The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
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IAEA
International Atomic Energy Agency

1936-48

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

7 - 25 April 2008

Lensless Imaging

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United States of America

Lensless Imaging

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)

Goals

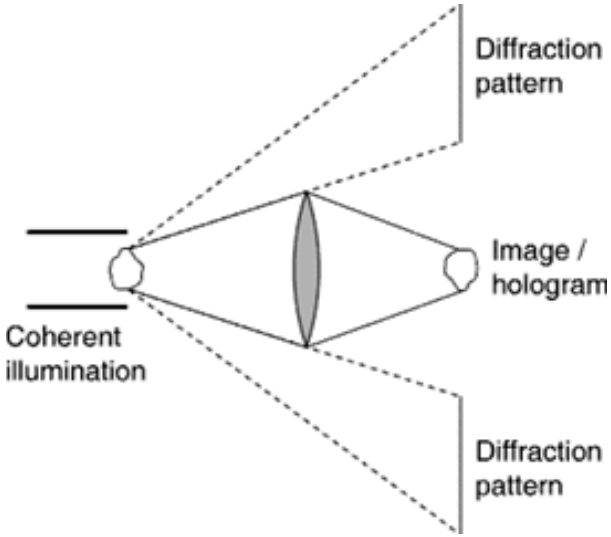
- 10 nm resolution (3D) in 1 - 10μm size biological specimens
  (small frozen hydrated cell, organelle; see macromolecular aggregates)
  Limitation: radiation damage!

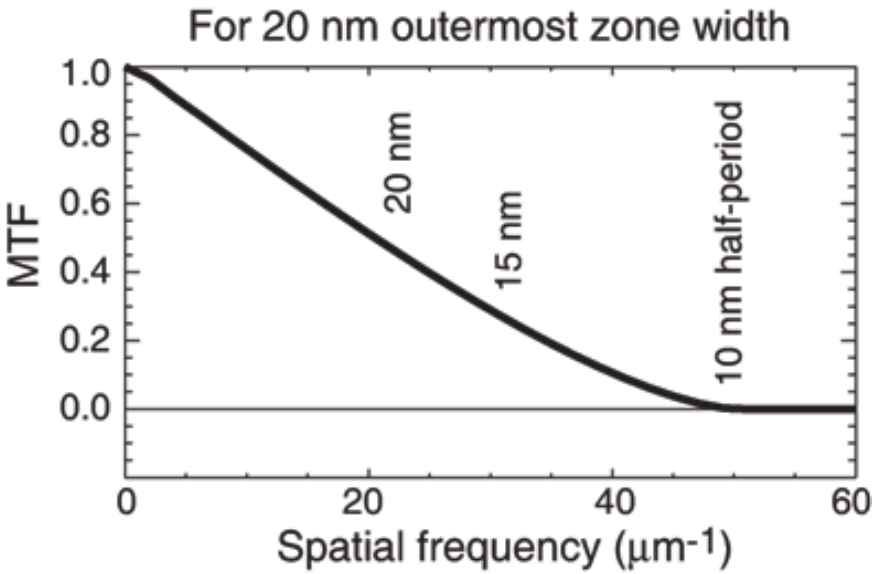
- <4 nm resolution in less sensitive nanostructures
  (Inclusions, porosity, clusters, composite nanostructures, aerosols…)
  eg: molecular sieves, catalysts, crack propagation

- 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% 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?





Many biological samples of the interest are…

- Non-crystalline : conventional crystallography
  not applicable
- Micron size scale : Electron microscopy can give sub nm resolution, but limited to 0.5 micron thick specimens.
  Often needs heavy metal staining
- Desire to see the living state, or as close as….. :
  light microscopy: image live cells at about 200 nm resolution

Looking for a method that works with minimal sample preparation at a resolution better than light microscopy

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"!

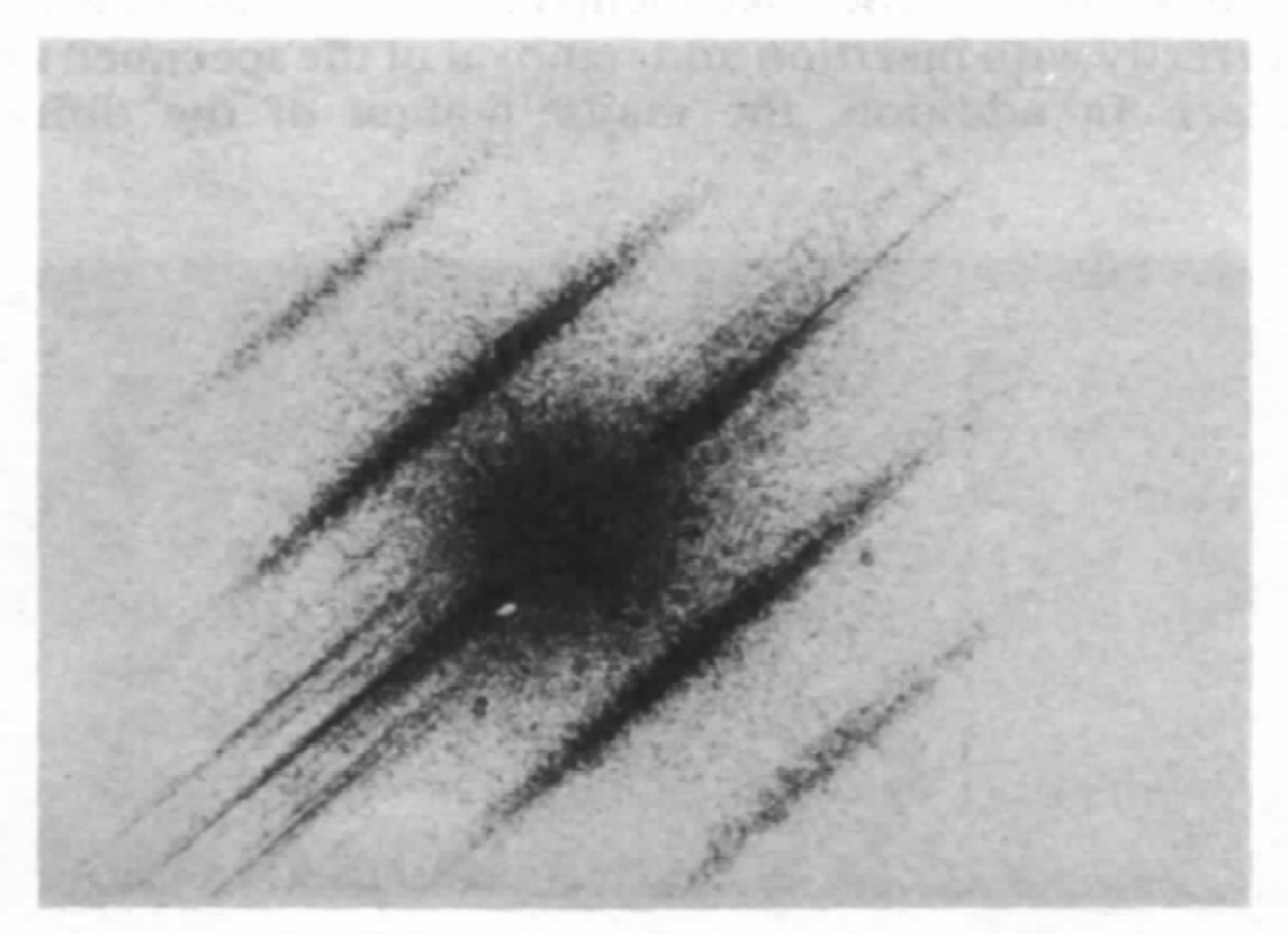
- Holographers do it – but they mix in a reference wave, need very high resolution detector or similar precision apparatus

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

History

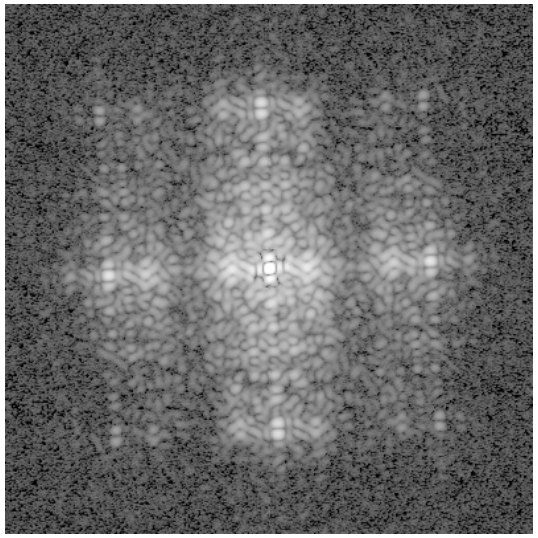
- Sayre 1952: Shannon sampling theorem in crystallography
- Gerchberg & Saxton, 1971: iterative phase retrieval algorithm in EM
- Sayre 1980: pattern stronger with soft X-rays; use SR to work without xtals!
- Fienup 1982: Hybrid Input-Output, support
- Bates 1982: 2x Bragg sampling gives unique answer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

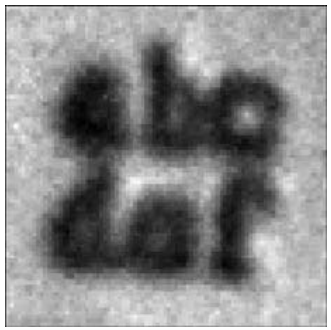
Diffraction pattern of a single diatom, 1987



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

λ=1.8 nm soft x-ray diffractio n pattern





Low angle data From optical micrograph

Scanning electron micrograph of object

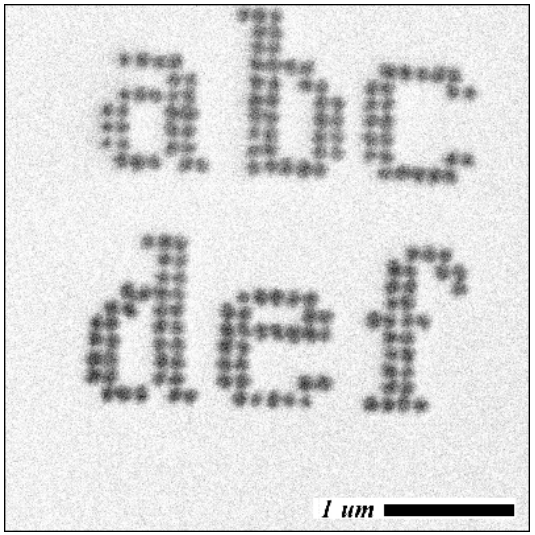


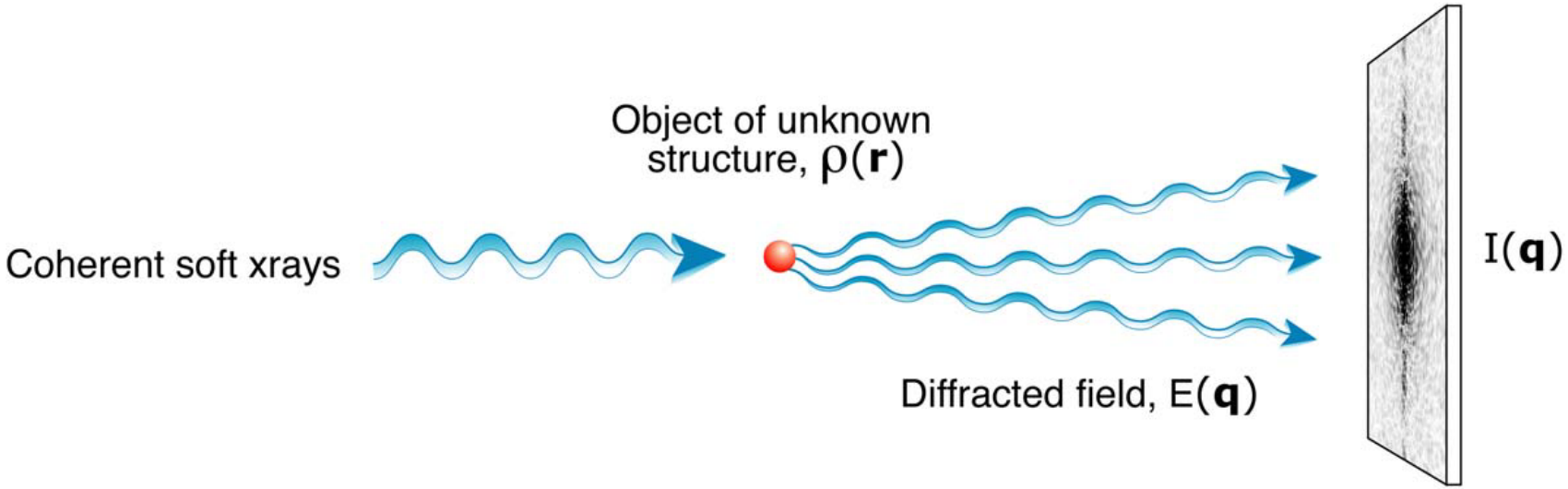
Image reconstructed from diffraction pattern (θ_{max} corresponds to 80 nm). Assumed positivity

