IMAGE FORMATION

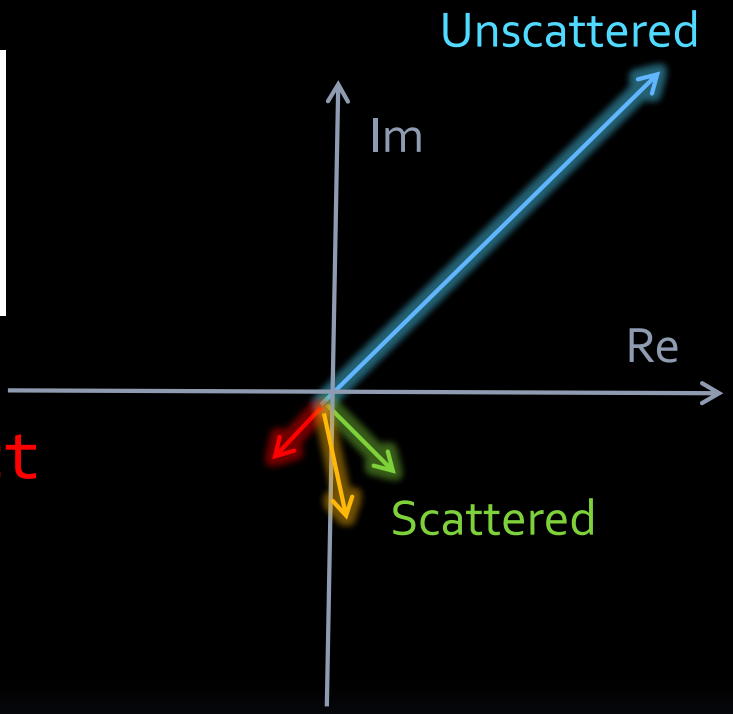
SUPPLEMENTARY

- Adding amplitude contrast to CTF
- Phase Plate

Contribution to CTF by real object with both phase and amplitude contrasts

Weak Phase Object

$$\sin\left(-\pi\Delta z\lambda k^2+\frac{\pi C_s\lambda^3k^4}{2}\right)$$
$$=\sin(\gamma(k))$$



Weak Amplitude Object

$$\cos(\gamma(k))$$

TOGETHER:

$$A\cos(\gamma(k))+\sqrt{1-A^2}\,\sin(\gamma(k))=\sin(\gamma(k)+\alpha)$$

Amplitude contrast coefficient

What do we want from phase plate ?

