Cryo-Electron Microscopy

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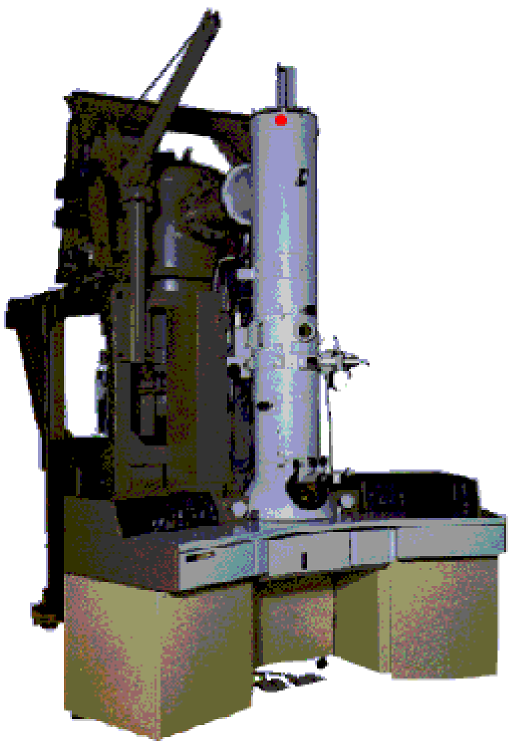
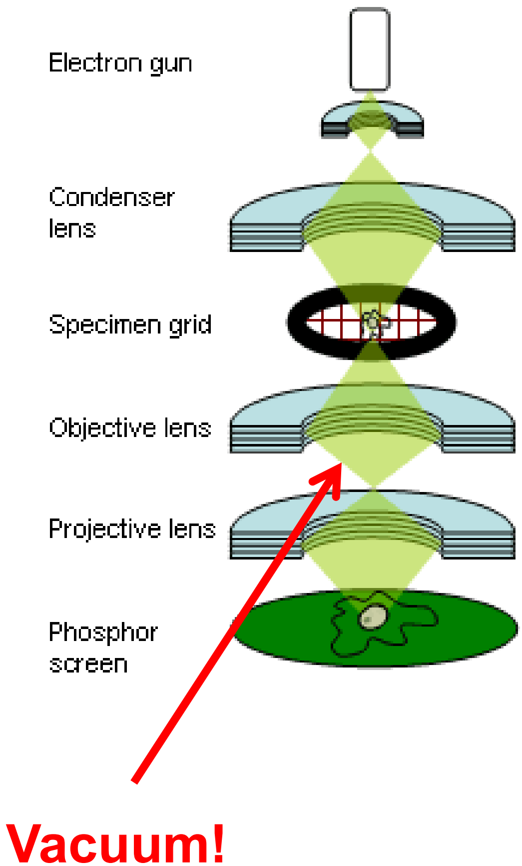
Transmission Electron Microscope





Electron source:
Thermal emission from heated cathode

Focussing:
Electro-magnetic Lenses

Detection:
Phosphor screen or CCD camera (former times: negative)

Pro & Con of cryo-EM

Pro

- Short wavelength => high resolution
- Strong interaction with materials => good contrast
- Electromagnetic lenses => standard optics (in contrast to X-ray crystallography)
- High intensity is easy to produce
- Inner structure of biomolecules is accessible

Con

- High vacuum requires special treatment of sample
- Sample has to be thin to avoid 100% absorption
- Electron beam damages biological samples => short measurements => low contrast of biomolecules

Microscope Optics

In order to see an "object" which is too small to be seen by our eyes, one needs to magnify the image. An example of magnifying an image by a lens is illustrated in Fig. 2.1:

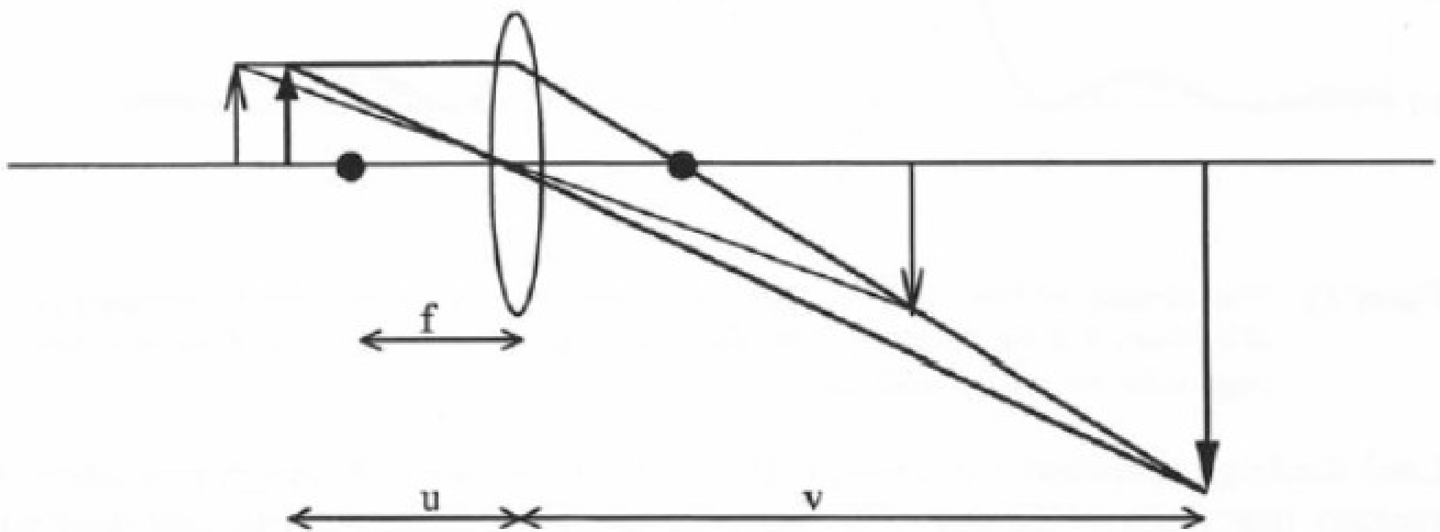
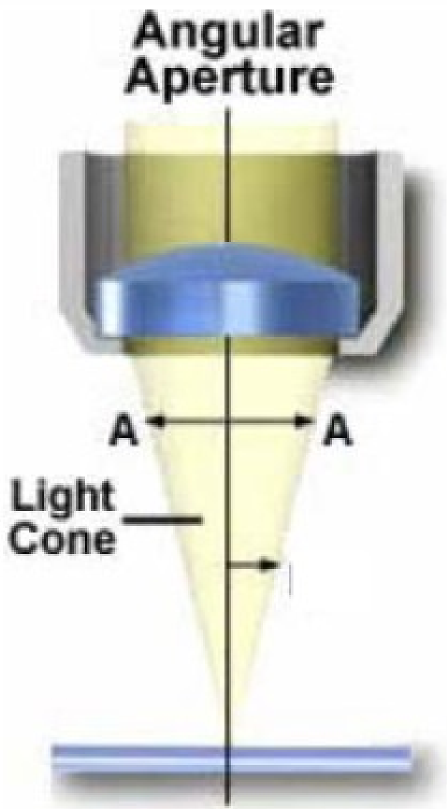
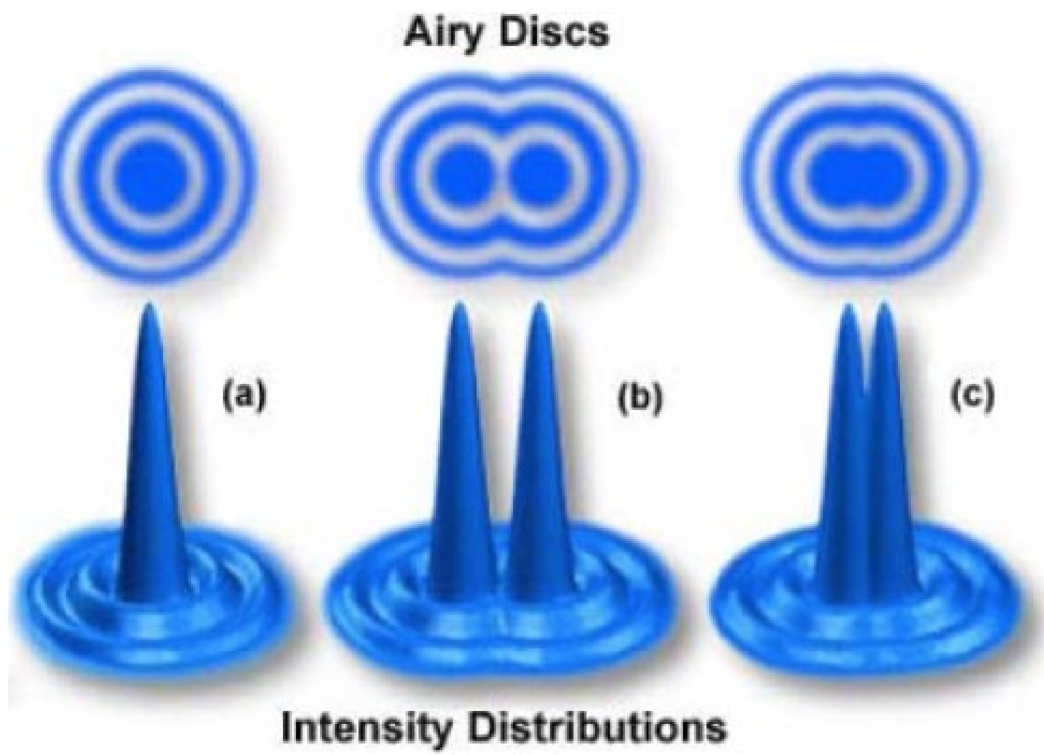


Figure 2.1 Ray diagram illustrating the formation of images by a lens. f = focal length, u = the distance between the object and the lens, v = the distance between the image and the lens.

Optical Resolution

An image cannot be endlessly enlarged due to the limit of the resolution. "Resolution" is the closest distance between two points on the object which can be clearly seen through the microscope to be separate entities.





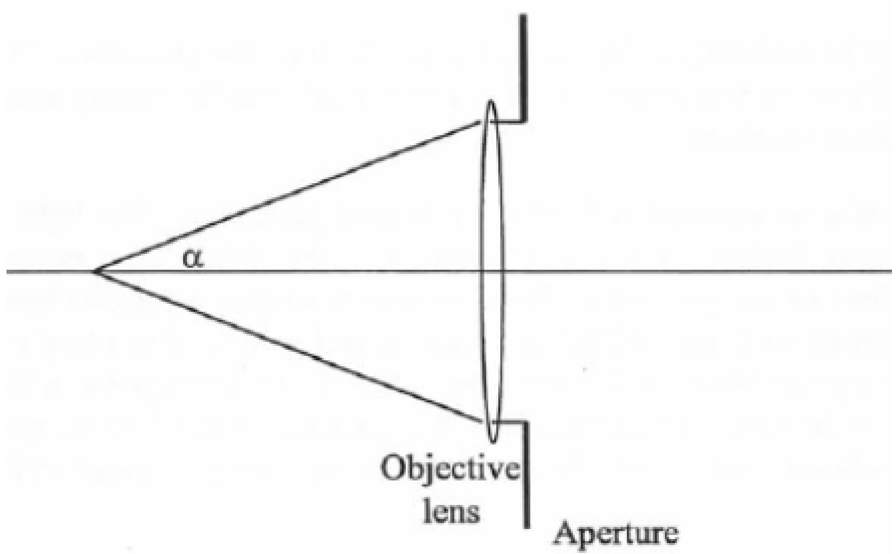
The central maximum of the Airy patterns is often referred to as an Airy disk, which is defined as the region enclosed by the first minimum of the Airy pattern and contains 84 percent of the luminous energy.

Rayleigh criterion

the intensity maximum of the Airy disc from one point coincides with the first minimum of the Airy disc from the second point, then the two points can be just resolved. The Rayleigh resolution can be derived from diffraction theory to be:

$$r = \frac{d}{2} = \frac{0.61\lambda}{\mu \sin\alpha} \qquad (2\text{-}2)$$

where λ is the wavelength of the light and μ is the refractive index of the medium between the object and objective lens. α is the semi-angle above which the light is stopped by the aperture, see in Fig. 2.3.



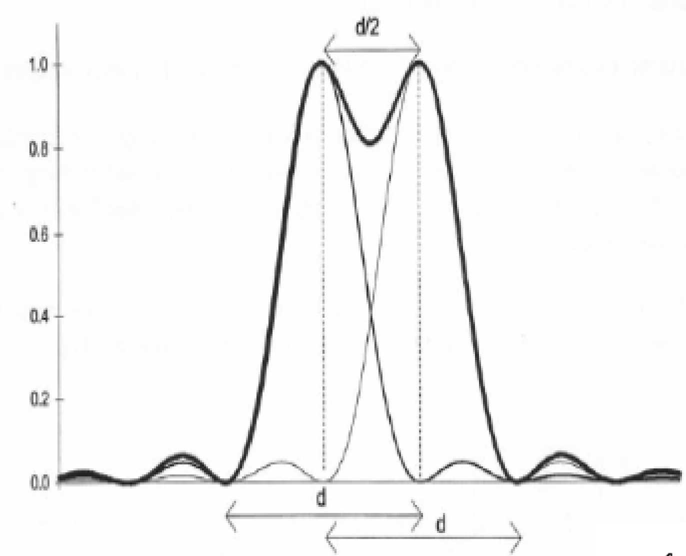
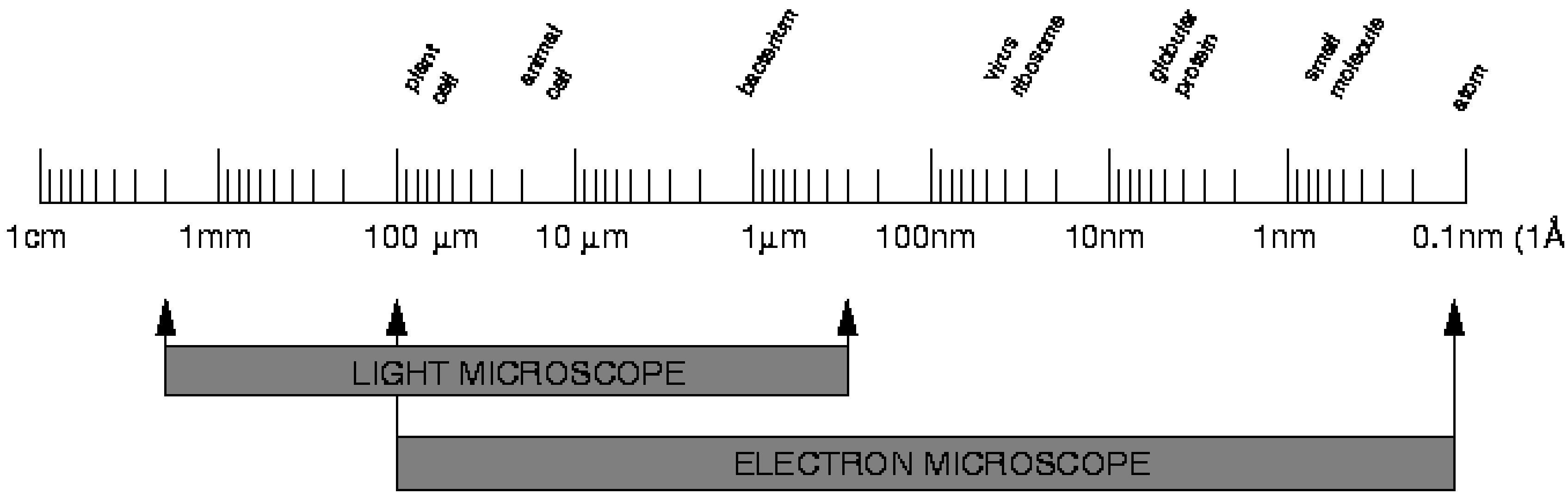


Figure 2.3 The definition of the semi-angle, α.

