The Abdus Salam International Centre for Theoretical Physics

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IAEA International Atomic Energy Agency

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School on Synchrotron and Free-Electron-Laser Sources and their Multidisciplinary Applications

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Diffraction Microscopy - basics

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Diffraction Microscopy - basics

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Outline

- Motivation
- Basic ideas
- Coherence
- The phase problem
- Solutions – holography
- Solutions – Diffraction microscopy
- Prior knowledge
- The apparatus
- First experiments
- Challenges

Why go lensless?

- A technique for 3D imaging of 0.5 – 20 μm isolated objects
- Too thick for EM (0.5 μm is practical upper limit)
- Too thick for tomographic X-ray microscopy (depth of focus < 1 μm at 10 nm resolution for soft X-rays even if lenses become available)
- Flash imaging: (Hajdu lecture)

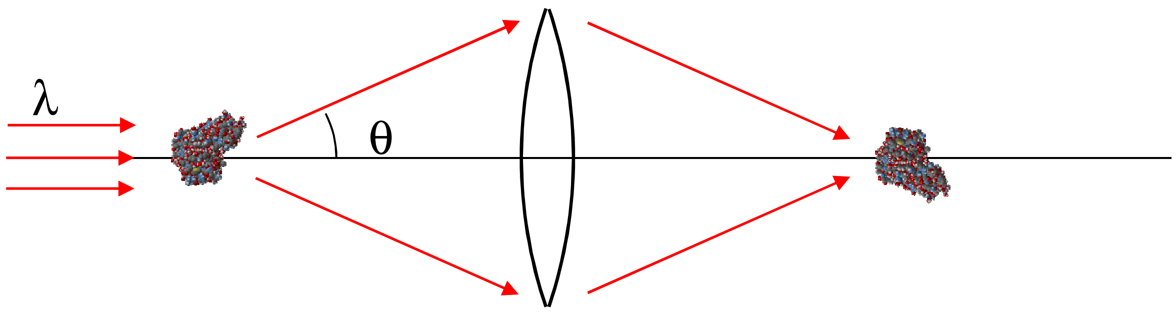
Goals @ synchrotrons

- 10 nm resolution (3D) in 1 - 10μm size biological specimens
  (small frozen hydrated cell, organelle; see macromolecular aggregates)
  Limitation: radiation damage!

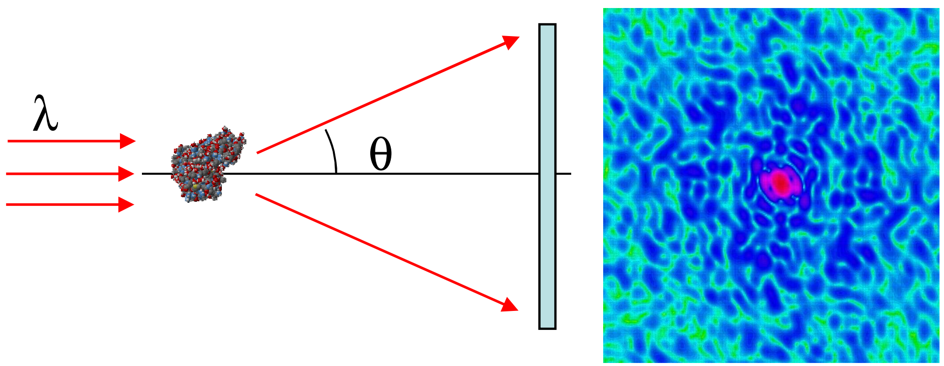
- <4 nm resolution in less sensitive nanostructures
  (Inclusions, porosity, clusters, composite nanostructures, aerosols…)
  eg: molecular sieves, catalysts, crack propagation

Alternatives to using a lens

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



Lenses have limitations. Do we really need them?



If you record the diffraction pattern, you lose the phase

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

Phase matters

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

Malcolm Howells at La Clusaz

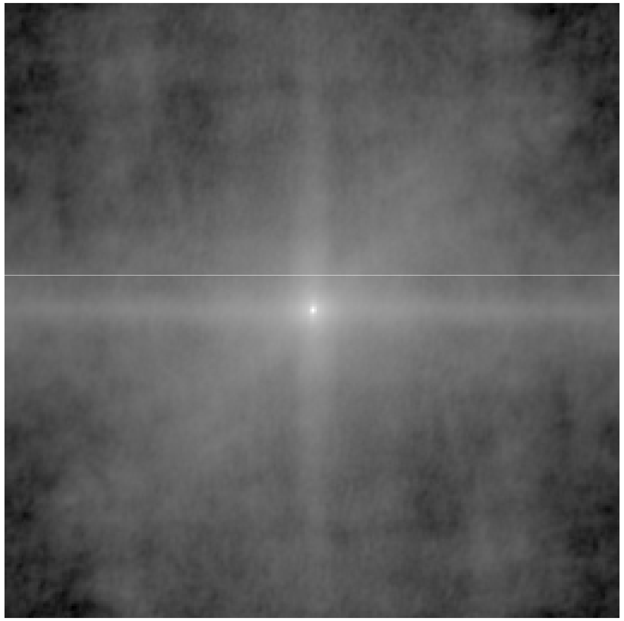


Image using only Fourier magnitudes

Image using only Fourier phases

C. Jacobsen

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"!

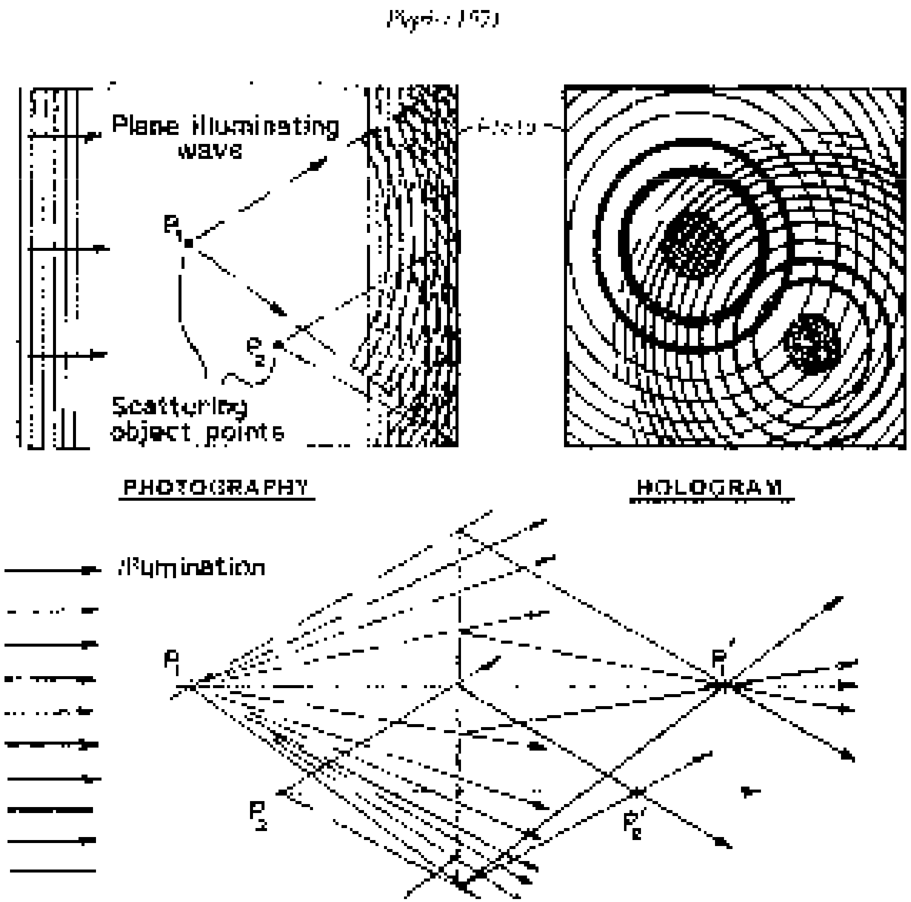
- Holographers do it – but they mix in a reference wave, need very high resolution detector or similar precision apparatus

- Crystallographers do it – but they use MAD, isomorphous replacement, or other tricks (plus the amplification of many repeats)

Holography

Gabor Nobel lecture 1971

in-line holography



First holography experiment with synchrotron radiation: Aoki, Ichihara & Kikuta, 1972

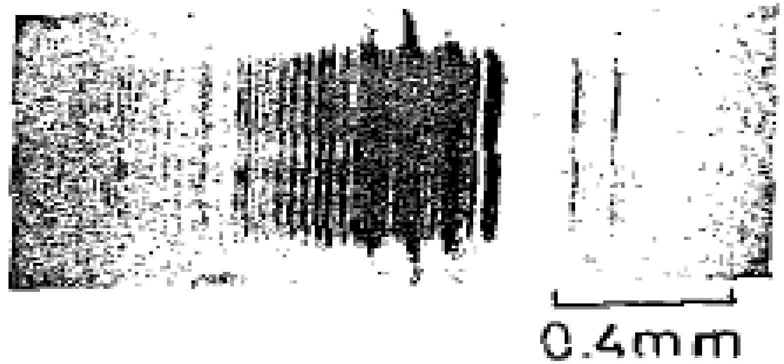


Fig. 2. An X-ray hologram.

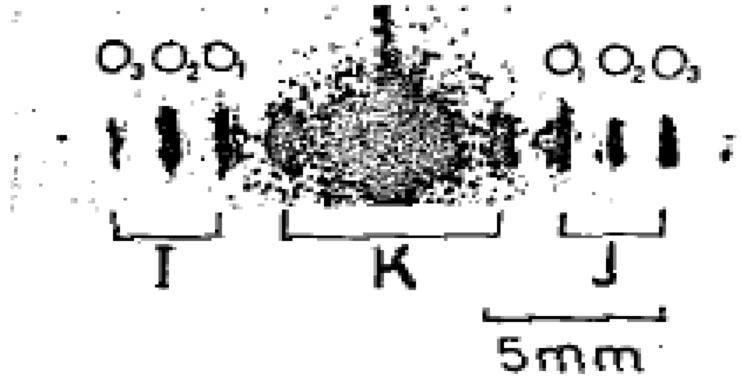
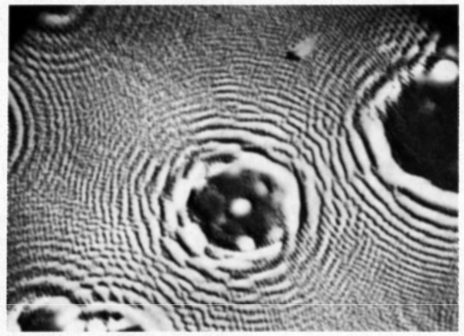


Fig. 3. Reconstructed images I and J, and the noise image K.

Aoki et al. Jap. J. Appl. Phy 11, 1857 (1972)

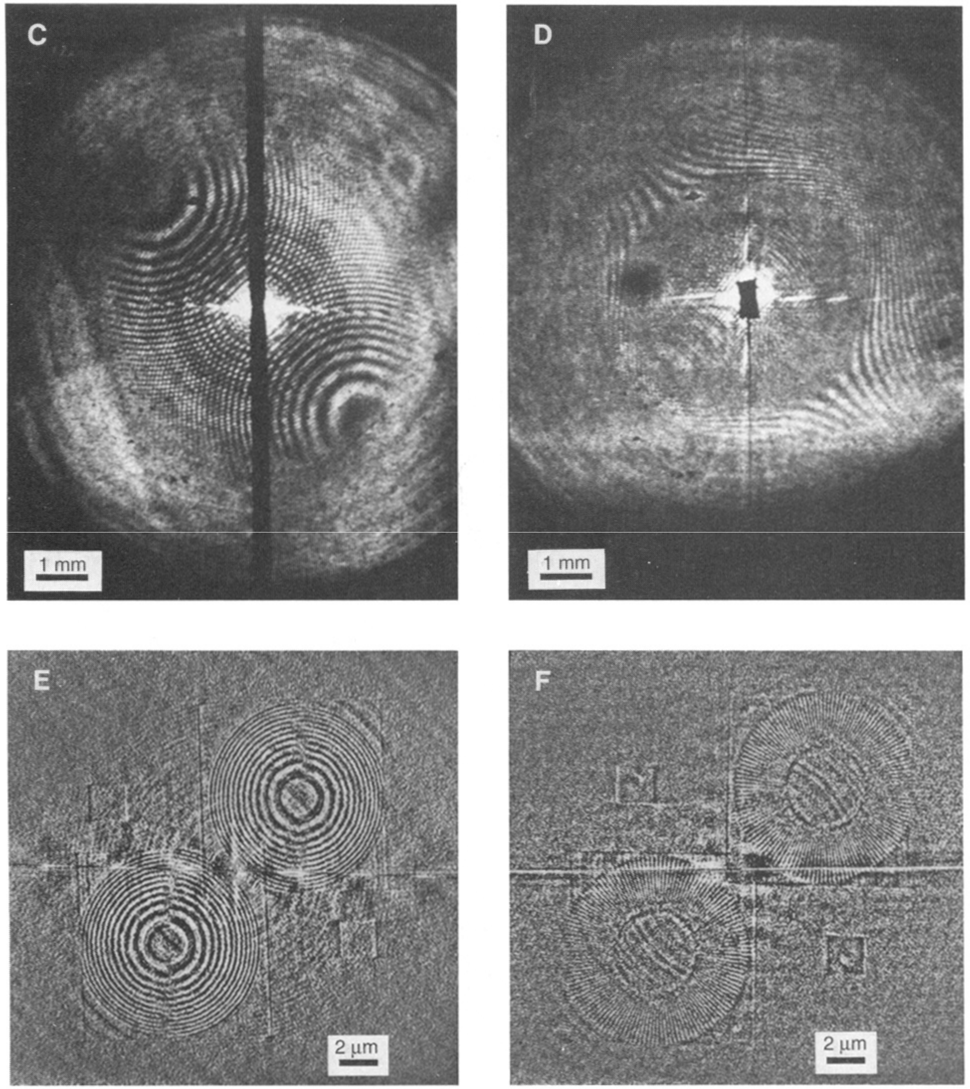
Holography

- Gabor holography
  - Encodes phase in fringes/speckles
  - Mimic reconstruction by computer
  - Requires high resolution detector
  - Aoki, Ichihara & Kikuta JJAP 11, 1847 (1972)
  - Howells, et al., Science 238, 514, (1987)
  - Not used much for high resolution imaging



- Fourier transform holography
  - Spherical reference wave spreads speckles
  - Simple reconstruction by inverse FT
  - How to get spherical reference?
  - McNulty et al., Science 256, 1009 (1992)

