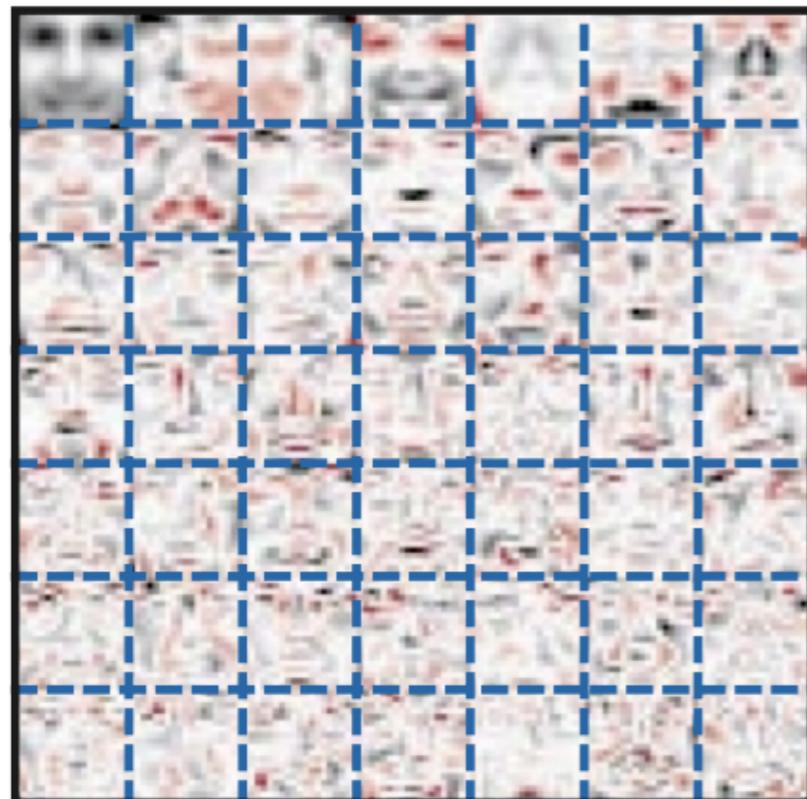
4 μm



Lignin and cellulose in 400 million year old chert: Boyce *et al.*, *Proc. Nat. Acad. Sci.* **101**, 17555 (2004), with subsequent pattern recognition analysis by Lerotic *et al.*, *Ultramicroscopy* **100**, 35 (2004).

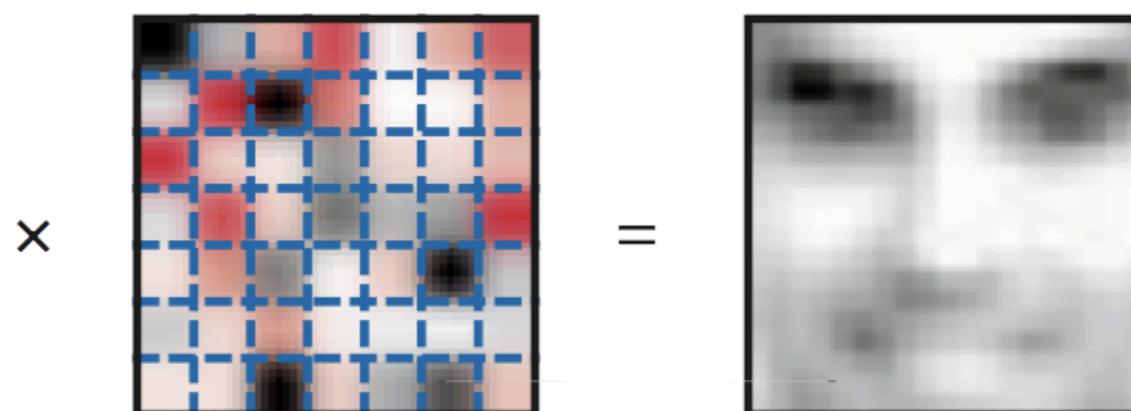
Avoiding negativity: non-negative matrix factorization

