CTF, micrograph evaluation

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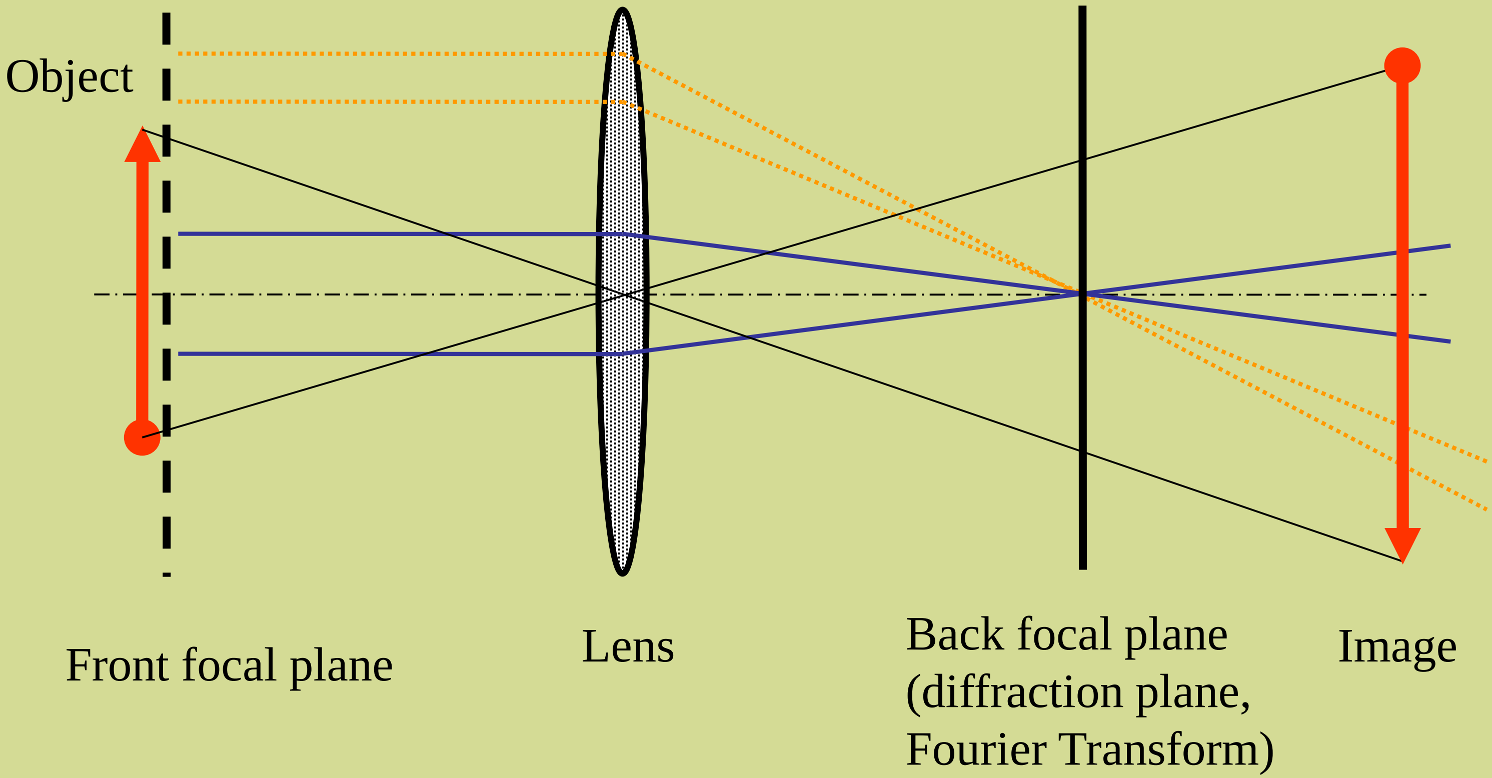
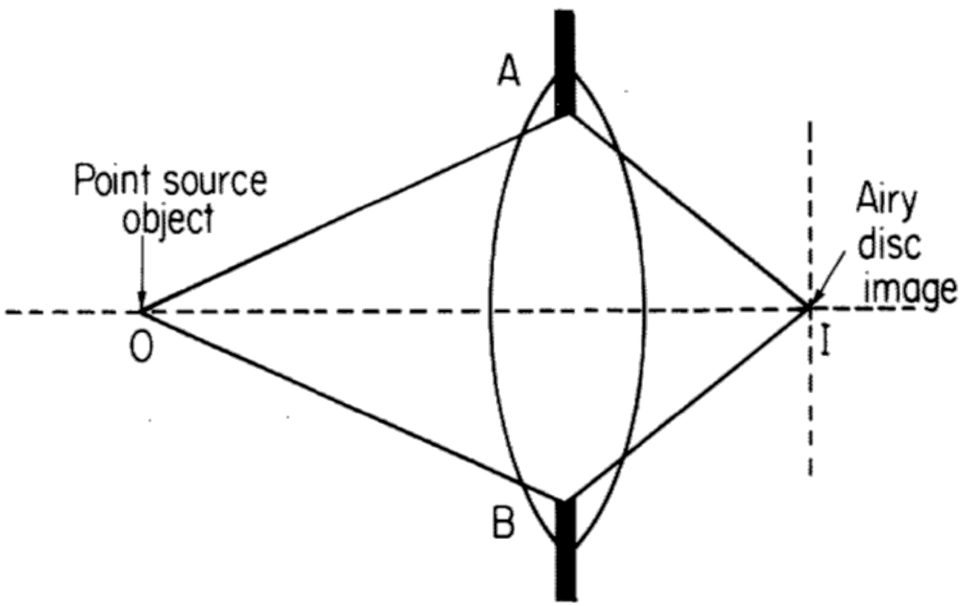
Lenses

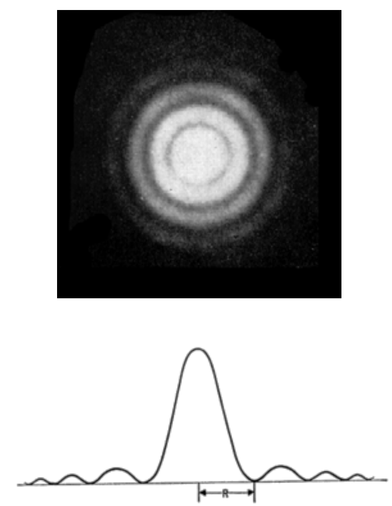

Object
Front focal plane
Lens
Back focal plane
(diffraction plane,
Fourier Transform)
Image

Optical systems – ideal vs. real

Ideal lens – object point -> point in the image

Real lens – object point -> smeared disk in the image





From Meek, 1st ed., Fig. 1.22, p.35
and Sjostrand, Fig. IV.18, p.115

For axially symmetrical lenses object points -> circular disks (Airy disks)

Phase vs. amplitude contrast

Phase:

A transparent object varies in refractive index and/or thickness, but does not cause amplitude changes in illumination.

A plane wave of uniform amplitude falls on the specimen and emerges with uniform amplitude A_0 but with phase variations over the plane surface.

$T(x,y) = A_0 \mathit{exp}[i\varphi(x,y)]$, for simplicity $A_0 = 1$

Assuming that the object is thin and the phase shift φ is small ($\varphi \ll 1$), the emerged wave can be described as

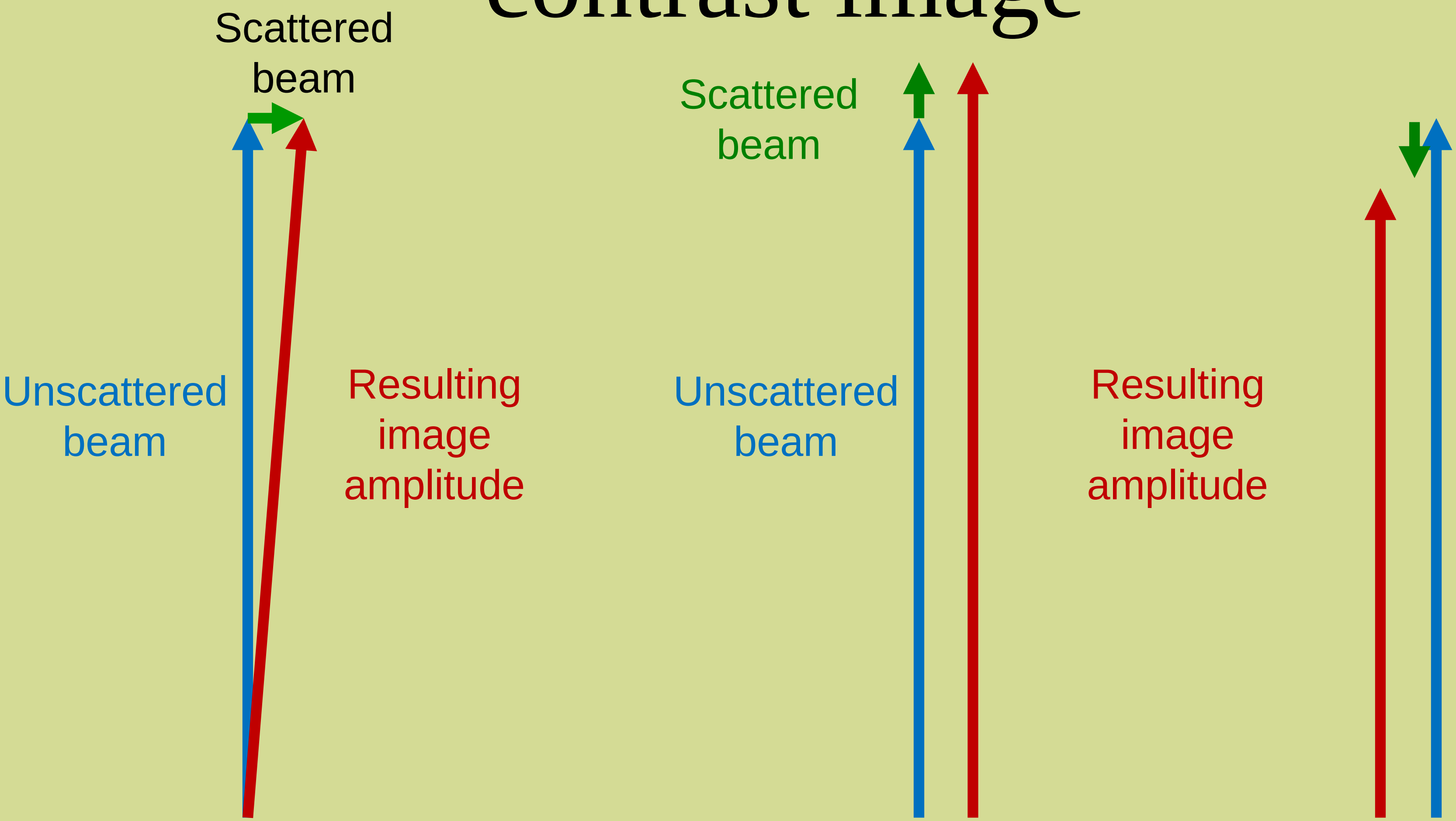
exp [if] $\approx 1 + i\varphi$, weak phase object;