Why electrons?



Visible Light:

λ = 400 – 600 nm

Electrons:

λ = 0.002 – 0.004 nm

Electrons are Both Particles and Waves

Electrons, like other particles, can be considered both as particles and waves. The wavelength of an electron depends on its velocity, v:

$$\lambda = \frac{h}{mv} \tag{2-3}$$

When an electron is accelerated through a potential difference U (volt), its wavelength can be calculated as follows:

$$\lambda = \frac{h}{(2em\mathrm{U} + \frac{e^2\mathrm{U}^2}{c^2})^{\frac{1}{2}}} = \left(\frac{1.5}{(\mathrm{U} + 10^{-6}\mathrm{U}^2)}\right)^{\frac{1}{2}} \text{ nm} \tag{2-4}$$

where h is Planck's constant, c is the speed of light, e and m are the mass and charge of the electron. Typical values of the electron wavelengths used in a TEM are:

U (kV)	100	200	300	400	500	1000
λ (Å)	0.0370	0.0251	0.0197	0.0164	0.0142	0.0087

The wavelength depends on the accelerating voltage used. The higher the acceleratin voltage, the shorter the wavelength.

Sample Preparation - Staining

⇒ To increase contrast: heavy atoms interact with electrons stronger than biomolecules (C, N, O, S, P)

- **Positive Staining**

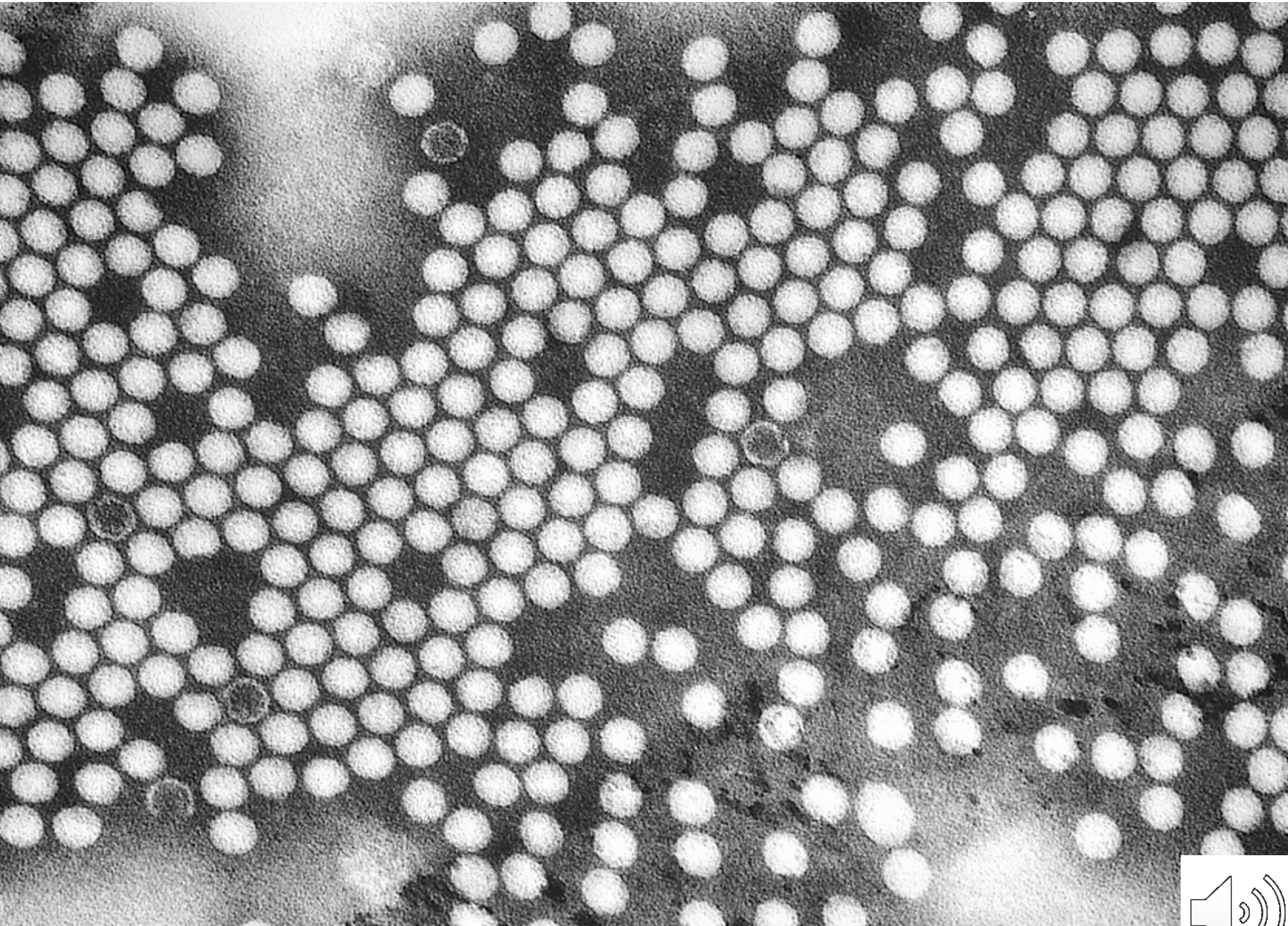
  treat sample with solution of salt like uranyl acetate, lead citrate, osmium tetraoxide – object is black on light background
- **Negative Staining**

  place sample on dried film of heavy metal salt – object is light spot on black background
- **Shadowing**

  spray thin layer of heavy metal on sample to produce a shadow

Disadvantage:

Size of stain reduces resolution to about 20-30 Å

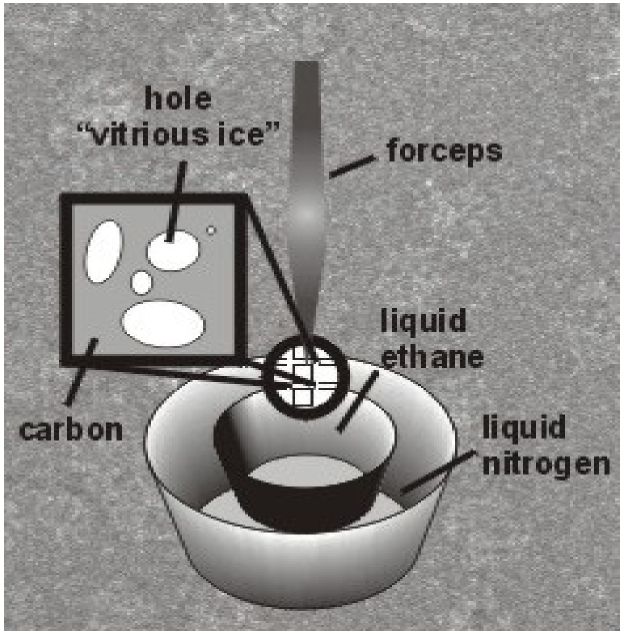


Alternative: Cryo-EM

- to avoid harsh staining which may change the structure of your sample

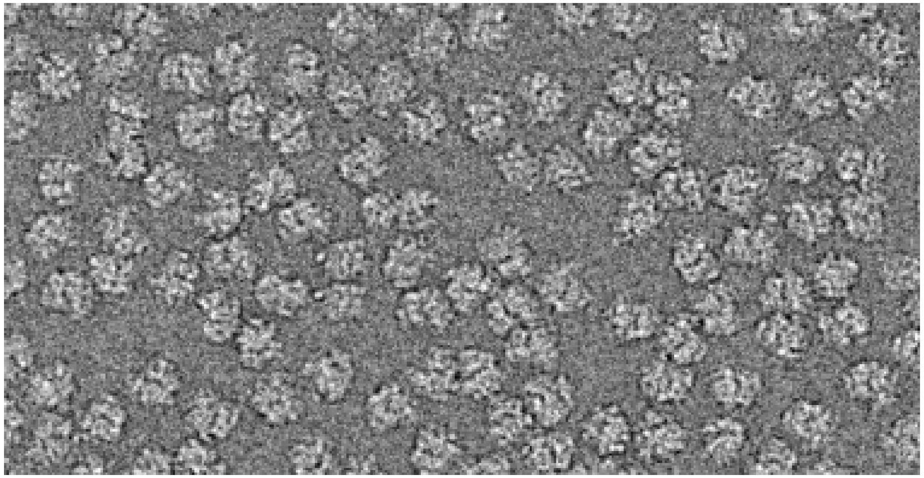
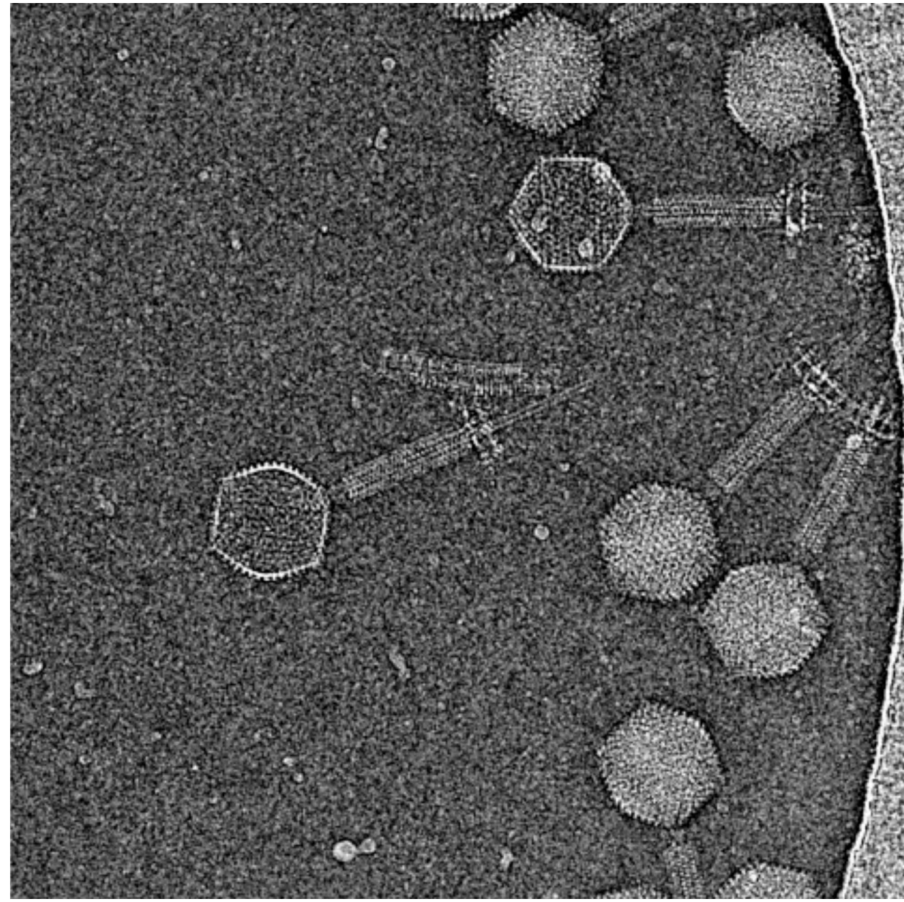
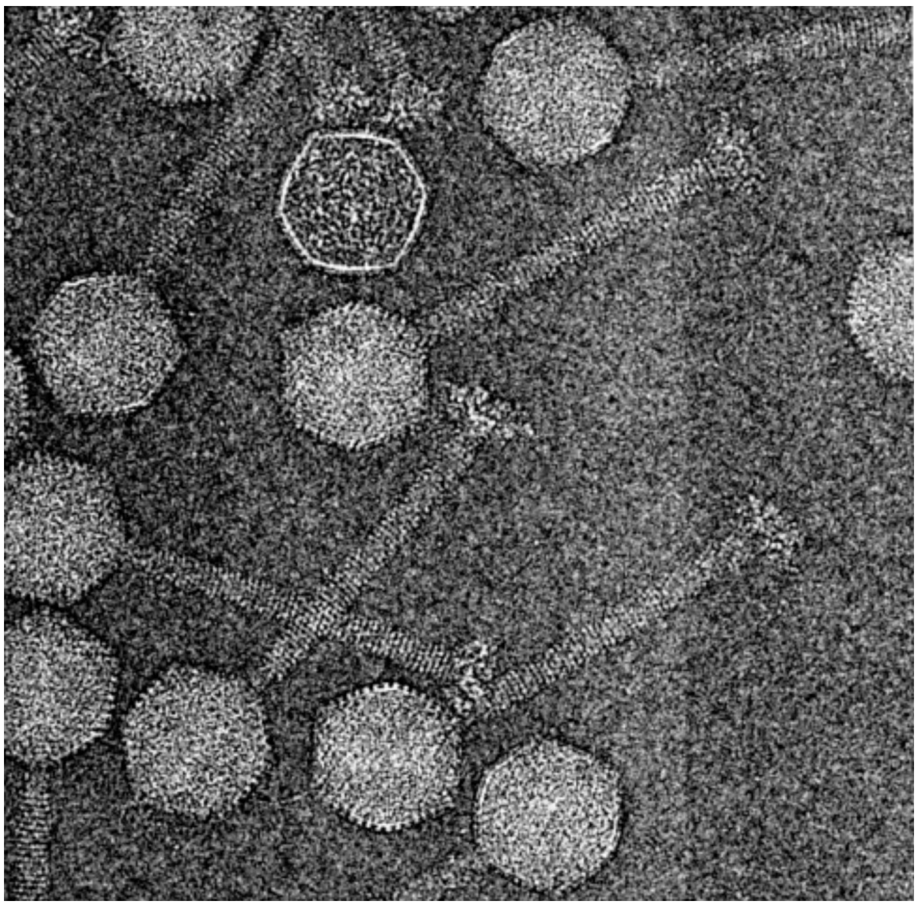
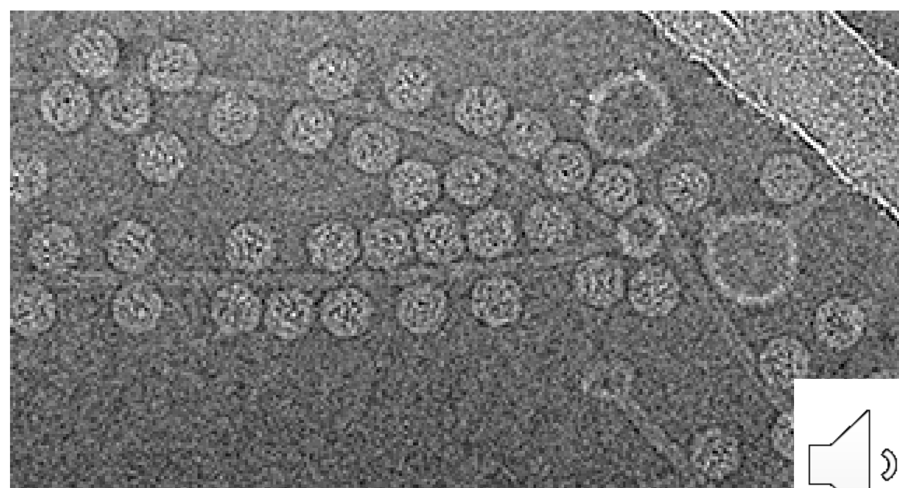
- stabilization of sample by rapid freezing of sample in liquid ethane to form vitreous ice
- into electron microscope at low temperatures to keep sample stable in hydrated state in vacuum

