In introduction to molecular electron microscopy

- Imaging macromolecular assemblies

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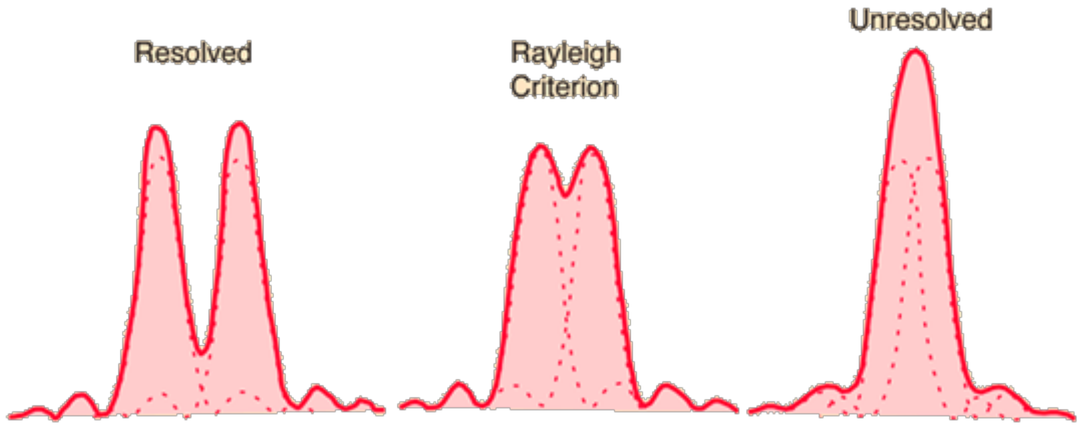
Resolution limit of an optical microscope system

Rayleigh criterion:

$$\sin\theta = 1.22\frac{\lambda}{D} \qquad \text{or} \qquad \Delta l = 1.22\frac{f\lambda}{D}$$



θ is the angular resolution, λ is the wavelength and D is the diameter of the lens aperture.

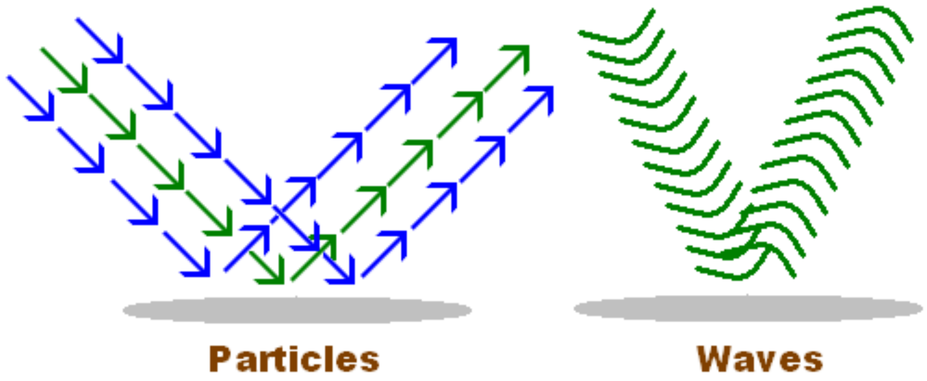
or: Δl is the spatial resolution, f is the focal length of an ideal lens.

Thus: The resolution of an light microscope system is limited by the wavelength of the light used. One of the ways to improve the resolution is to use light with shorter wavelength or use larger lens aperture.

Wave-particle duality of electron

It all started with the De Broglie's hypothesis:

$$\lambda = \frac{h}{p}$$



Particles and wave Reflected by a Mirror

λ is wavelength, h is Planck's constant, and p is momentum.

The original motivation of building an electron microscope came from the shorter wavelength of the electron.

Electron wavelength

Applying the principle of energy conservation to an electron (-*e*) traveled in voltage E_0:

$$eE_0 = \frac{h^2}{2m\lambda^2}$$

$$\lambda = \frac{h}{\sqrt{2meE_0}}$$

E_0 = acceleration voltage
λ = wavelength
h = Planck's constant

m = electron mass
e = electron charge

Electron wavelength

Take the relativity into consideration, the wave length is:

$$\lambda = \frac{h}{\sqrt{2m_0 e E_r}} \qquad E_r = E_0 + \left(\frac{e}{2m_0 c^2}\right) E_0^2$$

$$\lambda = \frac{1.22639}{\sqrt{E_0 + 0.97845 \times 10^{-6} E_0^2}}$$

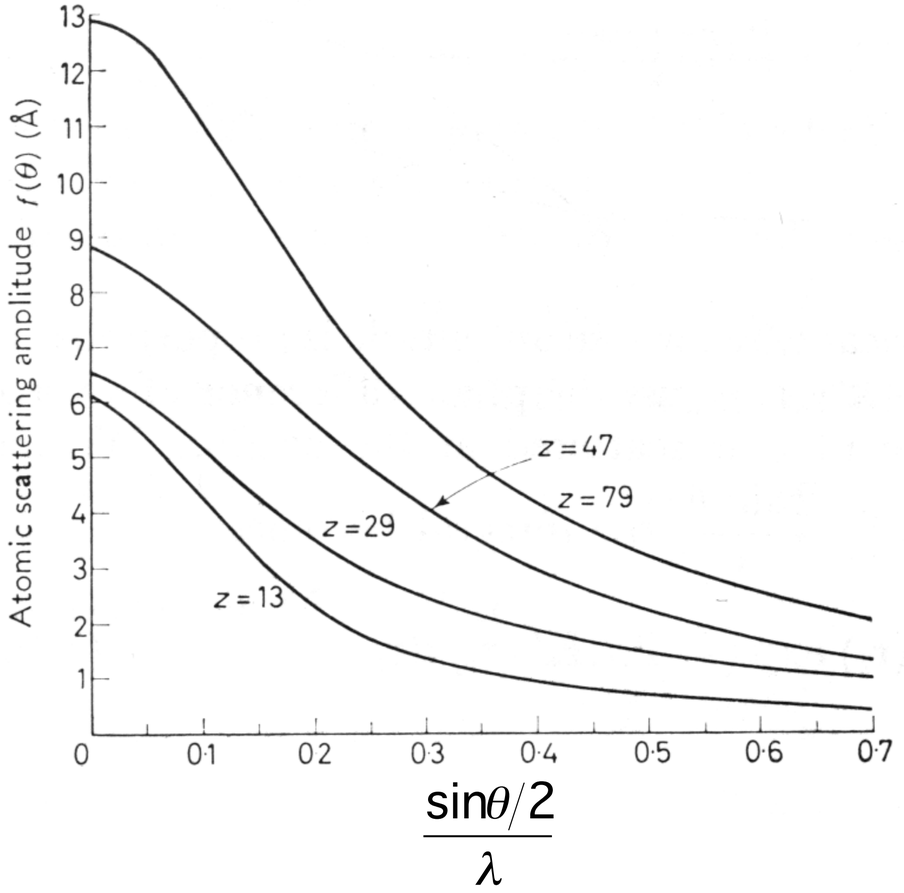
120kV λ=0.033Å; 200kV λ=0.025Å; 300kV λ=0.020Å;

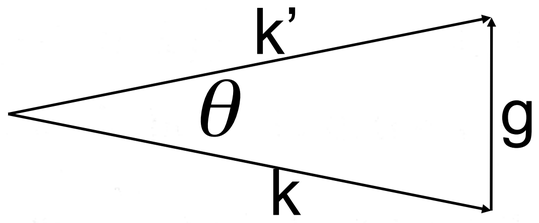
Note that these wavelength is considerably shorter than that used in X-ray crystallography, ~Å.

Atomic Scattering Factor for Electrons

Mott formula:

$$f_e(\theta) = \frac{me^2}{2h^2}\left(\frac{\lambda}{\sin\theta/2}\right)^2\left[Z - f_x(\theta)\right]$$





$$|\vec{g}| = \frac{2\sin\frac{\theta}{2}}{\lambda} = 2|\vec{k}|\sin\frac{\theta}{2}$$

Figure 4.6. Atomic scattering amplitudes as a function of $\sin\frac{1}{2}\theta/\lambda$ *for* Al($Z = 13$), Cu($Z = 29$), Ag($Z = 47$) *and* Au($Z = 79$)

Electron v.s. X-ray

As particles:

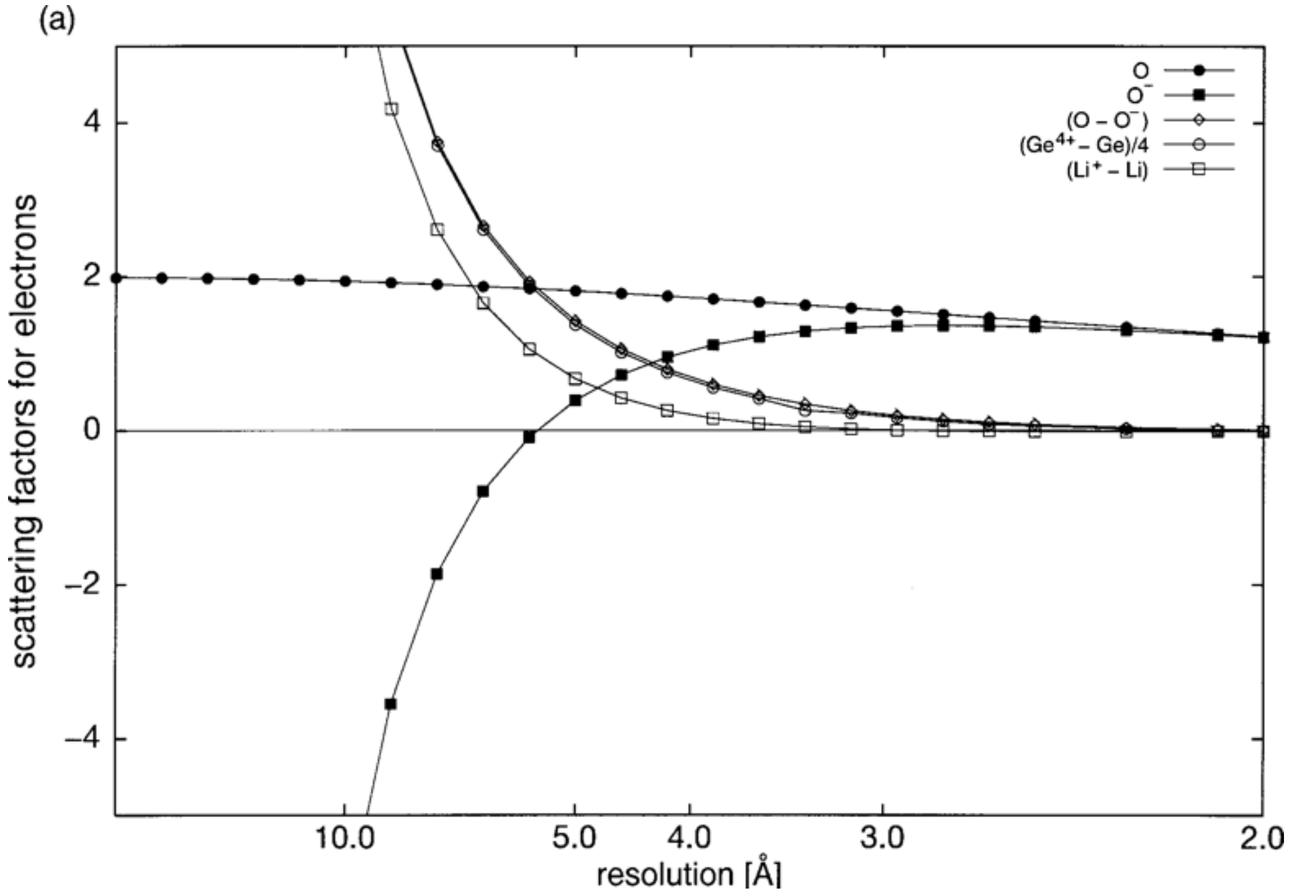
• Electrons interact with the potential field of an atom, inlcuding shell electrons and nucleus, X-rays interact with only shell electrons;

• Electrons have much larger scattering cross-sections than X-rays; multiple scattering is severer in electron scatterings than in X-ray diffraction; For biological sample, radiation damage is also severer than X-ray diffraction.

