Lensless imaging: recent achievements

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Outline

- Overview of X-ray diffraction microscopy
- Experimental setup
- Phasing algorithm: find phases of diffraction data
- Reconstruction of a freeze-dried yeast cell: validation, resolution…
- Reconstruction of frozen-hydrated yeast spores
- Conclusion

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

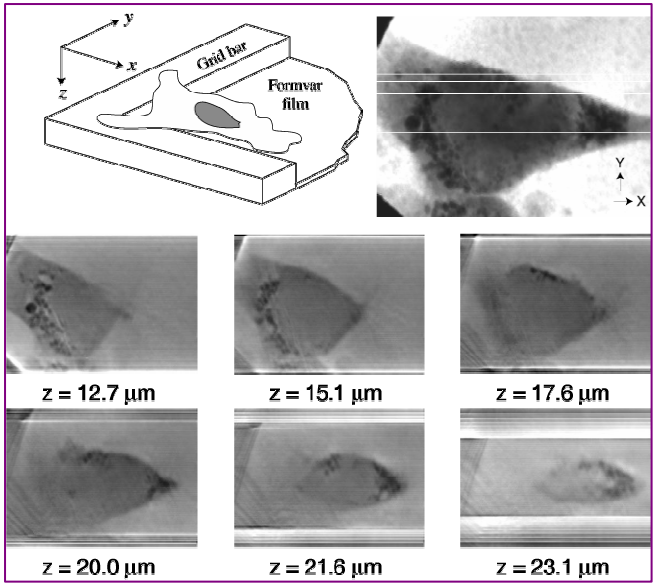
- 10 nm resolution (3D) in 1 - 10μm size biological specimens
  (small frozen hydrated cell, organelle; see macromolecular aggregates)
  Limitation: radiation damage!

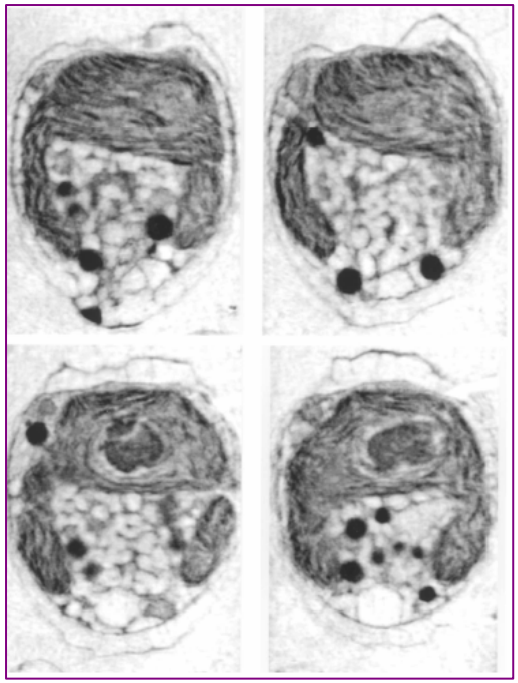
- <4 nm resolution in less sensitive nanostructures
  (Inclusions, porosity, clusters, composite nanostructures, aerosols…)
  eg: molecular sieves, catalysts, crack propagation

Soft x-ray tomography:

high throughput, high resolution by zone plate

- Frozen hydrated 3T3 cell by Wang *et al.*, *J. Micros.* **197**, 80 (2000)
- Frozen hydrated green alga by Weiss *et al.*,*Ultramicroscopy* **84** 185 (2000)



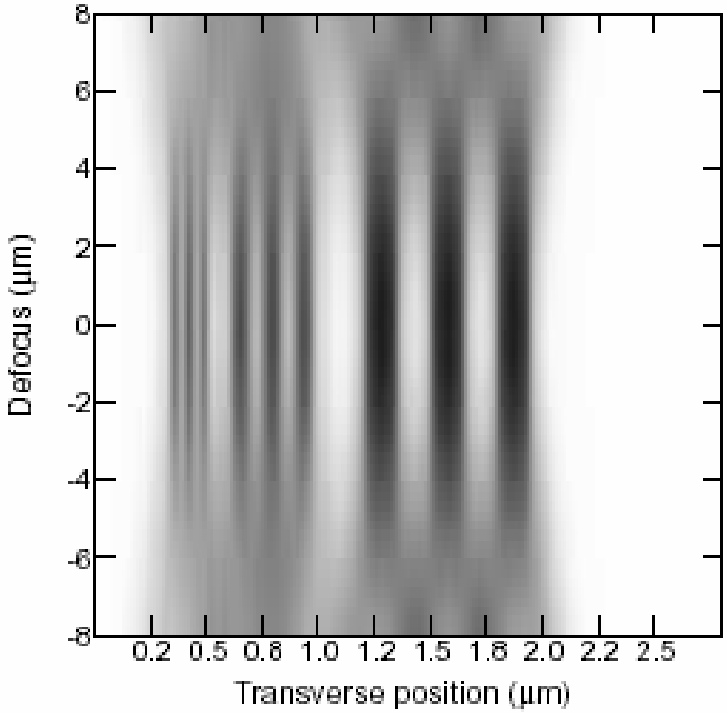


- Frozen hydrated yeast cells by Larabell and Le Gros, *Molec. Biol. Cell* **115**, 957 (2004)

Soft X-ray tomography limitations

- Assumption of straight line projections: all in focus
- Figure simulates imaging of 31nm, 71nm, and 143nm wide lines with 45 nm zp @ λ = 2.3 nm
- Depth of focus scales as $1/NA^2$
- For 22 nm zp,

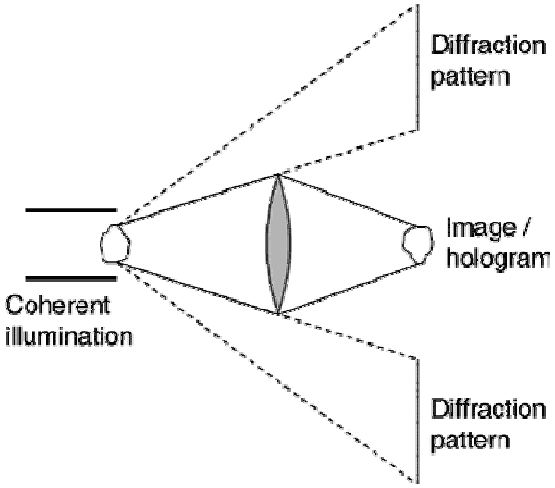
  thickness <2μm

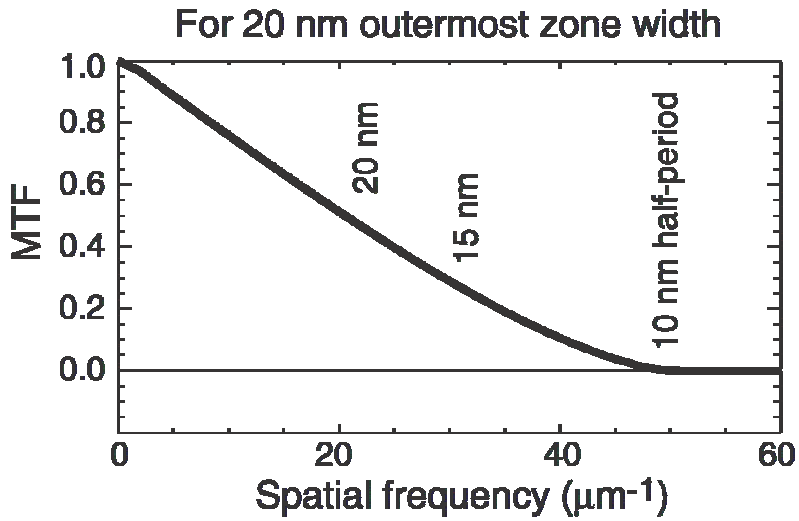


- For many specimens, radiation damage sets the ultimate limit on achievable resolution
- Lenses phase the signal, but lose the signal.

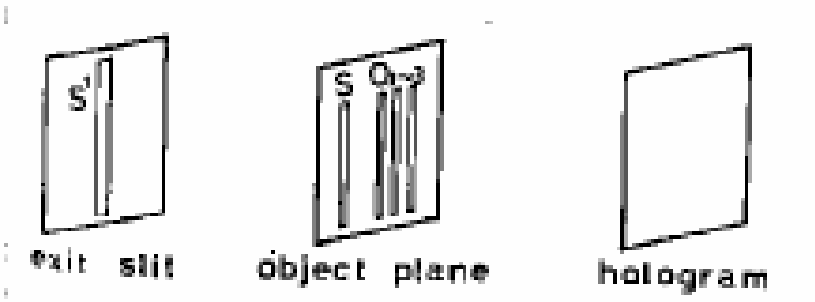
  Example: 20 nm zone plate with 10% efficiency, 50% window transmission, 20% MTF for 15 nm half-period:

  → 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?





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



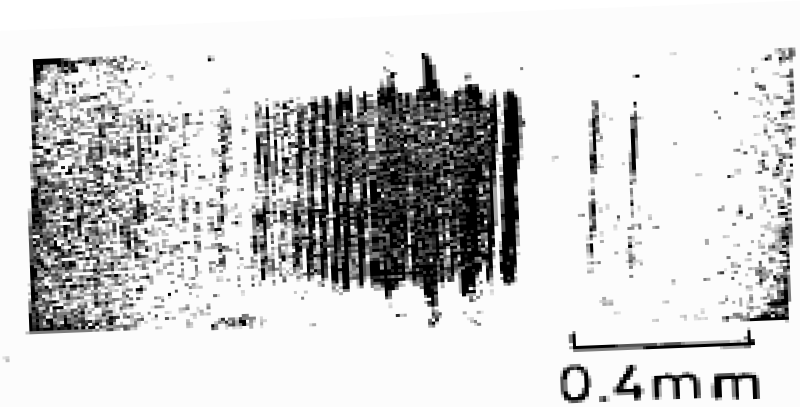


Fig. 2. An X-ray hologram.

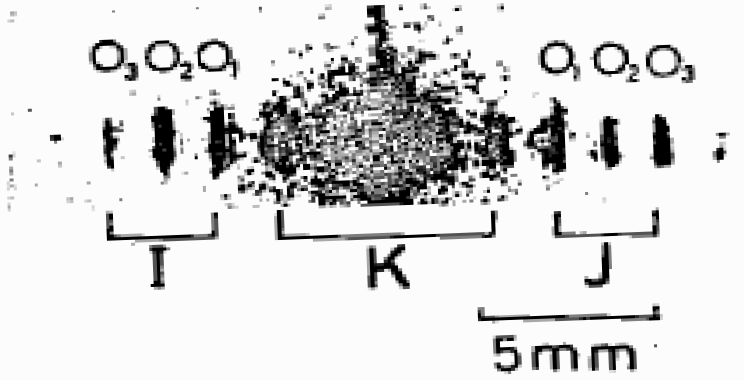
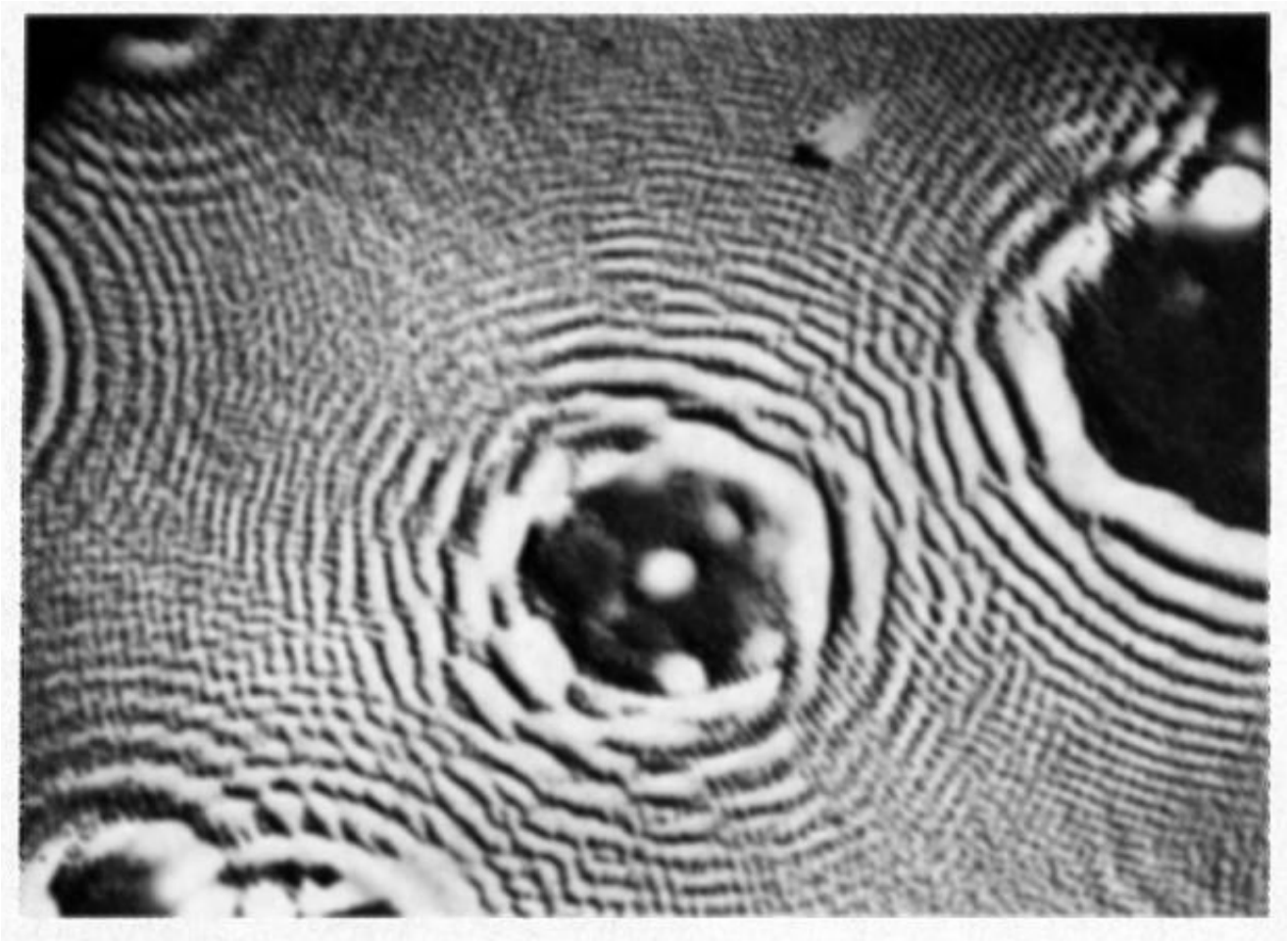


Fig. 3. Reconstructed images I and J, and the noise image K.

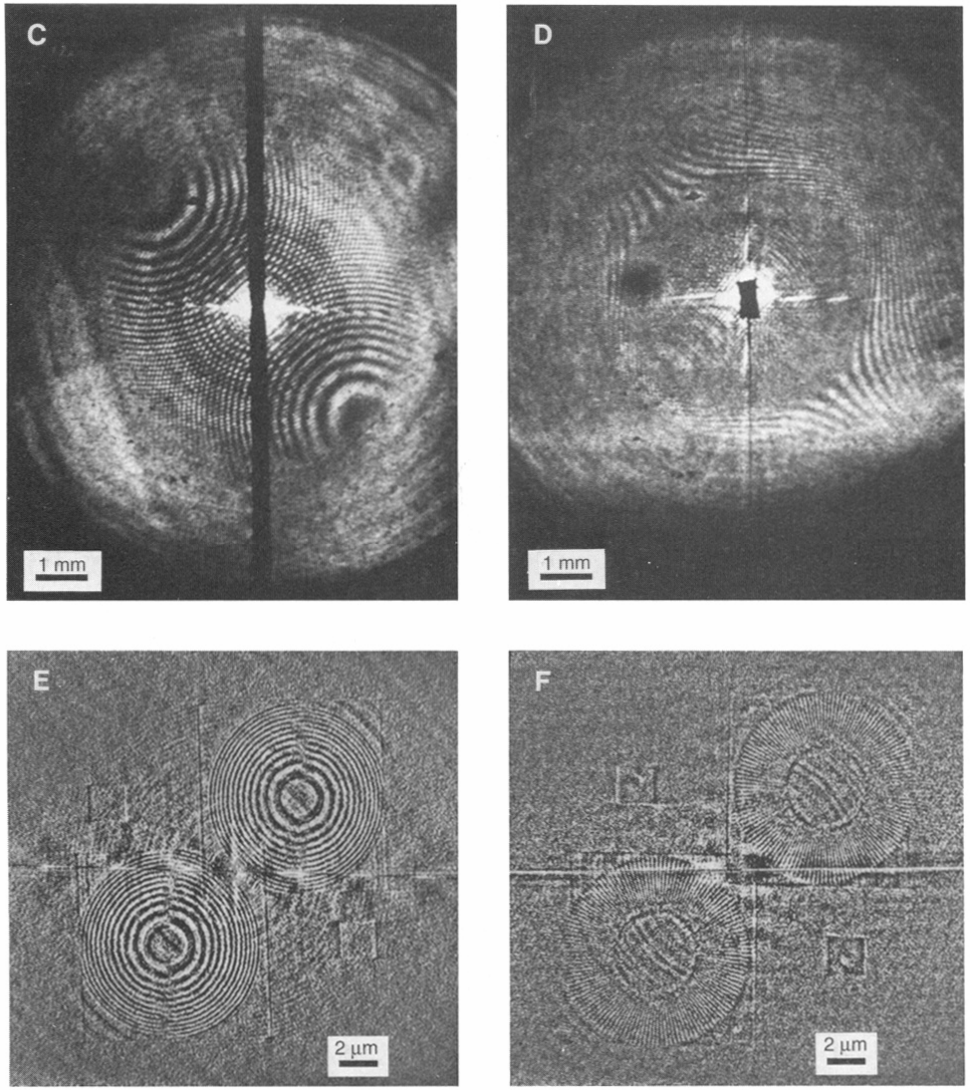
Aoki et al. Jap. J. Appl. Phy 11, 1857 (1972)

Gabor holography at the NSLS



Howells et al., Science 1987

Fourier transform holography at the NSLS



McNulty et al., Science 1992

Way around #2: diffraction imaging

D. Sayre, J. Miao, M. Howells, J. Spence, J. Kirz,….

Record diffraction patterns, retrieve phase

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

x-ray diffraction microscopy

advantage

- No lens limitation
- Phase and amplitude contrast

challenge

- Correct, unique solution from the phasing algorithm?