then $T(x,y) \approx 1 + i\varphi$; **Observed intensity** $T^2(x,y) = 1 - \varphi^2 \approx \mathbf{1}$

Amplitude:

$T(x,y) = A\mathit{exp}[i\varphi(x,y)]$; A varies, linear contrast transfer; even small variations are visible

Weak Phase <-> amplitude contrast image



Phase contrast

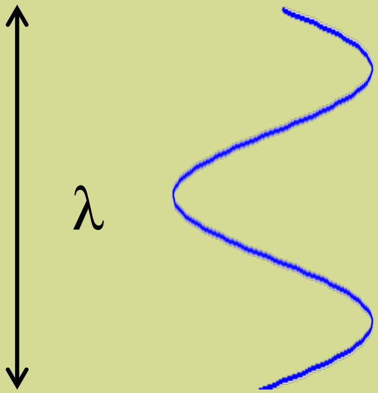
Amplitude contrast

$$\text{Amp(im)} = \sqrt{(\text{unscatt}^2 + \text{scatt}^2)}$$

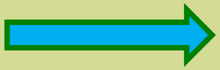
$$\text{Amp(im)} = \text{unscatt} +(-)\ \text{scatt}$$

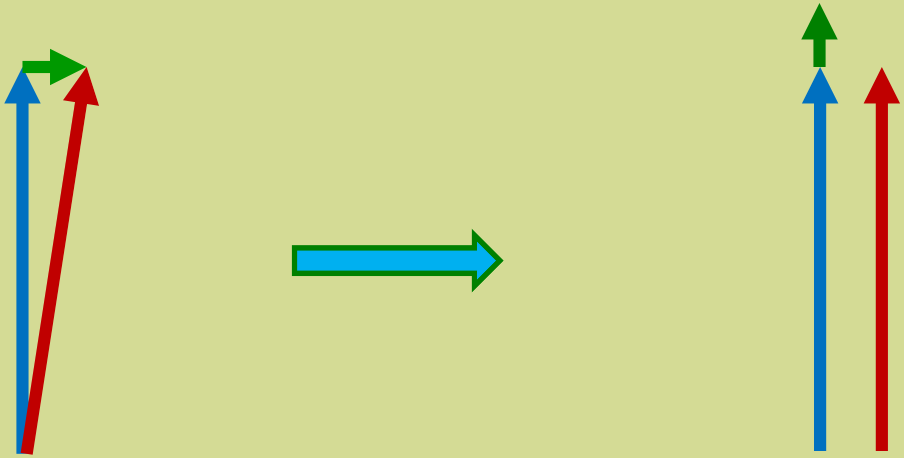
Phase plate

(phase -> amplitude contrast conversion)

