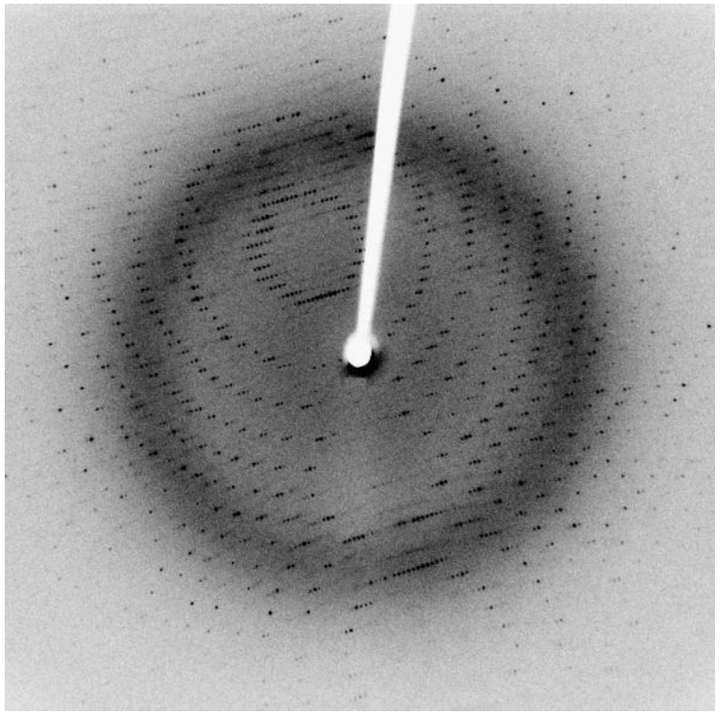
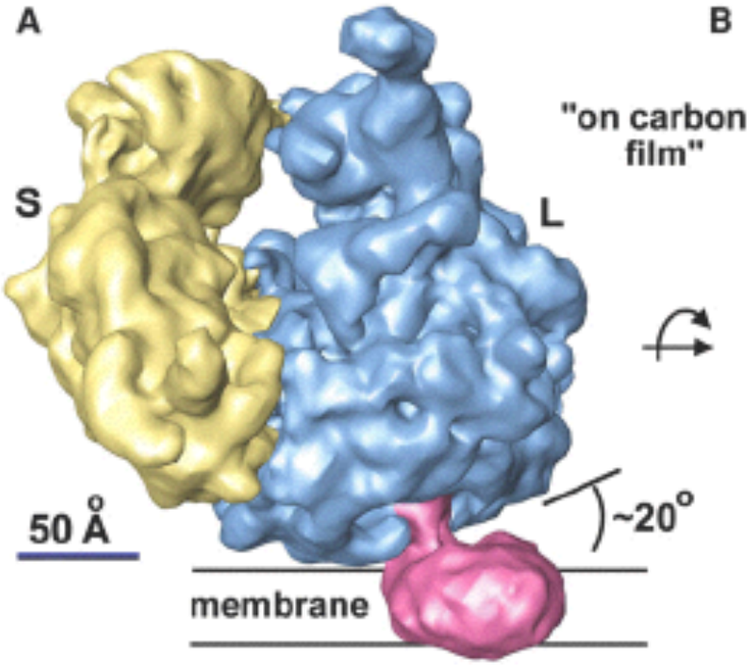
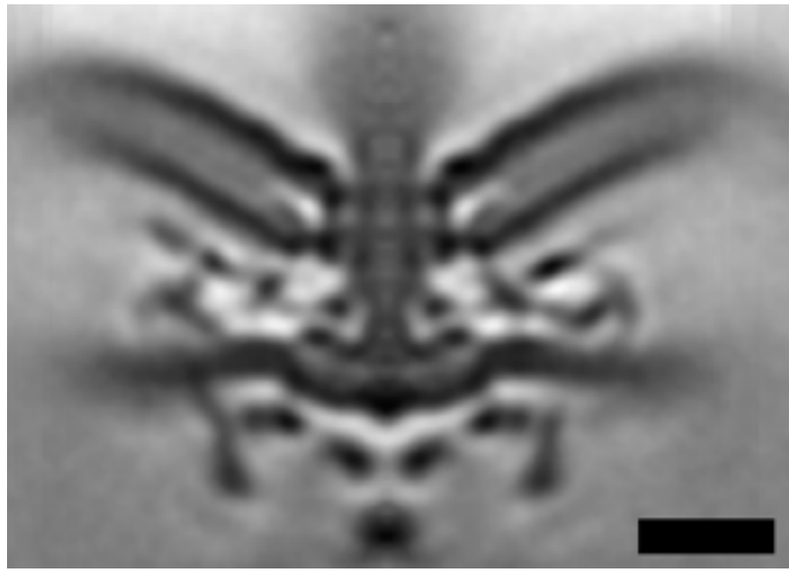
Introduction to structural biology techniques



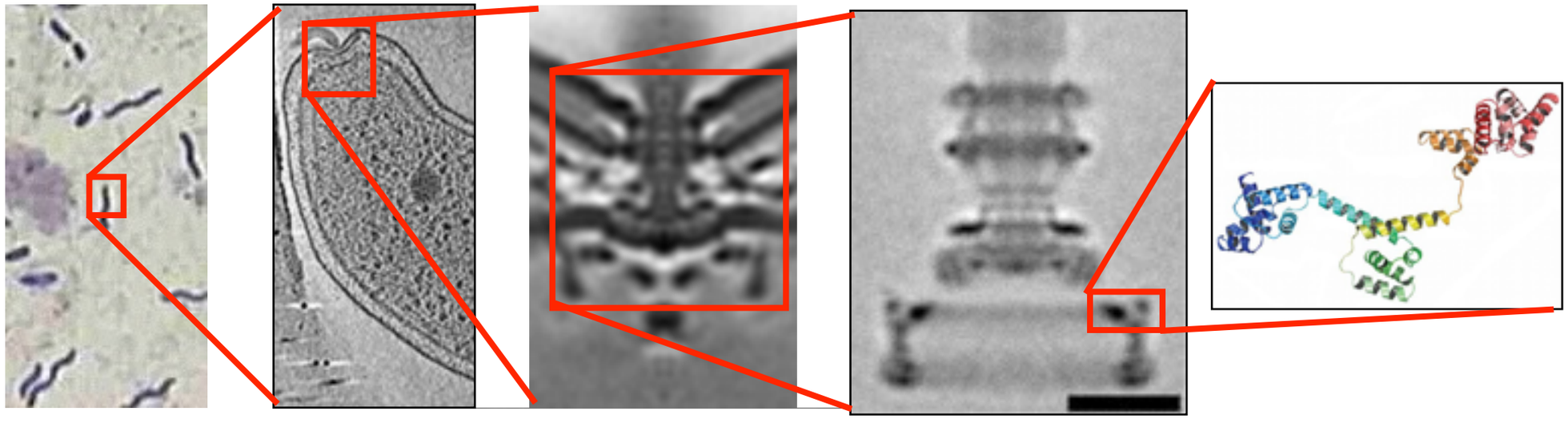


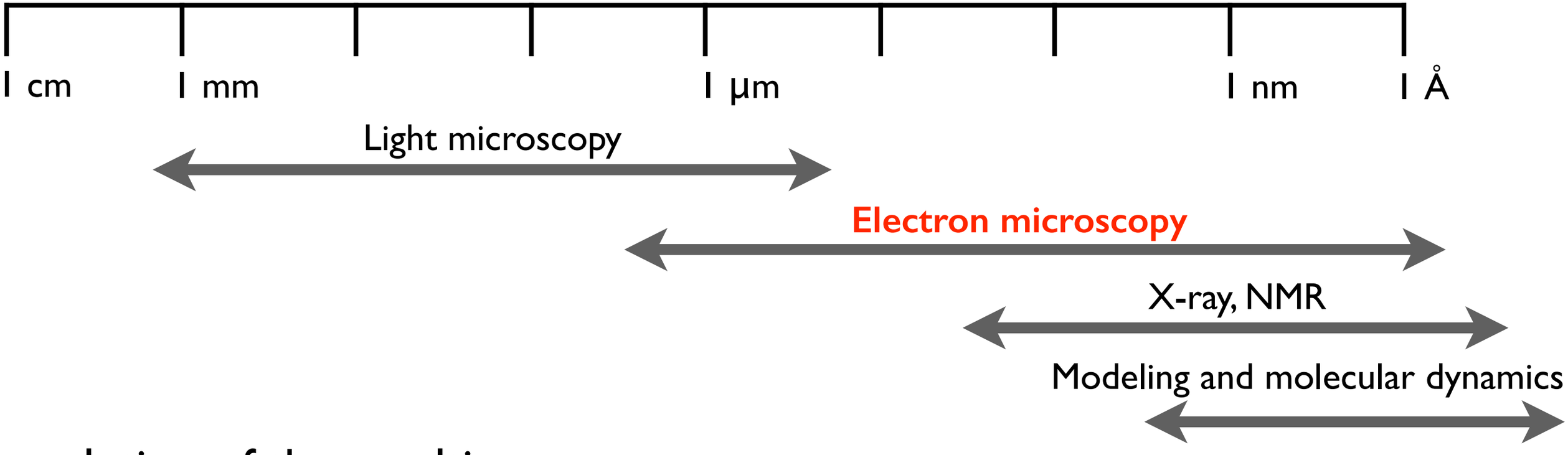


James C. Gumbart
Georgia Institute of Technology

NIH Hands On Workshop | Atlanta | November 7th, 2014

Structural biology continuum





resolution of the resulting image is limited by the wavelength of light used

$$d = \frac{\lambda}{2(n \sin \theta)}$$ **Abbe diffraction limit**

Four radiation types

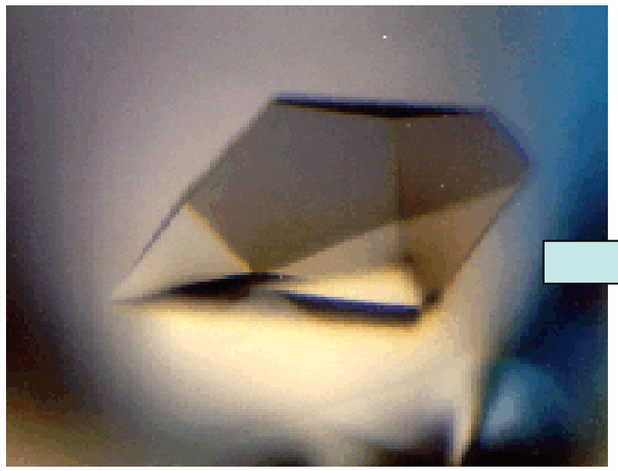
	Advantages	Disadvantages
Visible light	Low sample damage Easily focused Visible by eye	Long wavelengths
X-rays	Small wavelength (Angstroms) Good penetration	Hard to focus Damage sample
Electrons	Small wavelength (pm!) Can be focused	Poor penetration Damage sample
Neutrons	Low sample damage Small wavelength (pm)	How to produce? How to focus?

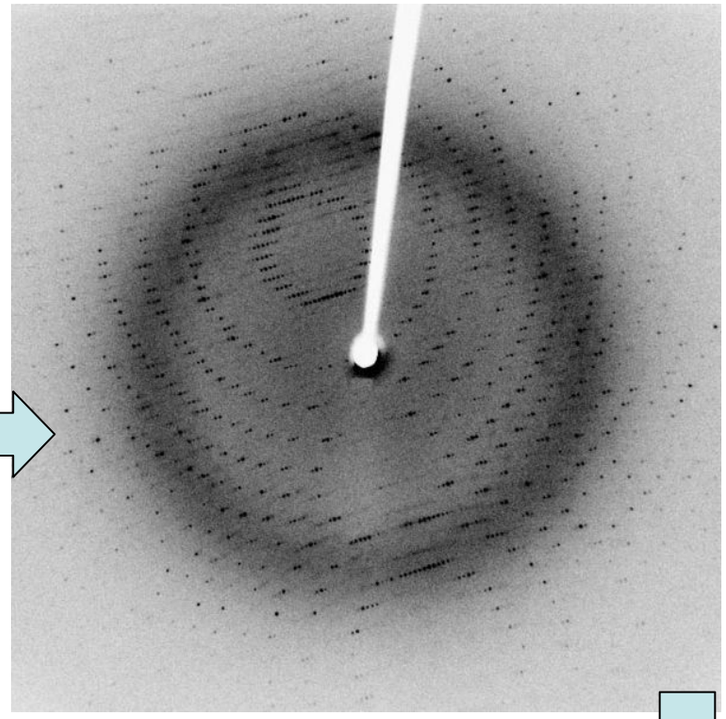
de Broglie, defends thesis in 1924, wins Nobel Prize in 1929

de Broglie wavelength: $\lambda = \frac{h}{p}$

10 keV electron → 0.01 nm wavelength

X-ray crystallography

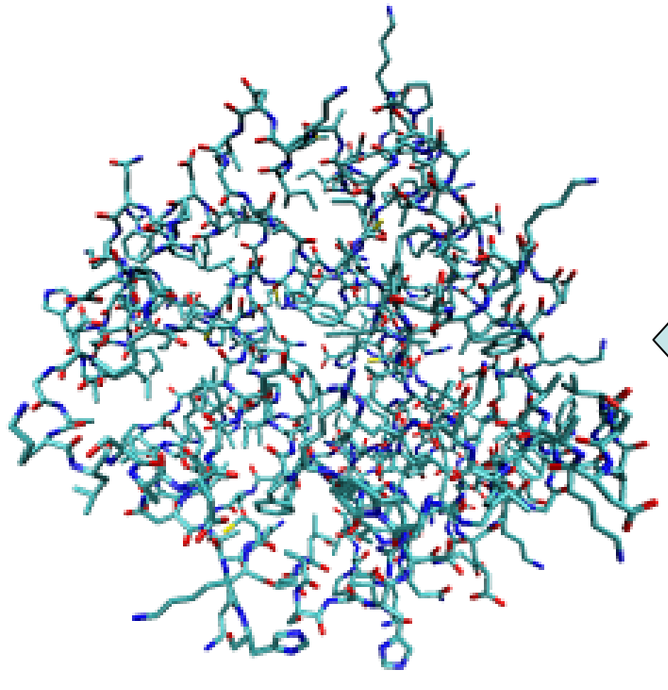


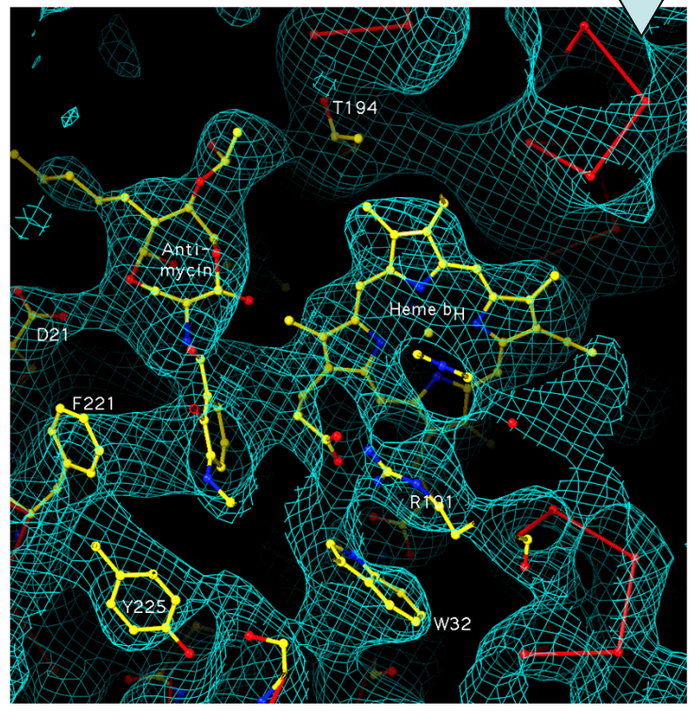


best resolution produced by X-rays, which have wavelengths on the scale of Ångstroms