- thickness of ice layer as small as possible!

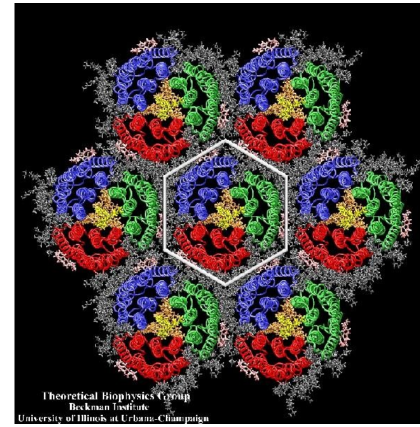


Advantage:

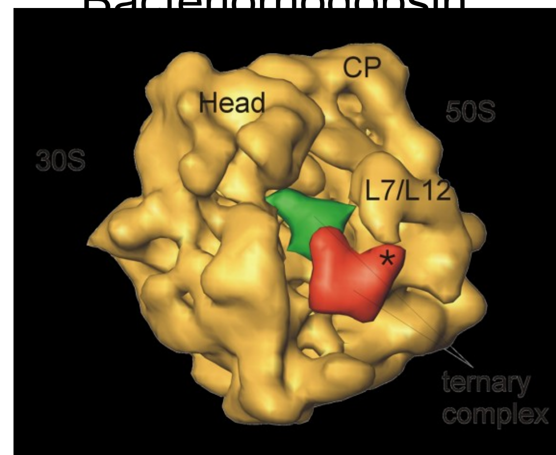
- sample structure is unchanged
- inner structures of molecules are accessible

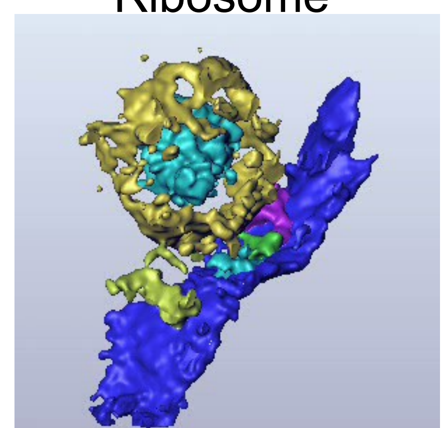
Types of Samples



Bacteriorhodopsin



Ribosome

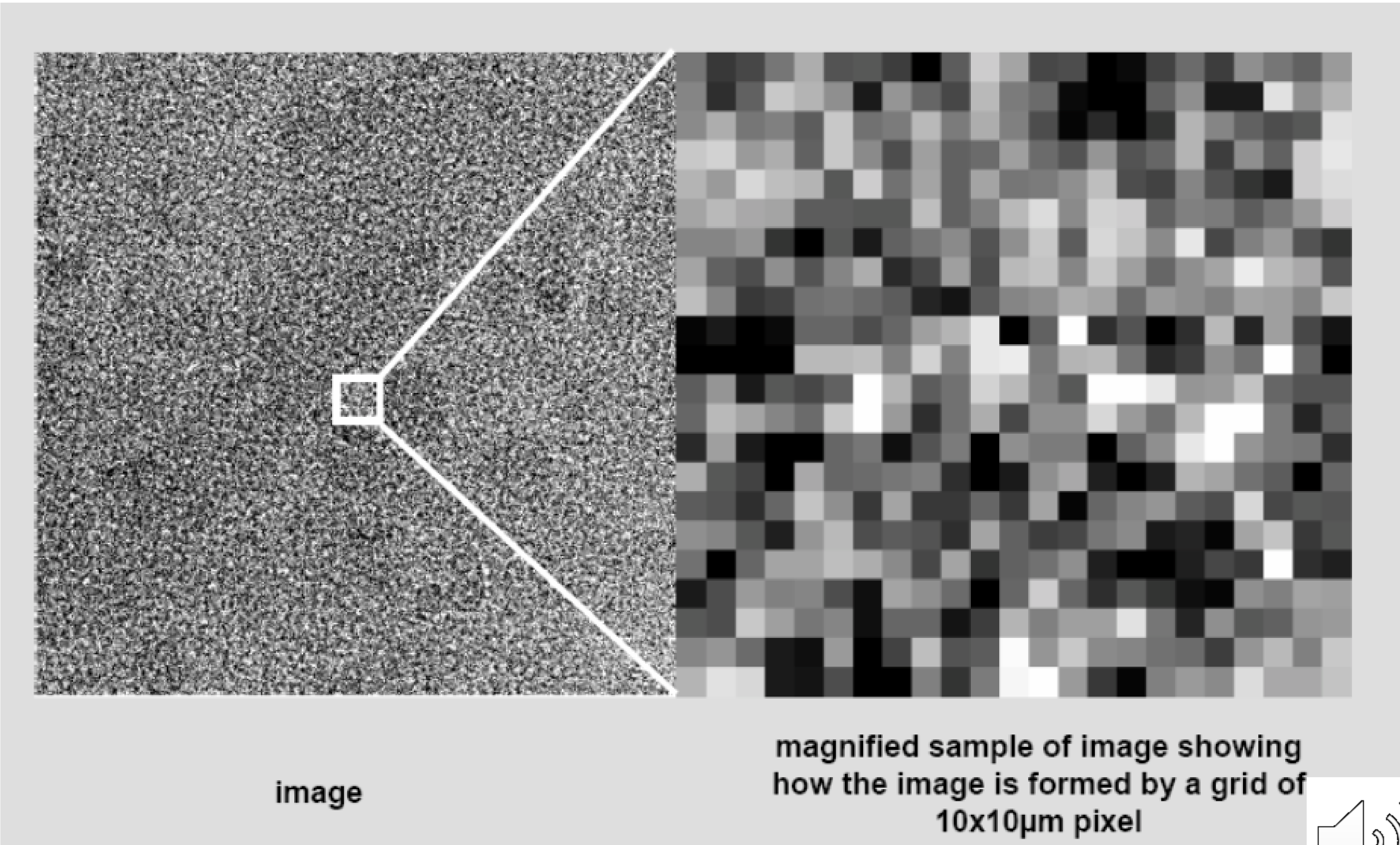


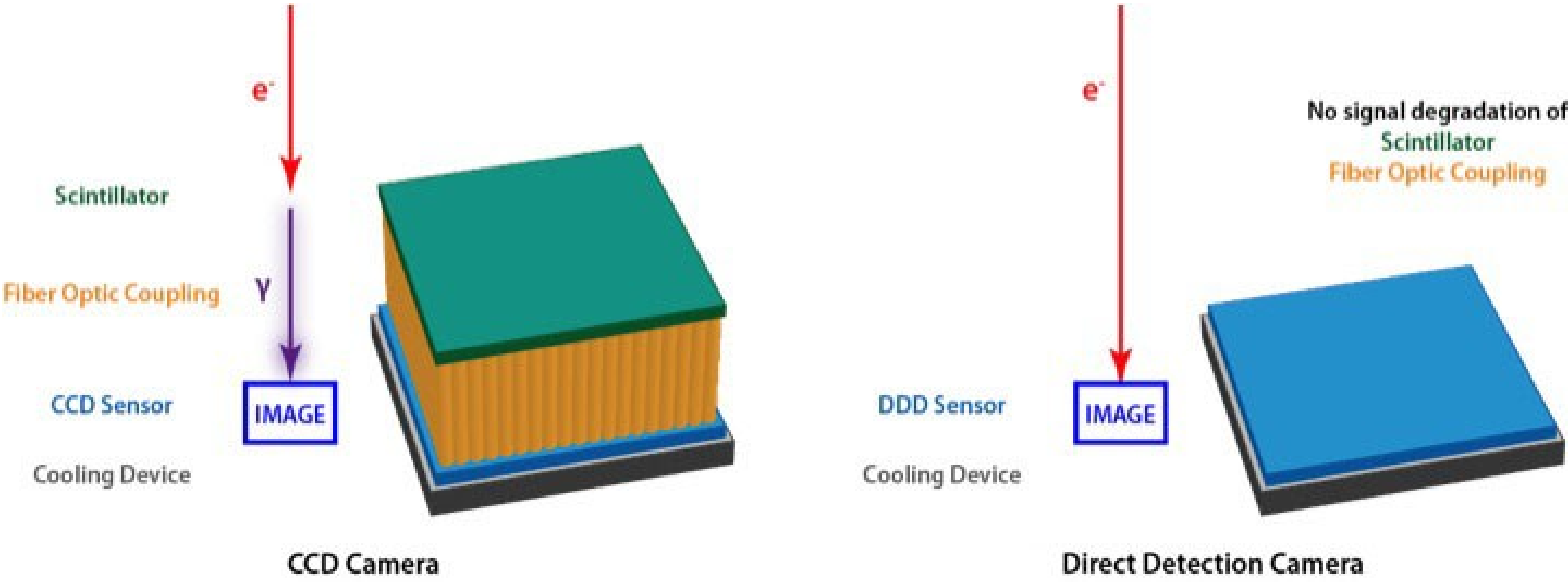
- Periodic arrangement
  => 2D electron crystallography
  small or membrane proteins < 200 kDa
  resolution up to 2.5 Å

- Random arrangement
  => single particle technique
  macromolecular complexes > 200 kDa
  up to atomic resolution

