Diffraction microscopy: present experiments at ALS, and future experiments at higher energies

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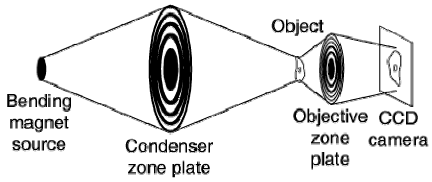
Zone plate microscopes

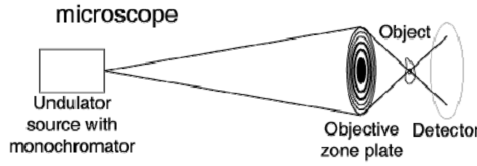
TXM
- Incoherent illumination; works well with a bending magnet, with fast imaging
- More pixels (e.g., 2048^2)
- Moderate spectral resolution in most cases

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

TXM: transmission x-ray microscope



STXM: scanning transmission x-ray microscope



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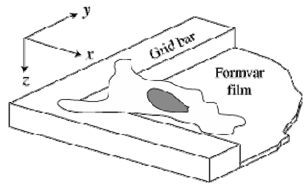
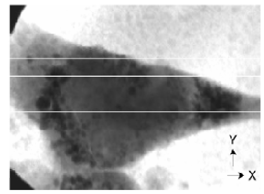
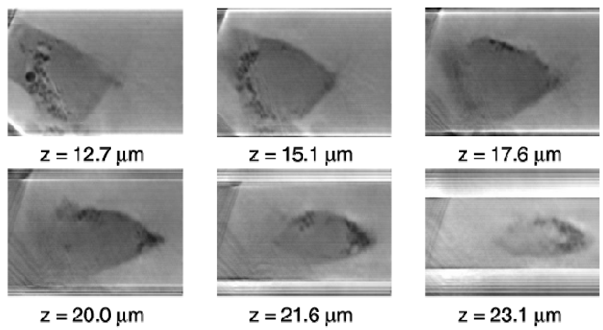
Tomography of a fibroblast

- Microscopes and tomography? No problem if object is within depth of focus, which for transverse resolution δ goes like $4\delta^2/\lambda$
- First demonstration of soft x-ray tomography: Haddad *et al.*, *Science* **266**, 1213 (1994); at X1A
- This example: Y. Wang *et al.*, *J. Microscopy* **197**, 80 (2000); cryoSTXM at X1A

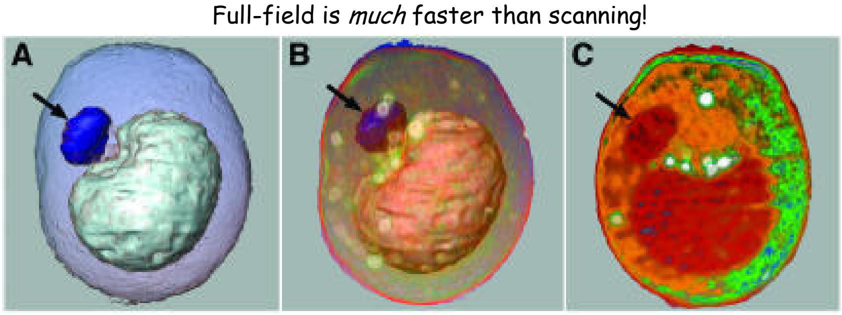




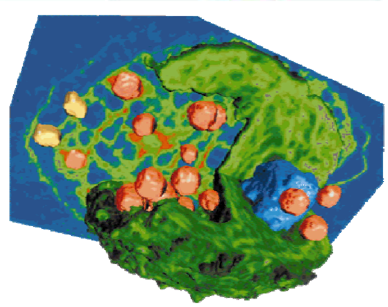


Tomography: full-field TXM

Full-field is *much* faster than scanning!



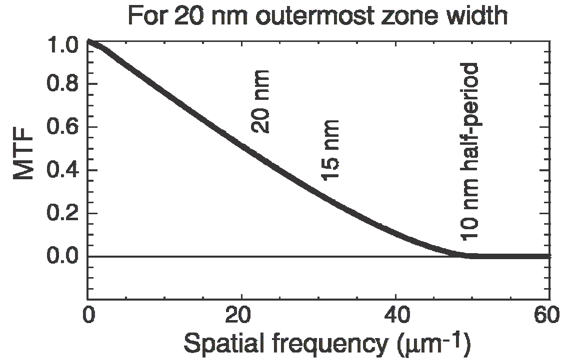
Frozen hydrated yeast *Saccharomyces cerevisiae*. C. Larabell and M. Le Gros, *Mol. Biol. Cell* **15**, 957 (2004). ALS/UC San Francisco.



Frozen hydrated alga *Chlamydomonas reinhardtii*: D. Weiß, G. Schneider, *et al.*, *Ultramicroscopy* **84**, 185 (2000). Göttingen/BESSY I. Newer results at BESSY II.

