THE UNIVERSITY of TEXAS

SCHOOL OF HEALTH INFORMATION SCIENCES AT HOUSTON

History of 3D Electron Microscopy and Helical Reconstruction

For students of HI 6001-125

"Computational Structural Biology"

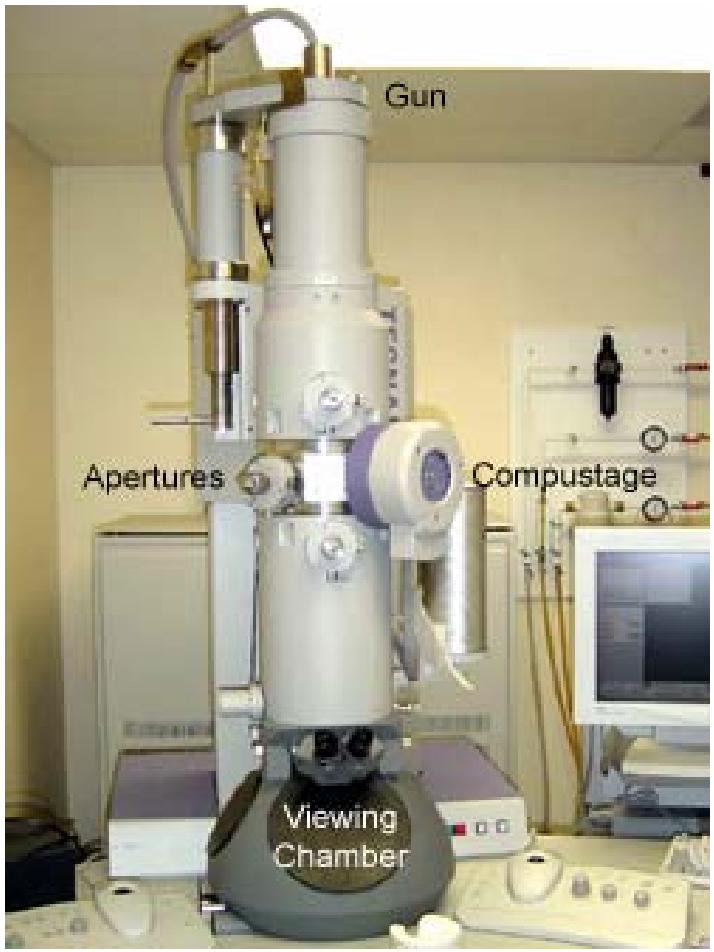
Willy Wriggers, Ph.D.

http://biomachina.org/courses/structures/07.html

The Electron Microscope

- Transmission Electron Microscope (TEM)

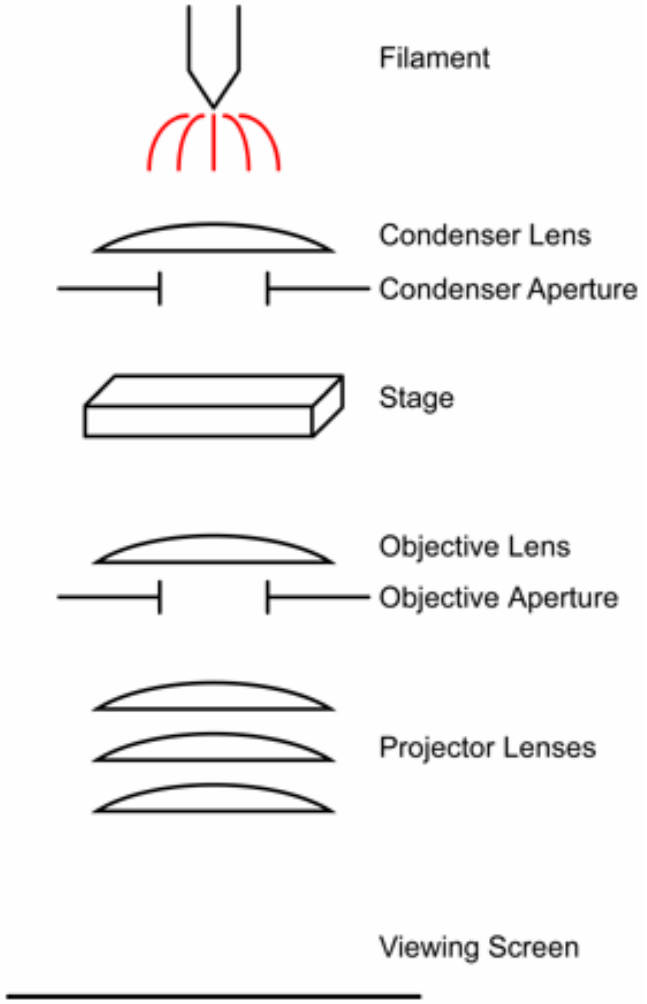
  - Detects internal structure of sample
  - Thin samples, so beam is not entirely absorbed
  - Cryo-EM: mostly phase object
  - Projection of 3D structure onto 2D screen (actually, projection of electrostatic potential)



http://cryoem.berkeley.edu/~nieder/em_for_dummies
http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

The Electron Microscope

- Electron gun: superheated metal filament emit electrons, collated into beam by thermionic and field emission gun
- Lenses: magnetic coils tuned to focus electron beam, to magnify image and to sharpen contrast
- Stage: holds sample, may be tilted by goniometer
- Aperture: limits size of electron beam.
  - Condenser aperture: maintains size of electron beam.
  - Objective aperture: controls contrast



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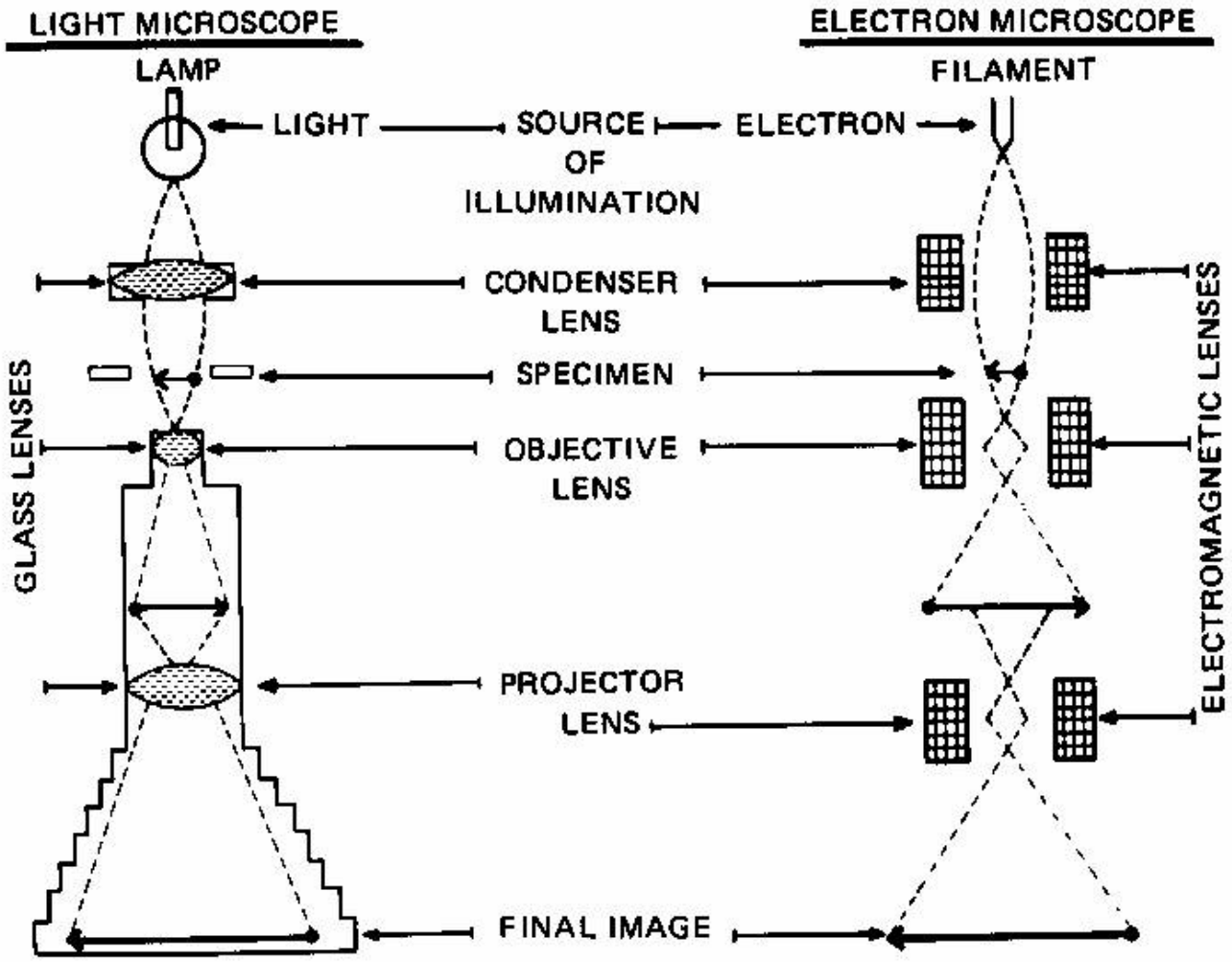
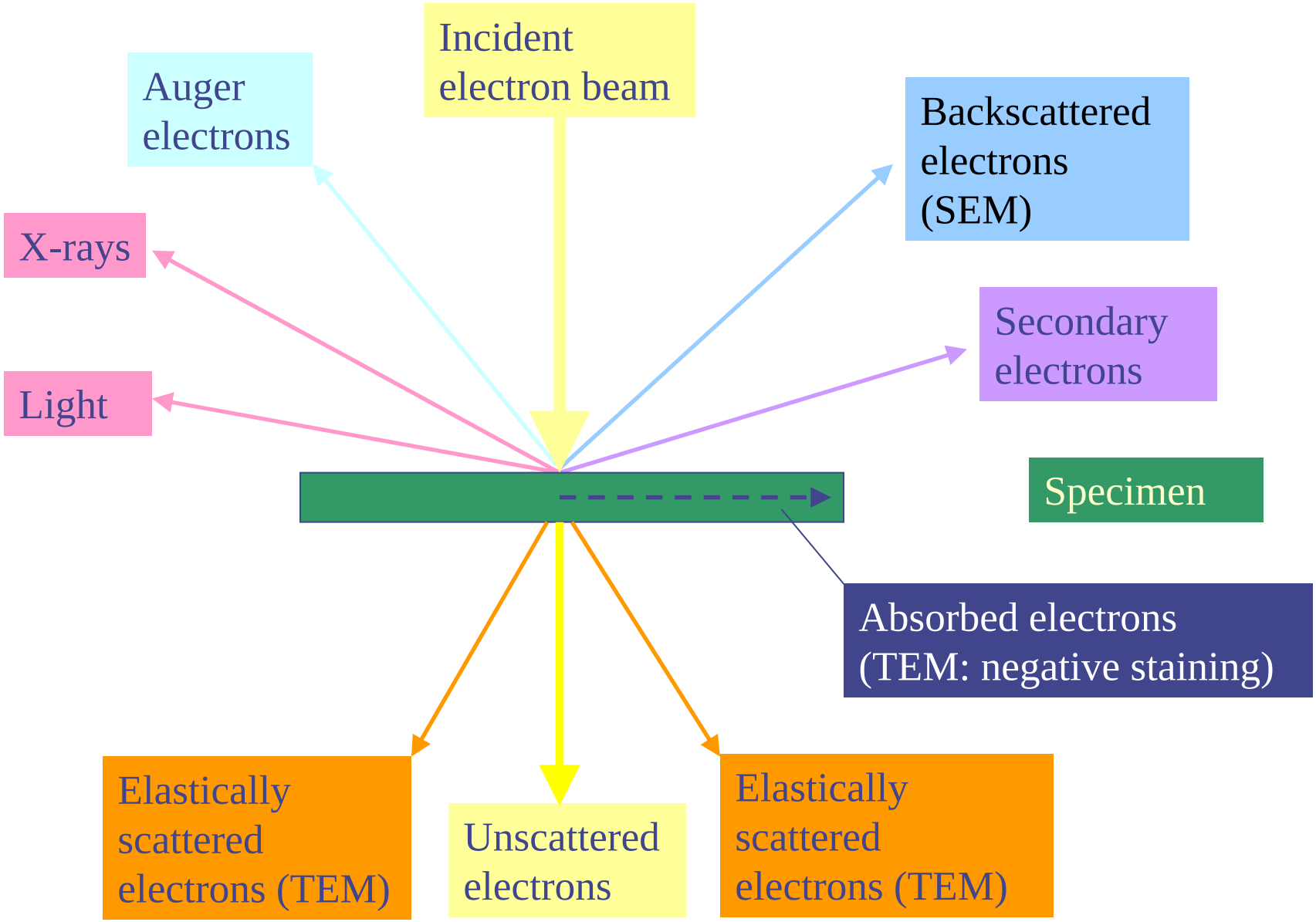
http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