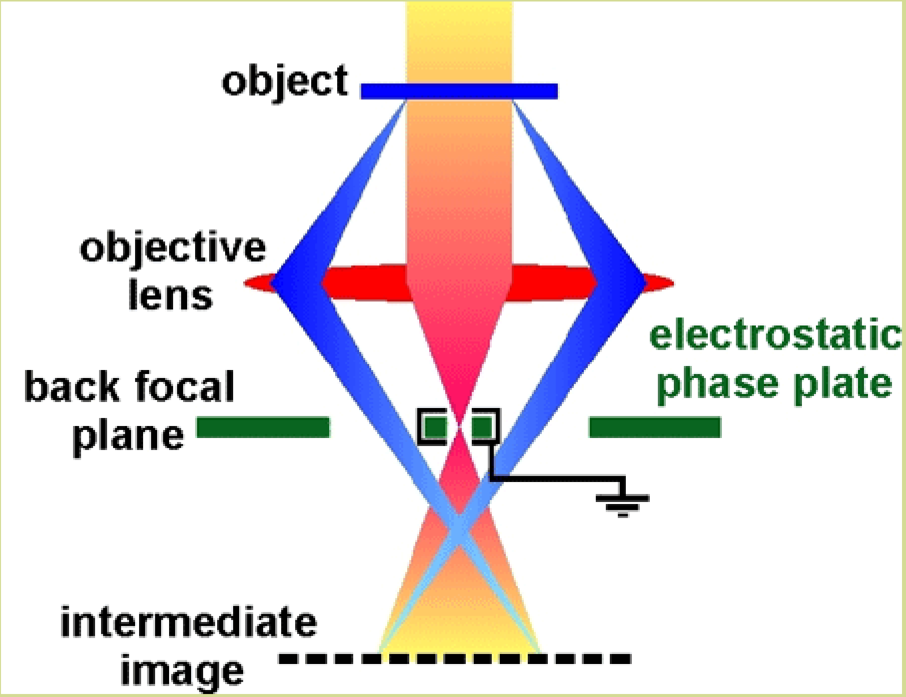


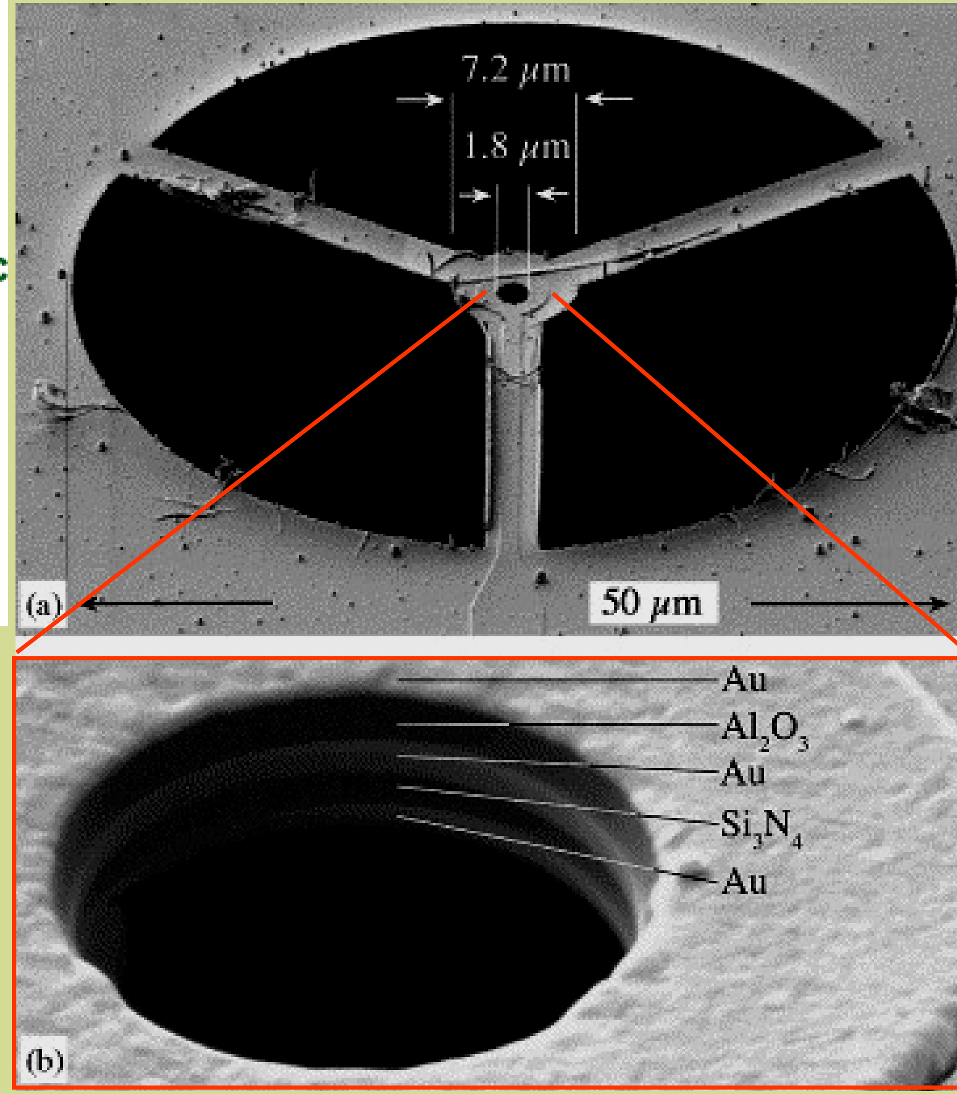
$\Delta = \frac{1}{4} \lambda$

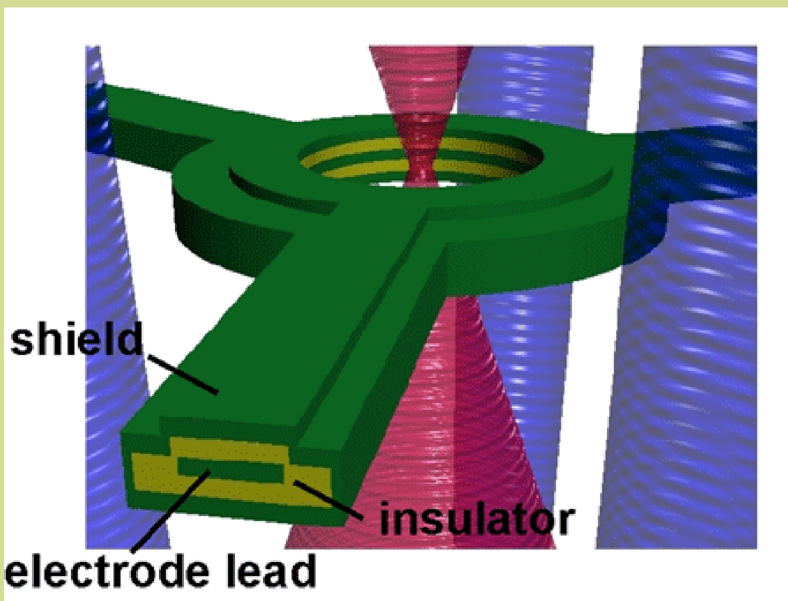
Phase contrast  Amplitude contrast



R. Schröder.







Majorovits E, Barton B, Schultheiss K, Perez-Willard F, Gerthsen D, Schroder RR. Ultramicroscopy. 2007 107(2-3):213-26.

Phase plates

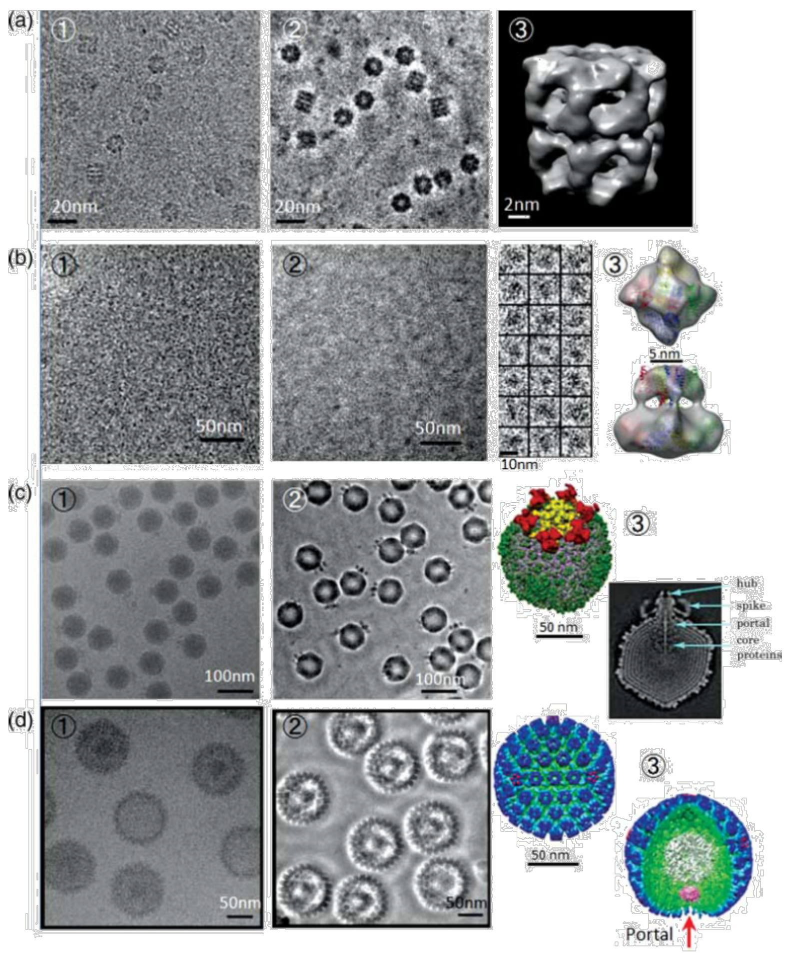
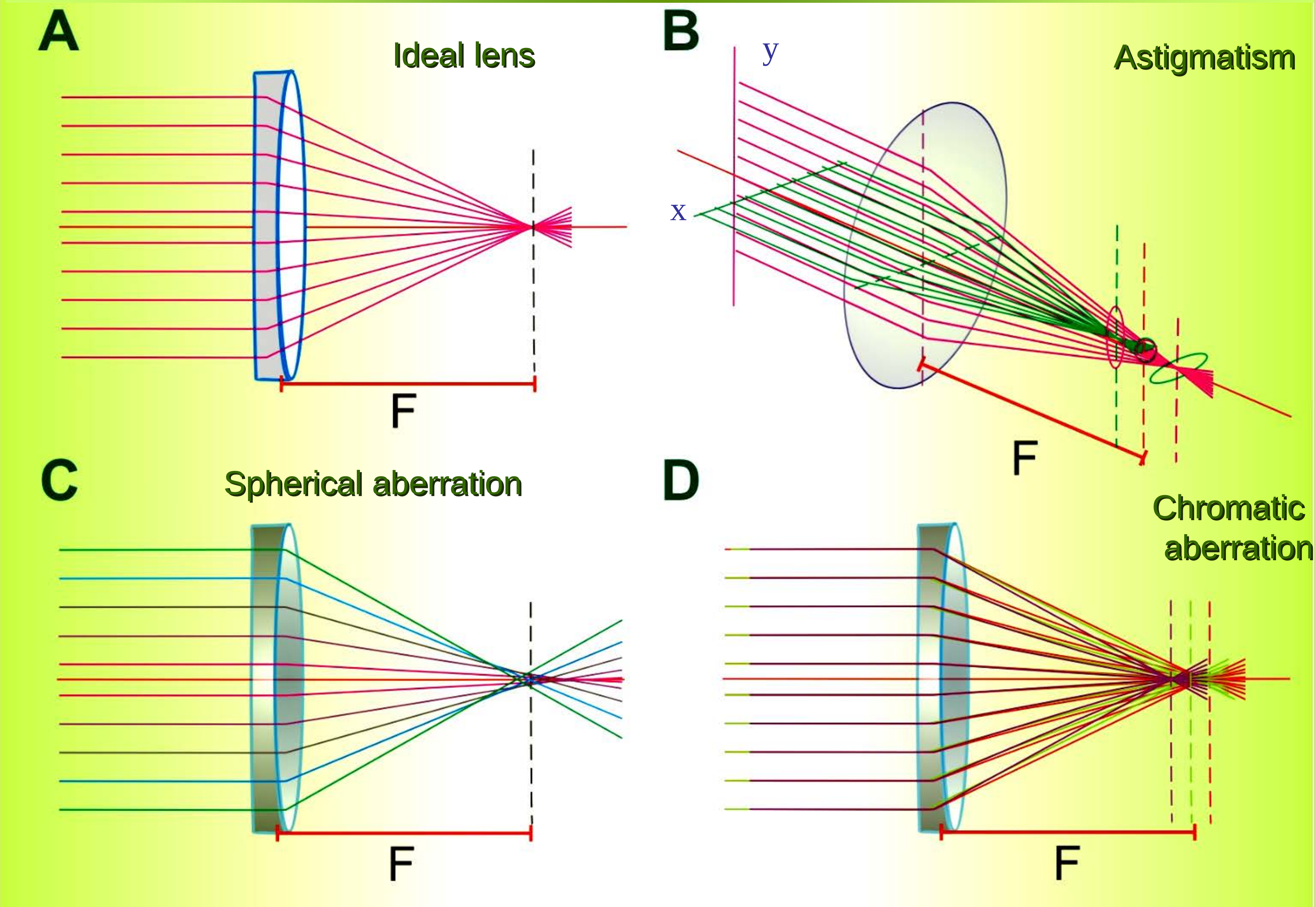


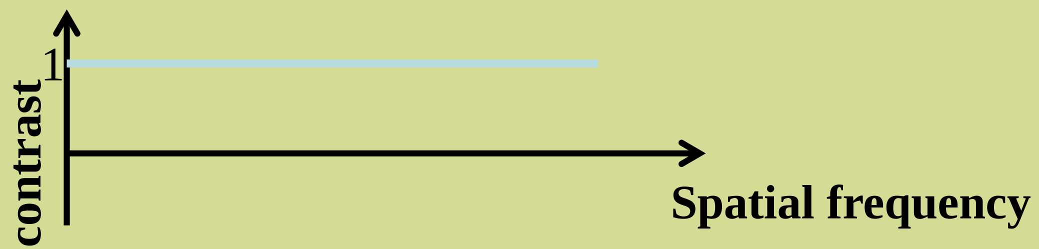
Fig. 9. Four examples of single-particle analysis based on cryo-electron microscopy. (a) A protein, GroEL (1: DPC image (300 kV), 2: ZPC image (300 kV), 3: 3D model) [from Fig. 2 of ref. 25]. (b) A membrane protein, TRPV4 (1: DPC image (300 kV), 2: ZPC image (300 kV), 3: 3D model) [from Figs. 3 and 6 of ref. 26]. (c) A bacteriophage, epsilon 15 (1: DPC image (200 kV), 2: ZPC image (200 kV), 3: 3D model) [from Figs. 2 and 3 of ref. 27]. (d) A capsid of herpes simplex virus type I (1: DPC image (200 kV), 2: ZPC image (200 kV), 3: 3D model) [from Figs. 1 and 2 of ref. 29].