Basic principles

- Single object, plane wave incident, scattered amplitude is Fourier transform of (complex) electron density f(**r**)

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

- Assume: Born Approximation
- Assume coherent illumination

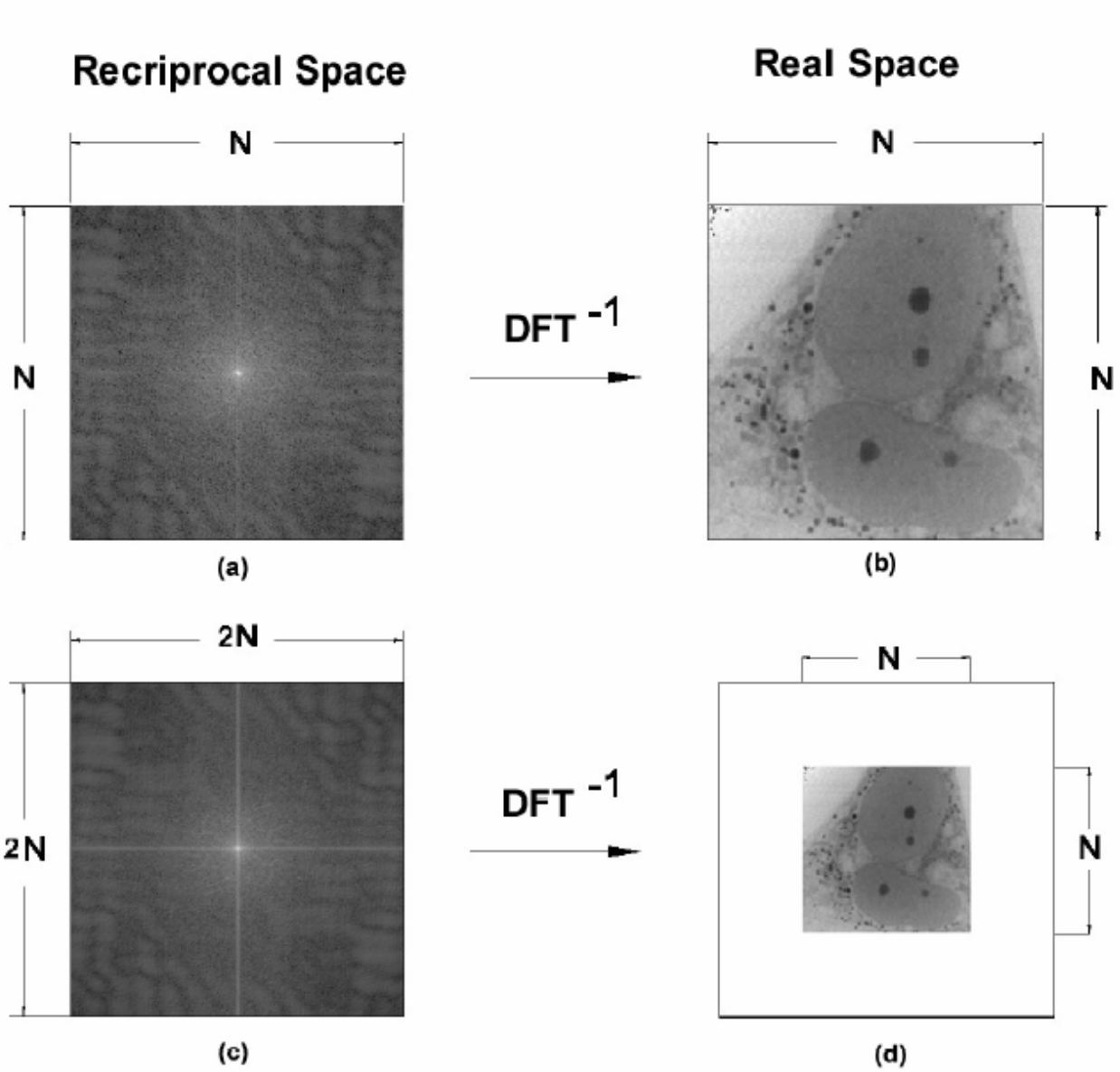


"Oversampling":

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

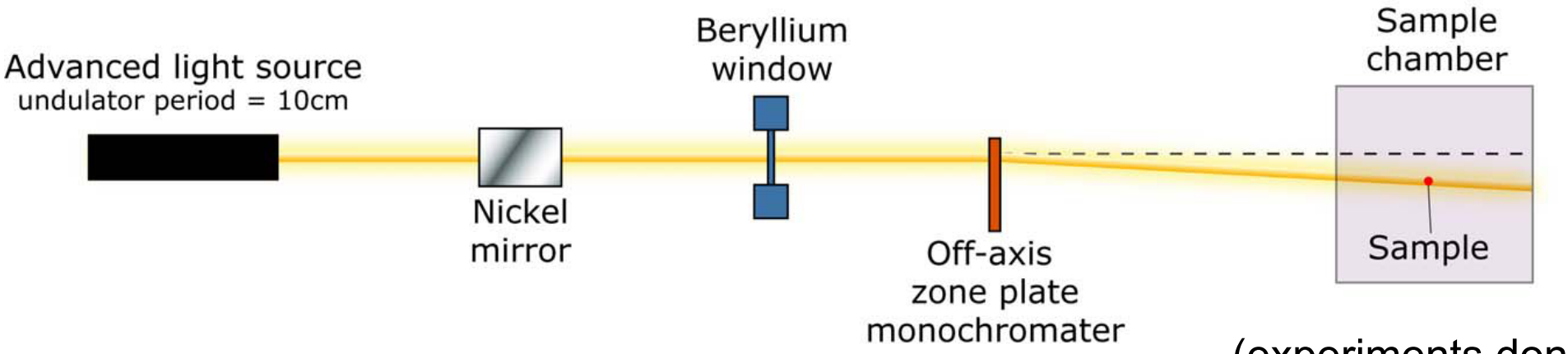
Finer sampling;
larger array;
smaller transform;
"finite support"

(area around specimen
must be clear!)



Experimental setup

Advanced Light Source beamline 9.0.1

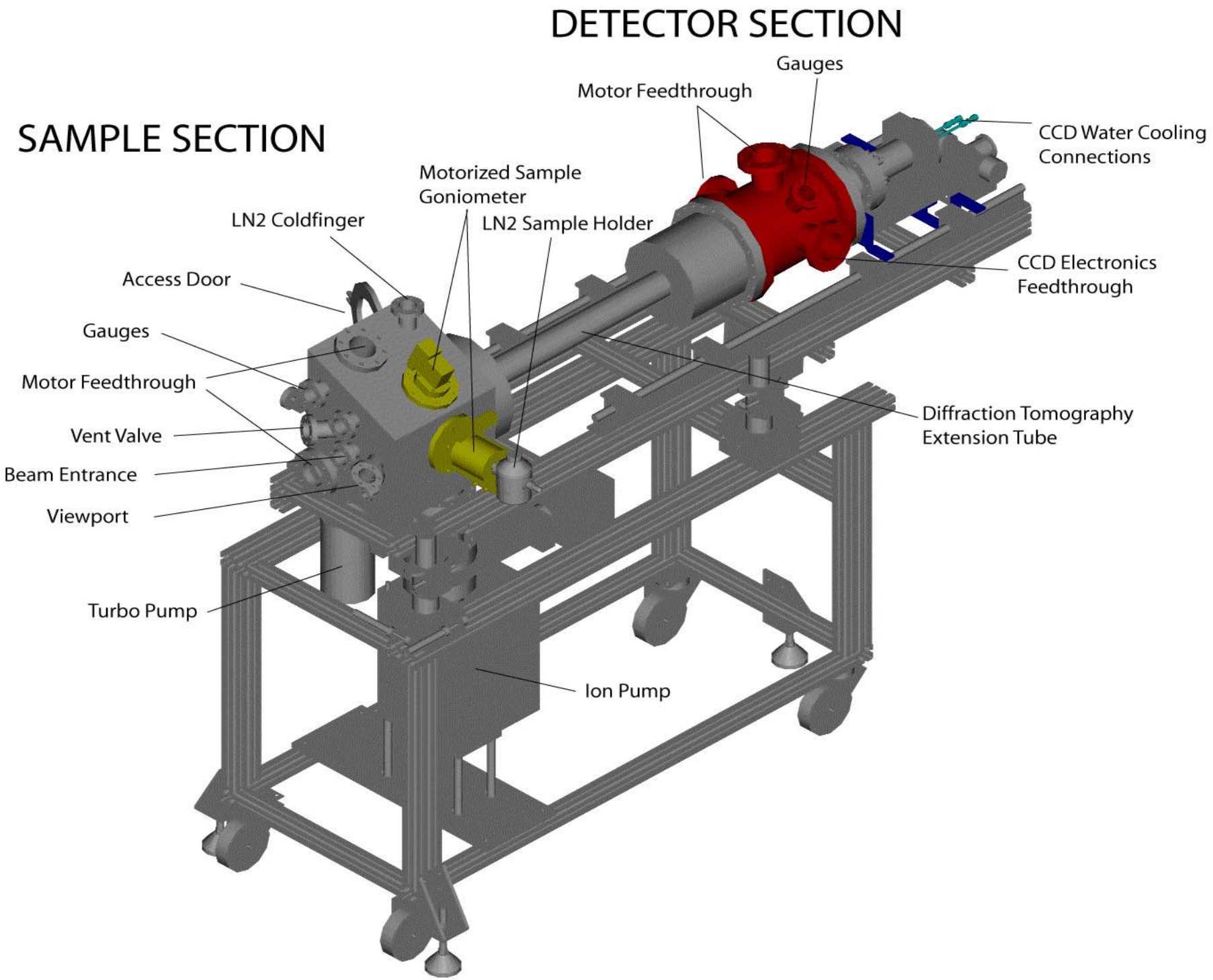


(experiments done at 520, 750 eV)