As wave:

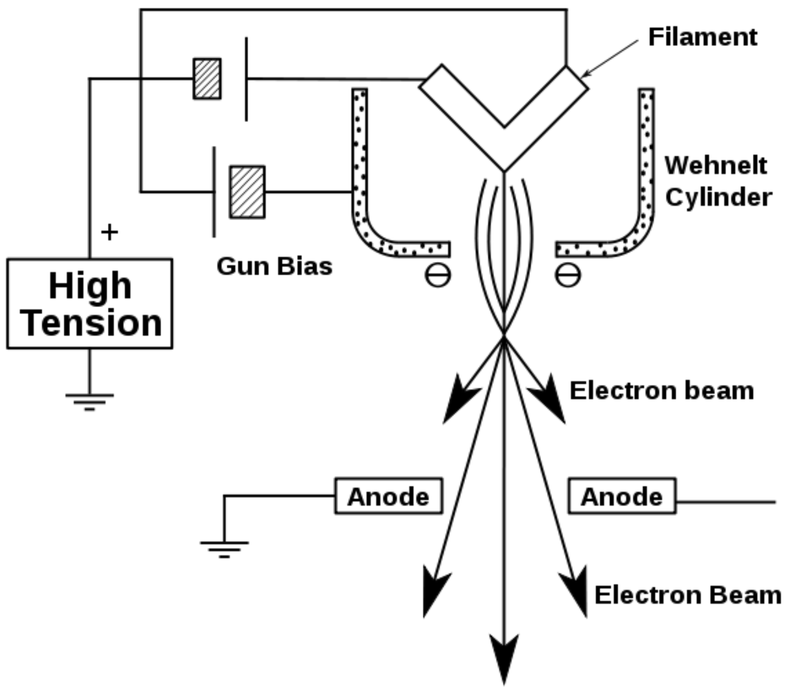
• Electrons can be focused by electromagnetic lens, X-ray can not be focused by lens;

Electron v.s. X-ray

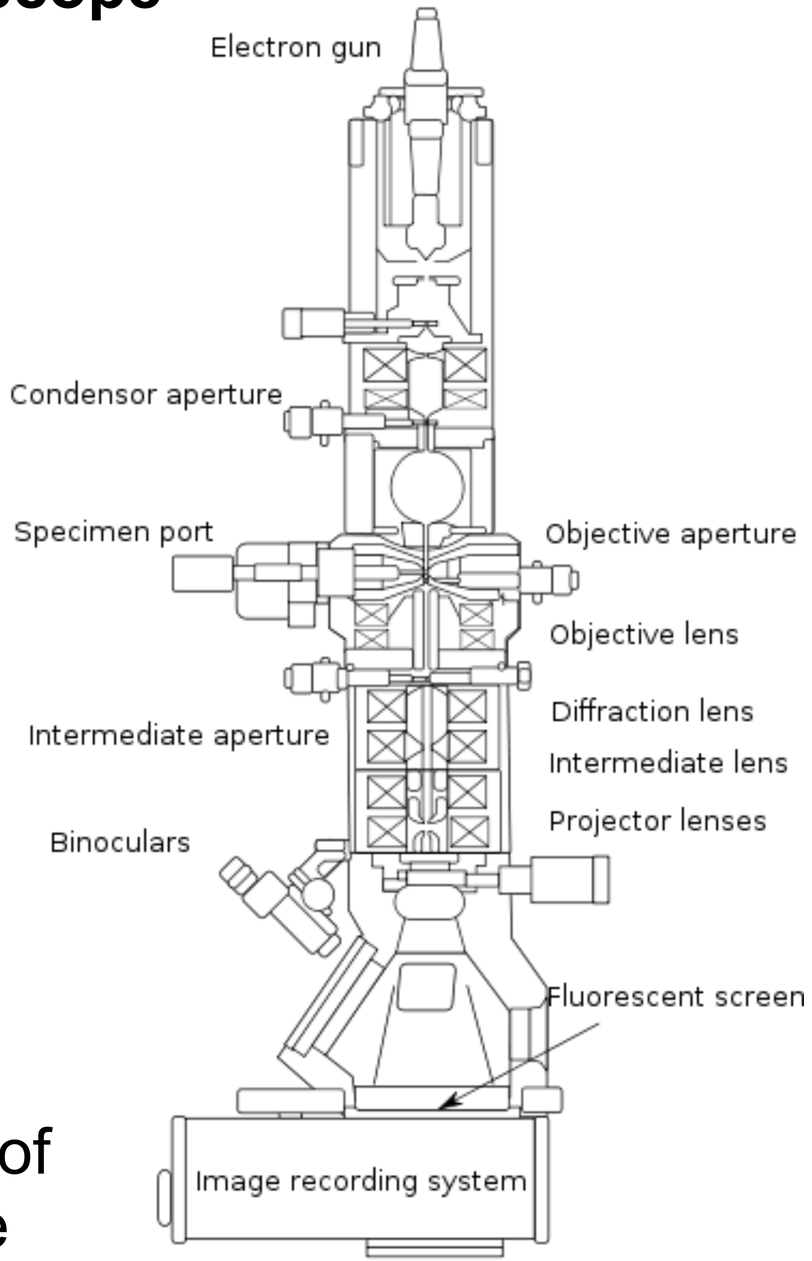


Mitsuoka et al. (1999) JMB, **286**, 861-882.

Electron Microscope

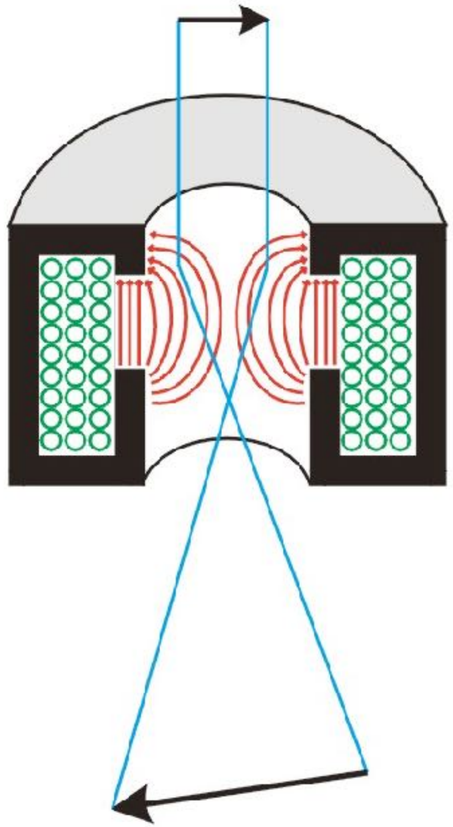


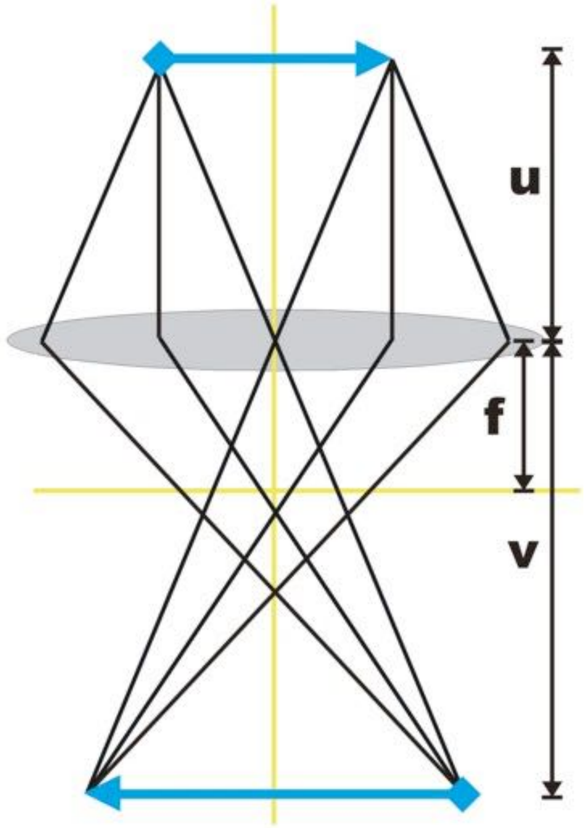
electron gun



a schematic drawing of electron microscope

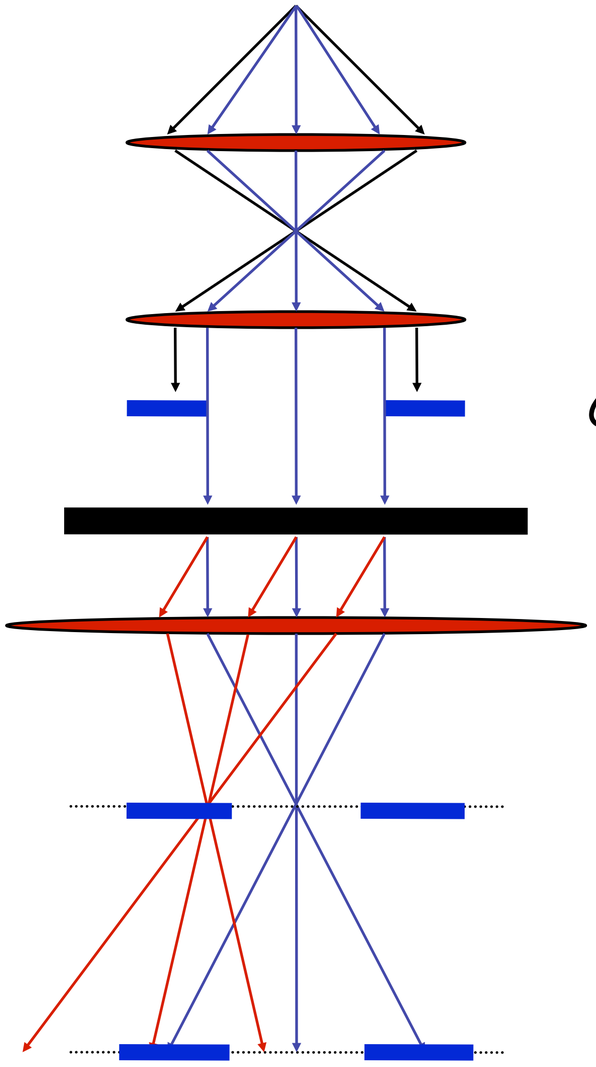
Electromagnetic lens





* The focal length of a electromagnetic lens can be easily adjusted by changing the lens current.