Aberrations in optics

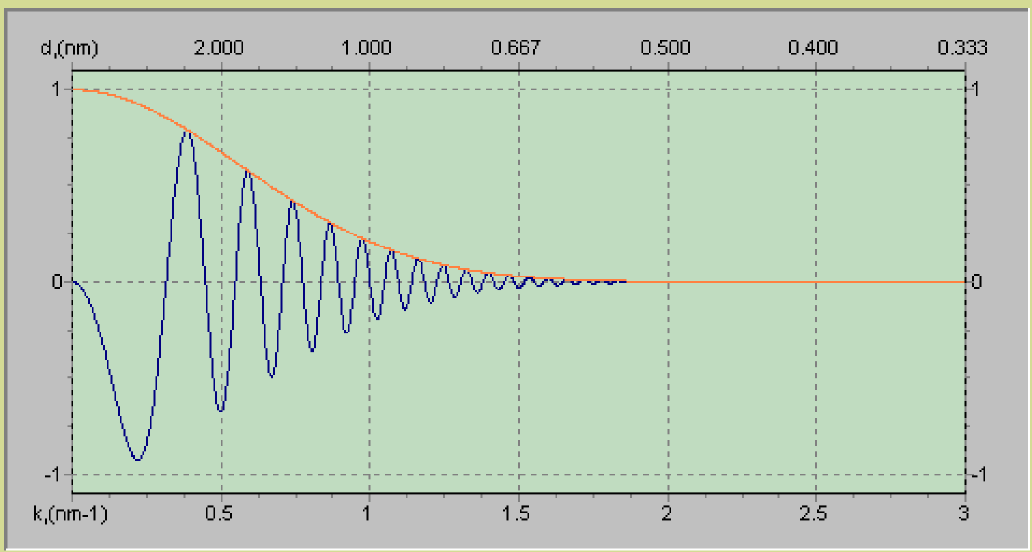


Contrast Transfer Function (CTF) of a microscope

Ideal microscope



Real microscope



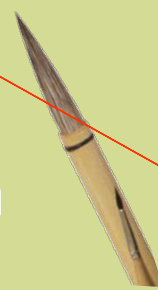
Contrast Transfer Function (CTF) of the microscope

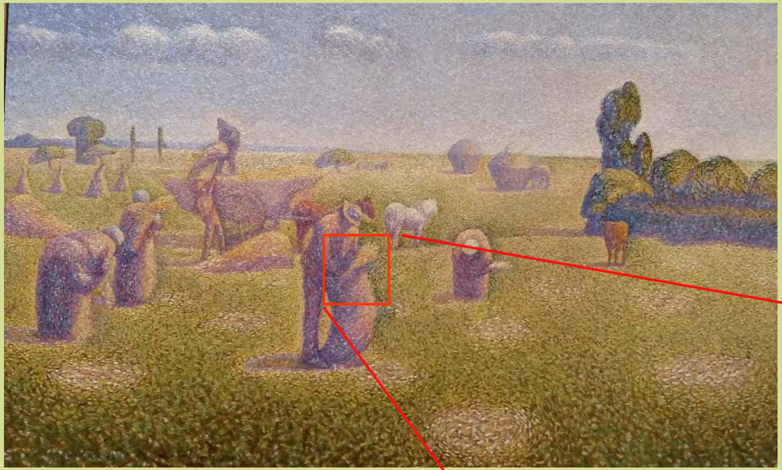
- $\rho_{im} = \rho_{obj} \otimes PSF$ (*real space*). *PSF* - Point Spread Function
- CTF = ***F***(PSF)
- $\rho_{im} = \rho_{obj} \otimes \boldsymbol{F}^{-1}$(CTF) (*real space*). $\boldsymbol{F}^{-1}$(CTF) - Point Spread Function
- $I_{im} = I_{obj} \bullet H$, where H = CTF • envelopes (*reciprocal, or diffraction, or Fourier space*)
- $$CTF(\omega) = \sqrt{(1-a^2)} \bullet \sin(\Gamma(\omega)) + a \bullet \cos(\Gamma(\omega))$$

  $\Gamma(\omega) \approx -\frac{1}{4} C_s \omega^4 + \frac{1}{2} \Delta z \omega^2$
- Astigmatism:
  $\Delta z \Rightarrow \frac{1}{2} (\Delta z_{max} + \Delta z_{min} + 2(\Delta z_{max} - \Delta z_{min})\cos(2\gamma))$;
  γ - angle from x-axis to major axis of astigmatism
- CTF is largely restorable except for places where it is close to zero

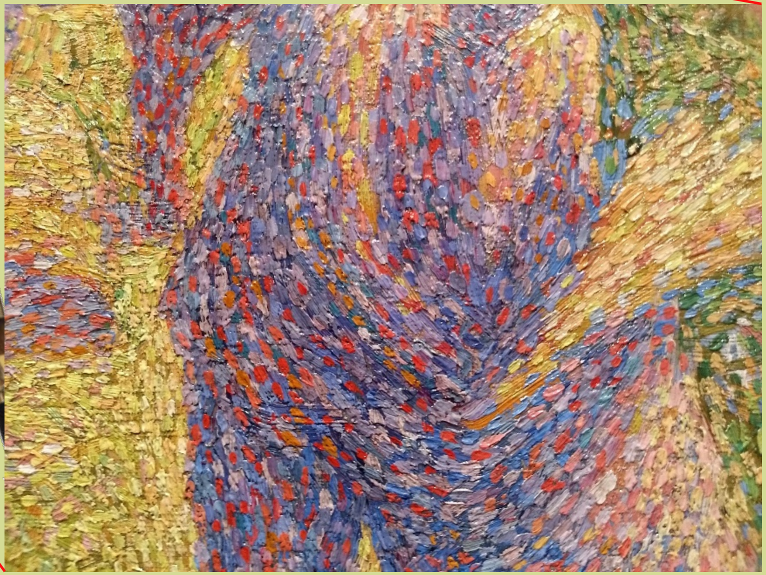
Point spread function

Thin pointed brush





Point spread function



Flat brush

Convolution

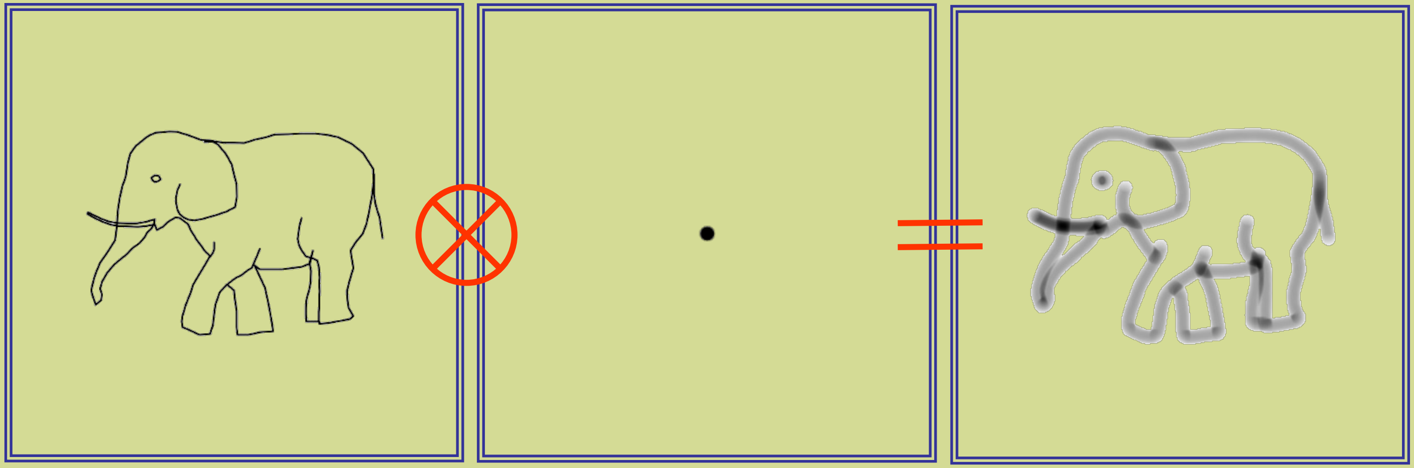
Convolution is a function describing distribution of one function by the other. It is defined as:

$$g(t) = \int g(x)h(t-x)dx = g(x) * h(x),$$

Or:

FT(J) = FT(G)*FT(H), where FT is Fourier transform

Point spread function and convolution



Convolution of two functions

Point Spread Function
PSF

Image space

Real space

Density

⇔

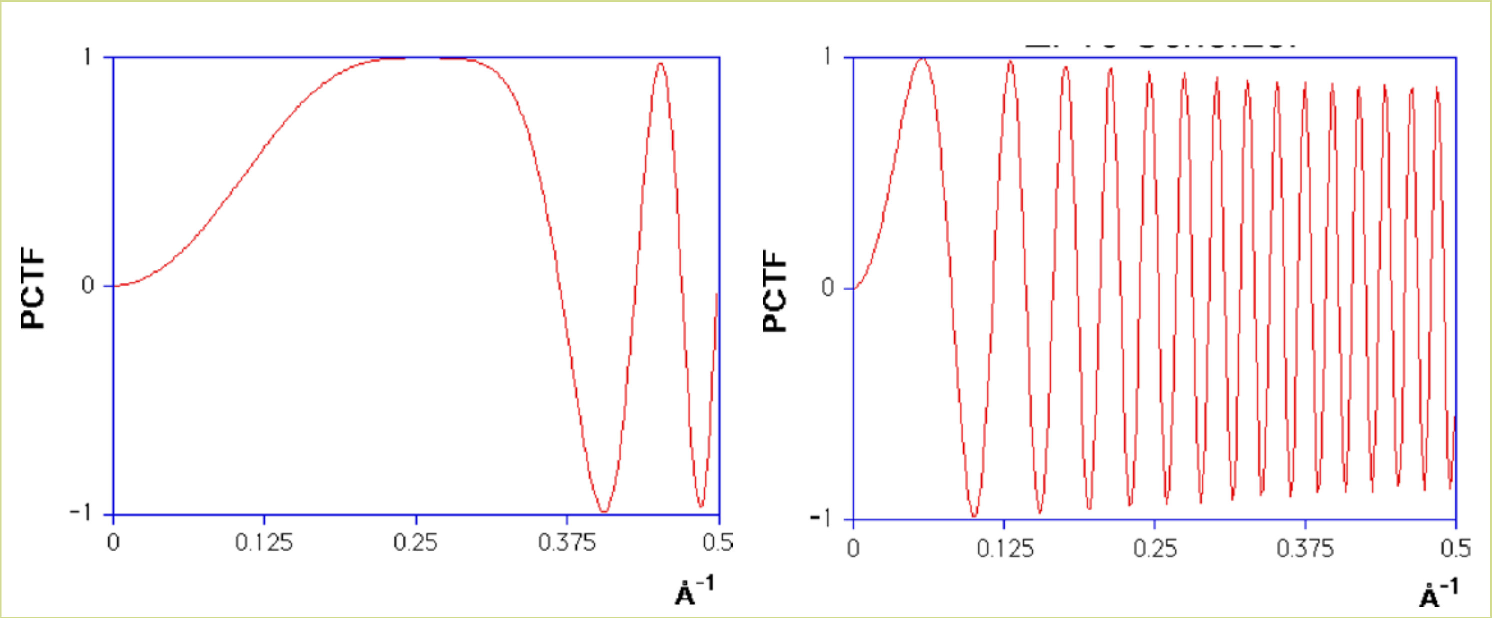
Contrast Transfer Function
CTF

Fourier space

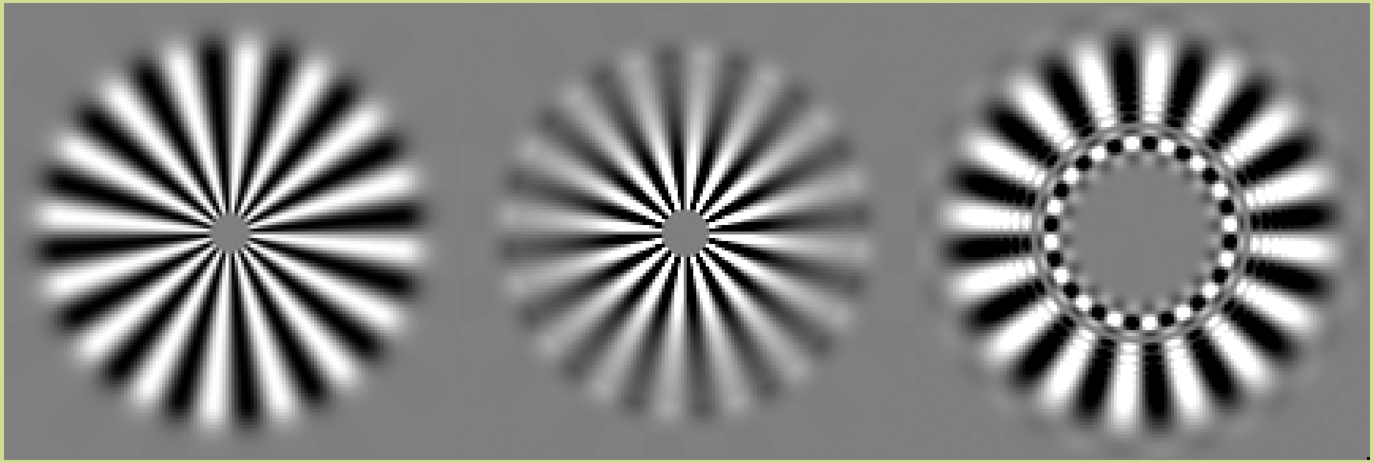
Reciprocal space

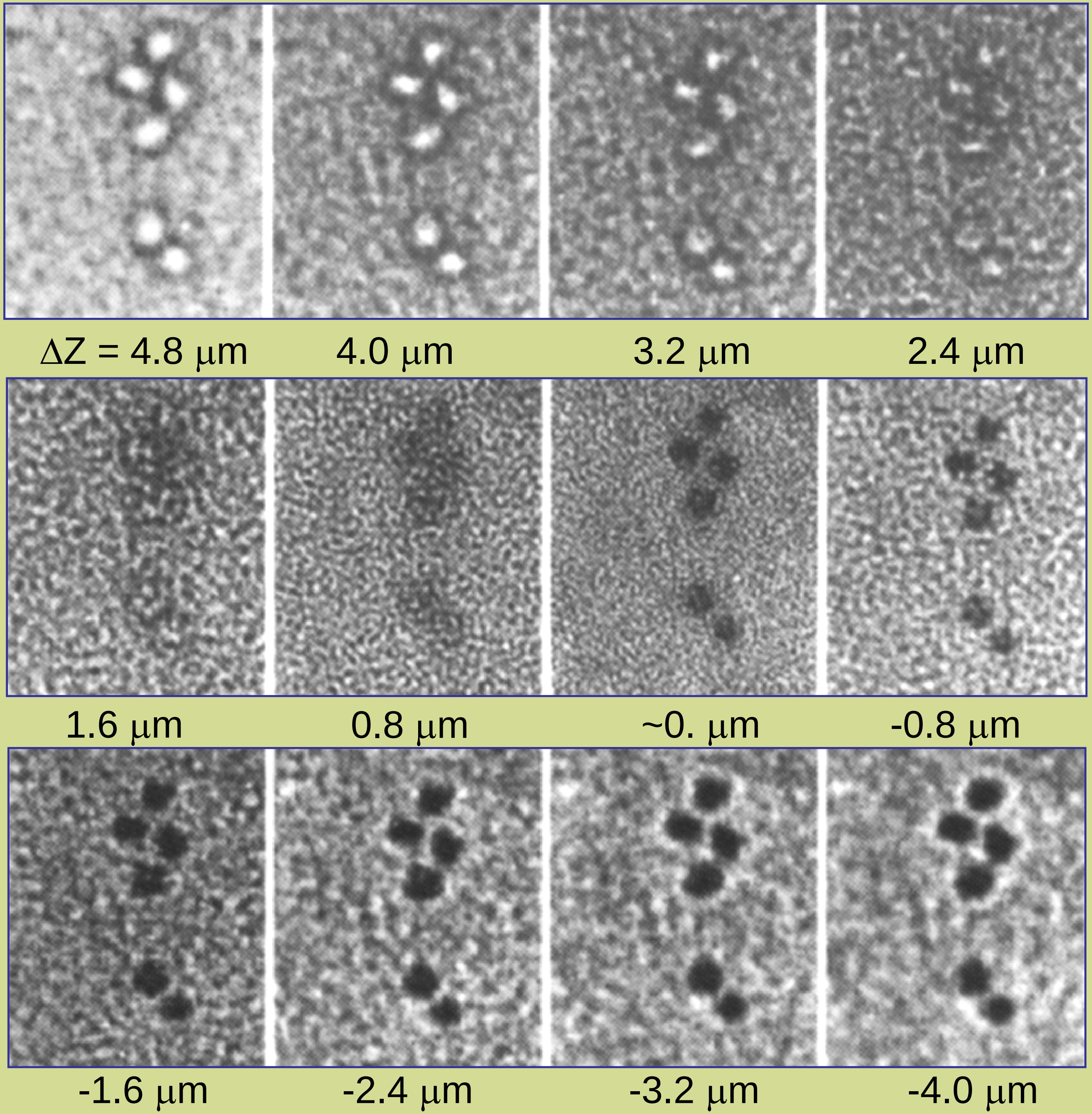
Amplitudes and phases

Δ: 1 Scherzer Δ: 10 Scherzer

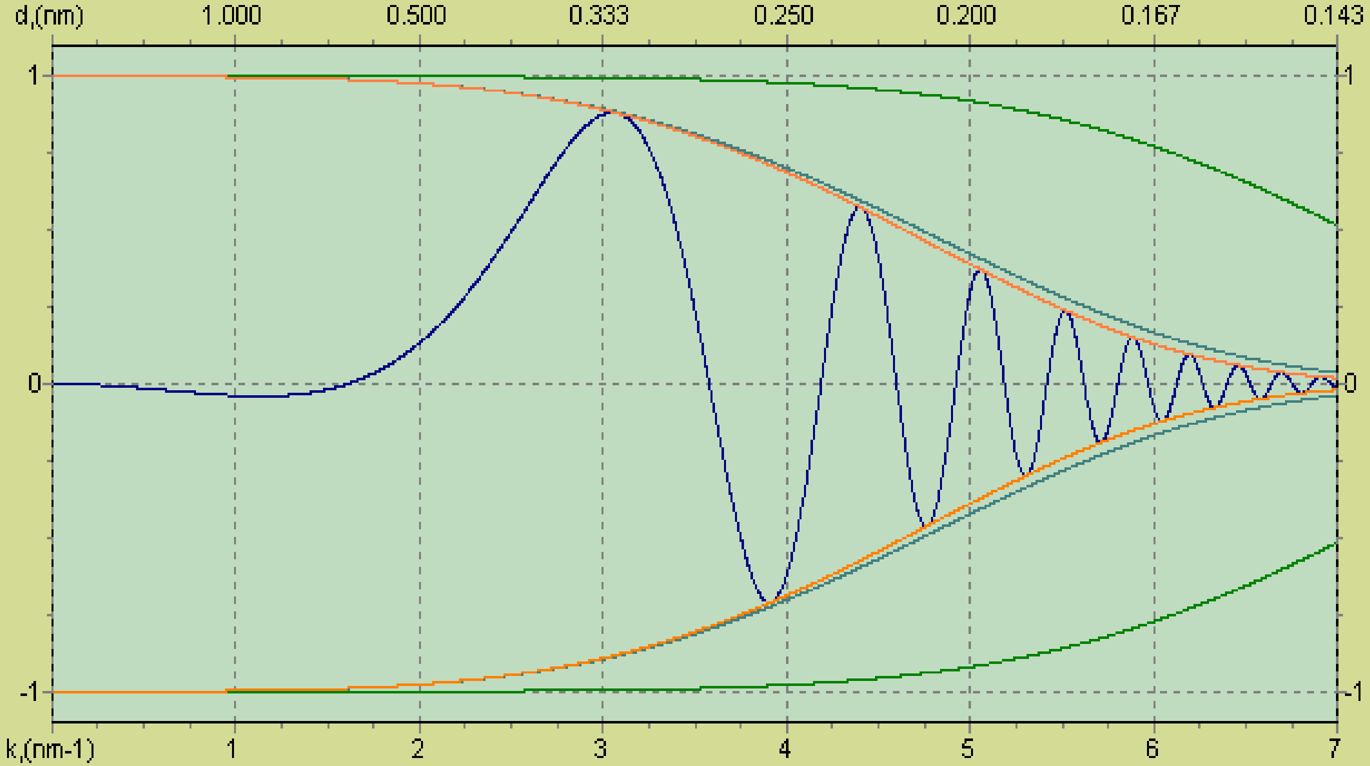


original Δ: 1 Scherzer Δ: 10 Scherzer



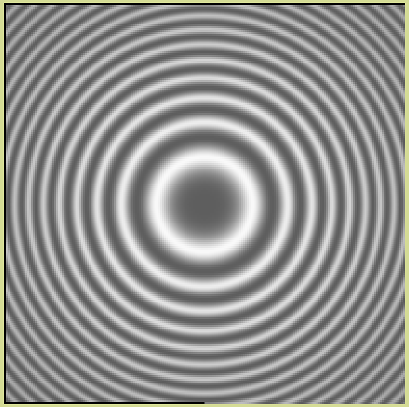


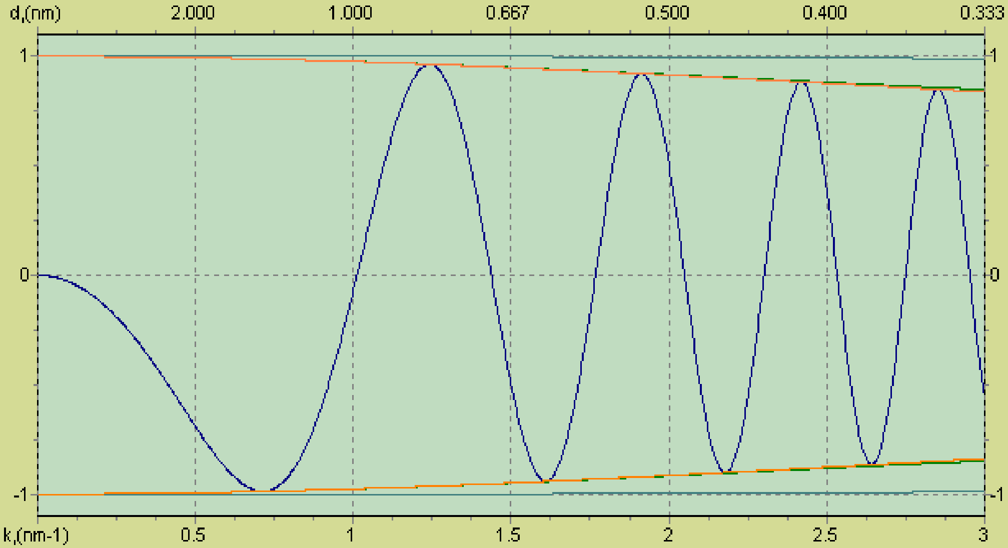
Defocus series of ferritin molecules on a carbon support film, V=100keV

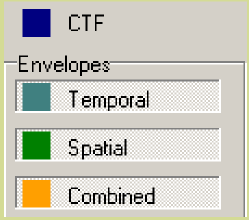