Motivation: high resolution on dose-limited specimens

- With 20 nm zone plates, have 5x loss for efficiency, 5x loss for modulation transfer function (MTF) at 15 nm feature size. (Tilted zone plates will help).
- Can we avoid this 25x signal loss, and go beyond the limits of available optics?



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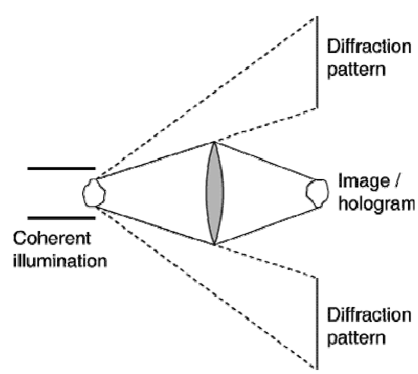
Imaging and scattering

- X ray scattering: Fourier space $|S(q)|^2$
- Microscopy: real space $\rho(x)$
- Relate spatial frequencies to positions x with q

$$q = \frac{\theta}{\lambda}$$

- Far-field relationship: Fourier transform
- One real space pixel affects all Fourier space pixels, and vice versa!



$$S(q) = \int \rho(x)\, e^{i2\pi x q}\, dx$$

$$\rho(x) = \int S(q)\, e^{-i2\pi q x}\, dq$$

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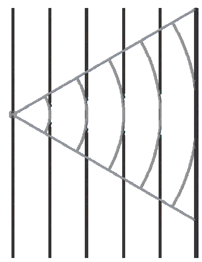
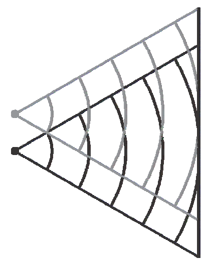
X-ray holography

- Holography gives amplitude *and* phase of object
- Reference wave should be stronger than object wave (i.e., not an atom): $I = (o+r)(o+r)^* = oo^* + rr^* + or^* + o^*r$
- In holography, you must do *something* at high resolution



Plane reference wave:
detector sets resolution limit



Spherical reference wave:
point source sets resolution limit

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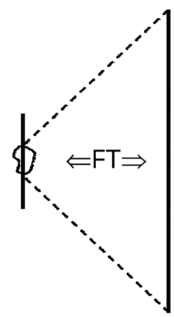
Imaging using x-ray diffraction from non-periodic specimens

- Proposed by Sayre (in Schlenker, ed., *Imaging and Coherence Properties in Physics*, Springer-Verlag, 1980)
- Previous experiments by Sayre, Kirz, Yun, Chapman, Miao
- Reconstruction: Fourier transform relationship between real space and Fourier space

Real space: can we make assumptions about the object?



Fourier space: we can measure the Fourier magnitudes

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Phase matters

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

Malcolm Howells at La Clusaz

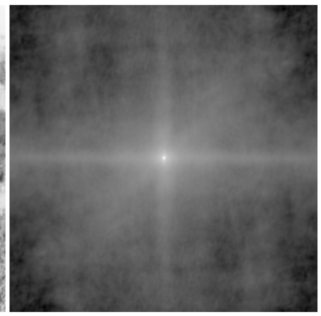

Image using only Fourier magnitudes

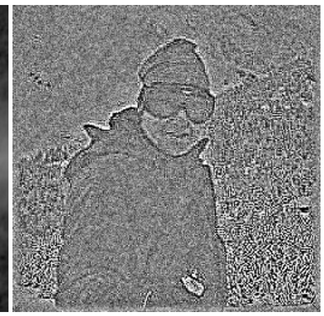

Image using only Fourier phases

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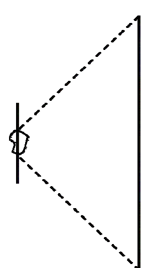
How to recover the missing info?

- Fourier space: half of the information is missing (phases)
- Real space: can only have unknowns in half of the area (or less)
- "Oversampling" ↔ Finite support constraint
- This larger real-space array is *properly* Shannon sampled

Real space: finite support (or other constraints)



Fourier space: magnitudes known, but phases are not

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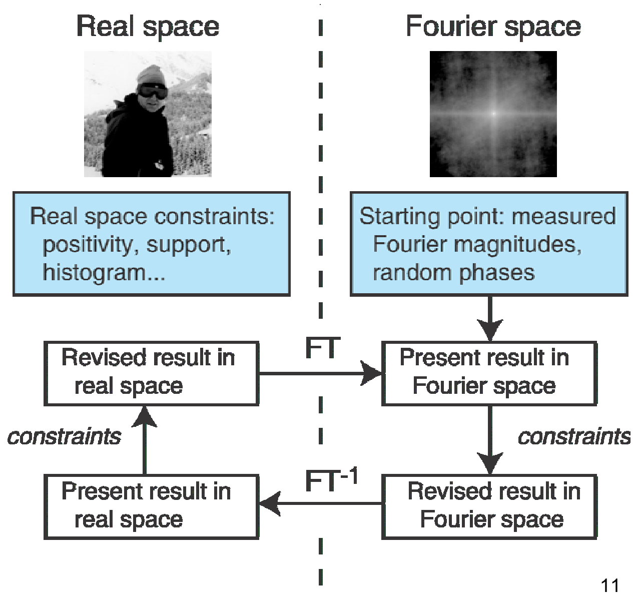
Iterative algorithms