Comparison of Optics



http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

Specimen-Beam Interaction



Sample Preparation

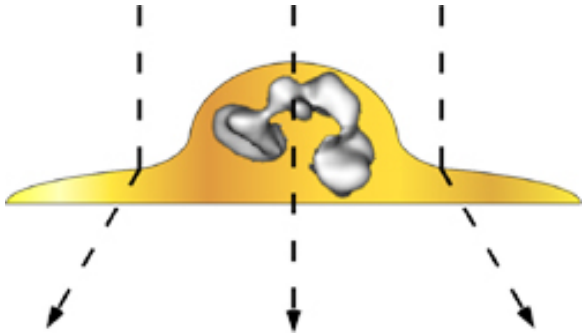
Chamber must be in vacuum, so evaporation must be eliminated

NEGATIVE STAINING

- Blotting sample dry through negative staining with heavy metal salts that absorb electrons

Pros: may improve contrast, can absorb more radiation, easy preparation

Cons: lower resolution, disturbs particles



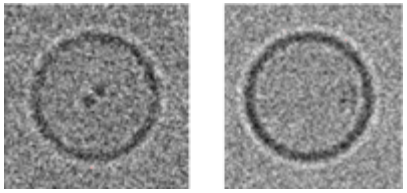
http://cryoem.berkeley.edu/~nieder/em_for_dummies/

CRYO-EM

- Freezing in liquid ethane
- Quickly solidify water in amorphous solid (vitreous ice)

Pros: preserves shape, decreases noise by freezing vibrations, preserves hydrated state

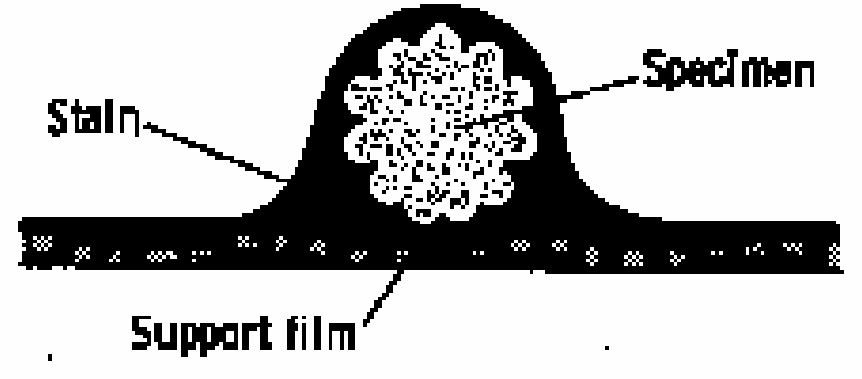
Cons: extensive preparation, low contrast, cubic ice

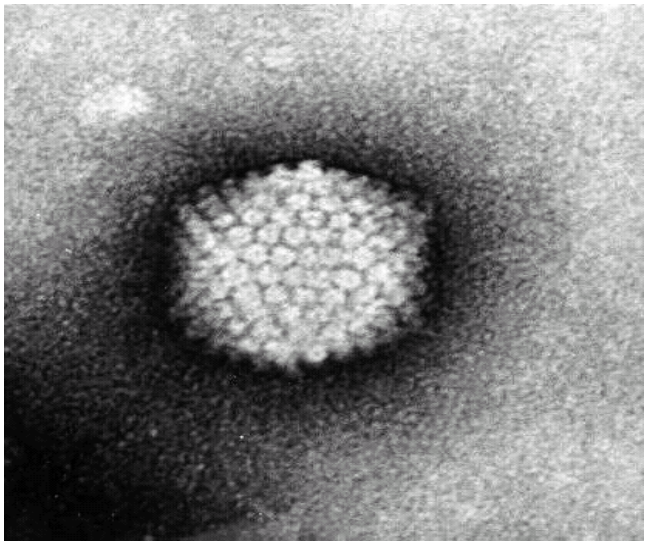


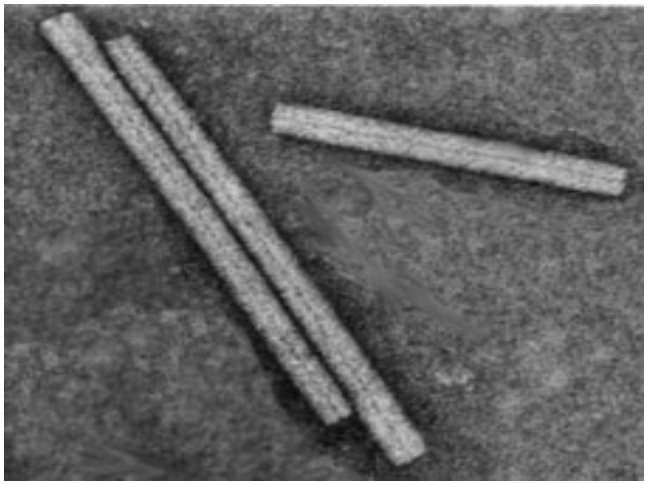
http://info.med.yale.edu/cmphysiol/sigworth/cryoem.html

http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

Negative Staining



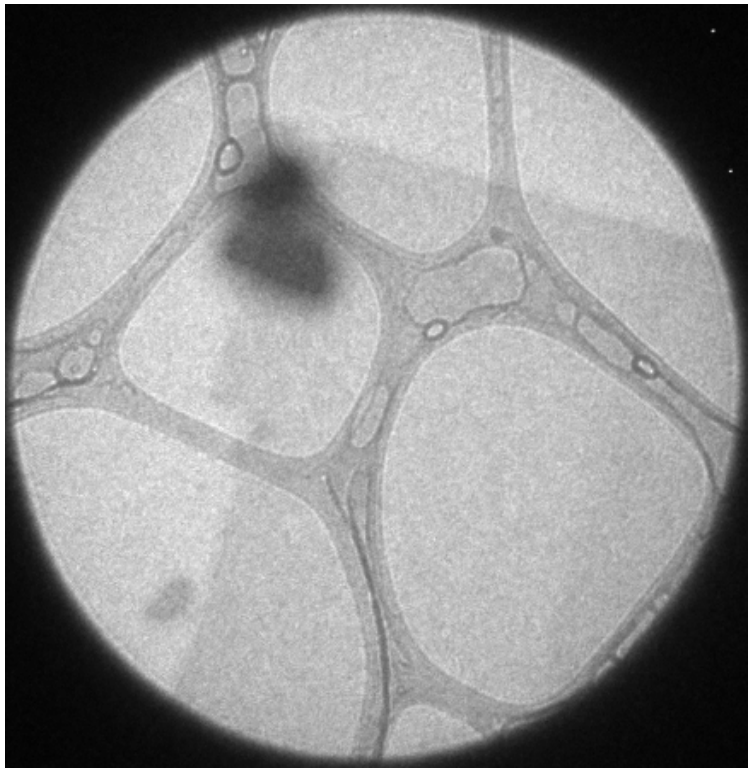




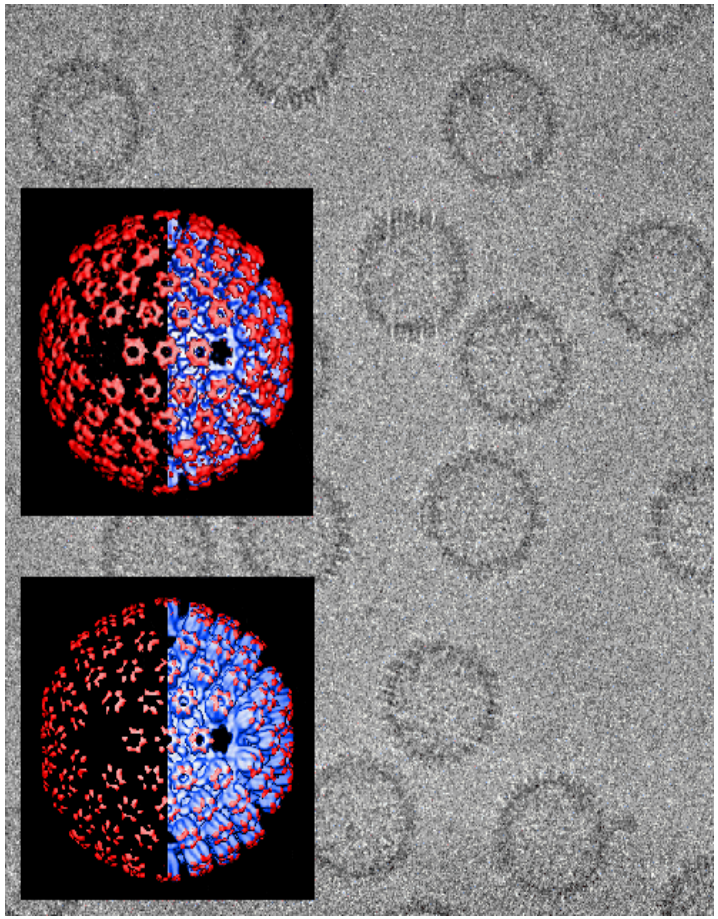
Bob Horne (Cambridge)