- Large Organelles (Golgi, ER), whole cells
  => tomography
  resolution > 4 Å

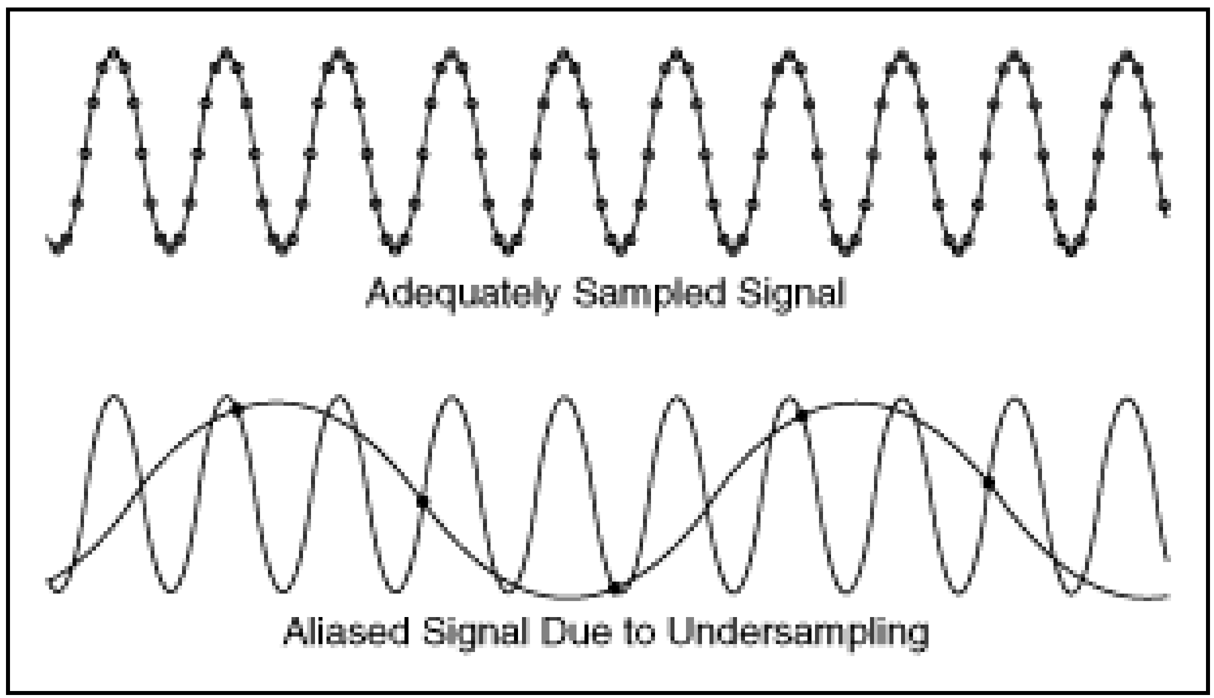
Digitization of Recorded Image

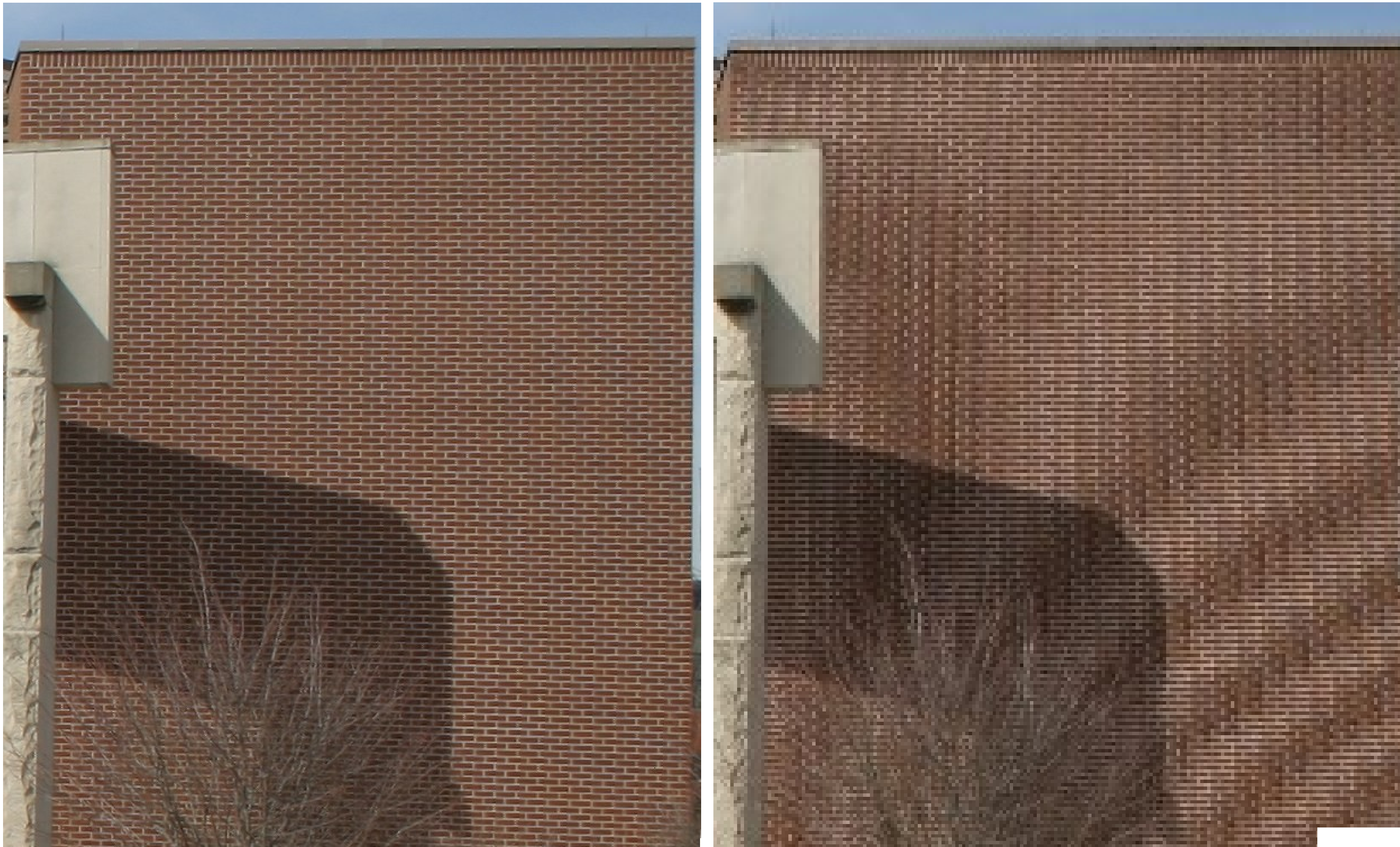



e-
Scintillator
Fiber Optic Coupling
γ
CCD Sensor
IMAGE
Cooling Device
CCD Camera
e-
No signal degradation of
Scintillator
Fiber Optic Coupling
DDD Sensor
IMAGE
Cooling Device
Direct Detection Camera

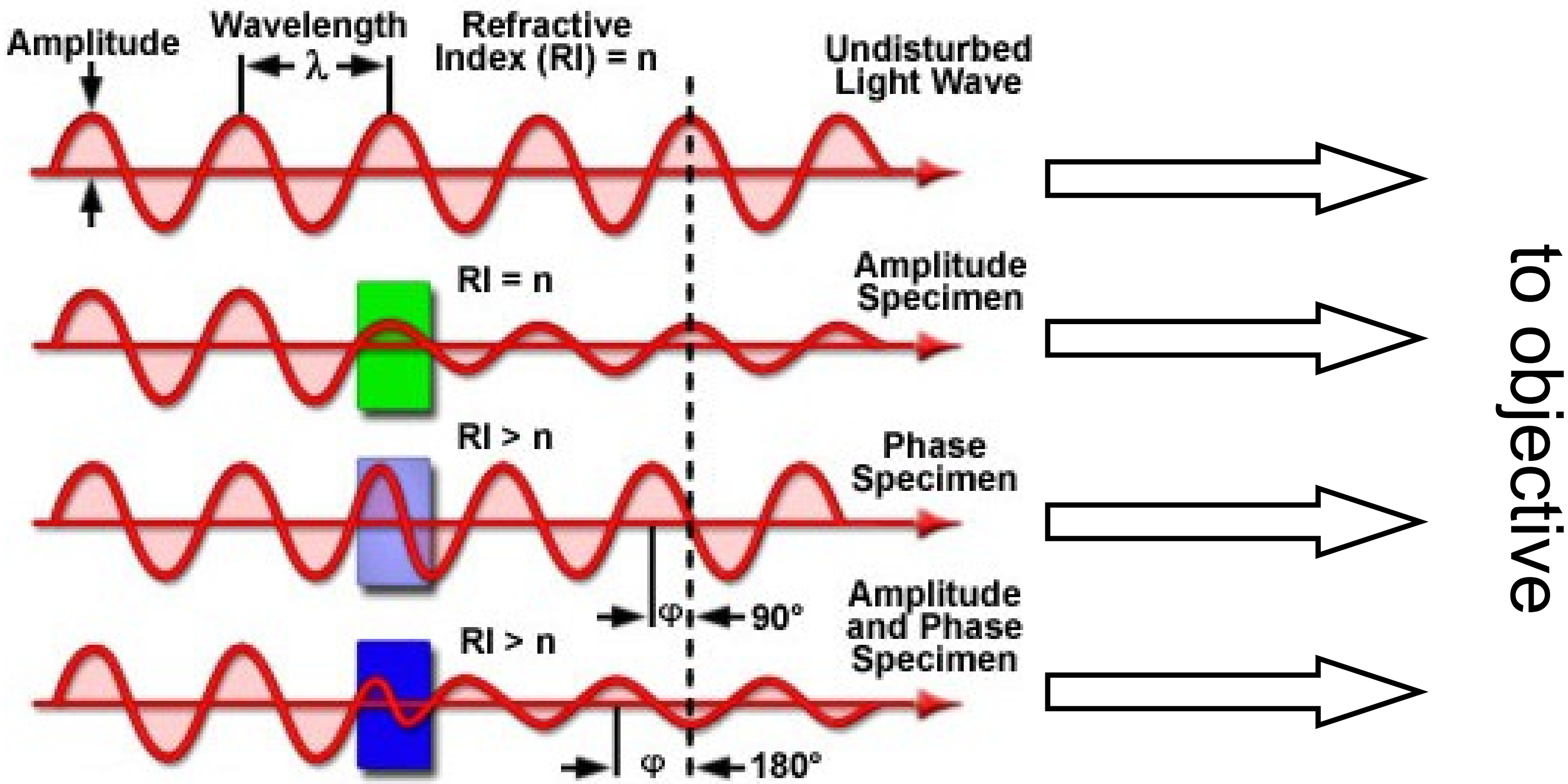
For a given sampling frequency, the maximum frequency you can accurately represent without aliasing is the Nyquist frequency, which equals one-half the sampling frequency, as shown by the following equation.

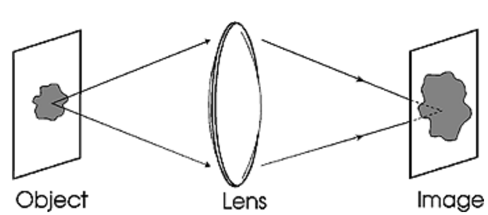
$$f_N = \frac{f_s}{2}$$



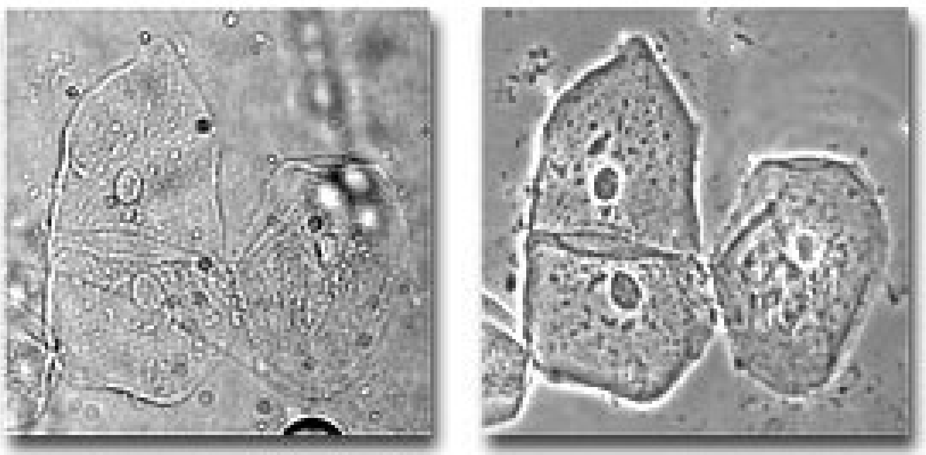


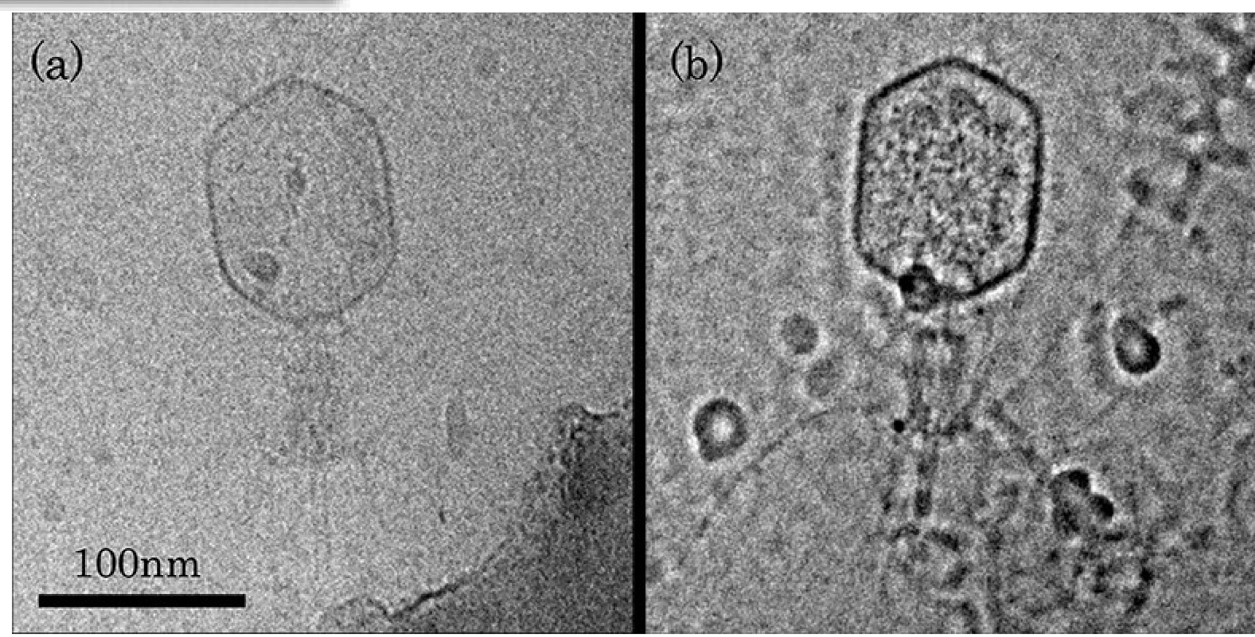
Macromolecules in water / vitreous ice are phase objects