Optic system in an electron microscope



Electron source

Condenser lens C1

Condenser lens C2

Condenser aperture

specimen

Objective lens

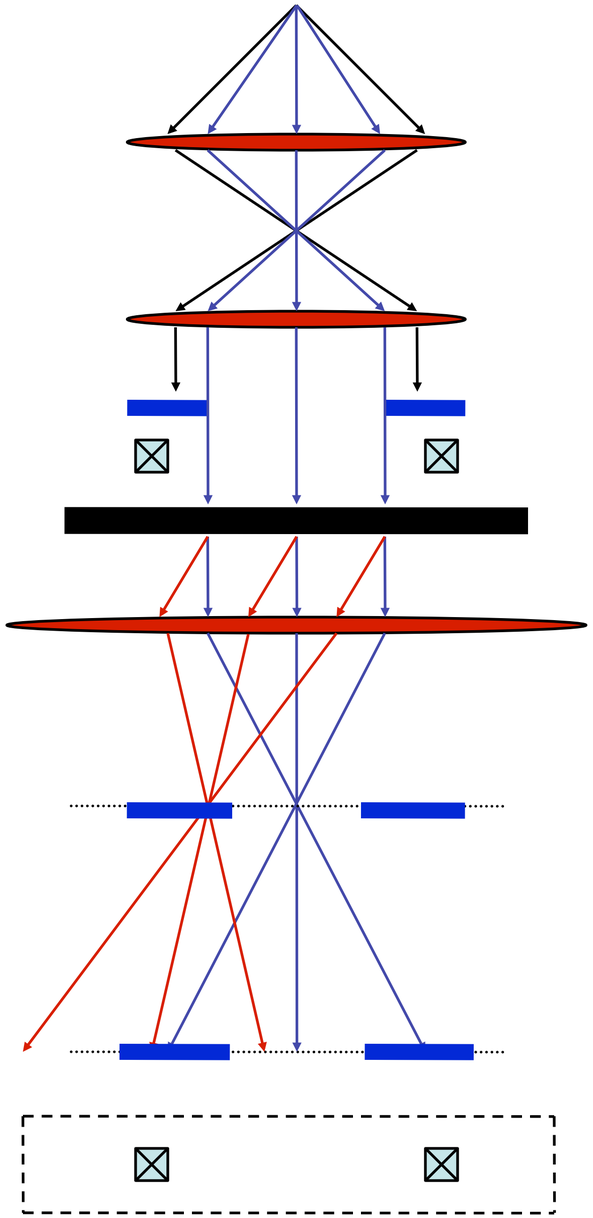
Back focal plane

Objective aperture

Back image plane

Select area aperture

Additional lens in the electron microscope



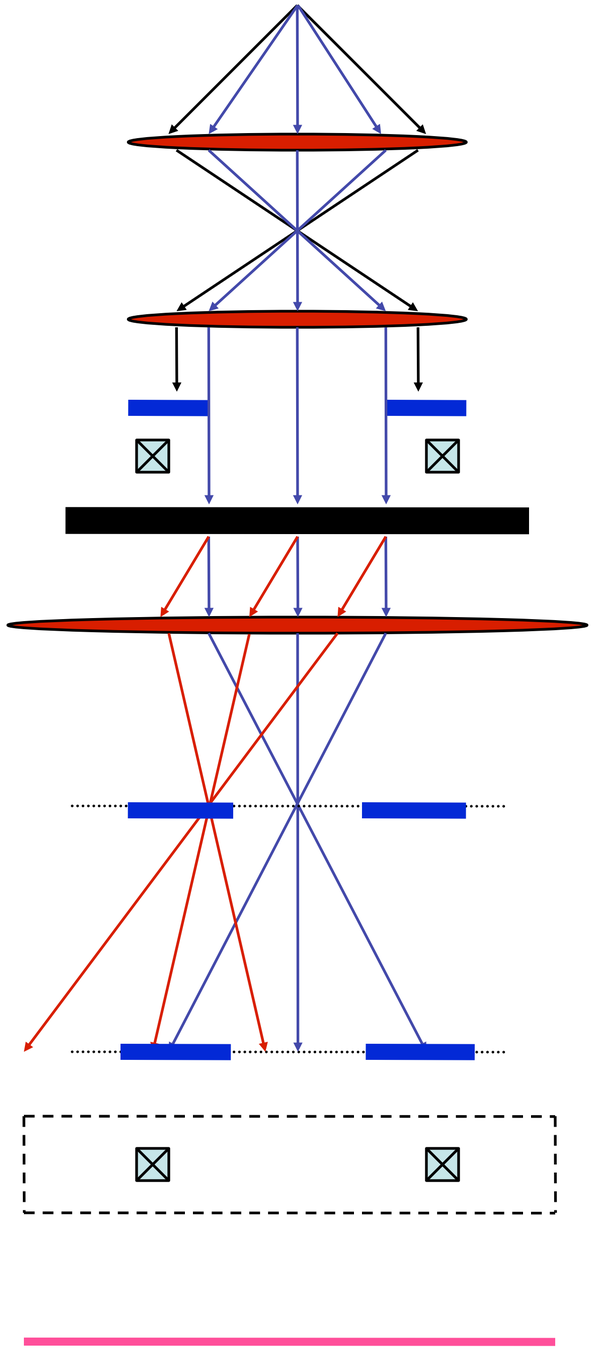
Beam shift coils

Projection lens system

Image shift/diffraction shift coils

screen

Image mode



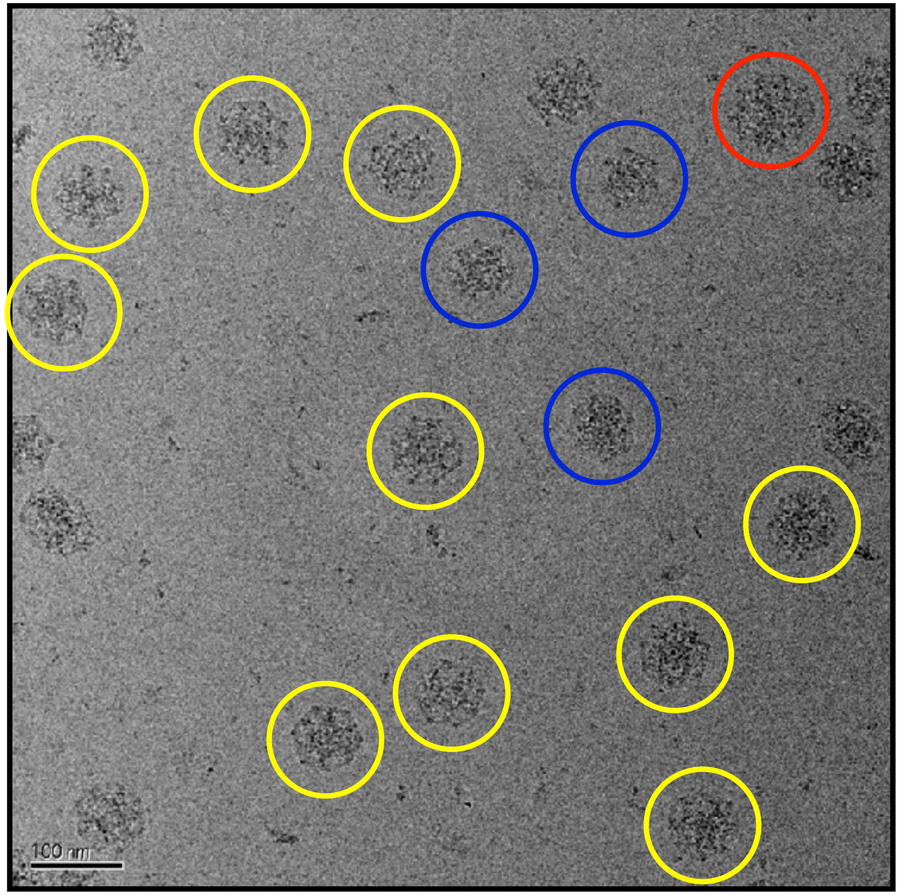
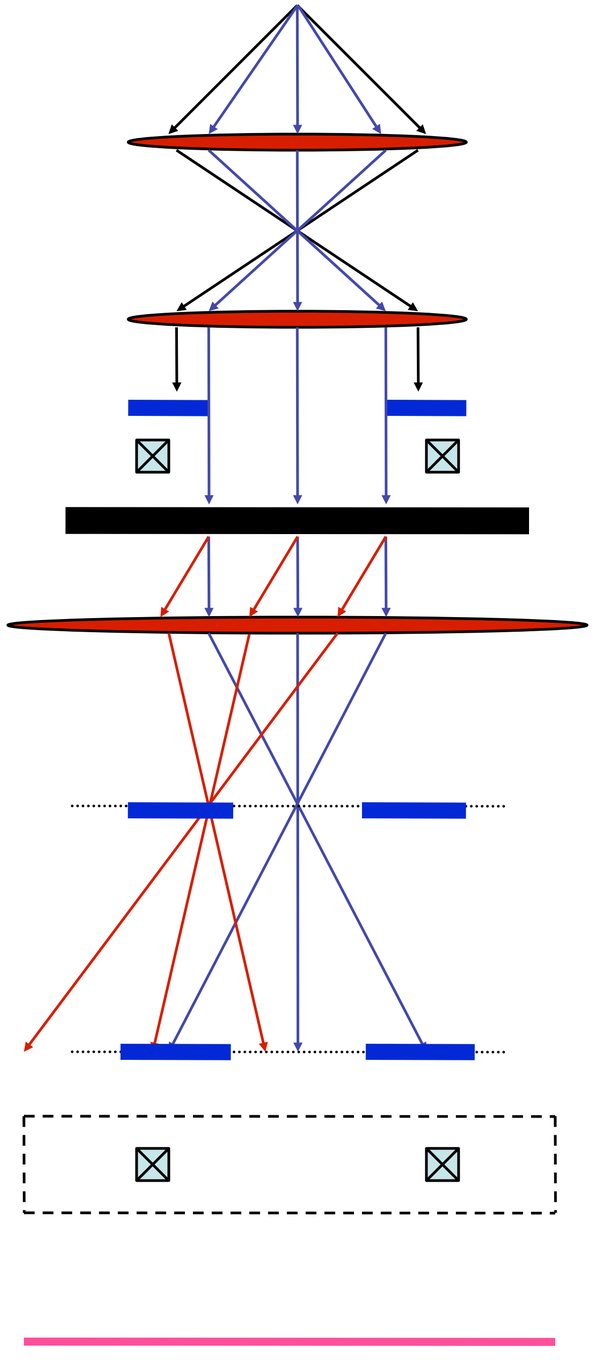
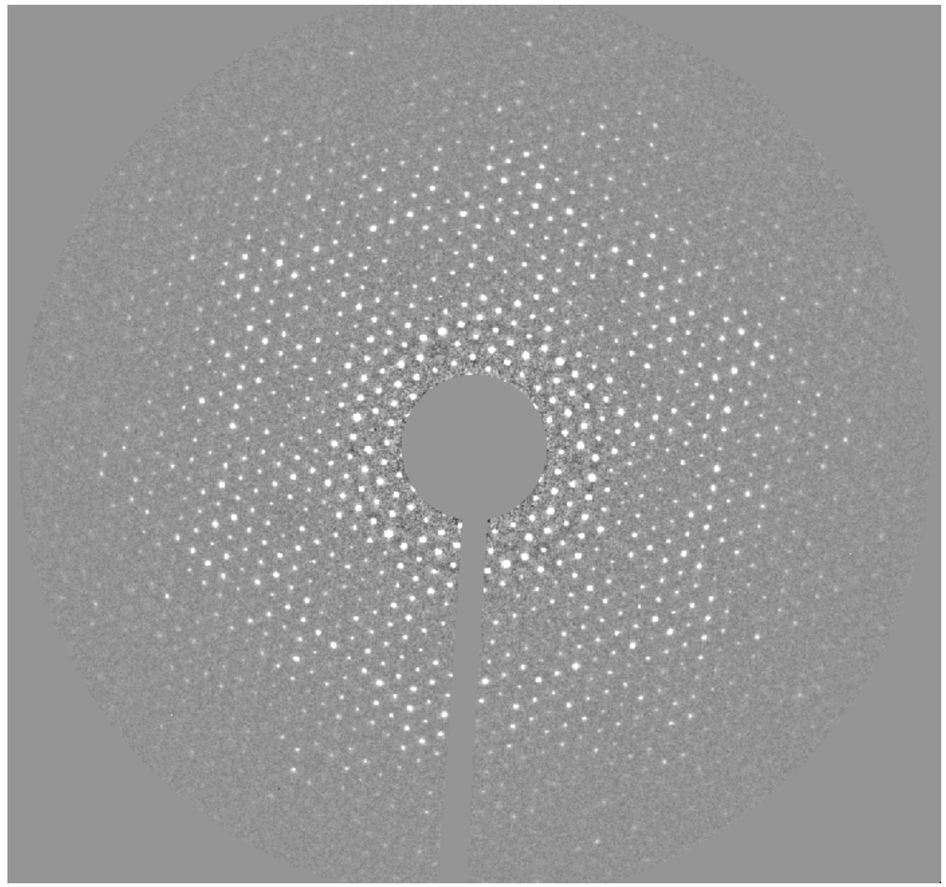