PCA

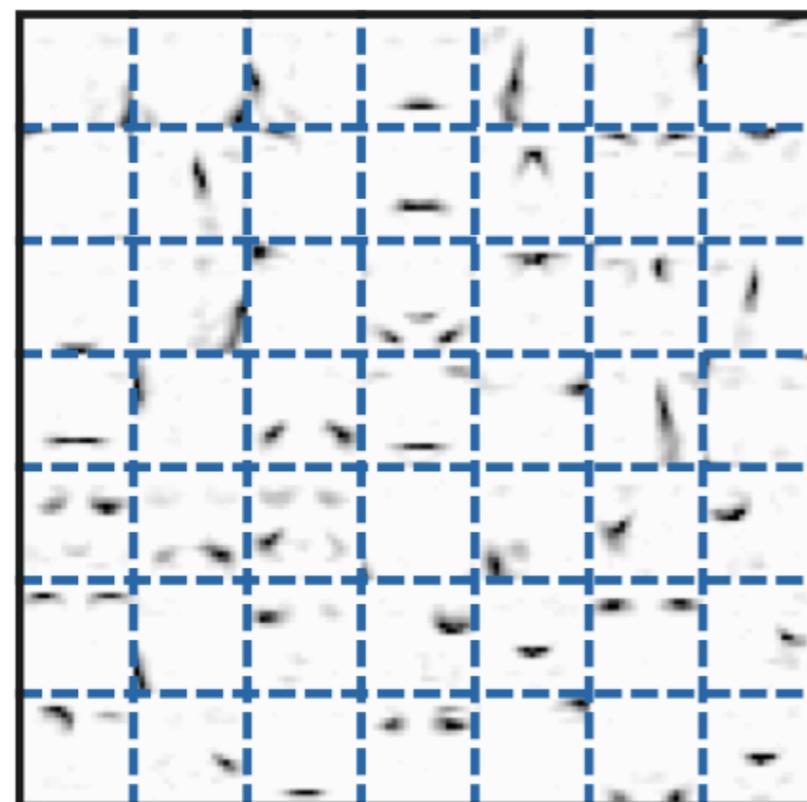


Lee and Seung, *Nature* **401**, 788 (1999)

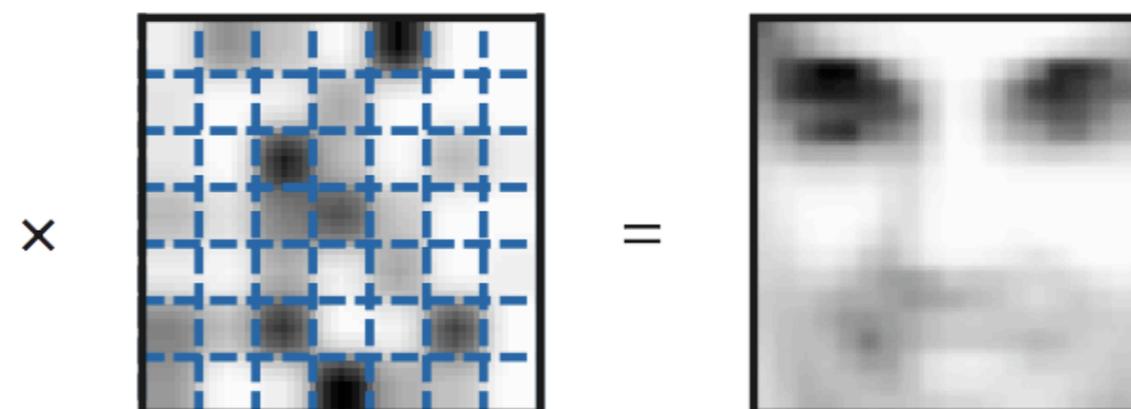
Principal component analysis



NMF



Non-negative matrix factorization



Non-negative matrix factorization

- Iterative procedure:

$$\mu_{N \times S} := \mu_{N \times S} \cdot \left(\frac{D_{N \times P}}{\mu_{N \times S} \times t_{S \times P}} \times t_{P \times S}^T \right)$$