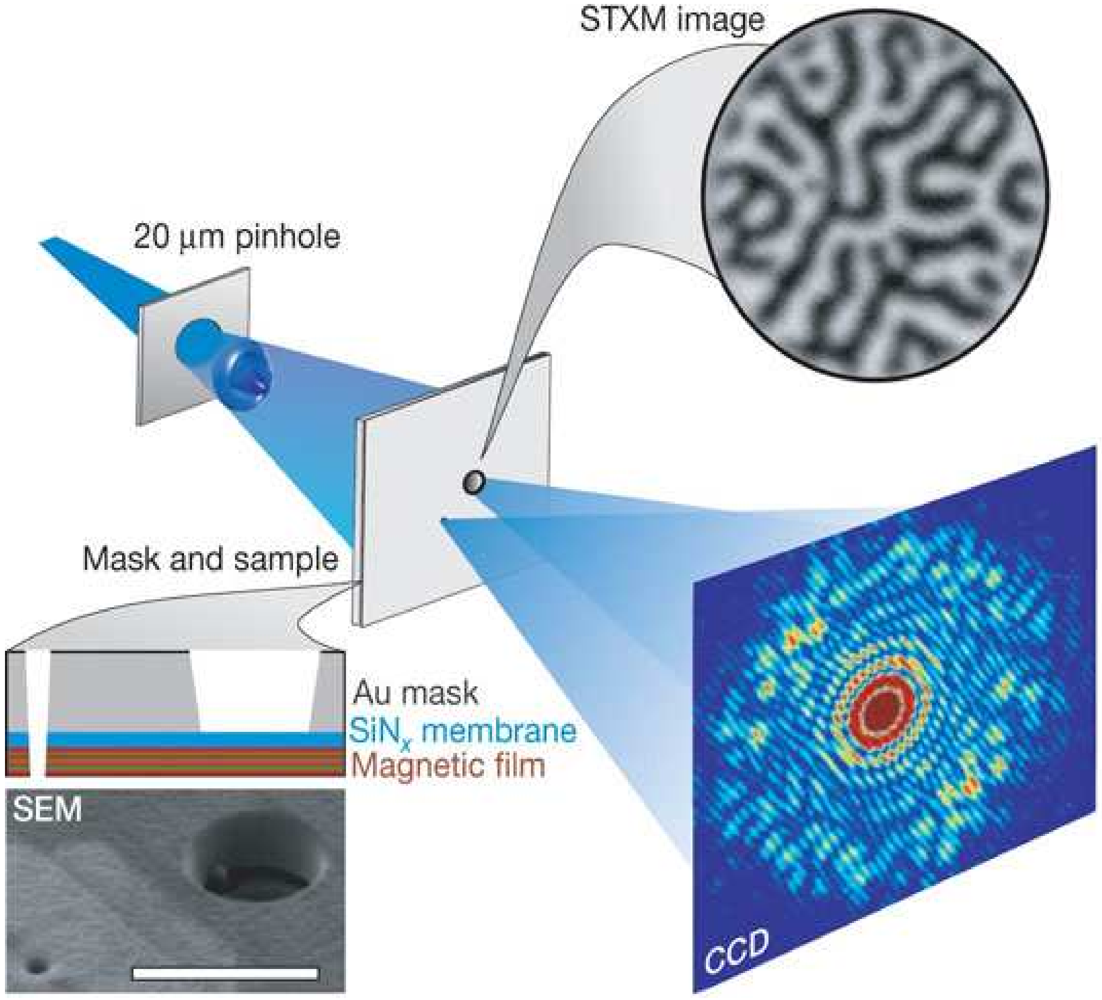
Fourier transform holography at the NSLS



McNulty et al., Science 1992

Fourier transform holography at BESSY

S. Eisebitt, J. Lüning, W. F. Schlotter, M. Lörgen, O. Hellwig, W. Eberhardt and J. Stöhr Nature 432, 885-888(2004)

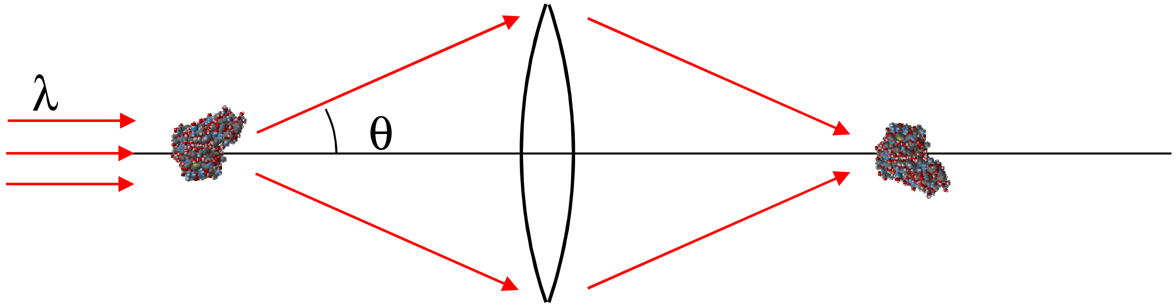


Fourier transform holography

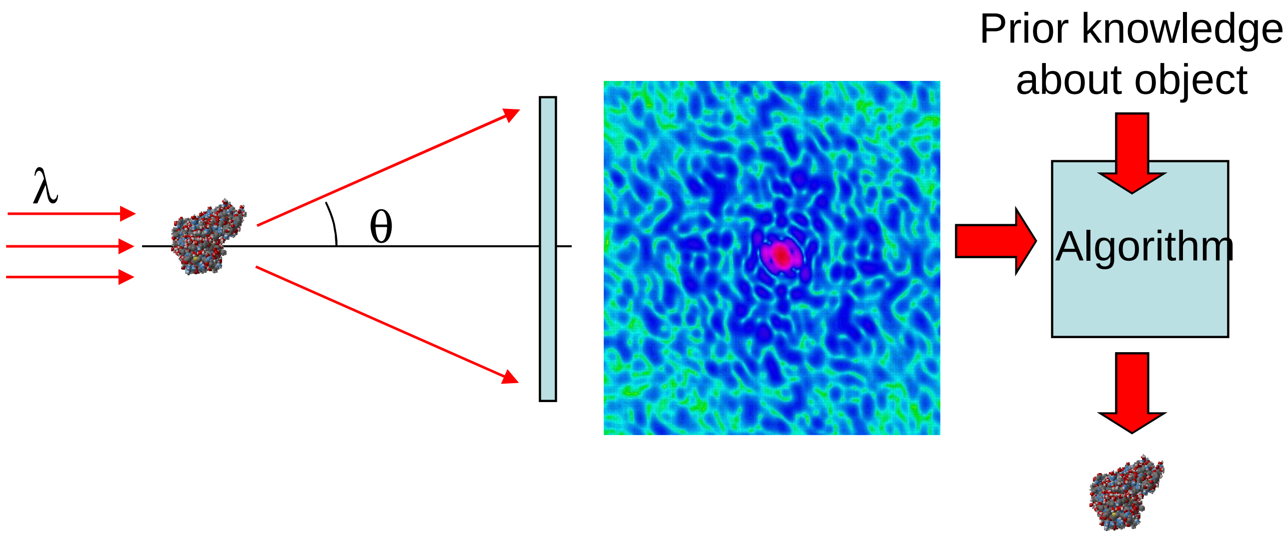
- Size of pinhole sets resolution
- How to get enough photons through?
- Do we really need a reference wave?

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

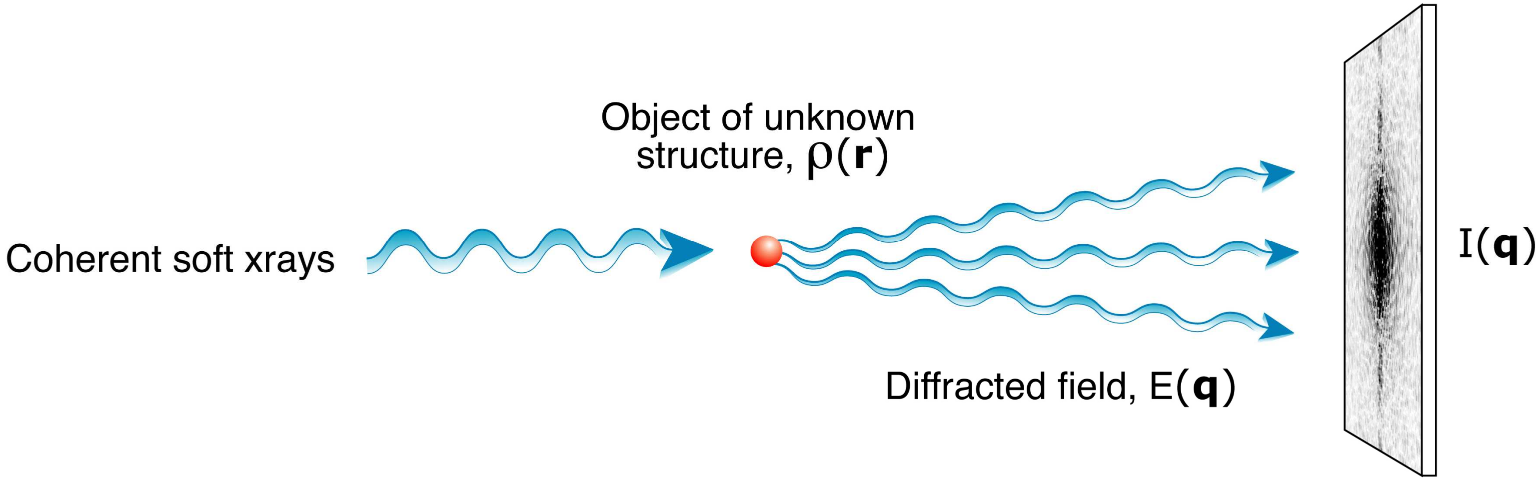
Idea of David Sayre

Basic principles

- Single object, plane wave incident, scattered amplitude is Fourier transform of (complex) electron density $f(\mathbf{r})$

$$F(\mathbf{k}) = \int f(\mathbf{r})\, e^{-2\pi i\, \mathbf{k} \cdot \mathbf{r}}\, d\mathbf{r}$$

- Assume: Born Approximation
- Assume coherent illumination



Creating coherent beams

- Life before lasers
- Temporal coherence
  - spectral lines or grating monochromators
  - measure of temporal coherence: $\lambda/\Delta\lambda$
- Spatial coherence (plane or spherical waves)
  - slits or pinholes "spatial filter"
  - Impose $\Delta x \cdot \Delta\theta < \lambda$ in each dimension
  - As gets λ shorter, acceptance becomes smaller

X-ray sources

- X-ray tubes
  - electron bombardment of solid target
- Synchrotron light sources
  - bending magnets
  - wigglers
  - undulators
- High harmonic generation
- Free electron lasers

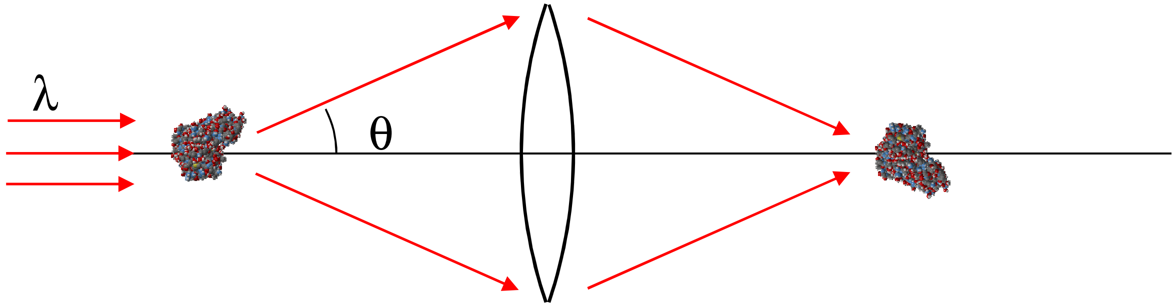
X-ray sources

$\Delta x \cdot \Delta y \quad \Delta\Omega$

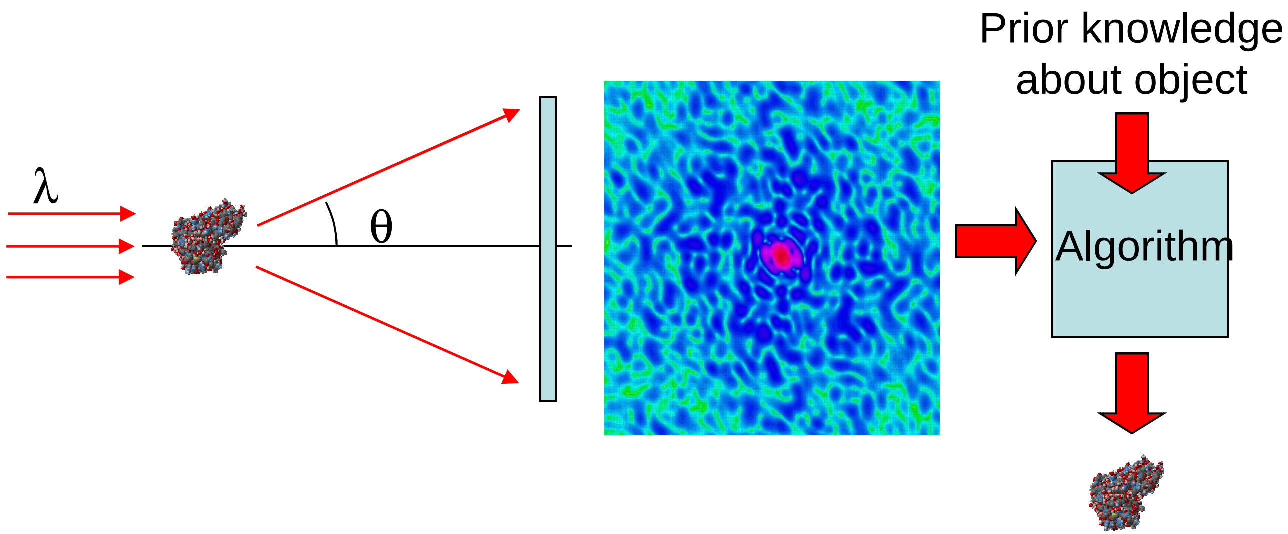
- X-ray tubes — $0.1 mm^2 \cdot 4\pi \sim 10^{14}\lambda^2$
- Synchrotron light sources (bend magn) — $0.01 mm^2 \cdot 10^{-6} \sim 10^{6}\lambda^2$
- Undulators — $0.01 mm^2 \cdot 10^{-8} \sim 10^{4}\lambda^2$
- FELs — Can be mostly coherent

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

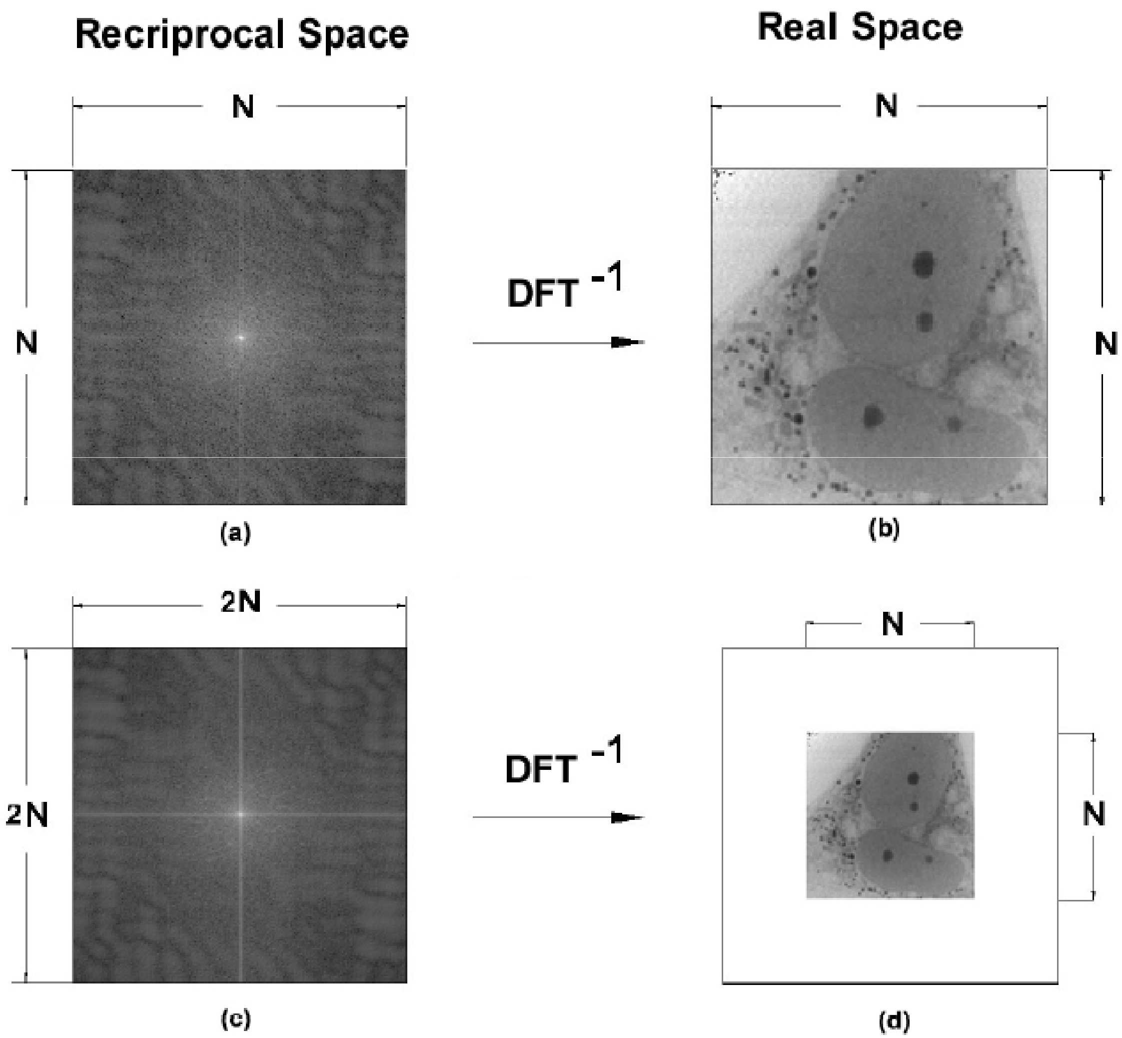
Where does prior knowledge come from?