(inverse) Fourier transform

$$f(\mathbf{r}) = \int \frac{d\mathbf{q}}{(2\pi)^3} F(\mathbf{q}) e^{i\mathbf{q}\cdot\mathbf{r}}$$

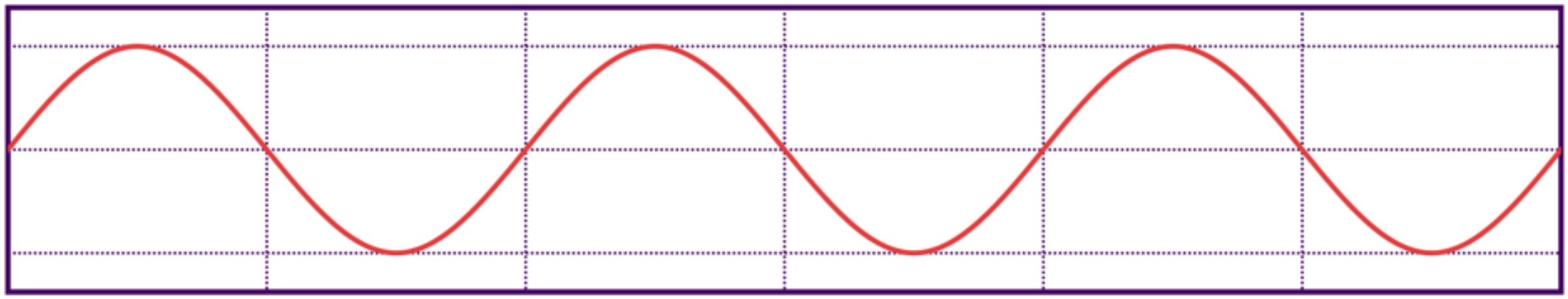




RCSB PDB
PROTEIN DATA BANK

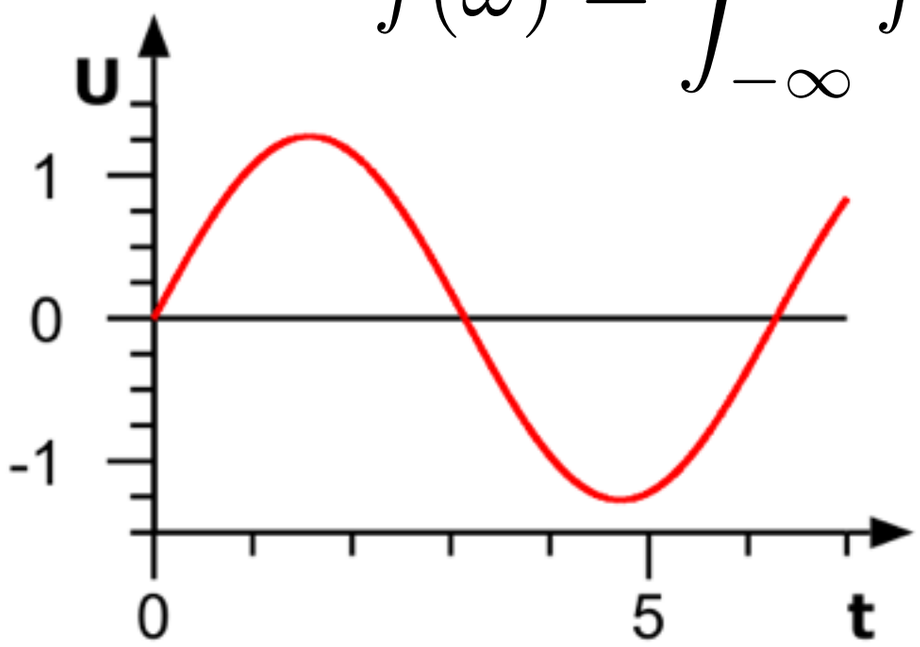
Fourier transforms

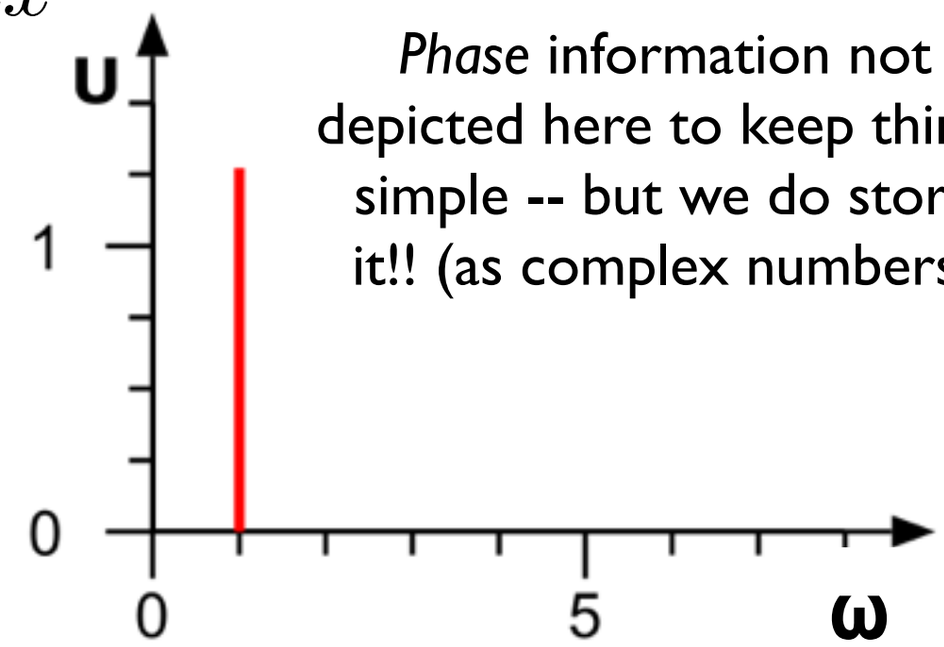
properties defining a sine wave:
amplitude, wavelength (or frequency), phase shift, and direction



Waves can be represented in frequency ("*reciprocal*") space

$$\hat{f}(\omega) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i \omega x} dx$$

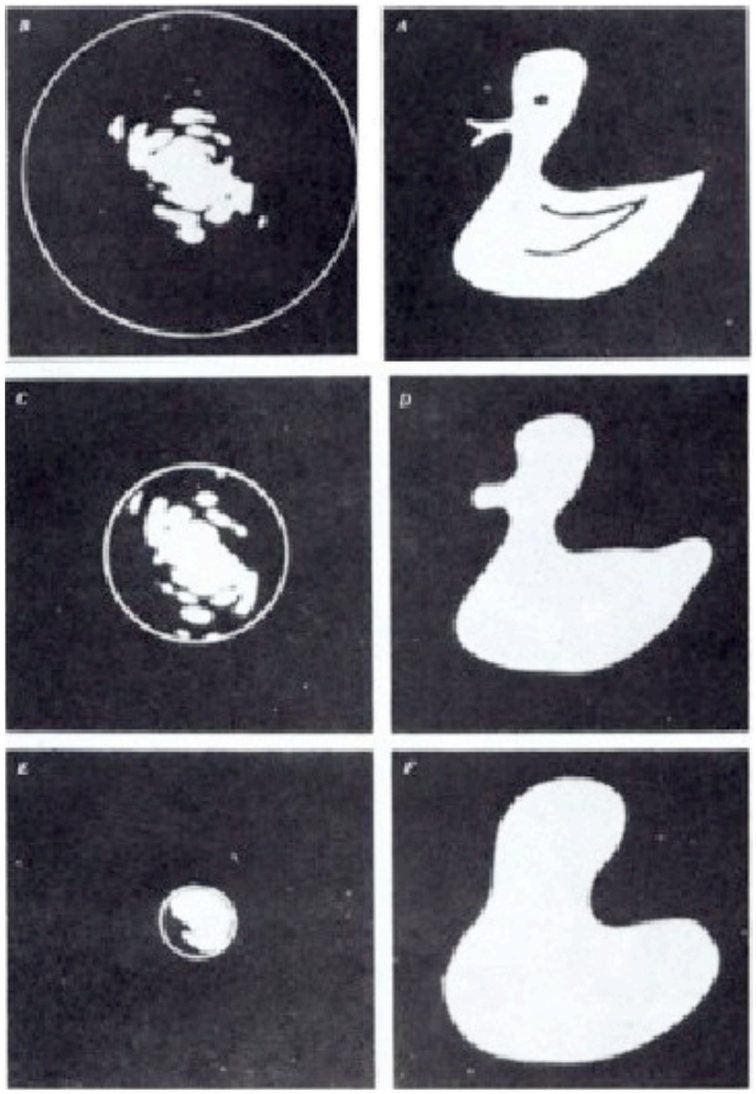


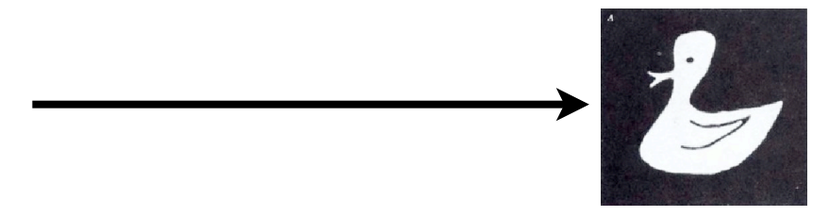


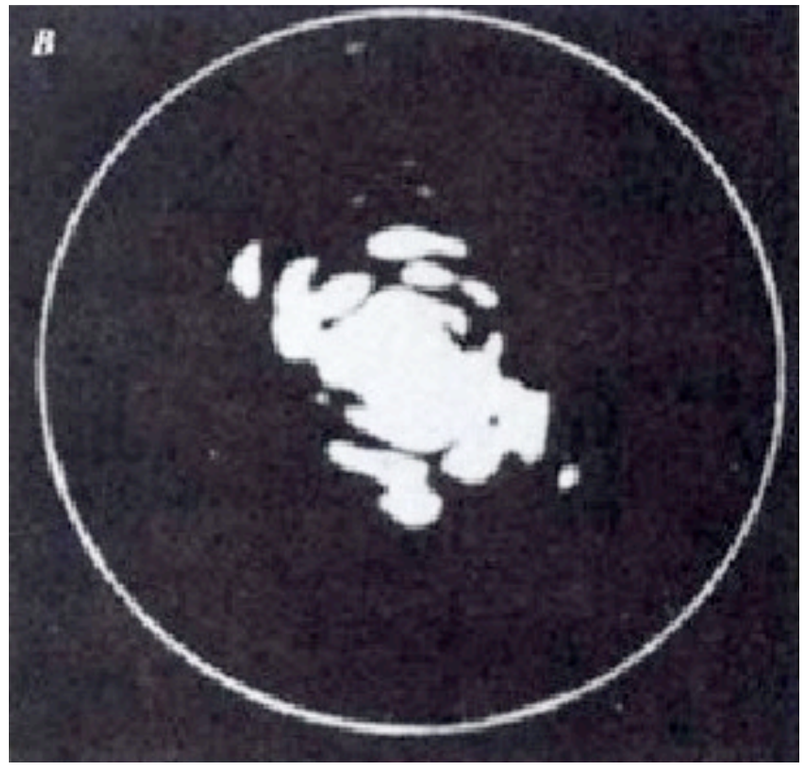
Phase information not depicted here to keep things simple -- but we do store it!! (as complex numbers)

diffraction patterns

diffracted X-rays (or electrons) produce
a Fourier transform of the original object





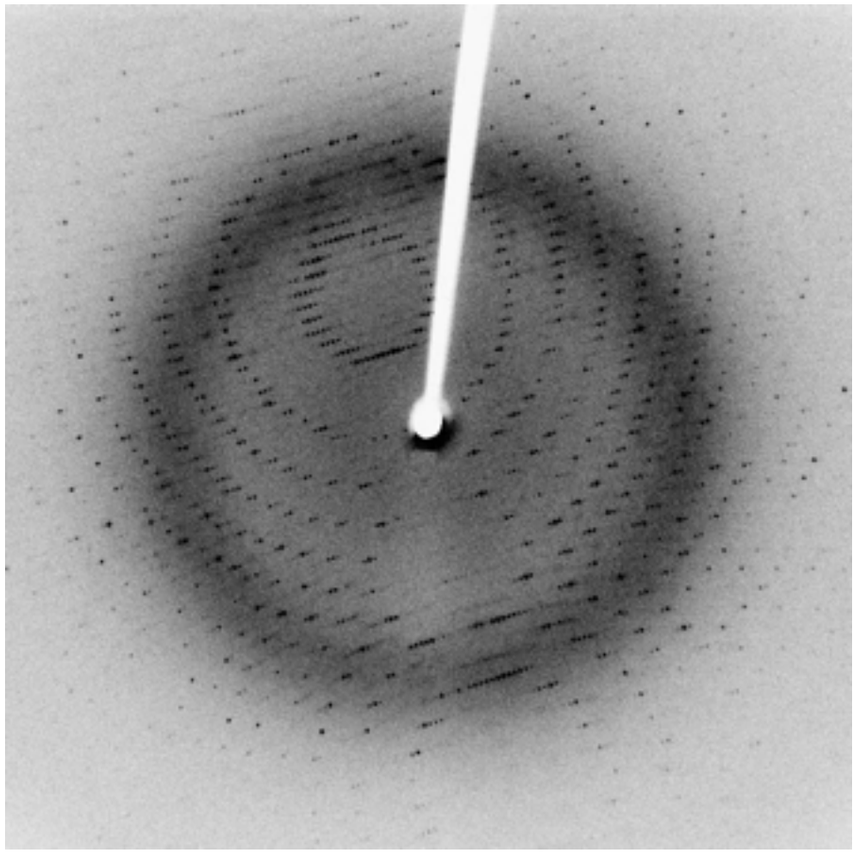


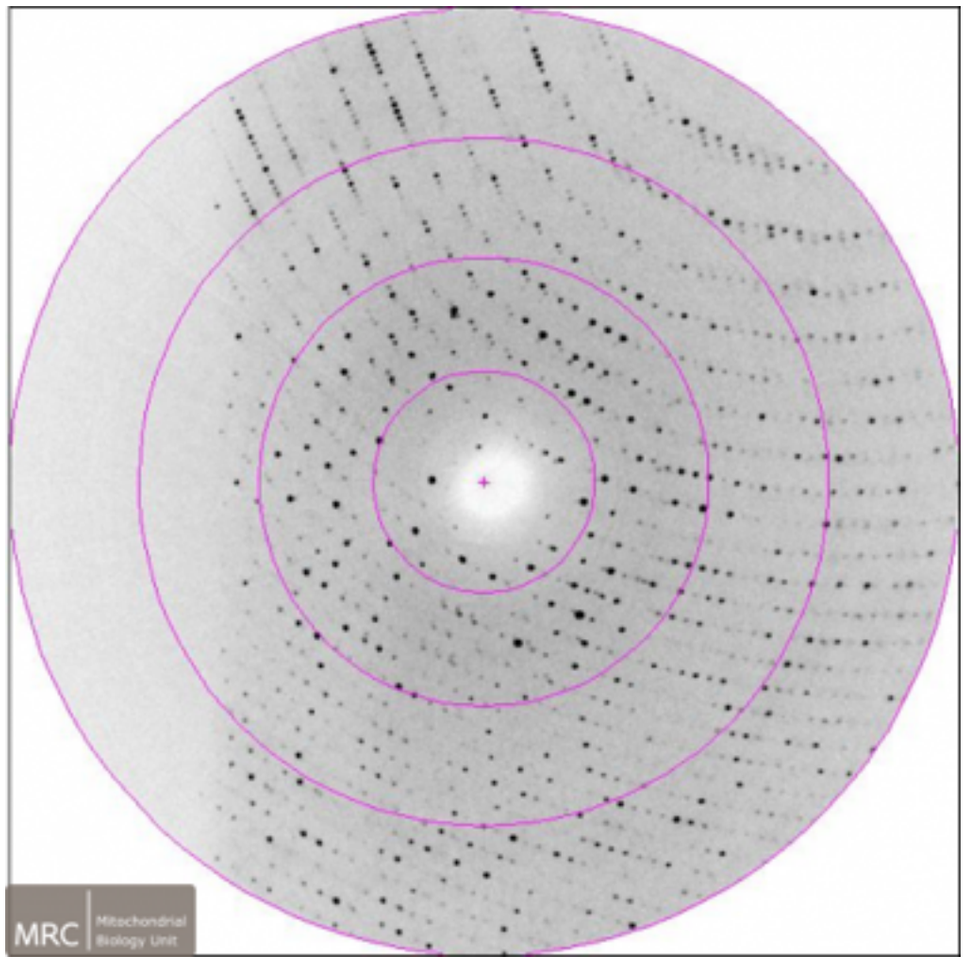
intensity of diffracted photons
(but not phases!)