Recall complete mixing of information between real and Fourier space:

$$S(q) = \int \rho(x)\, e^{i2\pi x q}\, dx$$

$$\rho(x) = \int S(q)\, e^{-i2\pi q x}\, dq$$

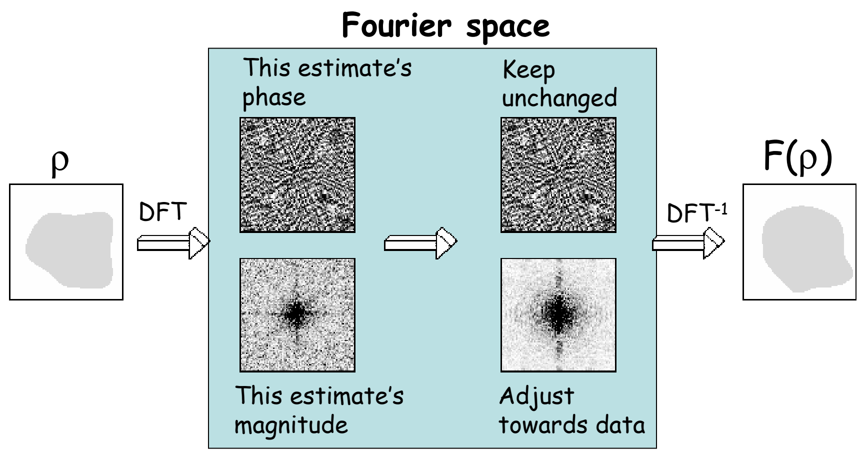
Microscopy, space imaging…



Iterative phase retrieval

- Gerchberg & Saxton: iterate between image and diffraction pattern magnitudes in electron micrographs to find image phase. *Optik* **34**, 275 (1971)
- Fienup: diffraction data only plus support constraints, and control theory insights. *Applied Optics* **21**, 2758 (1982)
- Elser: difference map, and histogram constraints. *J. Opt. Soc. Am.* **A 20**, 40 (2003)
- Works much better in 2D than 1D!
- Works even better in 3D than 2D!
- Coupling of solution in orthogonal directions

Fourier modulus constraint



(Enju Lima, Stony Brook)

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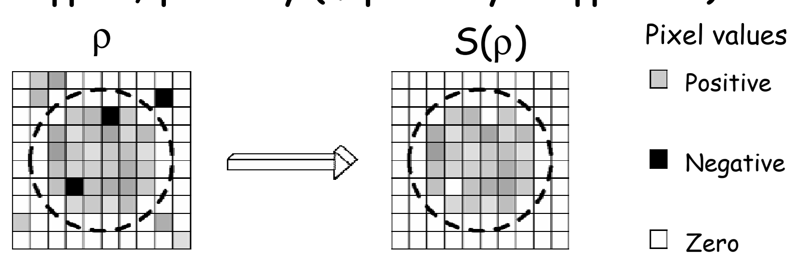
Real space constraints

Support, positivity (if positivity is applicable)



Histogram constraint

$\rho_i \rightarrow H_i$

(V. Elser)

$\rho =$ | 0 | 4 | 2 | 1 | 13 | 0 |

$H = \{10,7,2,0,0,0\}$

$H(\rho) =$ | 0 | 7 | 2 | 0 | 10 | 0 |

(Enju Lima, Stony Brook)

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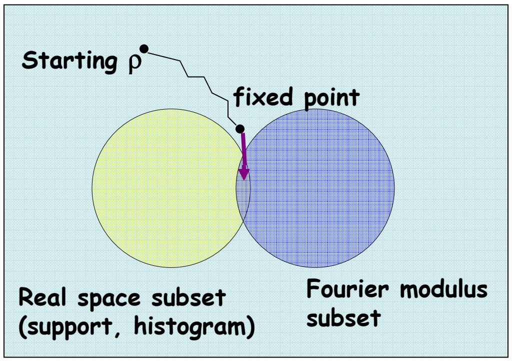
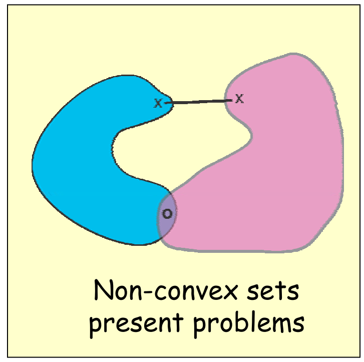
A picture of an iterative solution





Elser: project both real space and Fourier modulus constraints back to real space. *J. Opt. Soc. Am.* **A 20**, 40 (2003)

(Enju Lima, Stony Brook)

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What about complex objects?