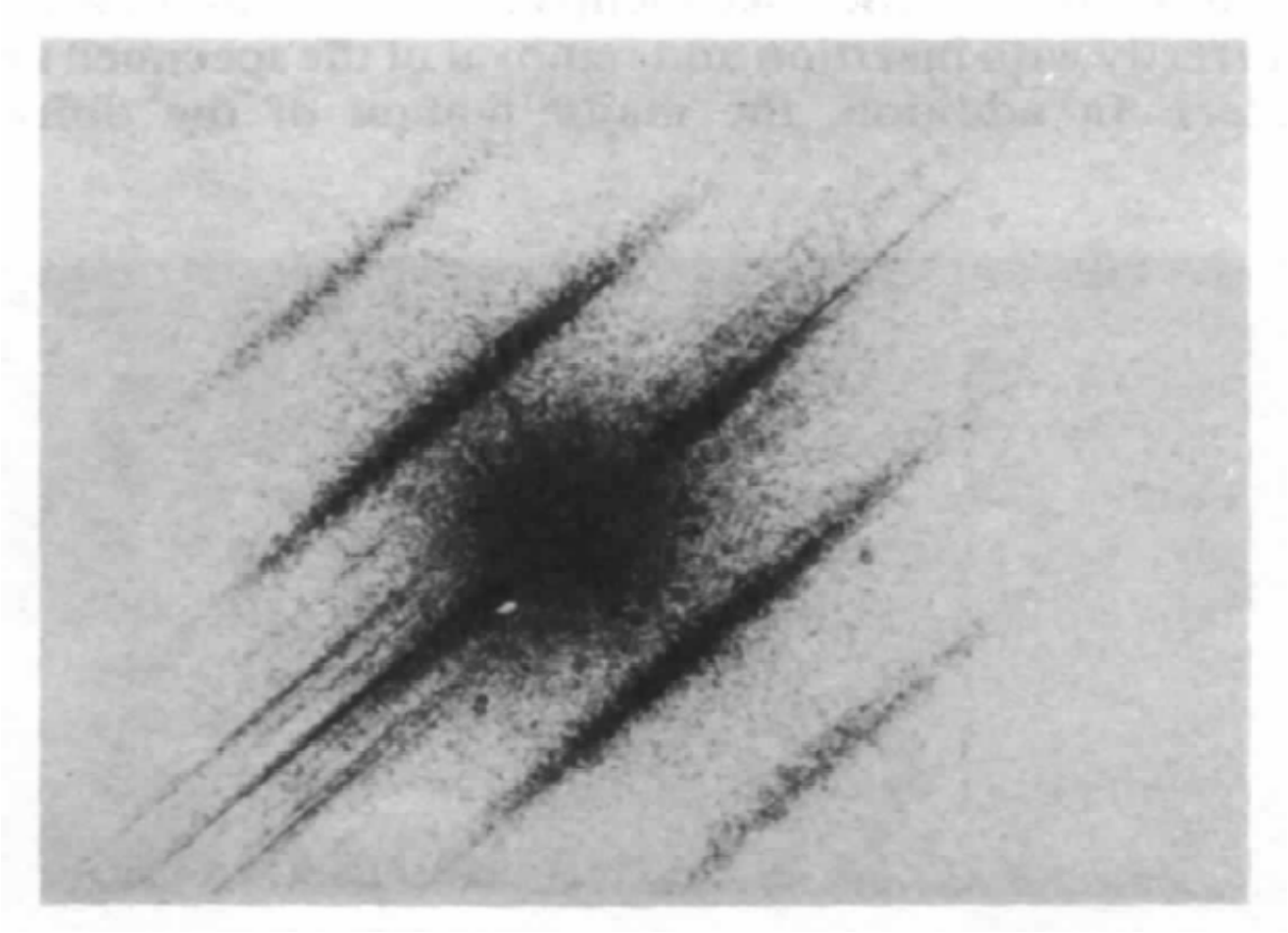
Image reconstruction from the diffraction pattern

- 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

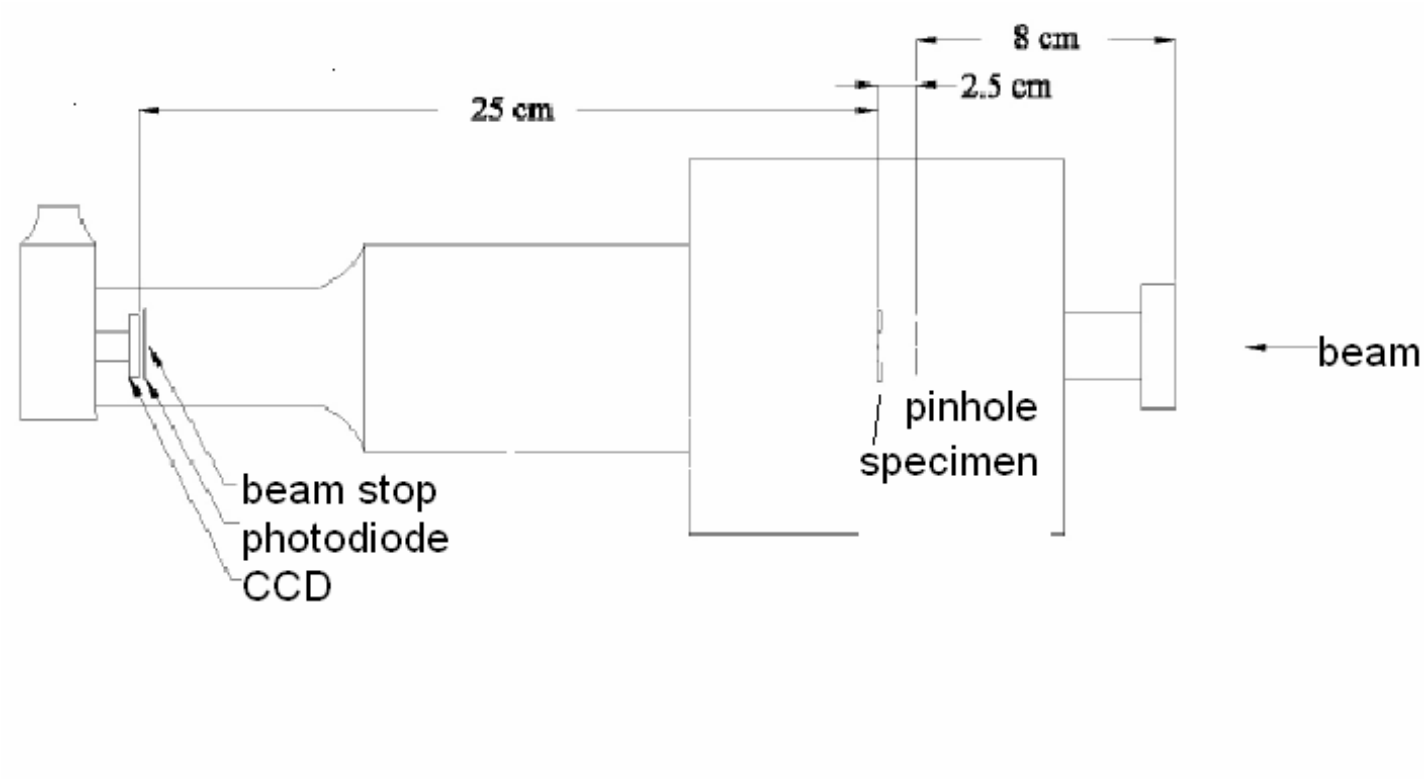
Diffraction pattern of a single diatom, 1987



Yun, Kirz & Sayre, Acta A.

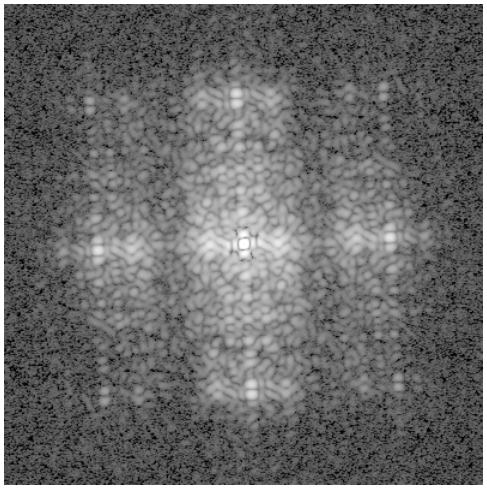
Modern era

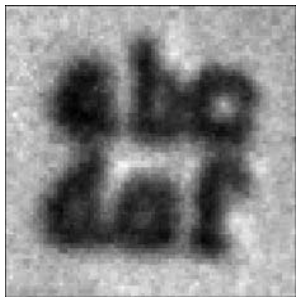
- 1998: Sayre, Chapman, Miao: oversampling & Fienup algorithm for X-rays
- 1999: first experimental demonstration in 2D



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

λ=1.8 nm soft x-ray diffraction pattern





Low angle data From optical micrograph

Scanning electron micrograph of object

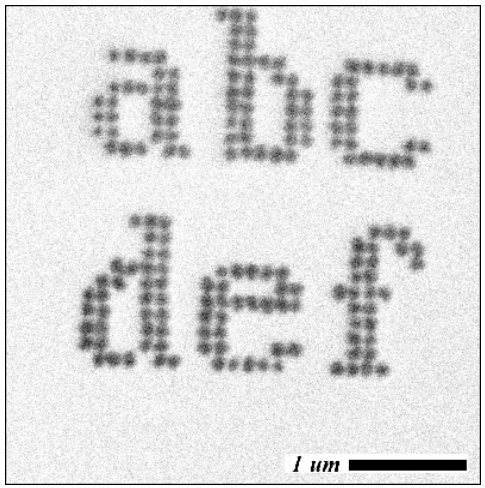


Image reconstructed from diffraction pattern (θ_{max} corresponds to 80 nm). Assumed positivity

Basic principles

- Single object, plane wave incident,

  scattered amplitude is Fourier transform of (complex) electron density f(**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:

  for object size a, resolution d,

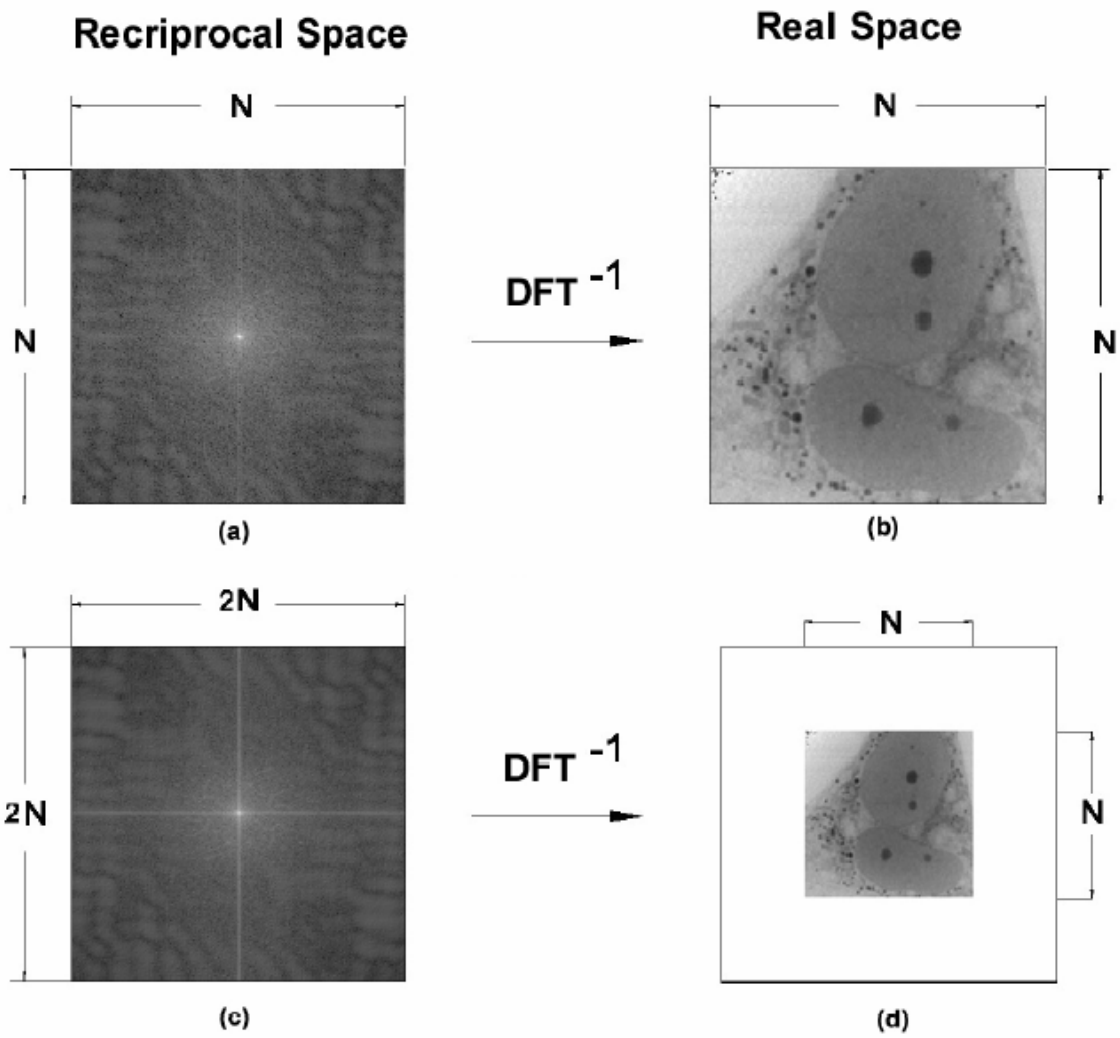
  - spatial coherence $\delta\theta < \lambda/4a$
  - temporal coherence $\delta\lambda/\lambda < d/4a$

"Oversampling":

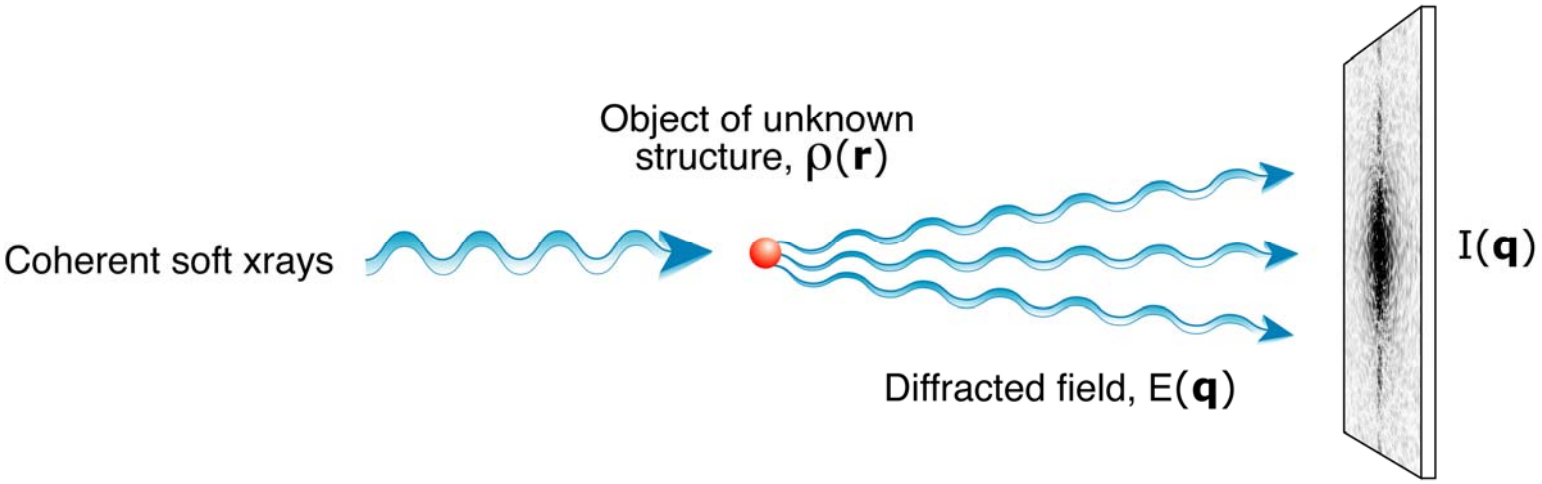
Non-crystals:
pattern continuous,
can do finer sampling
of intensity

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

(area around specimen
must be clear!)



X-ray Diffraction microscopy(XDM)



$E(\mathbf{q}) = \mathrm{FFT}\{\rho(\mathbf{r})\} \leftrightarrow \rho(\mathbf{r}) = \mathrm{FFT}^{-1}\{E(\mathbf{q})\}$

Where $E(\mathbf{q}) = |E(q)| * \exp(-i\varphi)$

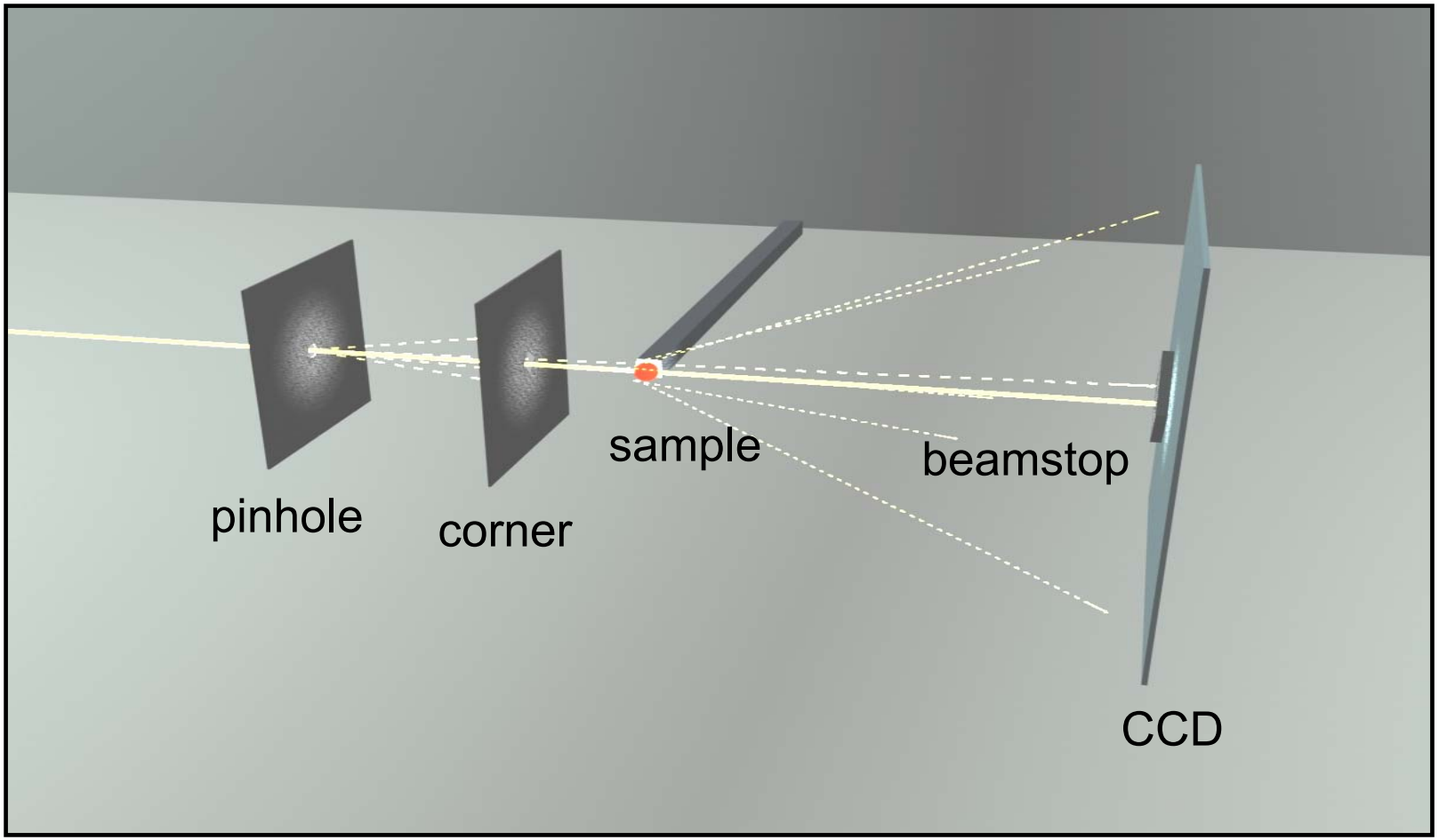
Lost phases are recovered by algorithmic procedure