Image mode

Clathrin coat

Diffraction mode





Diffraction mode

bacteriorhodopsin

EM images are projections

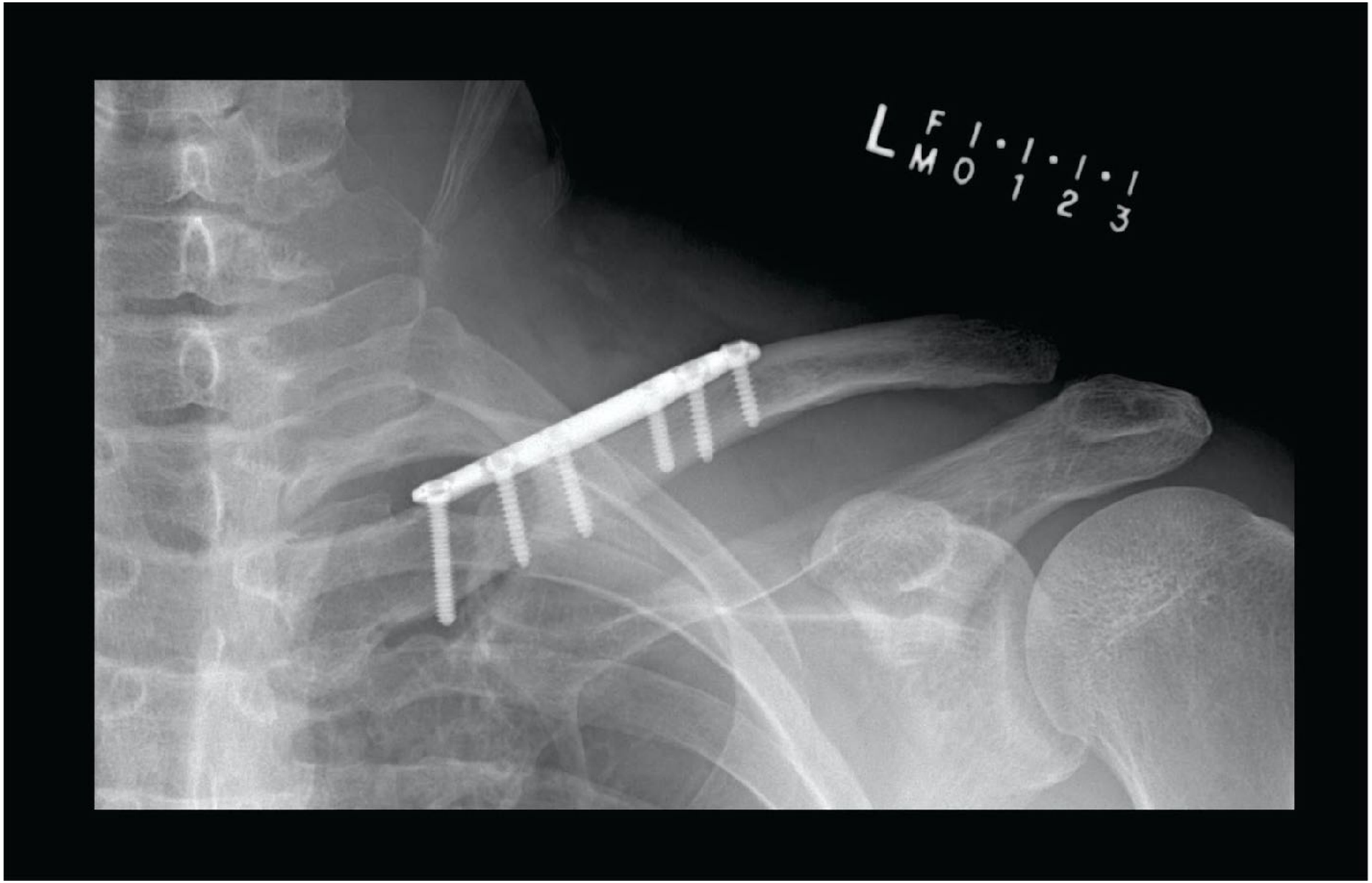


Image formation

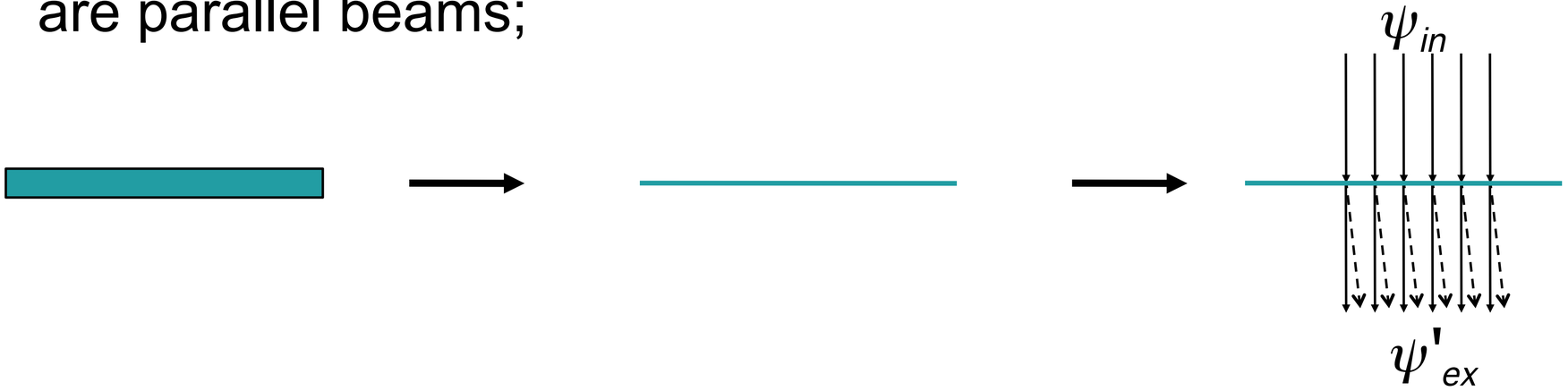
The image formation in the electron microscope can be treated as two separate processes:

1) The interaction of the incident beam with the specimen, described by the weak-phase object approximation, which is the theory used mostly to describe the image formation of thin specimen with light elements, such as a biological sample.
2) The propagation of the electron beam from exit plane of the specimen to the back image plane of the focus lens.

Weak-phase object approximation

This is a highly simplified theory based on the so-call weak-phase object, which is a very thin specimen formed mostly by low- and medium-weight molecules.

Suppose: 1) the specimen is very thin so that $\Phi(\vec{r},z)$ can be approximated by $\Phi(\vec{r})$; 2) both in-coming and exiting beams are parallel beams;



$\Phi(\vec{r},z)$ $\qquad$ $\Phi(\vec{r}) = \int \Phi(\vec{r},z)dz$ $\qquad$ $\psi_{ex}' = \psi_{in}\exp(i\Phi(\vec{r}))$

$$\psi_{ex}' = \psi_{in} \exp(i\Phi(\vec{r})) \tag{2}$$

$$\psi_{ex}' = \psi_{in}\left[1 + i\Phi(\vec{r}) - \frac{1}{2}\Phi^2(\vec{r}) + \ldots.\right] \tag{3}$$

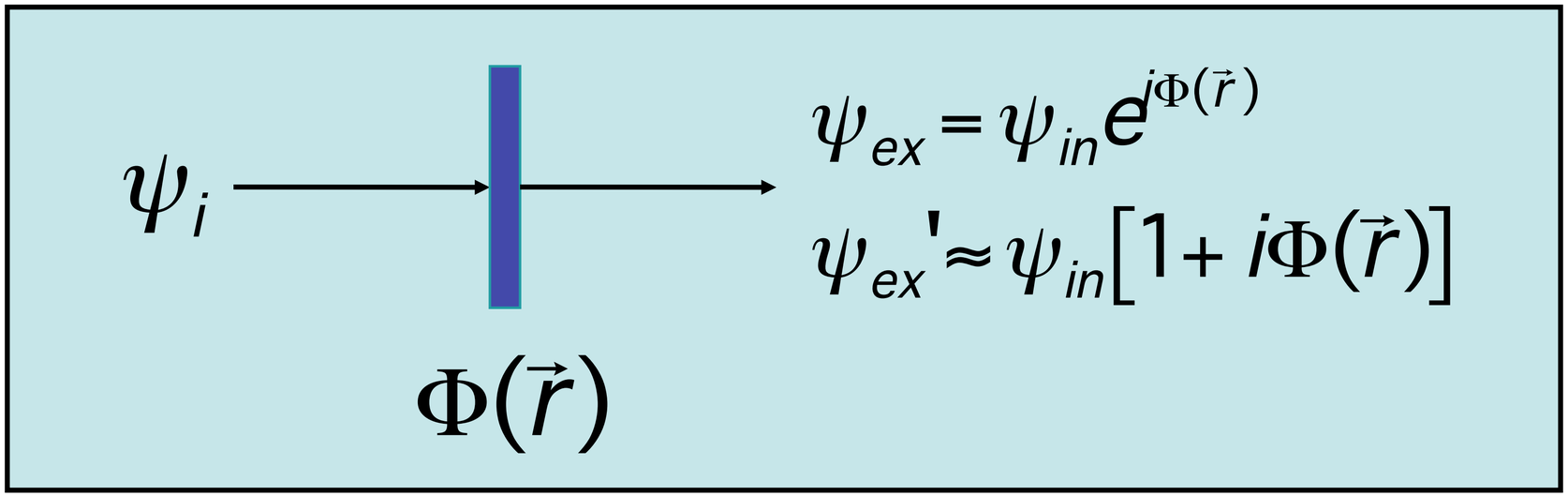
The first term in (3) represents the central unscattered beam, the second term the kinematically scattered beam and the higher terms are for the dynamical scattering. The weak phase object approximation assumes that $\Phi(\mathbf{r}) << 1$, the phase shift is so small that the following approximation will work:

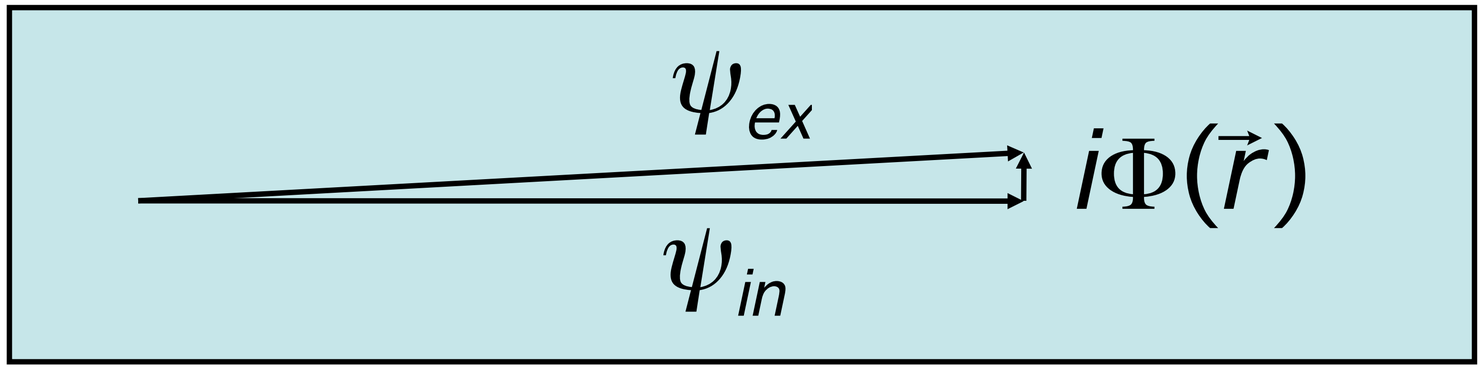
$$\psi_{ex}' \approx \psi_{in}\left[1 + i\Phi(\vec{r})\right] \tag{4}$$

Taking absorption into consideration:

$$\psi_{ex}' = \psi_{in} \exp(i\Phi(\vec{r}) - \mu(\vec{r})) \tag{5}$$

$$\psi_{ex}' \approx \psi_{in}\left[1 - \mu(\vec{r}) + i\Phi(\vec{r})\right] \tag{6}$$





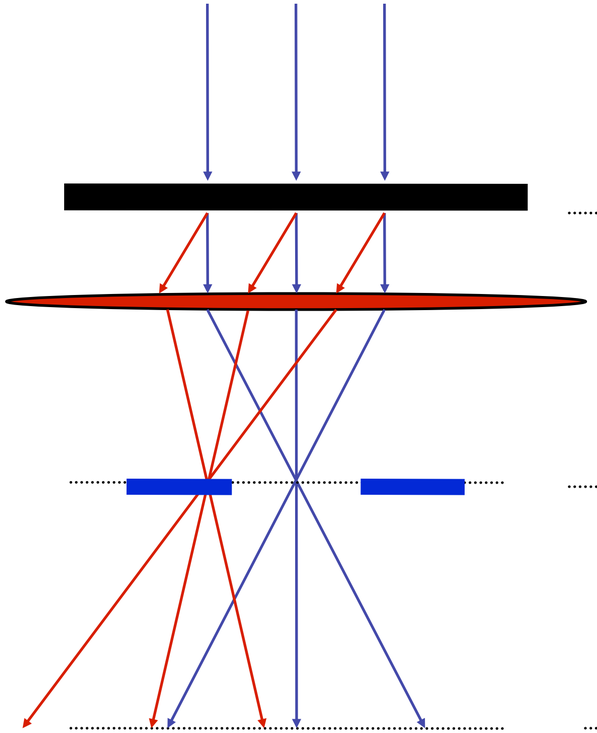
$$I_{ex} = |\psi_{ex}|^2 = |\psi_{in}|^2 = I_{in}$$

Image formation

At exit plane of specimen: $\psi'_{ex}(\vec{r}) \approx \psi_{in}\left[1 + i\Phi(\vec{r})\right]$

At back focal plane: $\Psi_{bf}(\vec{k}) = F\left[\psi'_{ex}(\vec{r})\right]$

At back image plane: $\psi_{im}(\vec{r}) = F^{-1}\left[\Psi_{bf}(\vec{k})\right]$



ψ'_{ex}

focal plane

$\Psi_{bf}(\vec{k})$

image plane

ψ_{im}

Image formation

The plane wave ψ' of exit-beam travel through objective lens to the back focal plane. The wave function at back focal plane of the objective lens is the Fourier transform of the exit wave:

$$\Psi_{bf}(\vec{k}) = F(\psi'_{ex}(\vec{r})) = F\left[1 + i\Phi(\vec{r})\right] = \delta(\vec{k}) + iF(\Phi(\vec{r})) \qquad (7)$$

However the lens aberration and defocusing generate an extra phase shift to the scattered beam:

$$\gamma(\vec{k}) = 2\pi\chi\vec{k}$$

$$\chi(k,\varphi) = \frac{1}{2}\lambda\left[\Delta z + \frac{1}{2}\sin 2(\varphi - \varphi_0)\right]k^2 + \frac{1}{2}\lambda^3 C_s k^4 \qquad (8)$$

Together with the aperture function A(***k***) the wave function at back focal plane will become:

$$\Psi_{bf}(\vec{k}) = F(\psi'_{ex})A(\vec{k})\exp(-2\pi i\chi\vec{k}) \tag{9}$$

Then, the wave function in the back image plane of the lens is the reverse Fourier transform of the wave function at back focal plane ($\otimes$ is for convolution):

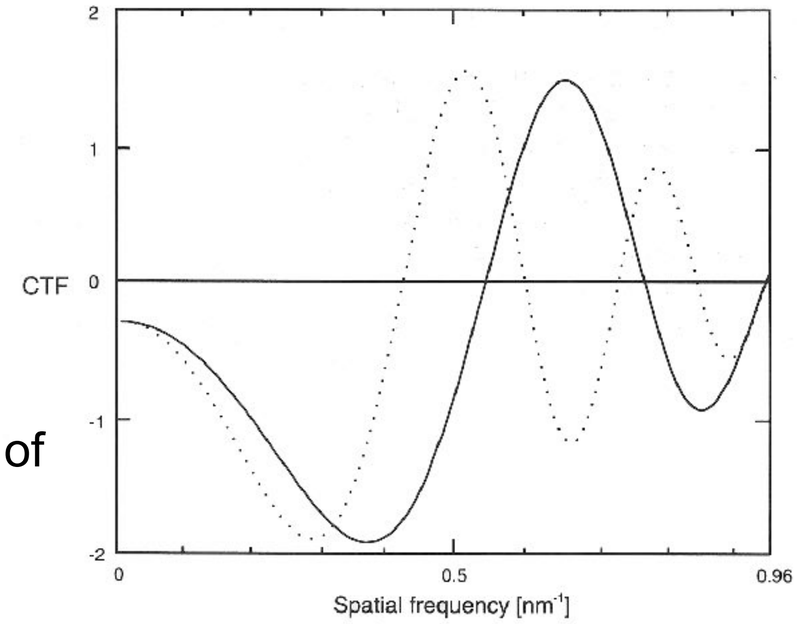
$$\begin{aligned}\psi_{im}(\vec{r}) &= F^{-1}\{F(\psi'_{ex})A(\vec{k})\exp(-2\pi i\chi\vec{k})\} \\ &= 1 + i\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes F^{-1}[\exp(-2\pi i\chi k)]\end{aligned} \tag{10}$$

The observed intensity in the image is then:

$$\begin{aligned}I_i(\vec{r}) &= \psi_i(\vec{r})\psi_i^* \\ &= 1 + 2\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes F^{-1}[\sin(-2\pi\chi k)]\end{aligned} \tag{11}$$

Contrast Transfer Function (CTF)

$$CTF = \sin(2\pi\chi k)$$



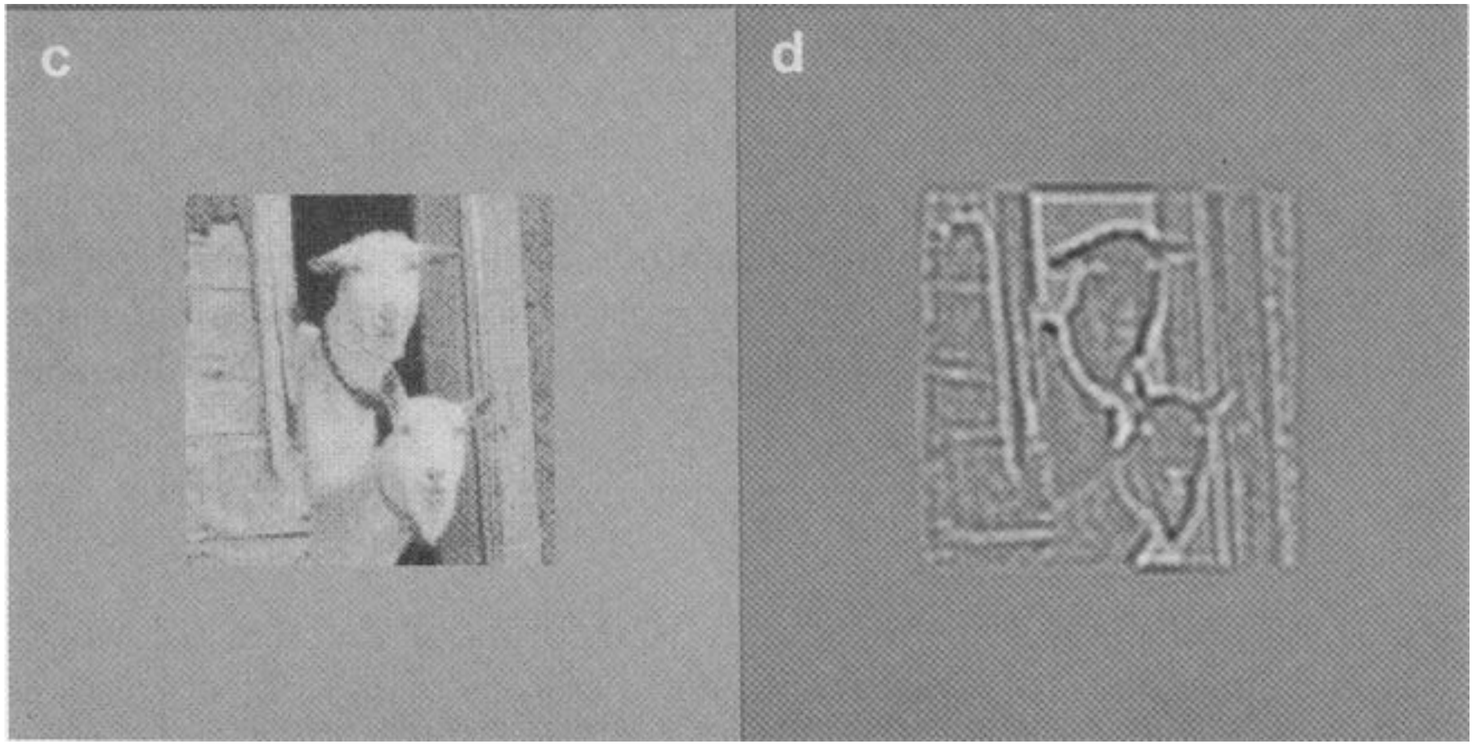
The intensity of a recorded image is directly related to the projection of specimen (good!) but modified by the FT of CTF (bad!).