- Iterative algorithms work best on objects with just one phase value (*e.g.,* real objects with uniform phase shift).
- Thicker biological specimens become complex objects (phase of exit wave can vary by more than $0 \Rightarrow \pi$).
- Strategies for truly complex objects:
  - A low-resolution image can be used to phase from low angles outwards [see *e.g.*, Fienup and Kowalczyk, *J. Opt. Soc. Am. A* **7**, 450 (1990)]
  - The object (or object and reference) can be isolated to two separated regions [see *e.g.*, Fienup, *J. Opt. Soc. Am.* **4**, 118 (1987)]

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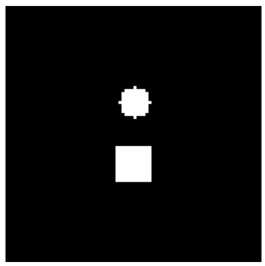
Iterative phasing: simple example

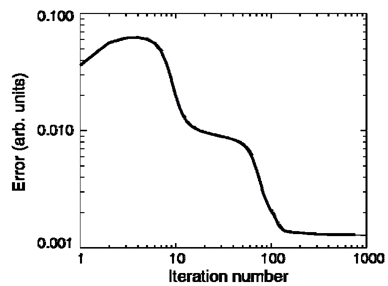
- High harmonic generation of XUV radiation from femtosecond lasers, illuminating two pinholes.
  - R. Bartels, A. Paul, H. Green, H.C. Kapteyn, M.M. Murnane, S. Backus, I.P. Christov, Y. Liu, D. Attwood, C. Jacobsen, *Science* **297**, 376 (2002)

Data



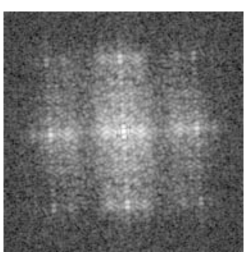
Support constraint (very loose)

[The movie]



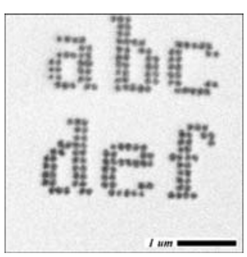
Reconstruction error

First x-ray demonstration



λ=1.8 nm soft x-ray diffraction pattern (left) with low-angle information from optical micrograph (right)

Scanning electron micrograph of object



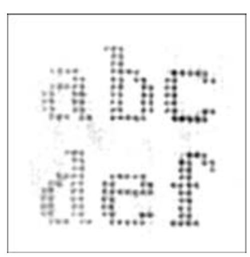
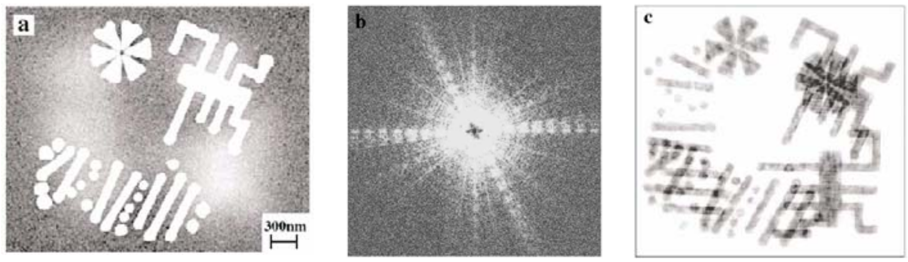


Image reconstructed from diffraction pattern (θ_{max} corresponds to 80 nm). Assumed positivity

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

Diffraction from a 3D object: 2D



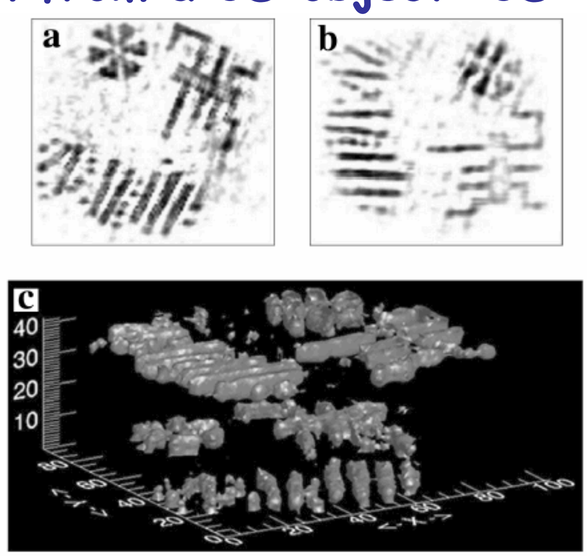
SEM 2D diffraction 2D reconstruction

- Two planar objects on windows, 1 μm apart
- 2D: CCD 'tiling' to give 1760^2 pixels, 8 nm half-period at edge (45 minutes@SPring-8; 2 Å)