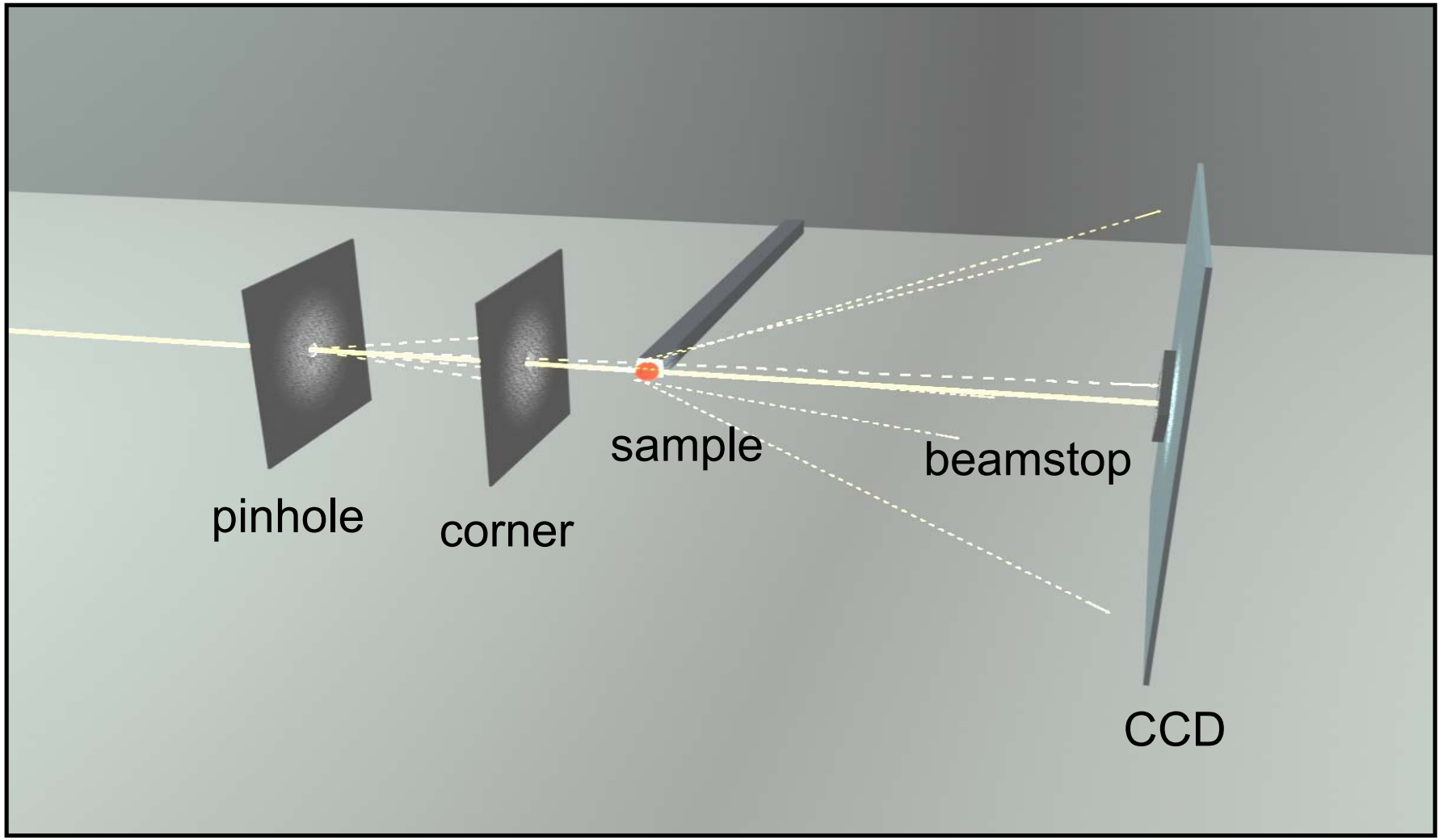
Yeast samples : 3 - 7 micron

Lateral coherence length : 15 micron

Oversampling ratio : 3 to 5

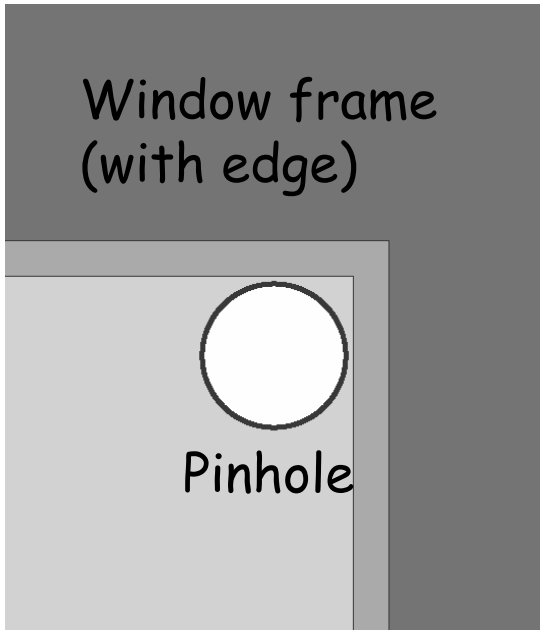

DETECTOR SECTION
SAMPLE SECTION
Gauges
Motor Feedthrough
CCD Water Cooling Connections
Motorized Sample Goniometer
LN2 Coldfinger
LN2 Sample Holder
CCD Electronics Feedthrough
Access Door
Gauges
Motor Feedthrough
Vent Valve
Beam Entrance
Viewport
Diffraction Tomography Extension Tube
Turbo Pump
Ion Pump

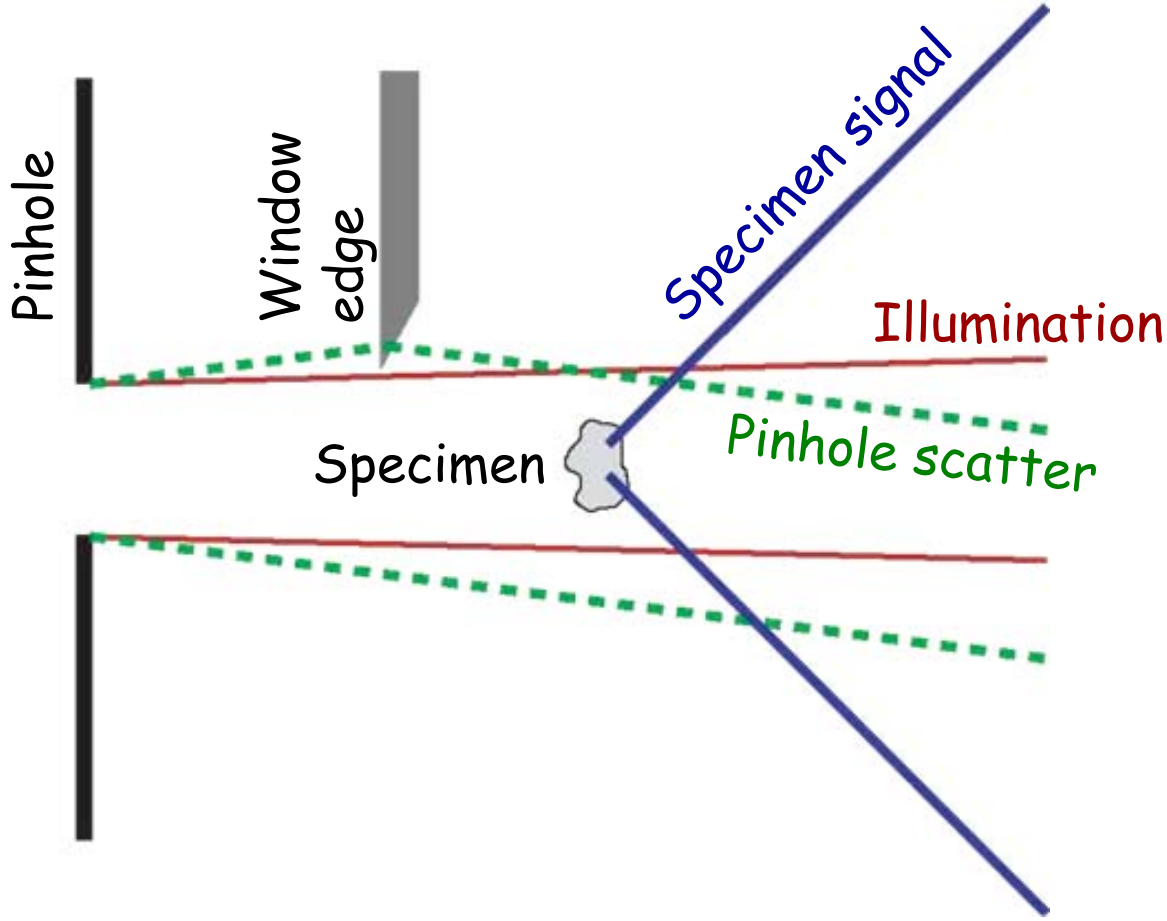
Inside vacuum chamber



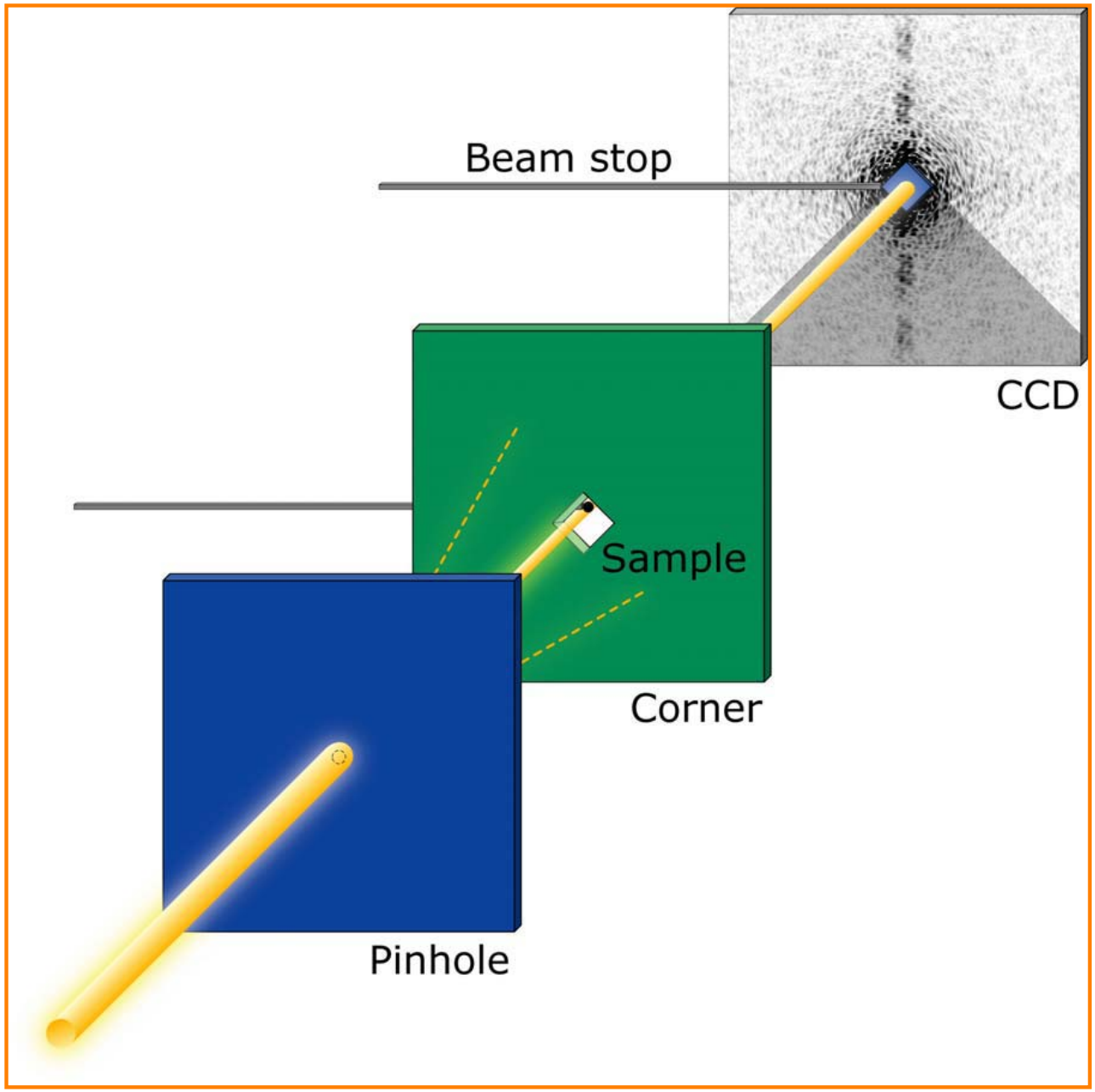
Removing scatter from pinholes

- Pinholes have scatter; can overwhelm weak diffraction.
- Use a “soft,” refractive corner to limit to one quadrant (idea due to H. Chapman, then at Stony Brook)