"Oversampling":

Non-crystals:
pattern continuous,
can do finer sampling
of intensity

Finer sampling;
larger array;
smaller transform;
"finite support"

(area around specimen
must be clear!)

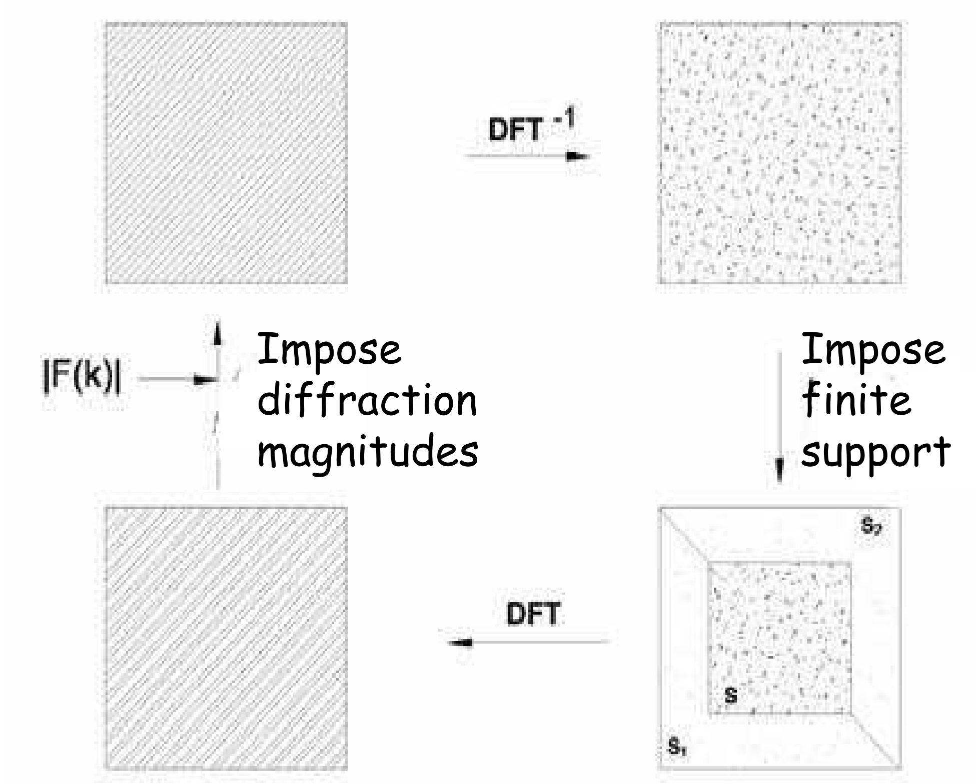


Reconstruction

Equations can still not be solved analytically

Fienup iterative algorithm

Reciprocal space Real space



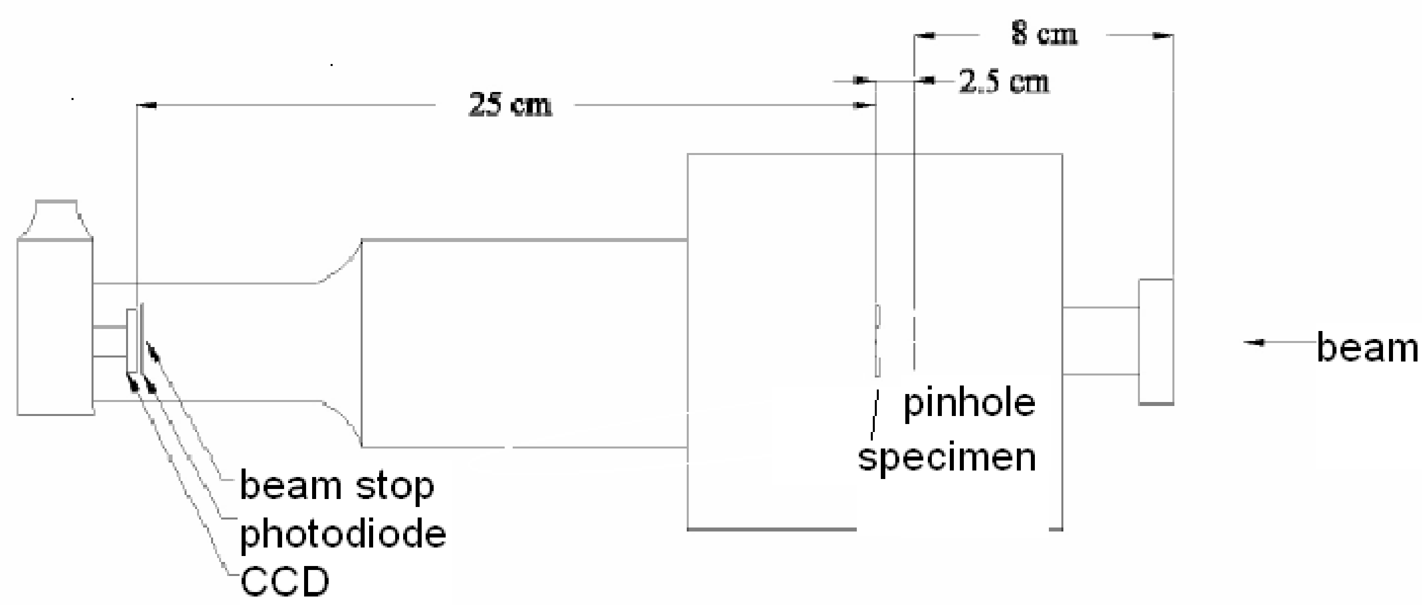
•Positivity of electron density helps!

History

- Sayre 1952: Shannon sampling theorem in crystallography
- Gerchberg & Saxton, 1971: iterative phase retrieval algorithm in EM
- Sayre 1980: pattern stronger with soft X-rays; use SR to work without xtals!
- Fienup 1982: Hybrid Input-Output, support
- Bates 1982: 2x Bragg sampling gives unique answer for ≥ 2 dimensions
- Yun, Kirz &Sayre 1984-87: first experimental attempts

Modern era

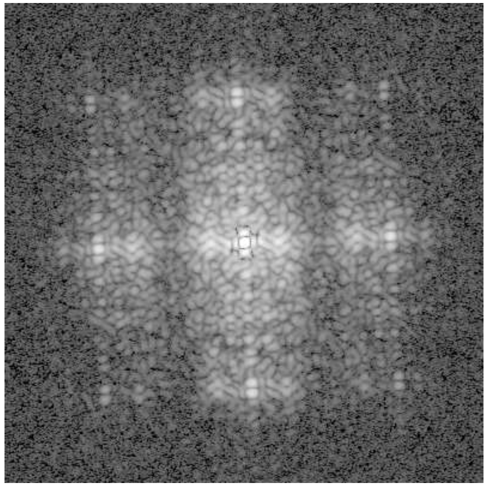
- 1998: Sayre, Chapman, Miao: oversampling & Fienup algorithm for X-rays
- 1999: first experimental demonstration in 2D

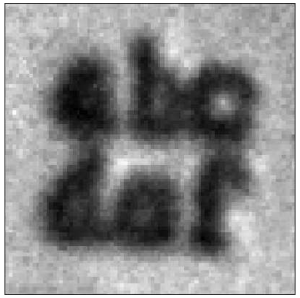


Miao, Charalambous, Kirz, Sayre, *Nature* **400**, 342 (1999).

Data collected at NSLS beamline X1B

λ=1.8 nm
soft x-ray
diffraction
pattern





Low angle data
From optical
micrograph

Scanning
electron
micrograph
of object

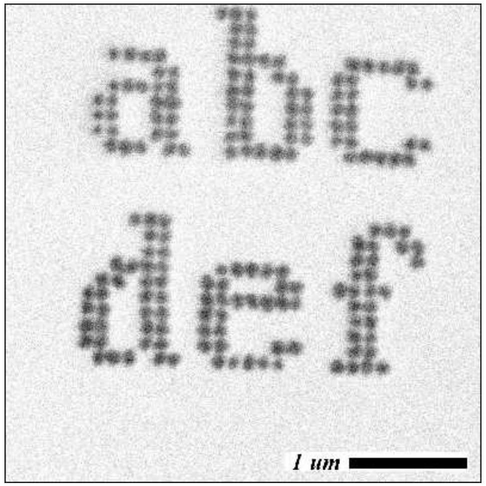


Image
reconstructed from
diffraction pattern
(θ_{max} corresponds to
80 nm). Assumed
positivity

Where we really want to be

- Collect a high resolution 3D data set in an hour or two
- Reconstruct reliably in a comparable amount of time

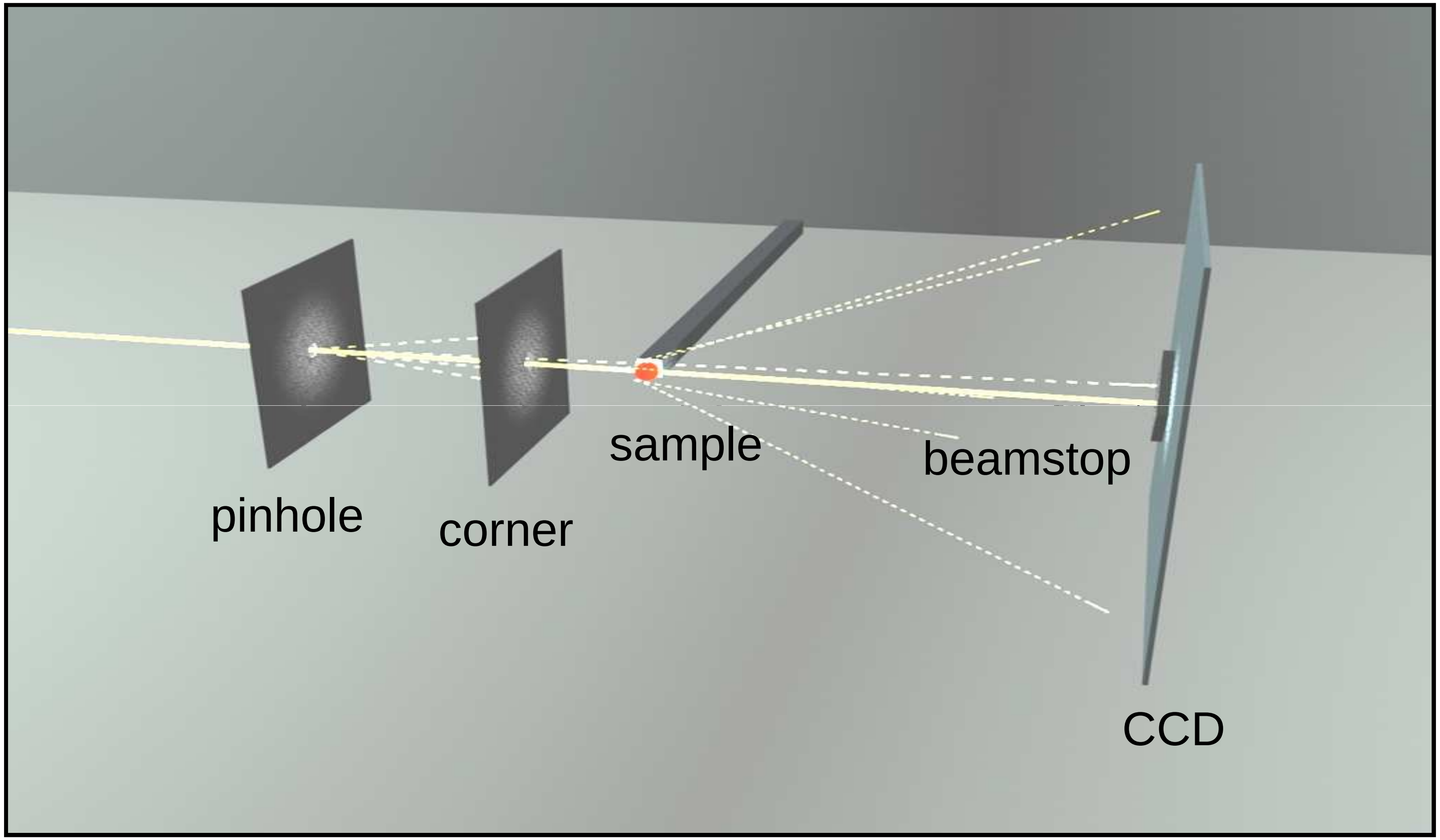
ic/ALSaerial/11-96

Challenges

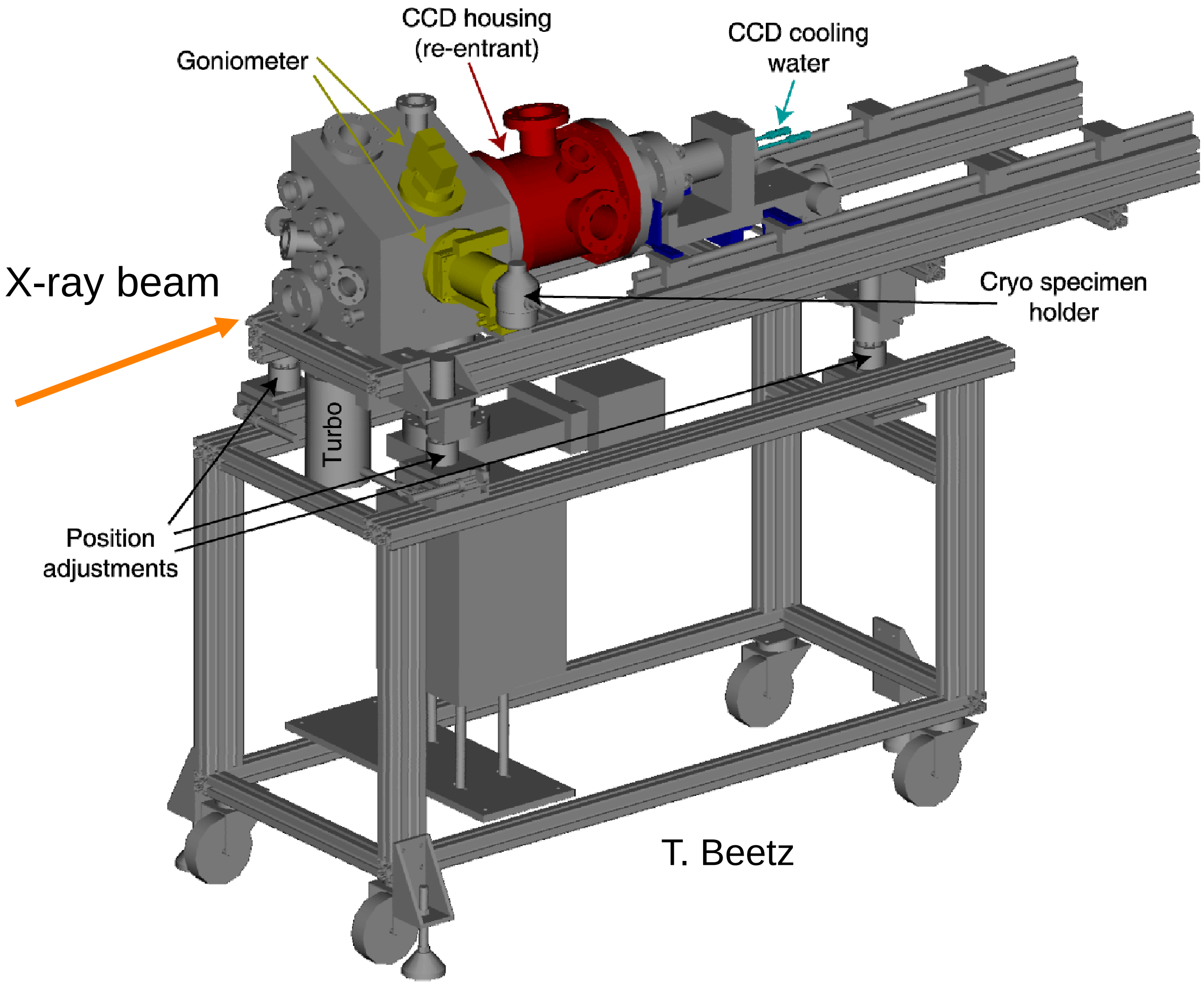
1/ recording the pattern

- Beamline to supply sufficient coherent photons
  - Eliminate higher orders: aperiodic undulator?
- Shielding detector from all but diffracted signal
- Aligning specimen with small beam-spot,
  - Keeping it aligned as specimen is rotated
- Minimizing missing data
  - (beam stop, large rotation angles, etc.)
- Dynamic range of detector
- Automation of data collection