$$I_i(\vec{r}) = \psi_i(\vec{r})\psi_i^*$$

$$= 1 + 2\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes F^{-1}(CTF) \qquad (12)$$

WebCTF: http://jiang.bio.purdue.edu/ctfsimu

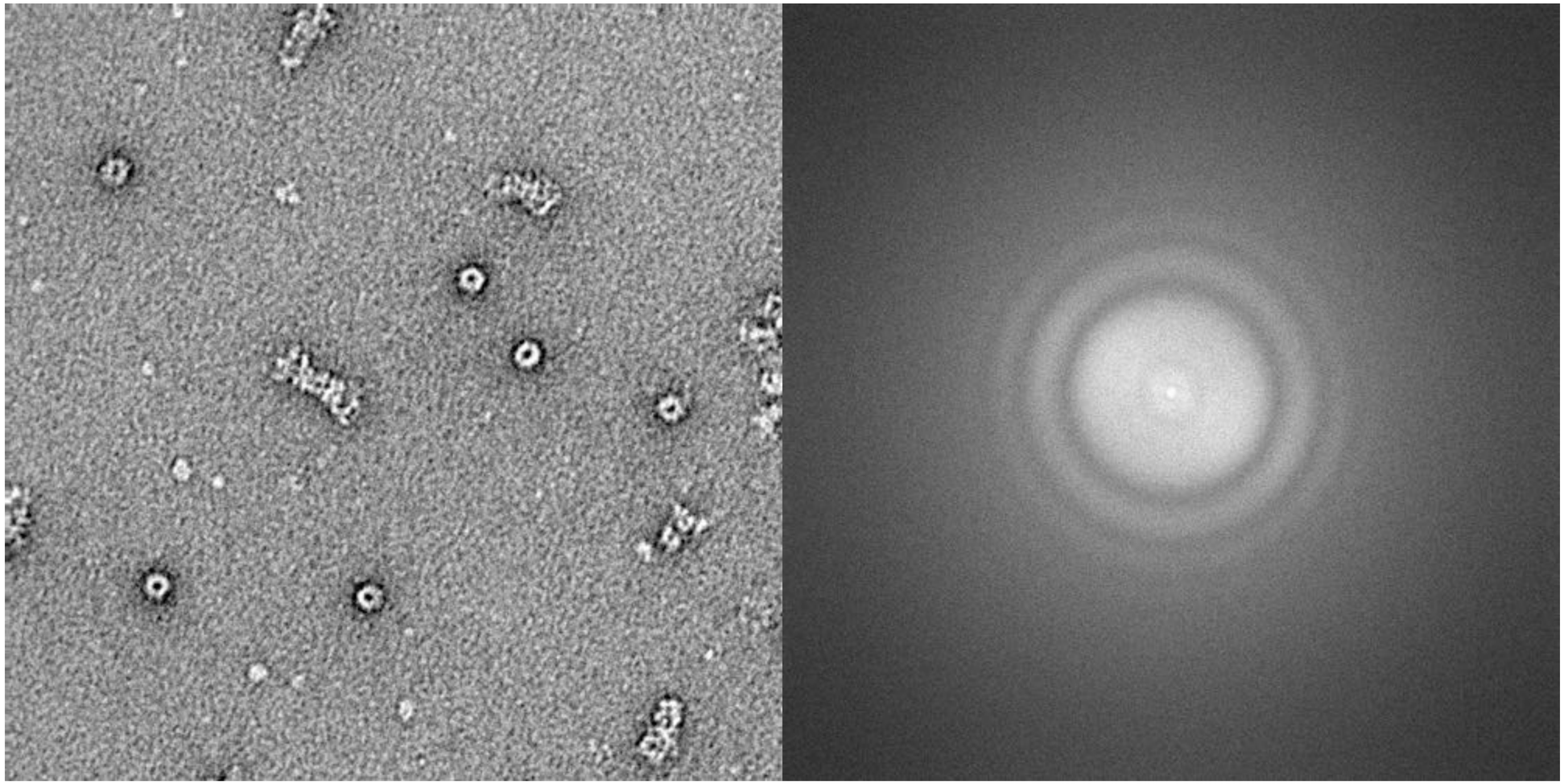
What is this CTF thing anyway and why do I care?



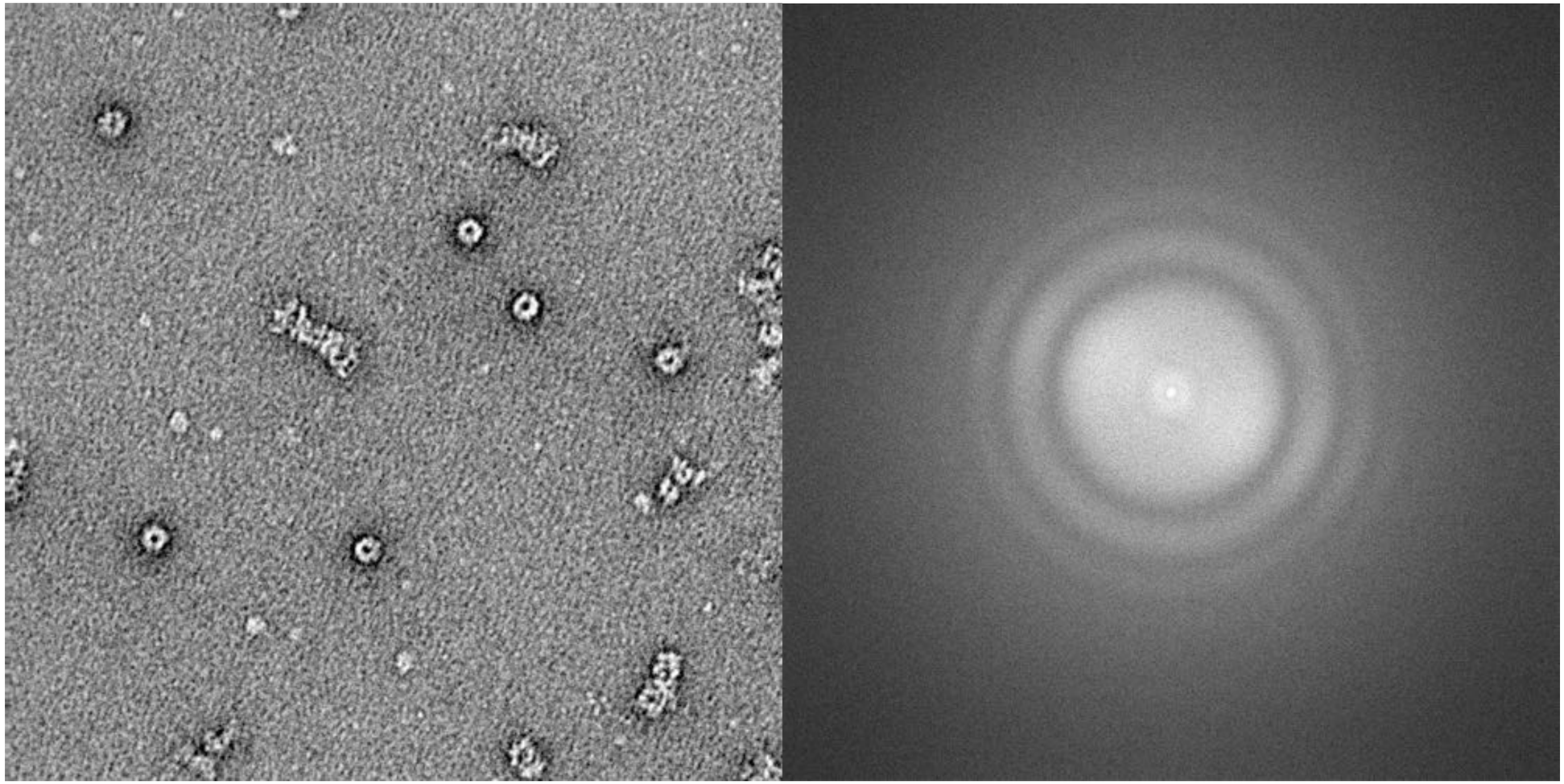
Distortions of CTF to the image are:
1) Contrast reverve of large area; 2) diminished contrast in large area; 3) edge enhancement and 4) appearance of fringes along the borders.