D. Sayre, *Acta Cryst.* **5**, 843 (1952),
Imaging processes and coherence in physics, pp 229 (1980)

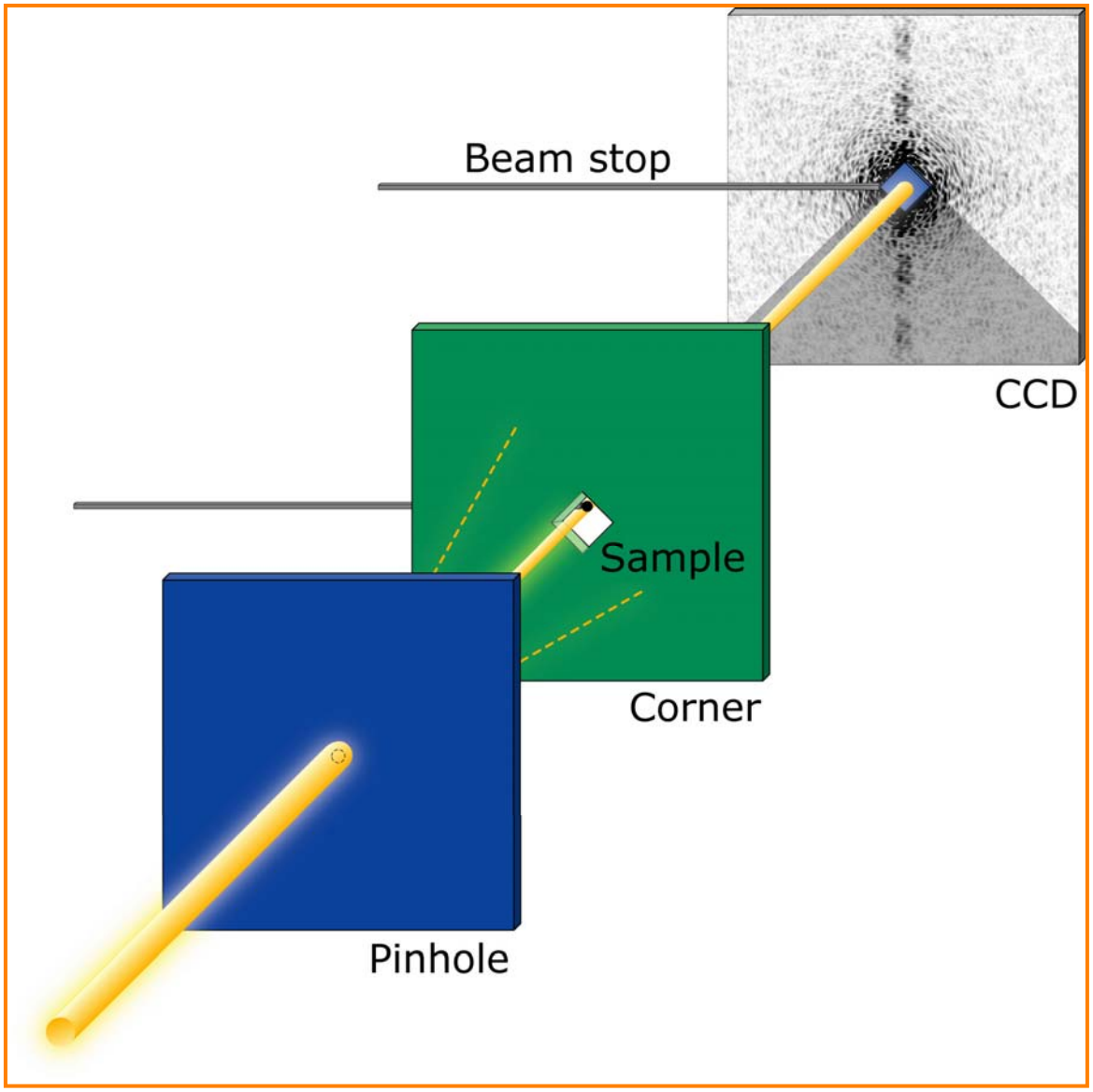
Gerchberg & Saxton, Optik **35**, 237 (1972)

Fienup, Appl. Opt. **21**, 2759 (1978)

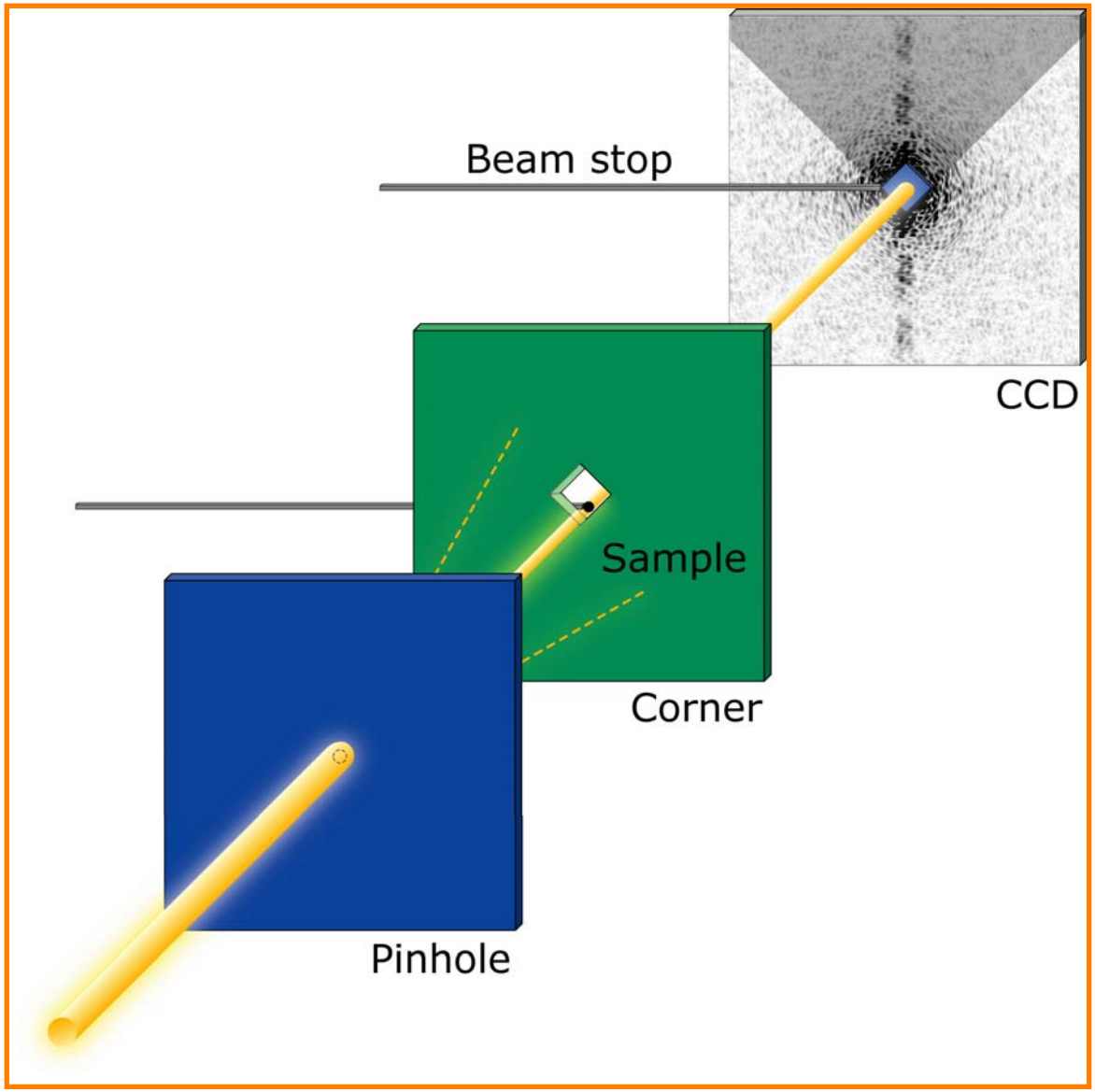
Inside vacuum chamber



Bottom half image



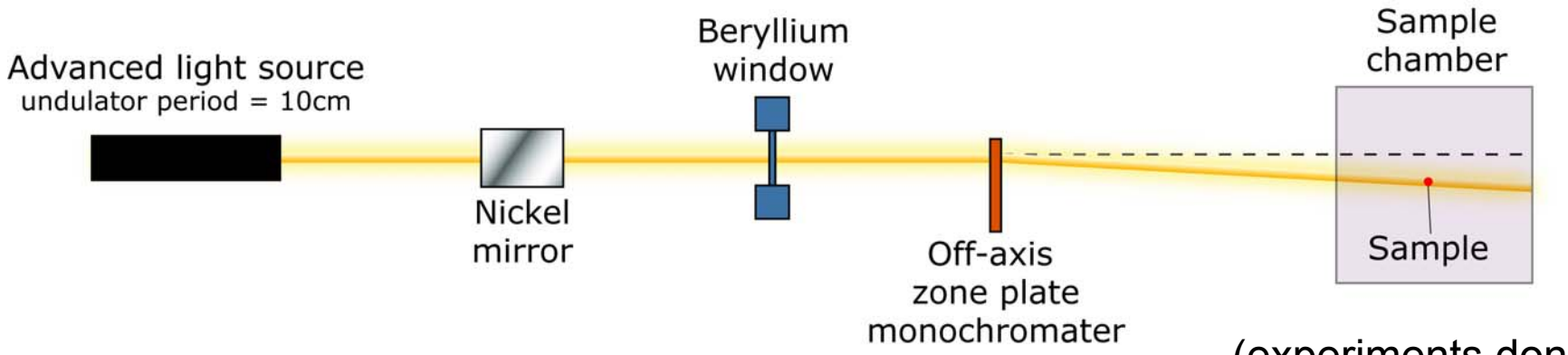
Top half image



jc/AL Saerial/11-96

Experimental setup

Advanced Light Source beamline 9.0.1



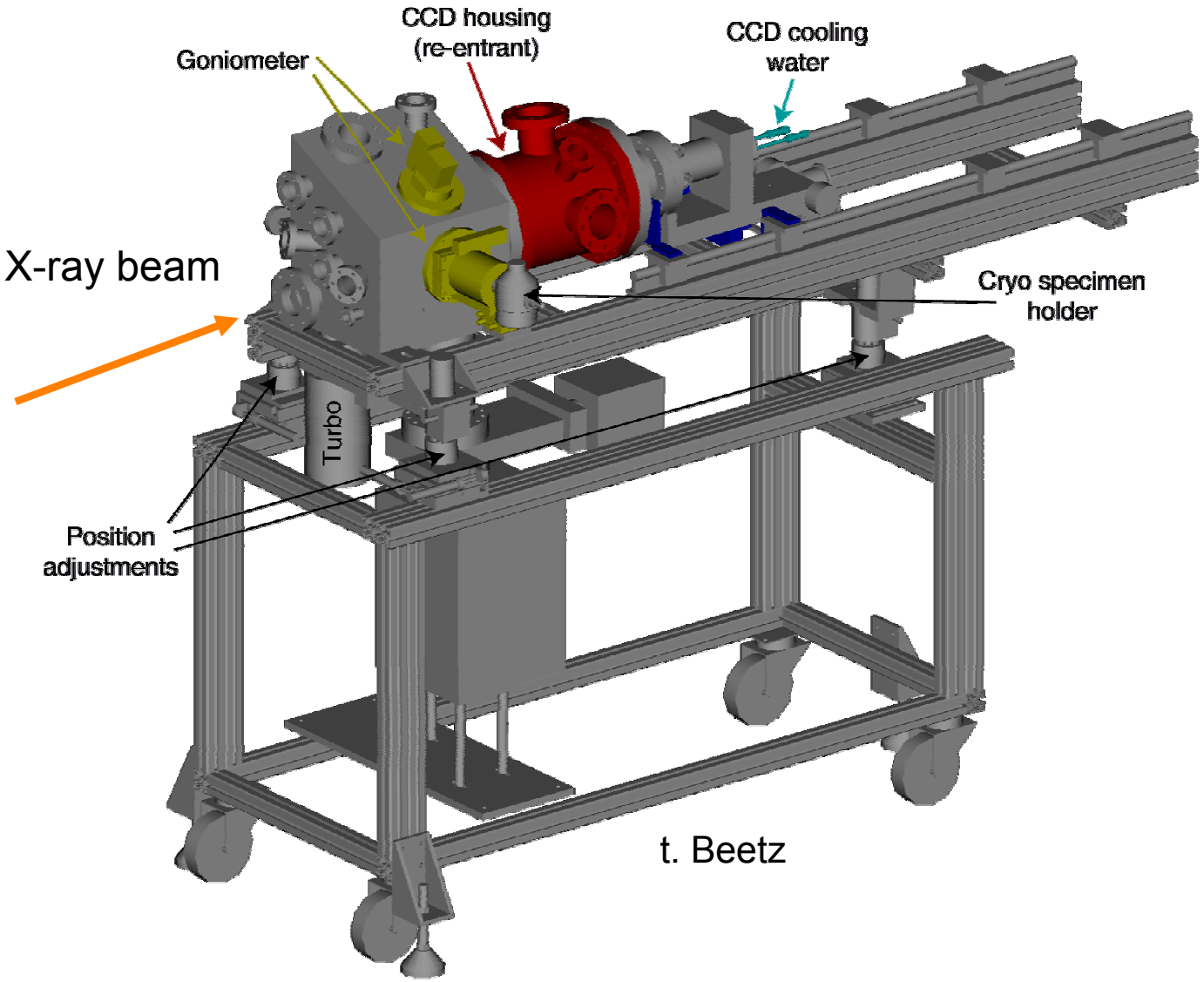
(experiments done at 520, 750 eV)

Yeast samples : 3 - 7 micron

Lateral coherence length : 15 micron

Oversampling ratio : 3 to 5

Diffraction Microscope by Stony brook and nsls



Gatan 630 cryo holder

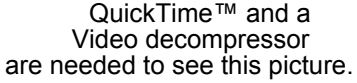
Algorithmic phase retrieval:

Impose known constraints
(information about the sample)

1. Fourier magnitude of sample -- measured
2. Shape of sample (support), shrink-wrap method
3. Positivity on electron density

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

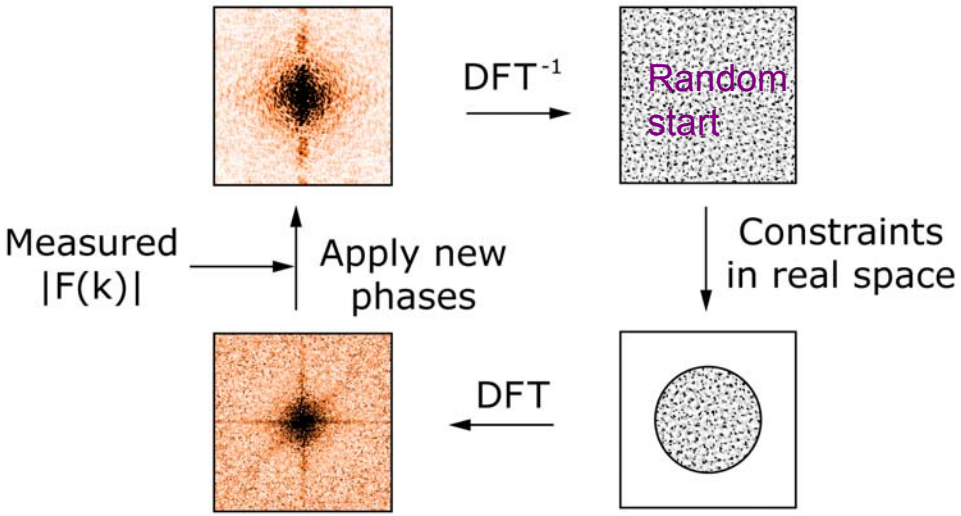
Marchesini *et al.*, *Phys. Rev. B* **68,** 140101 (2003)



algorithmic steps

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

hybrid input-output

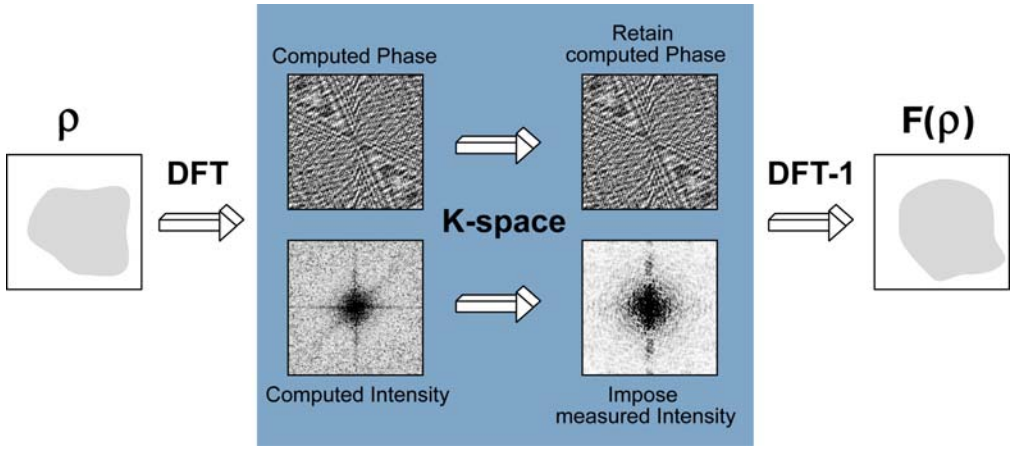


(Fienup, *Appl. Opt.* **21**, 2759 (1982))