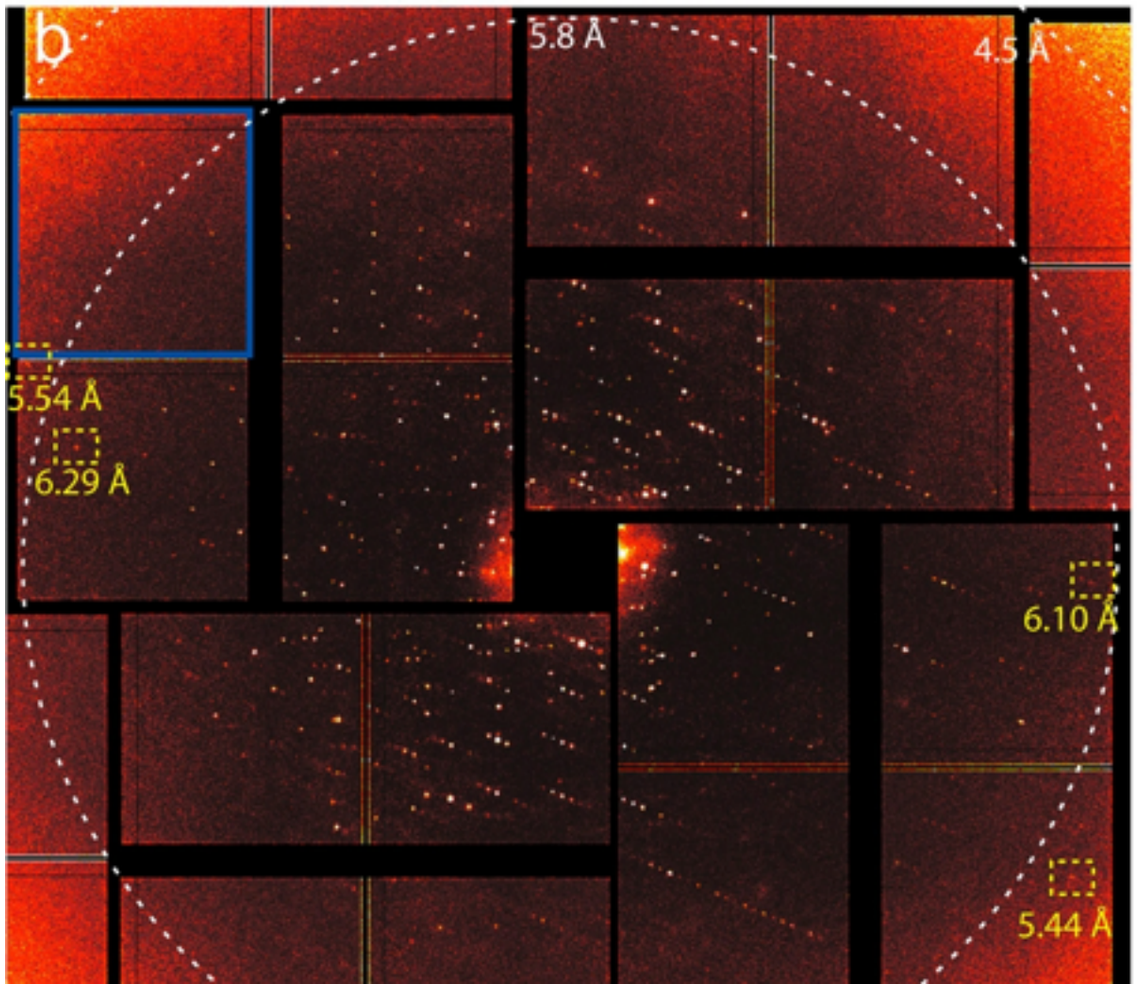
High "frequency" components contribute the details, and appear furthest from the origin

diffraction patterns

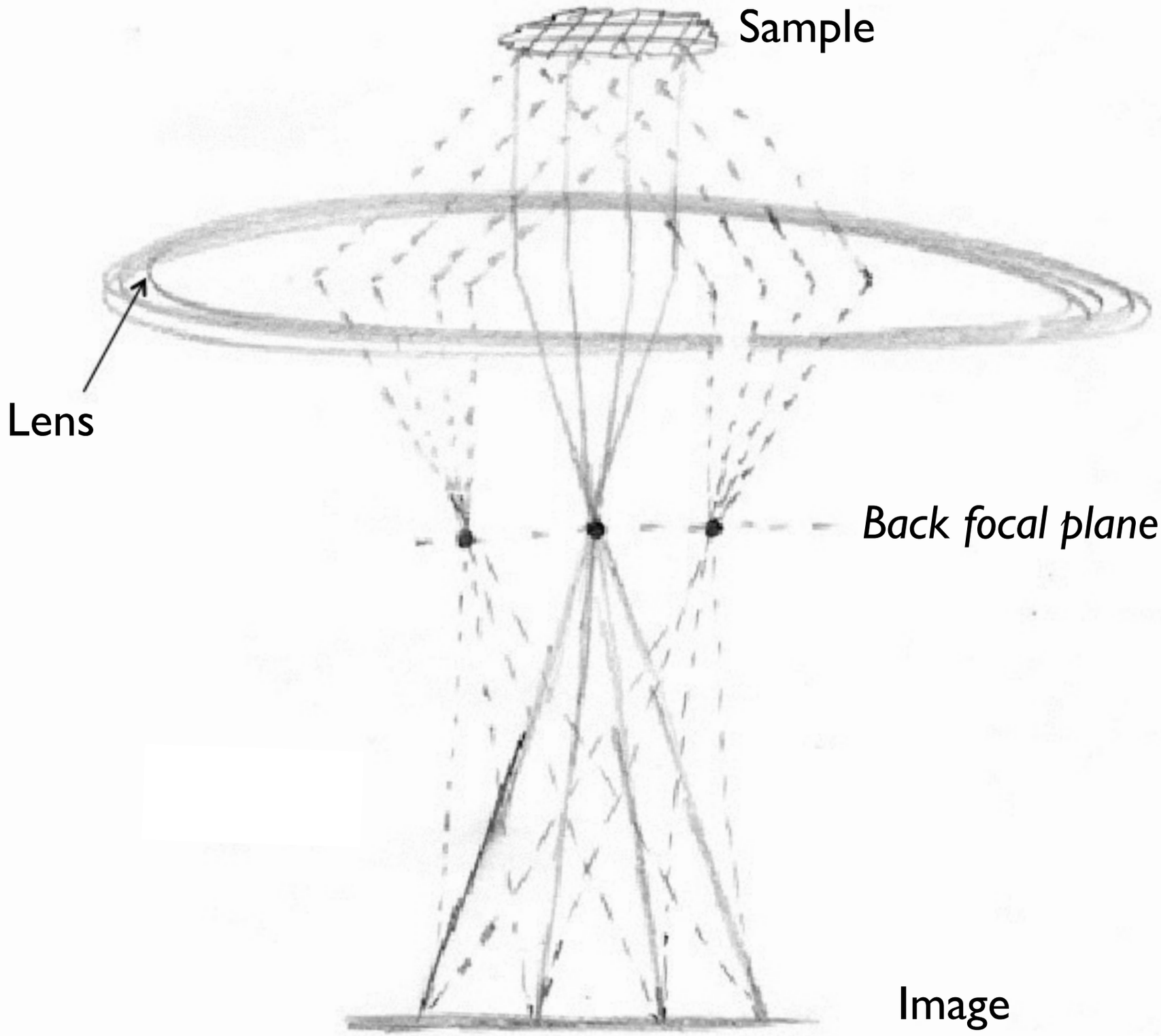
resolution determined by presence of data far from origin



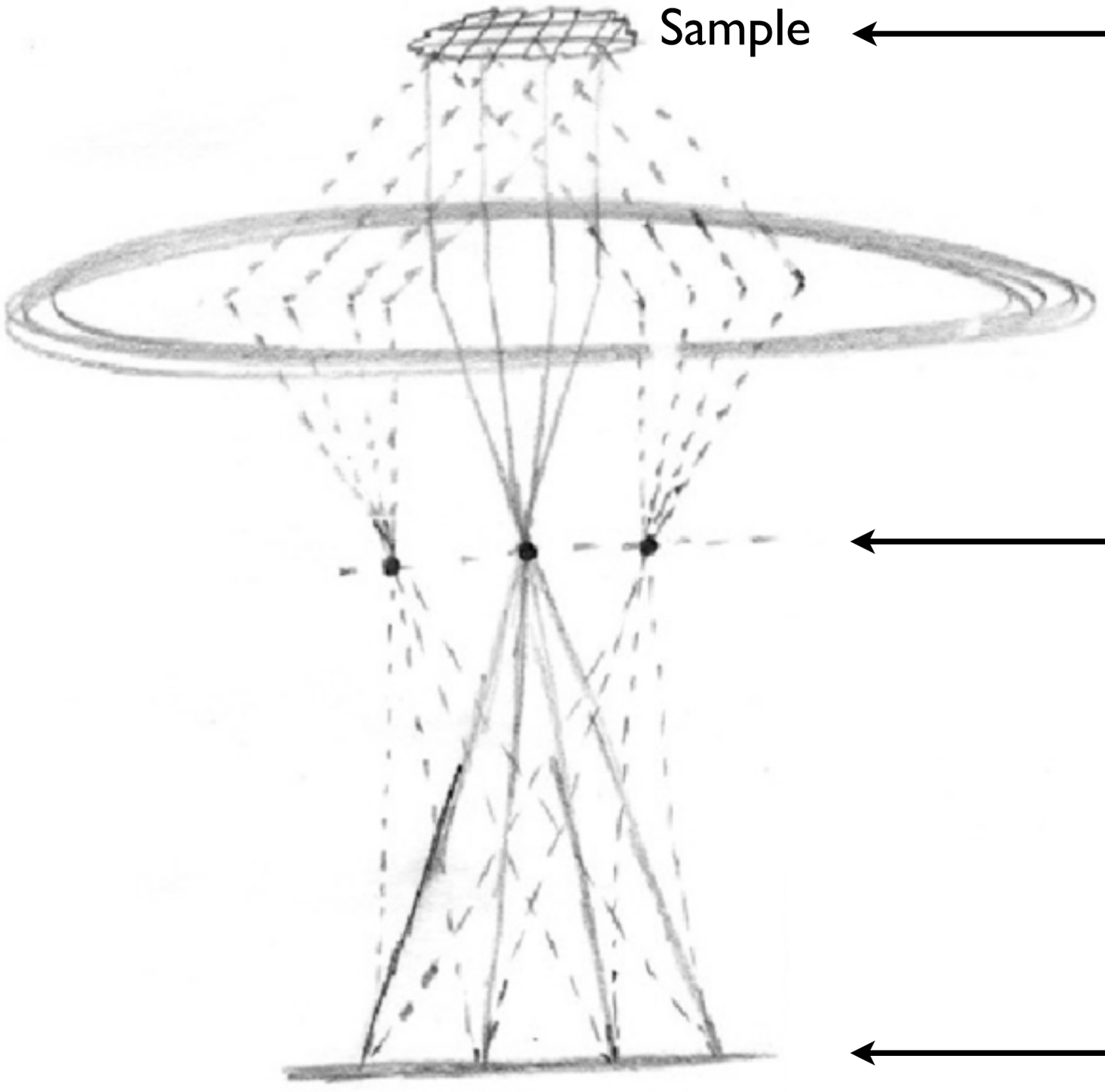




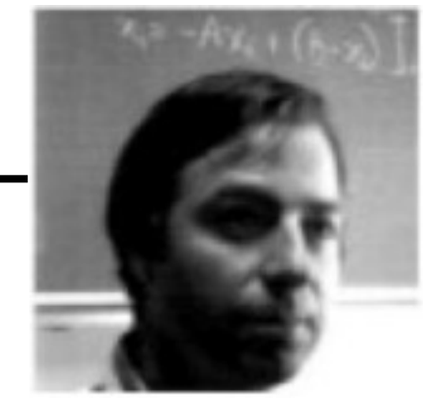
Before inverting reciprocal space back into an image, the diffraction pattern (i.e. Fourier transform) is focused at the **back focal plane**:



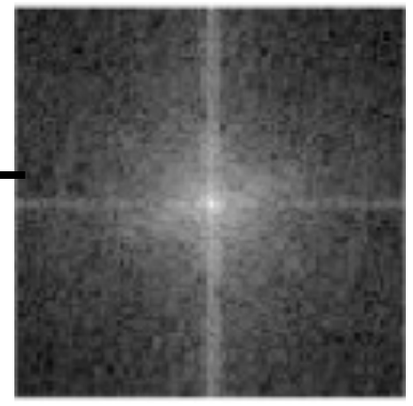
Sample



Lens



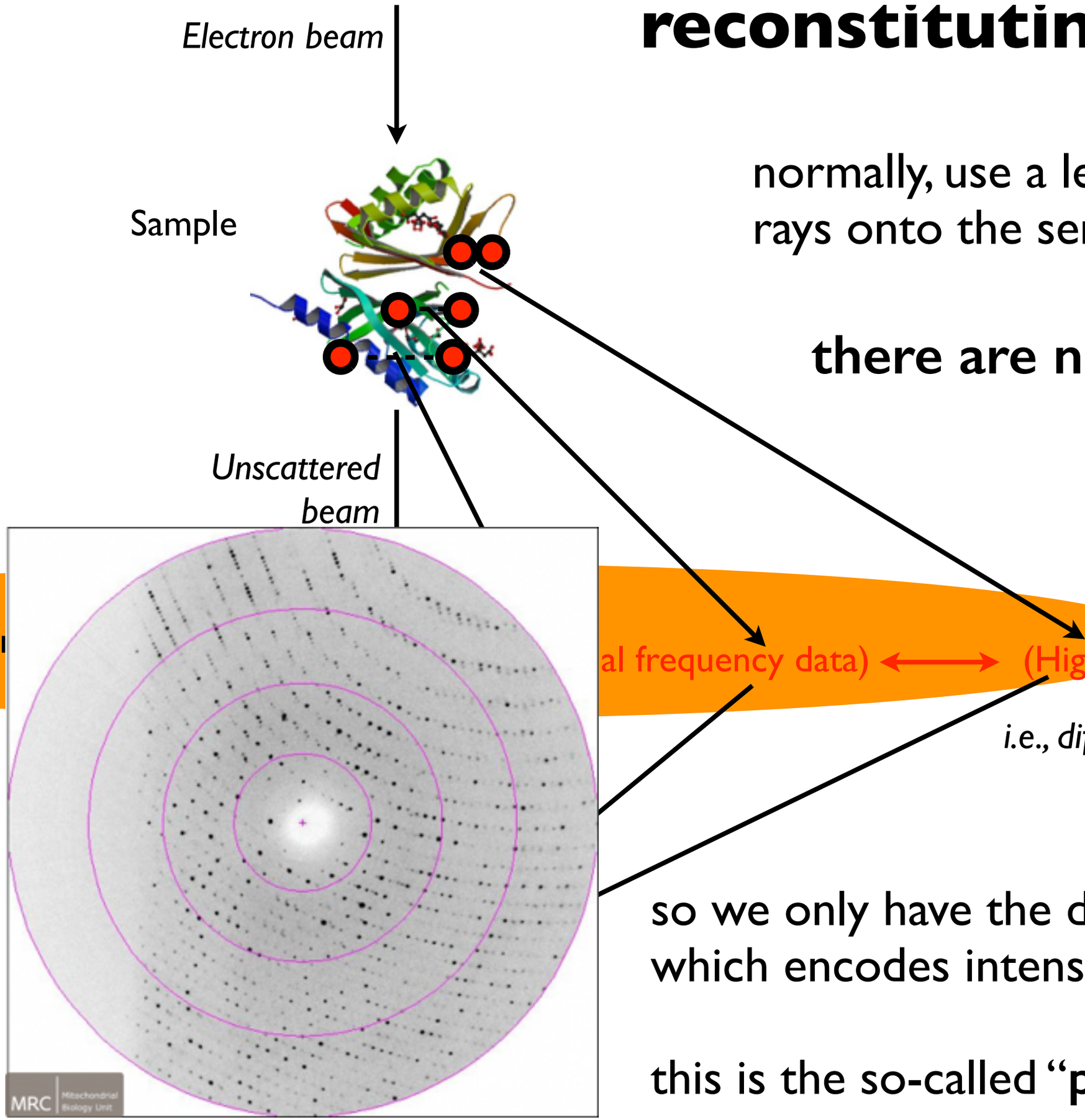
Sample



Back focal plane

Magnified image

reconstituting the image



normally, use a lens to refocus rays onto the sensor, *but*...