J. Miao, T. Ishikawa, B. Johnson, E. Anderson, B. Lai, K. Hodgson, *Phys. Rev. Lett.* **89**, 088303-1 (2002)

Diffraction from a 3D object: 3D

- Two planar objects on windows, 1 μm apart
- 3D: 21 angles (not ideal), 56 nm half-period at edge, 256^3 Fourier transform



J. Miao, T. Ishikawa, B. Johnson, E. Anderson, B. Lai, K. Hodgson, *Phys. Rev. Lett.* **89**, 088303-1 (2002)

Gatan 630 cryo holder

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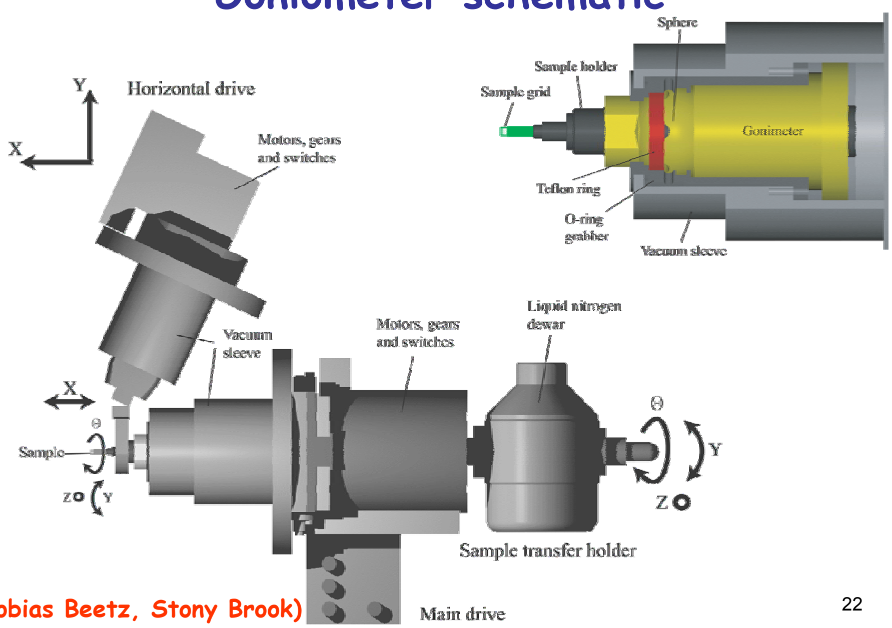
Goniometer schematic



(Tobias Beetz, Stony Brook)

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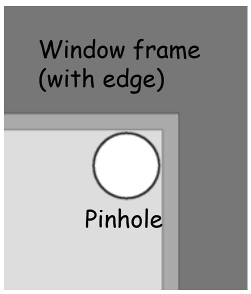
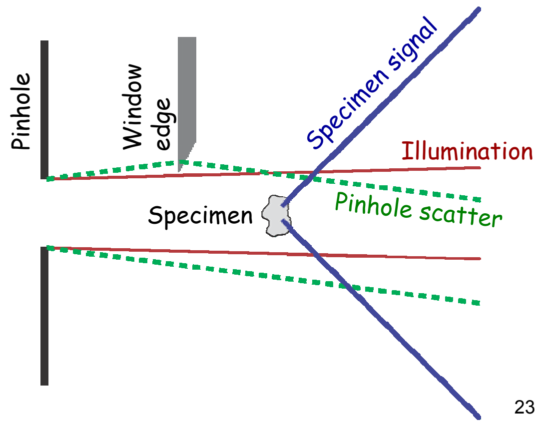
Removing scatter from pinholes

- 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)





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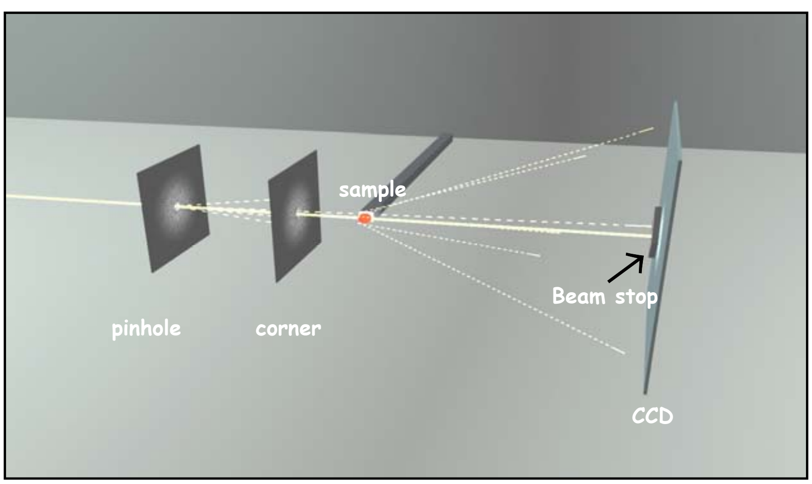
Experimental setup