From Joachim Frank

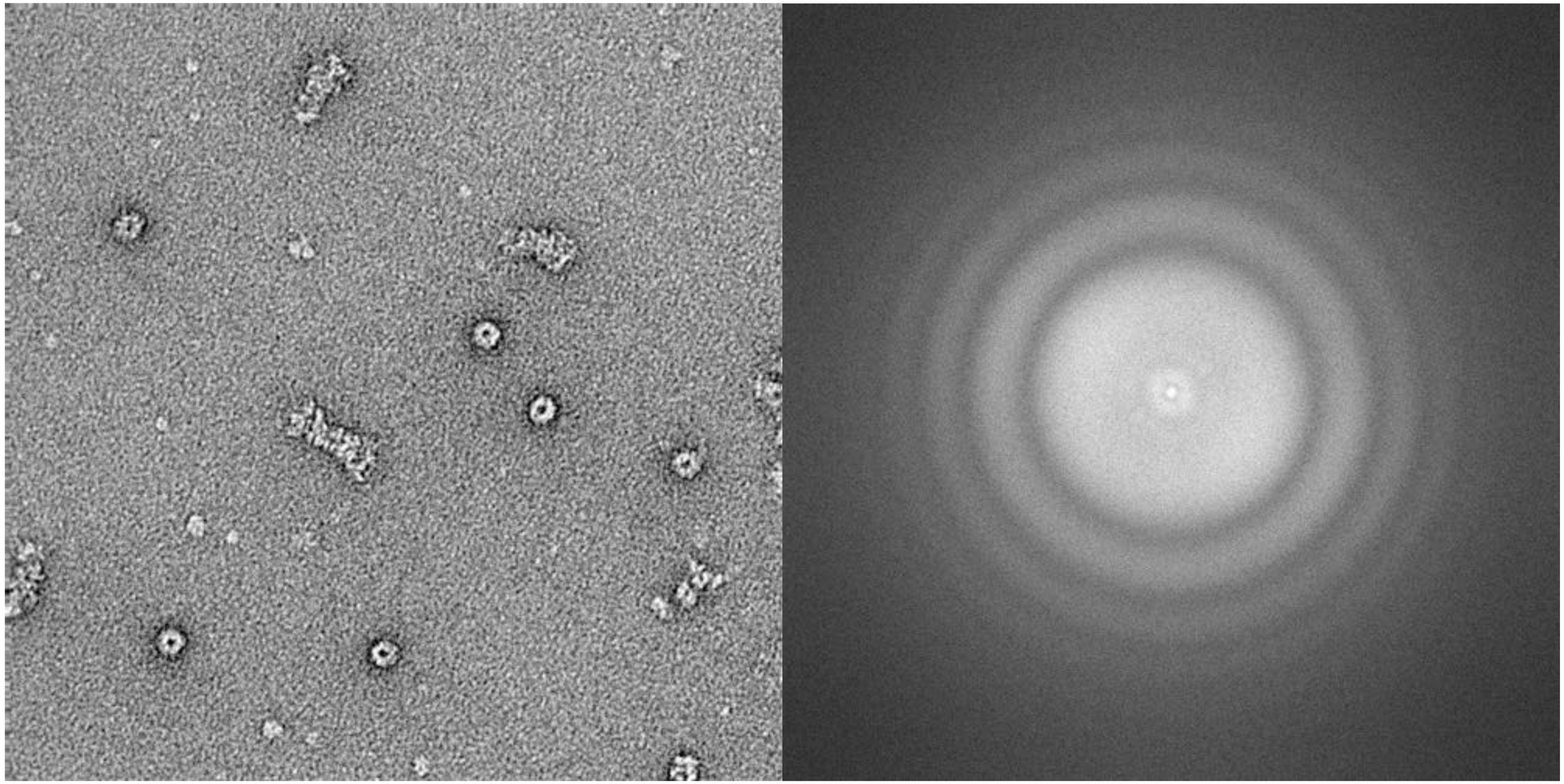
Defocus -2μm



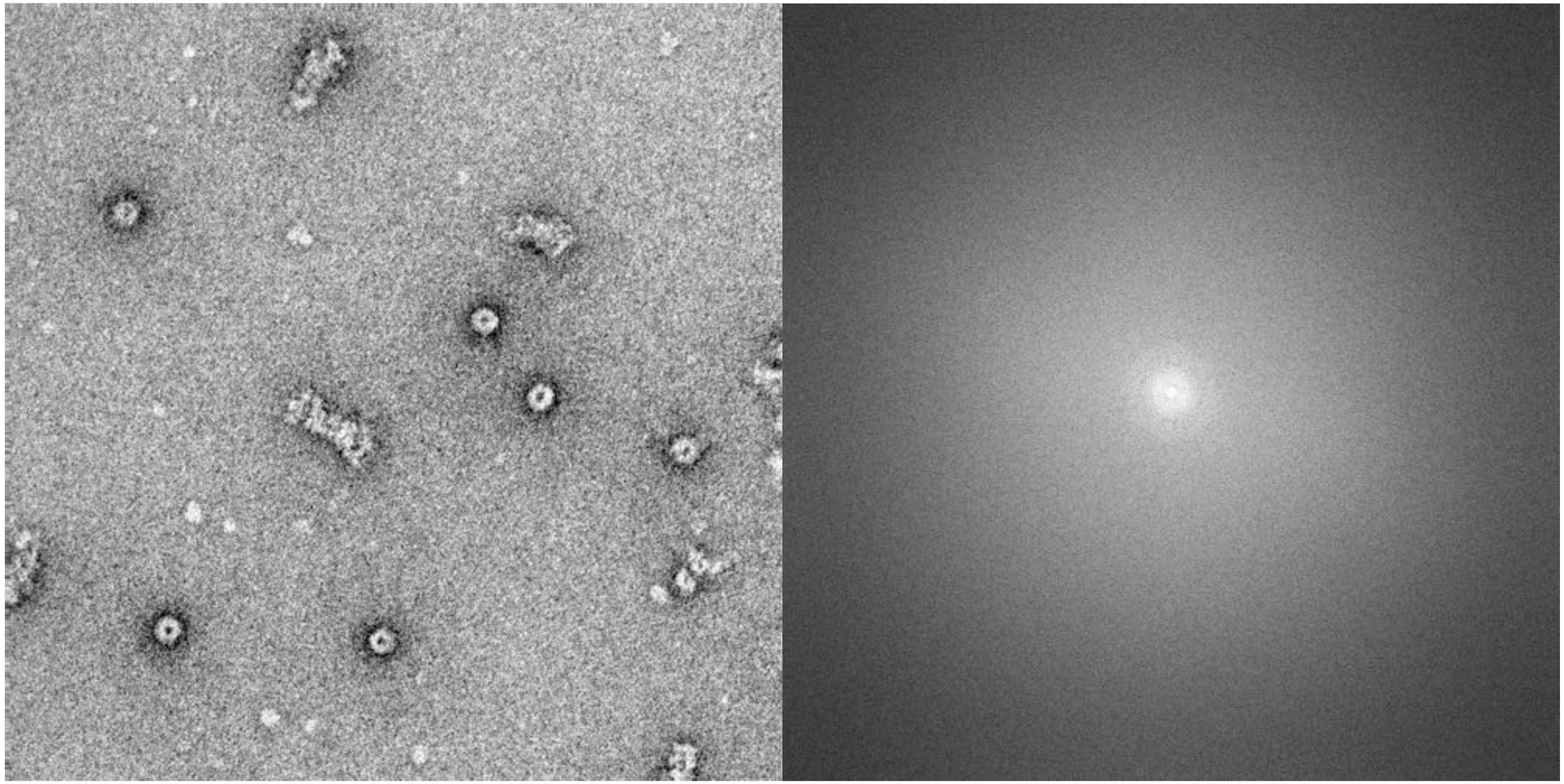
Defocus -1.5μm



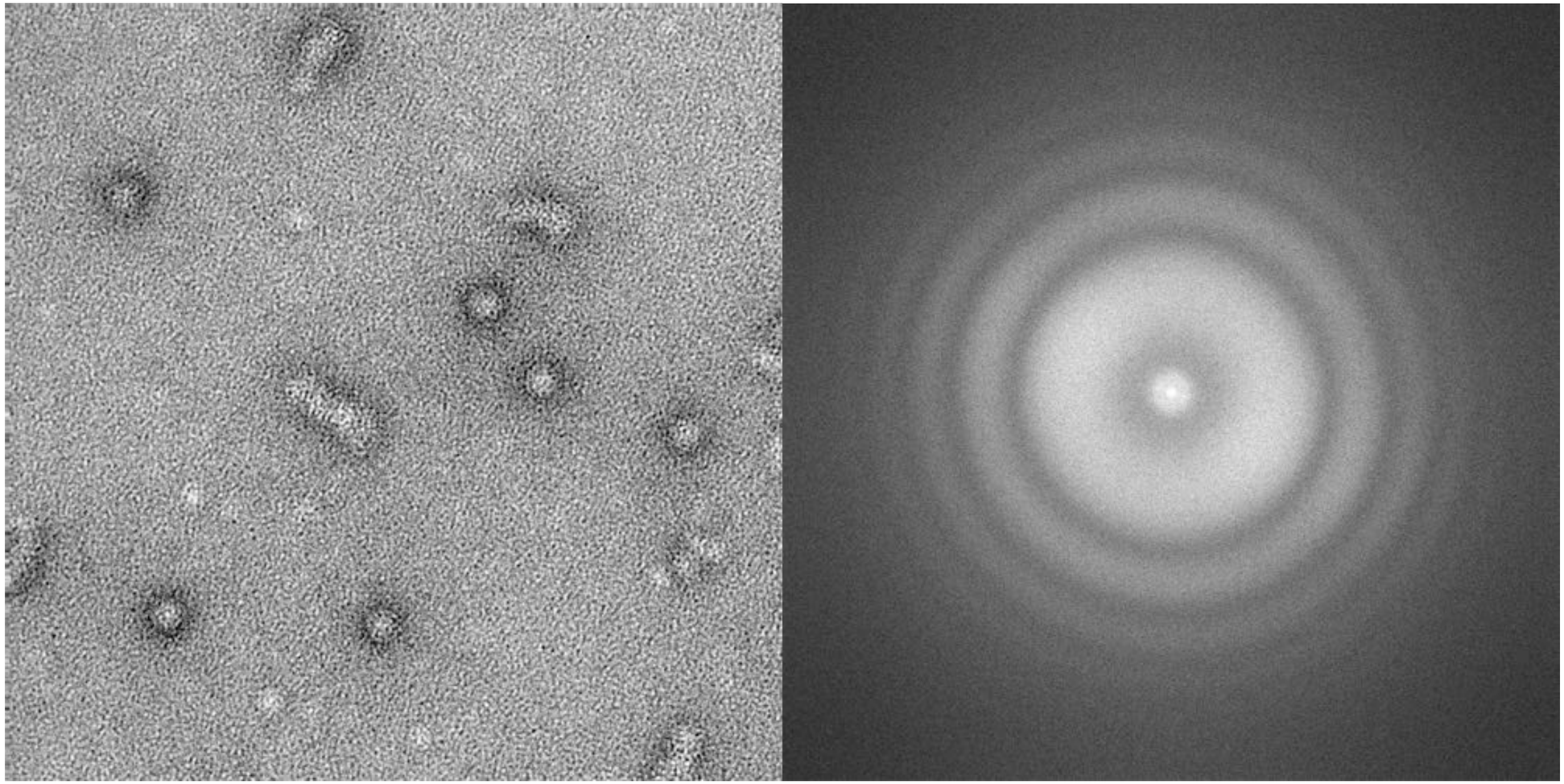
Defocus -1μm



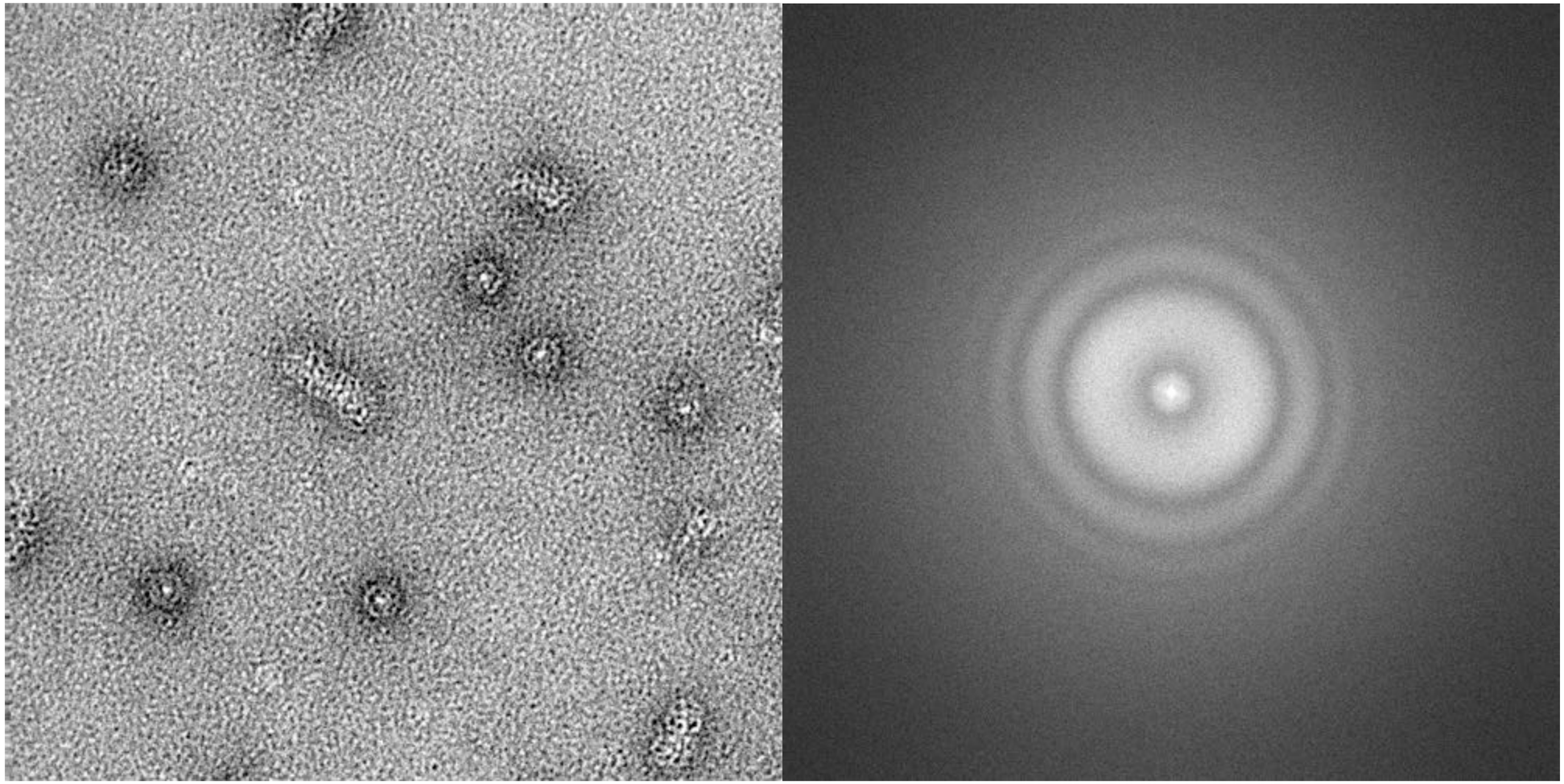
Defocus ~0μm



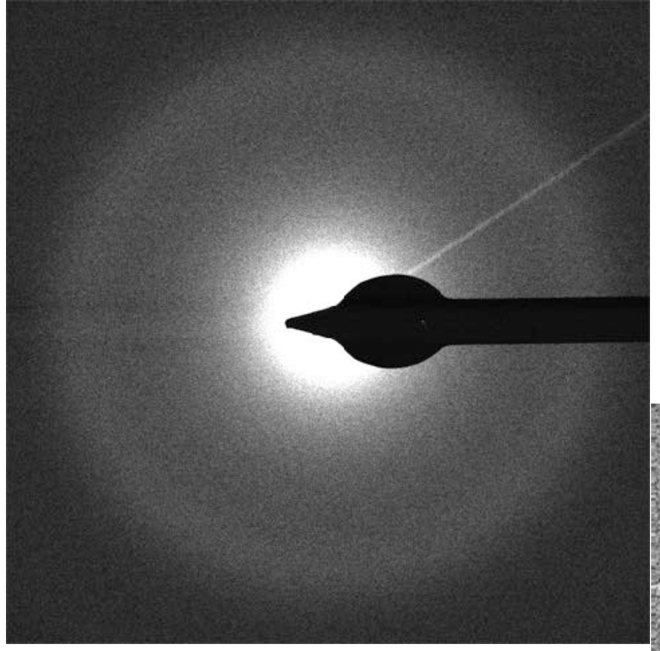
Defocus +1μm



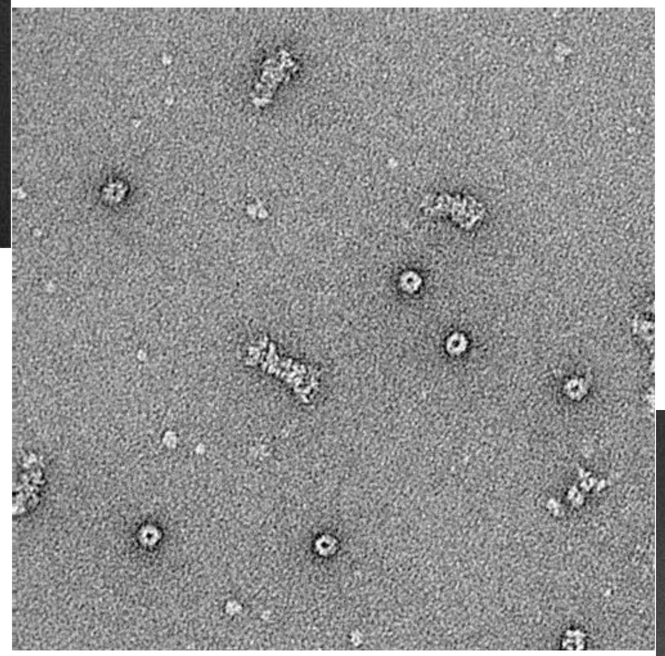