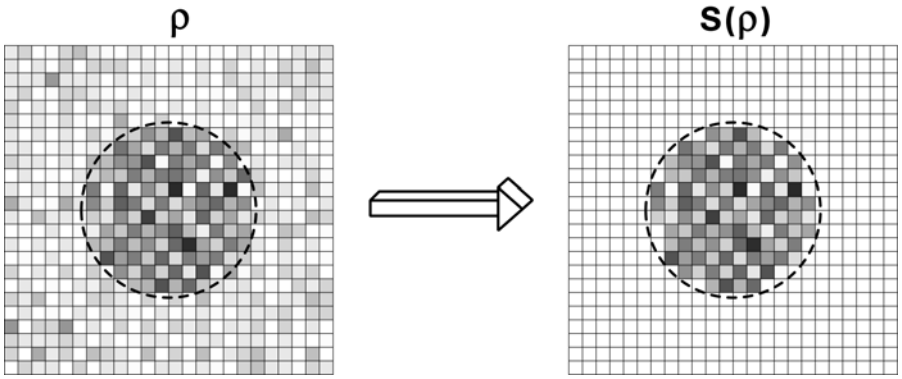
difference map: Elser, *J. Opt. Soc. Am. A* **4**, 118 (2002)

by adding the difference of two projections

1. Fourier magnitude constraint



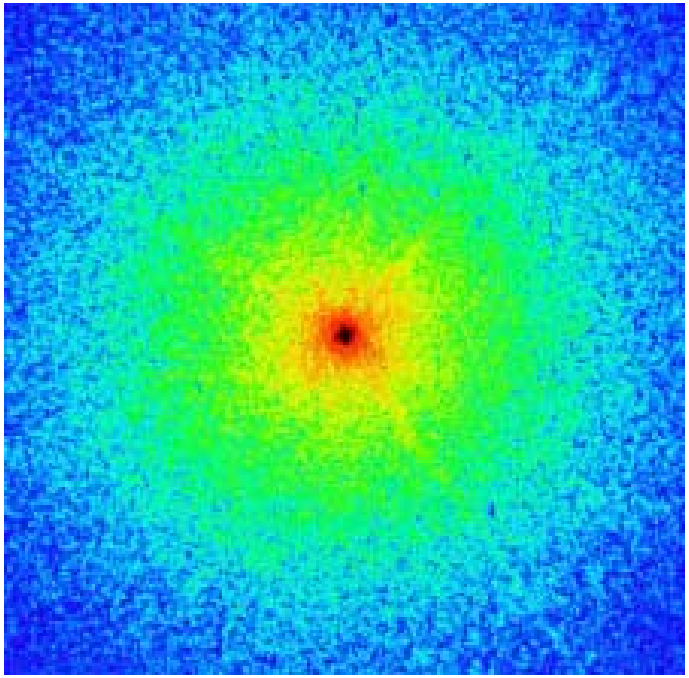
2. Support constraint

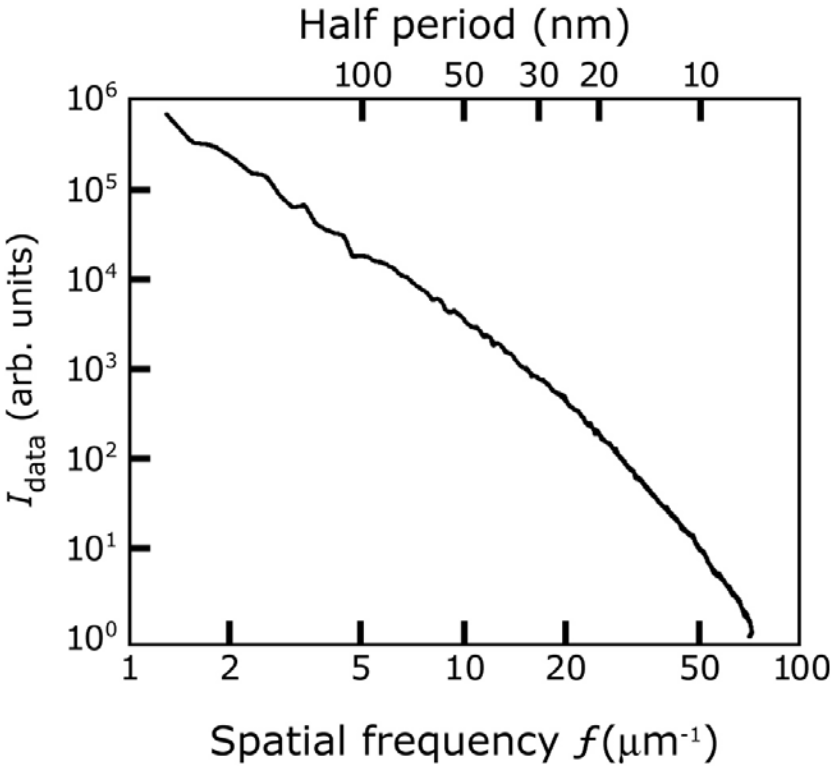


Zero-padding outside support by sampling the specimen finer than the Nyquist frequency (specimen plus support sampled *at* Nyquist)

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

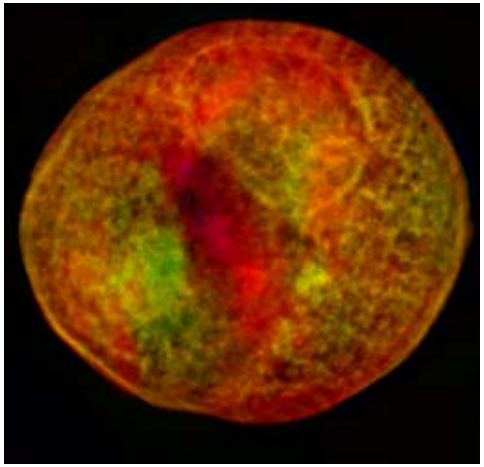




David Shapiro, stony brook, now at UC Davis

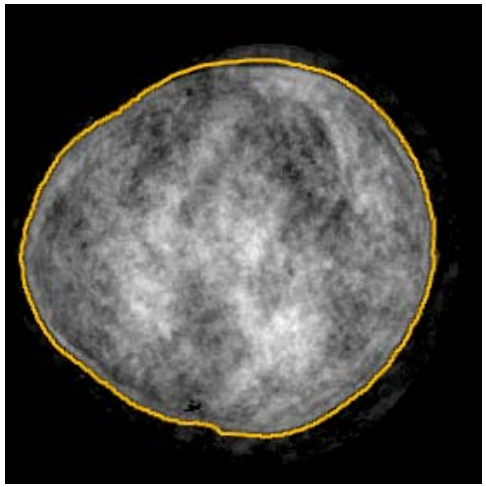
Summary of reconstruction details

- Final reconstruction was obtained by averaging iterates



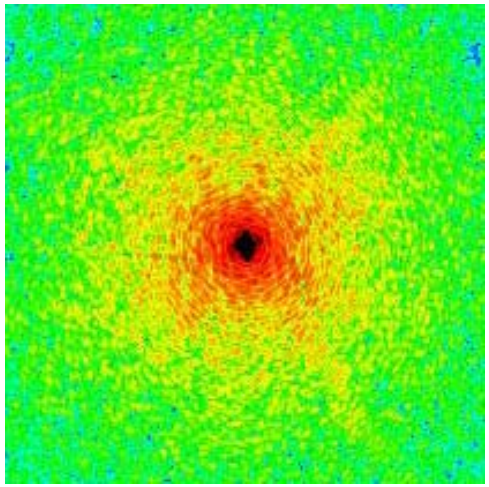
Summary of reconstruction details

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

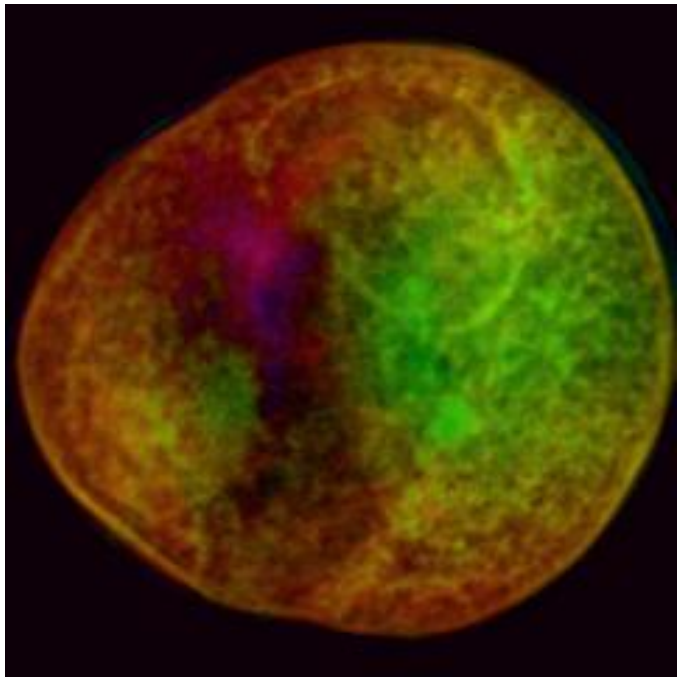


Summary of reconstruction details

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



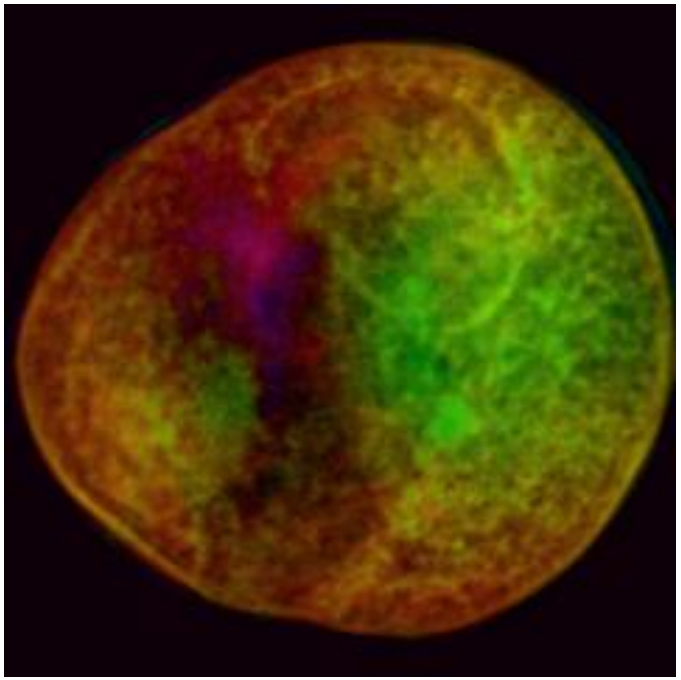
Reconstruction of complex image of FD yeast cell



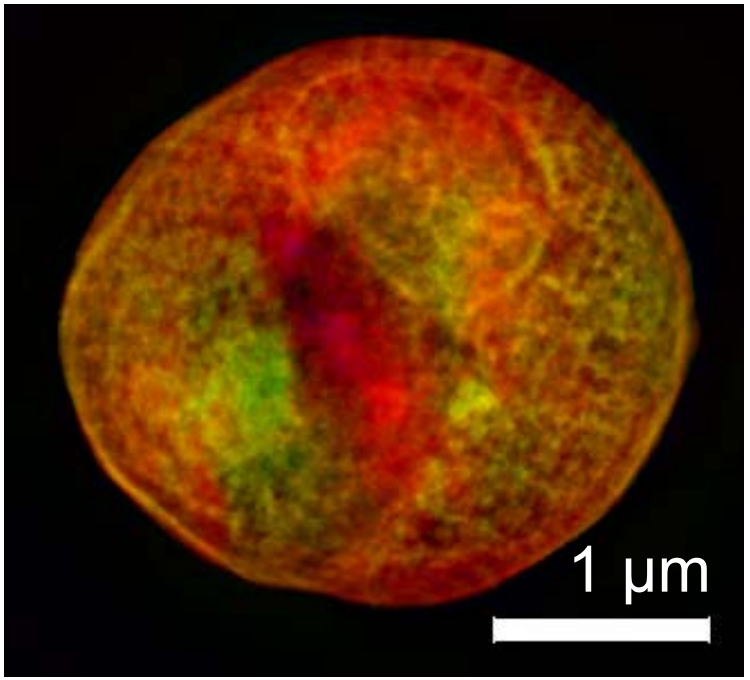
- algorithm: Difference map, beta=1
- 10,000 iterations
- Brightness - amplitude, hue - phase
- averaged over 100 iterates

Is the solution unique and faithful?

With averaging, reproducible reconstructions!



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

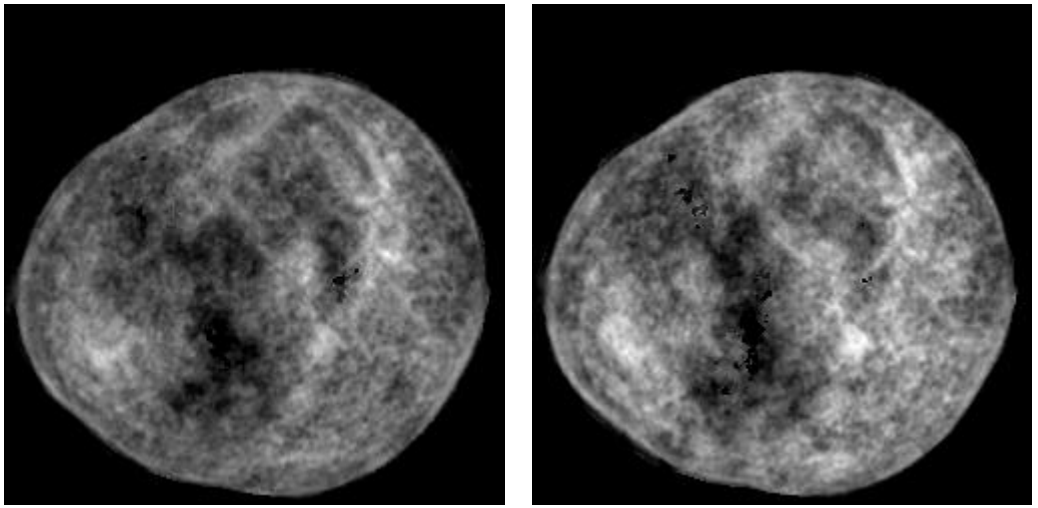


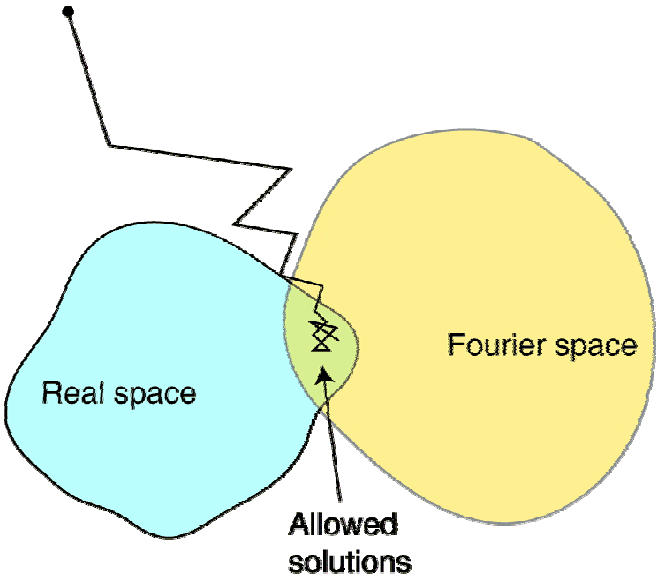
Cornell group: average of 980 iterates, 50 iterations apart

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

Iterative solutions “hop around”!

Two images (iterates) separated by 40 iterations



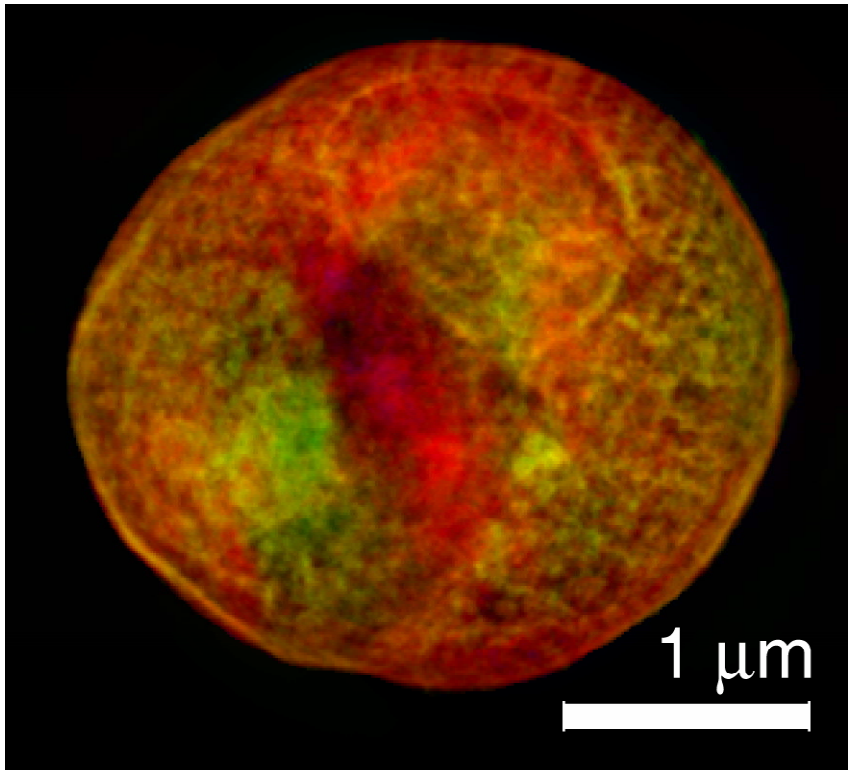


Noise in the data gives random fluctuations in the reconstructed image

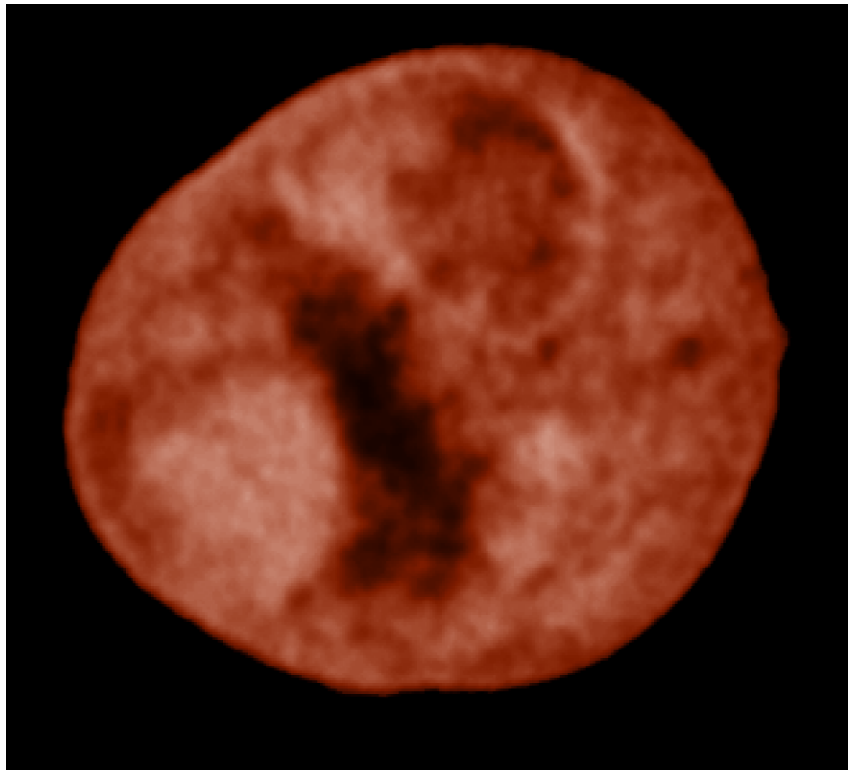
Averaging many iterates:

- reinforce reproducible information
- suppress non-reproducible information

Comparison with a microscope

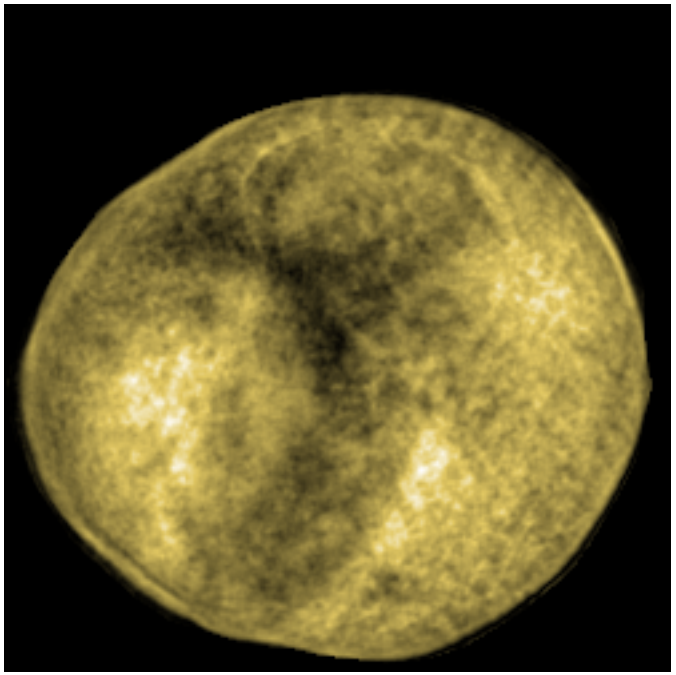


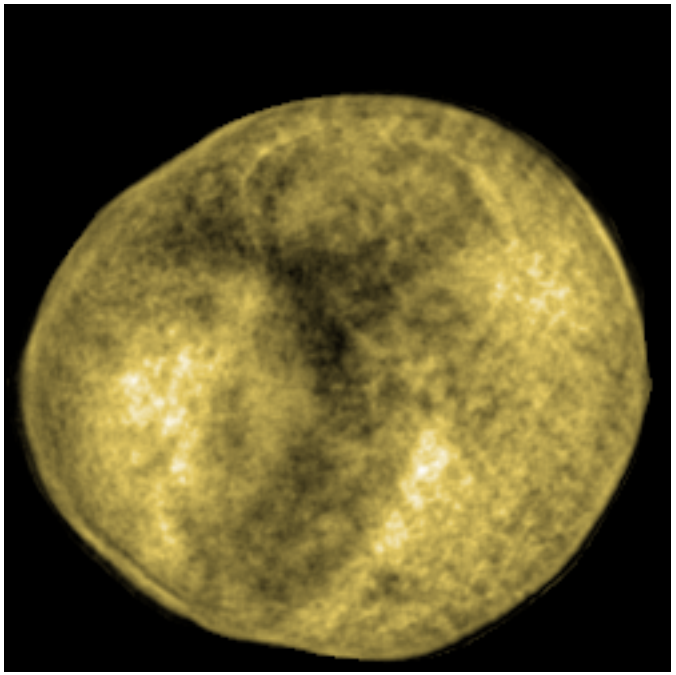
Diffraction reconstruction (data taken at 750 eV; absorption as brightness, phase as hue).



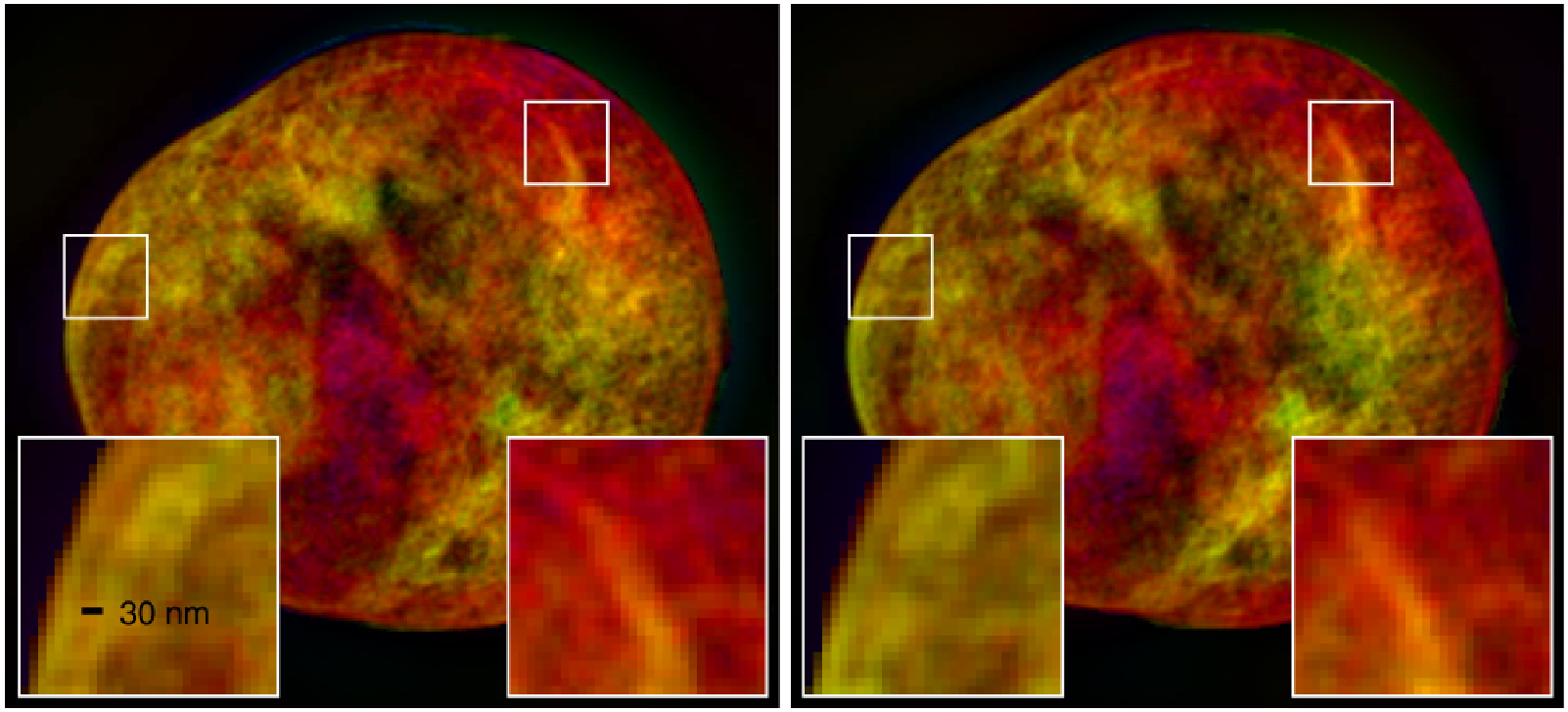
Stony Brook/NSLS STXM image with 45 nm Rayleigh resolution zone plate at 520 eV (absorption as brightness)

Two separate iterations with different random starts gives same reconstruction

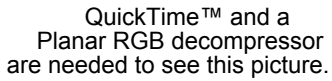




Reconstructions from data 1 degree apart show similar 30 nm structure



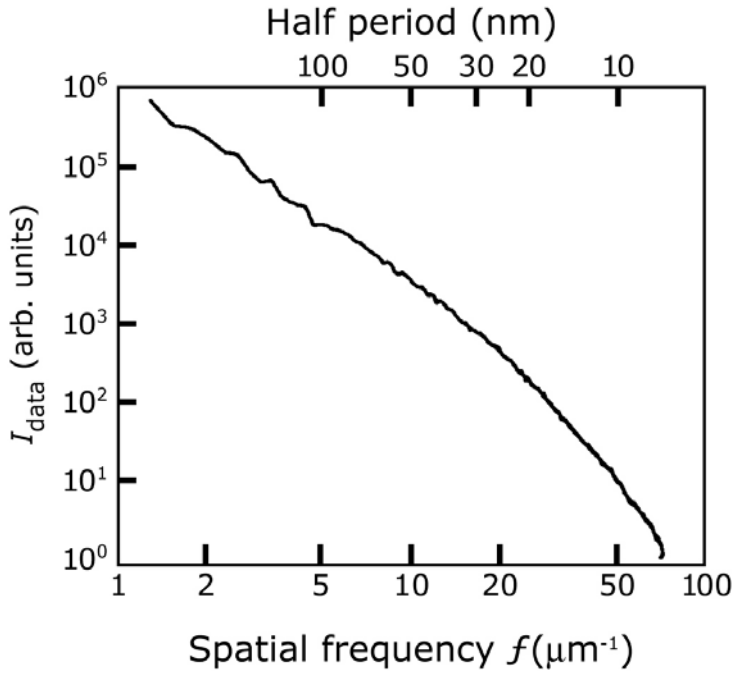
Movie: tilt from -3 to +5 degrees in 1 degree steps

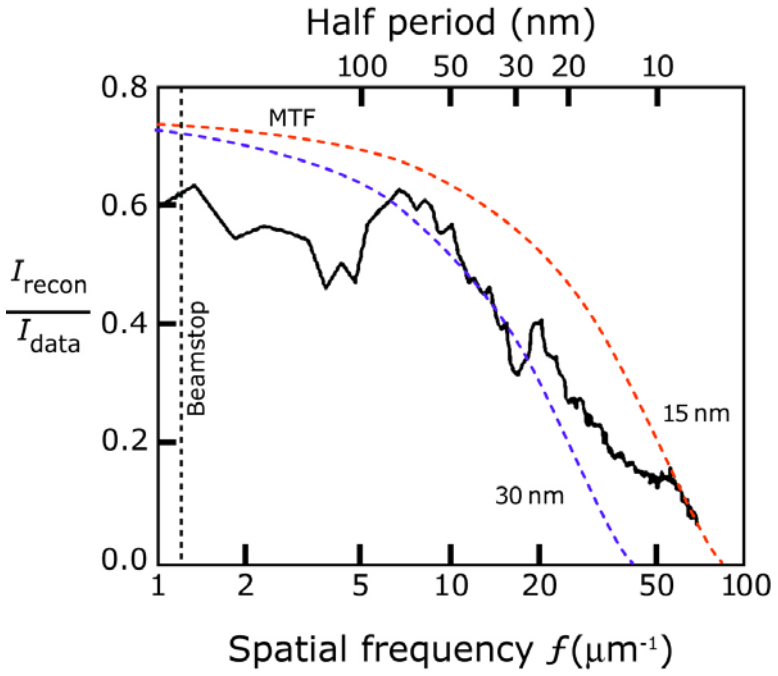


Pierre Thibault

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
  → analogous to the modulation transfer function





---> Reconstructed image at 30 nm resolution

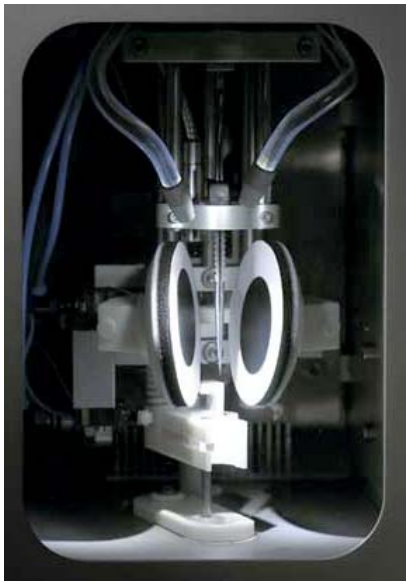
Reconstruction of frozen-hydrated yeast spores

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 ° C

XDM apart from Cryo-Em

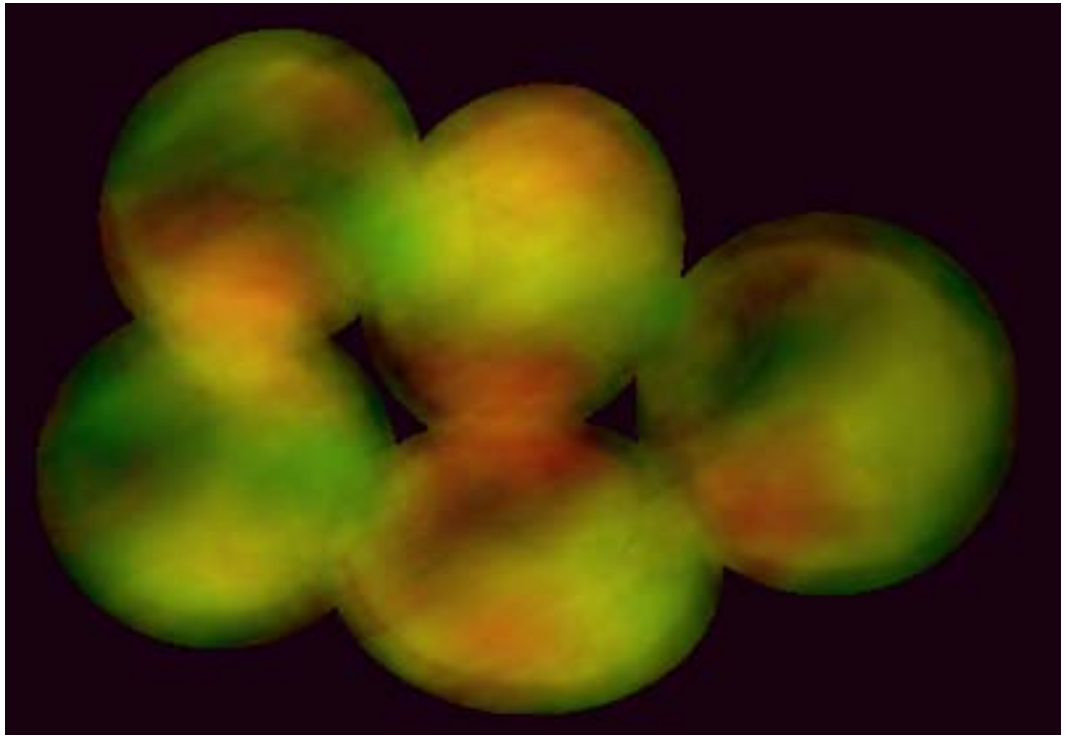
- larger sample, 3 - 7 micron
- ice thickness at 1 micron
- vitrification possible without high pressure freezing?



Commercial plunger
By FEI company

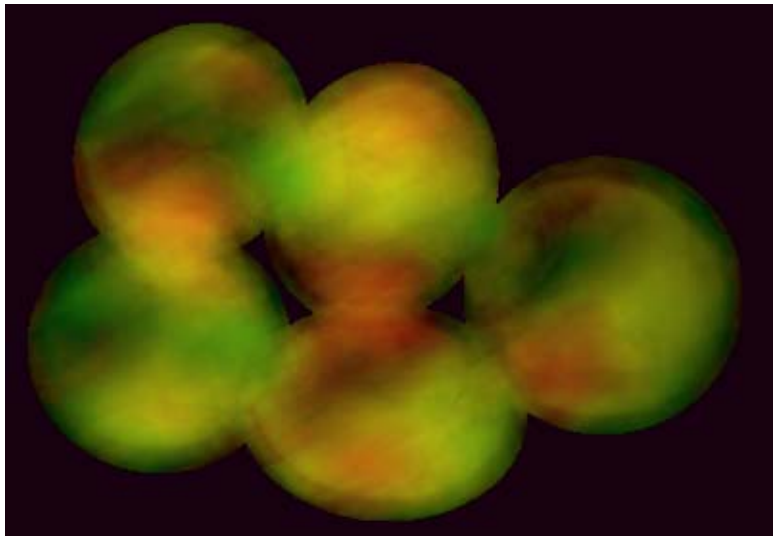
Initial reconstruction

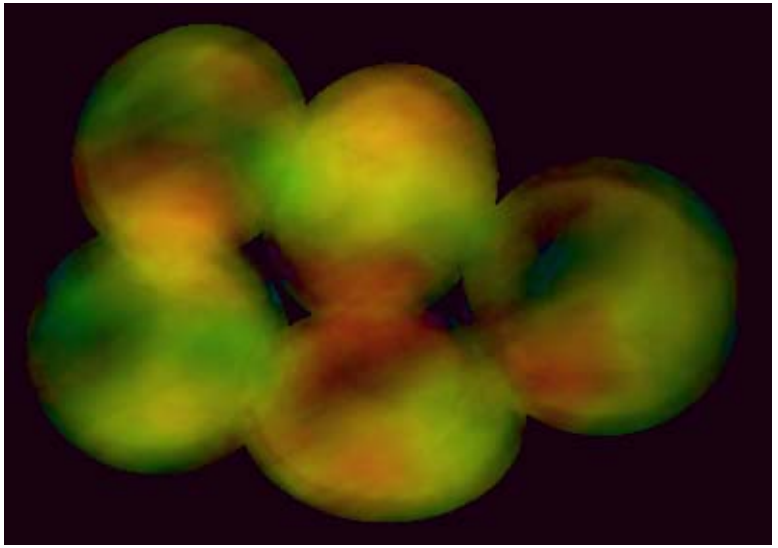
Shows a clump of 5 yeast spores, 8μ ∗ 5μ

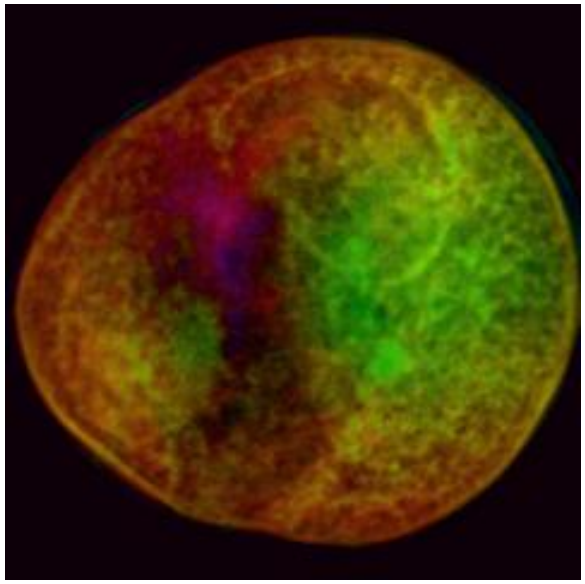


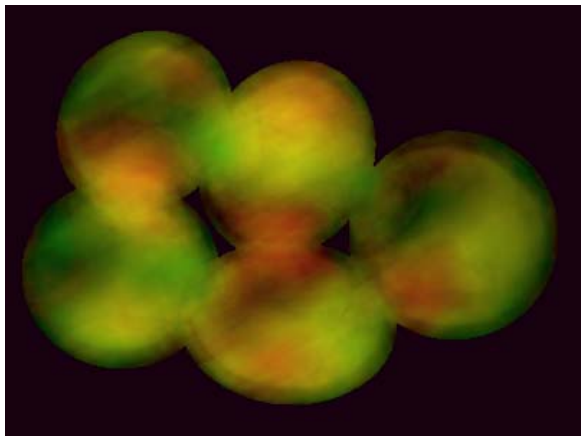
Is it reproducible?