ctfexplorer

CTF (blue) and envelope functions (green and light blue) vs. spatial frequency







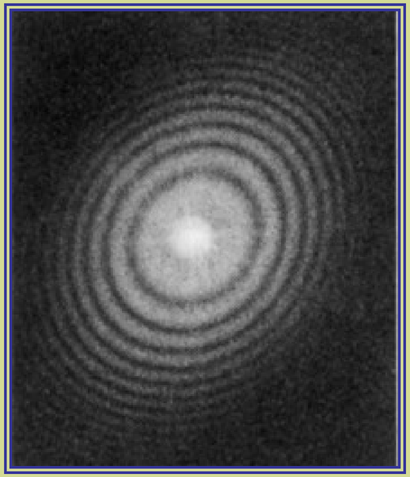
- CM 300 EM (FEI, 300 keV) image spectrum. 500 nm defocus.

Contrast Transfer Function (CTF) of a microscope

- Envelopes (envelope functions):
  - HT instability,
  - Lens current instability,
  - Spatial and temporal (energy spread) coherence of the primary beam.
  - Electromagnetic stray fields.
  - Vibrations.
- They all cause signal falloff at high resolution, some of them are defocus-dependent. These are destructive defects

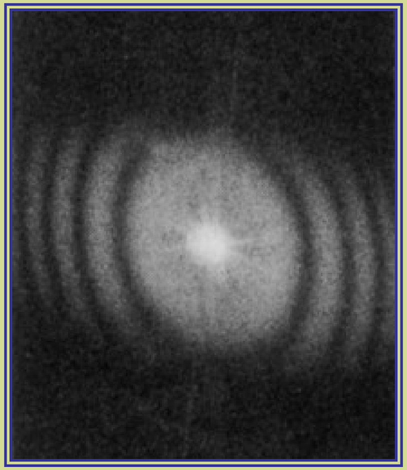
Examples of image power spectra with different defects