Cryo Prep using Holey Film

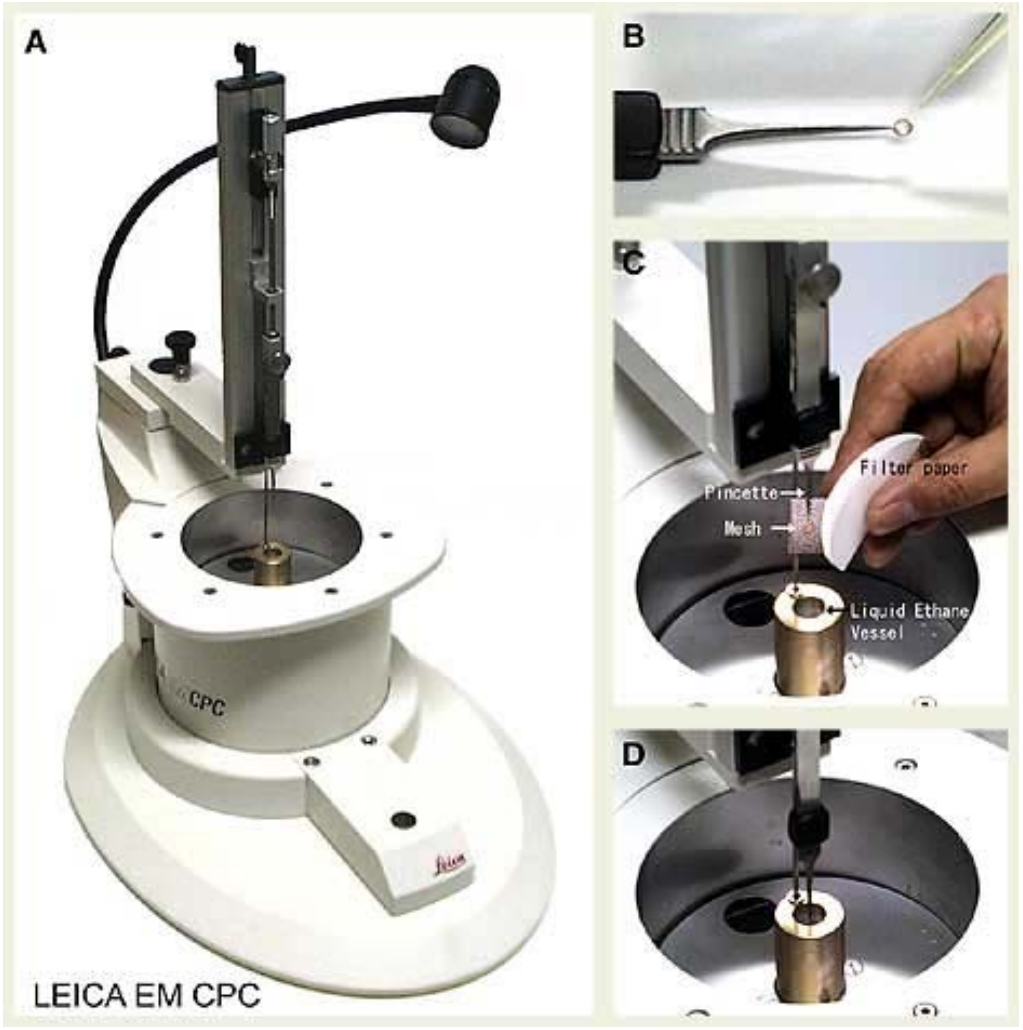
H. Fernandez-Moran
B. Glaeser
K. Taylor
J. Dubochet



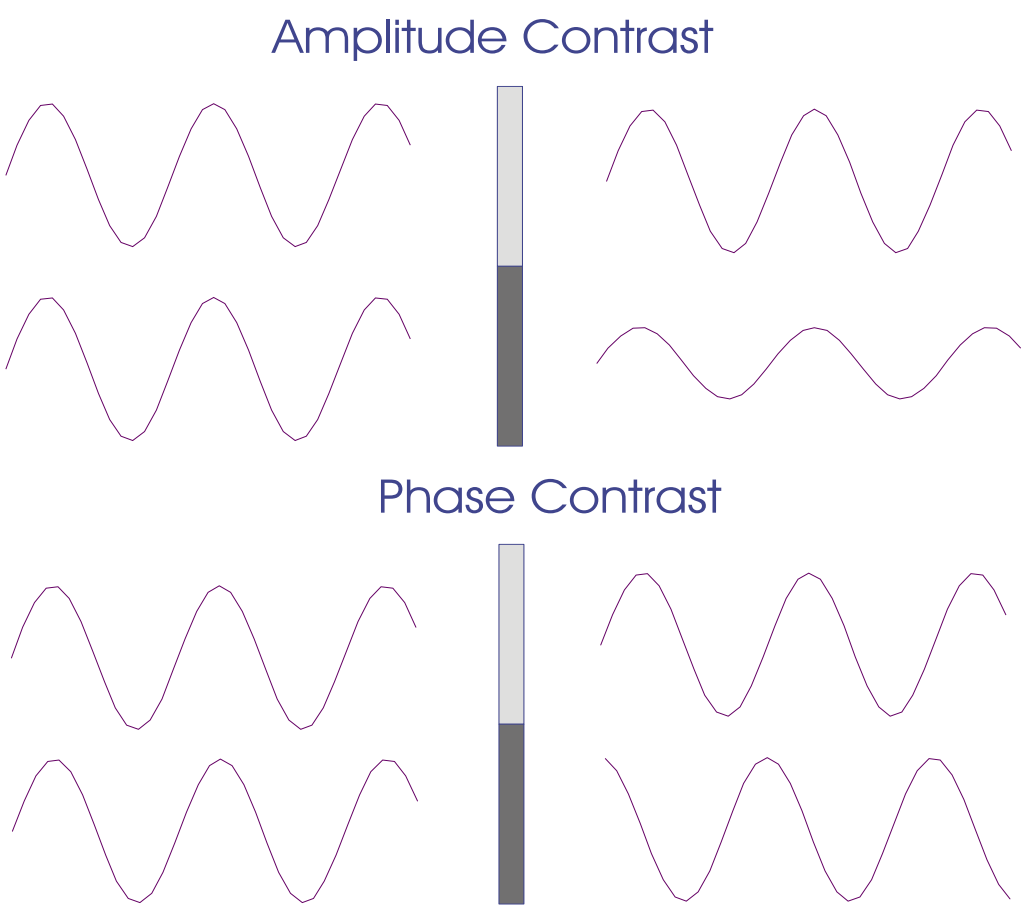
Aaron Klug



Flash Freeze in Liquid Ethane



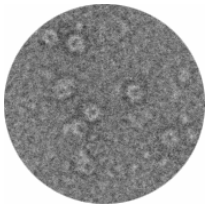
Specimen Contrast



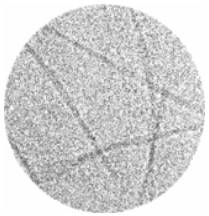
Cryo	neg. stain
10%	50%
90%	50%

http://www.cbem.bc.ic.ac.uk/ardan/bc/cryoem-20020507.ppt

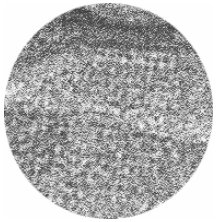
Types of Specimens



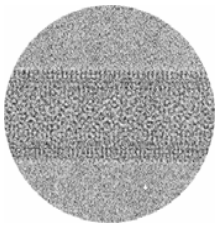
- Single Particles (Proteins, Ribosome)
  - No crystallization
  - Weak amplitude, no diffraction, alignment ambiguity, particle flexibility
  - ~7 angstroms



- Fibers and filaments (tubulin, collagen)
  - No crystallization, 2D distortion corrections, phase restrictions
  - Weak amplitude, no diffraction
  - ~9 angstroms



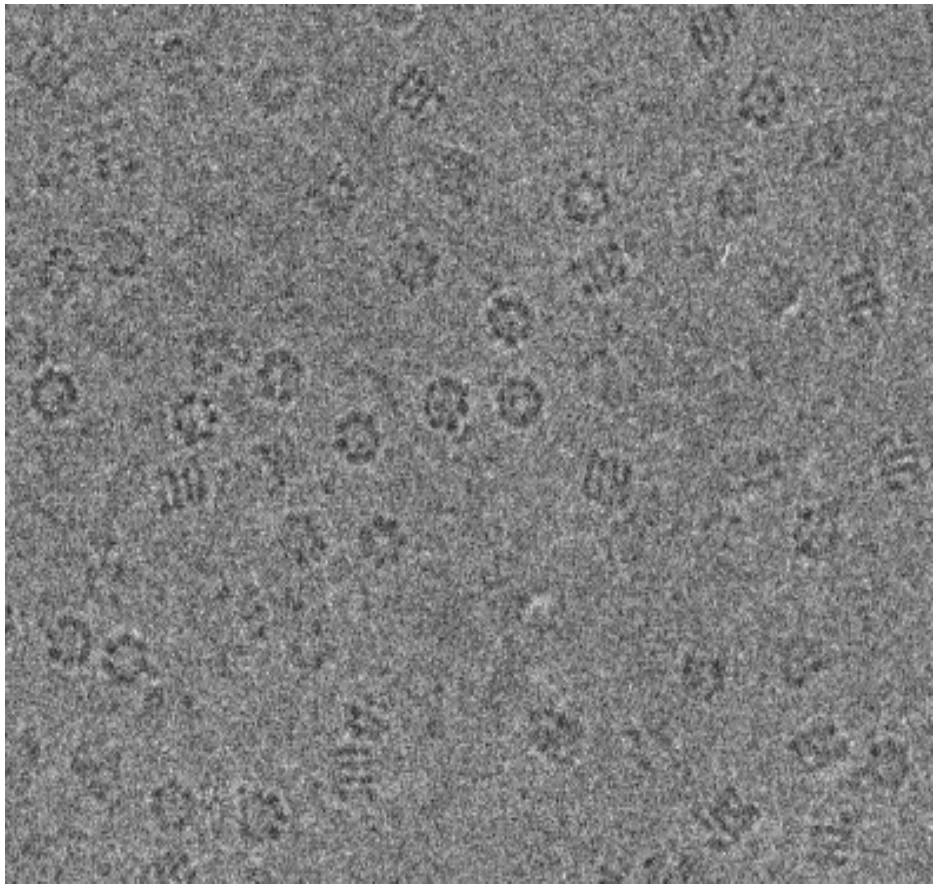
- 2D crystals (BR, AQP, LHCII)
  - Diffraction amplitudes, 2D distortion corrections, crystallographic methods
  - Crystallization, many tilts required, anisotropic data
  - ~3 angstroms