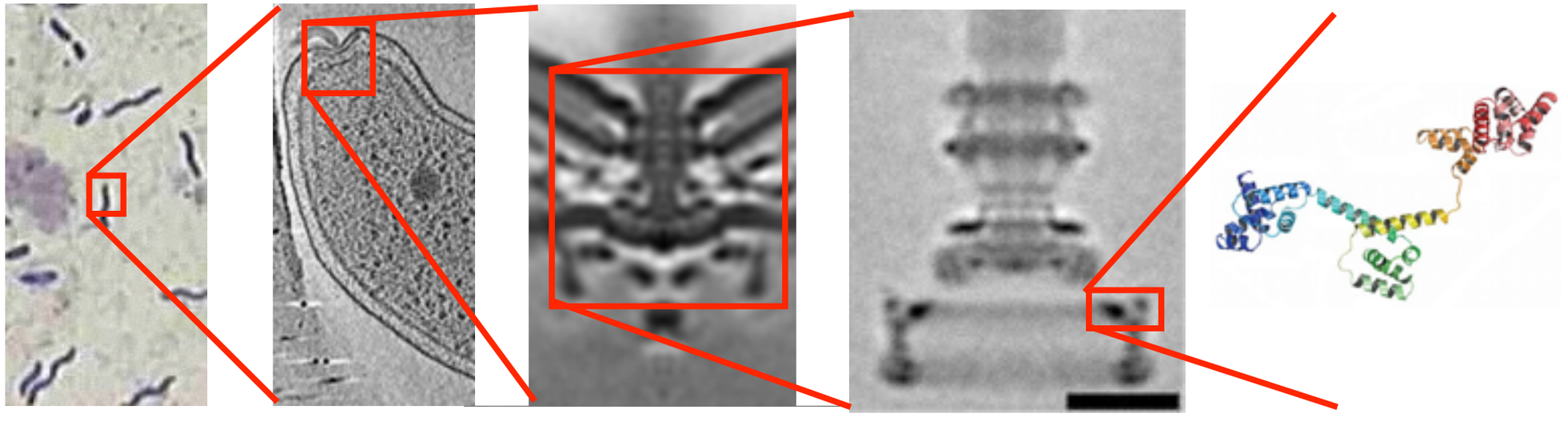
there are no X-ray lenses!!!

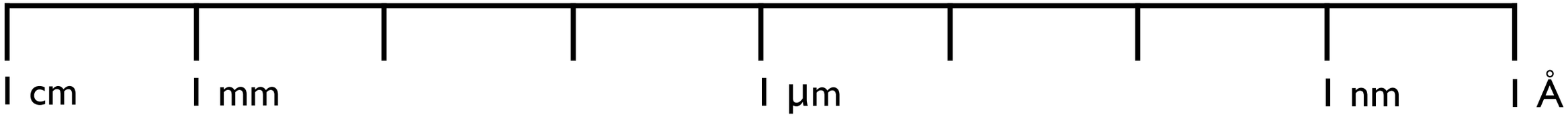
i.e., diffraction = Fourier transform

so we only have the diffraction pattern, which encodes intensities, but not phases

this is the so-called "**phase problem**"

Structural biology continuum





"Electron microscopy"

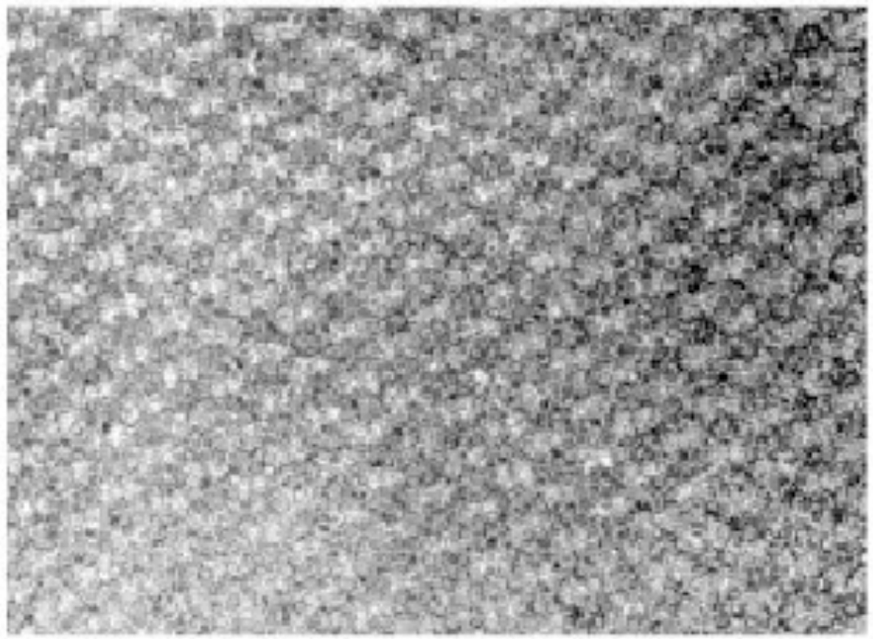
Electron cryo-tomography

Single particle analysis

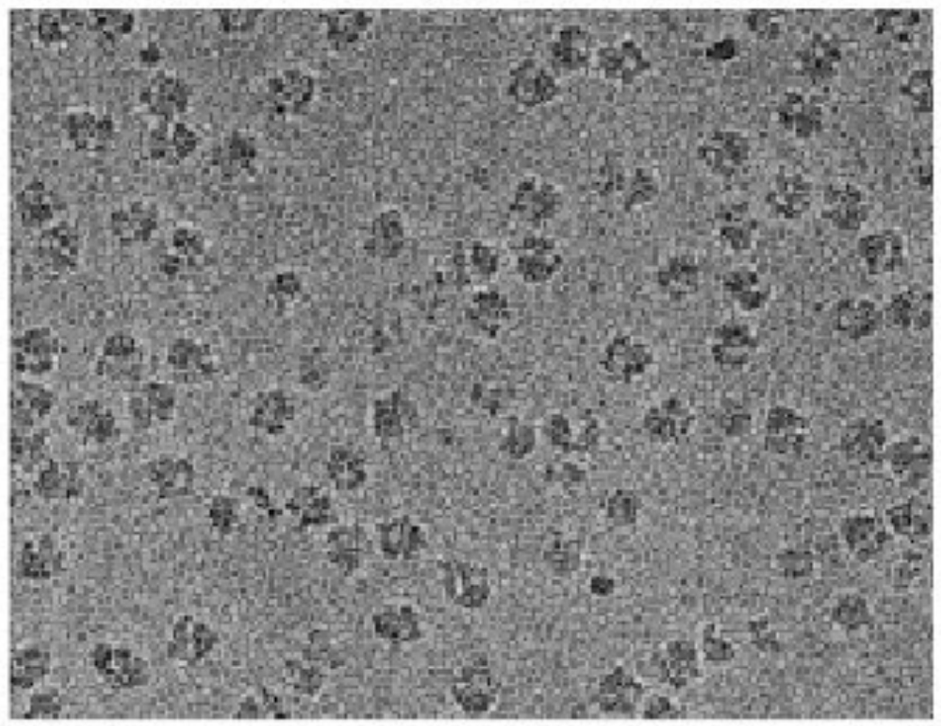
2-D Electron crystallography

Rough guide to “cryo-EM”:

Three flavors:

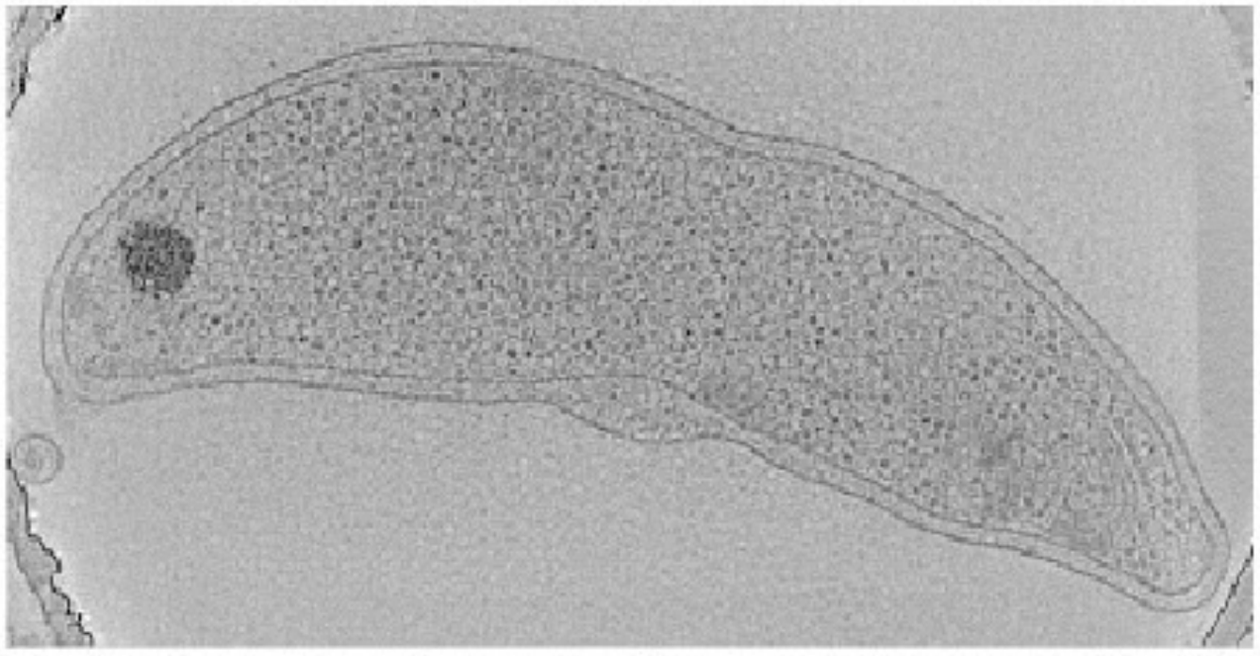


2D electron crystallography

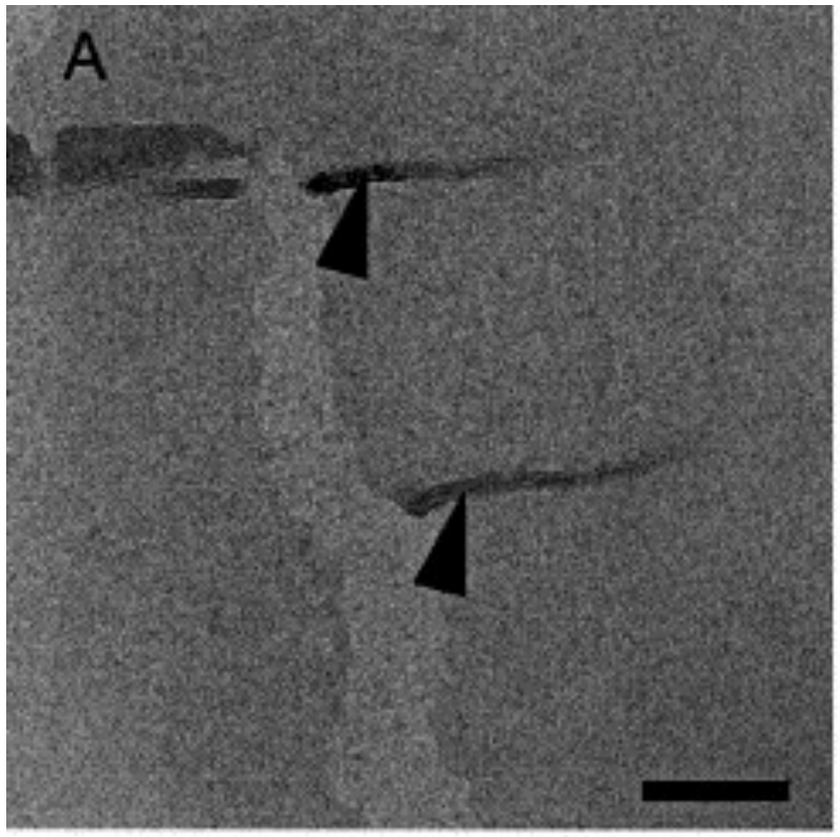


Single particle analysis

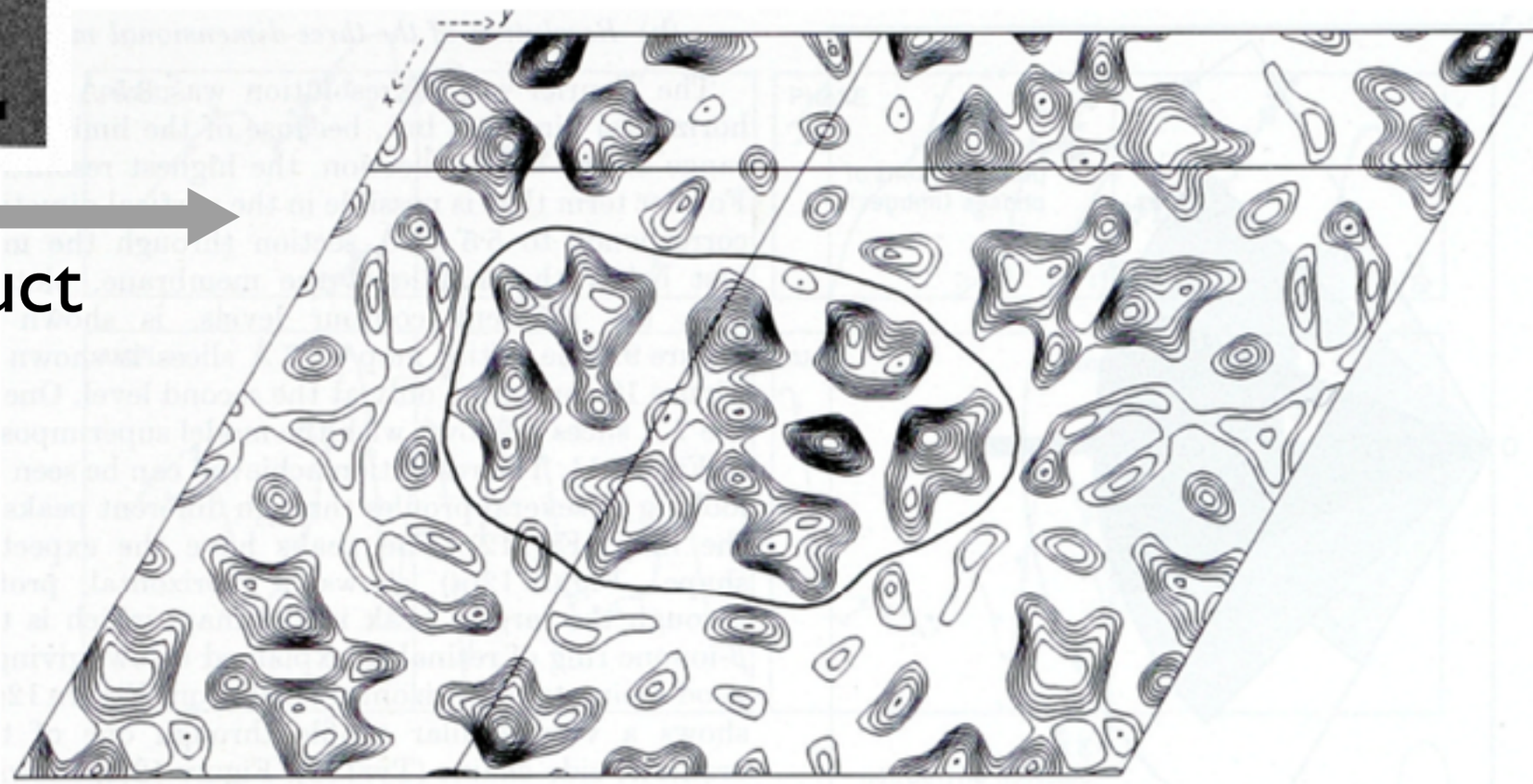
Electron cryo-tomography



2-D electron crystallography



Reconstruct

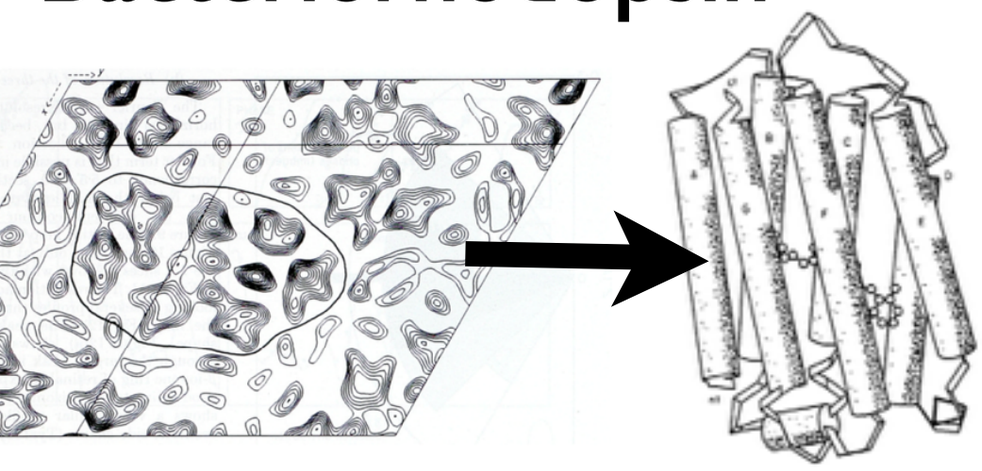


useful because phases aren't irretrievably lost

works better with smaller crystals than X-rays, but must be thin

Example structures

Bacteriorhodopsin

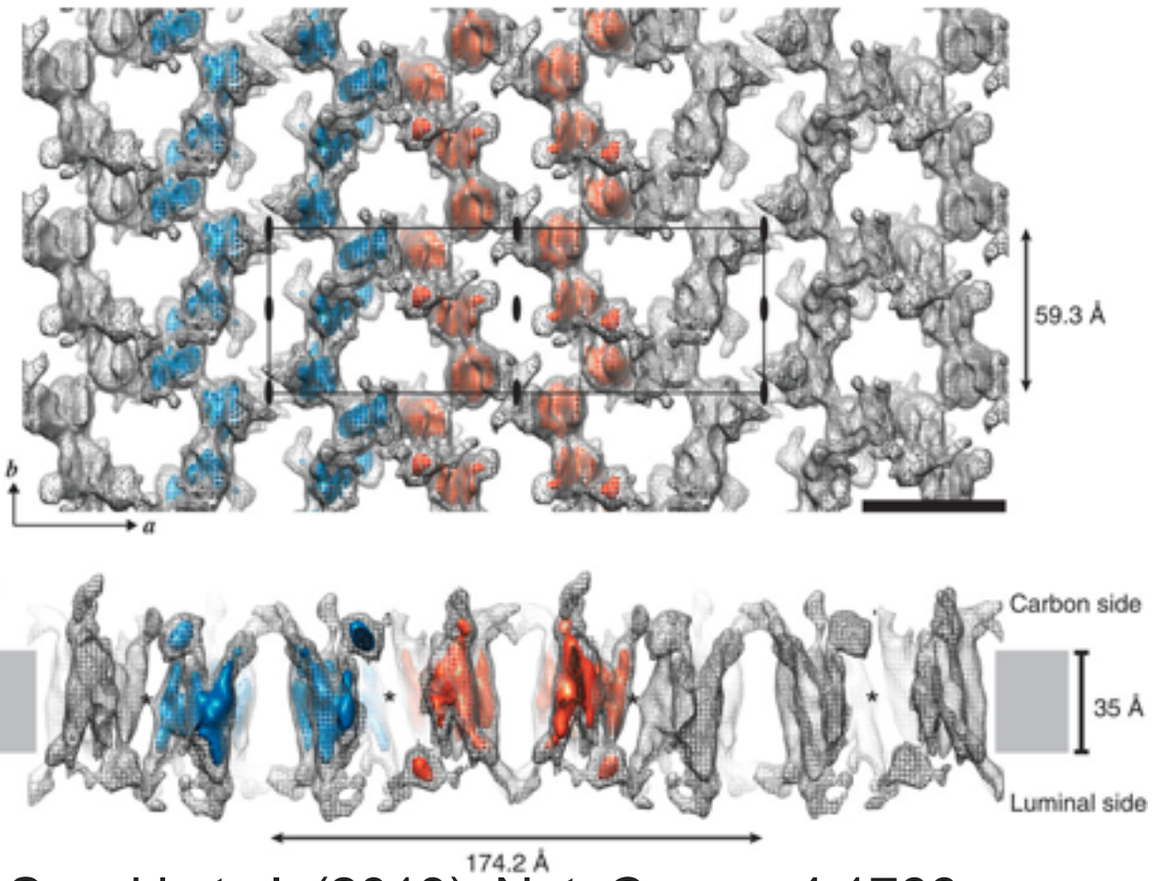


Henderson et al., 1990

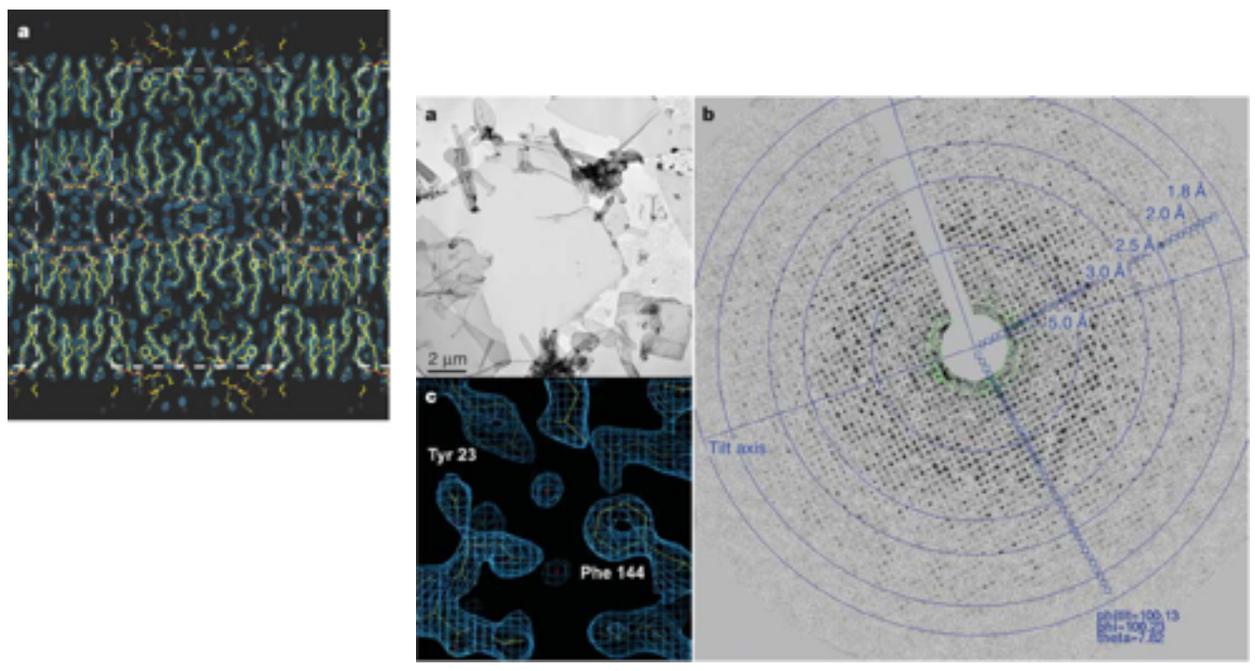
First ever electron crystallography structure, to 3.5 Å.

IP39 (~10Å)

Aquaporin 0 (1.9Å)



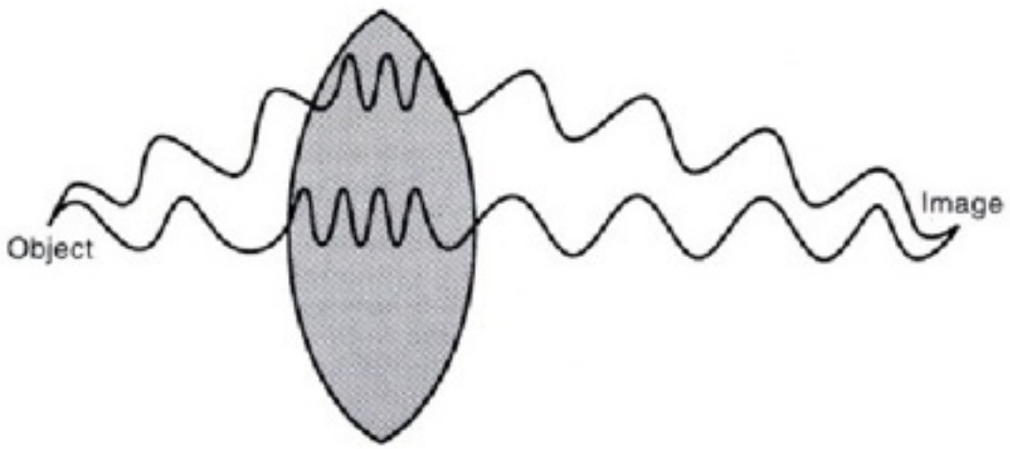
Suzuki et al. (2013) Nat. Comm. 4:1766.

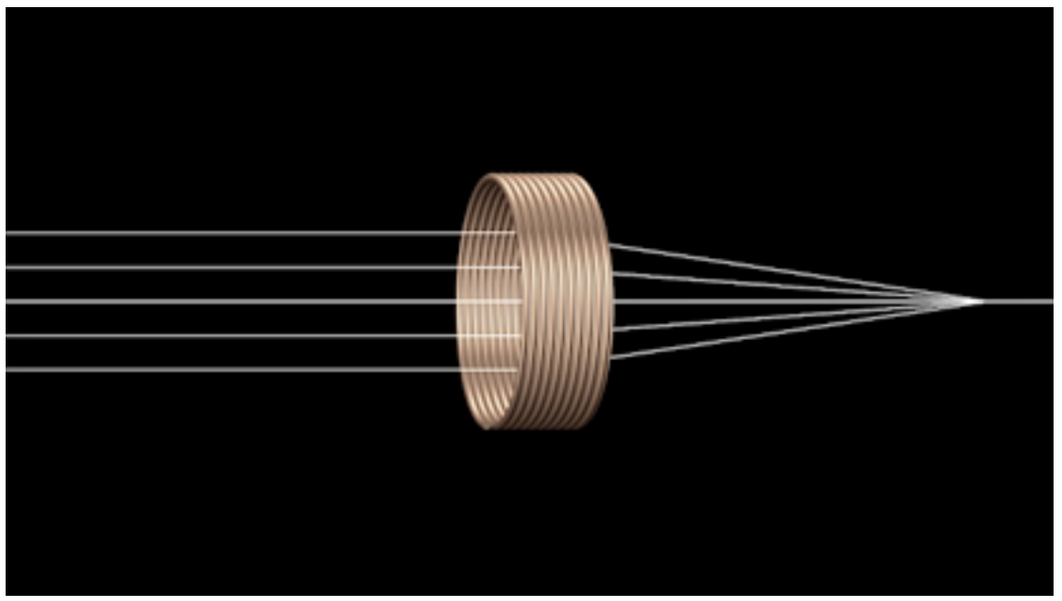


Gonen et al., Nature (2005) 438:633

electron lenses

Lenses "focus" divergent (diffracted) rays, allow production of image (including magnification)