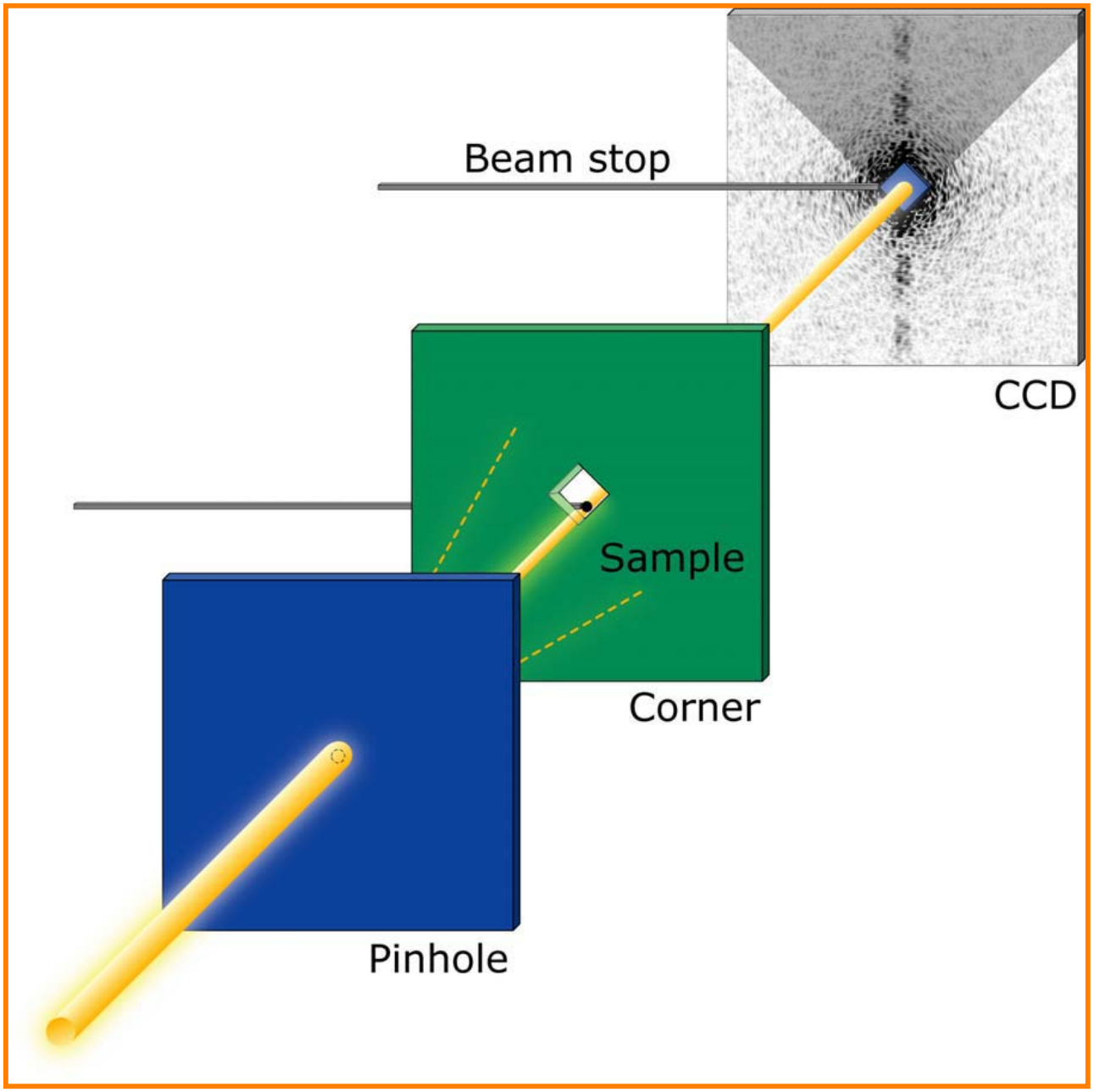




Bottom half image



Top half image

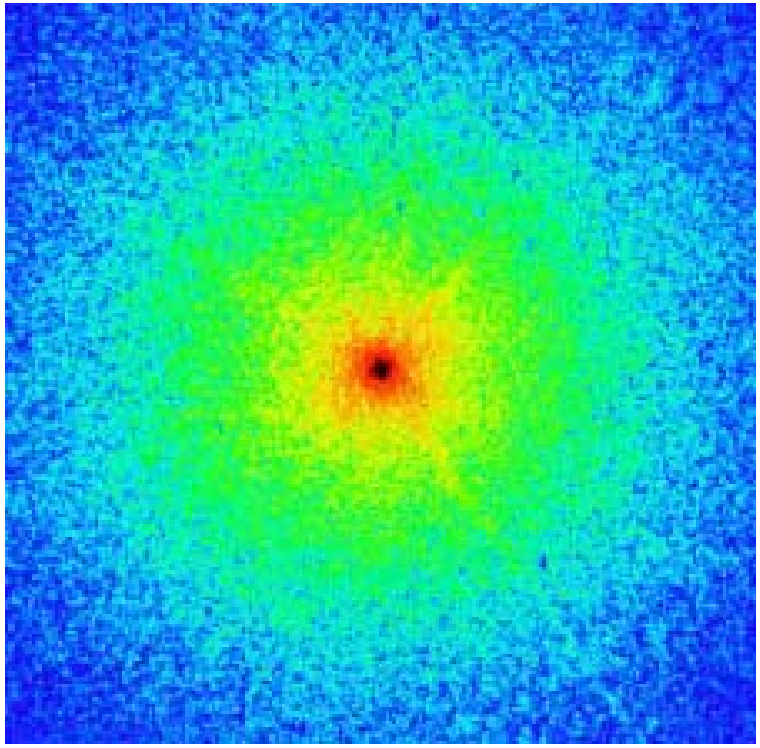


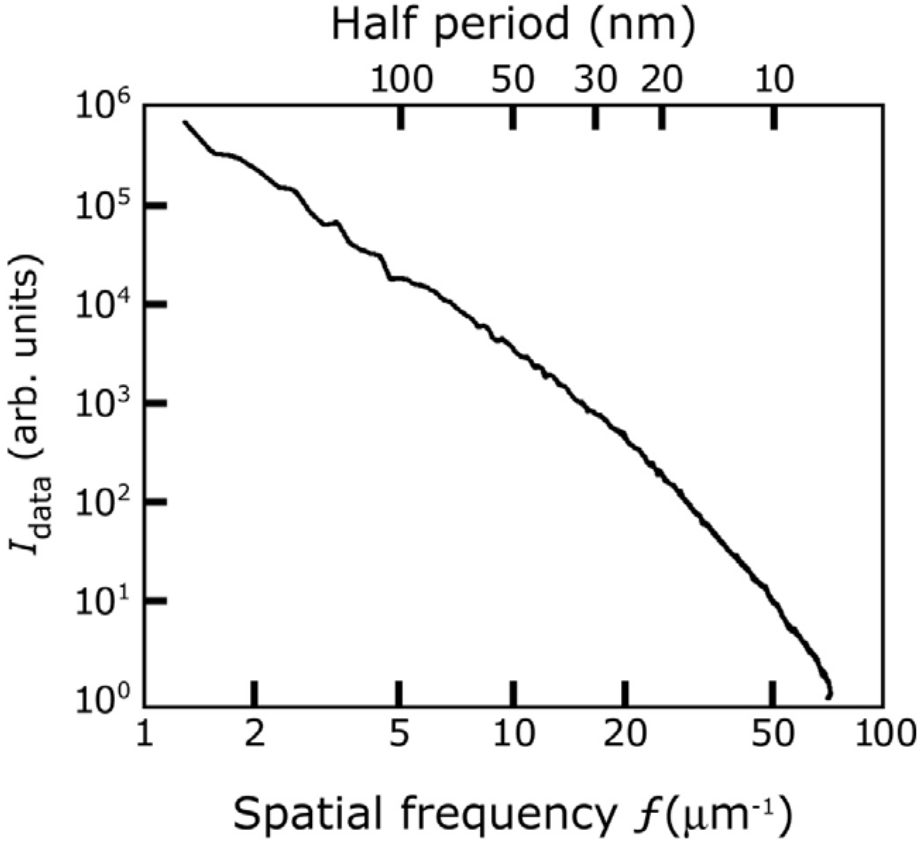
Gatan 630 cryo holder

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

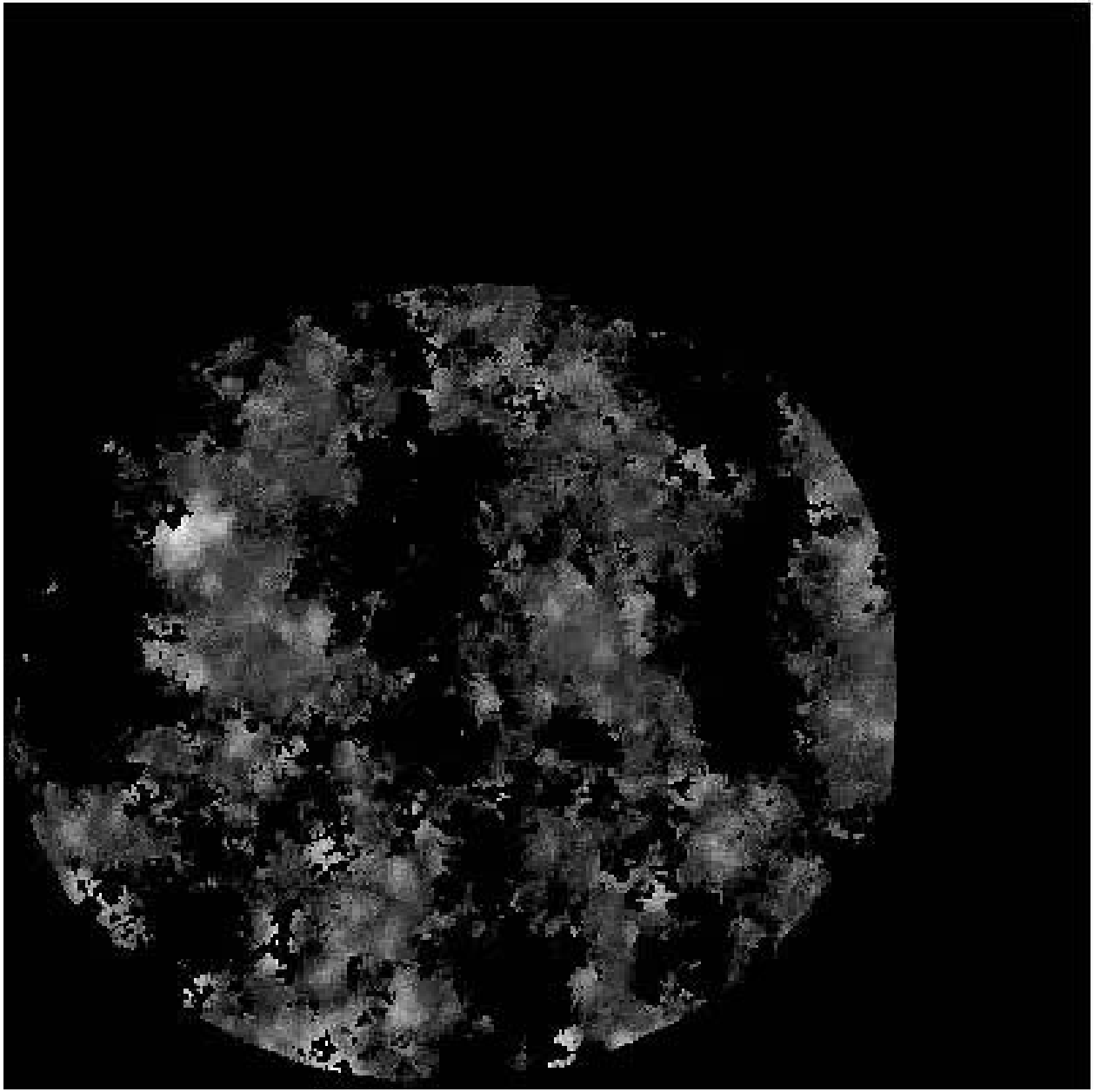
We do *not* claim that achieved resolution is given by maximum angle of data recording





David Shapiro, Stony Brook, now at UC Davis

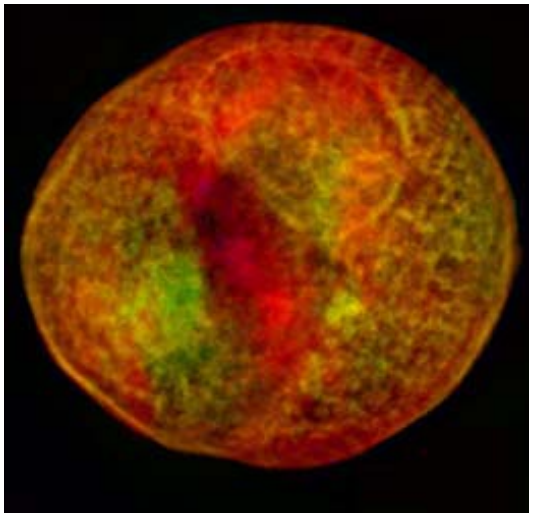
The reconstruction process



Enju Lima thesis

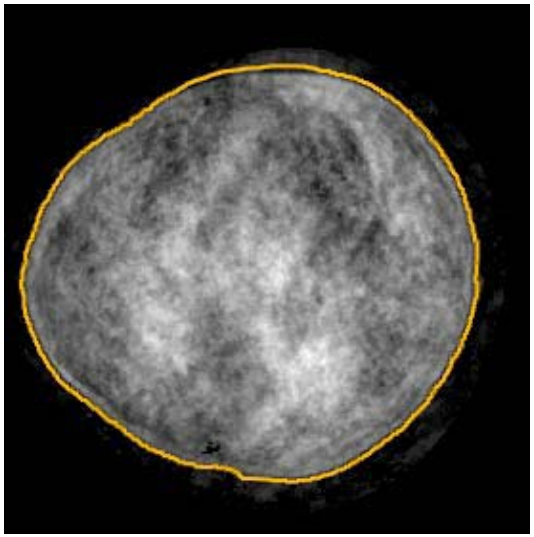
Summary of reconstruction details

- Final reconstruction was obtained by averaging iterates



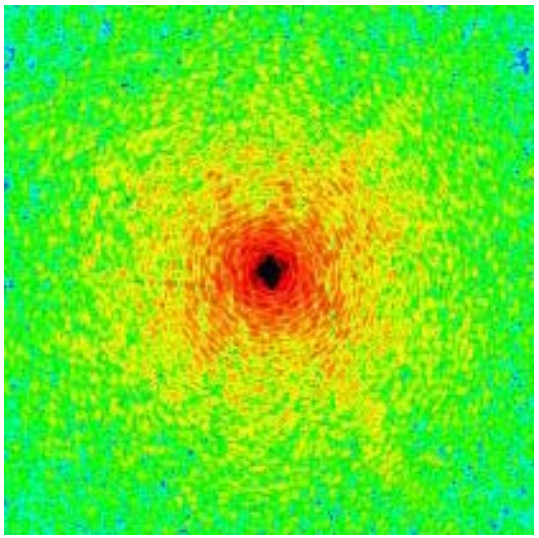
Summary of reconstruction details

- Final reconstruction was obtained by averaging iterates
- Support was calculated from the autocorrelation