Inside vacuum chamber

(Enju Lima, Stony Brook)

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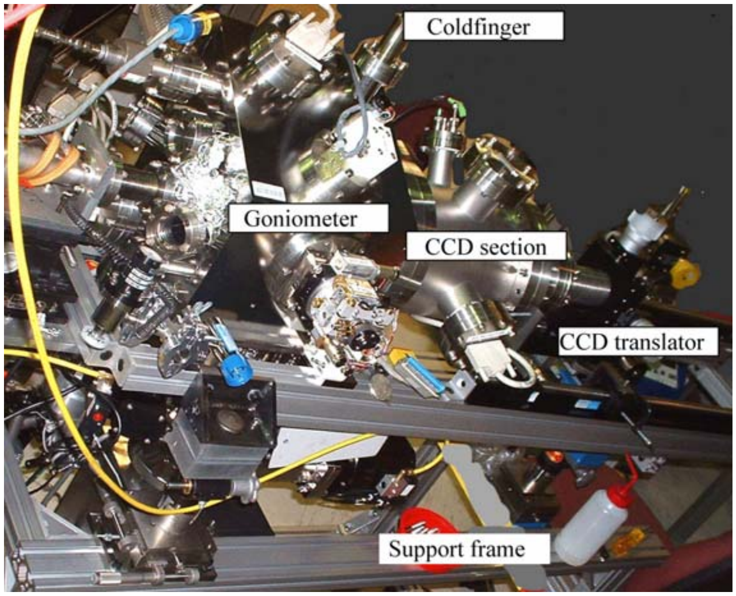
At ALS 9.0.1



(Tobias Beetz, Stony Brook)

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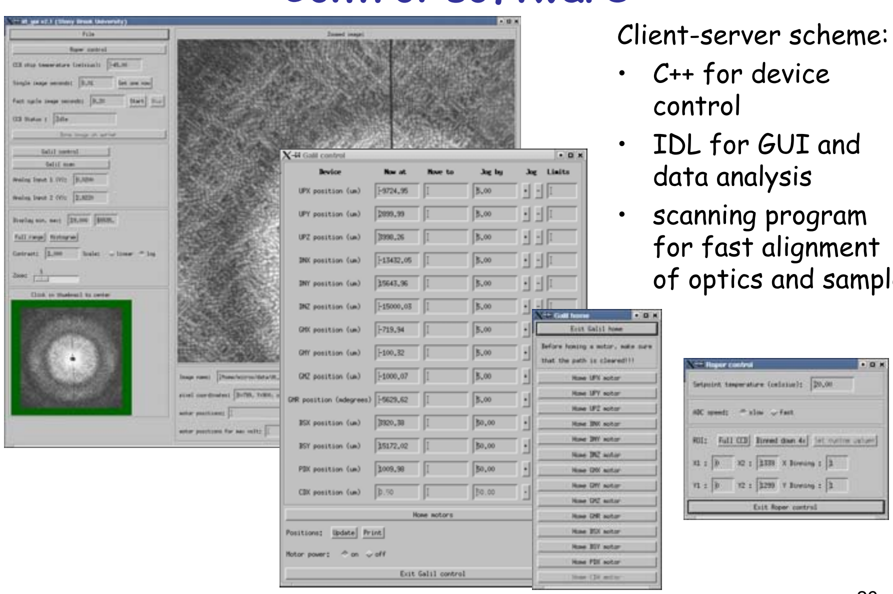
Control software



Client-server scheme:

- C++ for device control
- IDL for GUI and data analysis
- scanning program for fast alignment of optics and sample

(Tobias Beetz, Stony Brook)

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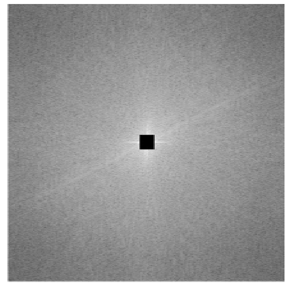
Effect of missing low spatial frequencies

Meghan Sumner



(Tobias Beetz, Stony Brook)

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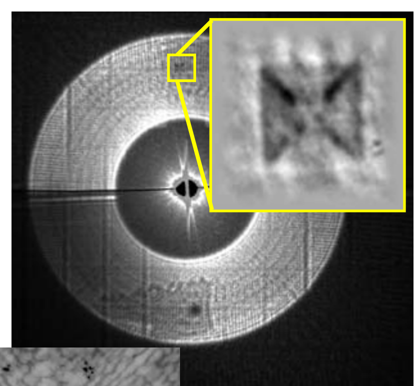
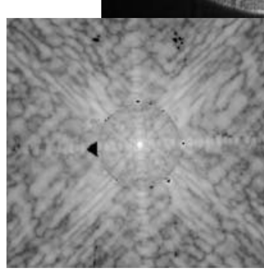
Zone plate imaging

Zone plate motorized for easy insertion & removal. However:

- 20 nm zone plate with central stop, JBX-6000 stitching errors (S. Spector)
- At 16 cm CCD distance; optical magnification gives only 100 nm pixels





(Tobias Beetz, Stony Brook)

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Latest results

- Several slides will go in here on latest unpublished results, including reconstructed images of yeast cells.