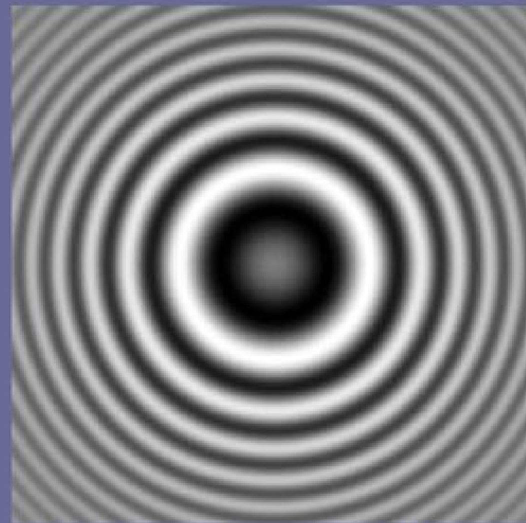
Phase Objects Require an Additional Phase Shift to be Seen





original object

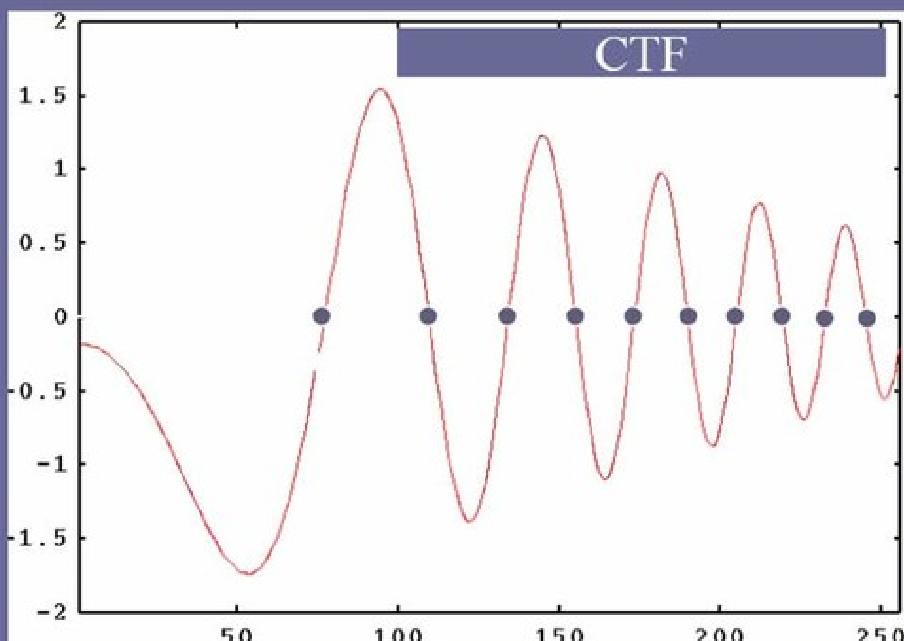
x



CTF for Δz = 2.500 µm

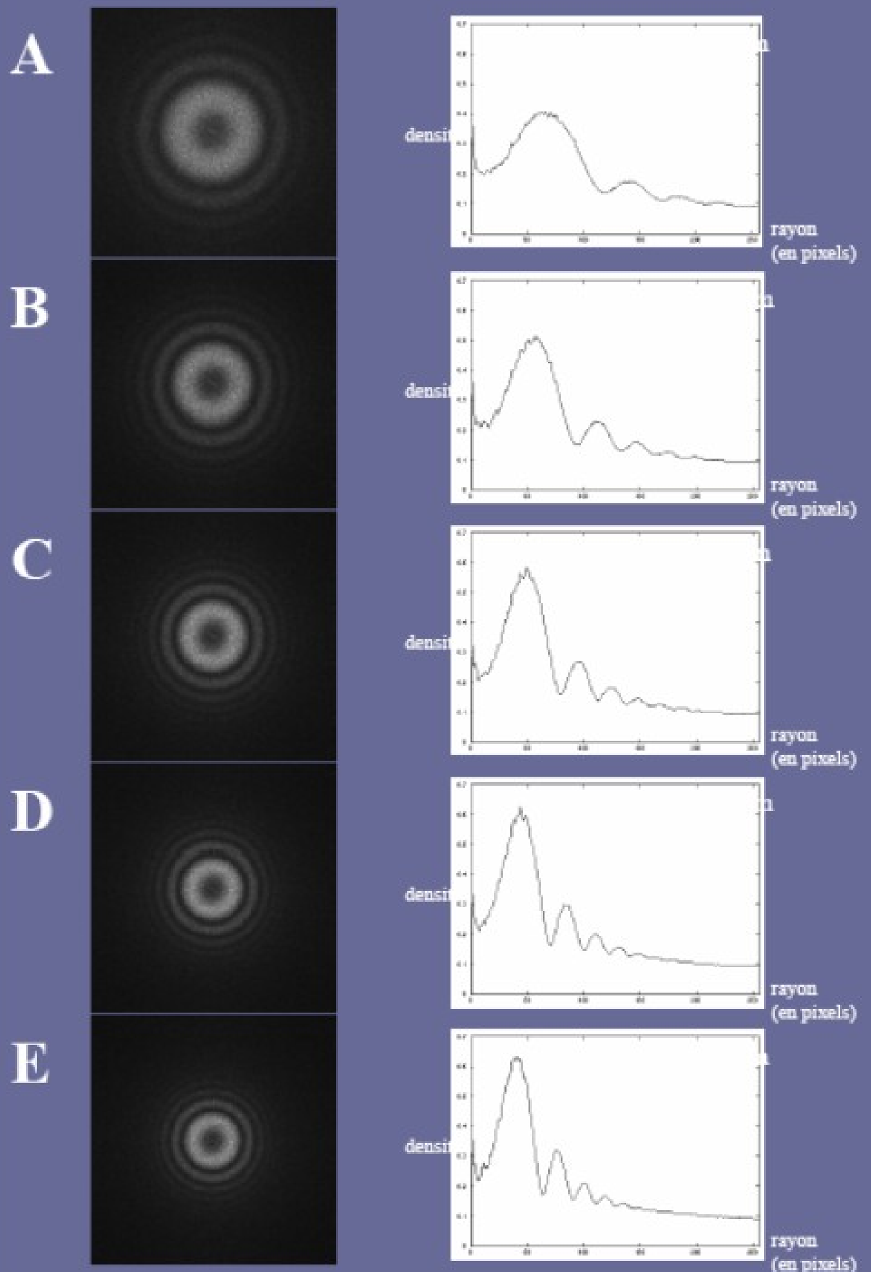
=

cryo-EM image

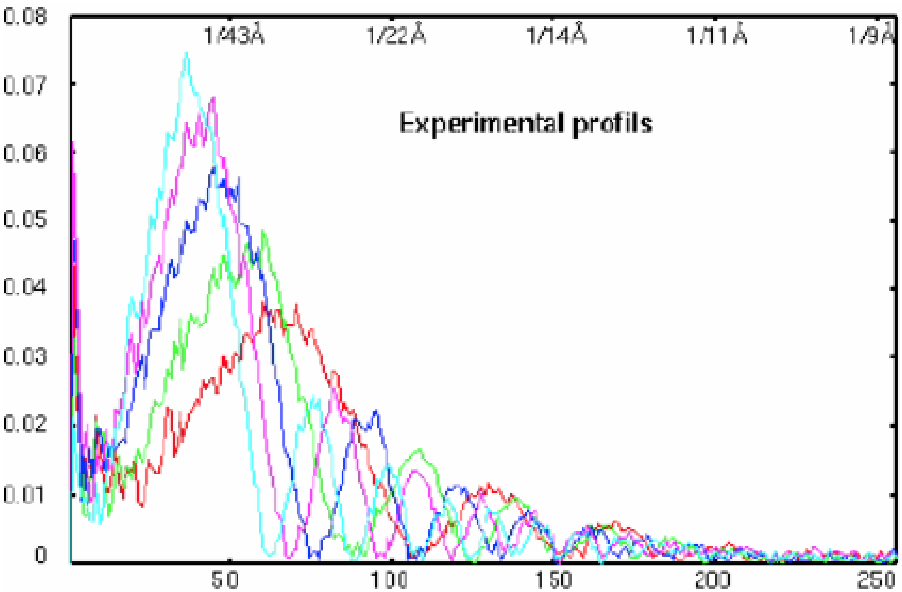


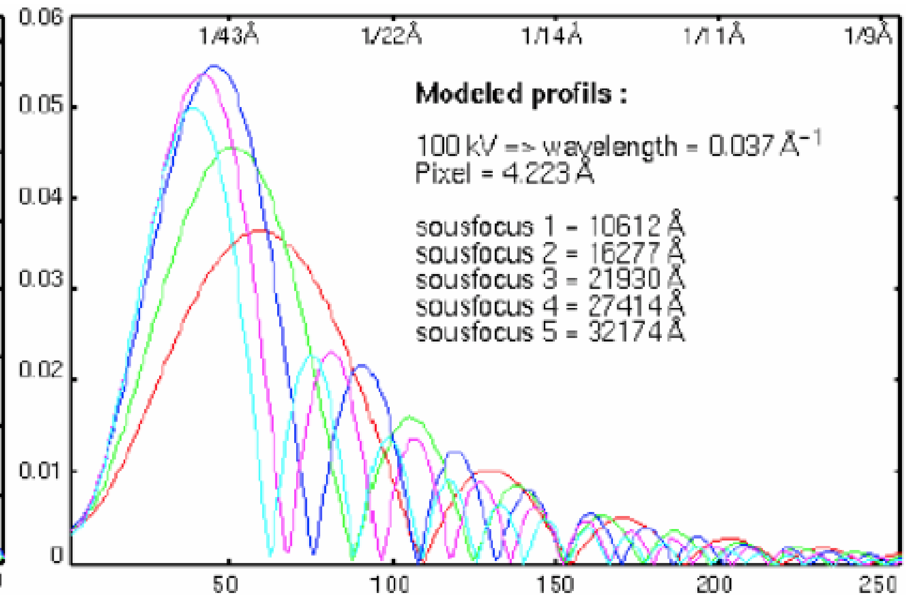
cryo-EM imag
contrast-invert

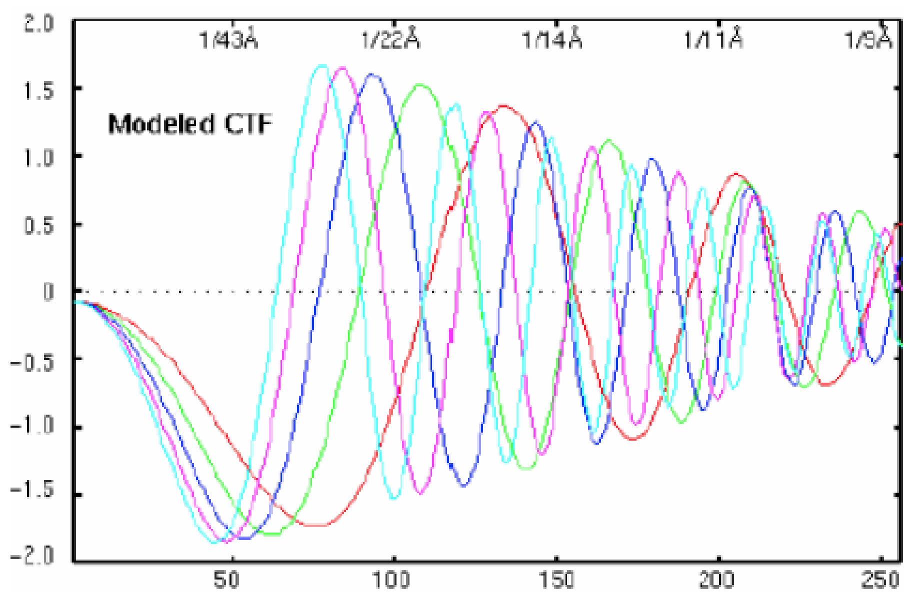
Gallery of Power Spectra at Different Defocus