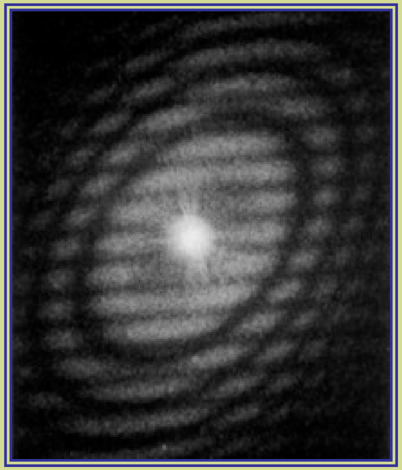
Large defocus, astigmatism



Strong astigmatism

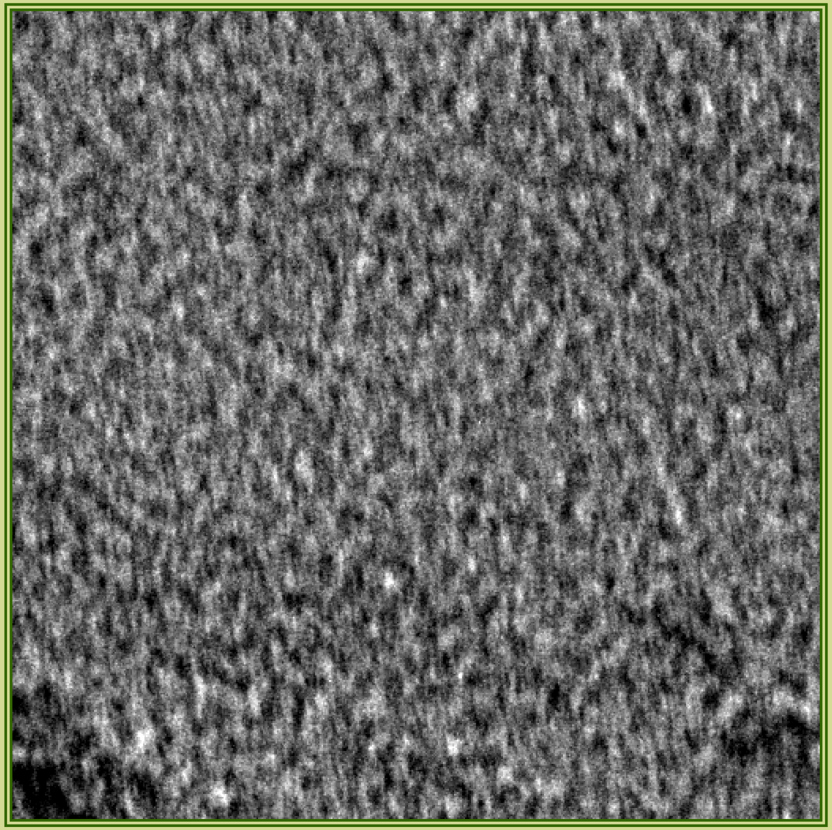
Specimen drift, astigmatism

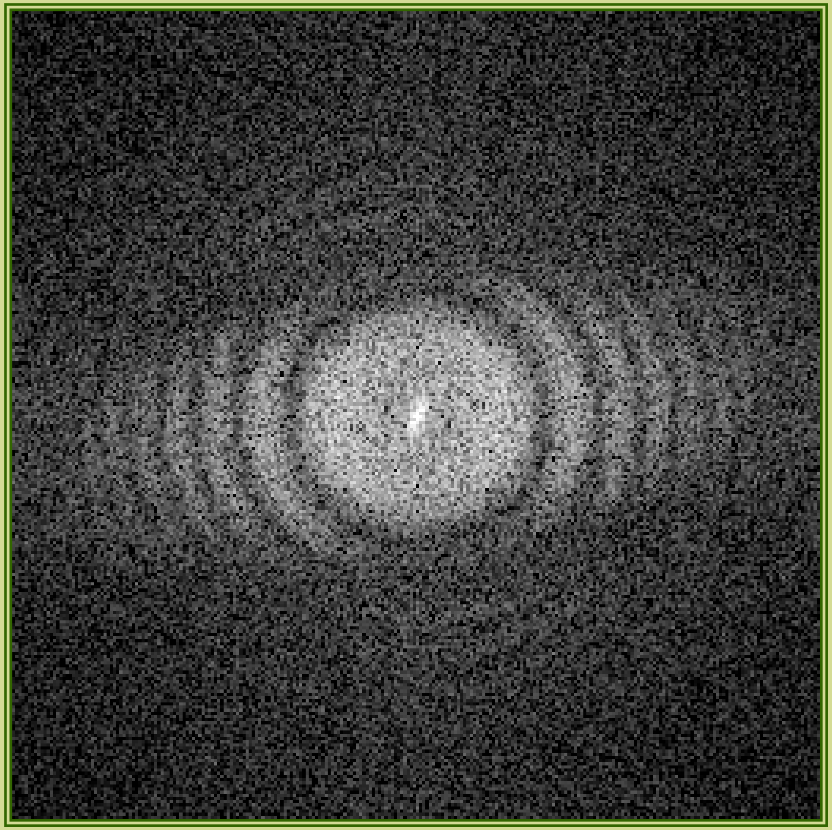




Sum of two images shifted relative to each other, astigmatism (Young fringes)

Drift



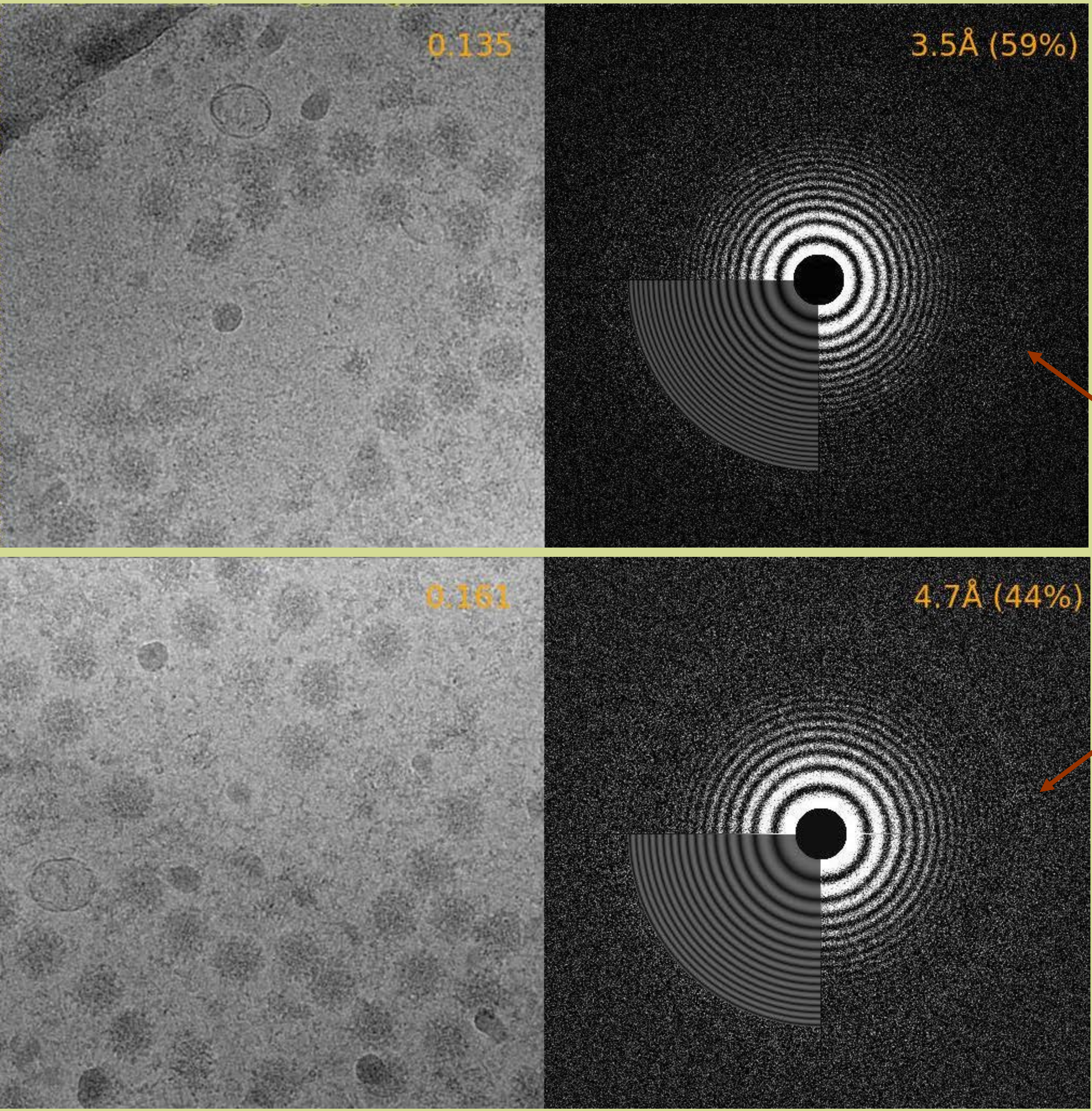


CTF correction; Wiener filtering

- $I_{im} = I_{obj} \bullet H$, where H = CTF • envelopes
- $$I_{restored} = \frac{I_{im}}{H}$$, where H ≠ 0 (no noise) or, better:

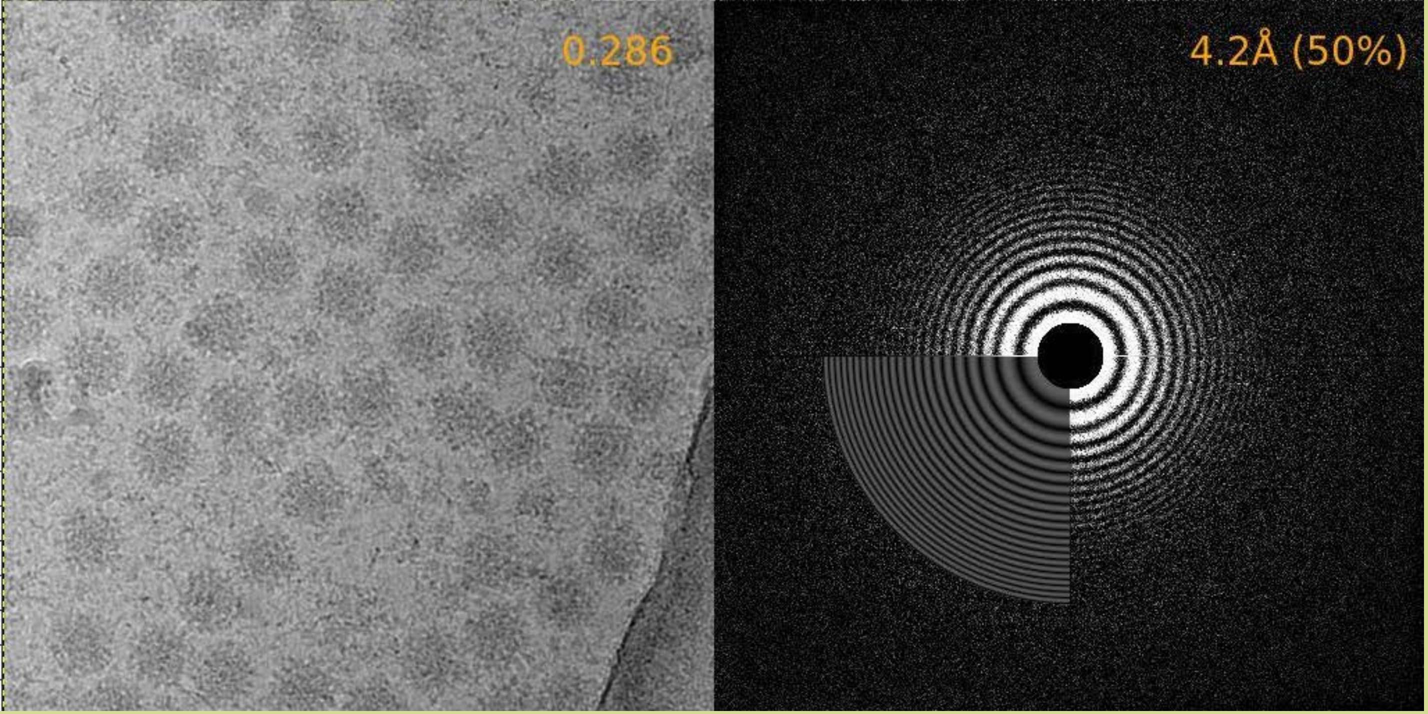
$$I_{restored} = \frac{I_{im} \bullet H}{H^2 + (\frac{N}{S})^2}$$, where N is noise, and S is signal

  - If signal is strong, then $I_{restored} = I_{im}/H$; but
  - If signal is weak then $I_{restored} \approx 0$
  - The latter formula is called "Wiener filter", an optimal filter