Reconstruction from two different random starts









Conclusion

Diffraction microscopy gives

- the complex valued image of a unstained freeze-dried yeast cell

- Frozen-hydrated yeast spores reconstruction in process

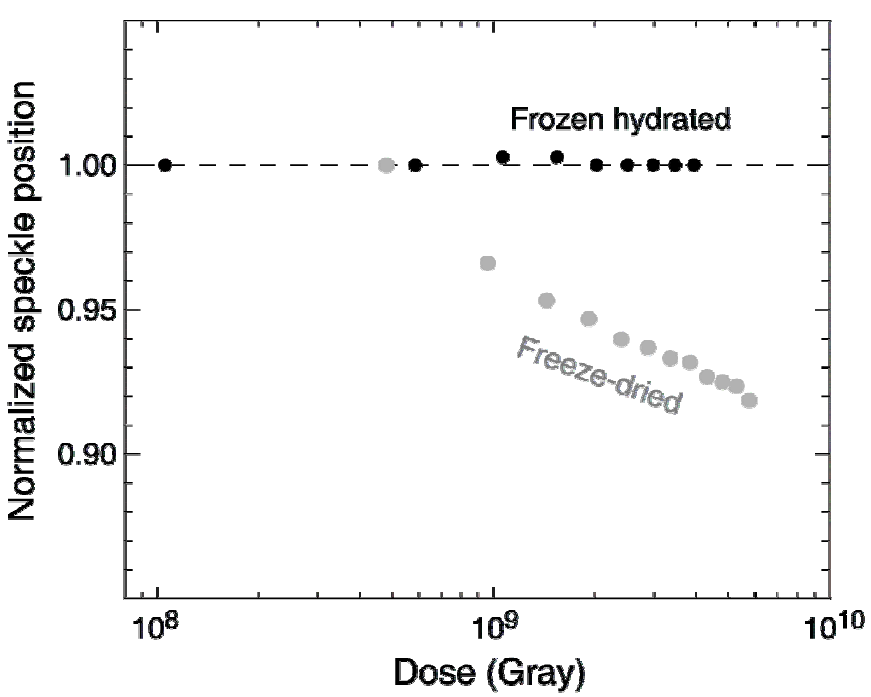
Work is sponsored by NIH and DOE
And many thanks to ALS staff, Bruce Futcher, Alison Coluccio, Agustin Avila-Sakar and Keith Lima

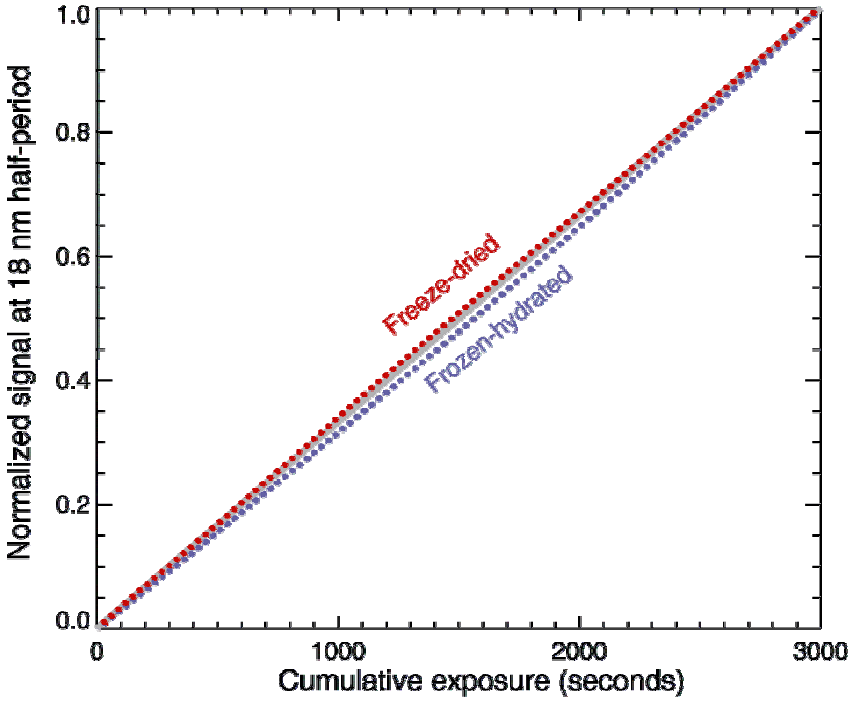
How can we believe the phasing?

- 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 two independent runs on the same data with different random starting phases.

Stability of frozen hydrated specimens

- D. Shapiro, PhD thesis





Challenges

1/ recording the pattern

- Beamline to supply sufficient coherent photons
- Shielding detector from all but diffracted signal
- Minimizing missing data
  - (beam stop, large rotation angles, etc.)
- Dynamic range of detector

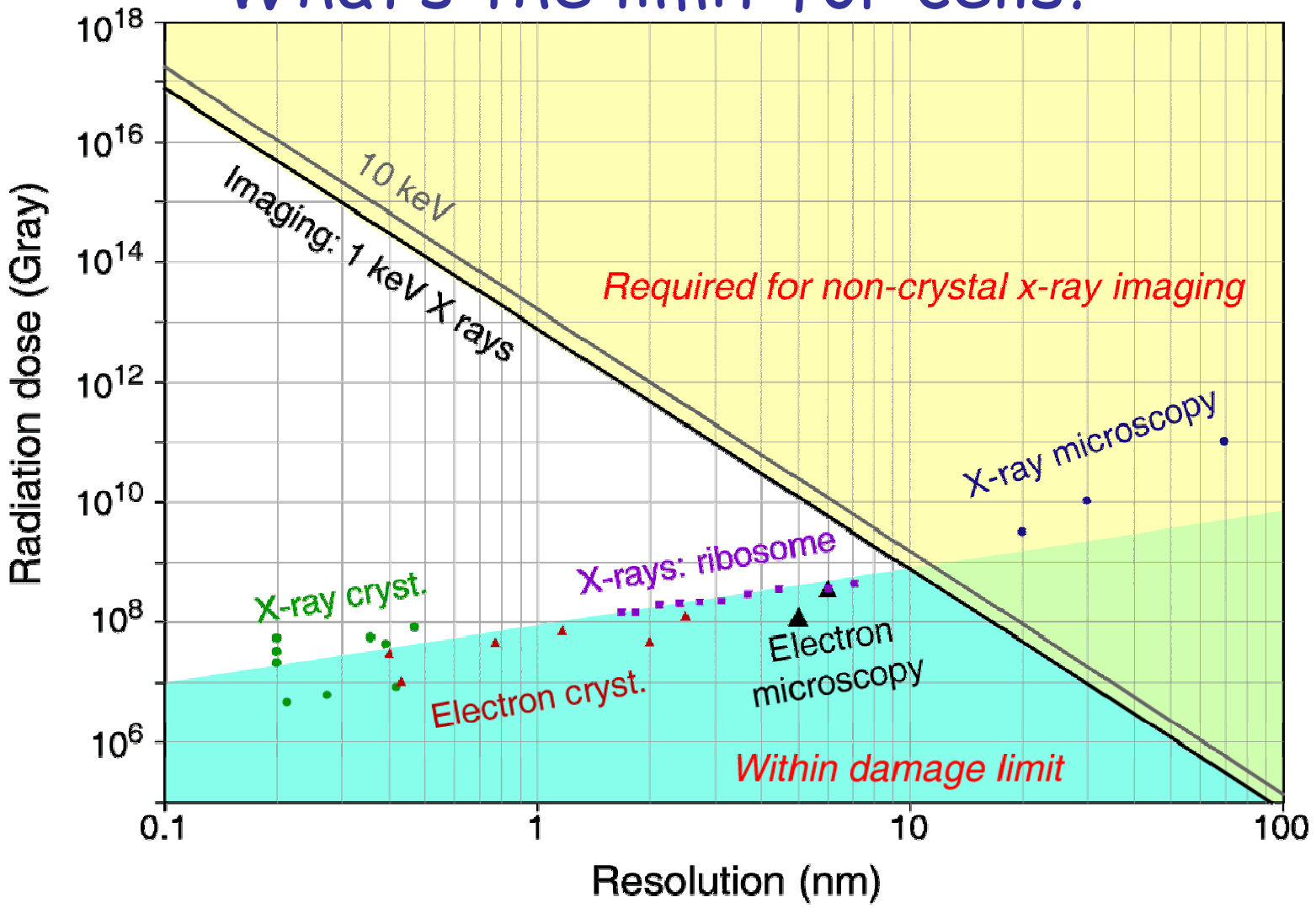
2/reconstruction

- How to avoid stagnation; local minima?
  - The enantiomorph problem
- How to tell whether algorithm converged?
  (easy when object known…)
  - Multiple random starts

3/ damage

- The ultimate limitation for radiation-sensitive materials only
- Dose fractionation (Hegerl and Hoppe 1976, McEwen 1995)

What's the limit for cells?

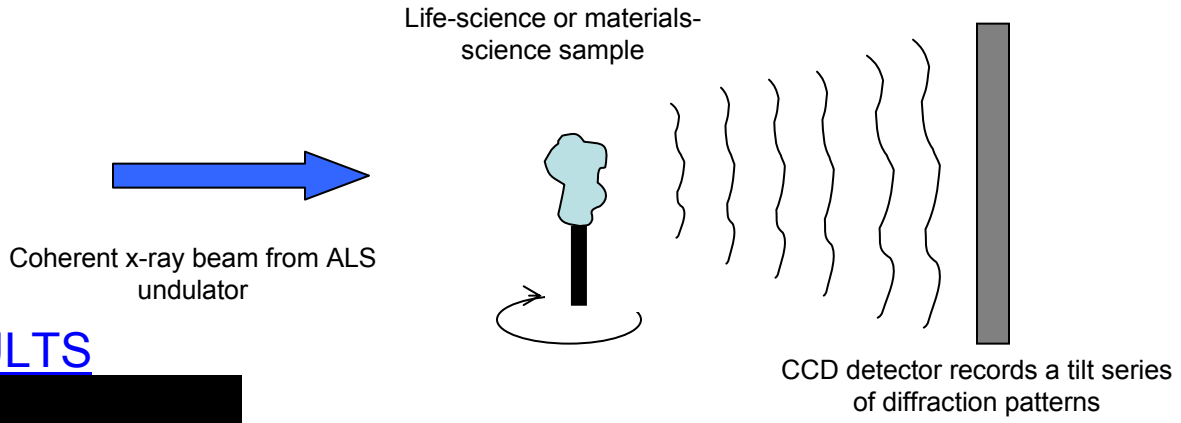


Howells *et al.*, *JESRP* (submitted)

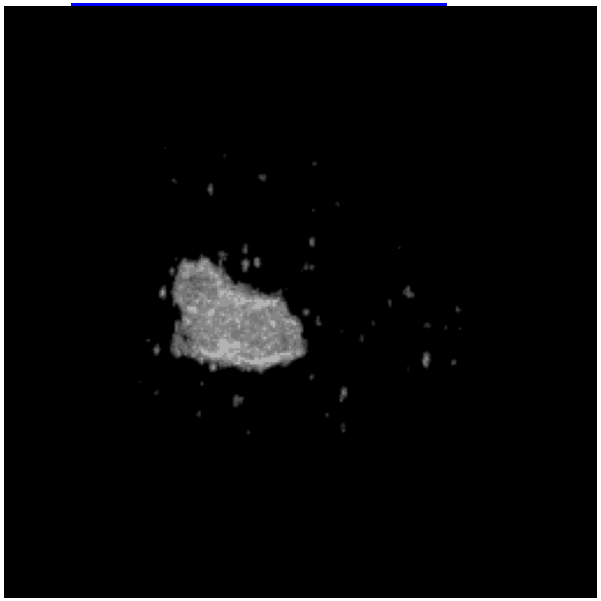
Recent Successes

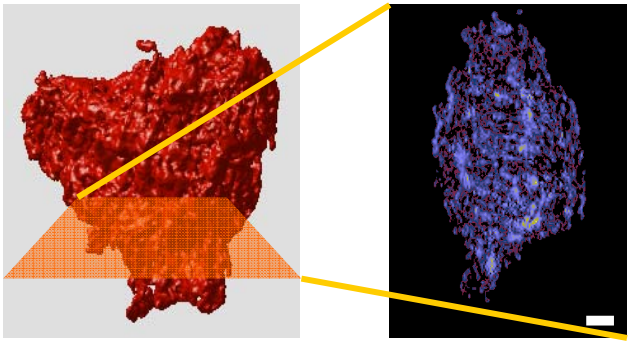
- Miao et al. (Stanford/Spring 8)
  - -First biological specimens (PNAS 100, 110, 2003)
  - -First 3D reconstruction (PRL 89, 88303, 2002)
- Howells, Spence, Chapman et al (ASU, LBL, LLNL)
  - –Reconstruction without low resolution image (Acta A59, 143 2003)
- Robinson et al. (Illinois)
  - -reconstruction of nano-crystal from structure of Bragg peak (PRL 87, 195505, 2001)

H. Chapman, A. Barty, M. Howells, S. Marchesini et al., LLNL, LBL



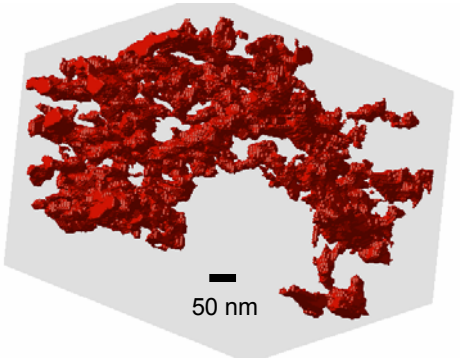
SOME RESULTS





3D isosurface

Cross-section (bar = 200nm)

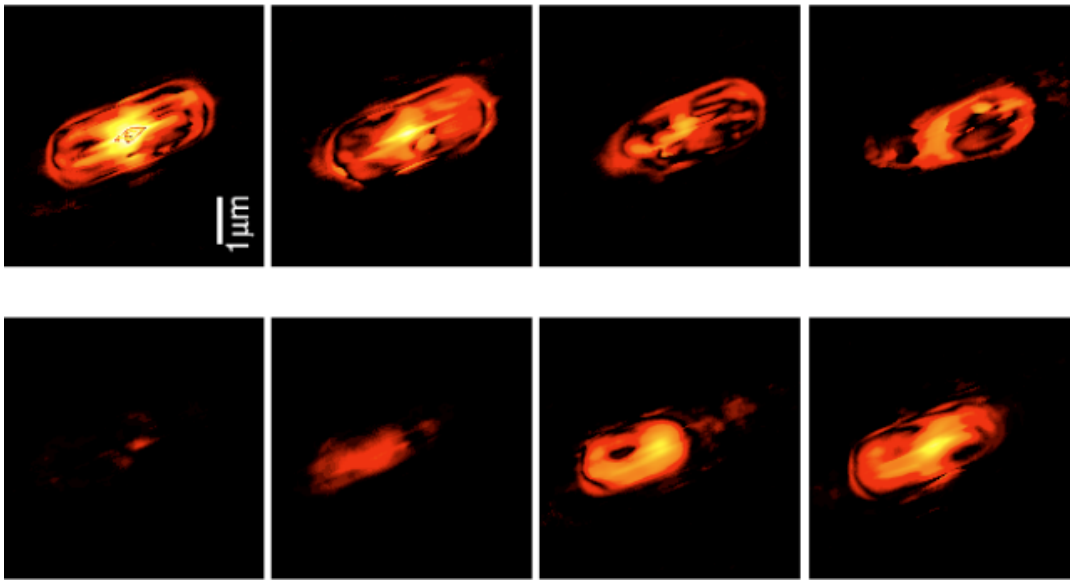


Res'n 20-30 nm

2-micron-wide particle of tantalum oxide foam of density about 0.1 gm/cm^3 which is about 1.2% of bulk density. The dataset of 280 views for the latter image was collected over two 8-hour shifts at 3.7 minutes per angle

In the past….

- J. Miao *et al.*: fabricated gold dot letters, *nature 400 (1999)*
  3D test objects, *Phys. Rev. B* **89,** 088303 (2002)
- G. Williams *et al.*: 3D imaging of microstructure in Au nanocrystals,
  Phys. Rev. Lett. ***90****, 175501 (2003)*
- H. Chapman *el al.:* 3D pyramid reconstruction, *JOSA A, in press may 2006*
- And more…



In the past….

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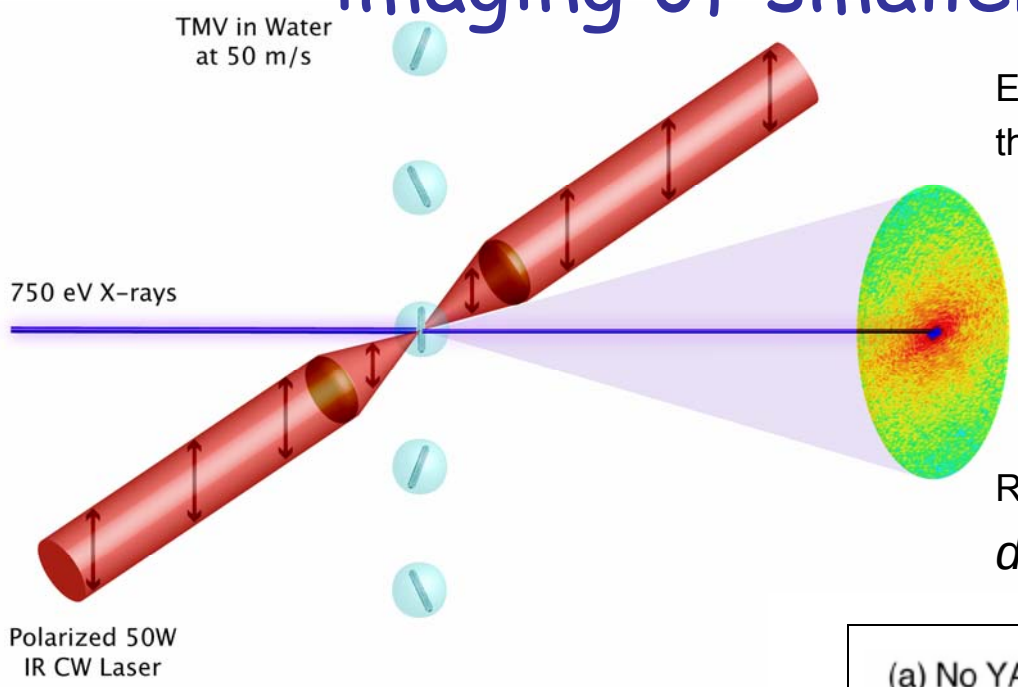
  Phys. Rev. Lett. ***90****, 175501 (2003)*
- H. Chapman *el al.:* 3D pyramid reconstruction, *JOSA A, in press may 2006*
- And more…

Conclusions

- Method of choice for micron-size specimens
- Damage will set limit on resolution for radiation-sensitive specimens
- Much progress on 2D problems, 3D just starting
- Surely an exciting prospect!