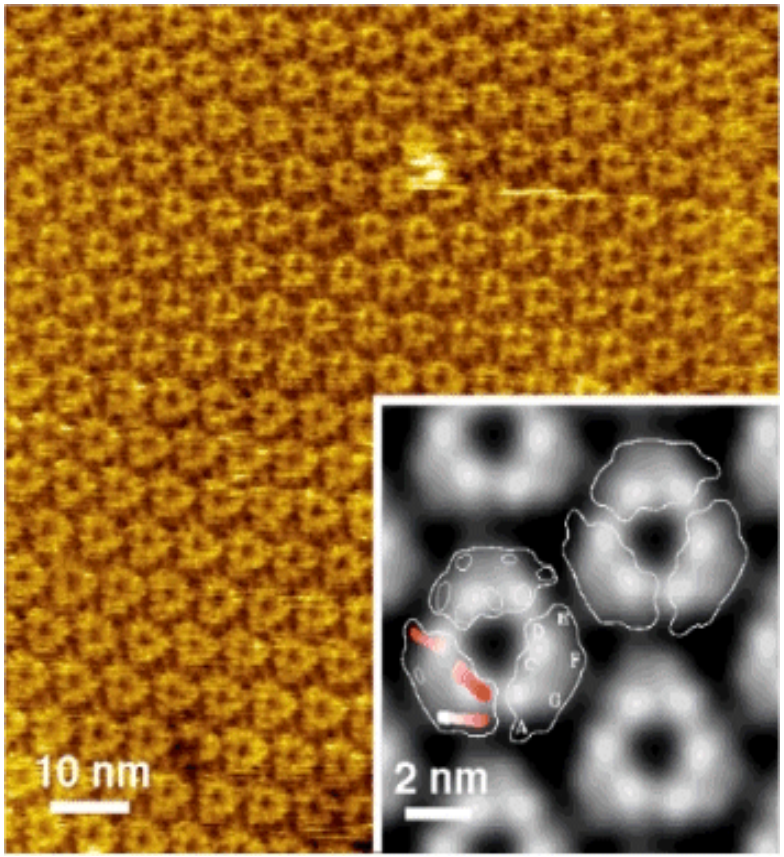
- Tubular crystals (AchR, Ca^{++}-ATPase)
  - Crystallization, No diffraction
  - Isotropic data, 3D distortion corrections, phase restrictions
  - ~5 angstroms

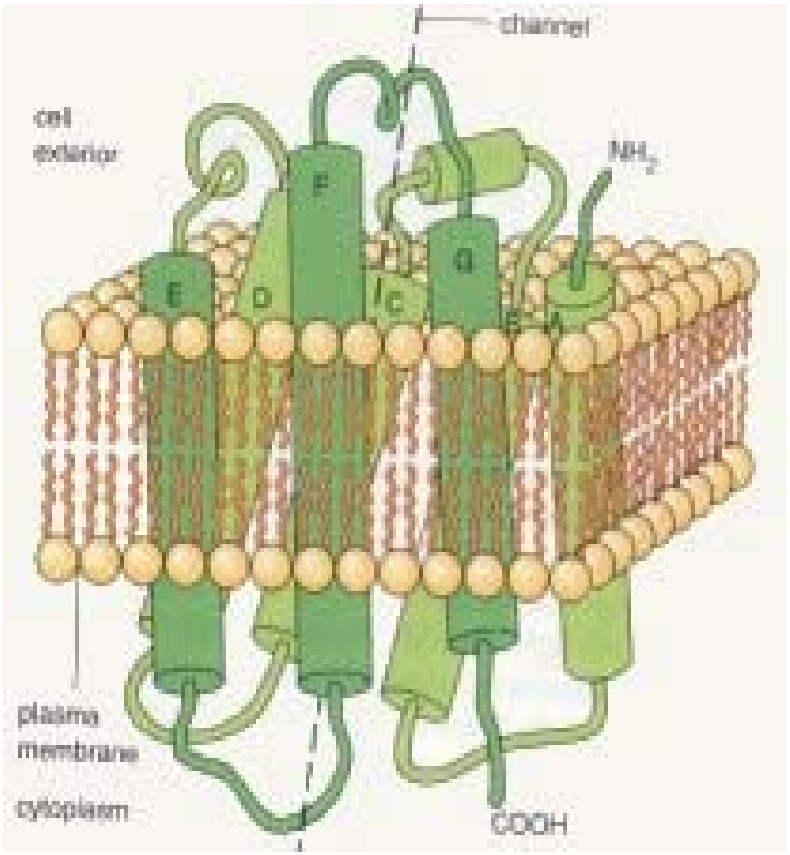
Single Particles

- Applicable to any protein or protein complex > 200kD
- Most common sample
- Number of software suites available
- Resolution ~9A (<7 with symmetry)



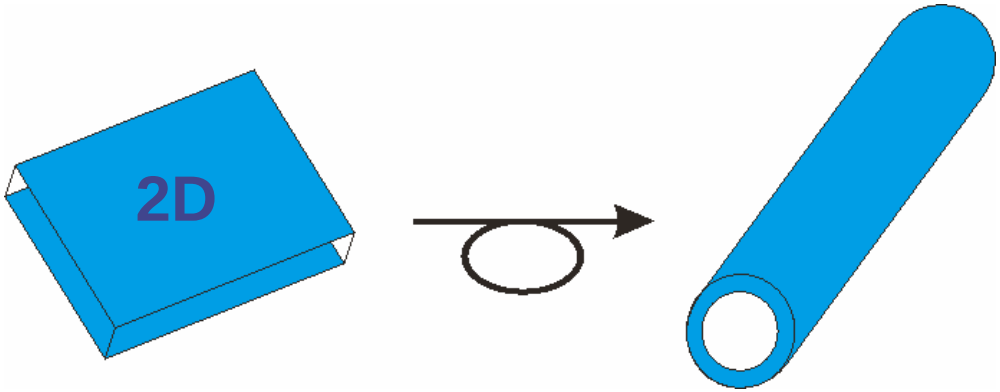
2D Crystals

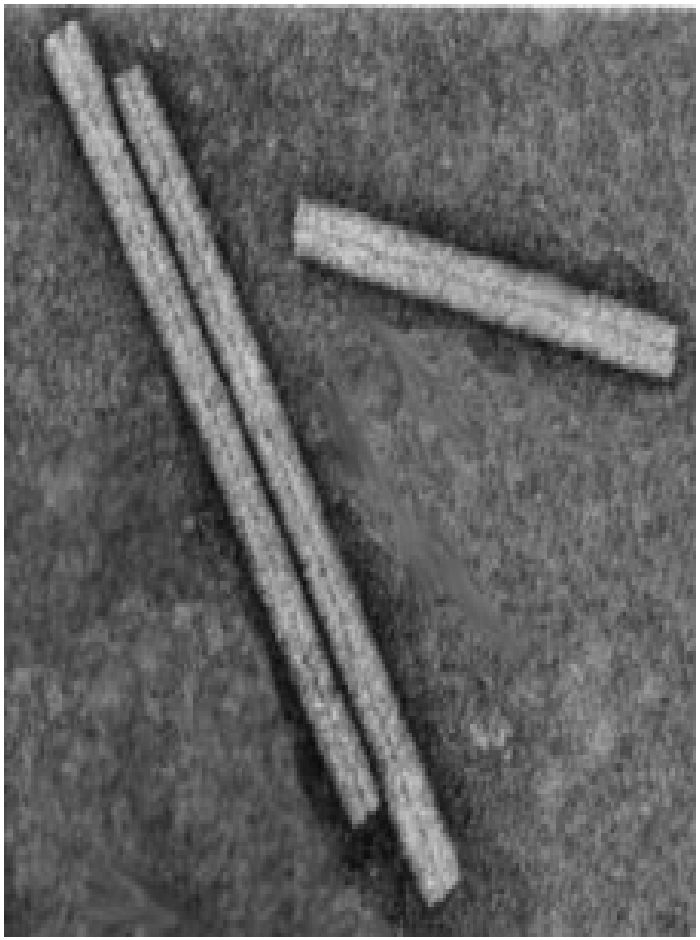




Henderson and Unwin

Tubular Crystals





Tubular vs. 2D or 3D Crystals

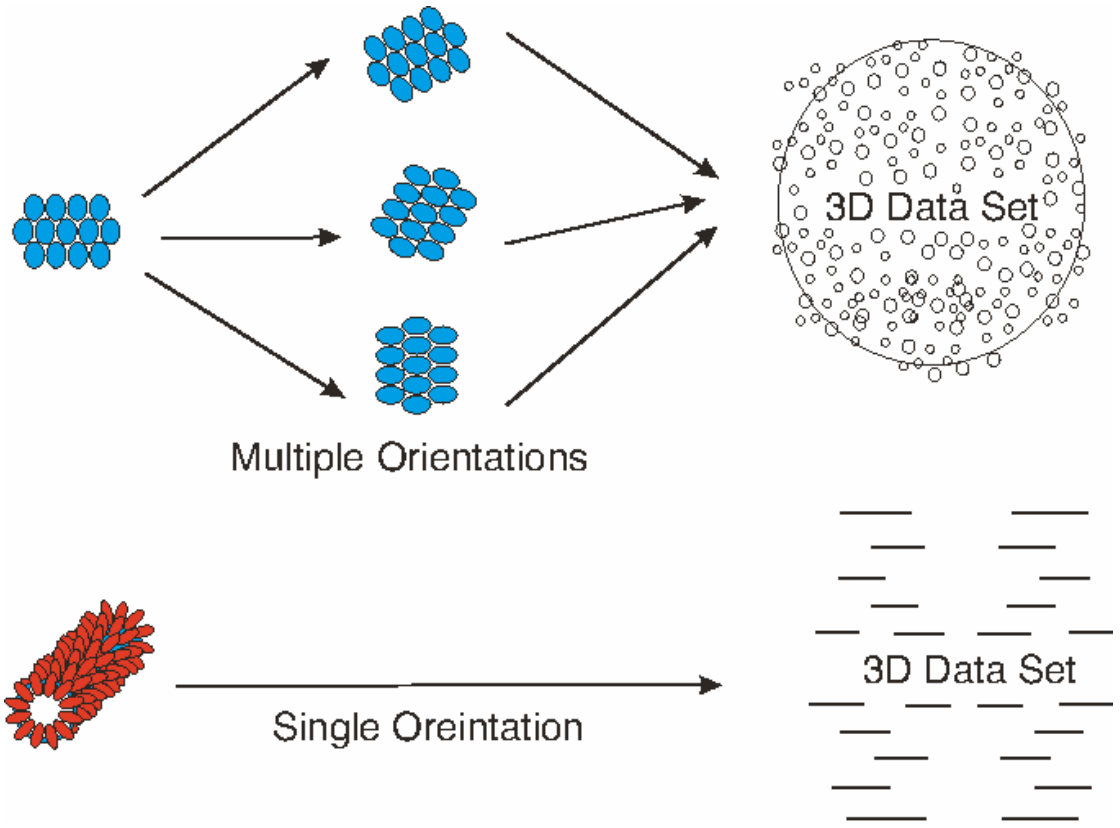
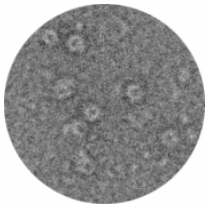