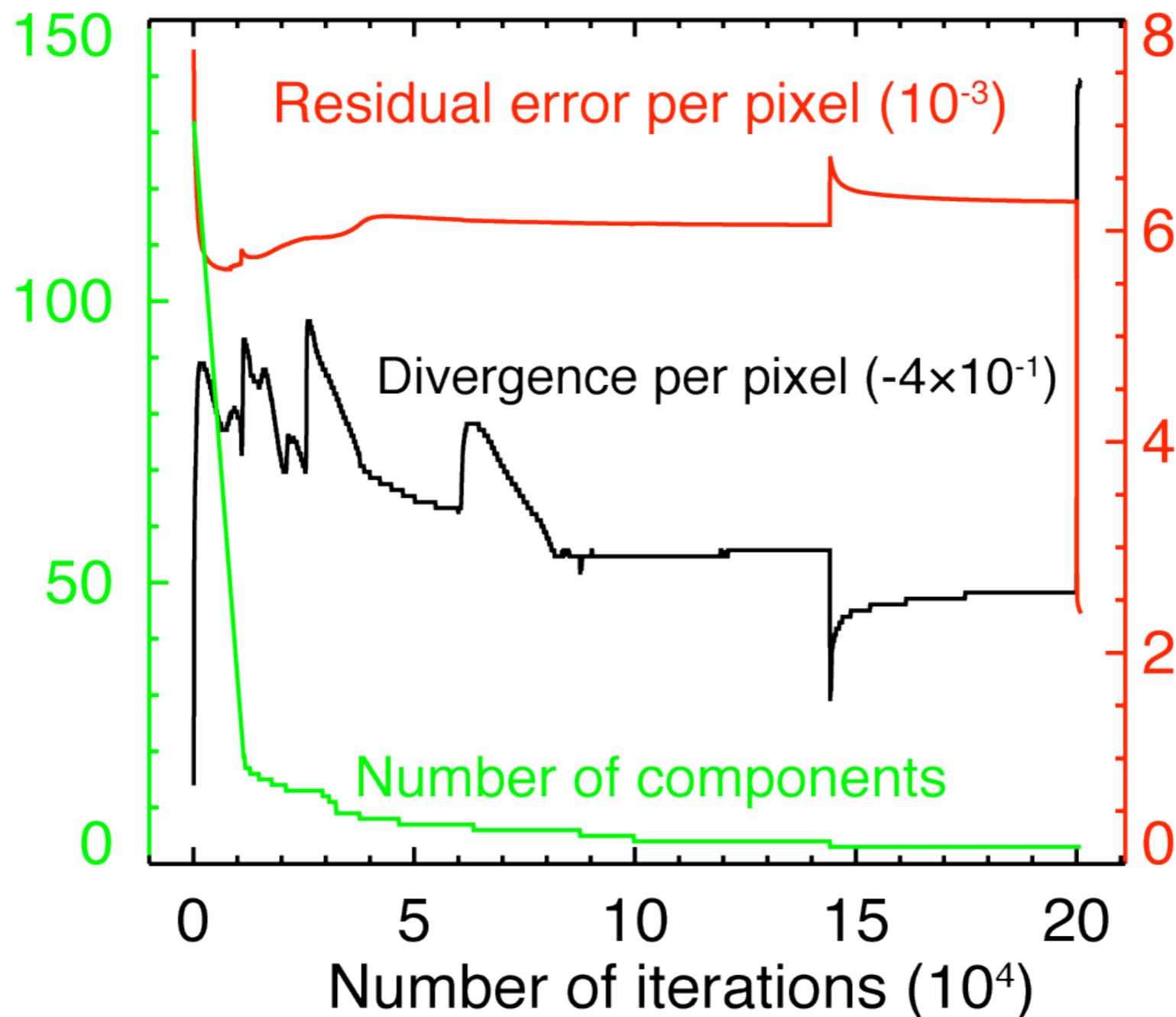
For those pixels where $\mu_{N \times S} \times t_{S \times P}$ gives larger values than those present in the data $D_{N \times P}$, this estimate update will drive $\mu_{N \times S}$ towards smaller values and vice versa.