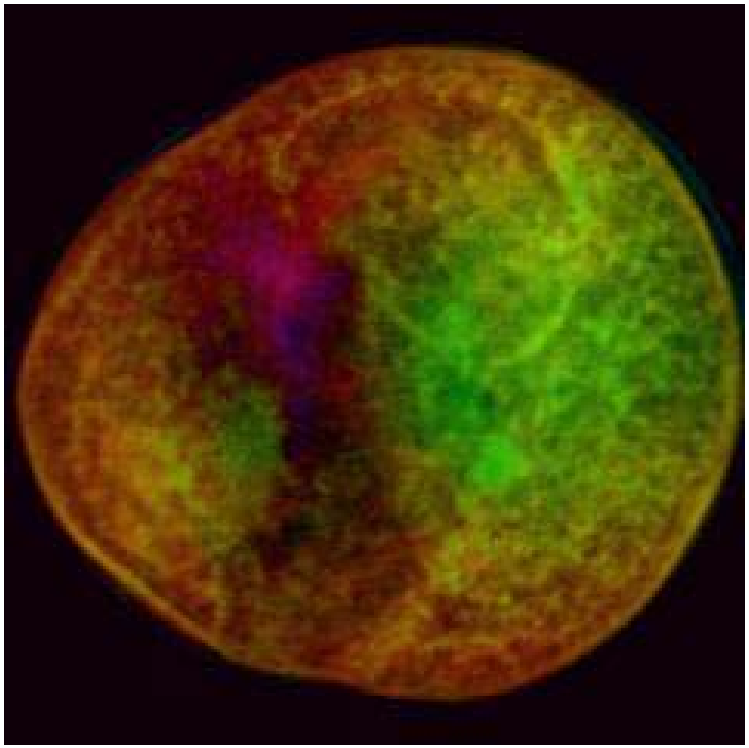


Summary of reconstruction details

- Final reconstruction was obtained by averaging iterates
- Support was calculated from the autocorrelation
- Missing data was recovered by the algorithm



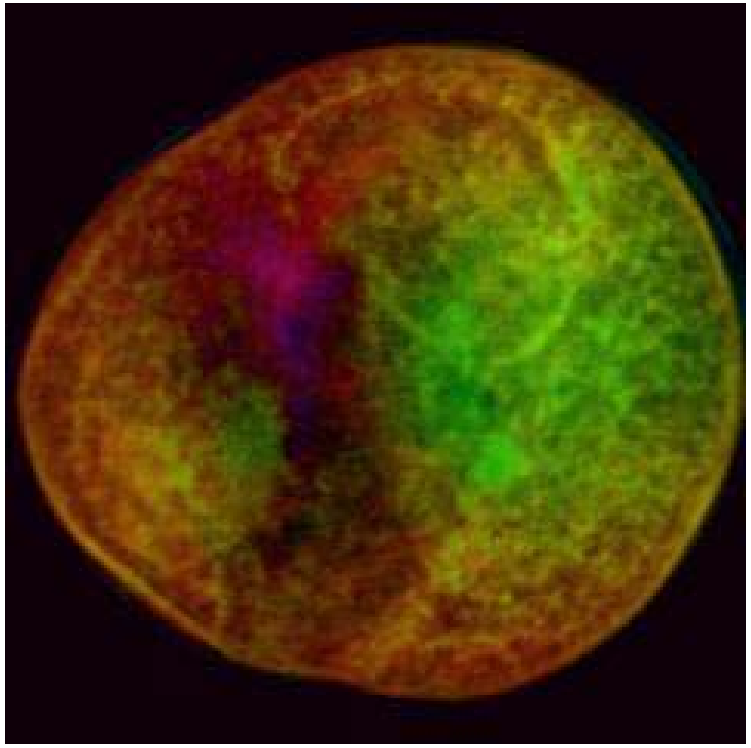
Reconstruction of complex image of FD yeast cell



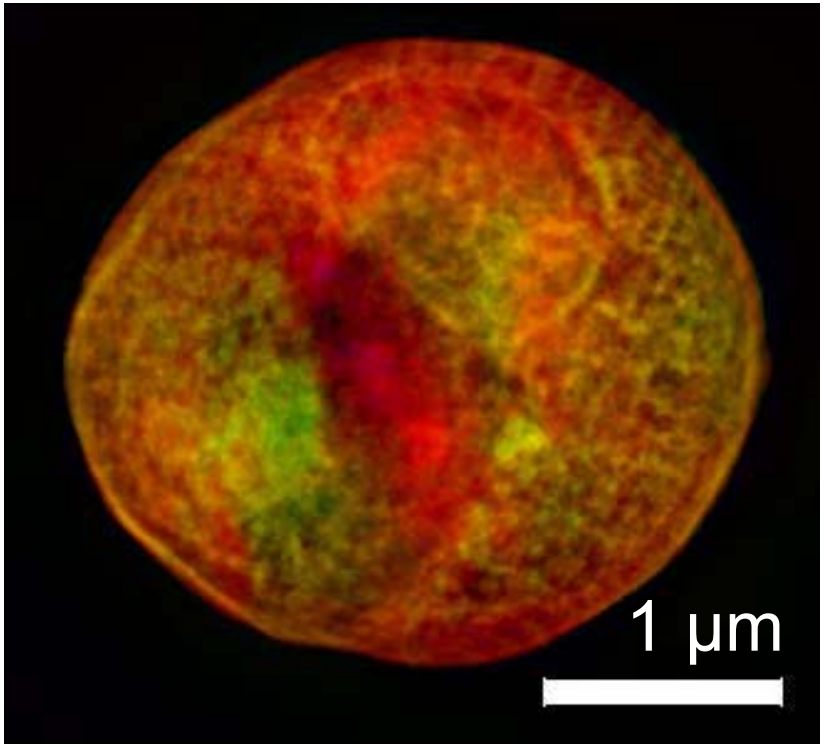
- algorithm: Difference map, beta=1
- 10,000 iterations
- Brightness - amplitude, hue - phase
- averaged over 100 iterates

Is the solution unique and faithful?

With averaging, reproducible reconstructions!



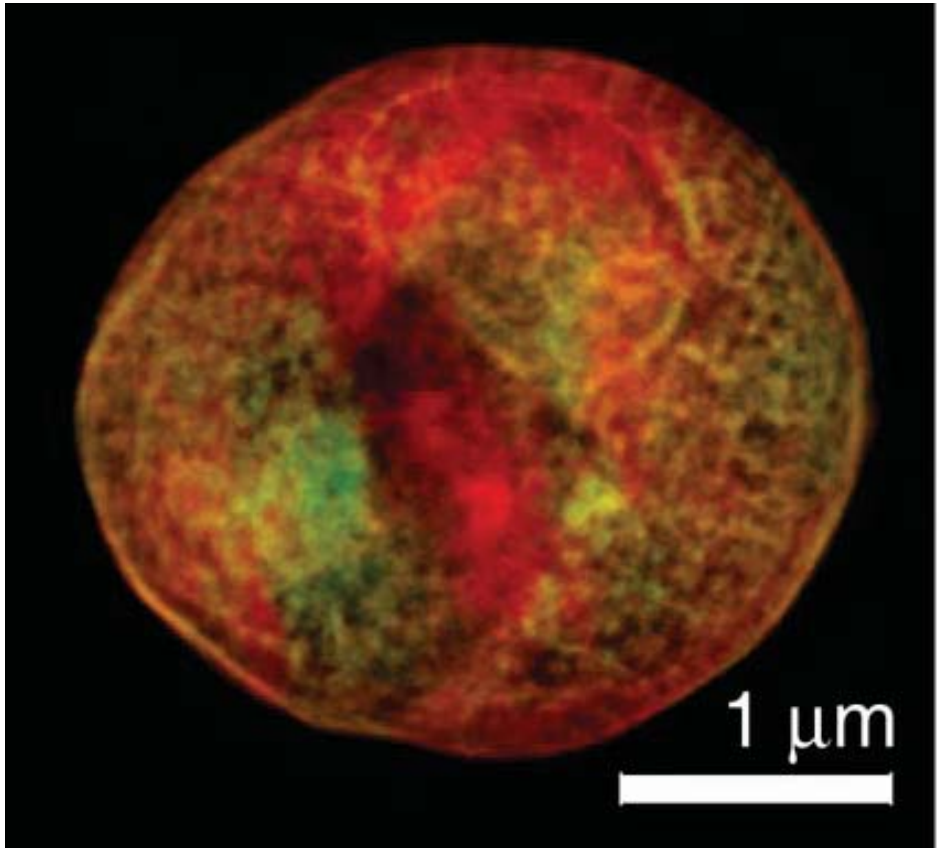
Stony Brook group: average of 100 Iterates, 40 iterations apart



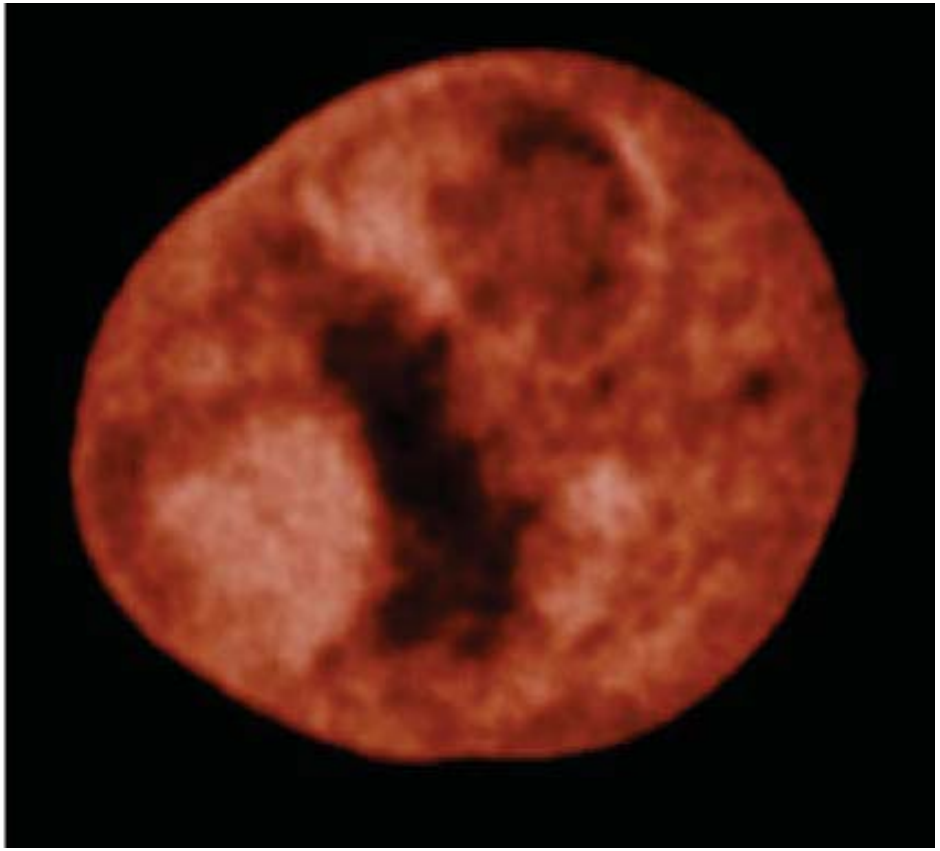
Cornell group: average of 980 iterates, 50 iterations apart

- Individual reconstruction programs with different starting random phases yield reproducible reconstruction!
- Hue difference from low mode?

Comparison with a microscope

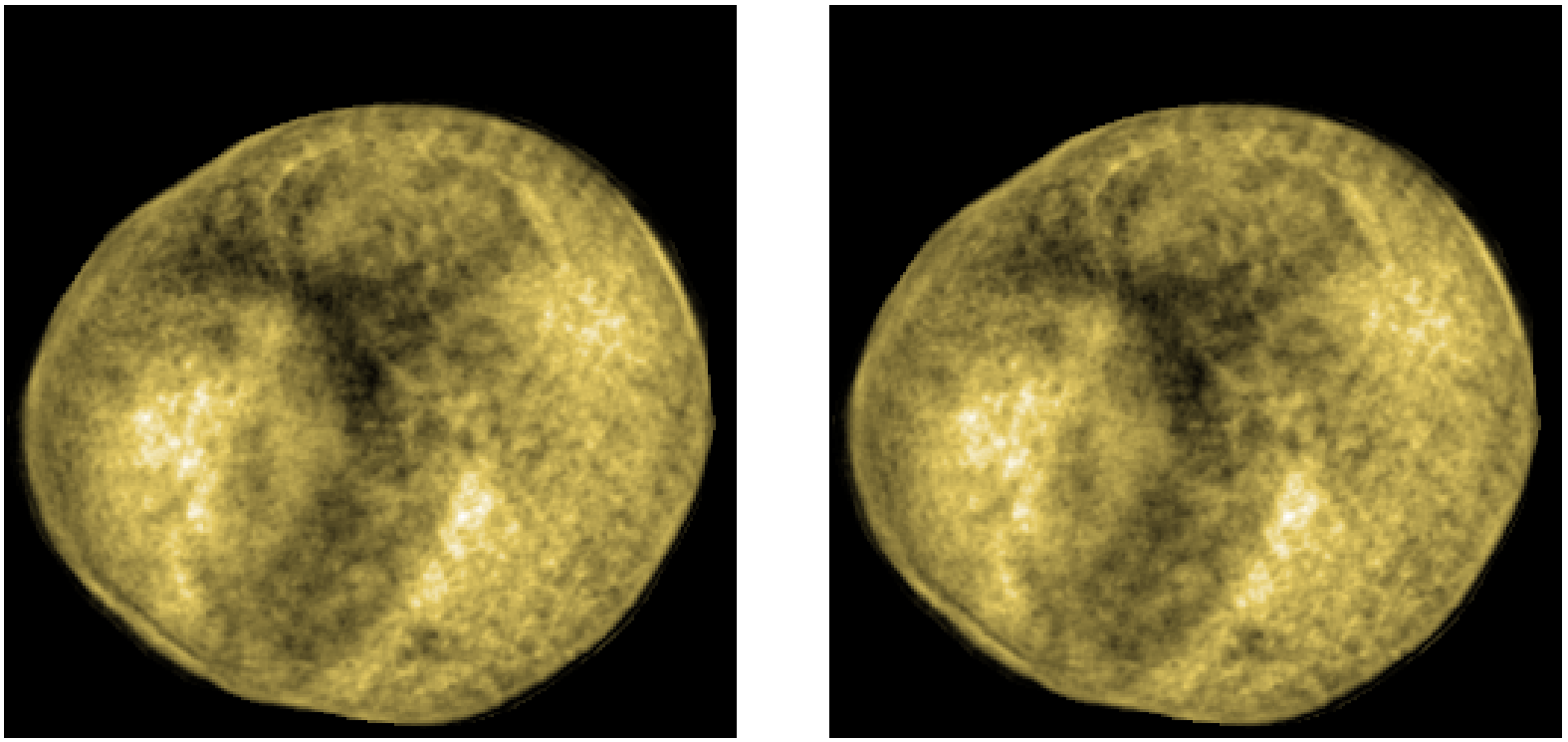


Diffraction reconstruction (data taken at 750 eV; absorption as brightness, phase as hue).