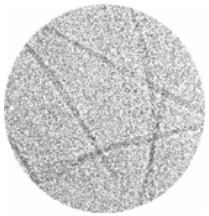
Image Recording

- Film
  - High density content (~20kx16k pixels)
  - Slow (development time, drying)
  - Requires digitization (scanning takes hours)
- CCD
  - Low density content (4kx4k pixels)
  - Fast (ms to sec)
  - Direct digital

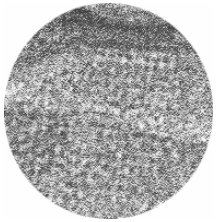
Processing Data



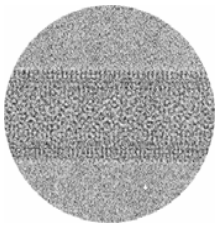
- Single Particles (Proteins, Ribosome)
  - Pick particles
  - Align
  - Classify, average and reconstruction



- Fibers and filaments (tubulin, collagen)
  - Pick segments determine symmetry
  - Align/rotate
  - Average

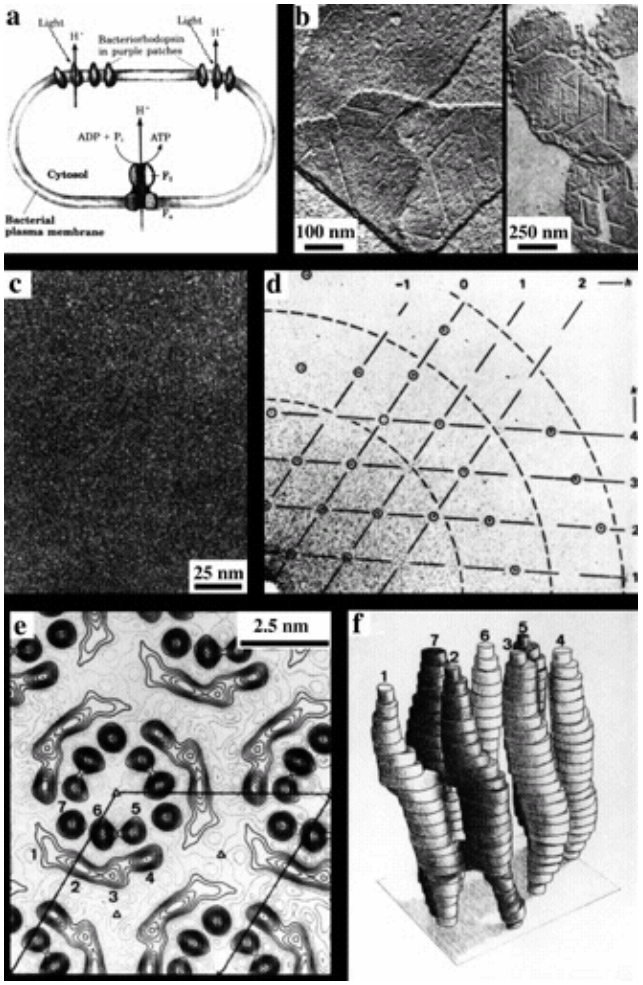


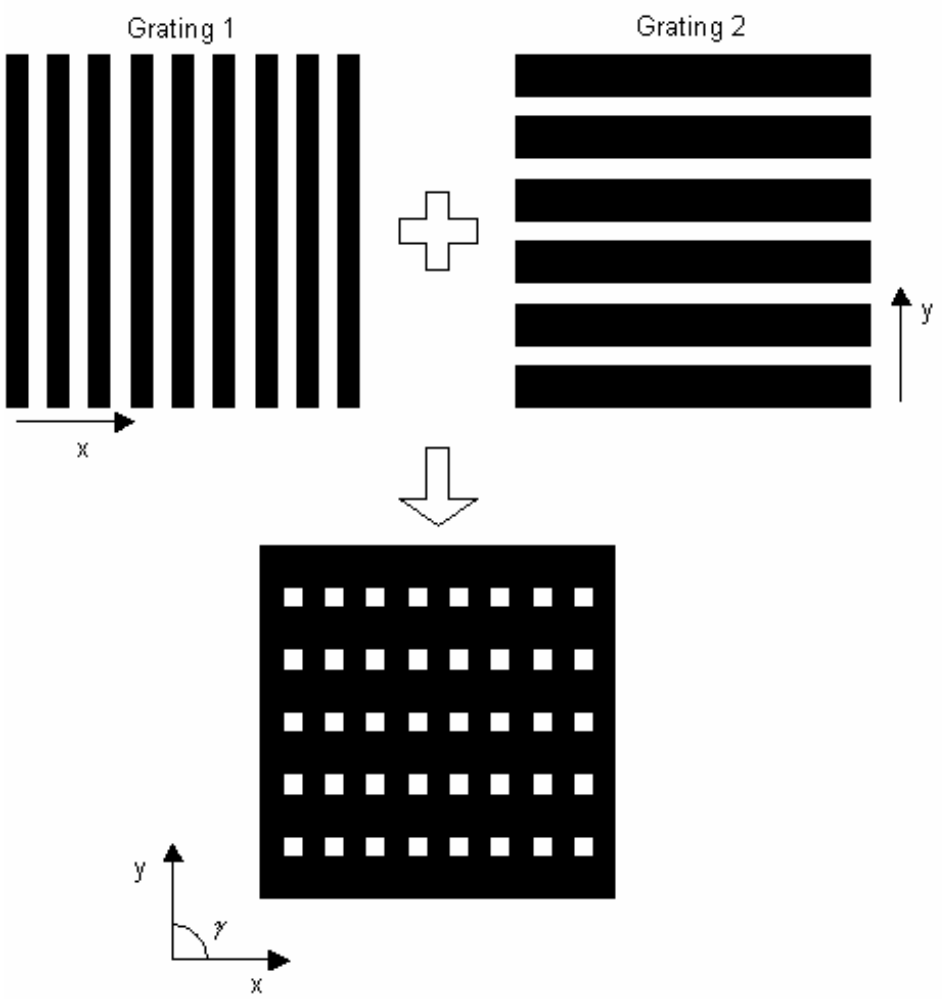
- 2D crystals (BR, AQP, LHCII)
  - Process images to achieve phases
  - Process diffraction data for amplitudes
  - Combine and refine as in X-ray

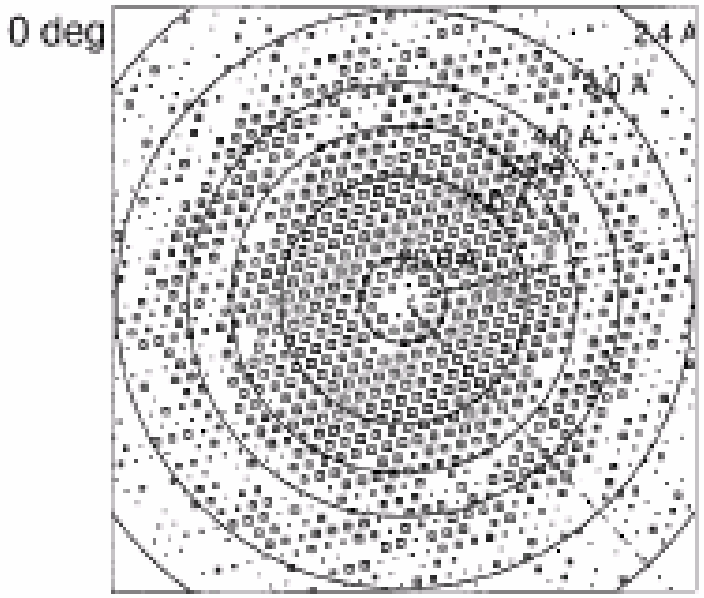


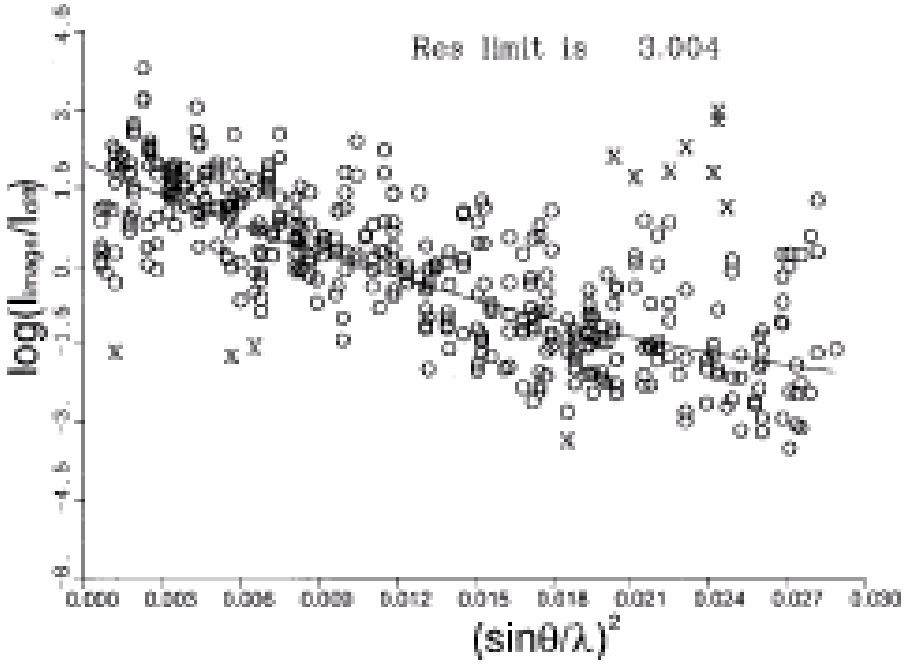
- Tubular crystals (AchR, Ca^{++}-ATPase)
  - Determine tube symmetry
  - Pick segments and distortion correction
  - Average and sum segments