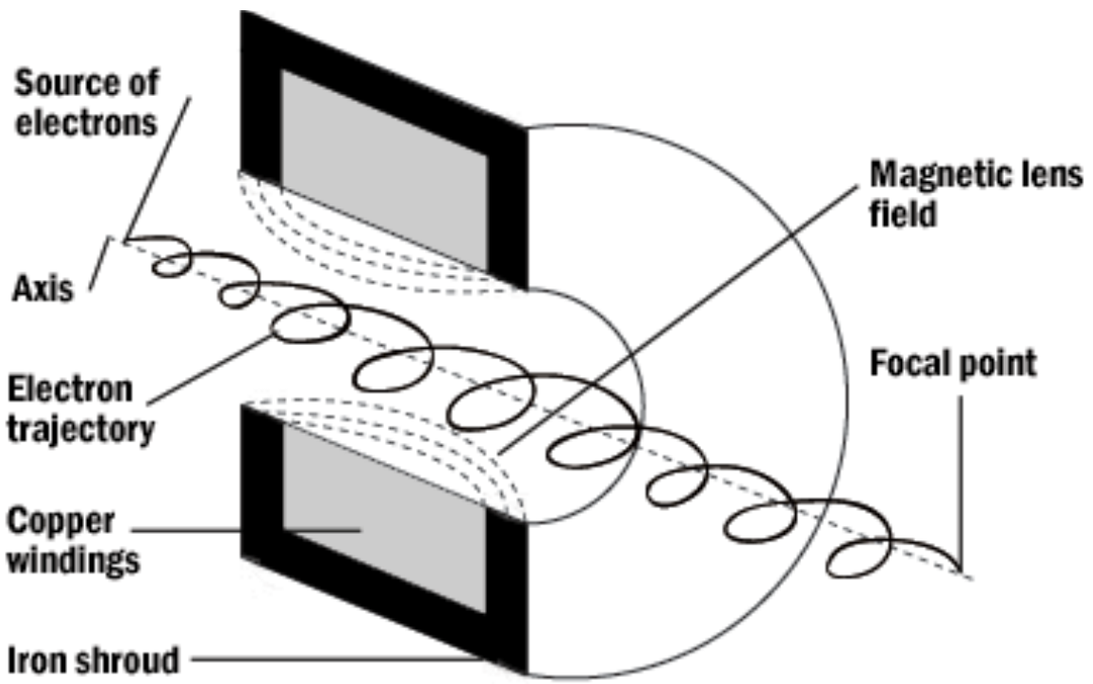




http://www.first-tonomura-pj.net/e/commentary/mechanism/index.html

For electrons, the "**lens**" is actually a **magnetic field**

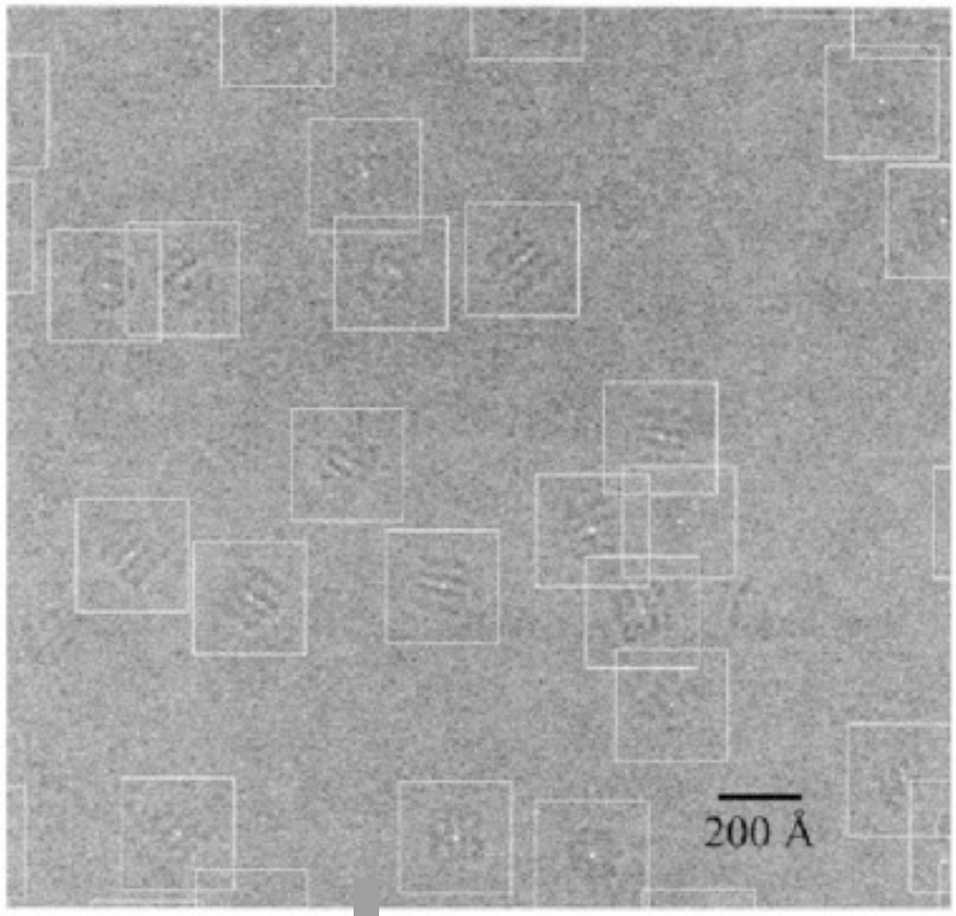
$$\mathbf{F} = -e\,(\mathbf{v} \times \mathbf{B})$$



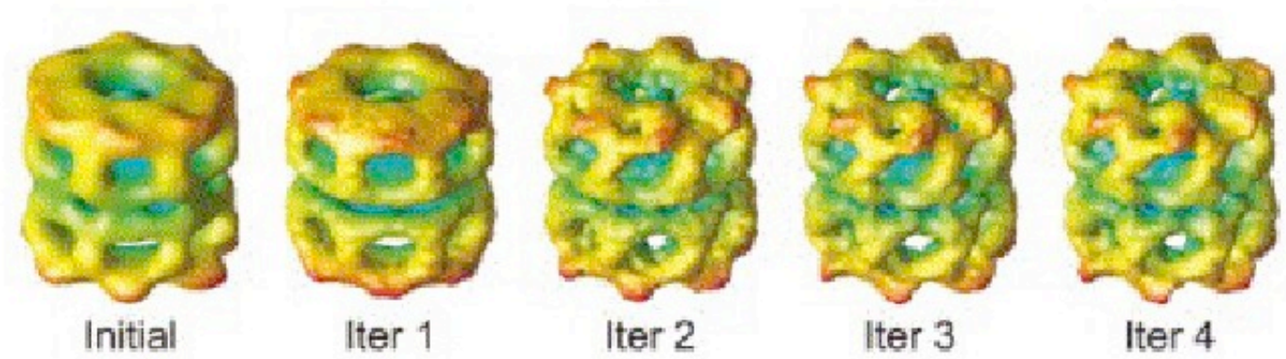
spiraling effect required to focus beam, but introduces unavoidable artifacts

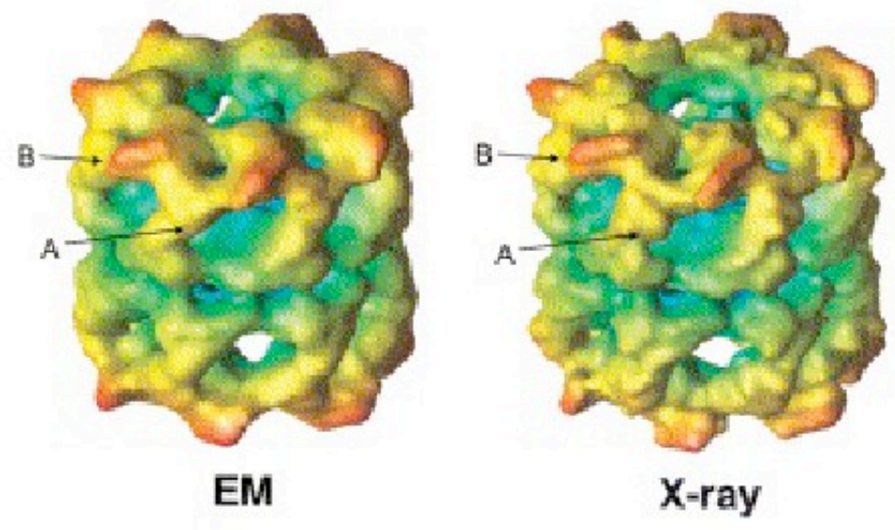
Single particle analysis (cryo-EM)

100 000's of ("identical") 2-D particles



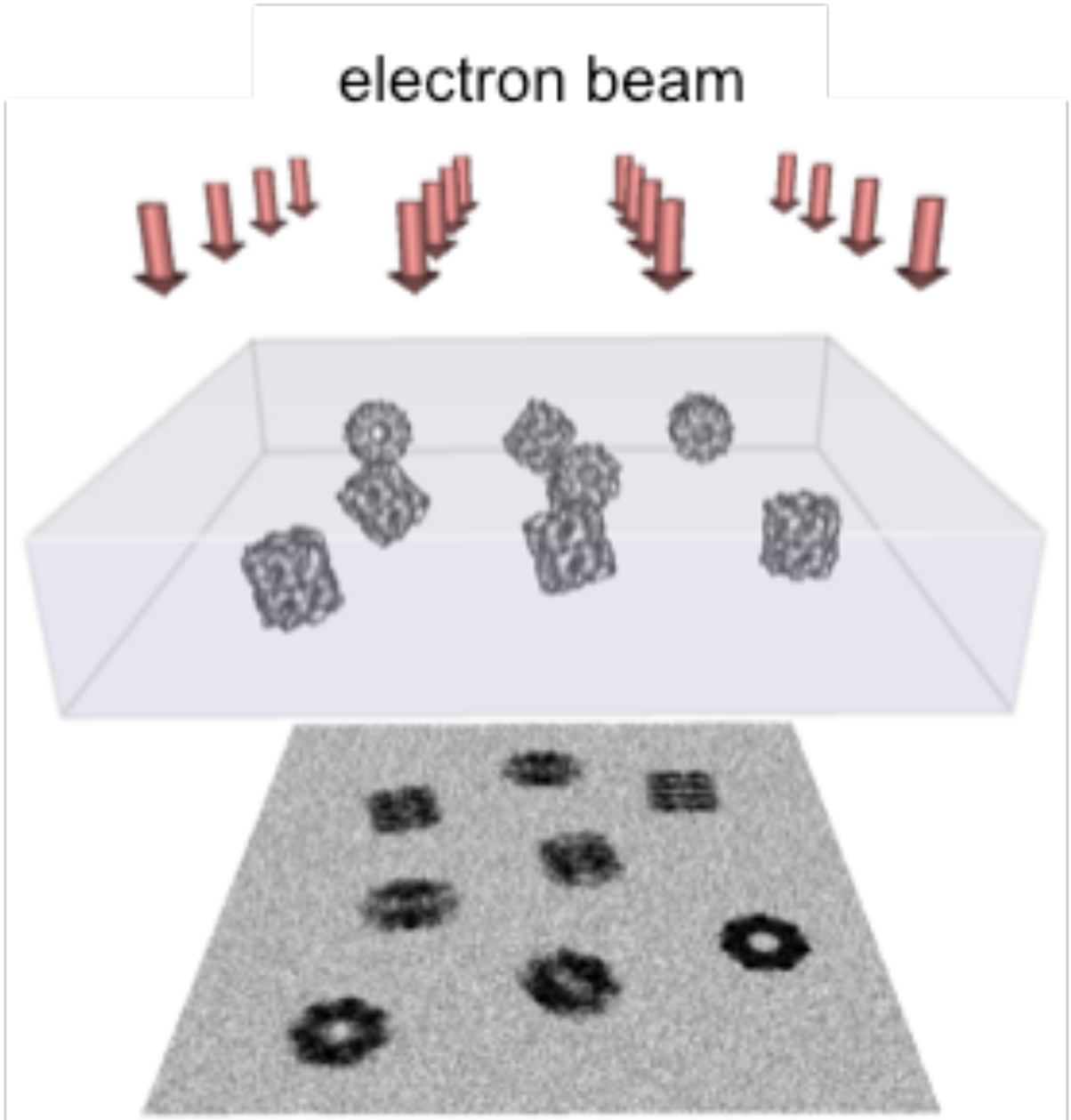
Align and average

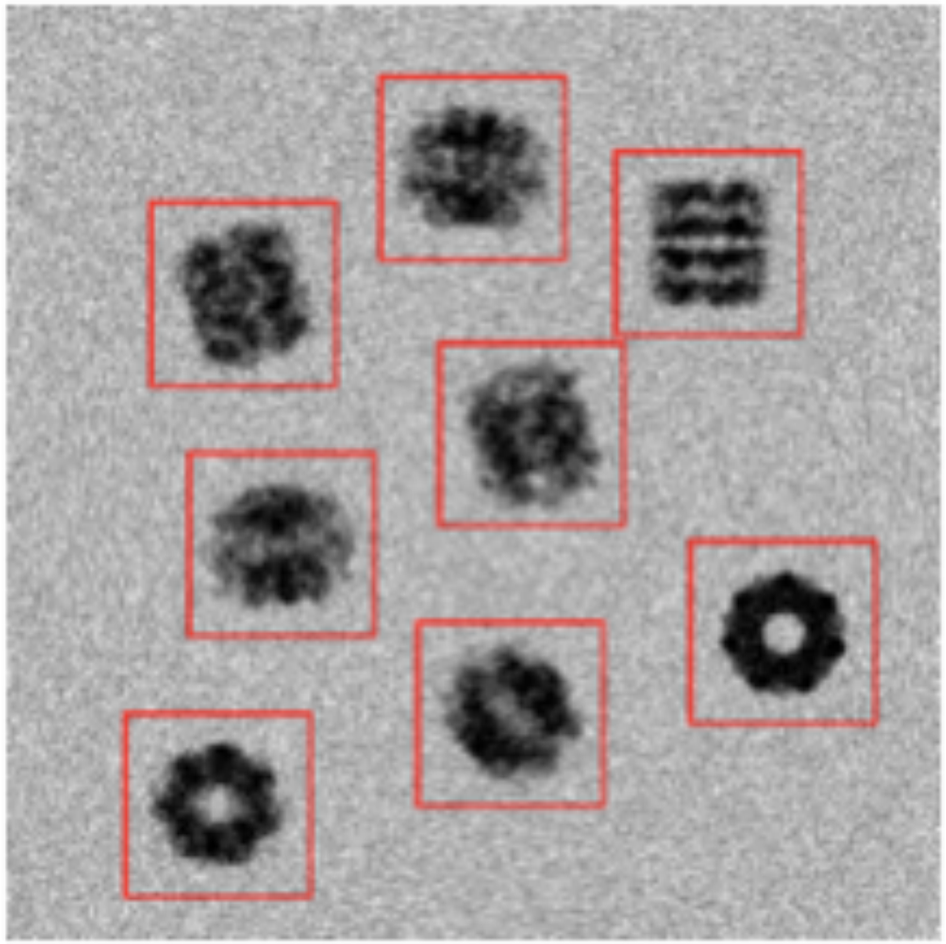




Ludtke et al., JMB 314:253 (2001)