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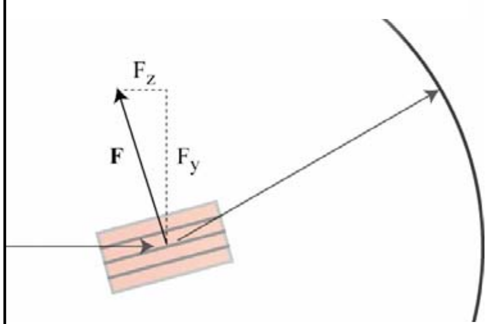
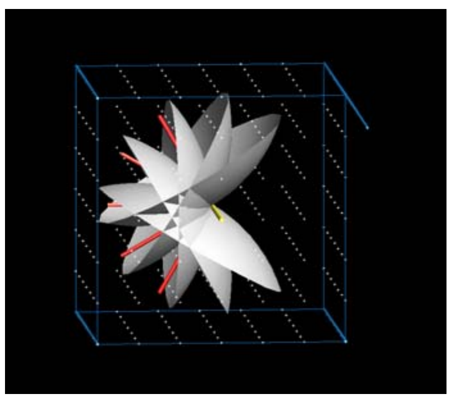
3D sampling considerations



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



Ewald sphere rotated about one axis only gives imperfect data filling in Fourier space

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3D: Computational considerations

- 2D is easy: 1024^2 FFT takes tenths of a second on a standard PC, and 100's of iterations take minutes
- 3D is hard.
  - 512^3 takes 5 minutes on a 1.8 GHz Athlon *per iteration*.
  - Memory requirements per 3D array: $(1K)^3$=1 Gbyte, times 4 for single-precision floating point, times 2 for before and after correction, times 2 for real and Fourier space: 16 GBytes!
  - Opportunity for parallelization! Xeon or G5 cluster

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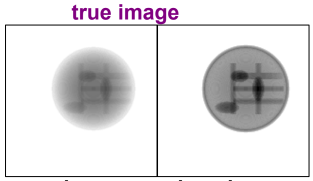
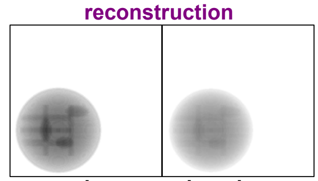
3D complex object simulations ("fake" 3D object of water, lipid membrane, protein rods and ellipsoids, including both absorption and phase shift)

true image



real imaginary

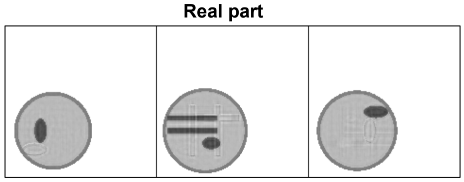
reconstruction



real imaginary

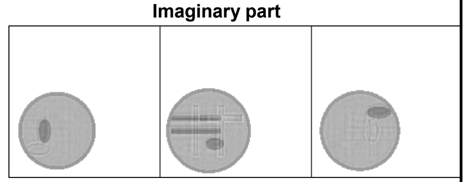
z axis slices of reconstruction at the end of 5000 iterations:

Real part



Z=25 z=35 z=65

Imaginary part



Z=25 z=35 z=65

More iterations needed than with pure real case to have the same quality reconstruction

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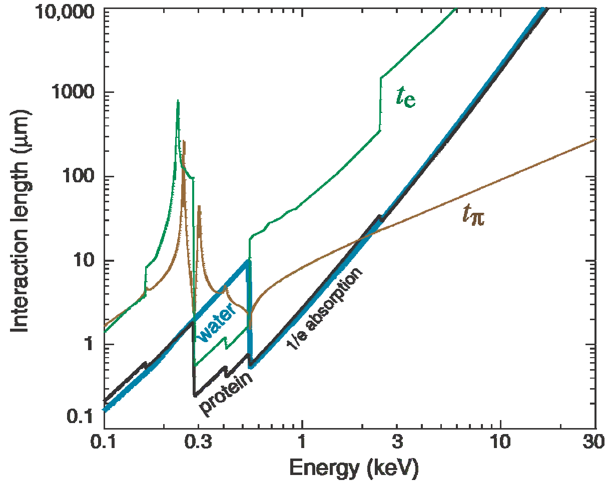
(Enju Lima, Stony Brook)