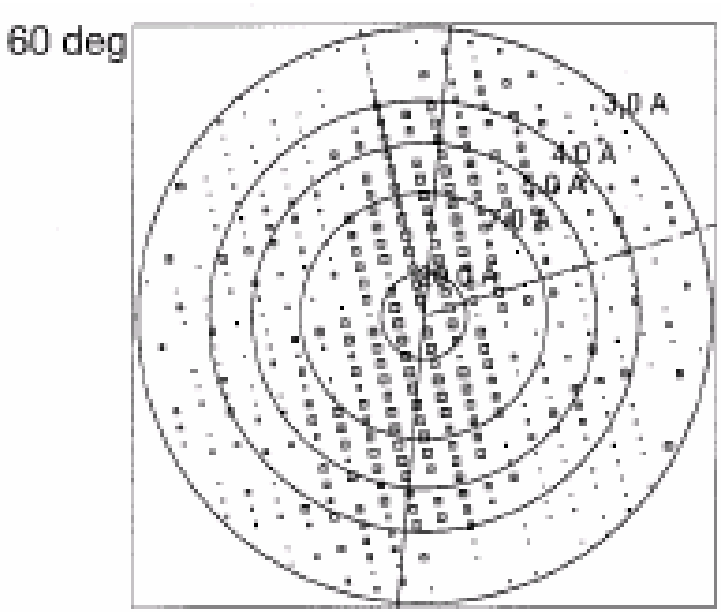
Data Processing Example: 2D Crystals

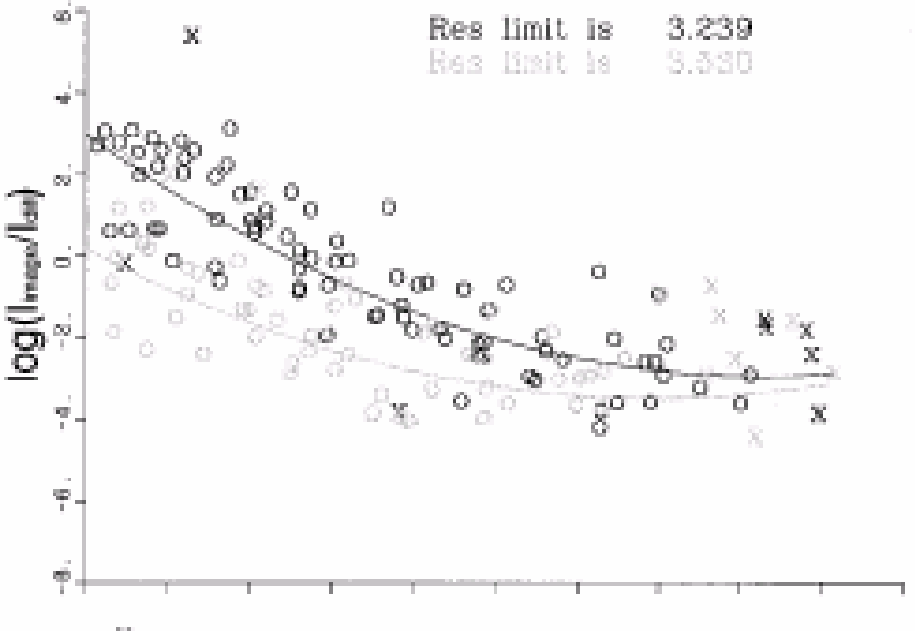




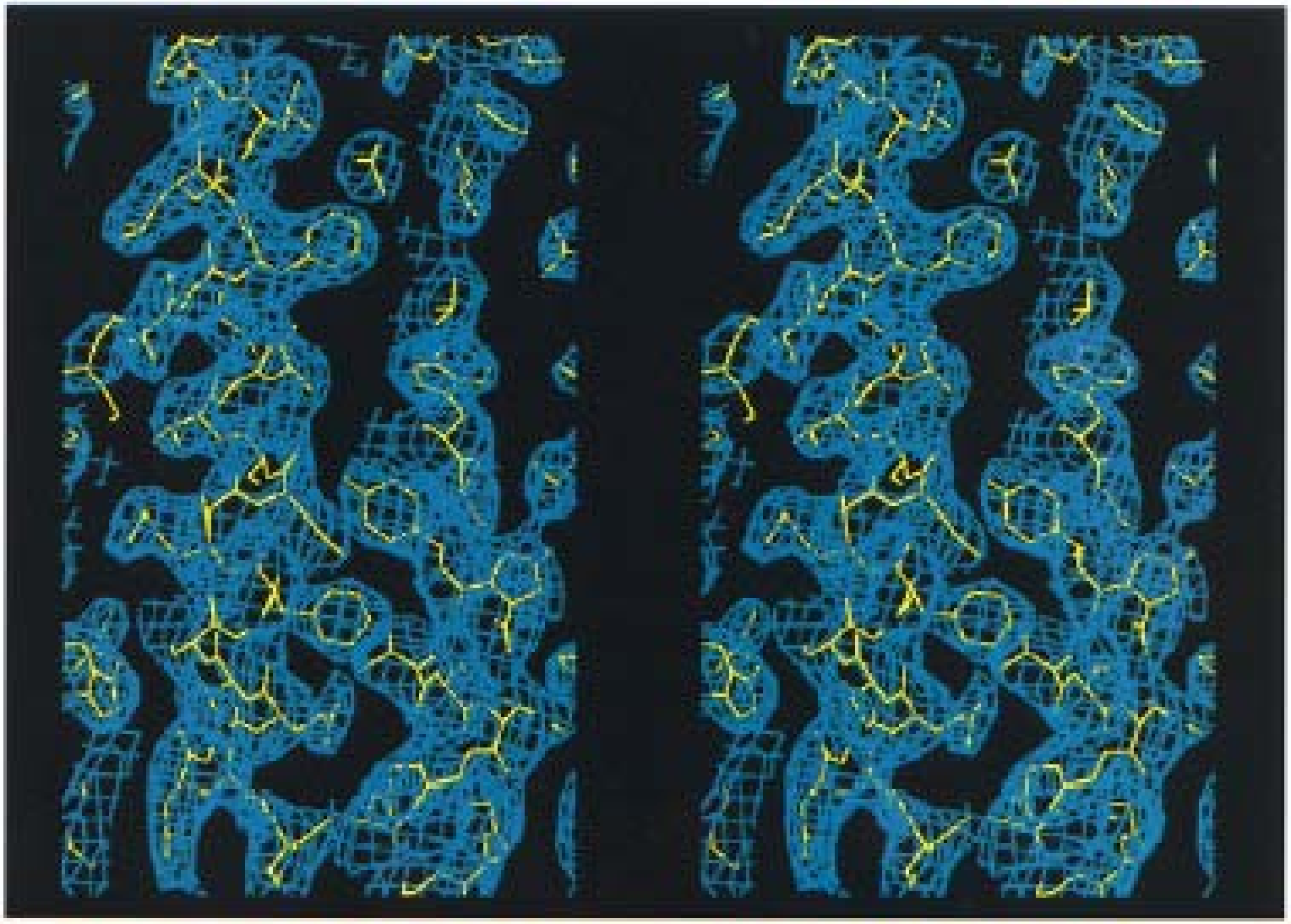








A Well-Refined EM Map

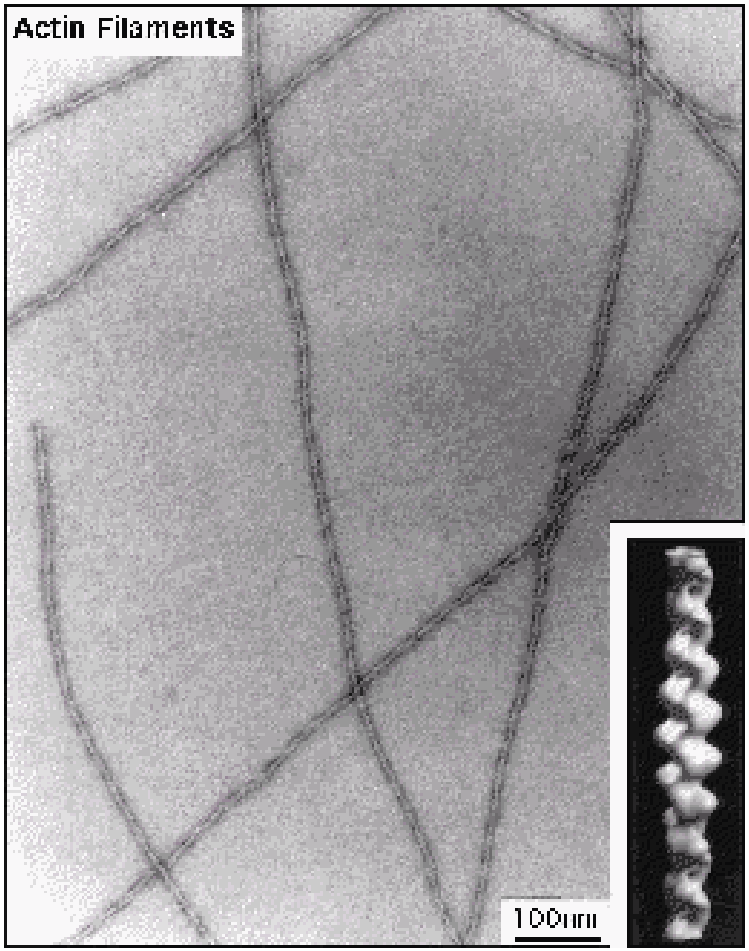


History of Electron Microscopy and 3D Reconstruction Methods

- 1950s: membrane topology of cellular structures, e.g. mitochondria
- 1950s: (Crick, Klug *et al*) FT of helical structures, selection rules
- 1964: (Parson and Martius) high resolution electron diffraction on fibers
- 1968: (DeRosier and Klug) first 3D structure determination of T4 Bacteriophage tail based on helical reconstruction
- 1970: (Crowther *et al*) first icosahedral viruses
- 1972 (Matricardi *et al*), 1974 (Taylor and Glaeser), 1975 (Unwin and Henderson): 2D crystals
- 1983 (Knauer *et al*): ribosome 3D reconstruction (asymmetric single particle)
- 1990 (Henderson *et al*): atomic resolution of bacteriorhodopsin (2D crystal)

Glaeser, J. Struct. Biol, 128, 3-14 (1999), Michael Moody chapter (see course web site)