sorting the data

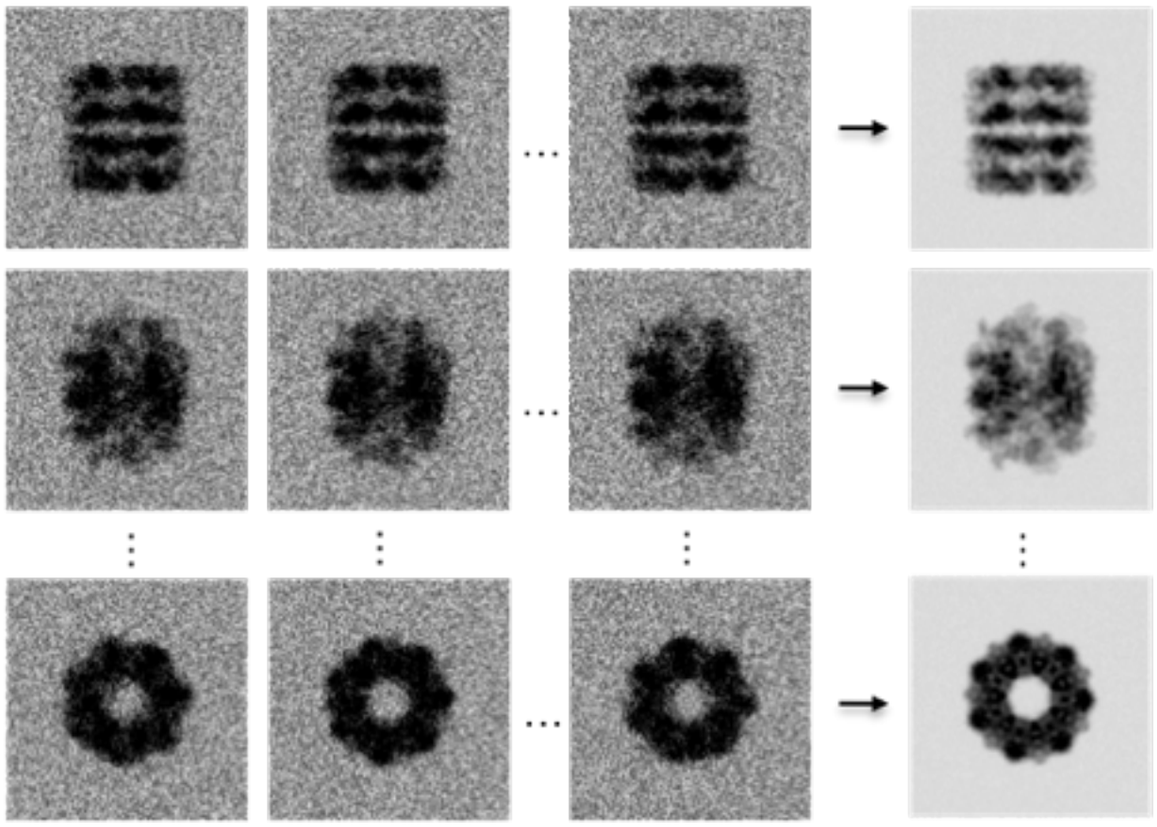


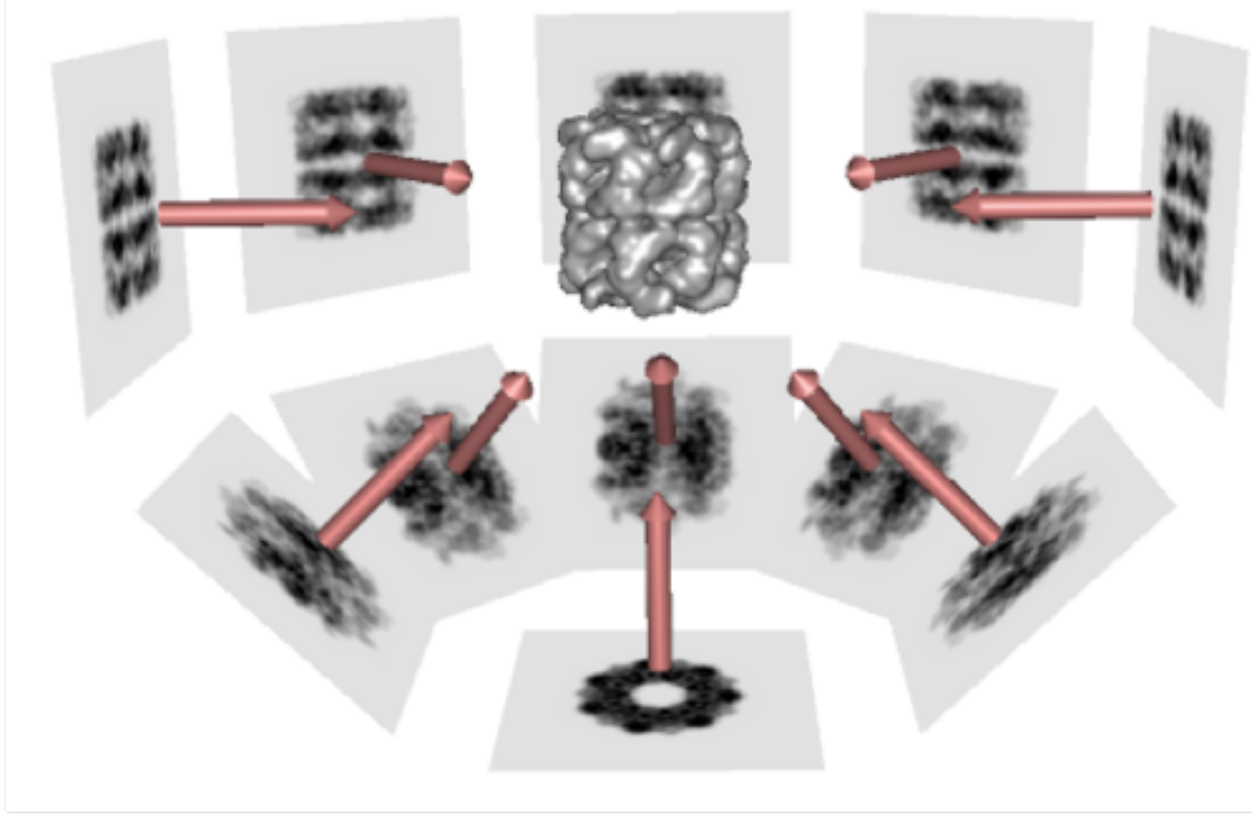


http://people.csail.mit.edu/gdp/cryoem.html

2D images are aligned and sorted computationally into classes representing homogeneous particles and perspectives

Class averages

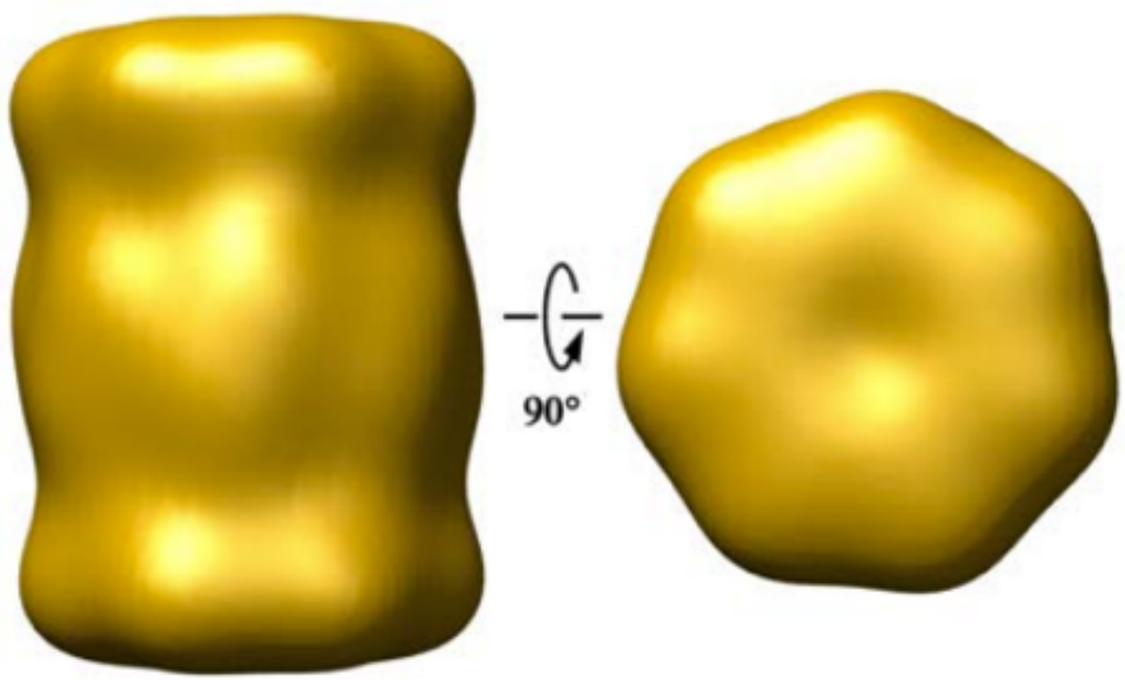


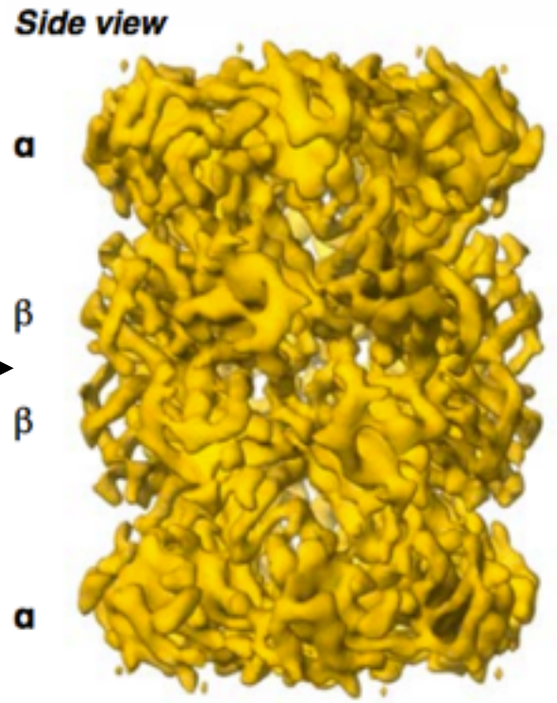


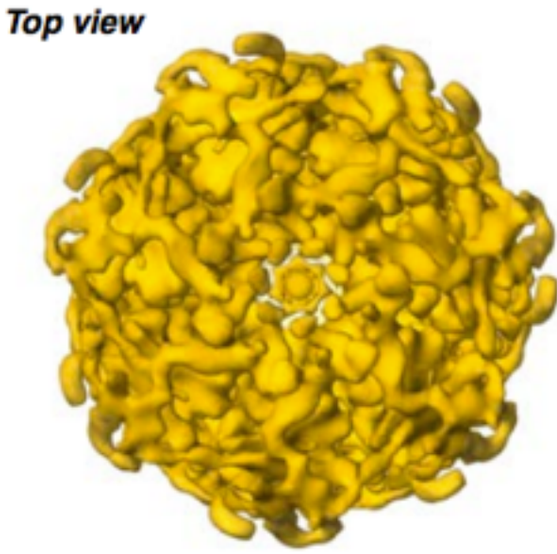
http://people.csail.mit.edu/gdp/cryoem.html

classes are then averaged and back-projected to produce 3D density map

iterative refinement





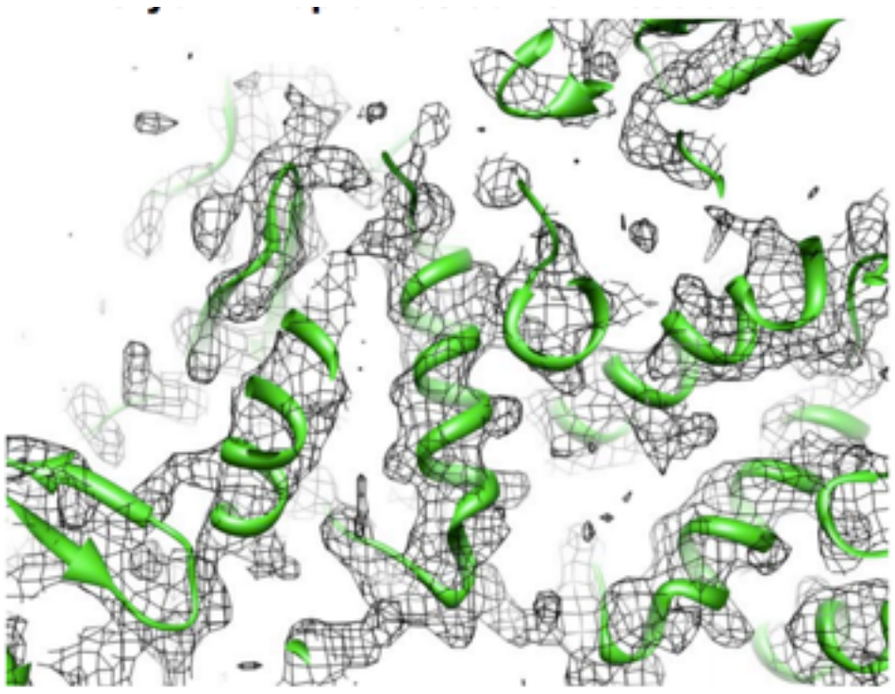


cryo-EM map of the proteosome (iteration 1)

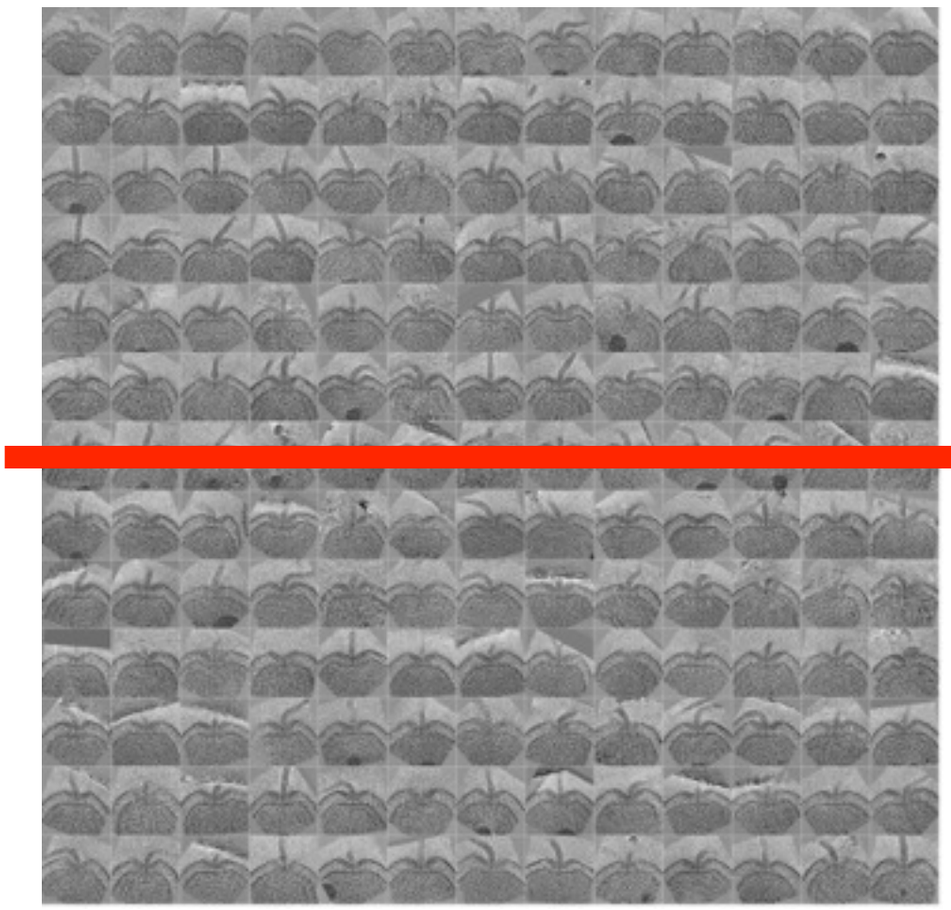
final map

back projection is iterative - need the model for **projection matching** with class averages

maps can have resolutions ranging from near-atomic (<5 Å) to 2-3 nm



map resolution



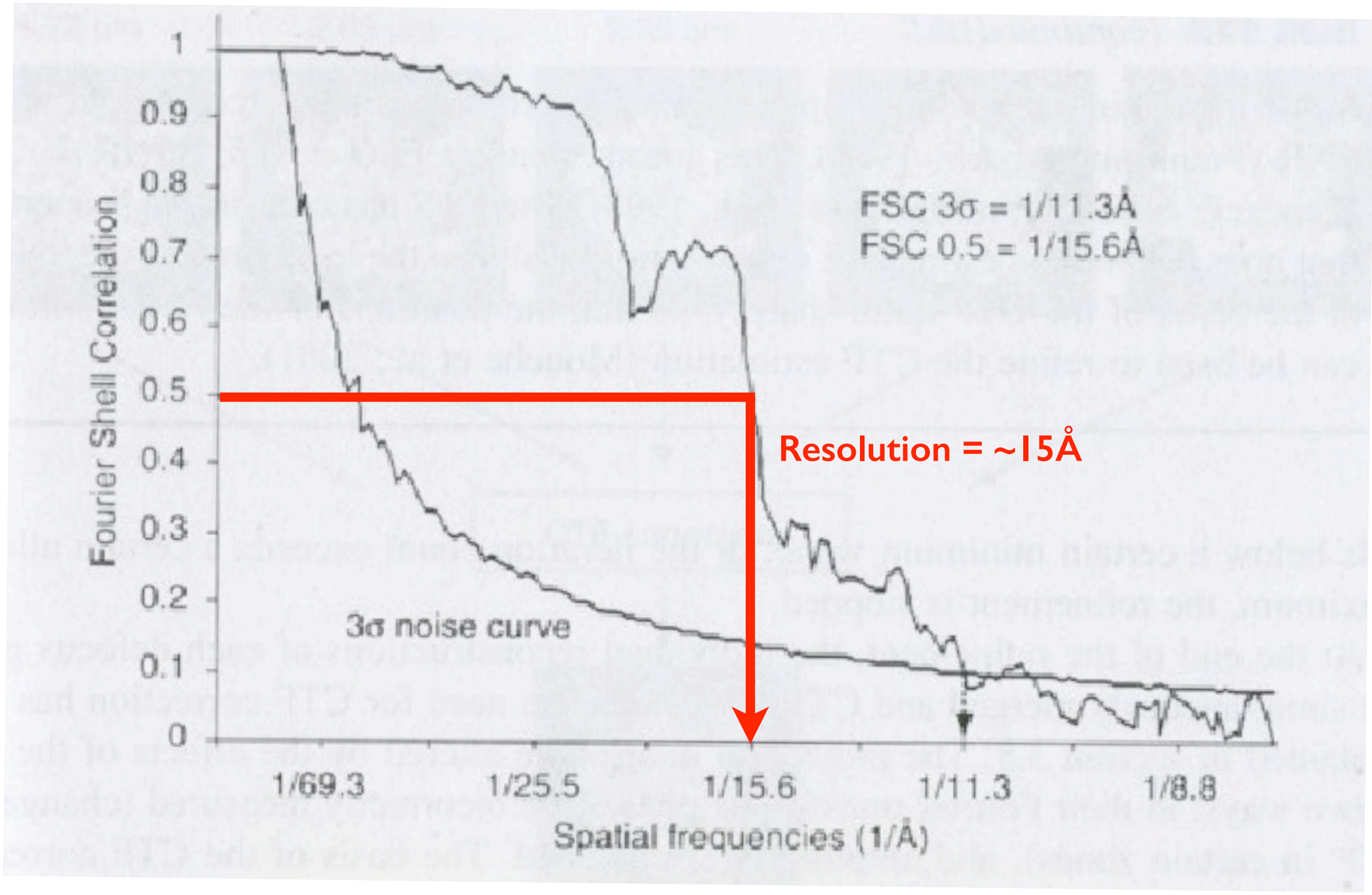
Split dataset in half, calculate two independent reconstructions