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

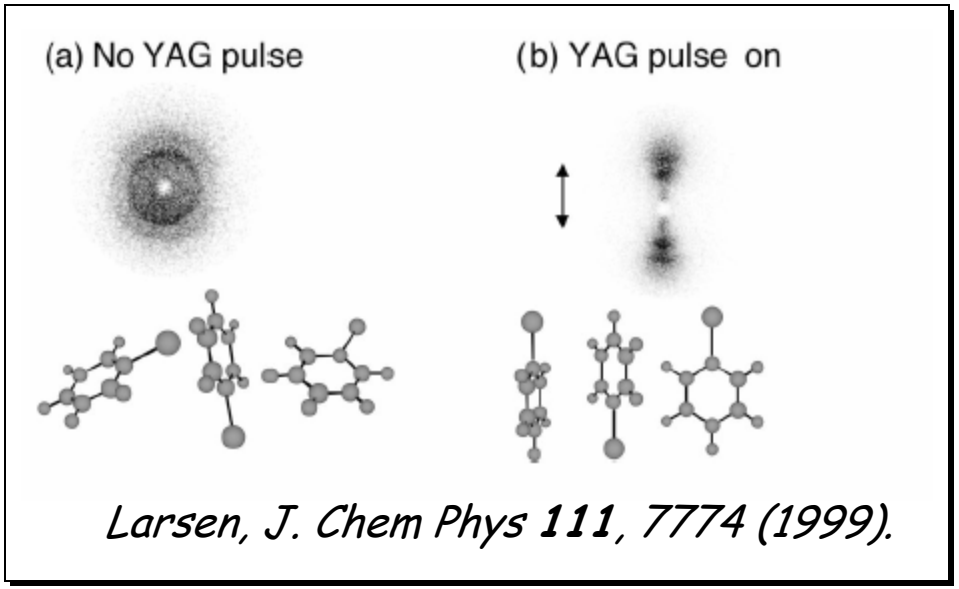
$\Delta\alpha$ - polarizability anisotropy in nm^3

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).*

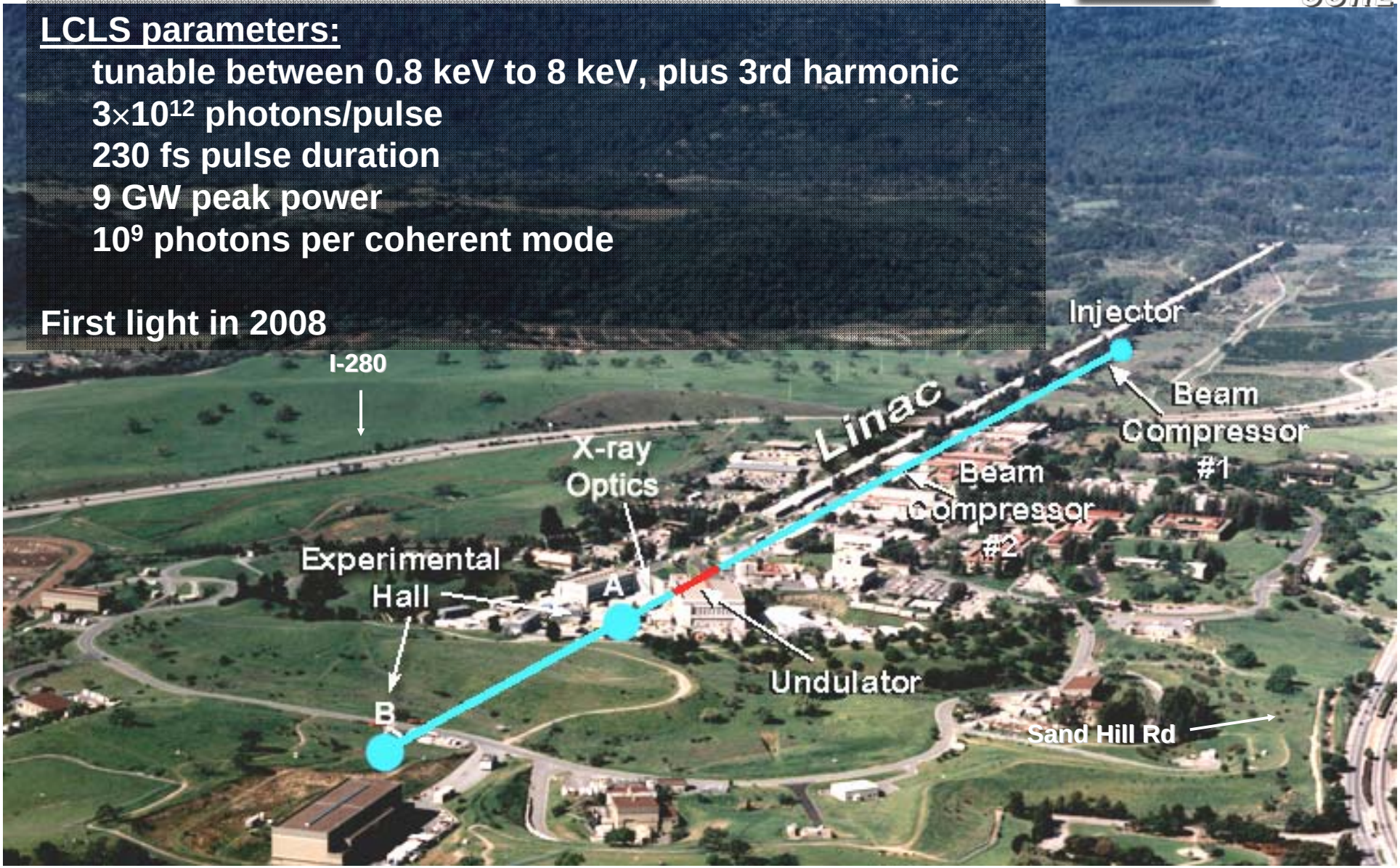
Stanford Linear Accelerator Center

SSRL

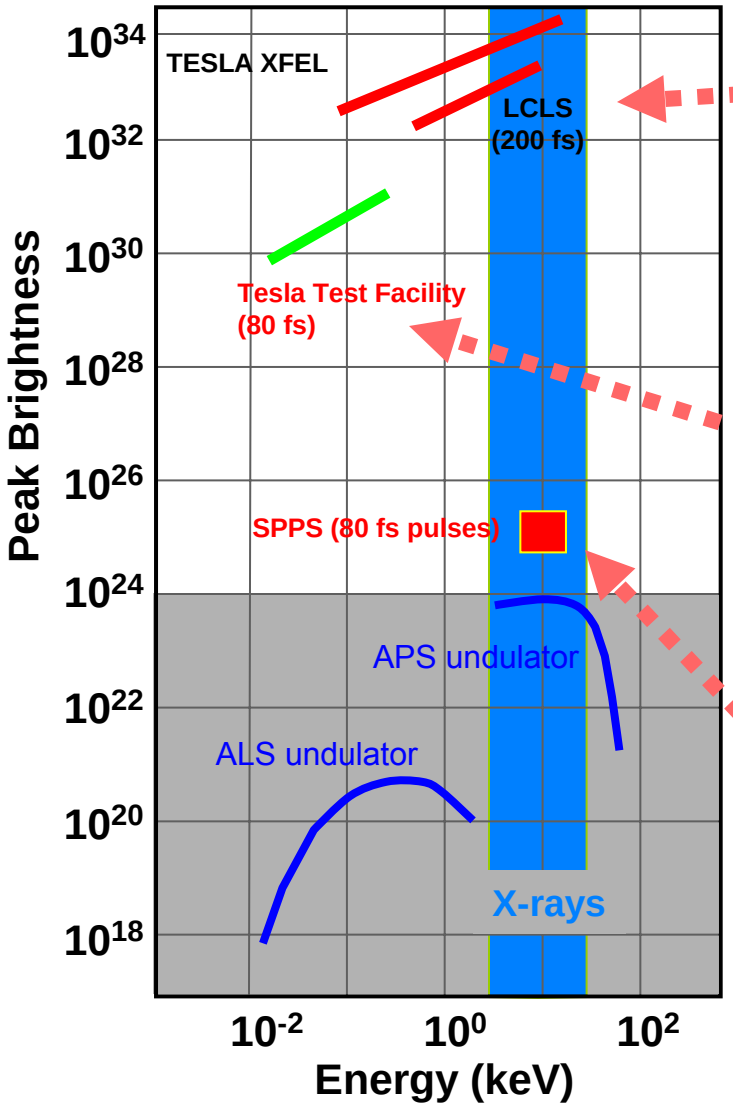
LCLS parameters:

- tunable between 0.8 keV to 8 keV, plus 3rd harmonic
- 3×10^{12} photons/pulse
- 230 fs pulse duration
- 9 GW peak power
- 10^9 photons per coherent mode

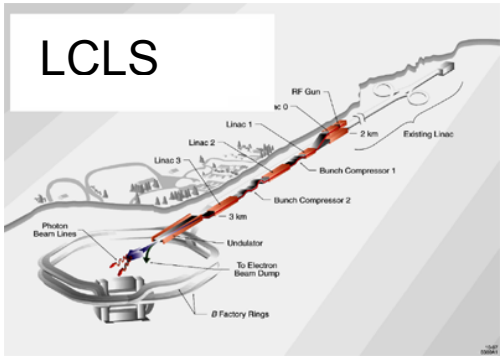
First light in 2008



We are entering a new era in x-ray science



APS=Advanced Photon Source (ANL)
ALS=Advanced Light Source (LBNL)



operational 2008

8 keV, 200 fs, 10^{12} photons

Linac Coherent Light Source, SLAC, Stanford

operational 2005

500 eV, 80 fs, 10^{13} photons

Tesla Test Facility DESY, Hamburg

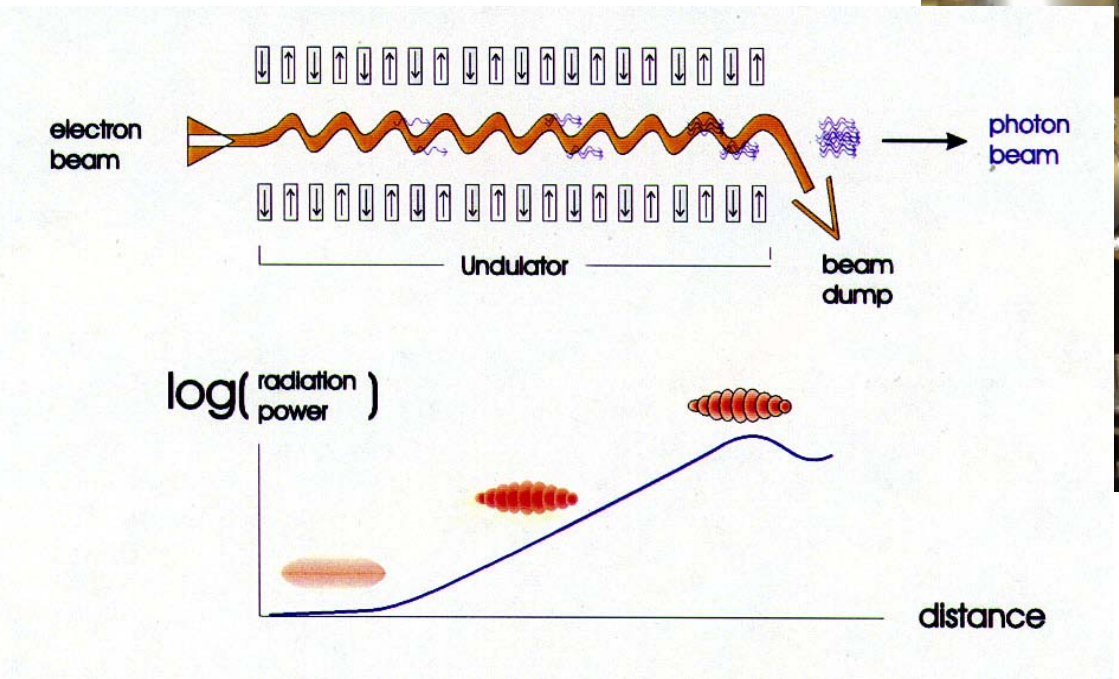
operational now

8 keV, 80 fs, 10^{7} photons

Sub-picosecond Photon Source, SLAC, Stanford

Coherent X-rays are produced by SASE

Self-Amplified Spontaneous Emission

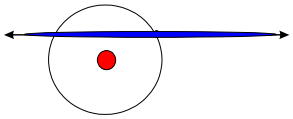
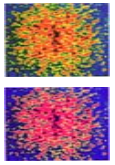
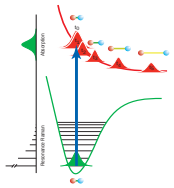
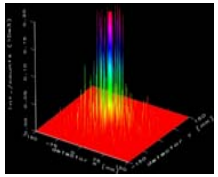
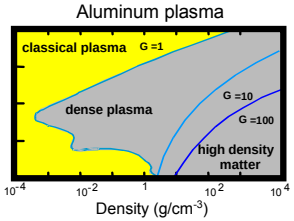


Advanced Photon Source

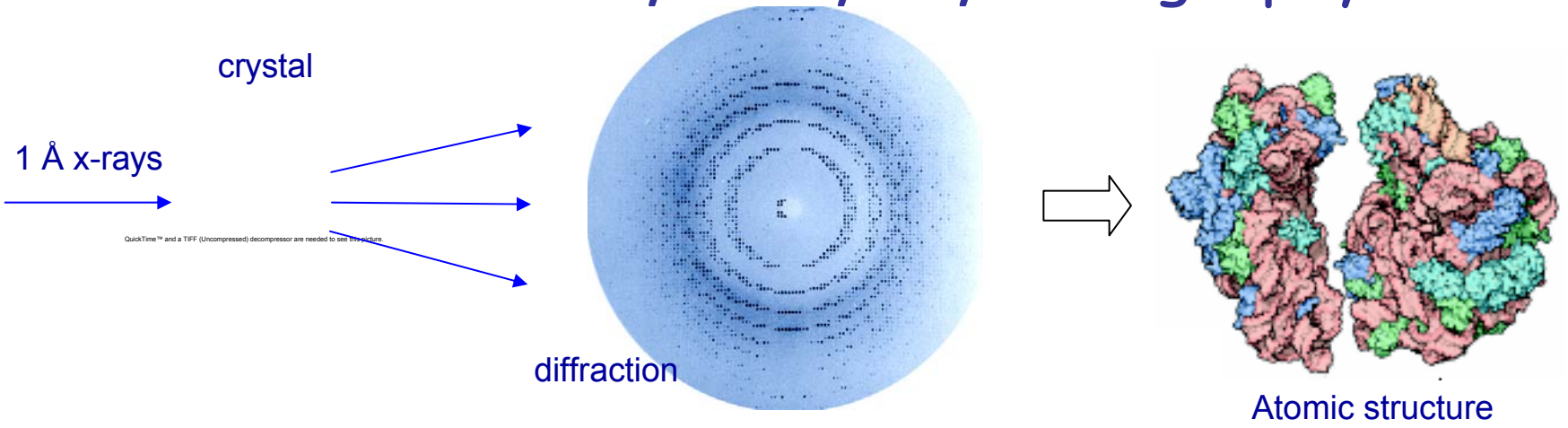
Electrons are bunched under the influence of the light that they radiate.
The bunch dimensions are characteristic of the wavelength of the light.

Excerpted from the TESLA Technical Design Report, released March 2001

LCLS Thrust Areas

	Team leader	Co-team leader(s)
 • AMO science	Lou Dimauro	Nora Berrah
 • Coherent scattering at the nanoscale	Brian Stephenson	Karl Ludwig
 • Pump/probe dynamics	Kelly Gaffney	David Reis Jorgen Larsson
 • Nano-particle and single molecule (non-periodic) imaging	Janos Hajdu	John Miao Henry Chapman
 • Pump/probe high energy density physics	Dick Lee	Phil Heimann

Today, the majority of molecular structures are determined by x-ray crystallography

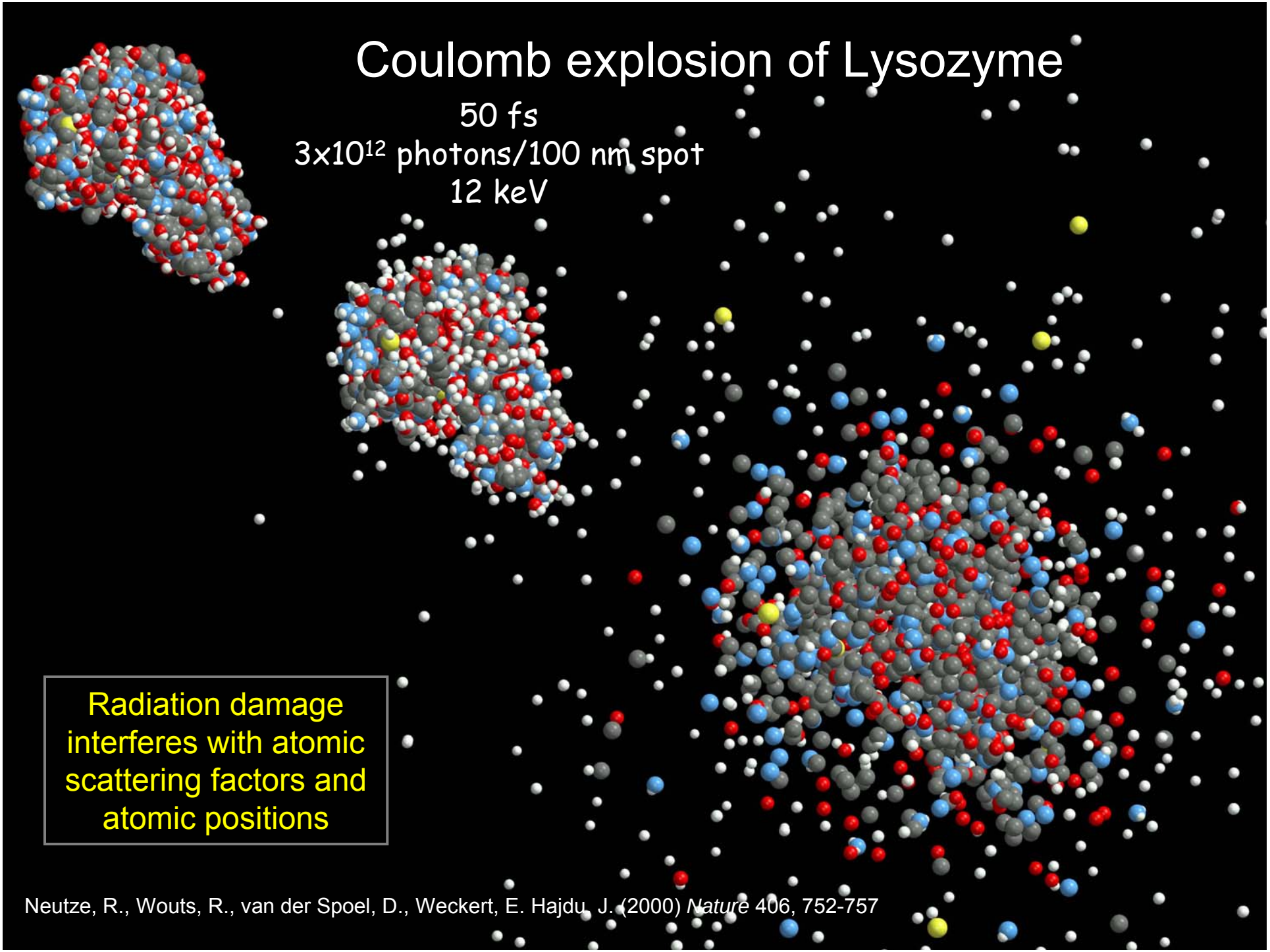


- **Radiation damage is spread out over 10^{10} identical unit cells**
- **Diffraction from unit cells adds up coherently to form strong Bragg peaks**
- **> 22,000 structures solved (in protein data bank), but ~4000 *distinct* structures**
- **The bottleneck is in growing crystals**

There are vast systematic unknowns in structural biology, even in the post genomics world. Less than 2 % of the human proteome is known

Protein structure using XFEL's?