Inside vacuum chamber

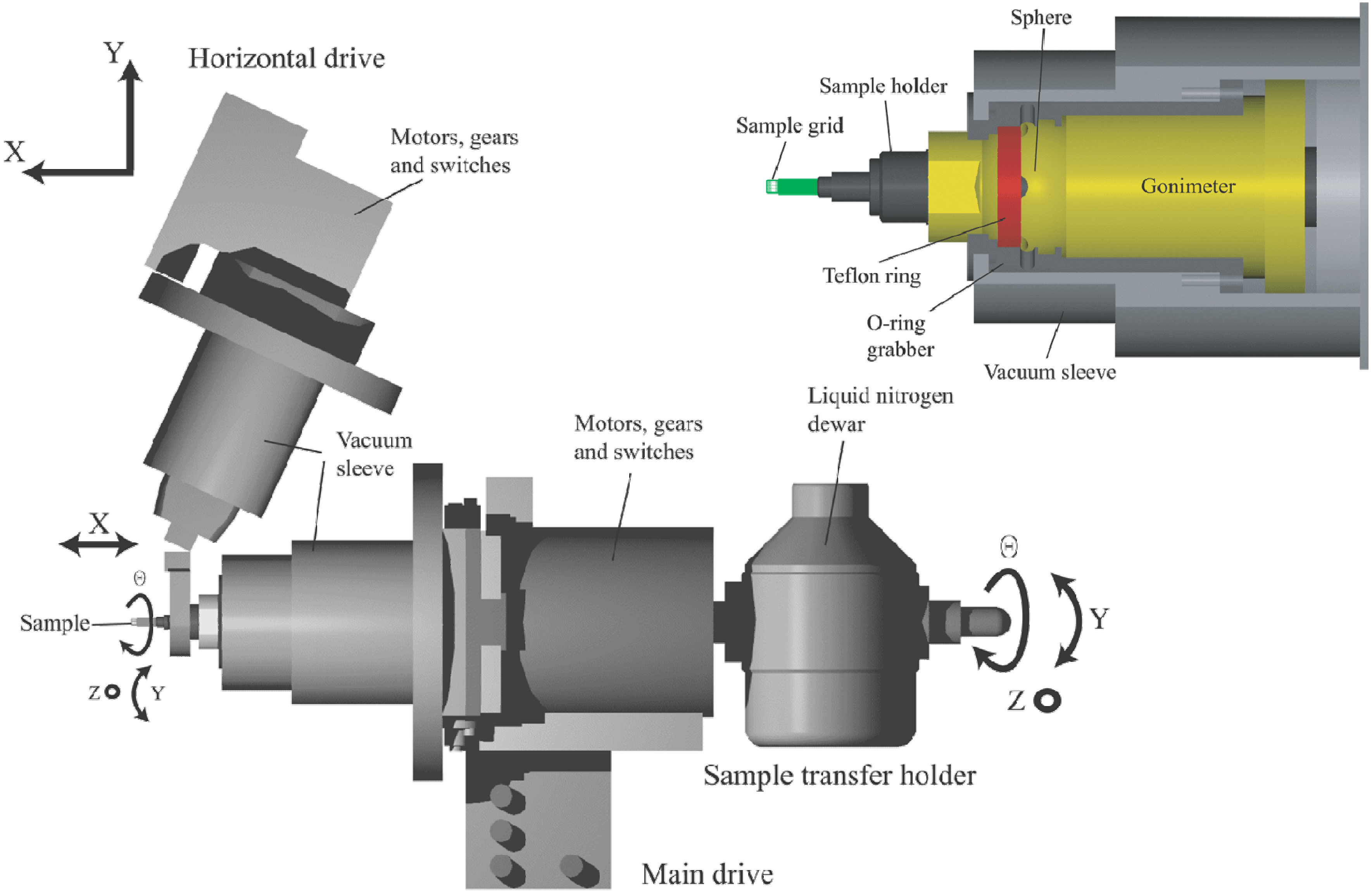


Diffraction Microscope by Stony Brook and NSLS



Gatan 630 cryo holder

JEOL 2000 goniometer schematic



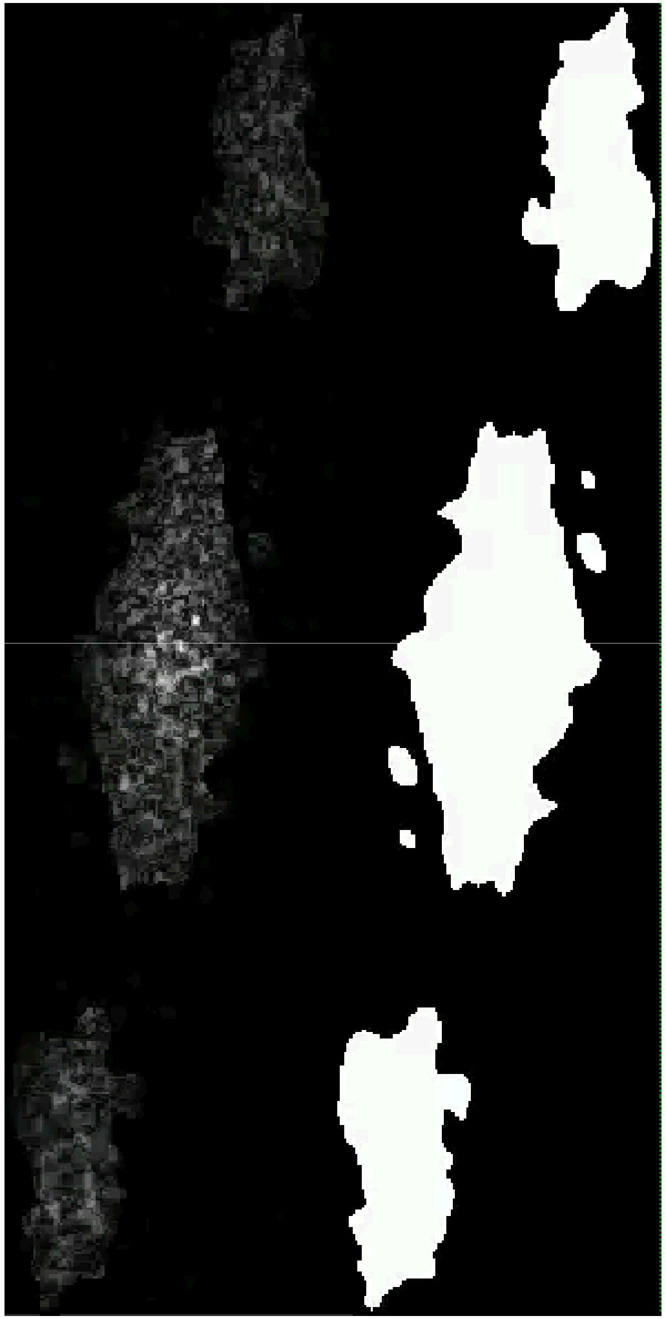
Challenges

2/reconstruction

- How to avoid stagnation; local minima?
  - The enantiomorph problem
- How to tell whether algorithm converged?
  - (easy when object known…)
  - Multiple random starts
- How to make best use of the data?
  - Of prior knowledge? (Fienup, Elser, Szöke)
- How to optimize use of computer resources?
  - Want many 1024^3 DFT
- Much work remains to be done!

When rough support is not available, it can be found from “Shrink-wrap”

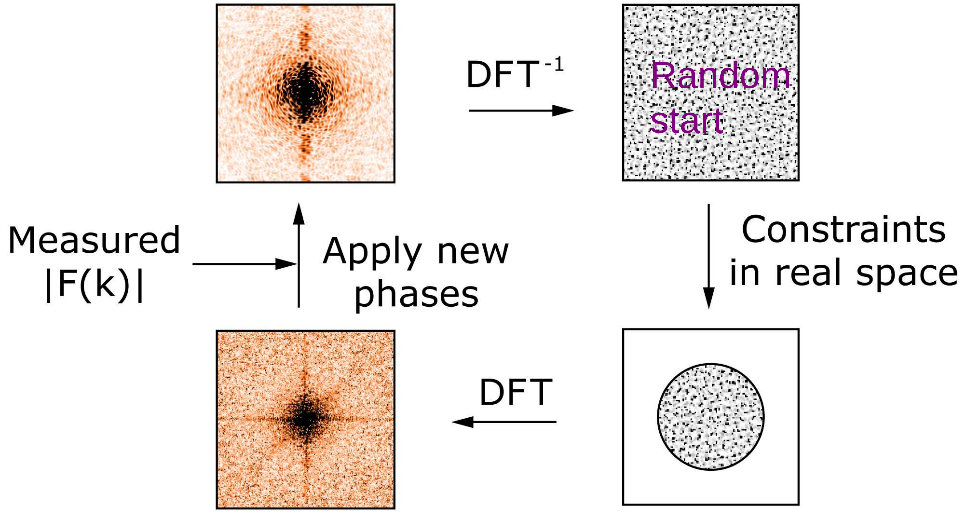
Marchesini *et al.*, *Phys. Rev. B* **68,** 140101 (2003)



algorithmic steps

- Algorithm starts with an image (random)
- Apply projections
- Iteratively modify image until converge

hybrid input-output



(Fienup, *Appl. Opt.* **21**, 2759 (1982))

difference map: Elser, *J. Opt. Soc. Am. A* **4**, 118 (2002)

by adding the difference of two projections

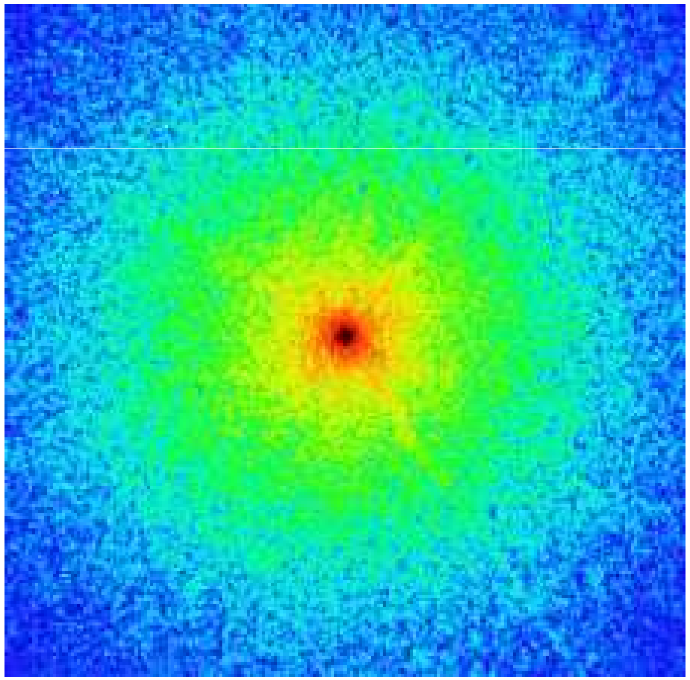
Comments

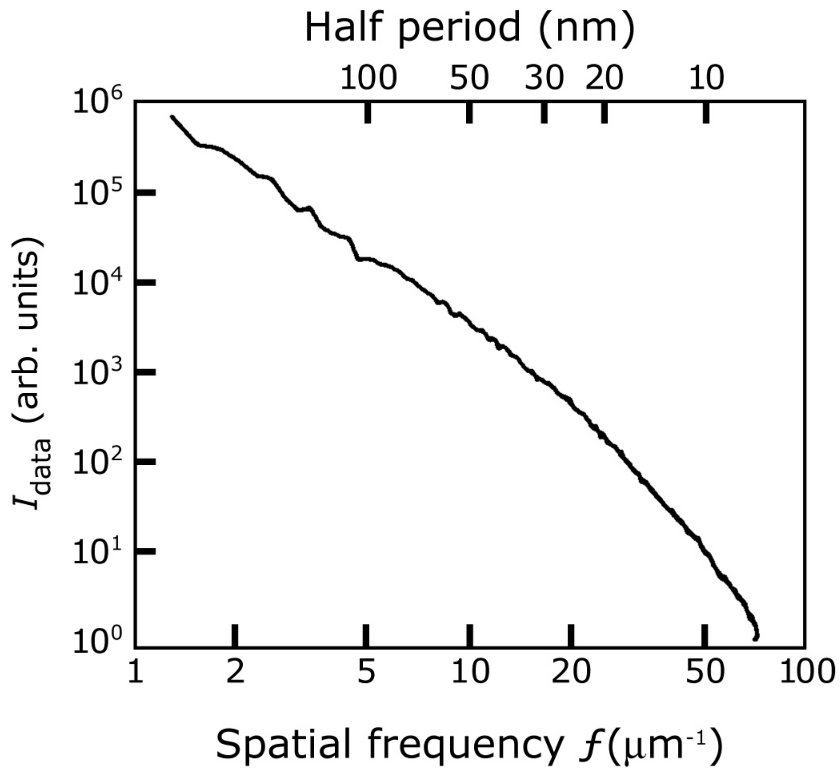
- Works perfectly for perfect, complete data
- Algorithm often requires thousands of iterations, stagnates sometimes
  - (Enantiomorph problem)
- Works even better for 3D!
- Real data are rarely perfect, or complete

Diffraction data and its reconstruction 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