Align two structures, flip into reciprocal space (i.e., 3D FT), and calculate correlation co-efficients between bands of spatial frequency

Fourier shell correlation: $$FSC(r) = \frac{\sum_{r_i \in r} F_1(r_i) \cdot F_2(r_i)^*}{\sqrt{\sum_{r_i \in r} |F_1(r_i)|^2 \cdot \sum_{r_i \in r} |F_2(r_i)|^2}}$$

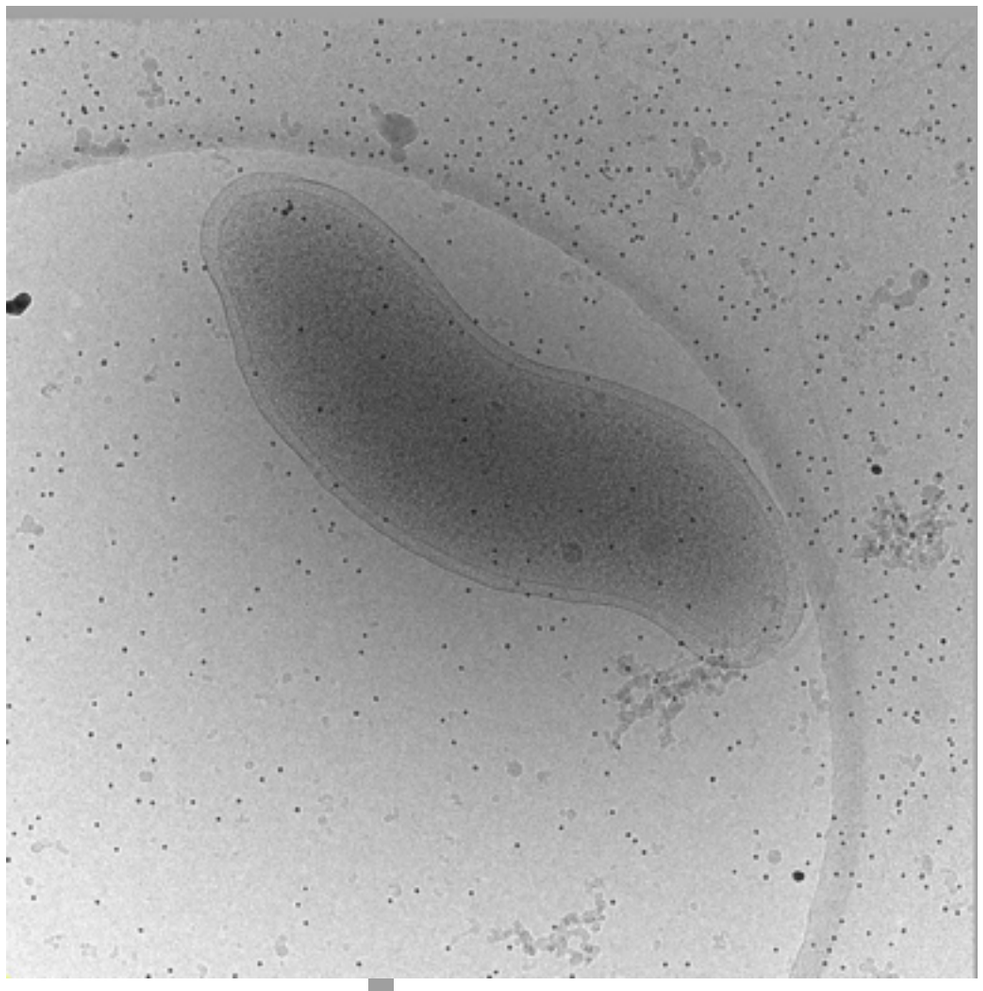
map resolution

FSC between two halves of the data set



Electron cryo-tomography

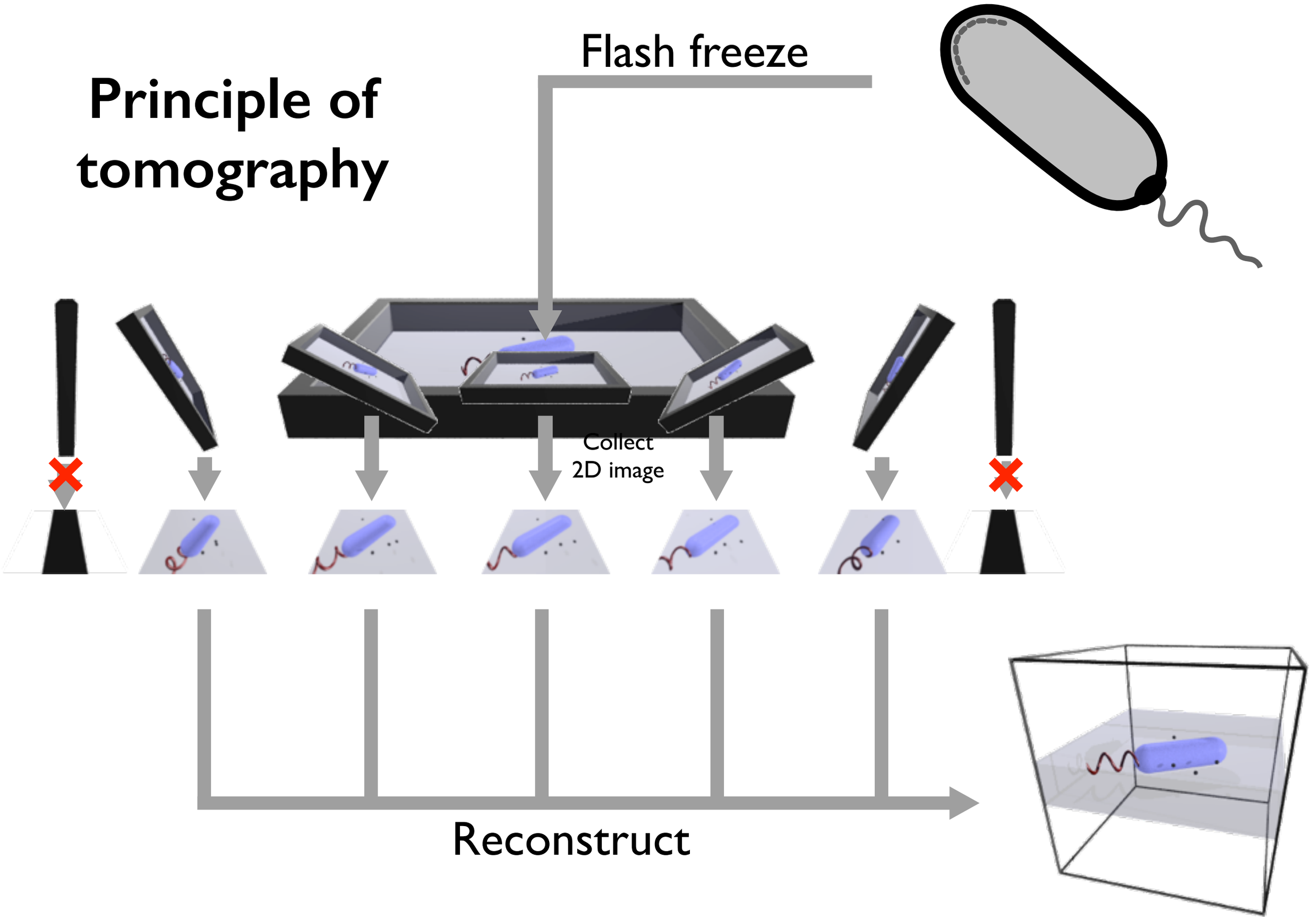
~100 2D images



Reconstruct

3D tomogram

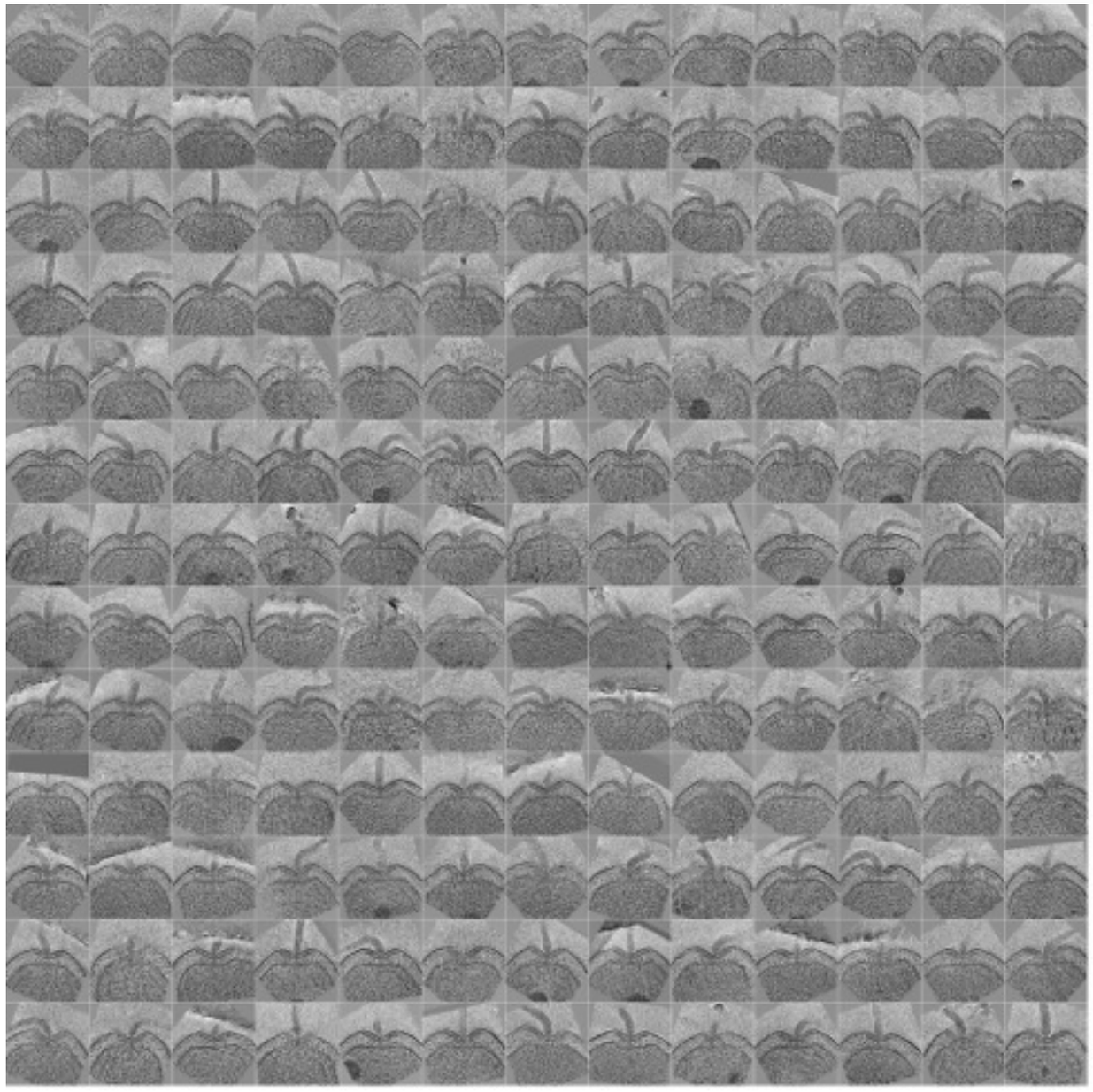
Principle of tomography

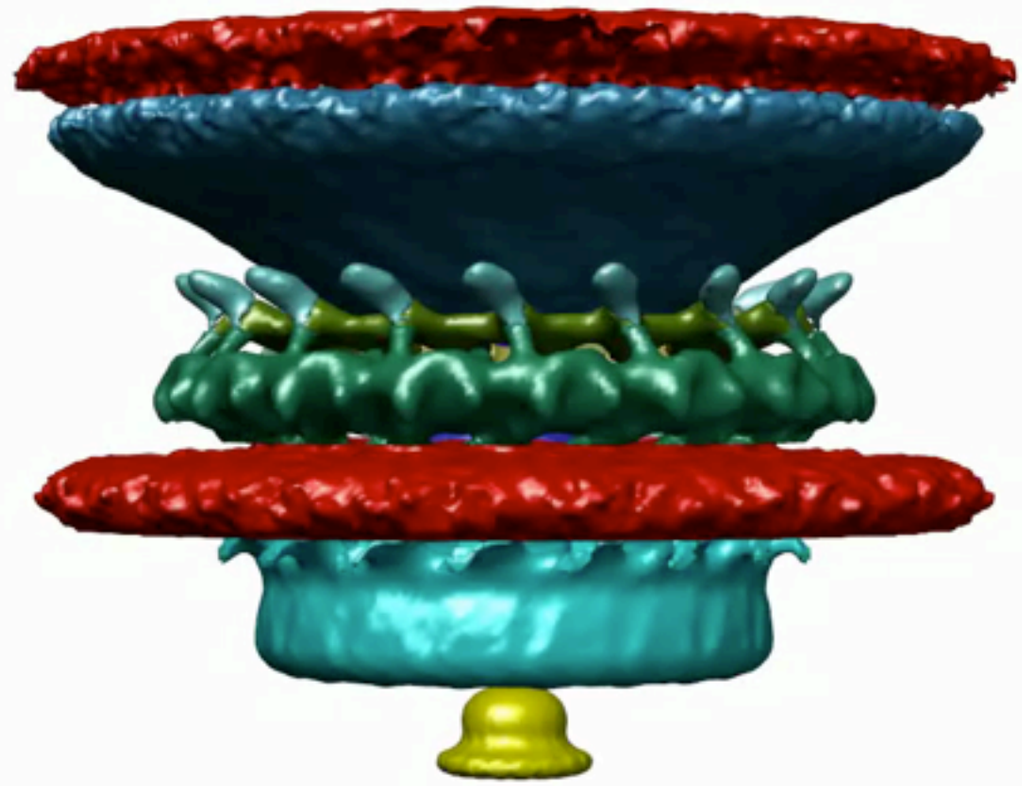


Baumeister et al., *Trends in Cell Biology* **9**:81

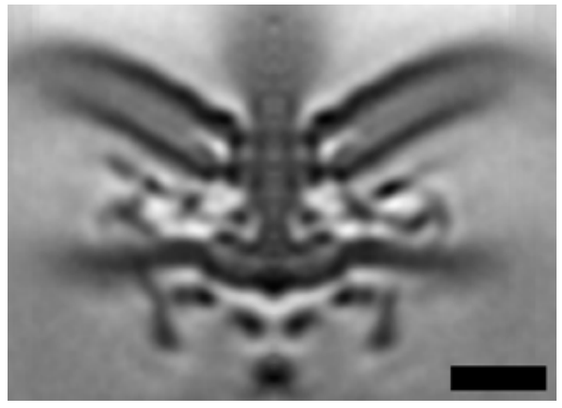
Subtomogram averaging

100's of ("identical") 3-D particles





Align and average



Identifying cellular features