History of Helical Reconstruction



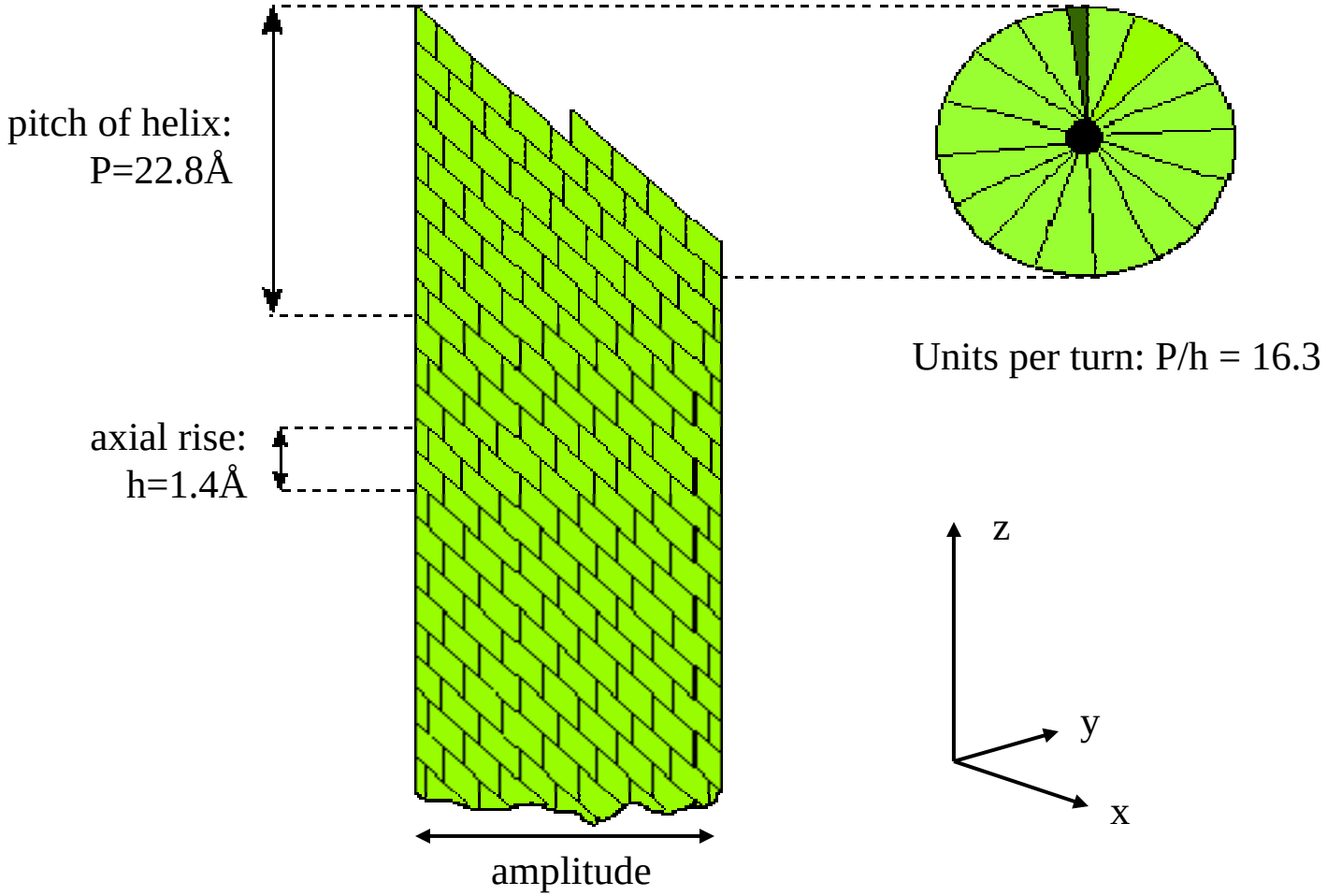
Historically, the first reconstruction method, we start with this approach.

See also homework assignment!

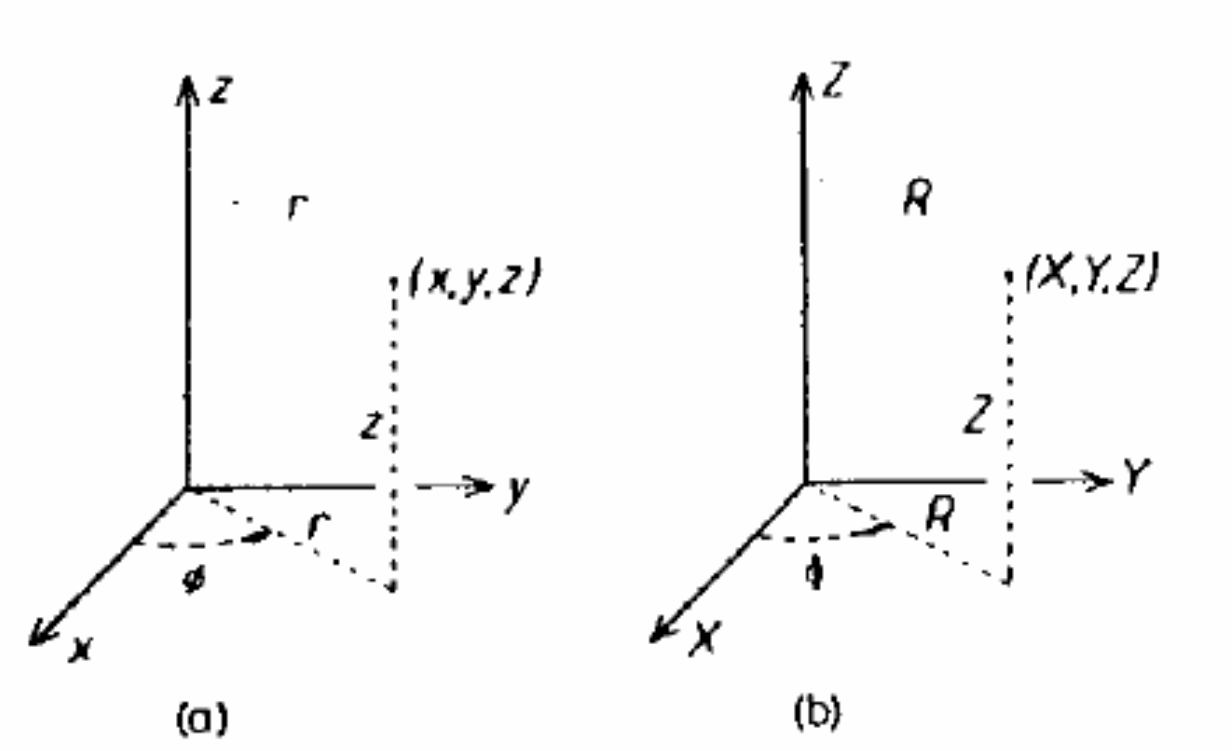
We follow here pp. 190-194 in the Moody chapter (most comprehensive overview)

Helix Geometry

- helix can be defined mathematically by three parameters: **amplitude** (diameter), **axial rise** h (z-distance covered by one subunit) & **pitch** P (z-distance covered by each complete turn of the helix)



Cylindrical Coordinate System



Real Space Reciprocal Space

Michael Moody chapter (see course web site)

Bessel Functions

We wish to make the best use of the fact that the helix is periodic in both ϕ and z. We note that a two-dimensional repeating structure which is periodic in x and y gives a particularly simple transform—it exists only at the points of the reciprocal lattice in the XY-plane. Now the transform of that two-dimensional repeating structure contains the term $\exp[2\pi i(Xx + Yy)]$; so we expect a similar simplification if a similar form in Φ and Z could somehow be introduced into Equation (13). Z and z are already in the correct combination, and we need only introduce the required change into Φ. This is possible through use of the generating function for Bessel functions (Watson, 1958):

$$\exp\{\tfrac{1}{2}u(t - t^{-1})\} = \sum_{n=-\infty}^{\infty} t^n J_n(u) \tag{14}$$

from which the substitution $t = i\exp(iv)$ yields:

$$\exp(iu\cos v) = \sum_{n=-\infty}^{\infty} \exp\{in(v + \pi/2)\} \cdot J_n(u) \tag{15}$$

Michael Moody chapter (see course web site)

Bessel Functions

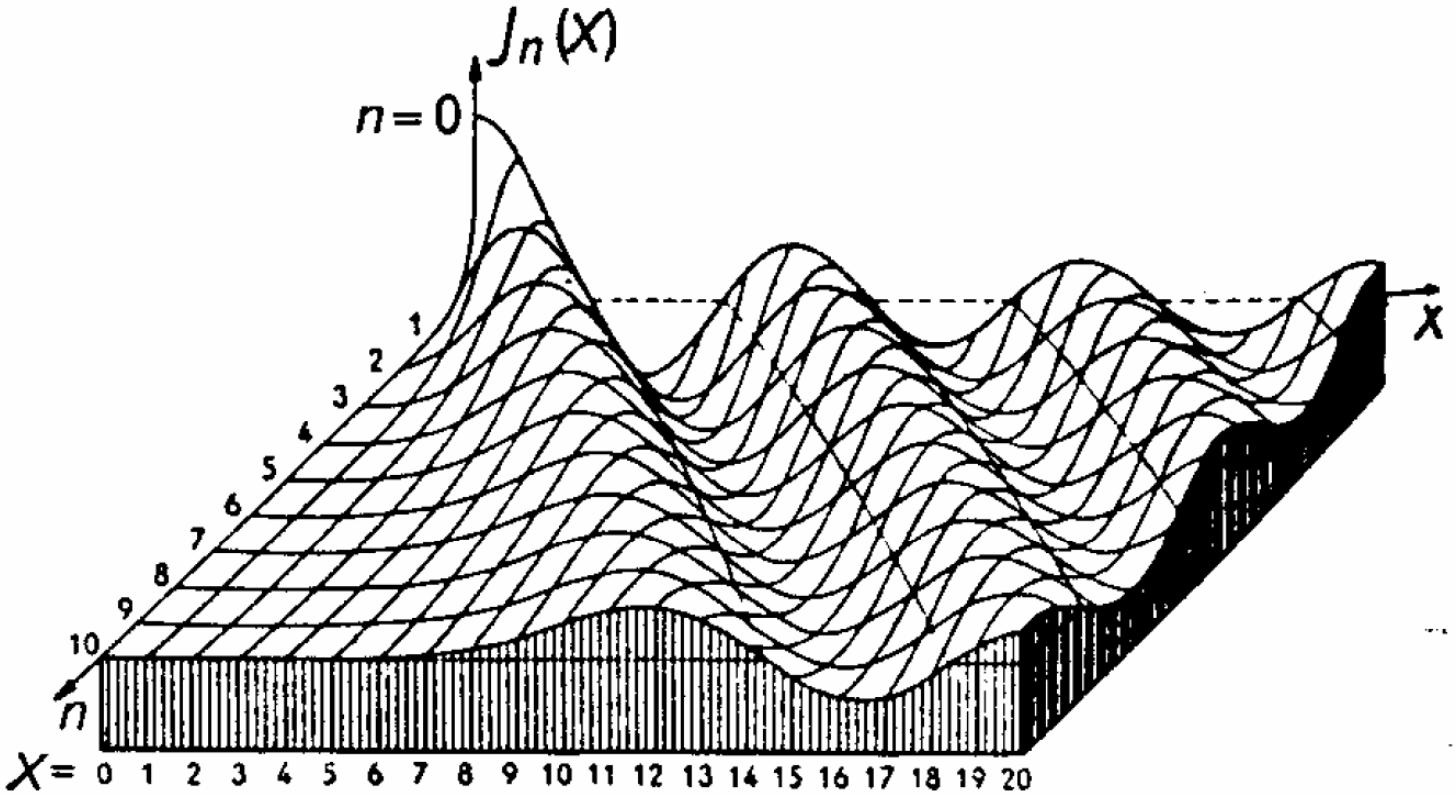


Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)

Cochran et al, 1952 (see course web site)