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

Two separate iterations with different random starts gives same reconstruction

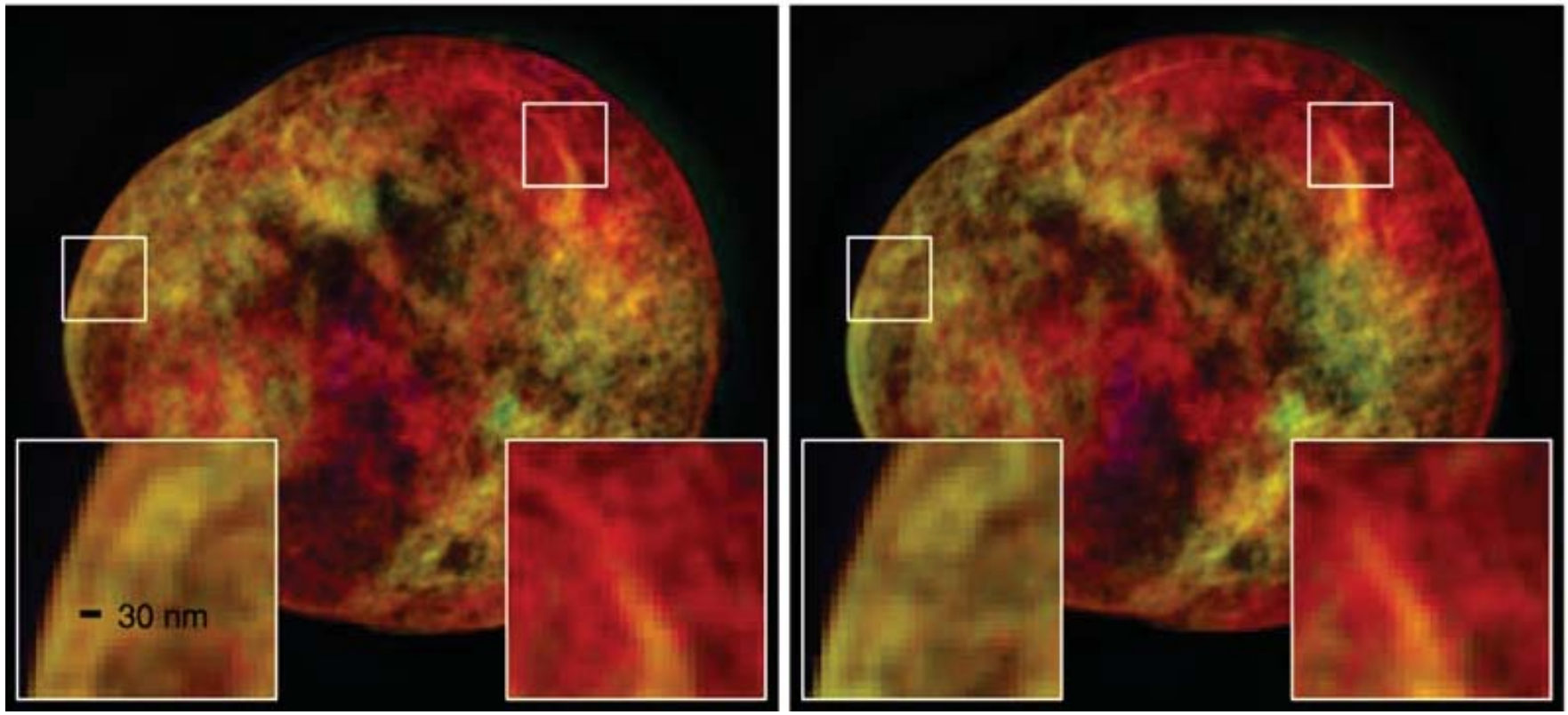


- **How to handle missing data in the center:**

  Patching from STXM image : not successful
  - stxm gives absorption contrast
  - xdm gives absorption and phase contrast

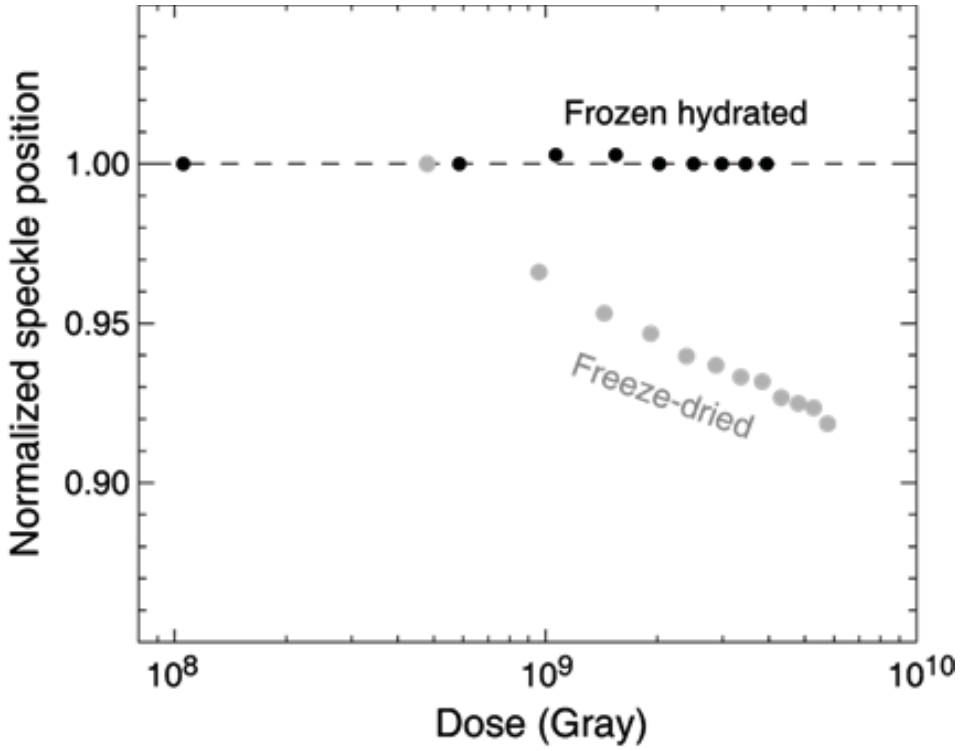
  Better to find it via algorithm, but need to be careful:
  - if missing data unconstrained during the iteration, one starts to see unwanted effects, for example: Gaussian blurring effect
  - solution: apply upper limit in the magnitude

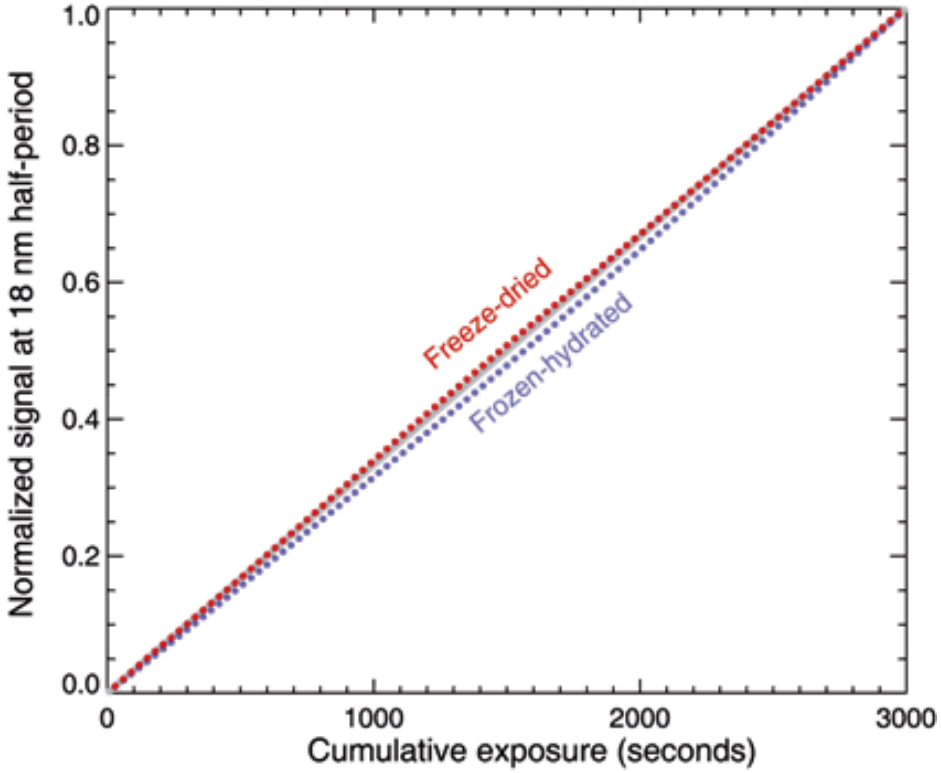
Reconstructions from data 1 degree apart show similar 30 nm structure



Stability of frozen hydrated specimens

- D. Shapiro, PhD thesis





Reconstruction of frozen-hydrated yeast spores

Frozen-hydrated state:

- gives less structural artifact
- radiation hardy - 3D reconstruction
- vitrified ice state required - tricky business
- cooling rate > 10,000 K/s
- Temperature < -140 ^{o} C

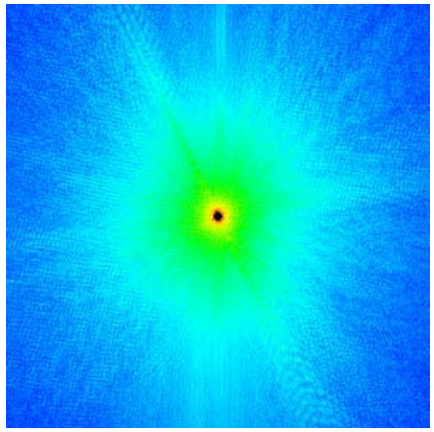
XDM apart from Cryo-Em

- larger sample, 3 - 7 micron
- ice thickness at 1 micron
- vitrification possible without high pressure freezing?