Defocus +2μm



Diffraction, image and power spectra

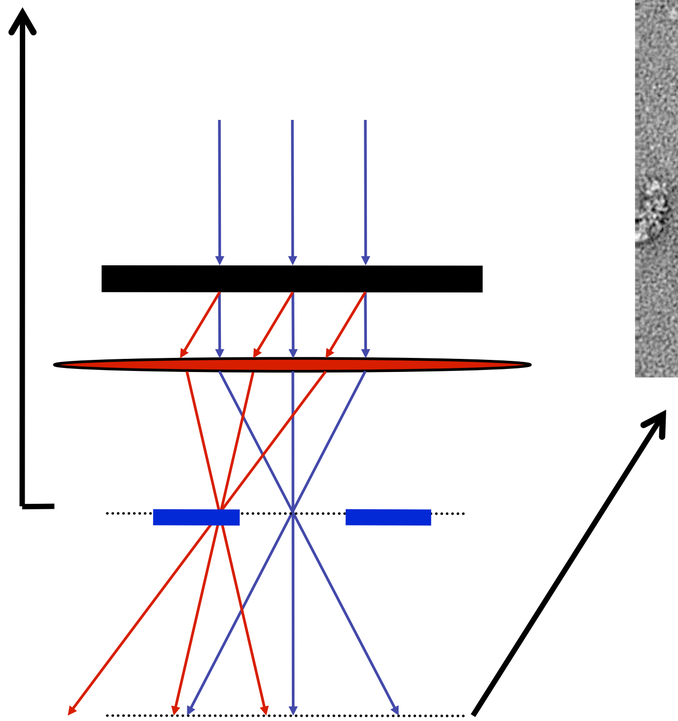


diffraction

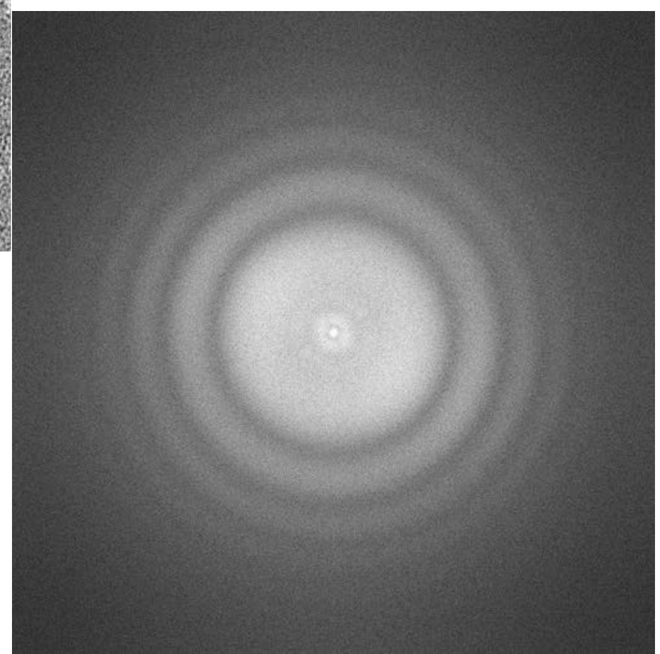


iamge

Fourier power spectrum

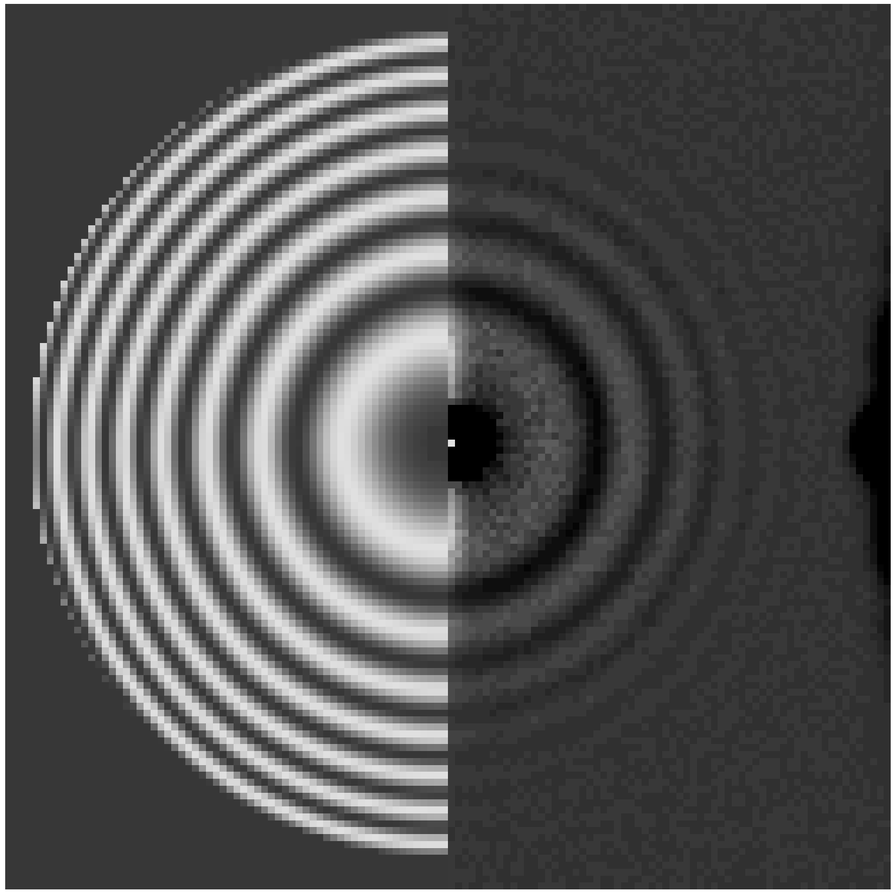


FT



Determine CTF

Image power spectrum



Model

Experiment

E = 120 kV, Δf = 21000 Å, C_s = 2 mm, A = 0.15