Phase contrast: soft and hard x rays

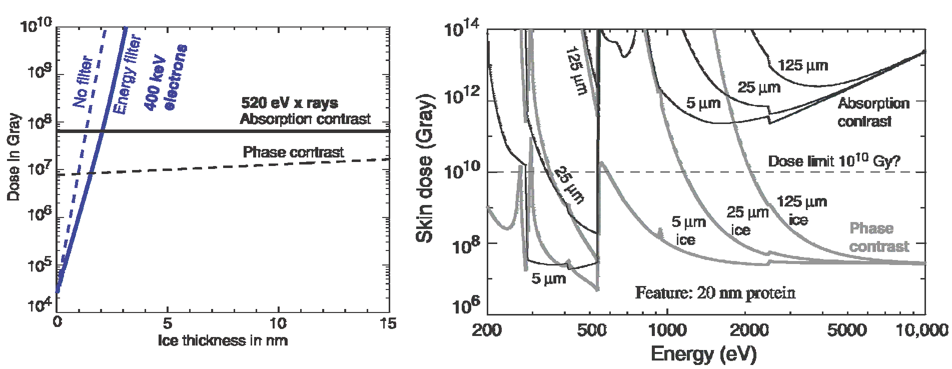
t_e is thickness for $1/e$ transmission difference

t_π is thickness for π phase shift



X rays and thick specimens

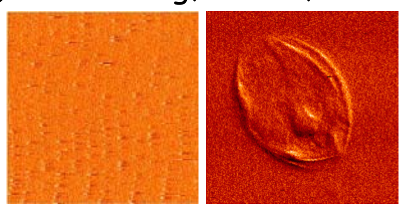
X-rays: better for thicker specimens. Sayre *et al.*, *Science* **196**, 1339 (1977); Schmahl & Rudolph in **X-ray Microscopy: Instrumentation and Biological Applications** (Springer, 1987)



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

Phase contrast for microprobes

- Fluorescence mapping for elemental signal; phase contrast for mass; thus quantitative concentrations
- Transmission detector can be used simultaneously during fluorescence scans.
- Applications: As hyperaccumulators (Kissell & Reeder, Stony Brook) and Twining, Baines, Fisher *et al.* studies



Absorption Differential phase

Diatom imaged at APS 2-ID-E (30 µm image field). B. Hornberger, C. Jacobsen (Stony Brook), and S. Vogt, J. Maser, D. Legnini (APS)

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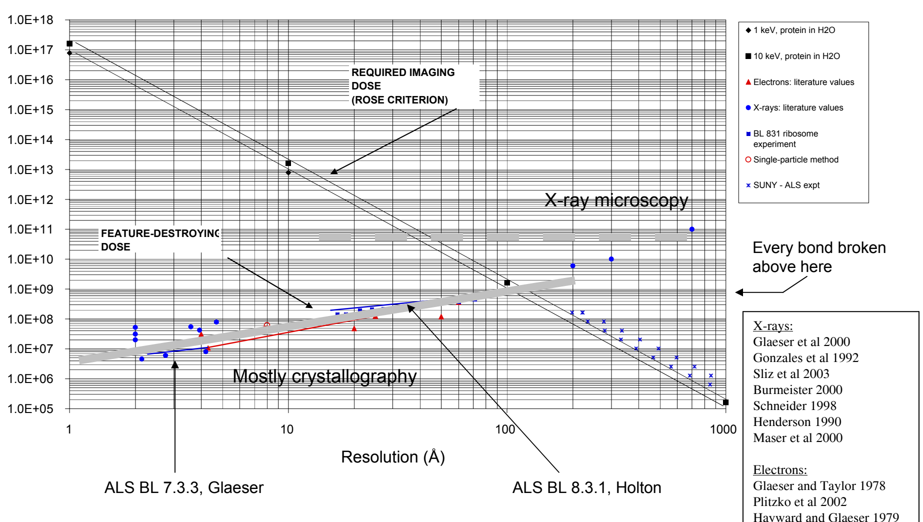
DOSE-RESOLUTION RELATIONSHIP FOR 3D IMAGING OF FROZEN-HYDRATED SAMPLES



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(Malcolm Howells, LBL)

Group effort!

Names in red are part of diffraction imaging effort

- Faculty: Chris Jacobsen and Janos Kirz
- Senior research support specialist: Sue Wirick
- Students: Tobias Beetz (now BNL), Holger Fleckenstein, Benjamin Hornberger, Xiaojing Huang, Bjorg Larson, Enju Lima, Mirna Lerotić, Ming Lu, Huijie Miao, David Shapiro
- Guest scientist: David Sayre
- Brookhaven National Lab: Aaron Stein, John Warren
- Cornell University: Veit Elser and Pierre Thibault
- Lawrence Berkeley Lab: Malcolm Howells
- Lucent Technologies/NJNC: Don Tennant
- Support: NIH, NSF, DoE, NASA

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