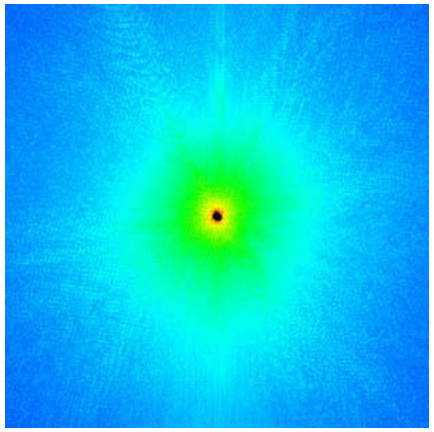
Commercial plunger
By FEI company

Diffraction magnitude of frozen-hydrated spores

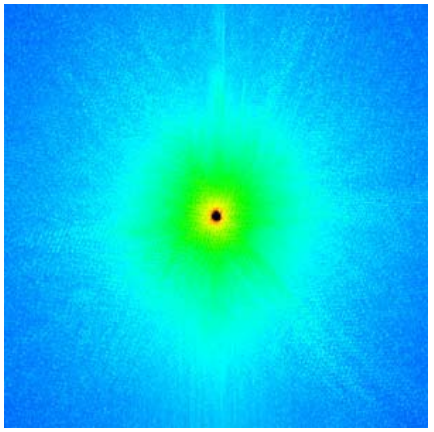
-5 degree



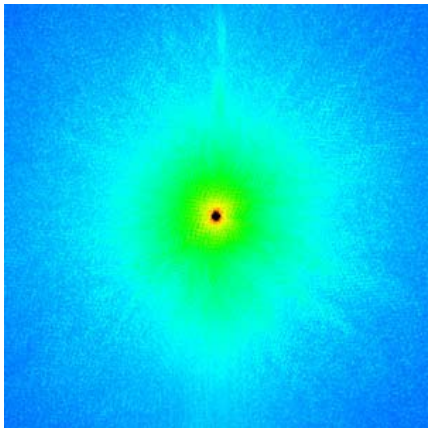
-15 degree



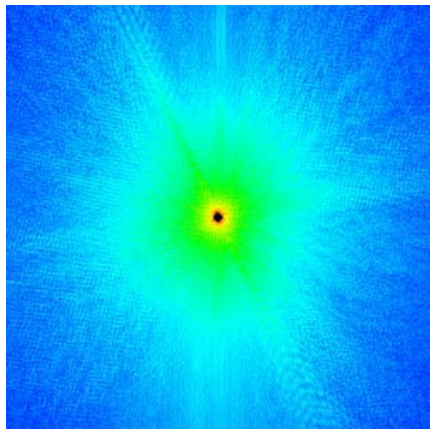
-25 degree



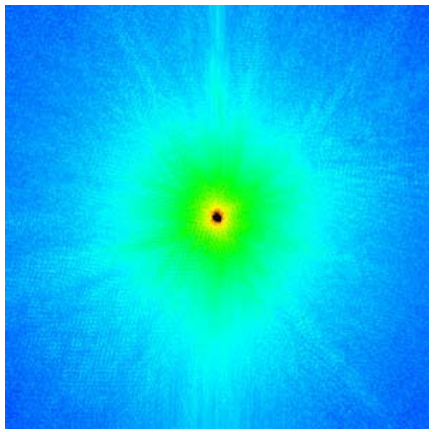
-35 degree



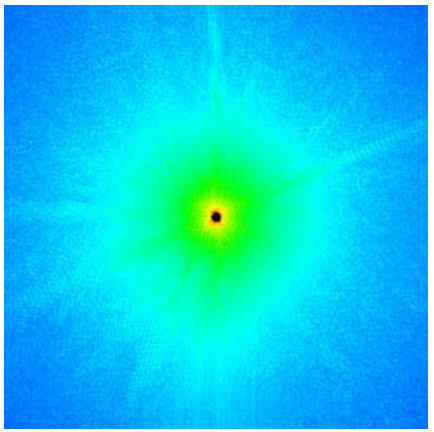
5 degree



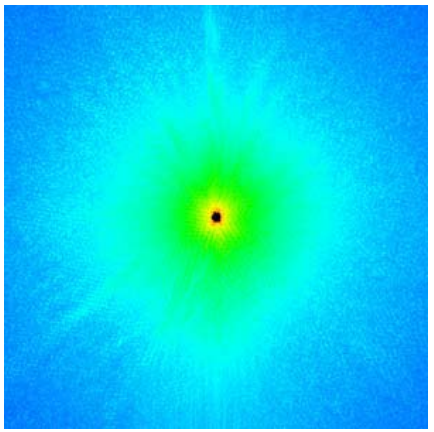
15 degree



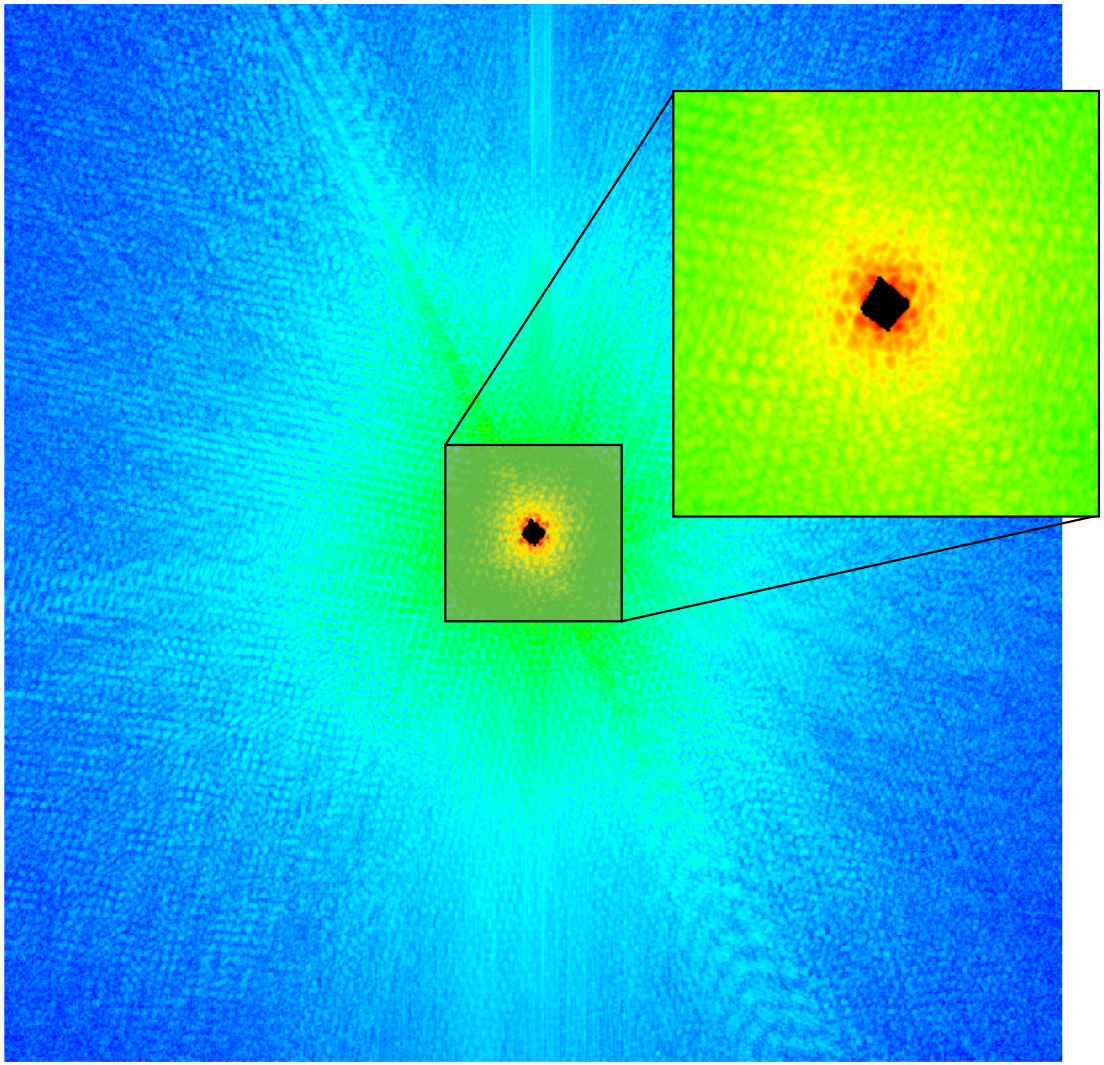
25 degree



35 degree



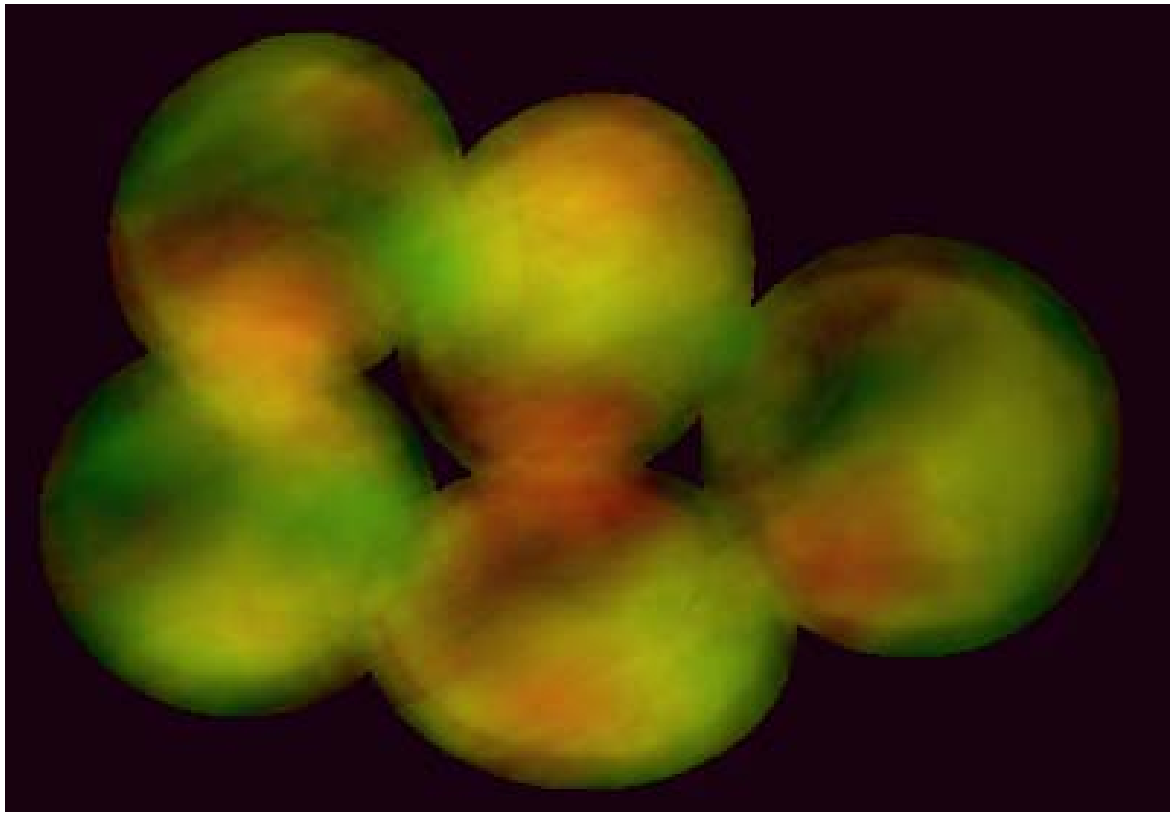
-5 degree data set



- specimen size ≈ 8 μ * 5 μ
- scattering grid bar not negligible
- # of missing speckles impose challenge on phasing

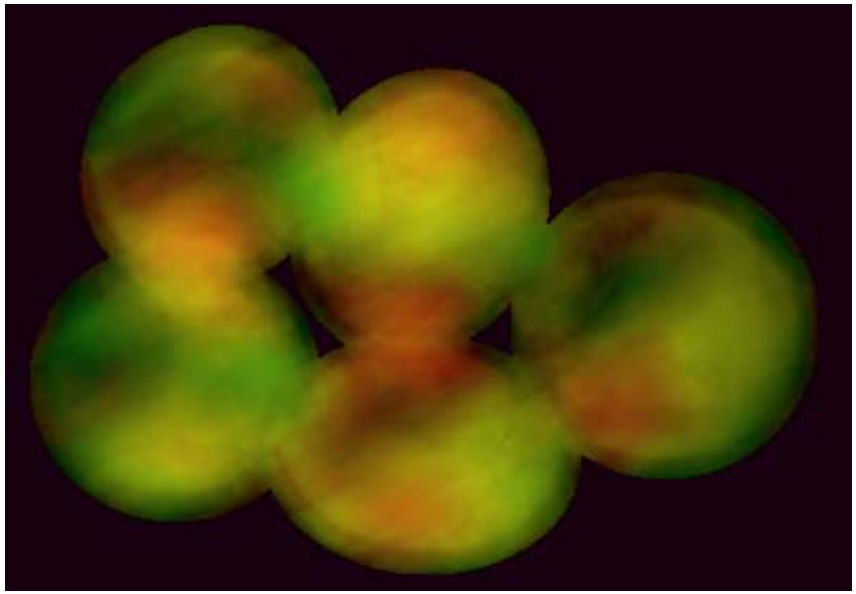
Initial reconstruction

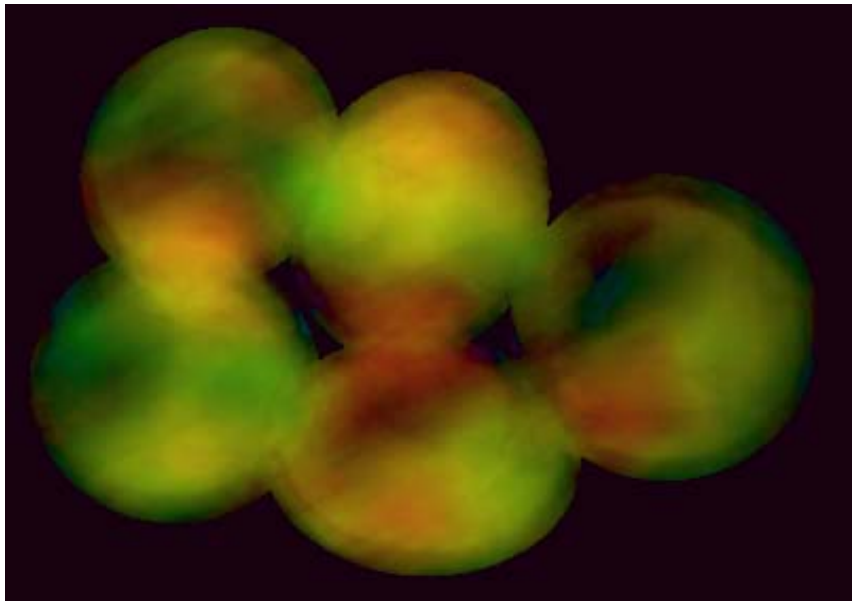
Shows a clump of 5 yeast spores, 8μ * 5μ



Is it reproducible?