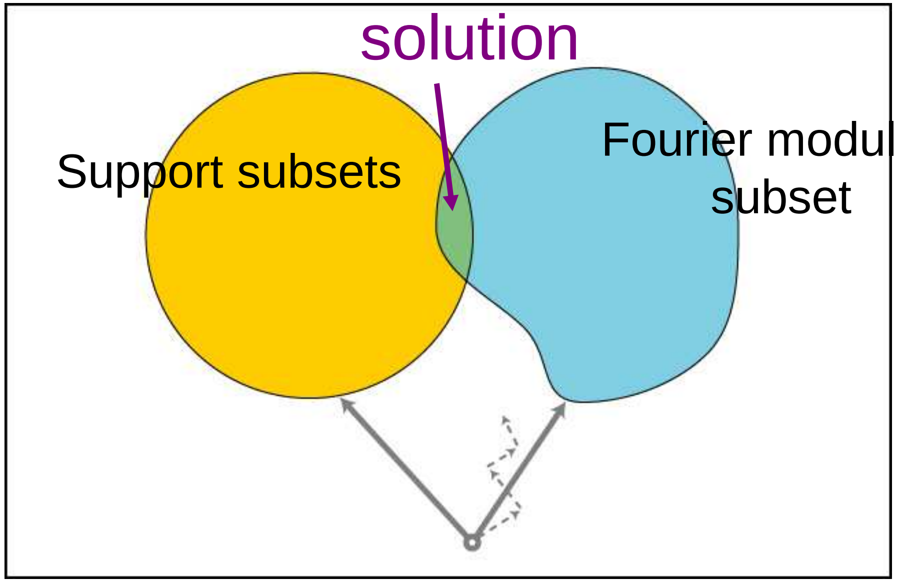




David Shapiro, Stony Brook, now at ALS

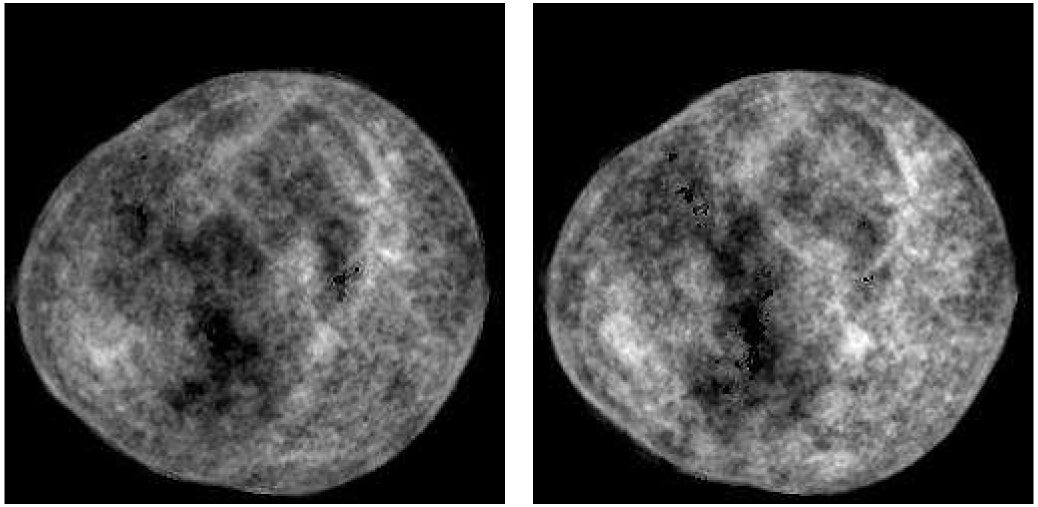
Impose known constraints
(information about the sample)

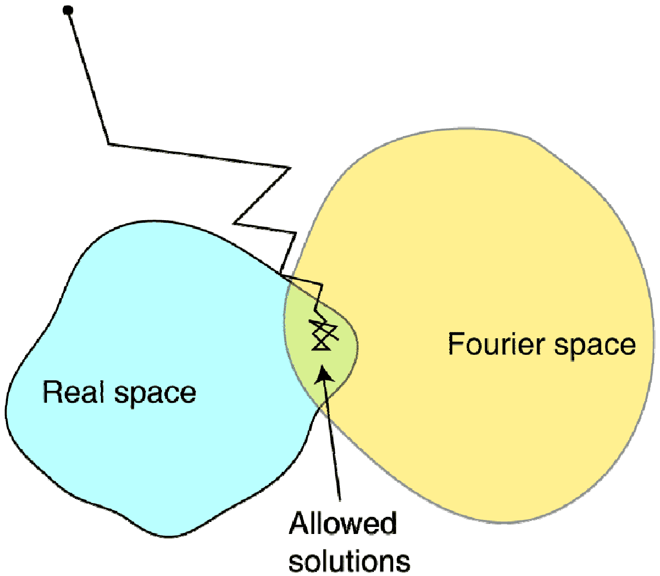
1. Impose measured Fourier magnitude
2. Impose sample boundary (support)



Iterative solutions "hop around"!

Two images (iterates) separated by 40 iterations





Noise in the data gives random fluctuations in the reconstructed image

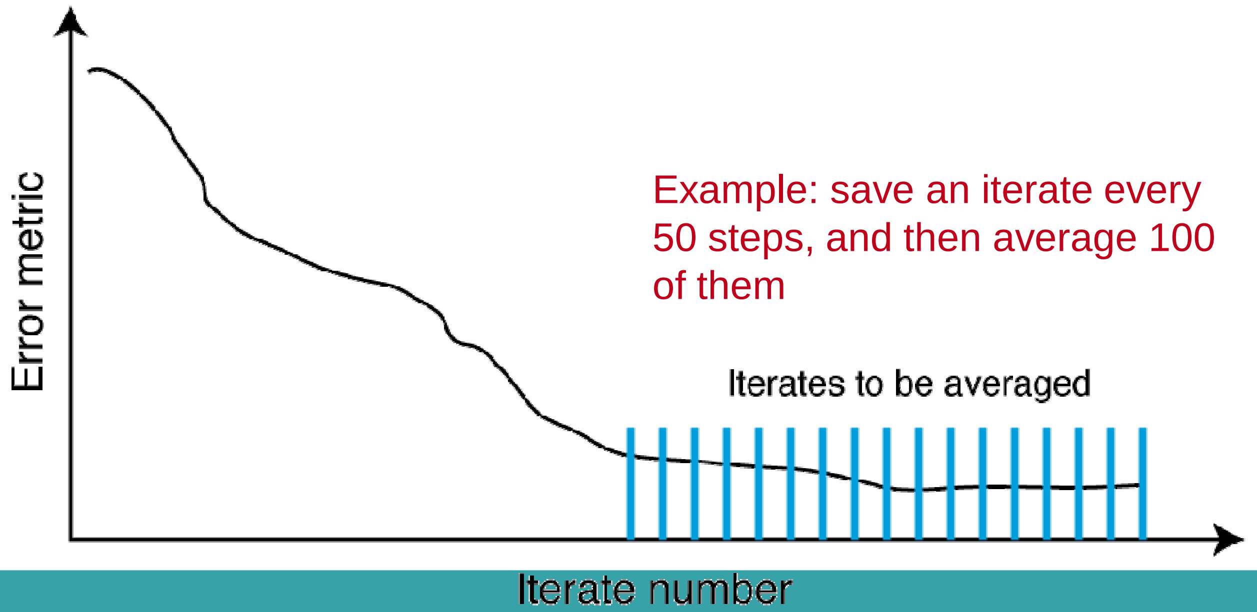
Averaging many iterates:

- reinforce reproducible information
- suppress non-reproducible information

D. Shapiro et al., Biological imaging by soft x-ray diffraction microscopy, *PNAS* **102** (43), 15343, (2005)

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)

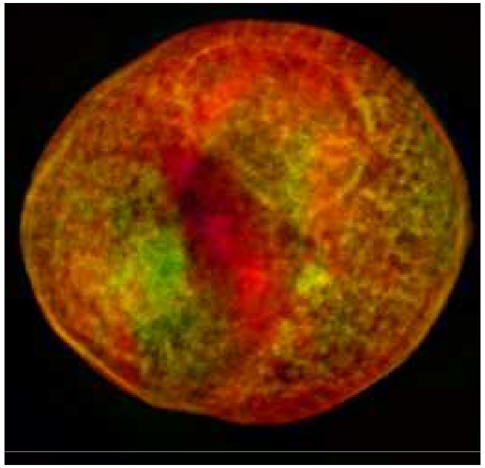


Summary of reconstruction details

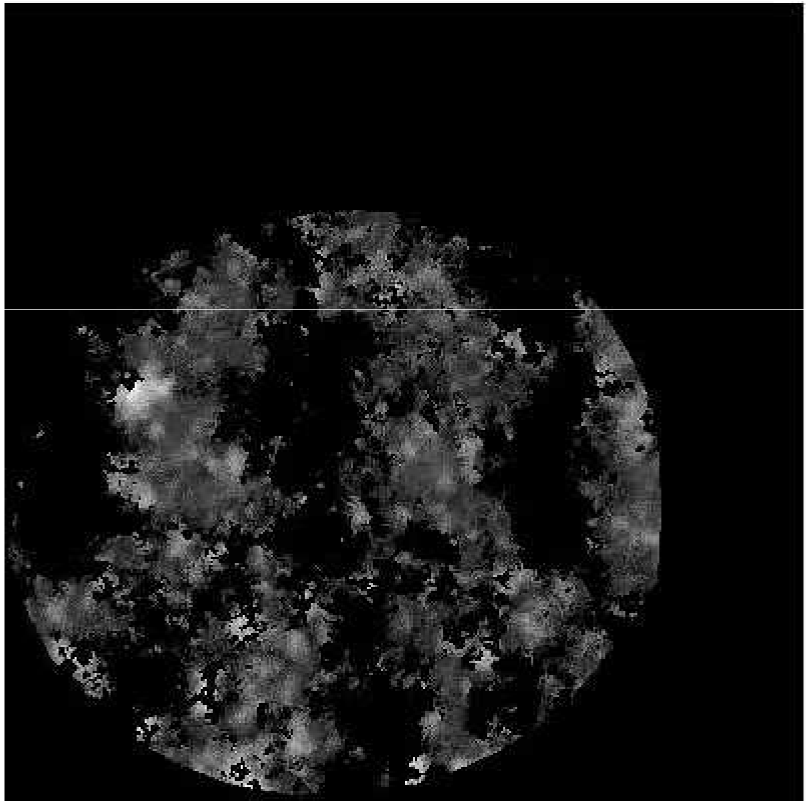
- Final reconstruction was obtained by averaging iterates

  10,000 iterations
  Brightness - amplitude, hue - phase

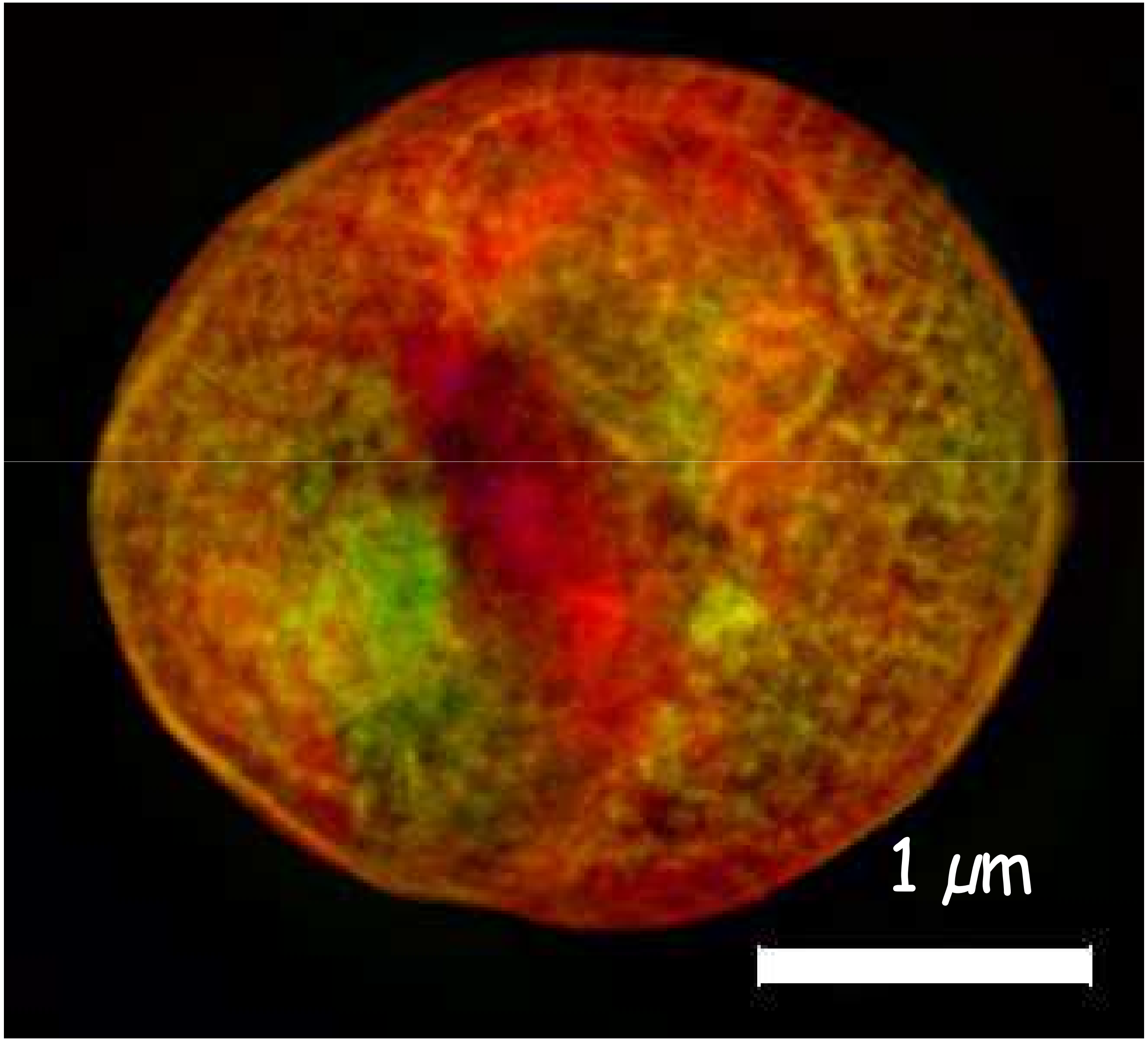
  averaged over 100 iterates



The reconstruction



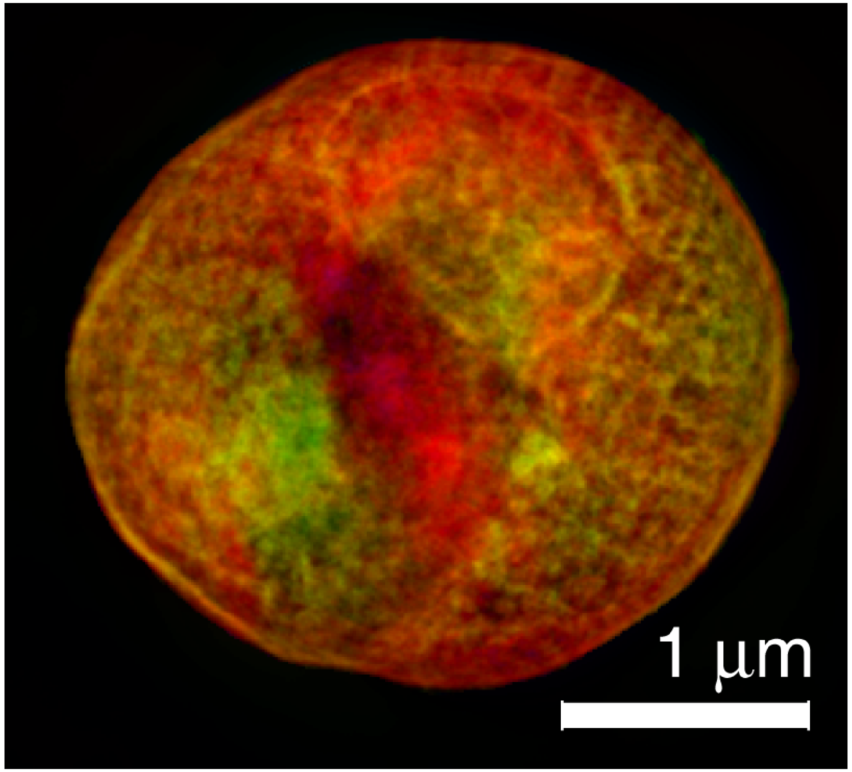
Reconstructed image



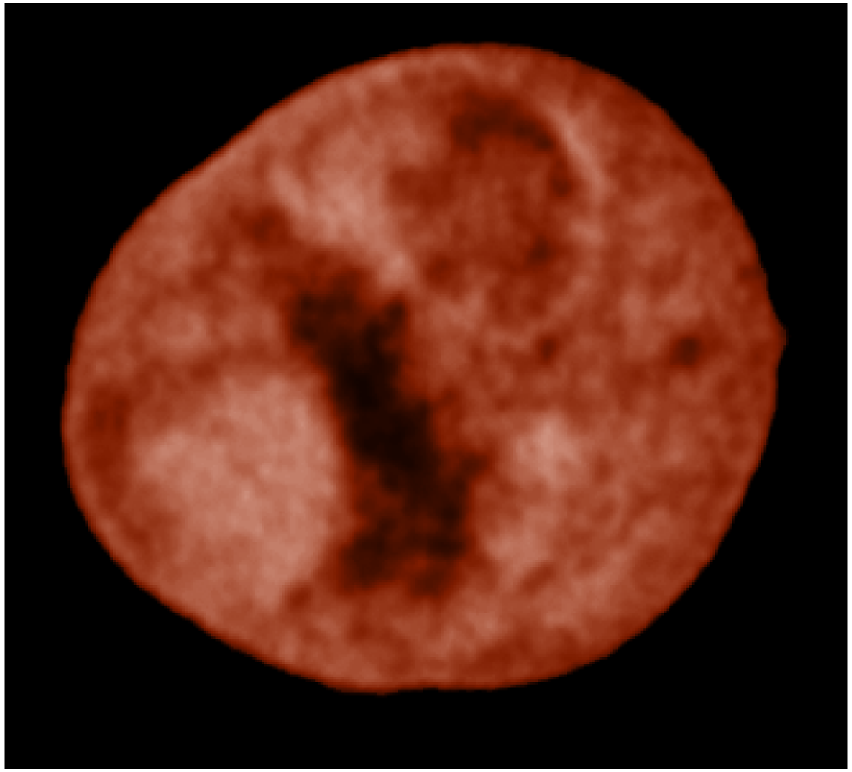
Shapiro *et al.*, *Proc. Nat. Acad. Sci.* **102**, 15343 (2005).