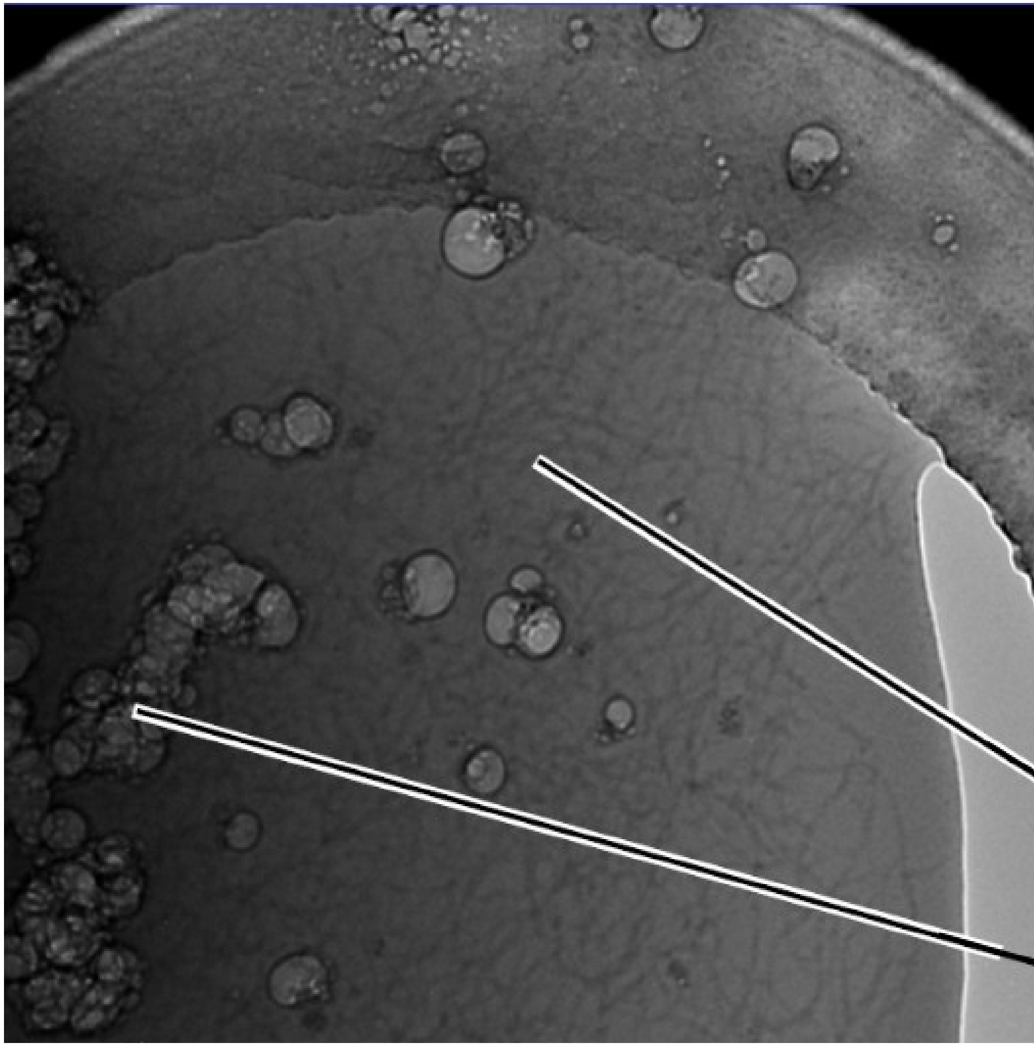
Multiple Defocus Groups







Bubbling: A Sign of Radiation Damage



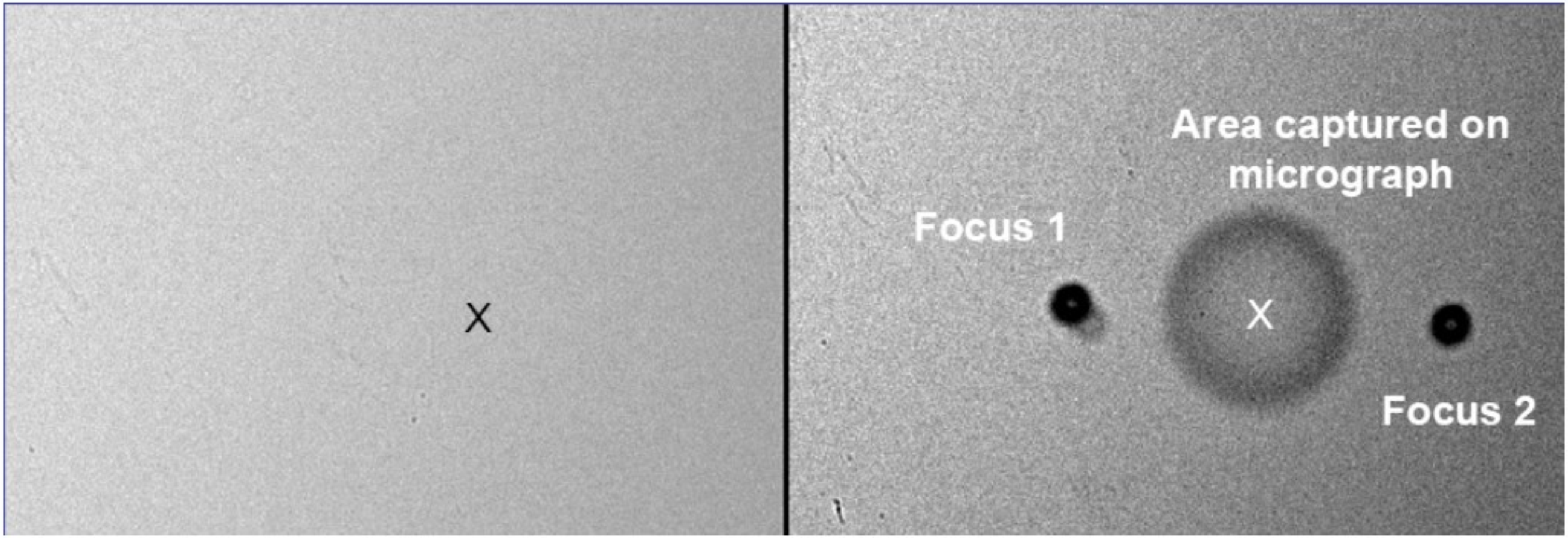
A sample of unstained amyloid materials after a few seconds of illumination with an electron beam.

While some fibers can still be detected, “bubbling” within the field of view indicates total destruction of the sample

amyloid fibers

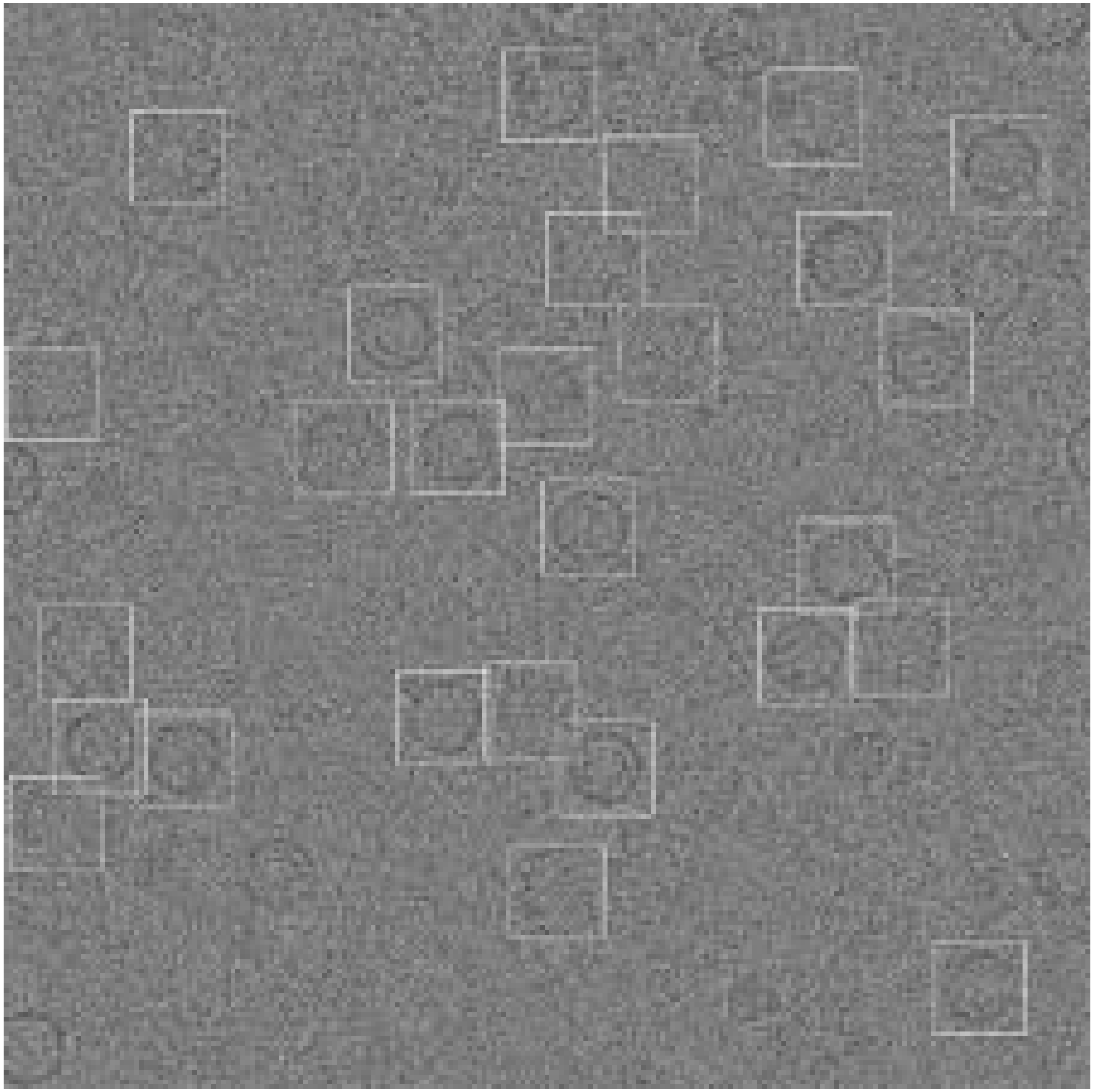
bubbles

Low-Dose Microscopy

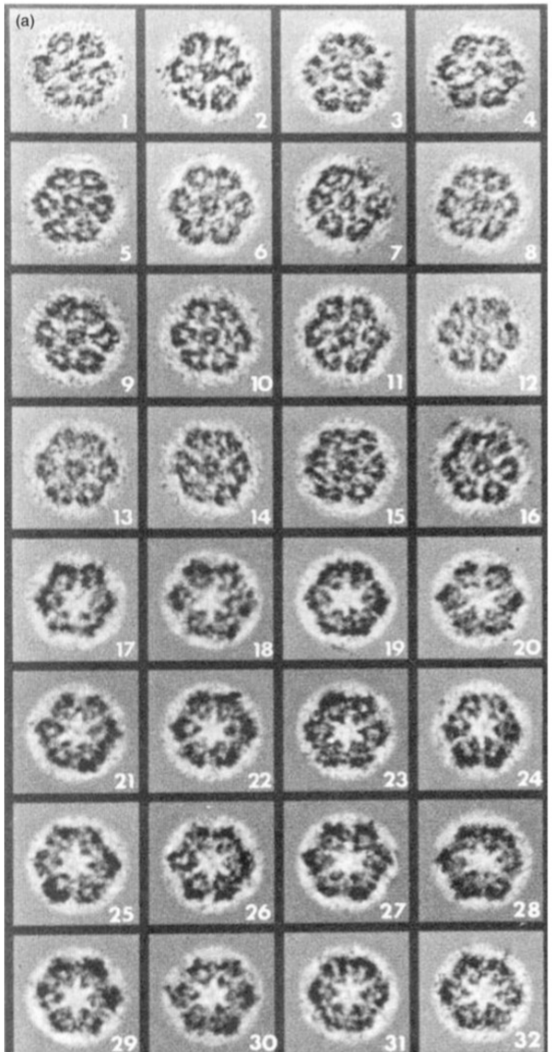


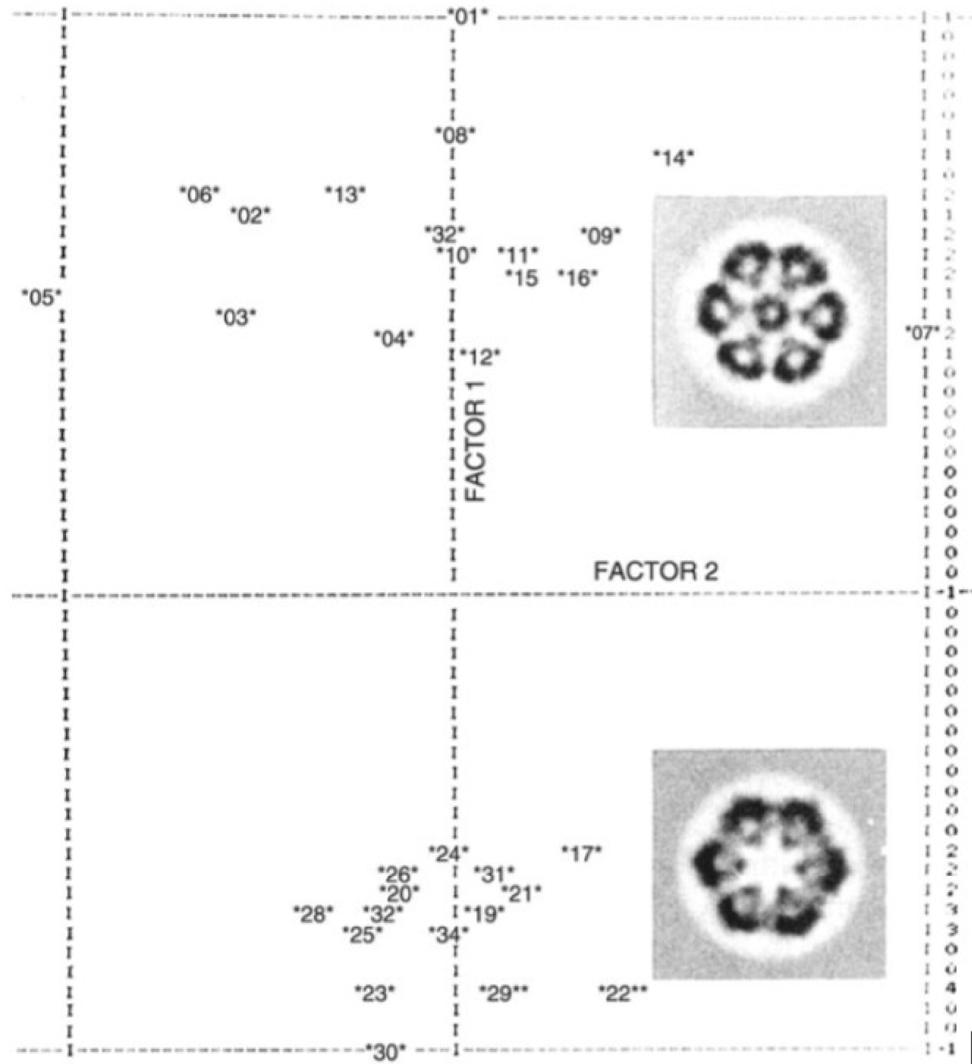
Appearance of trehalose dried down on a carbon film (left). The sugar allows to demonstrate how “low-dose” microscopy is done (right). Let X be the area of interest (for instance a crystal or virus/single particle). Prior to taking a picture some parameters such as “defocus” and “astigmatism” need to be adjusted. To avoid destruction of the specimen, any adjustments are made on small areas (Focus 1 and 2) located adjacent to the area that will be photographed. In the example, the trehalose burned as it was exposed at high magnification (220kx, Focus 1 and 2). Similarly, by exposing the area to be captured for about 30 seconds at 52,000 fold magnification.

Signal to noise ratio



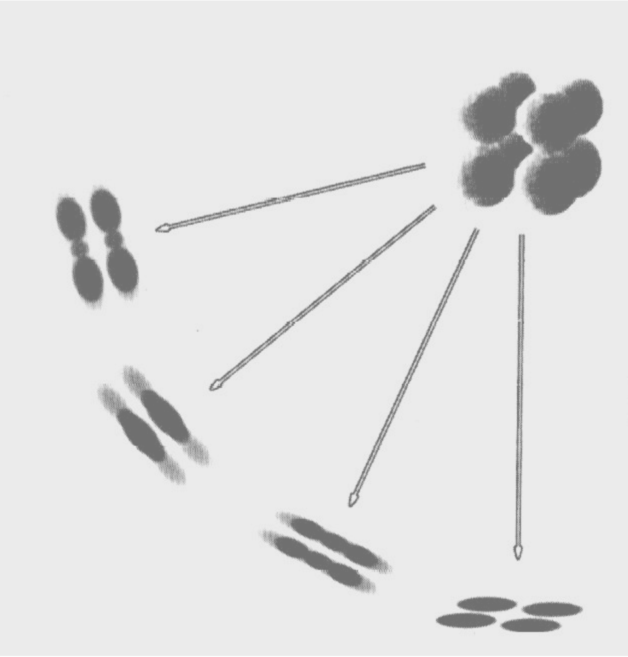
Classification and averaging (principal component analysis)





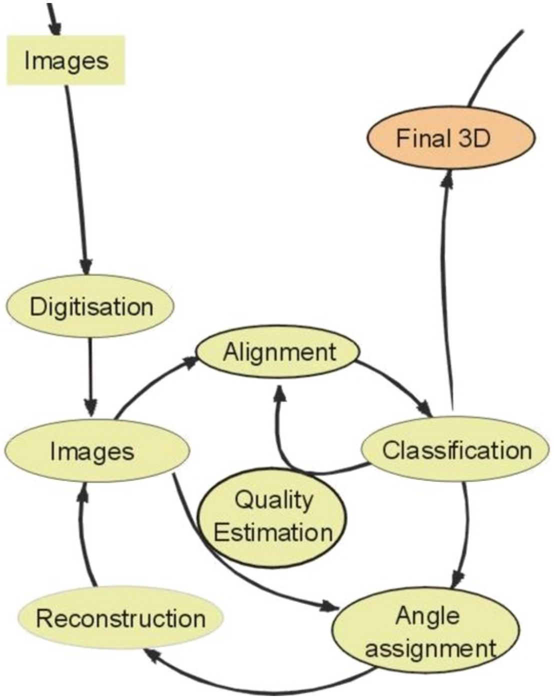
3D Reconstruction

When the angles between the different classes are known (estimated), a 3D model can be calculated.