$$t_{S \times P} := t_{S \times P} \cdot \left(\mu_{S \times N}^T \times \frac{D_{N \times P}}{\mu_{N \times S} \times t_{S \times P}} \right)$$

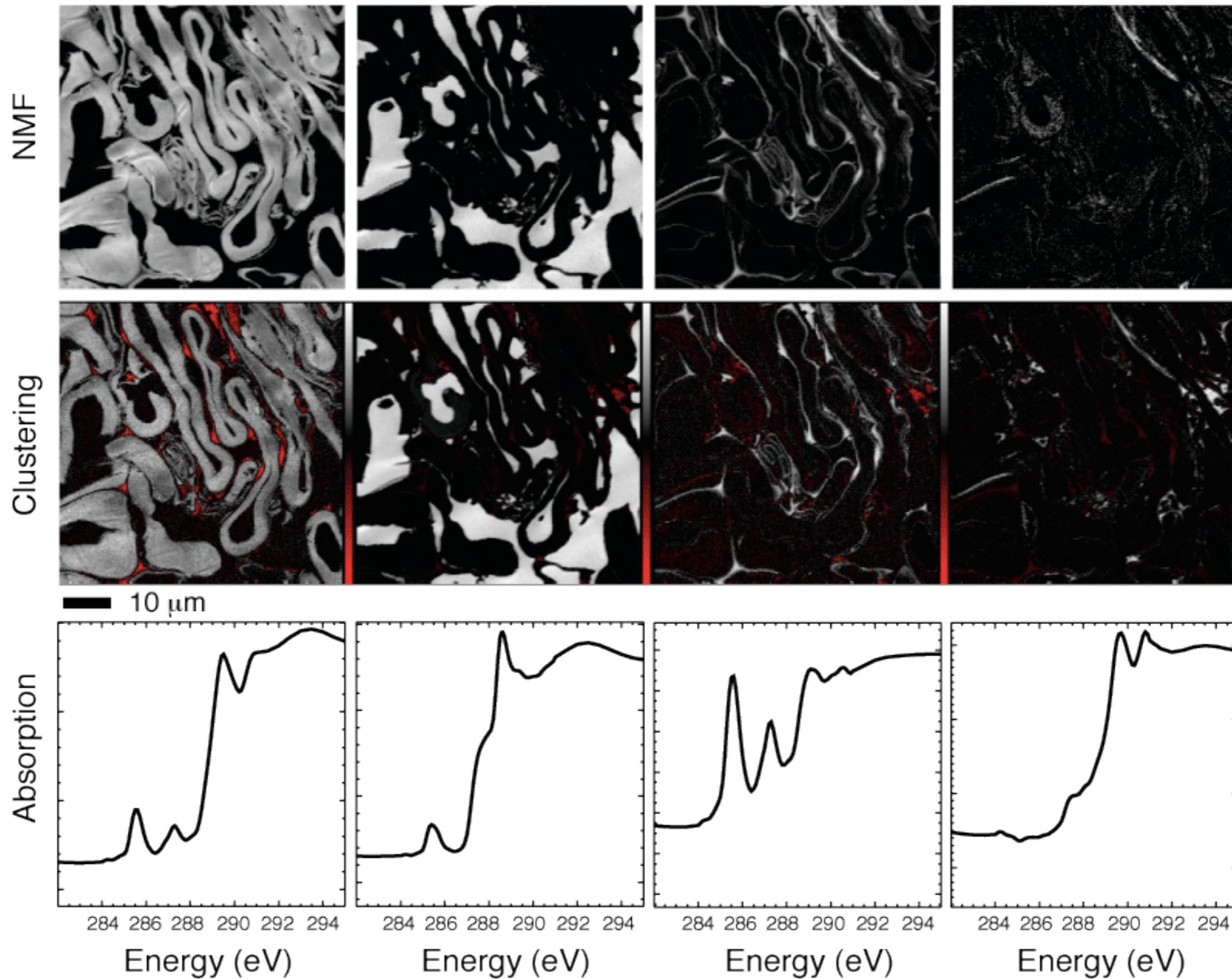
- Fleckenstein and Jacobsen (unpublished),
after Lee and Seung (1999)