Is the solution unique and faithful?

Comparison with a microscope

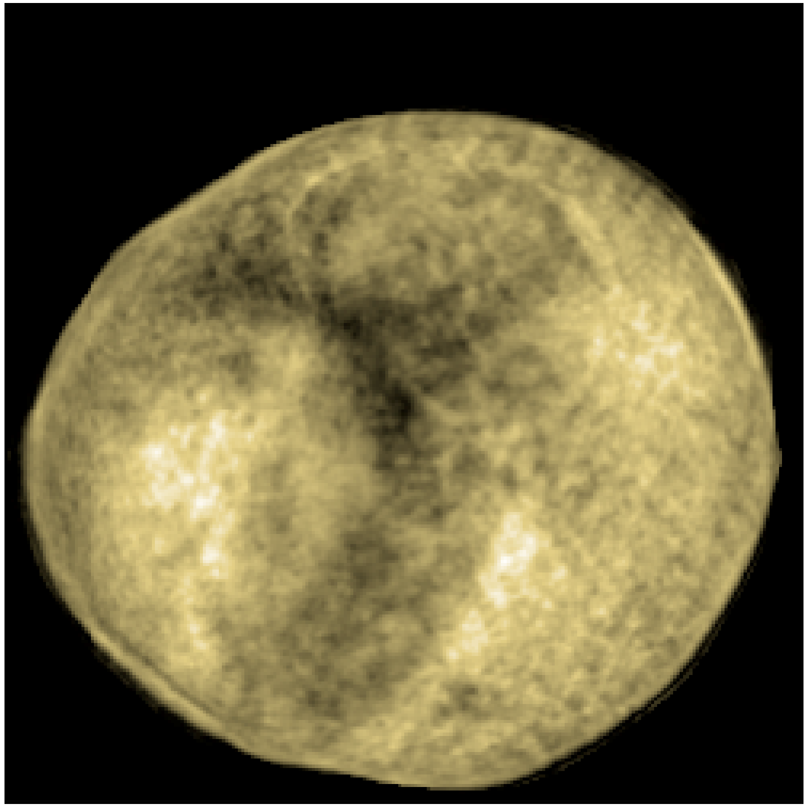


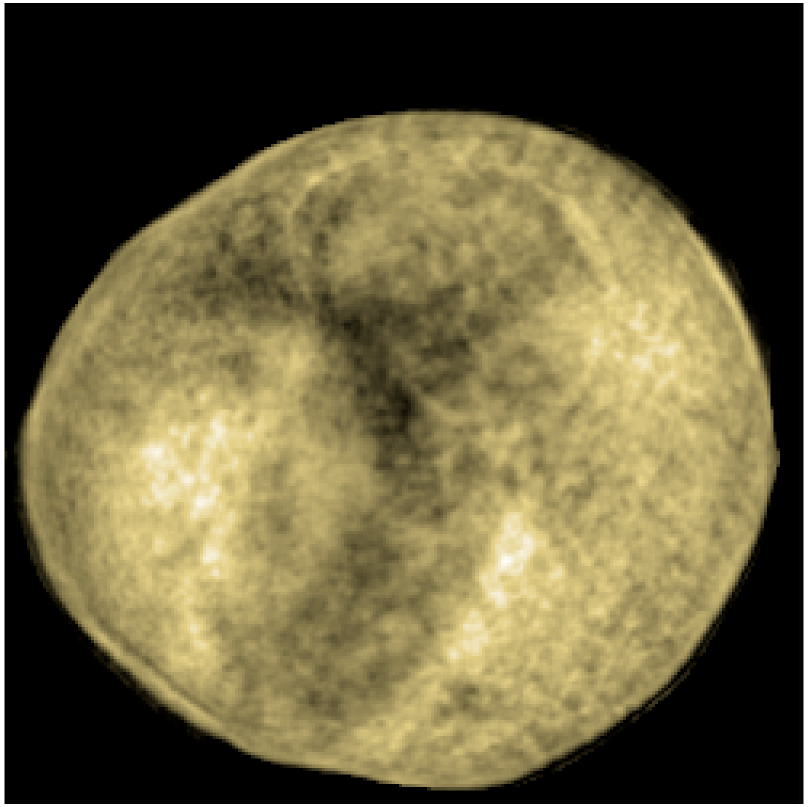
Diffraction reconstruction (data taken at 750 eV; absorption as brightness, phase as hue).



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

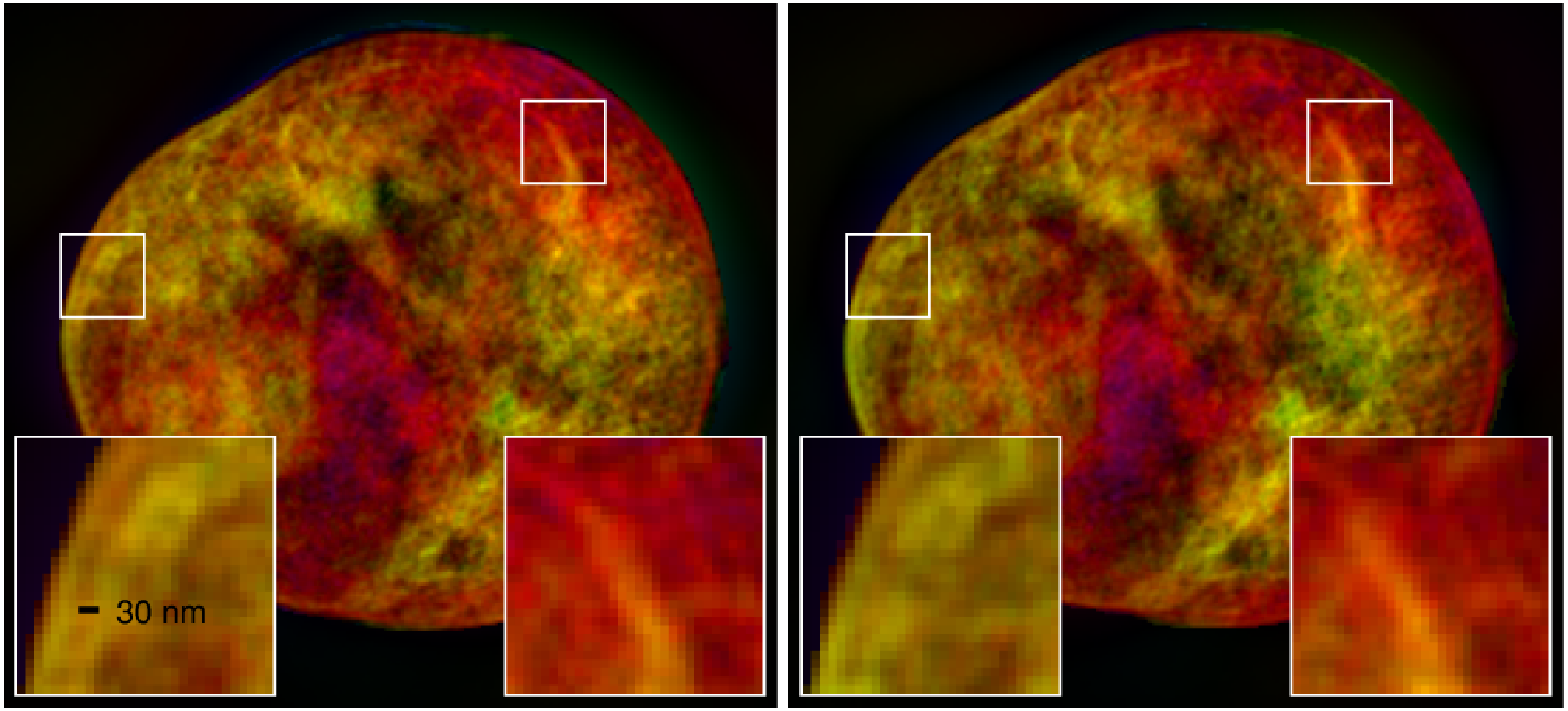
Different starting random phases



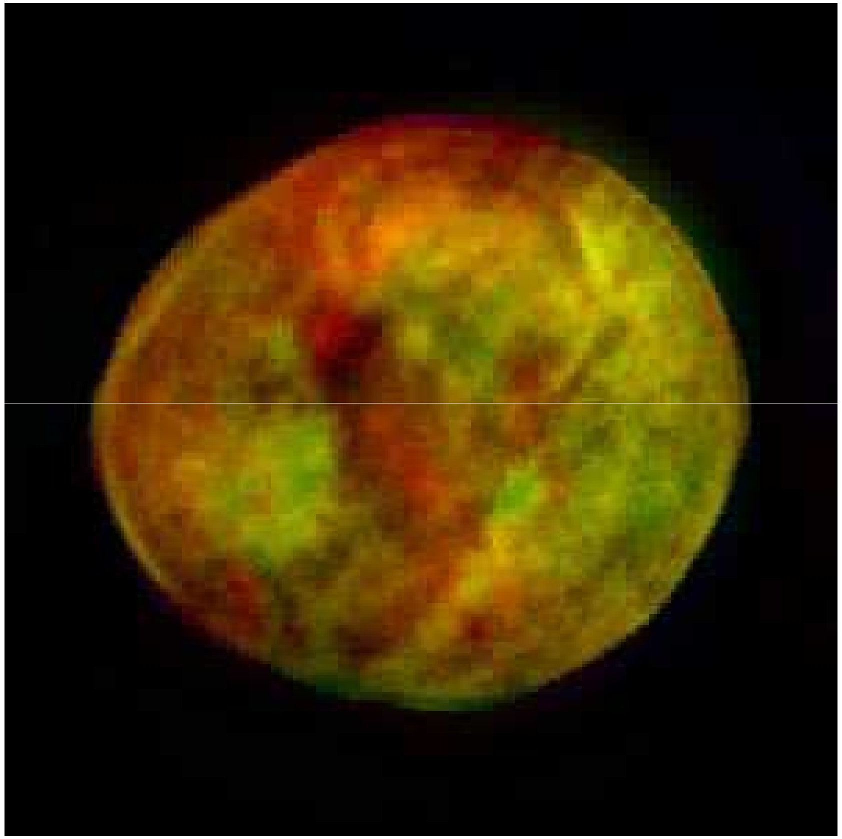


Two separate runs of algorithm with different random starting phases. In both cases, 125 iterates spaced 40 iterations apart were averaged (E. Lima).

Reconstructions from data 1 degree apart show similar 30 nm structure



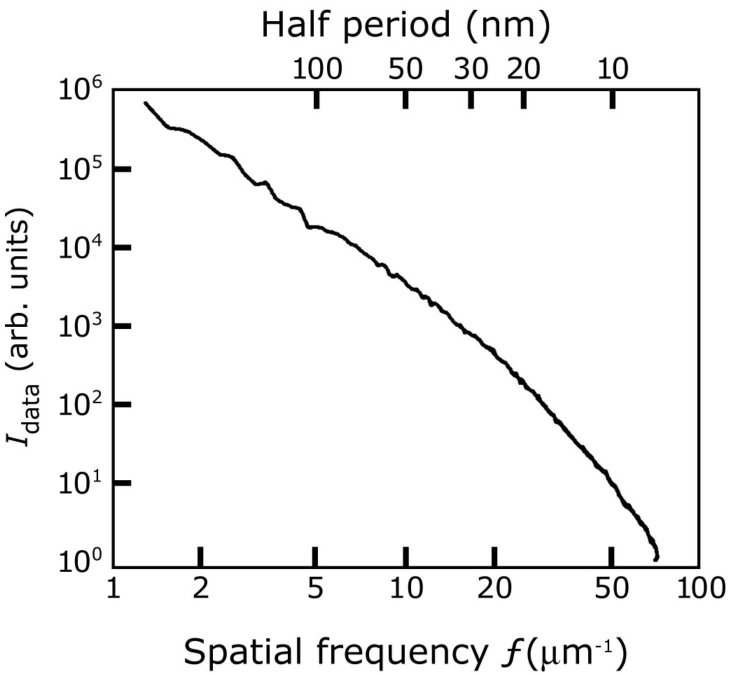
Movie: tilt from -3 to +5 degrees in 1 degree steps

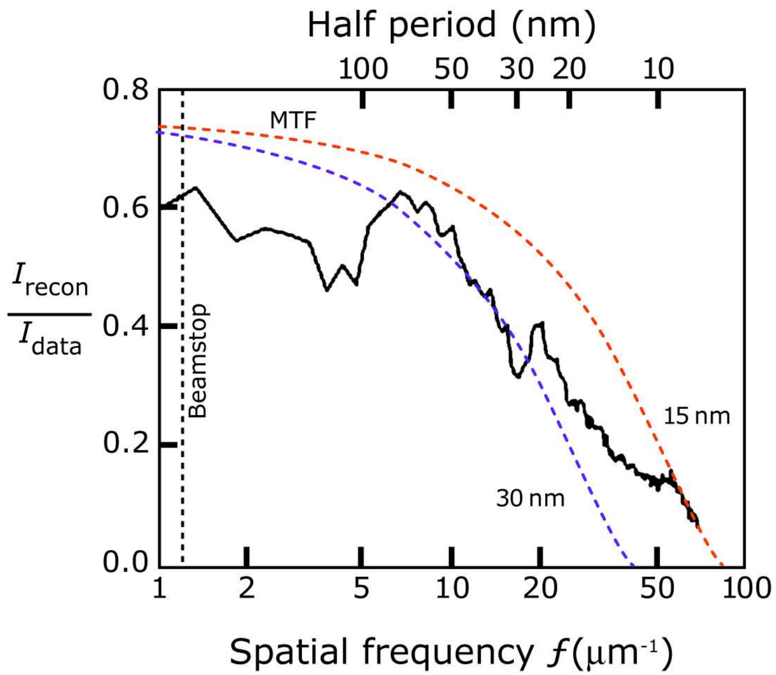


Pierre Thibault

What is the resolution?

- Data extends to an angle corresponding to 9 nm half-period but is it all equally well phased?
- Fourier intensity of reconstructed solution versus raw data
  $\rightarrow$ analogous to the modulation transfer function





---> Reconstructed image at 30 nm resolution

How can we believe the phasing?

- By understanding the nature of solution finding and averaging iterates (Elser and Thibault).
- By comparing reconstruction with a microscope image.
- By getting similar images from separate data sets from tilts 1º apart.
- By getting similar images from independent runs on the same data with different random starting phases.