Reconstruction from two different random starts

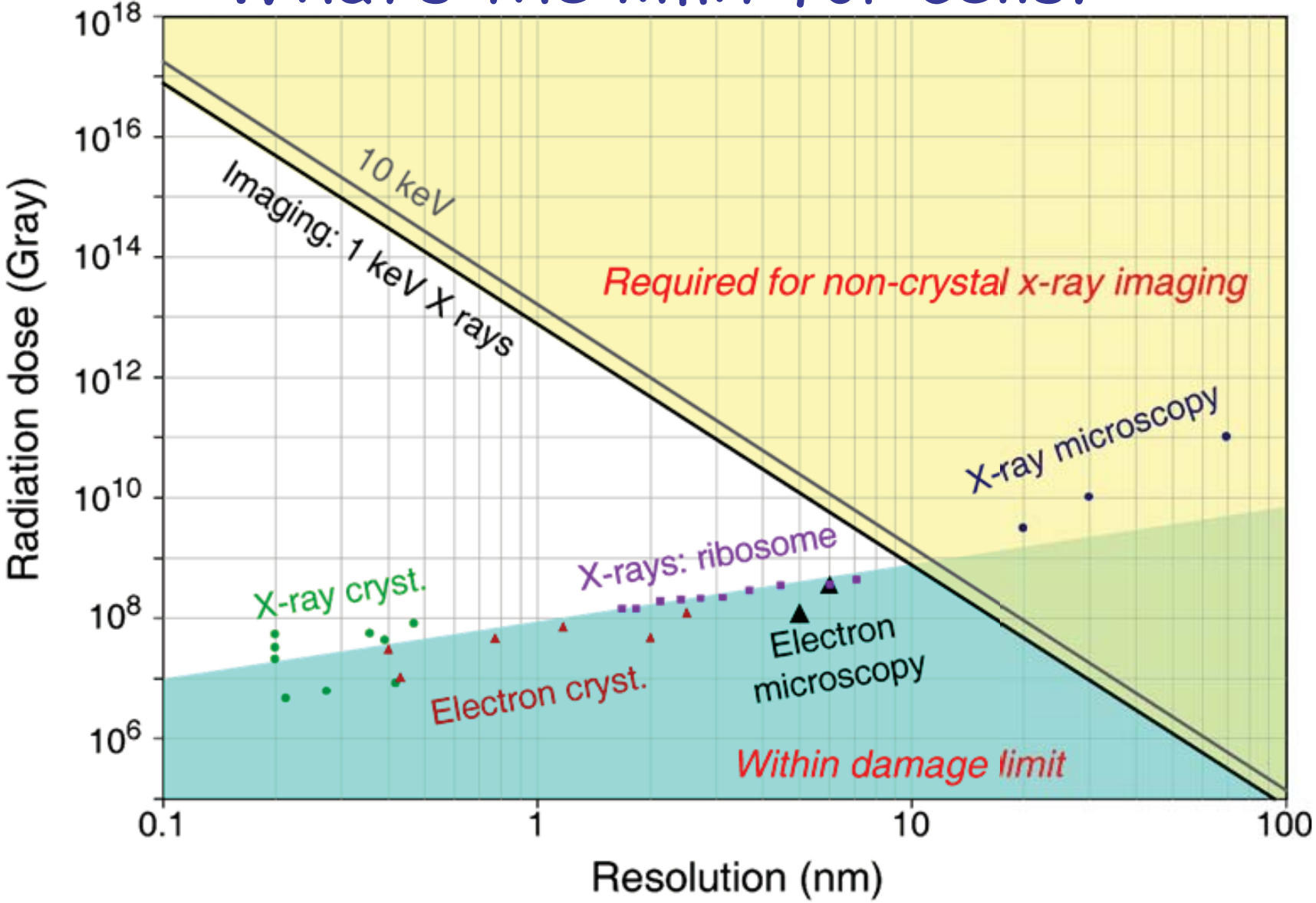




Challenges

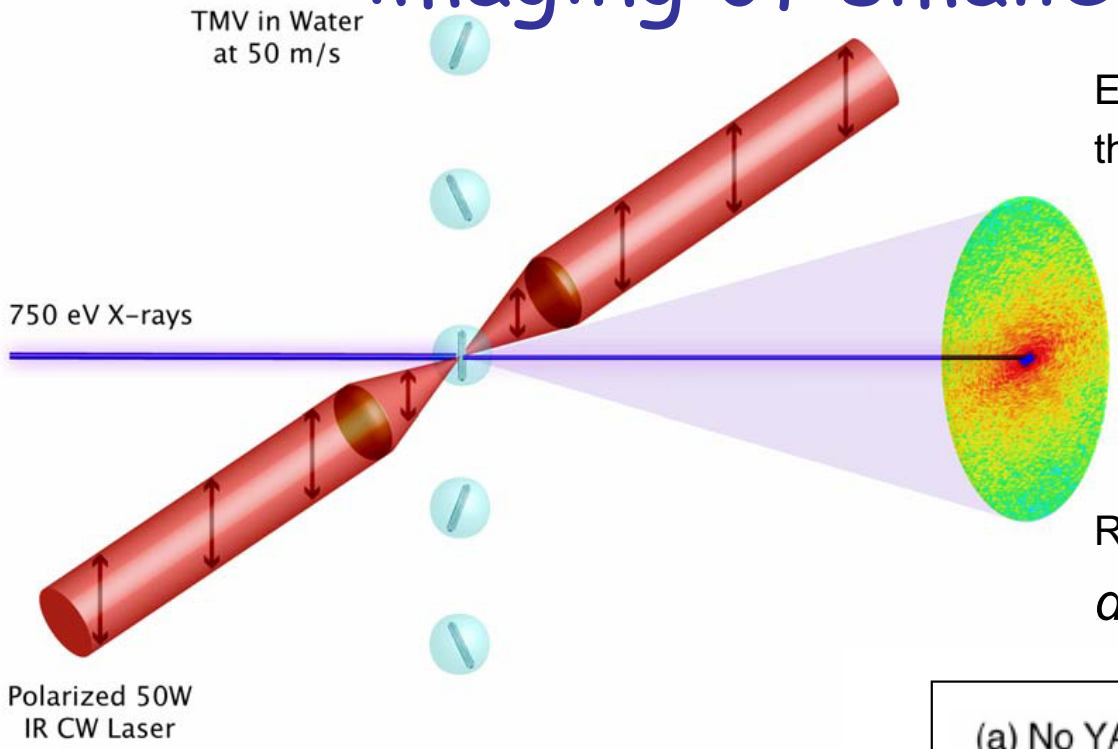
- Biological samples are weak scatterers
- Reducing background
- Finding the support
- Missing data
- Born Approximation violated
- Radiation damage, contamination, …

What's the limit for cells?



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

Laser alignment of molecules will allow the imaging of smaller molecules



Equipartition of rotational potential energy with thermal energy gives

$$\left\langle \Delta\theta^2 \right\rangle = \frac{T}{3\times10^{-8} I \Delta\alpha}$$

T - temperature in K

I - laser power in W/cm^2

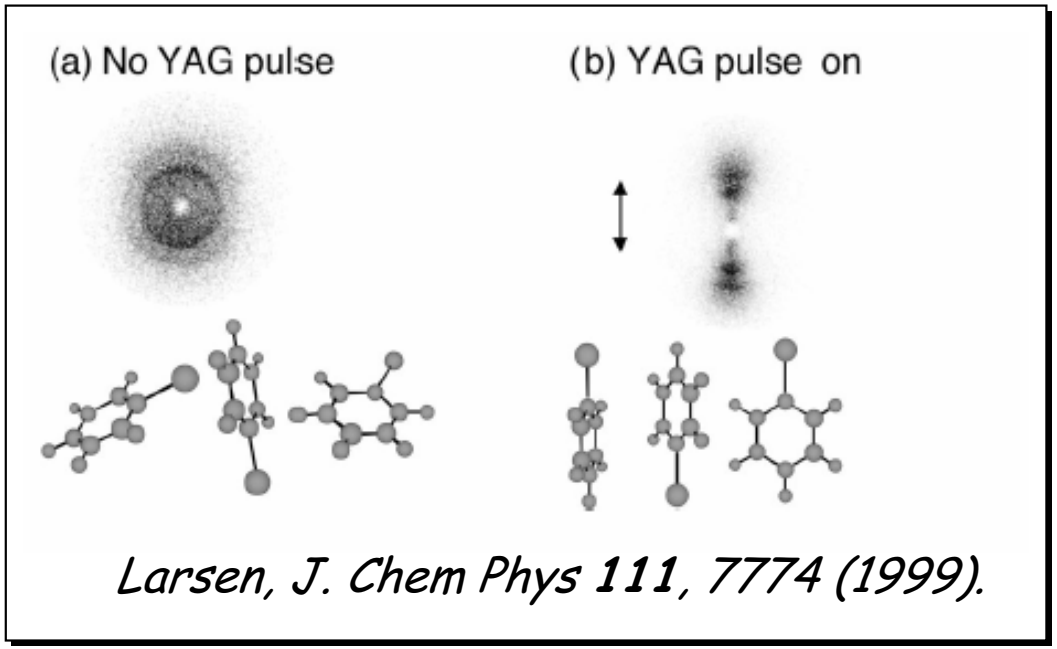
$\Delta\alpha$ - polarizability anisotropy in nm^3

Resolution is limited by the degree of alignment:

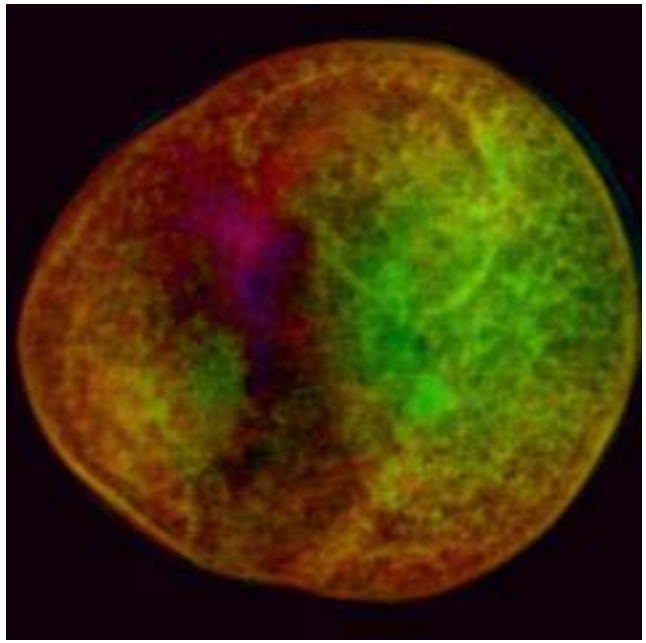
$d = (L/2)\,\Delta\theta$

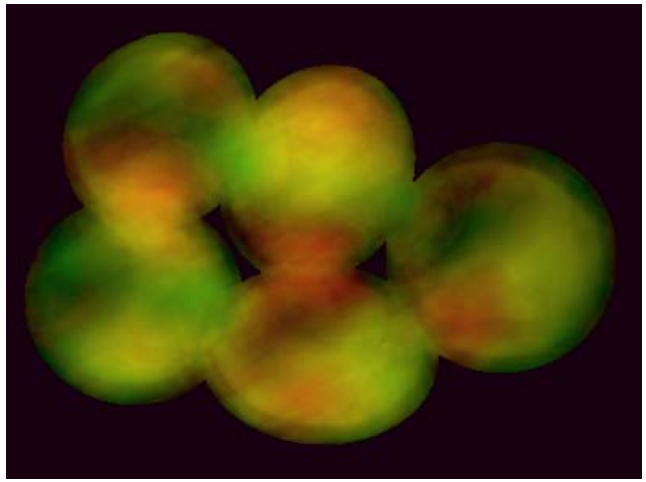
J.C.H. Spence and R.B. Doak, Phys. Rev. Lett. ***92****, 198102 (2004)*

J.C.H. Spence et al., Acta Cryst. A ***61****, 237 (2005)*



Larsen, J. Chem Phys ***111****, 7774 (1999).*





Conclusion

Diffraction microscopy gives

- the complex valued image of a unstained freeze-dried yeast cell
- Frozen-hydrated yeast spores reconstruction in process

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