Fourier-Bessel Transform

In this last equation, v is no longer the argument of the cosine, but occurs as the combination nv in the exponential. Setting $u = 2\pi Rr$ and $v = (\Phi - \phi)$ in Equation (15), then Equation (13) gives us:

$$F(R, \Phi, Z) = \sum_{n=-\infty}^{\infty} \exp\{in(\Phi + \pi/2)\} \int_0^{\infty} J_n(2\pi Rr) 2\pi r \left[\frac{1}{2\pi} \int_0^{2\pi} \int_{-\infty}^{\infty} f(r, \phi, z) \exp\{i(2\pi Zz - n\phi)\} \, dz \, d\phi \right] dr \tag{16}$$

Michael Moody chapter (see course web site)

The (In)Famous little g's and Big G's

Because of the simplicity of the $z-Z$ transform, this is always performed first, so all the useful "n-transforms" are functions of Z. However, there are two states for the radial variables (r or R), so there are two of these intermediate "n-transforms". The first is the expression in square brackets in Equation (16):

$$g_n(r, Z) = \frac{1}{2\pi} \int_0^{2\pi} \int_{-\infty}^{\infty} f(r, \phi, z) \exp\{i(2\pi Z z - n\phi)\, dz\, d\phi \qquad (17)$$

The complete r-integral of Equation (16) is our second "n-transform":

$$G_n(R, Z) = \int_0^{\infty} g_n(r, Z) J_n(2\pi R r) 2\pi r\, dr \qquad (18)$$

Michael Moody chapter (see course web site)

The Forward Transform

Using $G_n(R, Z)$, the polar coordinate F.T. of Equation (16) may be written more simply as:

$$F(R, \Phi, Z) = \sum_{n=-\infty}^{\infty} \exp\{in(\Phi + \pi/2)\} G_n(R, Z) \tag{19}$$

Using the orthogonality of complex exponentials, this equation may be solved for $G_n(R, Z)$, giving:

$$G_n(R, Z) = \frac{1}{2\pi i^n} \int_0^{2\pi} \exp(-in\Phi) F(R, \Phi, Z)\, d\Phi \tag{20}$$

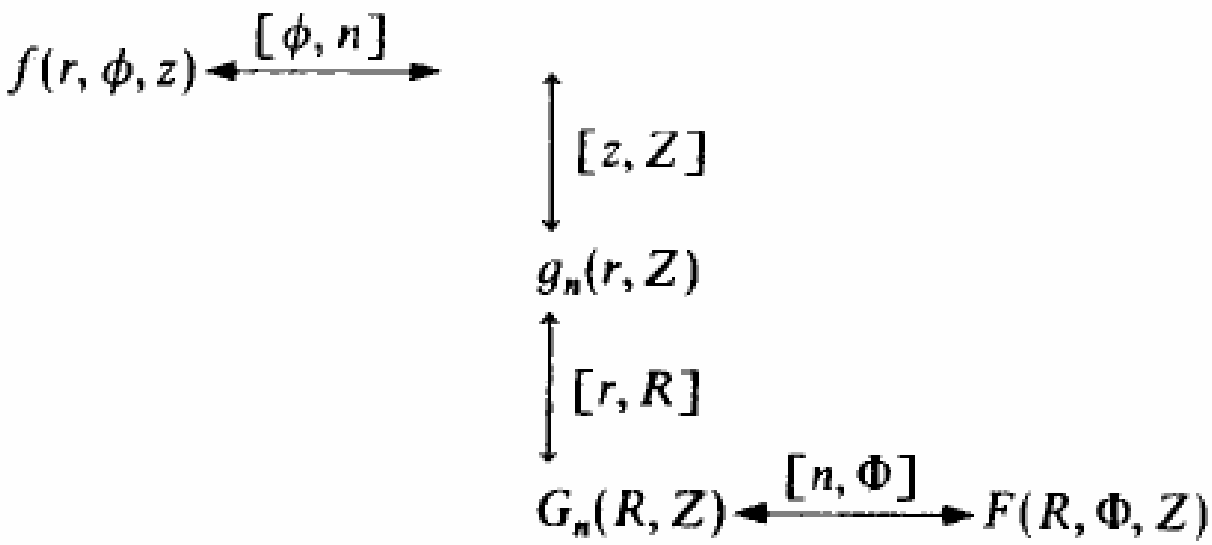
Equation (18) represents $G_n(R, Z)$ as a Hankel transform of $g_n(r, Z)$. Using the inversion theorem for these transforms (see the comments following Equation (27)), we also have

$$g_n(r, Z) = \int_0^{\infty} G_n(R, Z) J_n(2\pi R r)\, 2\pi R\, dR \tag{21}$$

Note: Hankel Transform = Fourier-Bessel Transform

Michael Moody chapter (see course web site)

Forward Transform Pathway



(22)

Michael Moody chapter (see course web site)

Inverse Transform for 3D Reconstruction

$$f(r,\phi,z) = \sum_{n=-\infty}^{\infty} \exp\{in(\phi - \pi/2)\} \int_0^{\infty} J_n(2\pi Rr)2\pi R \times \left[\frac{1}{2\pi}\int_{-\infty}^{\infty}\int_0^{2\pi} F(R,\Phi,Z)\exp\{-i(2\pi Zz + n\Phi)\}\,d\Phi\,dZ\right] dR \quad (25)$$

2D -> 3D!

Derivation in Moody chapter, pp. 192-193.

This requires indexing of 2D diffraction pattern (layer lines), see subsequent video lecture by Moody.

Michael Moody chapter (see course web site)

Helical Selection Rule for Allowed Peaks

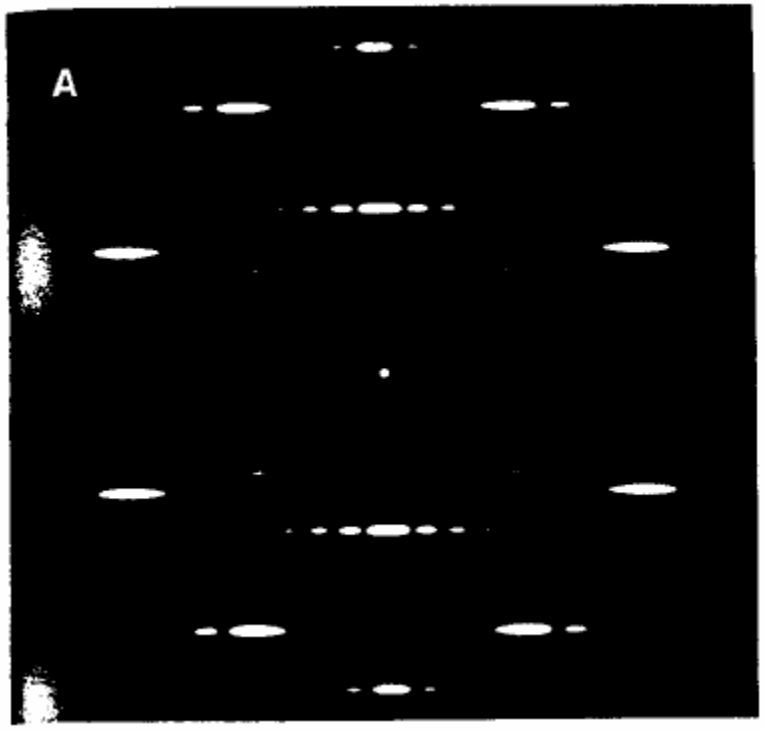
If there is an exact z-repeat of length c, i.e. if $f(r, \phi, z) = f(r, \phi, z + c)$, then $2\pi Zc$ must be a multiple of 2π, or $Zc = L$ (L = any integer). Substituting this in Equation (29), we

$$L = um + tn \tag{30}$$

where u ($= c/h$) is the number of units per repeat, and t ($= c/P$) is the number of turns per repeat. Equation (30) is a linear equation with integral coefficients and two variables (m, n). Given one solution (m_1, n_1), the next is $(m_1 + j, n_1 - ju/t)$, where j is the smallest integer that makes $j(u/t)$ integral.

Michael Moody chapter (see course web site)

Helical Selection Rule: n-l Plot



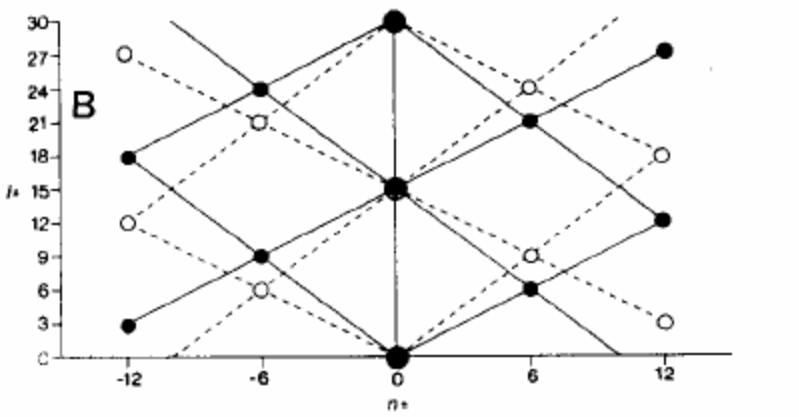


Fig. 10. The Fourier transform of a helix (a) gives a series of layer line reflections corresponding to its top and bottom that form two overlapping lattices. These can be most easily analysed in a (n,*l*) plot (b). This plot shows the lattice terms from the top (filled circles) and bottom (open circles) of the helix. Once the lattice has been identified, it can be used to deduce the helical symmetry, which in this case is six strands, each containing 15 subunits per turn.

Klug et al., 1958; Stewart, 1988 (see course web site)

References-Helical Reconstruction

- Cochran, Crick, & Vand, 1952 (FT of helix)
- Klug, Crick, & Wyckoff, 1958 (selection rule, n-l plot)
- DeRosier & Klug, 1968 (first ever 3D reconstruction from EM)
- Stewart, 1988 (great review of helical reconstruction technique)
- Moody, 1990 (of course)

- These references are downloadable online (see session web site).

References-Electron Microscopy

- Texts
  - Biophysical Electron Microscopy: Basic Concepts and Modern Techniques by U. Valdre (Editor), Peter W. Hawkes (Editor)
  - Three-Dimensional Electron Microscopy of Macromolecular Assemblies by Joachim Frank
  - Negative Staining and Cryoelectron Microscopy: The Thin Film Techniques by Robin J. Harris, James R. Harris
- Reviews
  - Henderson, R. The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules. *Q Rev Biophys* **28**, 171-93 (1995).
  - Glaeser, R. M. Review: electron crystallography: present excitement, a nod to the past, anticipating the future. *J Struct Biol* **128**, 3-14 (1999).
  - Stowell, M. H., Miyazawa, A. & Unwin, N. Macromolecular structure determination by electron microscopy: new advances and recent results. *Curr Opin Struct Biol* **8**, 595-600 (1998).
- Web
  - http://em-outreach.ucsd.edu/web-course/toc.html
  - http://ncmi.bcm.tmc.edu/%7Estevel/spintro/siframes.htm
  - http://cryoem.berkeley.edu/~nieder/em_for_dummies/