Iterative Process:

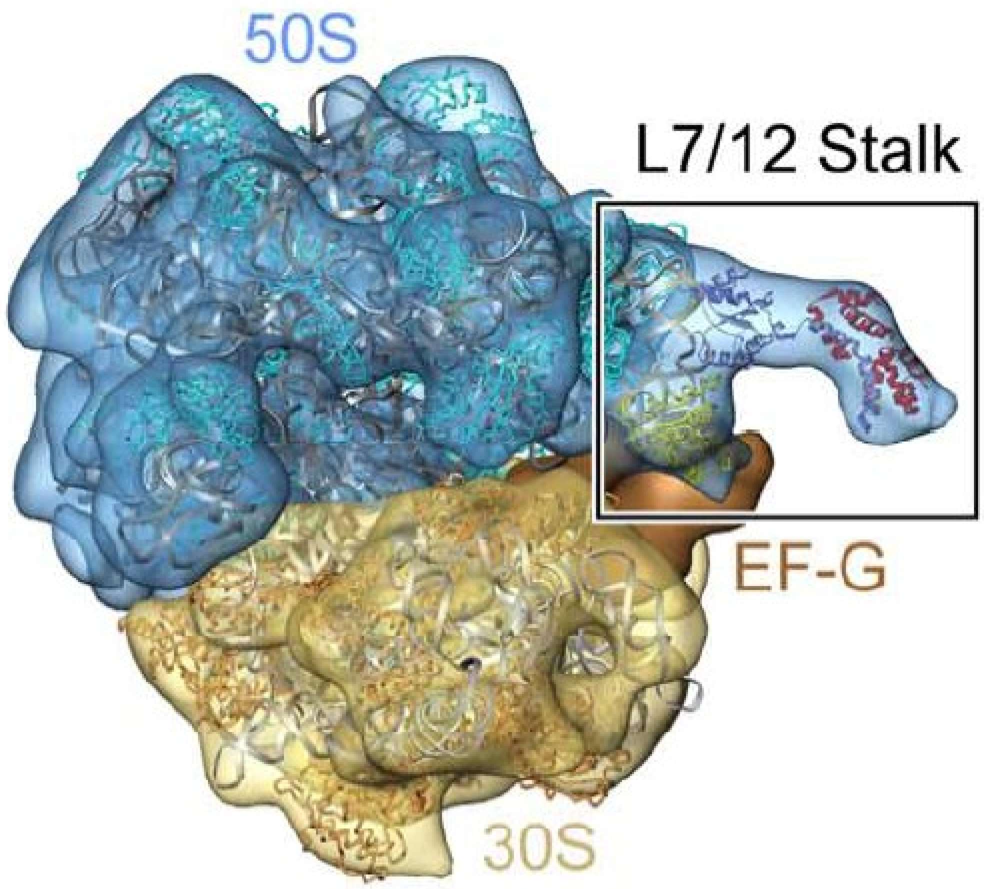
3D model is used to generate 2D images which are fed into statistical analysis of images (alignment and classification).

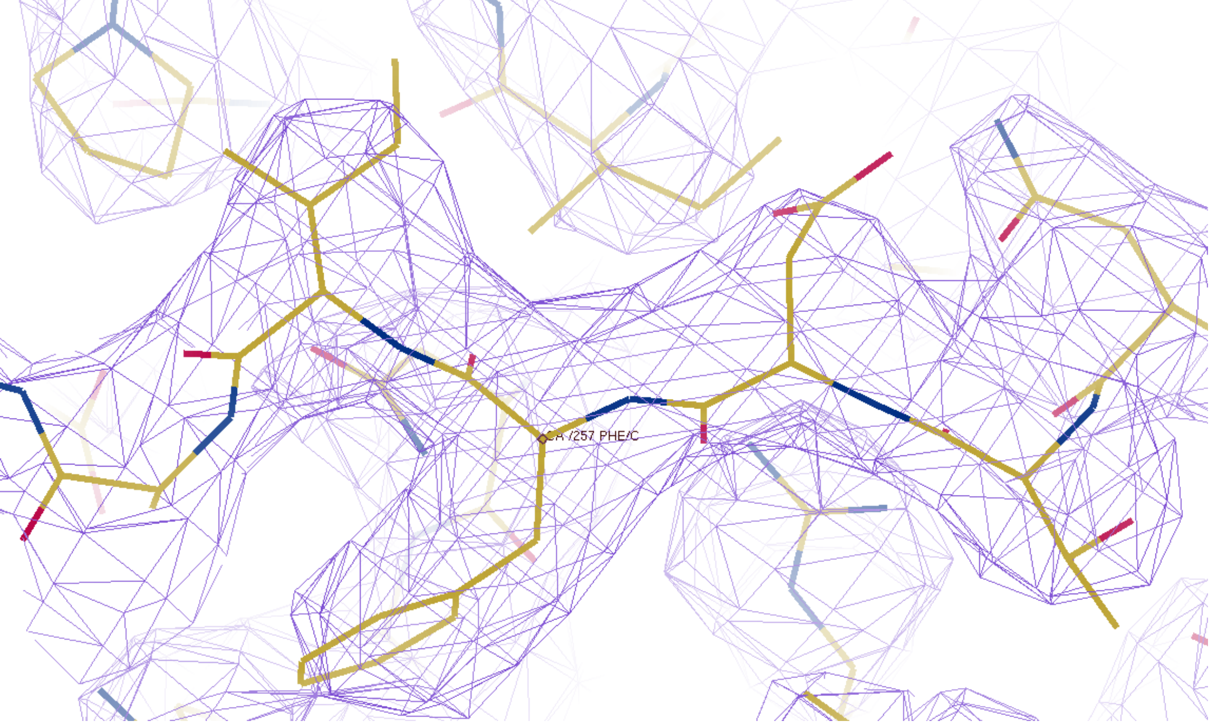


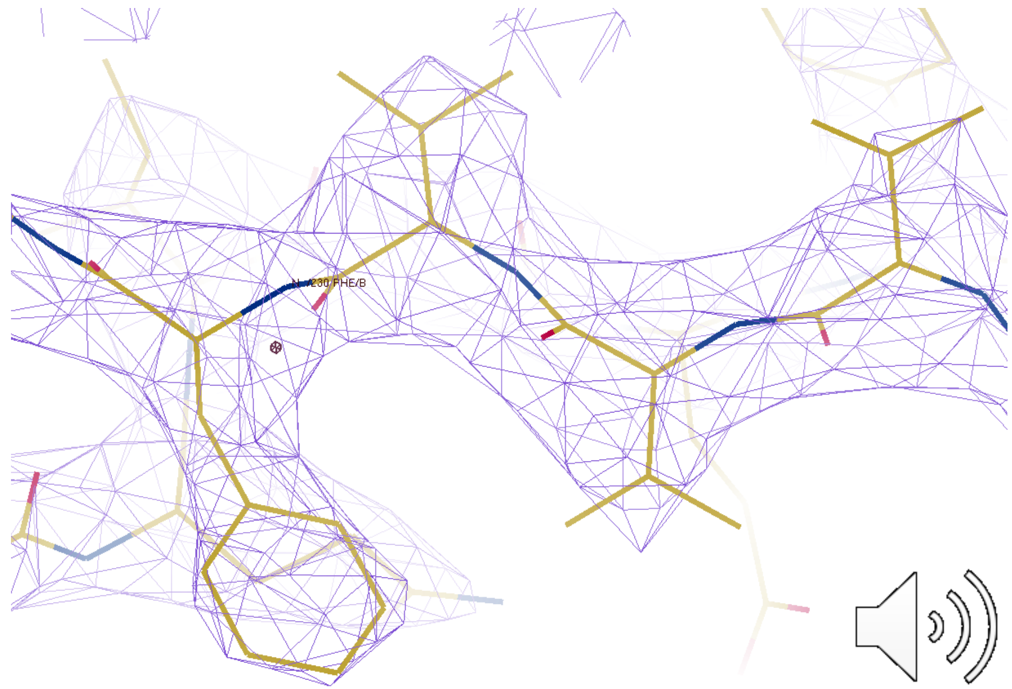
And then?