NMF: many, many iterations

- We've been trying to speed it up...
- Bigger hammer: computation on graphics processors



Wood data: Michette, Phanopolous *et al.*

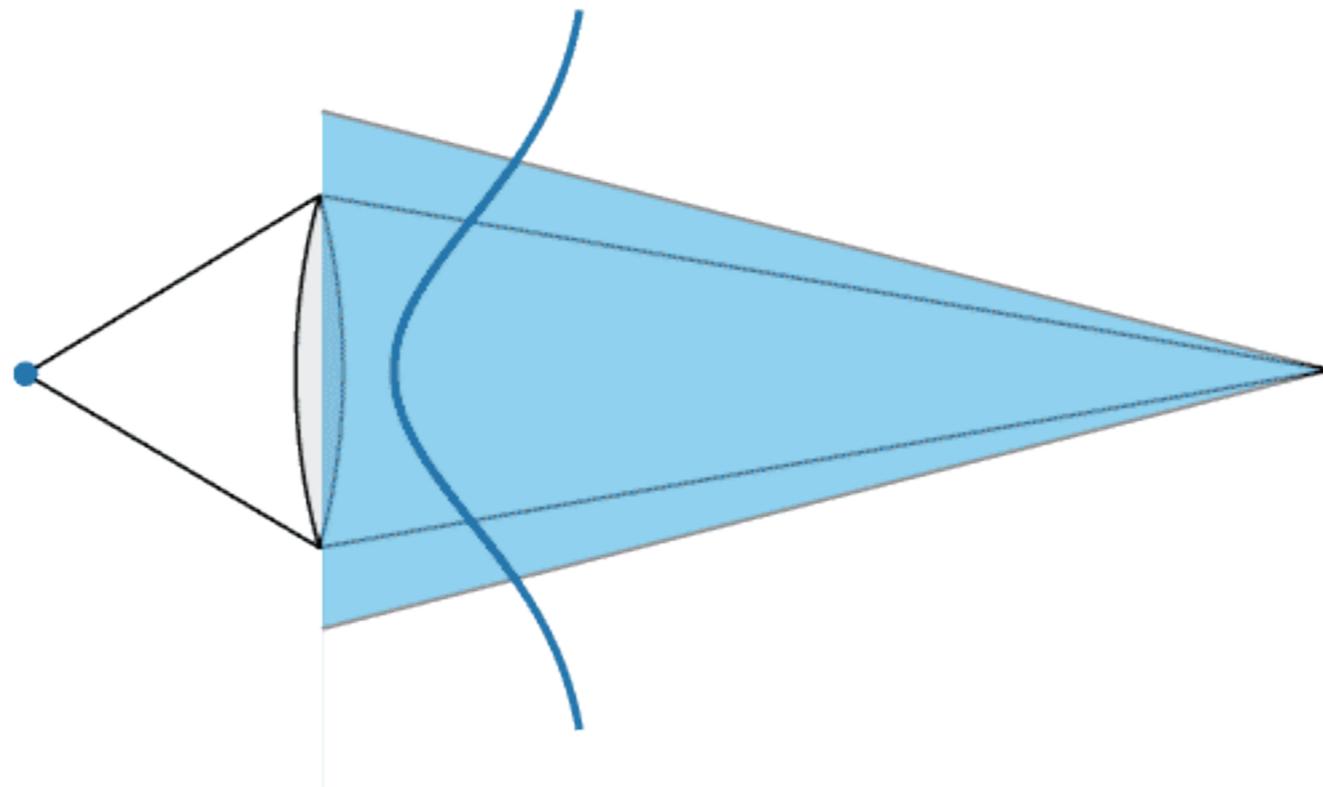


Getting the most bang per photon

- Use the strongest contrast mechanism (for example, phase contrast).
- Use the most efficient optical system (scanning, or lensless).
- Make the sample as robust as possible (cryo).
- Extract your information from complex data.