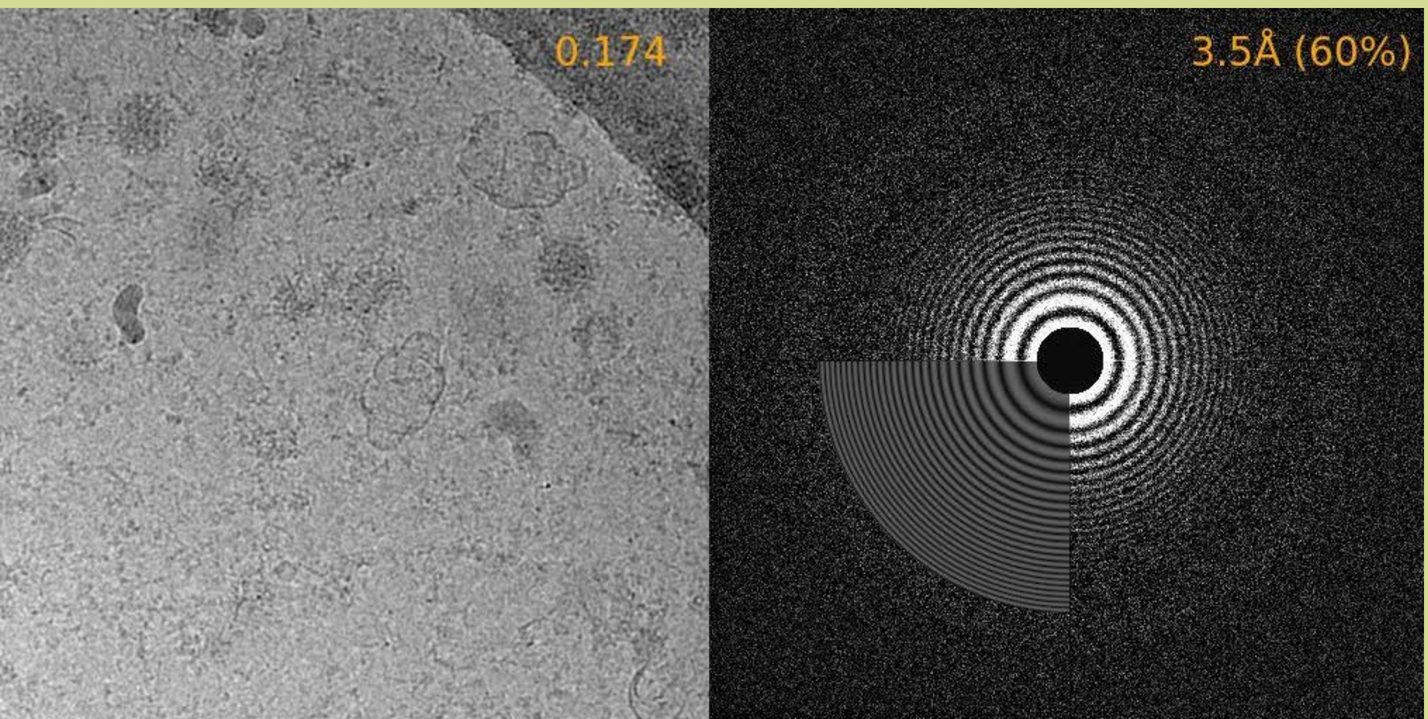


CTFFIND,
frame alignment

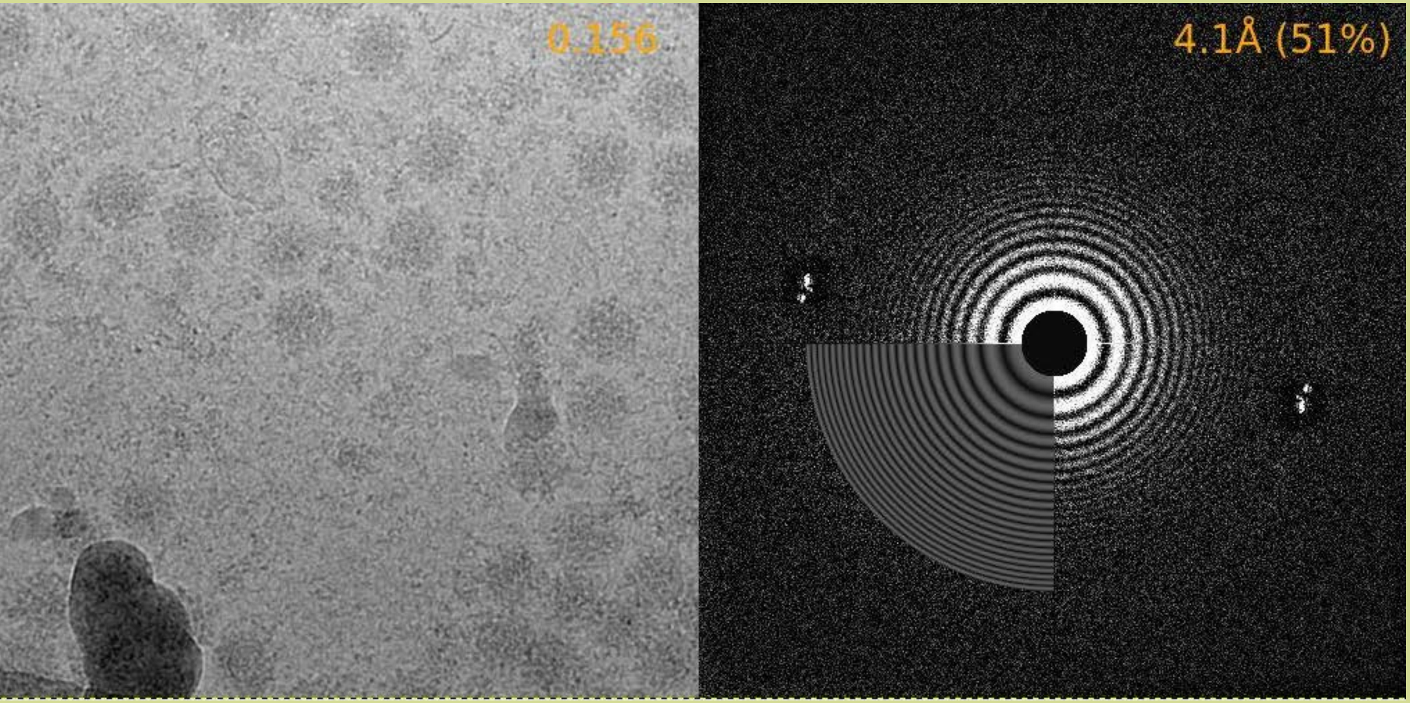
Water scattering ring



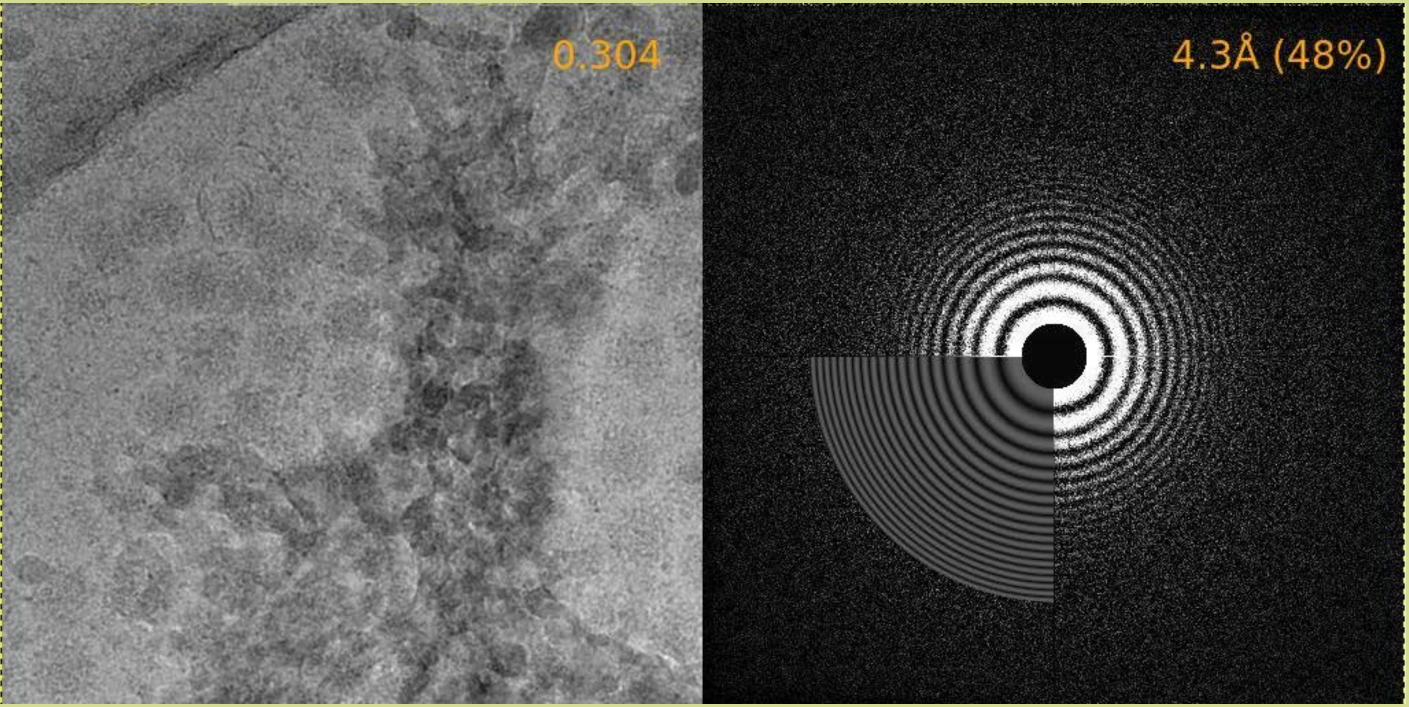
Good image



Good image,
No sample



Crystalline ice present



Heavy contamination

References

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Zheng, S. Q., E. Palovcak, J.-P. Armache, K. A. Verba, Y. Cheng and D. A. Agard (2017). "MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy." Nature Methods **14**: 331.