Challenges:

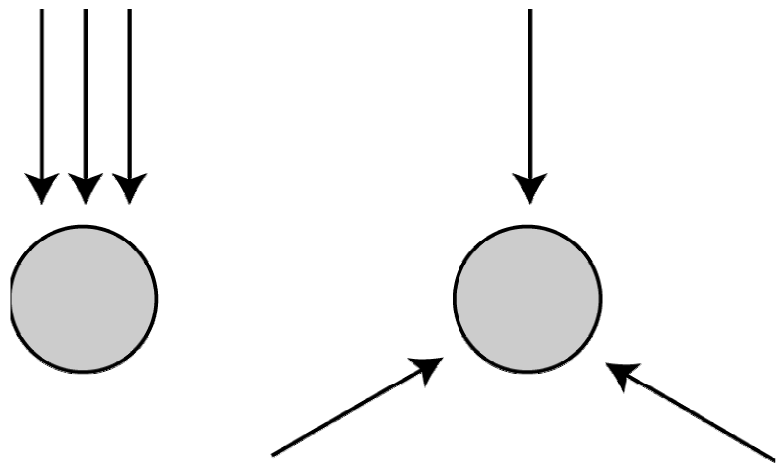
3/ damage

- The ultimate limitation for radiation-sensitive materials only
- Dose fractionation

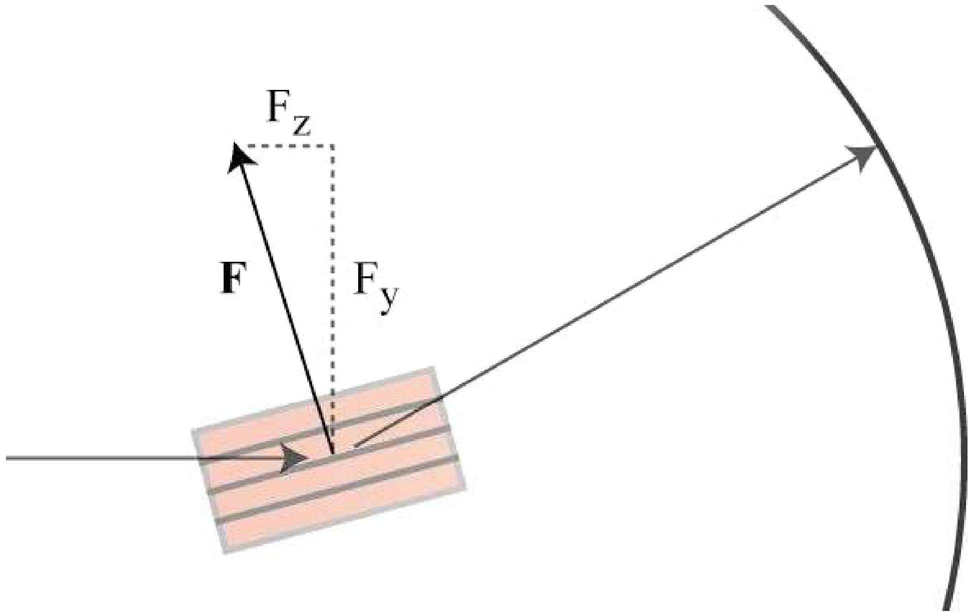
  (Hegerl and Hoppe 1976, McEwen 1995)

Dose fractionation

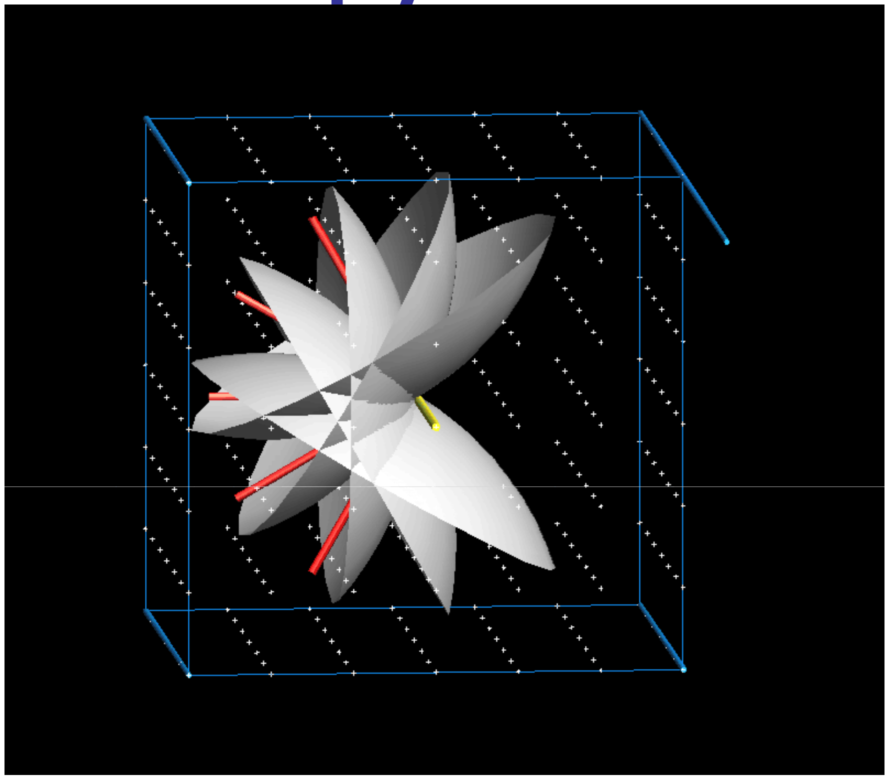
- You can divide the number of photons needed for a good 2D view into 3D views.
- Hegerl and Hoppe, *Z. Naturforschung* **31a**, 1717 (1976); McEwen *et al*., *Ultramic.* **60**, 357 (1995).



Diffraction microscopy in 3D



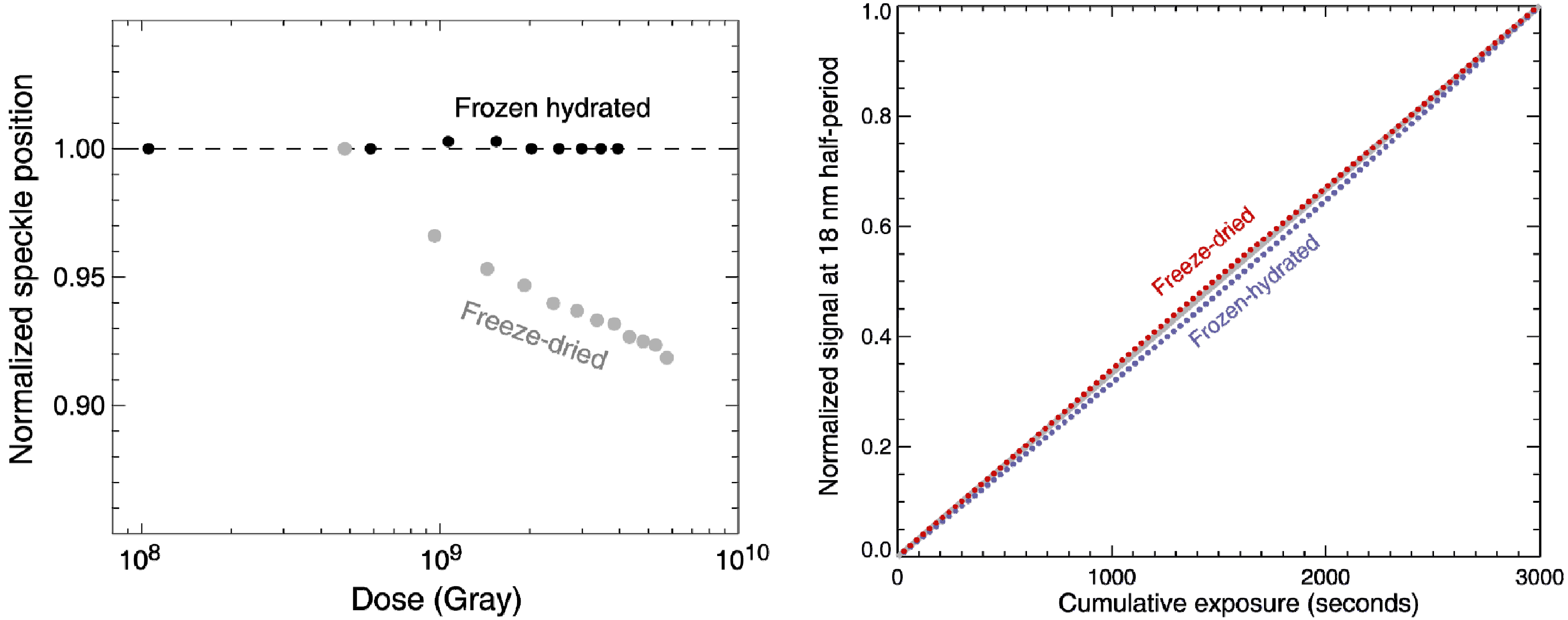
Bragg gratings that diffract to a certain angle represent a specific transverse and longitudinal periodicity (Ewald sphere)



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

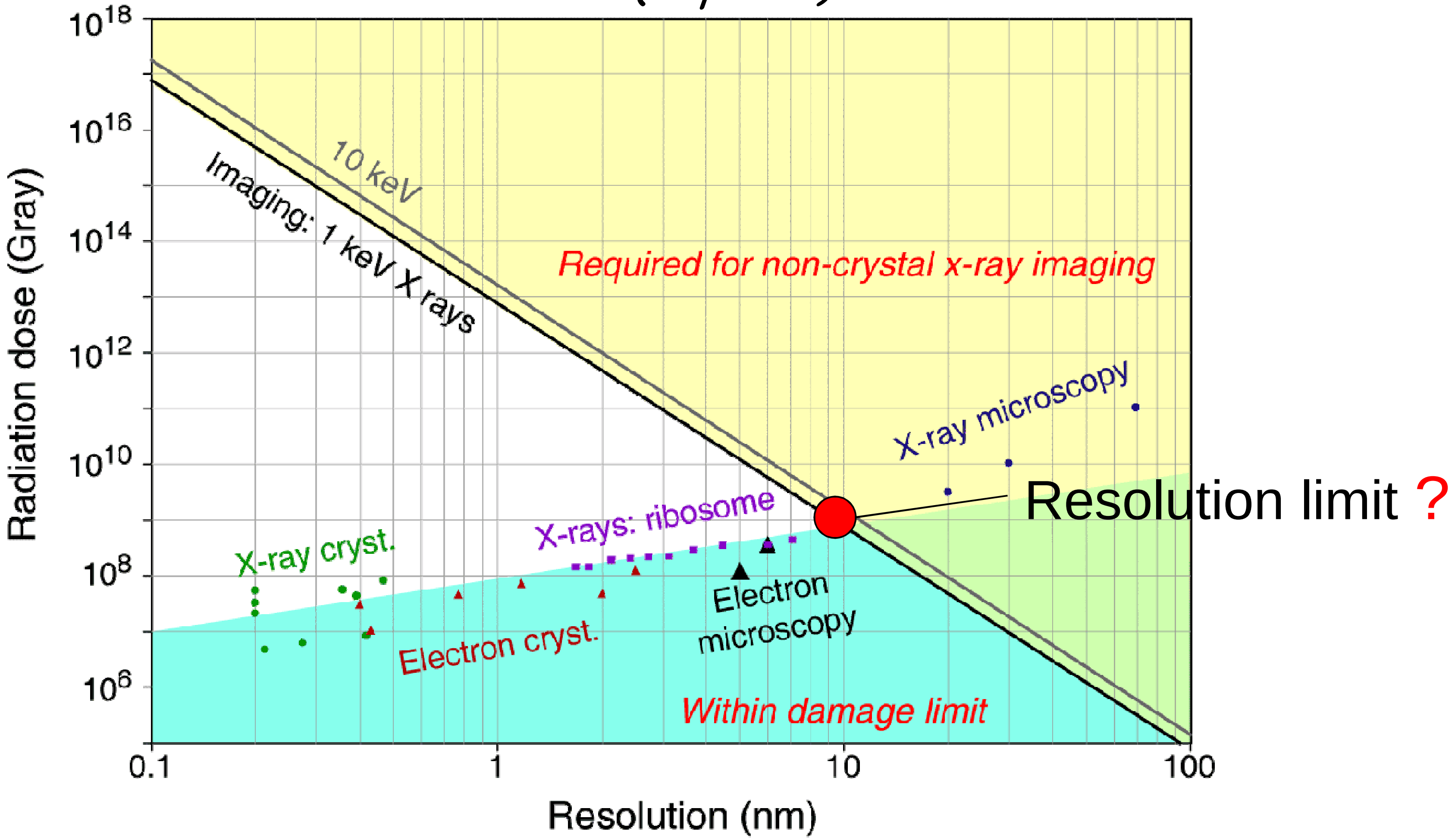
Stability of frozen hydrated specimens

- D. Shapiro, PhD thesis



The ultimate challenge

Radiation damage in biological samples in XDM:
Frozen hydrated state of protein by Howells *et al.*
JESRP (in press)



Inverse fourth power law of dose vs resolution: Dose ~ 1/resolution-size^4

Acknowledgements

- David Sayre
- Wenbing Yun
- Chris Jacobsen, Malcolm Howells
- Henry Chapman
- John Miao
- David Shapiro, Enju Lima, Stefano Marchesini
- Veit Elser & Pierre Thibault

- DOE/BES; NIH

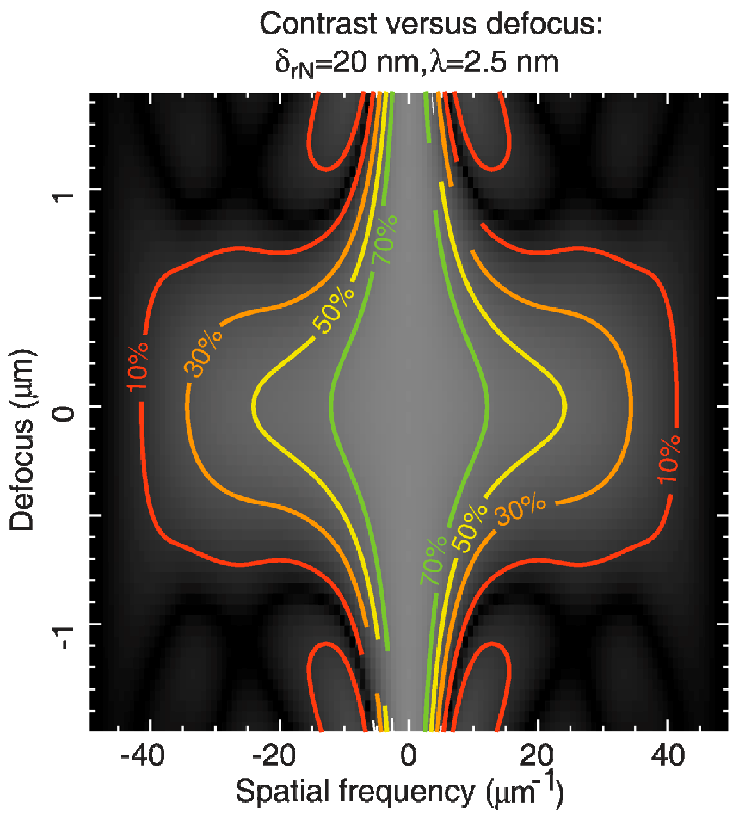
Conclusions

- Method of choice for micron-size specimens
- Damage will set limit on resolution for radiation-sensitive specimens