- 1999: Blundell & Johnson
  multiple identical copies
- 2000: Neutze et al. short pulse needed
- 2000: LCLS first experiments Hajdu et al.
- 2001: Miao, Hodgson & Sayre: Rubisco
- 2002: Huldt thesis: how to align patterns?
- 2004: LCLS SAC approves LOI
- 2005: proposal for MIE@ LCLS


Coulomb explosion of Lysozyme
50 fs
3x10^12 photons/100 nm spot
12 keV
Radiation damage interferes with atomic scattering factors and atomic positions
Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) Nature 406, 752-757

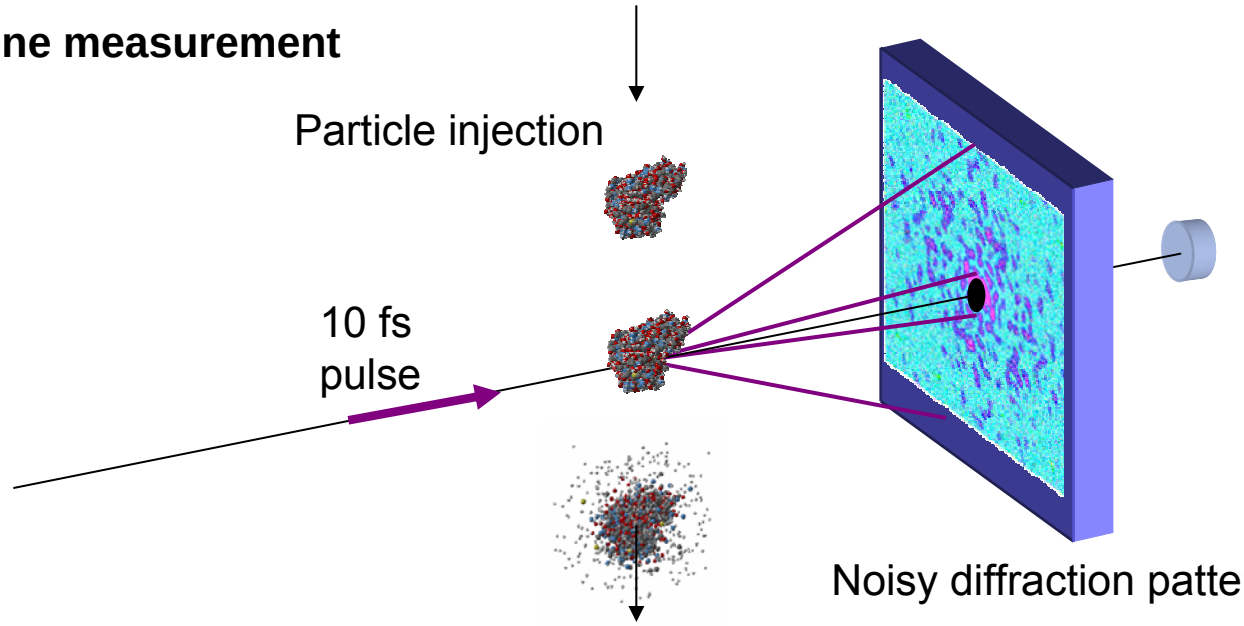
The collaborators:

USA: **Henry Chapman**, Keith Hodgson, Abraham Szöke, David Sayre, **John Miao**, Ian Robinson, James Fienup, Veit Elser, Janos Kirz, Ian McNulty, Lukas Novotny, Pascal Anger, Chris Jacobsen, David Shapiro, Enju Lima, Huije Miao, Keith Hodgson, Helmut Strey, Roger Falcone, Musahid Ahmed, John C.H. Spence, Eugene Ingerman, Henry Chapman, Stefan Hau-Riege, Hope Ishii, Stefano Marchesini, Rodney Balhorn, Henry Benner, Matthias Frank, Aleksandr Noy, Anton Barty, Brent Segelke, Richard London, Daniel Barsky, Peter Young, Richard Lee,
SWEDEN: David van der Spoel, Nicusor Timneanu, Martin Svenda, Gösta Huldt, Carl Caleman, Magnus Bergh, Sara Lejon, Alexandra Patriksson, Richard Neutze, Arjan Snijder, Susanna Tornroth, Jan Isberg, **Janos Hajdu**
PORTUGAL: Martha Fajardo, Nelson Lopes, Joao M Dias, Goncalo Figueira, Luis Silva, Ricardo Fonseca, Fabio Peano,
POLAND: Beata Ziaja,
FRANCE: Hamed Merdji, Philippe Zeitoun,
HUNGARY: Gyula Faigel,
UK: Carol Robinson,
GERMANY: Jochen Schneider, Edgar Weckert, Josef Feldhaus, Elke Plönjes, Thomas Möller, Christoph Bostedt, Ivan Vartaniants, Christian Schroer,
AUSTRALIA: Keith Nugent

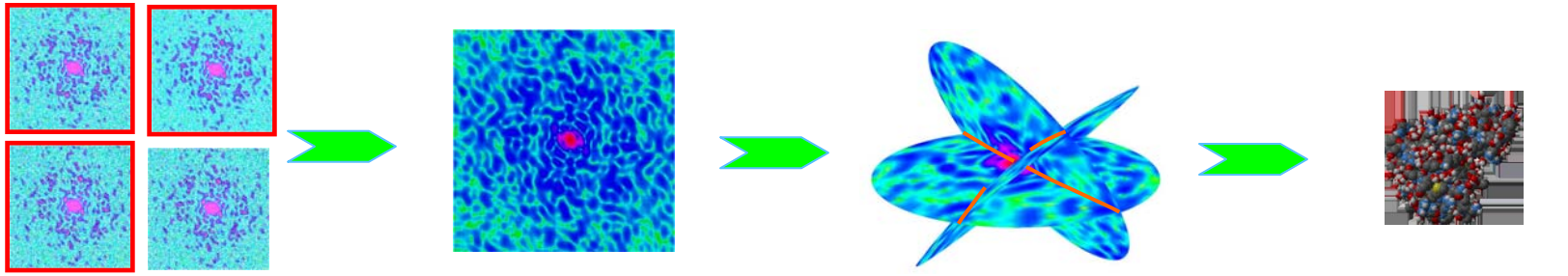
(30 Institutional Members)

X-ray free-electron lasers may enable atomic-resolution imaging of biological macromolecules

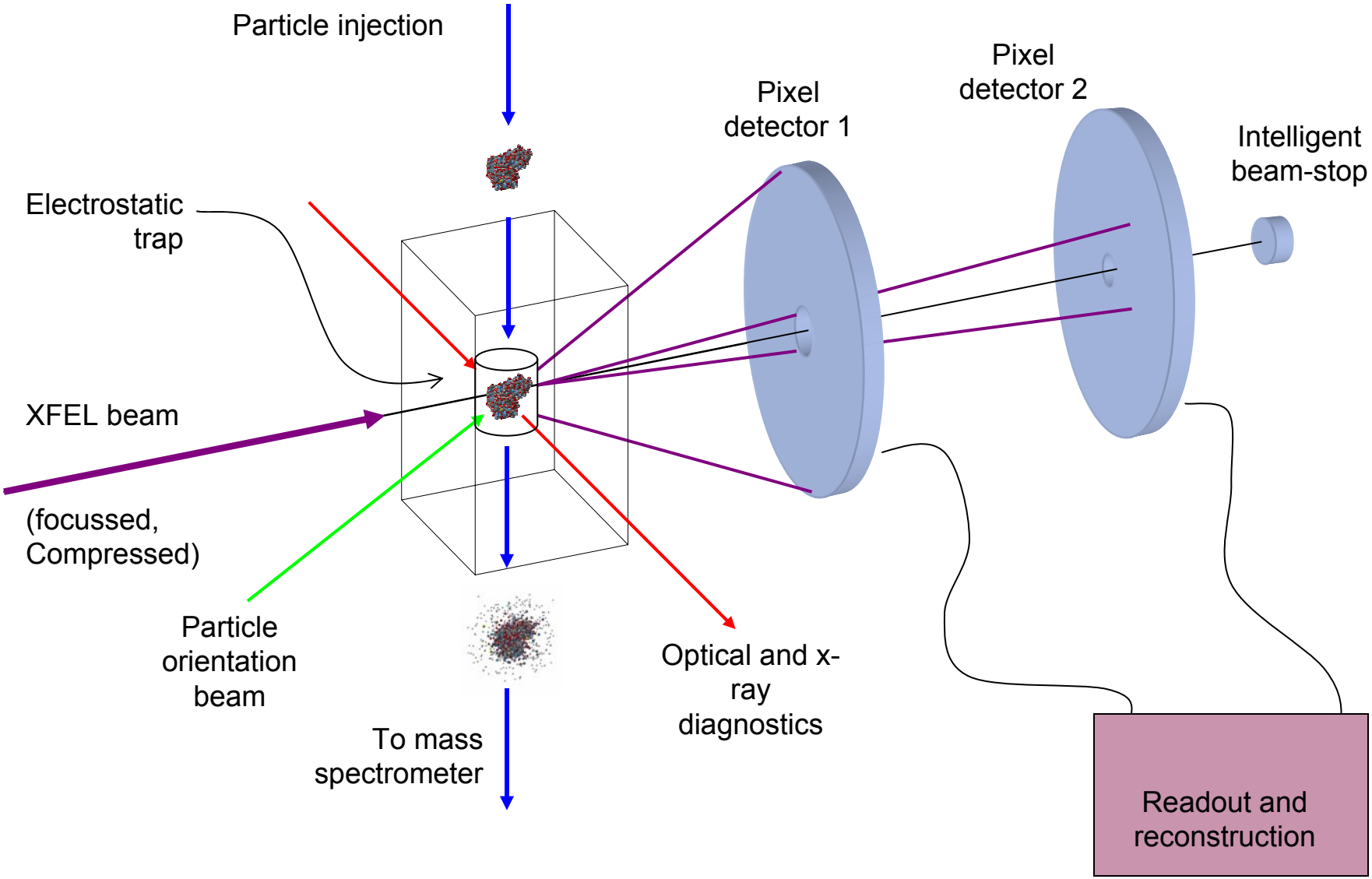
One pulse, one measurement



Combine 10^5-10^7 measurements



The LCLS interaction chamber and detector arrangement



Early experiments at LCLS

Development will take place in two chambers

	Injected particles	**Samples on substrates**
2008 800 eV 230 fs	Diffraction from a mist of particles at 800 eV • Validate damage models • Imaging demonstrations	Coherence characterization Diffraction from nanoparticles • Below-damage-threshold imaging • Magnetics (?)
2009 8 keV 230 fs	• Test damage mitigation (e.g. tamper) • Diffraction of aligned particles	• Single-shot 2D imaging Improvements in focusing, signal to noise
2010 8 keV 50 fs	Diffraction from single particles • Damage validation • 3D imaging of simple particles	Single-shot biological cells Membrane crystals

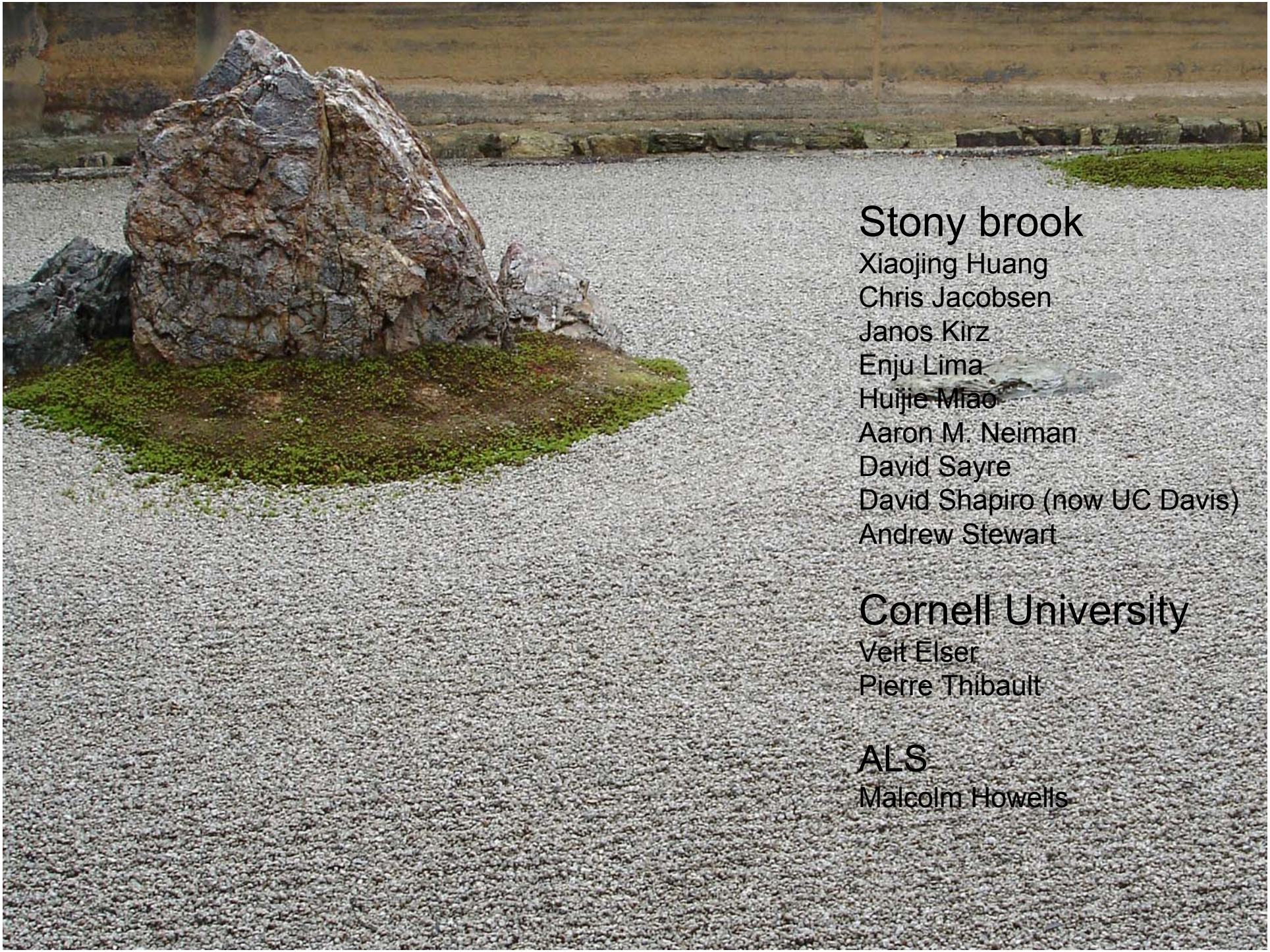
Conclusions

- High risk – high pay-off project
- Challenges: pulse length, focusing, injection/alignment, diagnostics, recording, sorting, reconstruction
- Team working on every aspect: theo & exp't
- It will not do single membrane proteins on day 1!

Acknowlegements

- Janos Hajdu & Henry Chapman
- David Sayre
- Chris Jacobsen, Malcolm Howells, David Shapiro, Enju Lima
- Veit Elser & Pierre Thibault

- DOE/BES; NIH



Stony brook

Xiaojing Huang
Chris Jacobsen
Janos Kirz
Enju Lima
Huijie Miao
Aaron M. Neiman
David Sayre
David Shapiro (now UC Davis)
Andrew Stewart

Cornell University

Veit Elser
Pierre Thibault

ALS

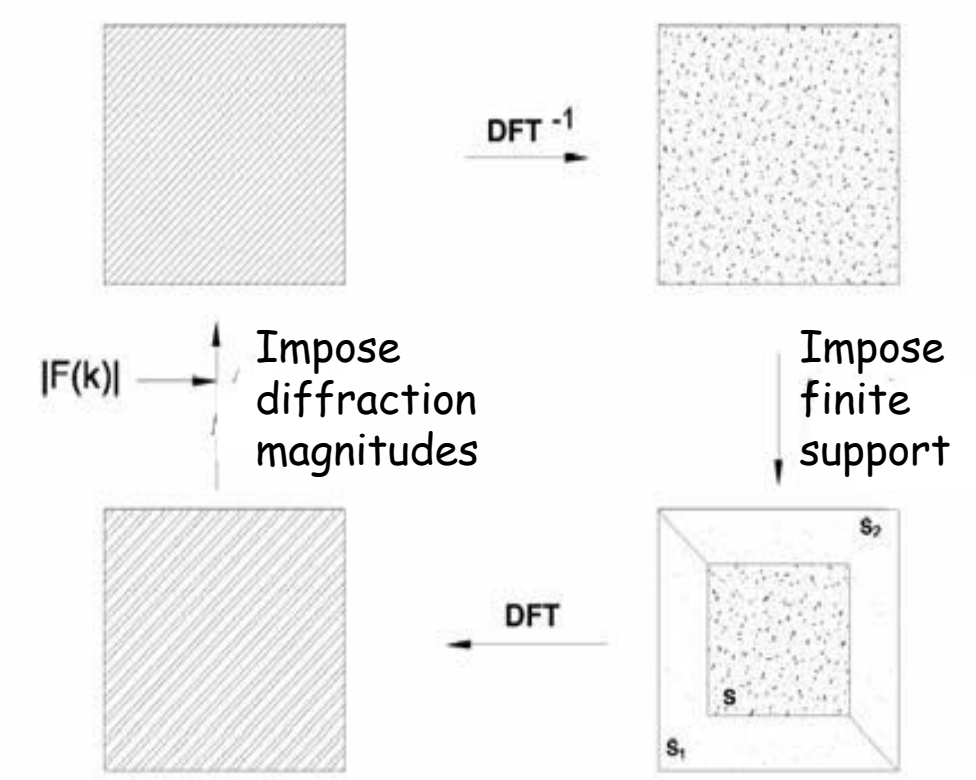
Malcolm Howells

Reconstruction

Equations can still not be solved analytically

Fienup iterative algorithm

Reciprocal space Real space



•Positivity of electron density helps!