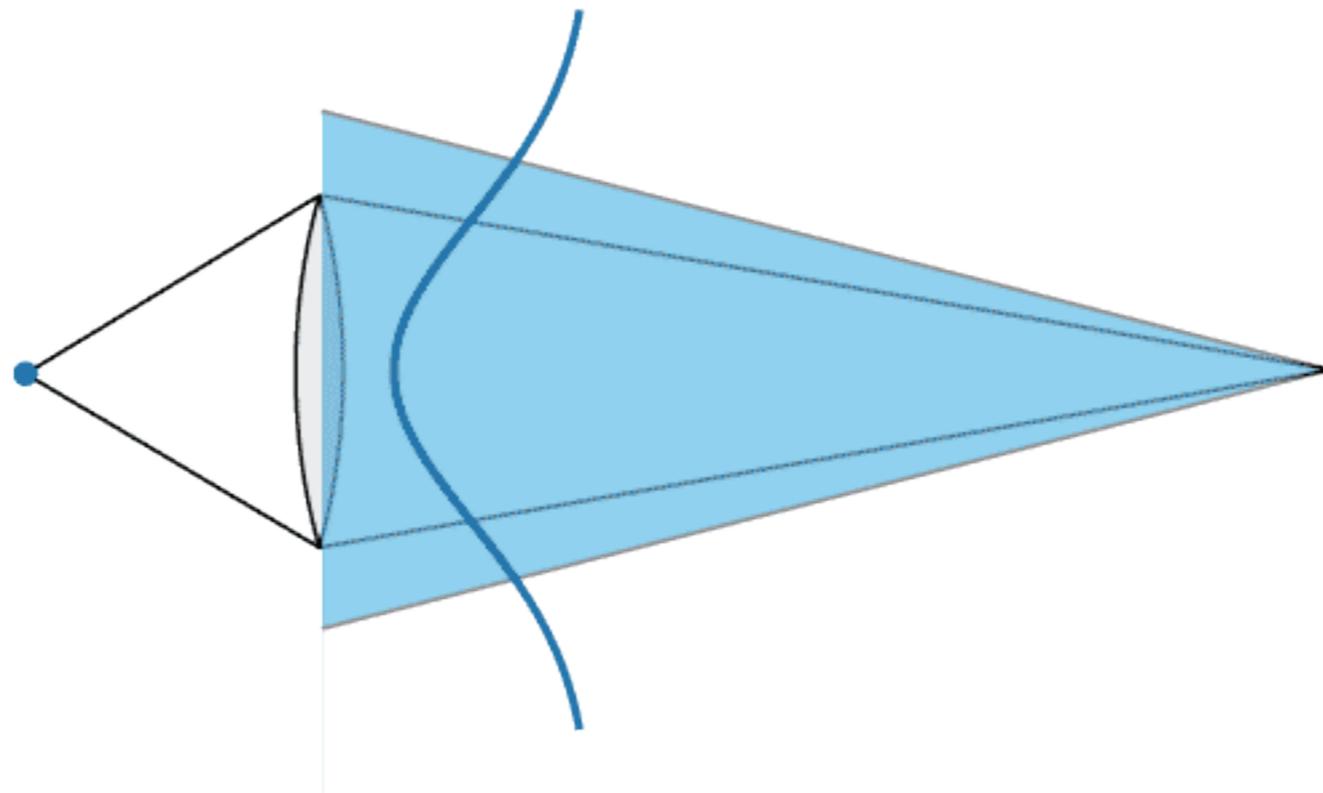
Angle stability requirements

- Image position does not shift, but flux accepted by beamline does.



Position stability requirements

- Shifts in the beam position produce both intensity fluctuations and image position fluctuations.



If multimode, prefer higher beta straight?