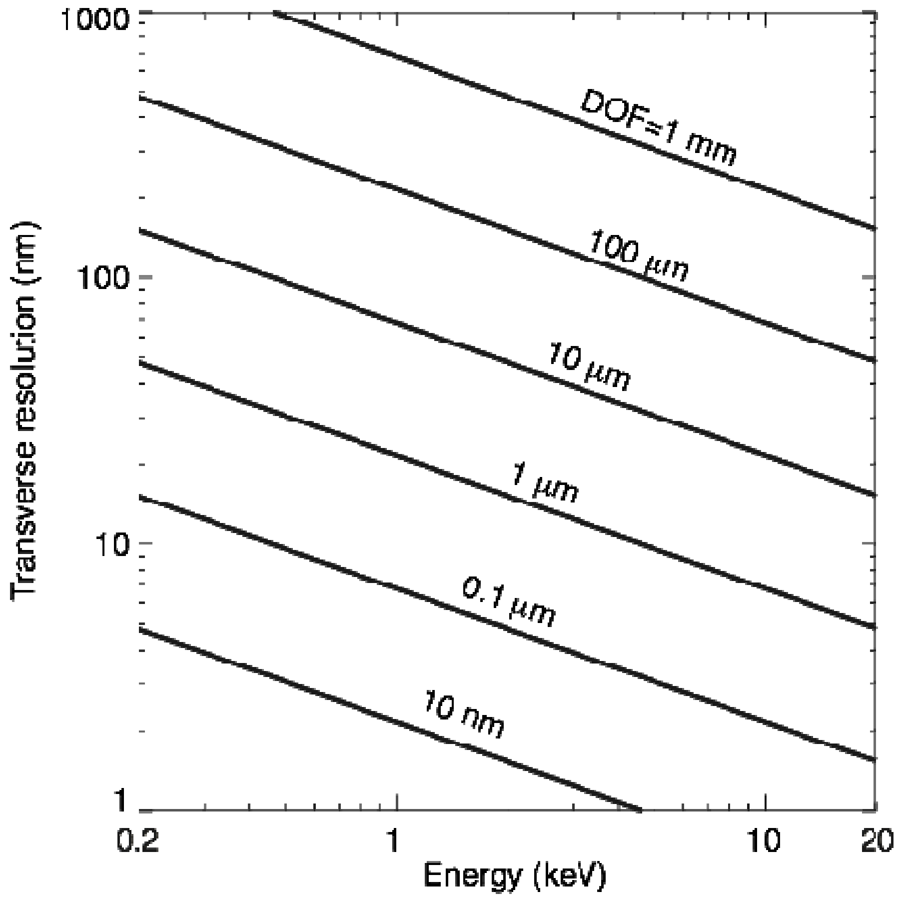
3D imaging with lenses

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

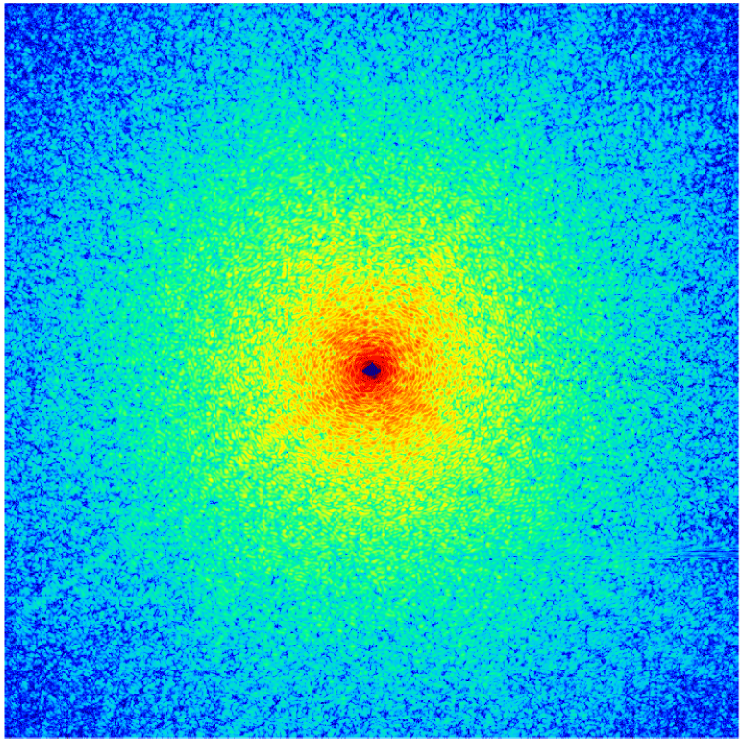


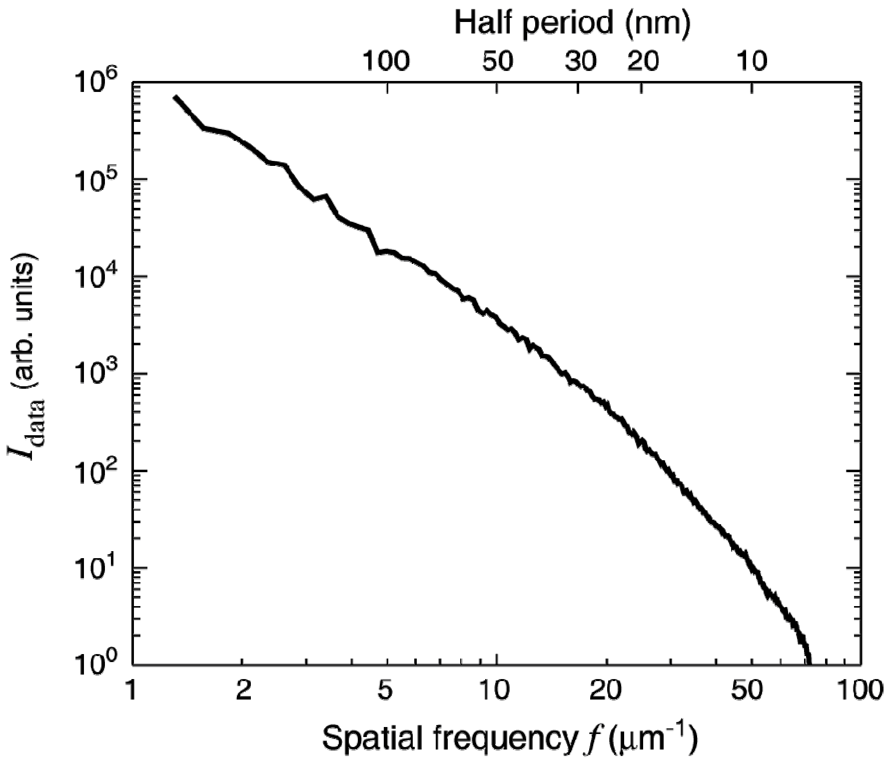
Through-focus deconvolution with lenses:

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

Diffraction from a yeast cell

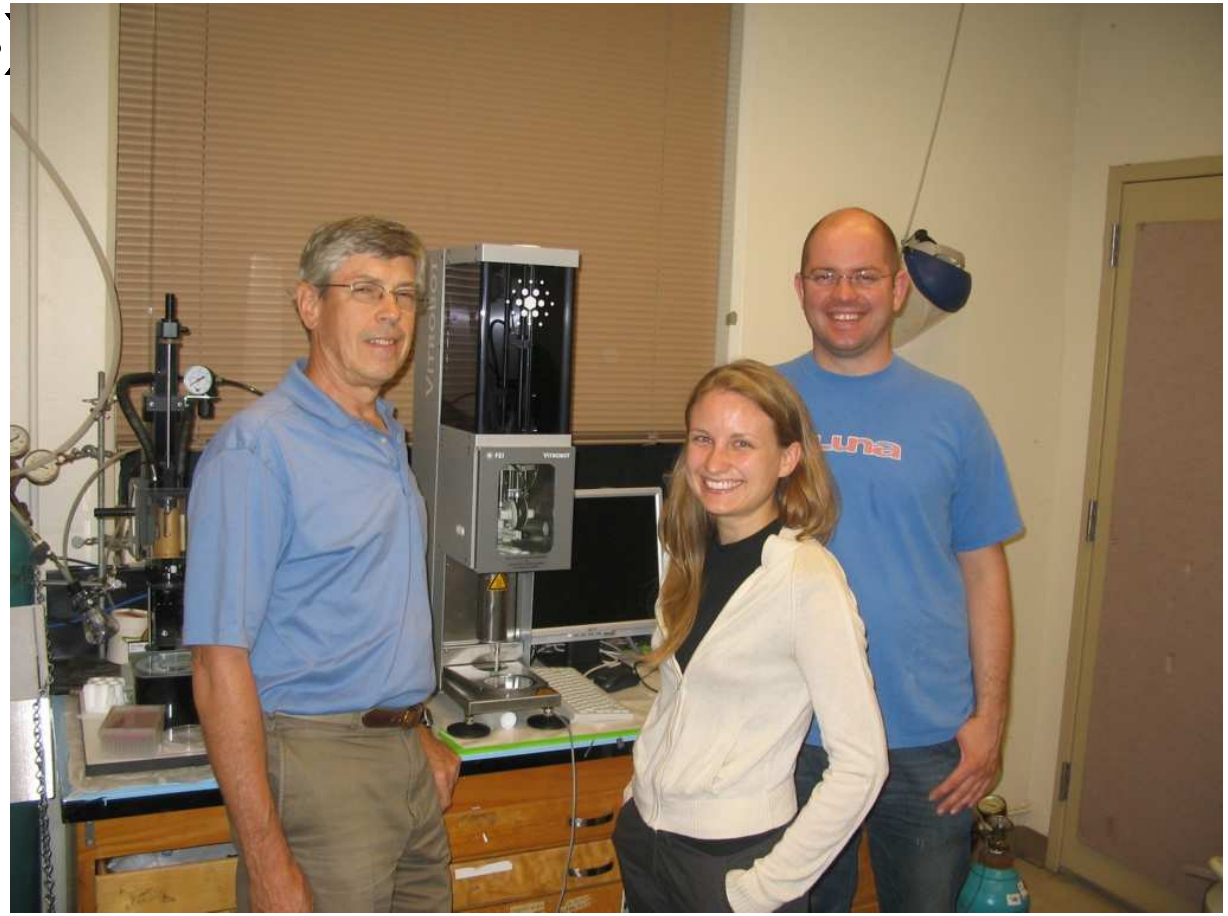
- Shapiro, Thibault, Beetz, Elser, Howells, Jacobsen, Kirz, Lima, H. Miao, Nieman, Sayre, *Proc. Nat. Acad. Sci.* **102**, 15343 (2005). ALS beamline 9.0.1 operated at 750 eV
- Total dose to freeze-dried, room temperature, unstained cell around 10^8 Gray
- Oversampling ratio is about 9 in each dimension





Frozen hydrated!

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



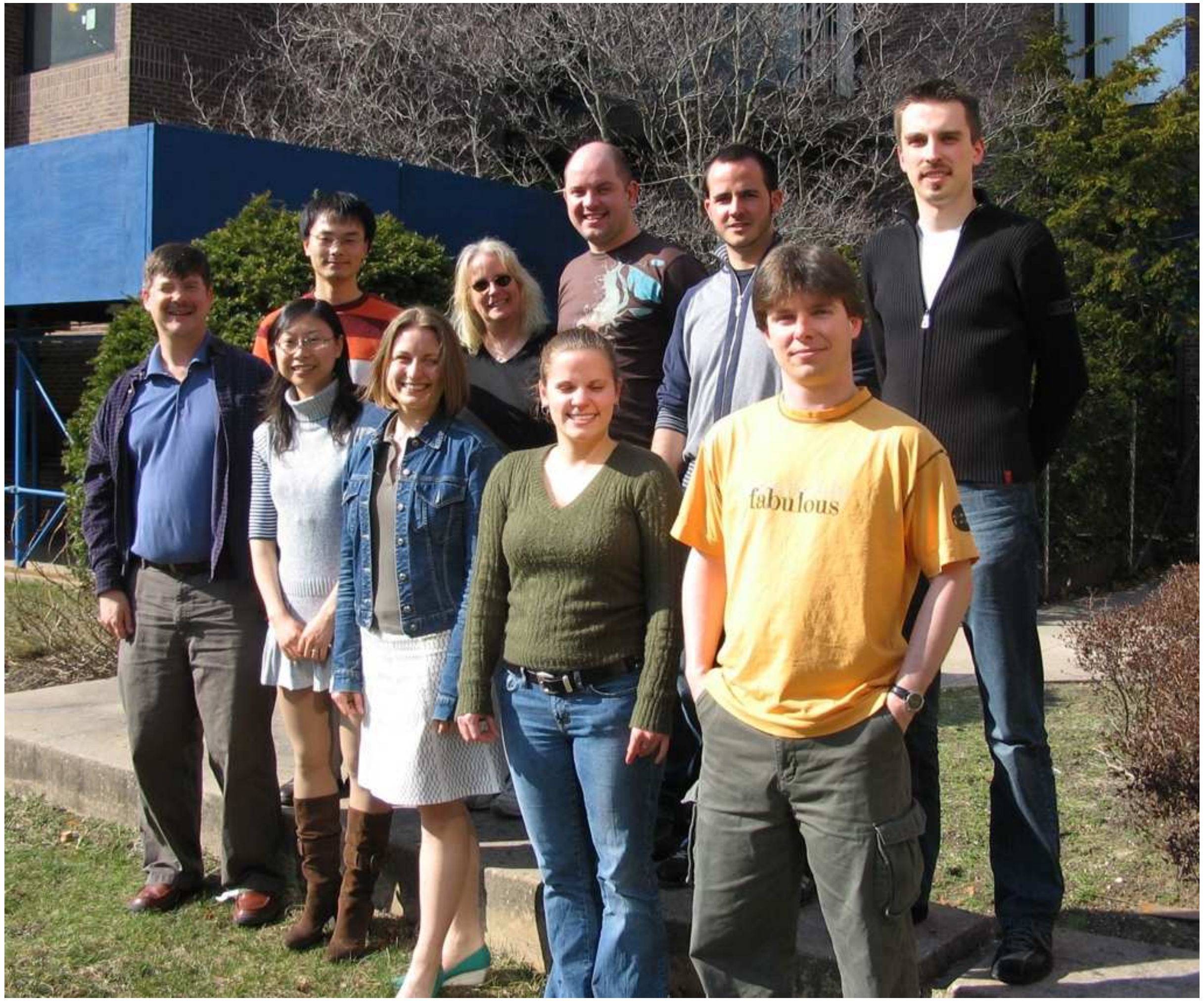
Ken Downing, Bjorg Larson, and Andrew Stewart.
Thanks also to Eva Nogales and her lab!

“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.

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!**

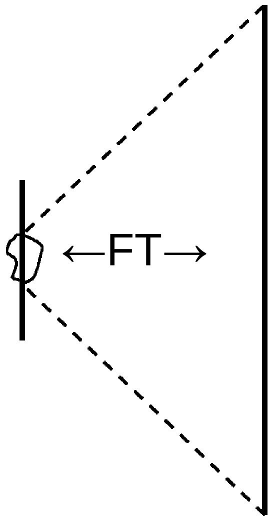


Funding: DoE (method development), NIH (application to cells)

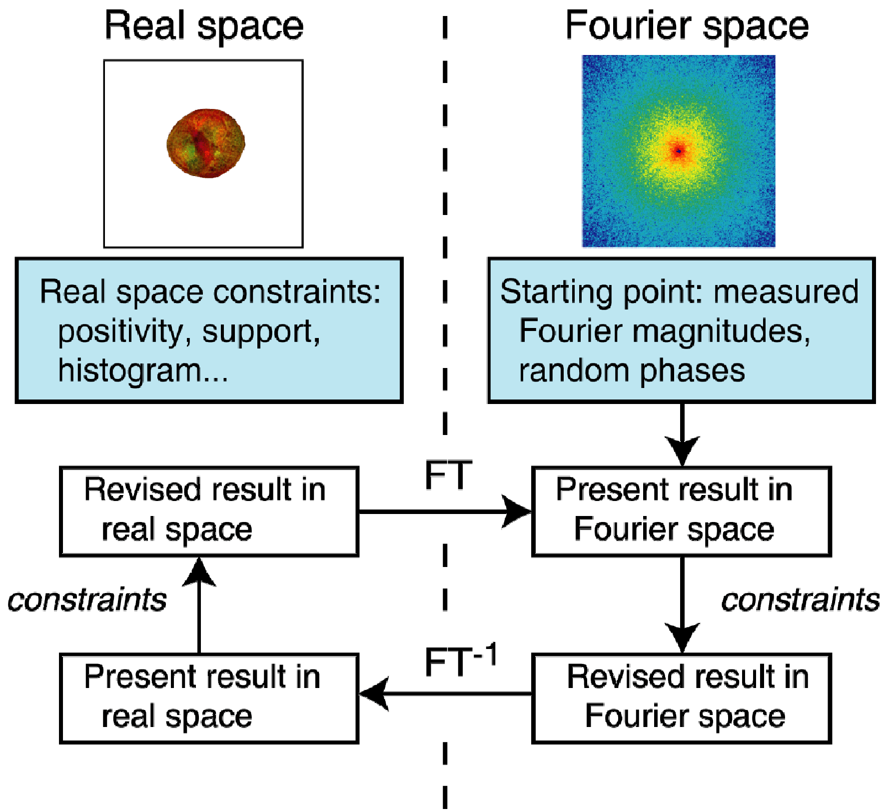
What if no real-space image?

•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.