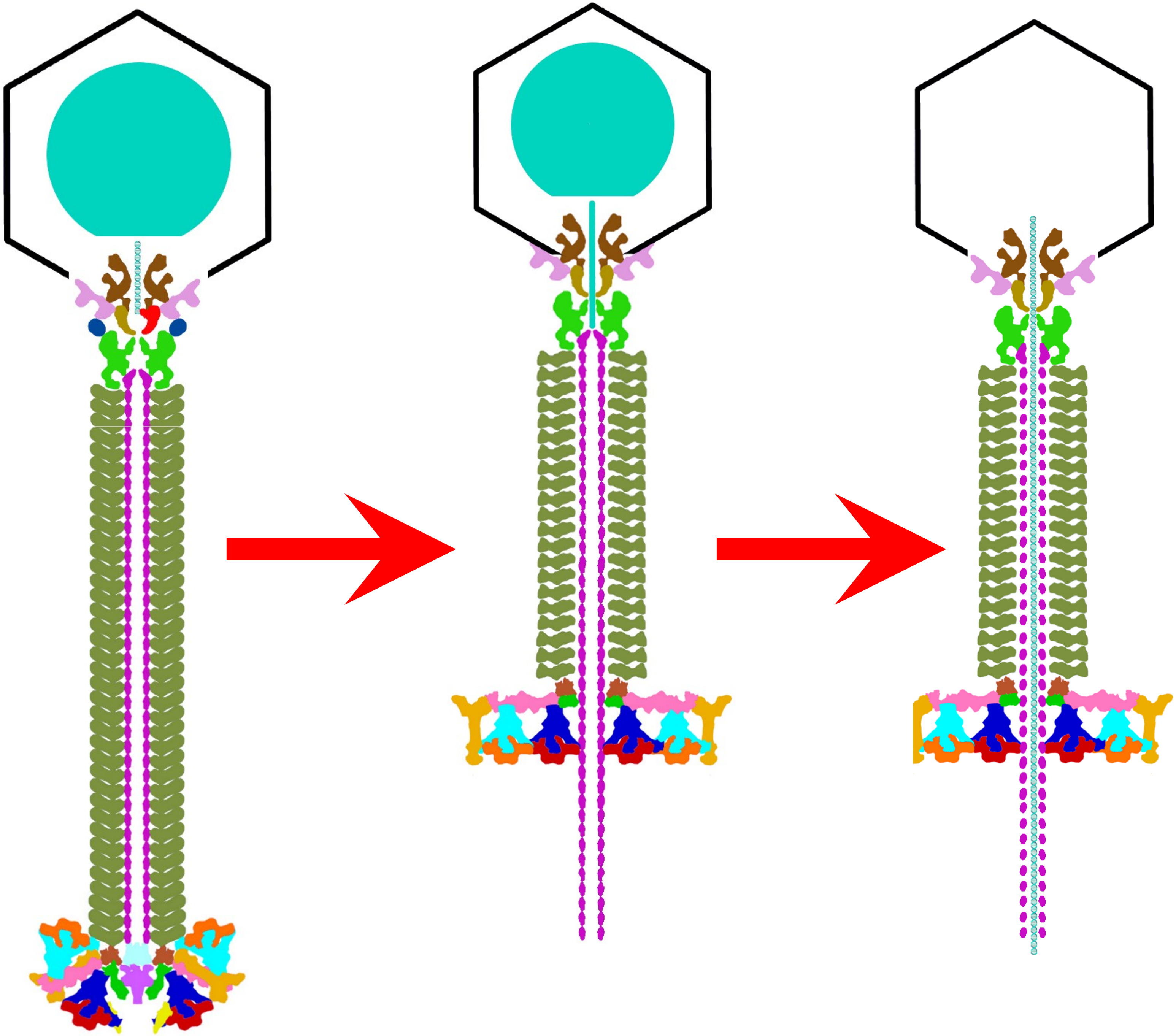
Try to interpret 3D map,

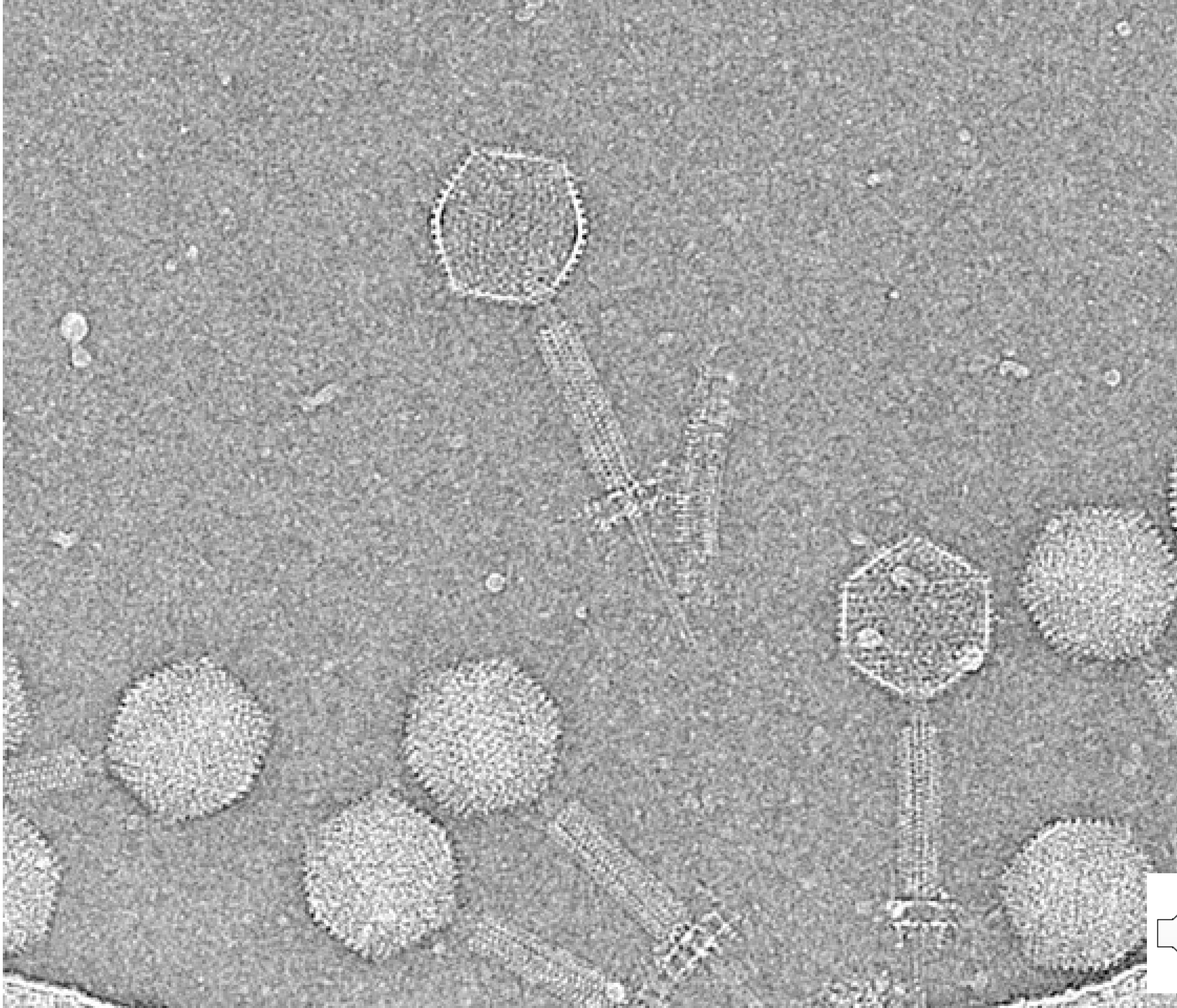
e.g. try to fit known crystal structures into electron density map

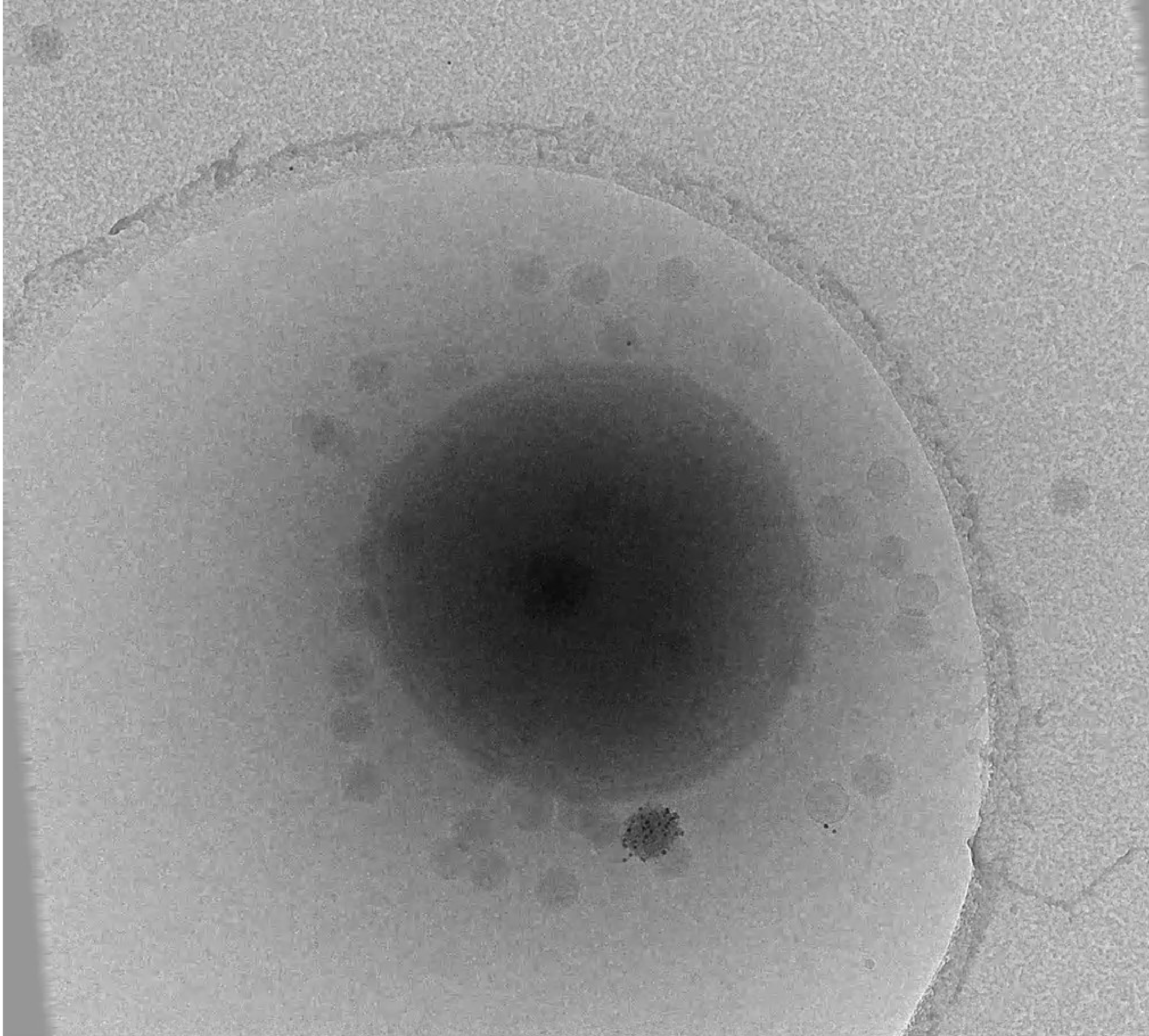



CA /257 PHE/C

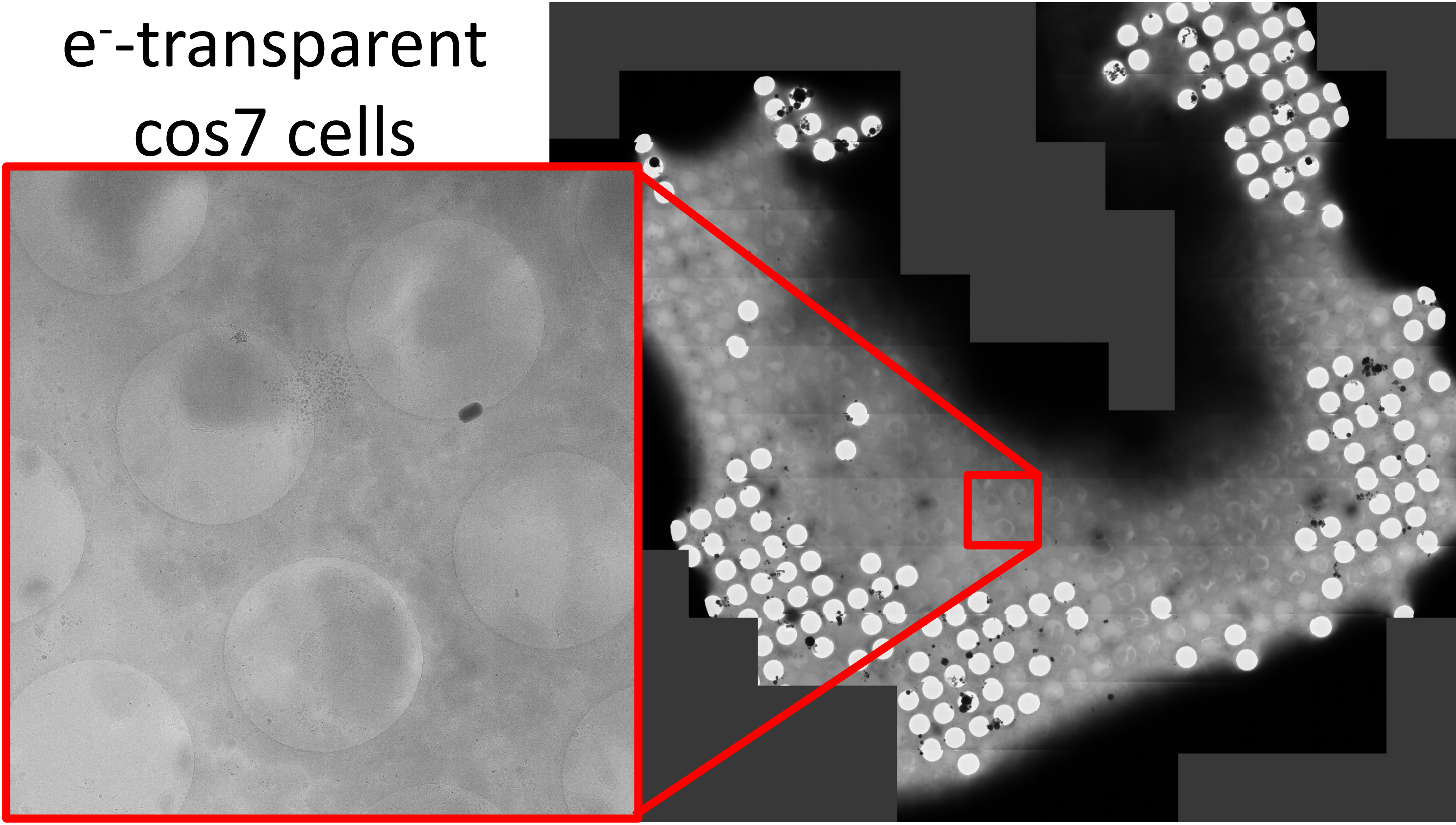

N /230 PHE/B



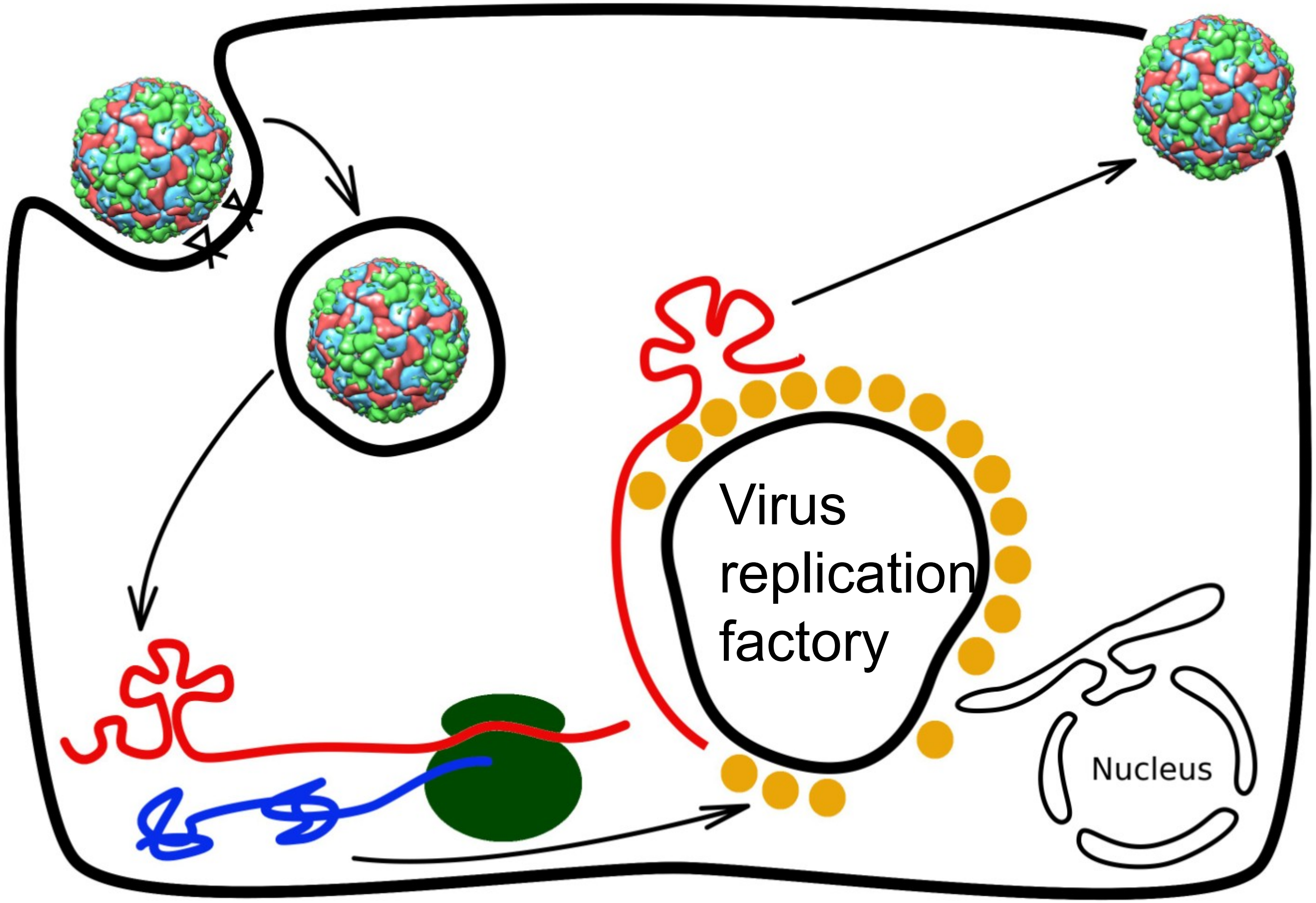




e^--transparent cos7 cells



Infection cycle of enteroviruses



Endosomes 30 min post-infection