Phase Contrast

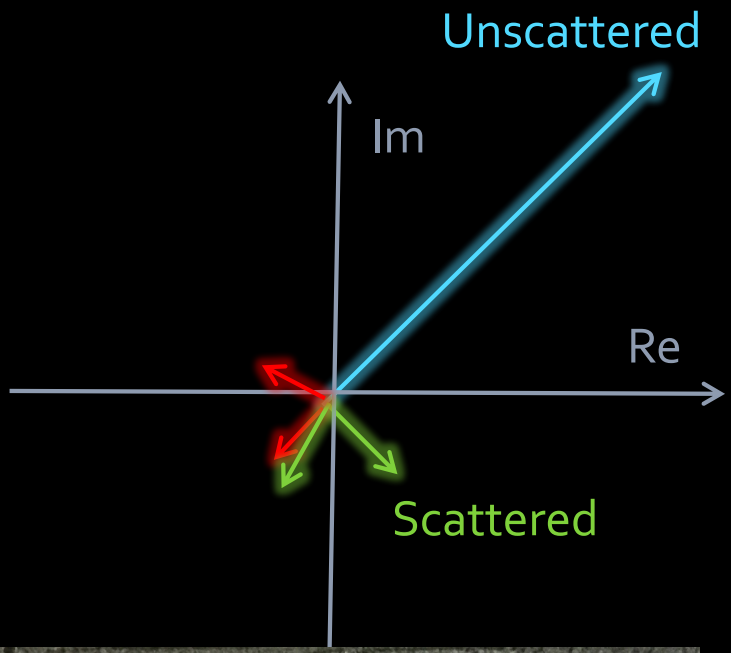
$\sin(\gamma(k))$

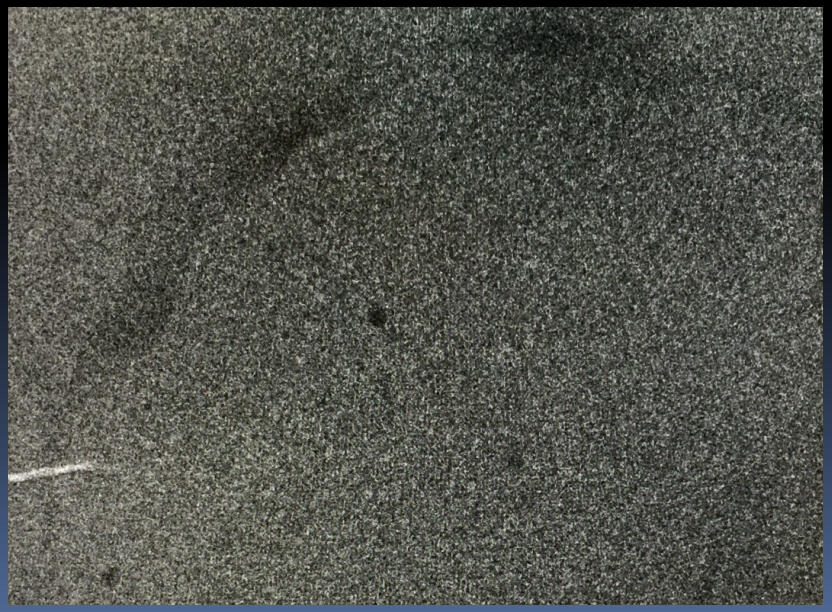
+ Amplitude Contrast

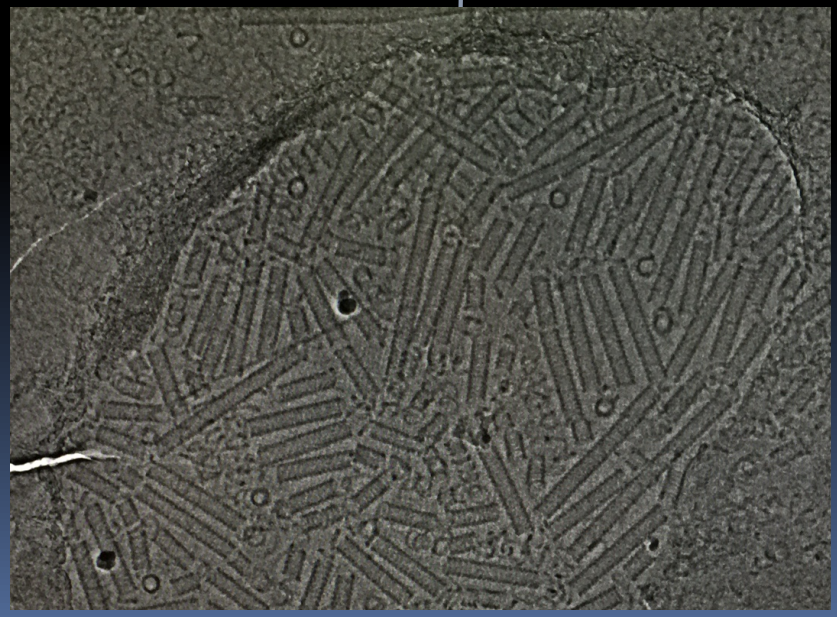
$\sin(\gamma(k)+\alpha)$

+ Phase Plate

$\sin(\gamma(k)+\alpha+\varphi)$

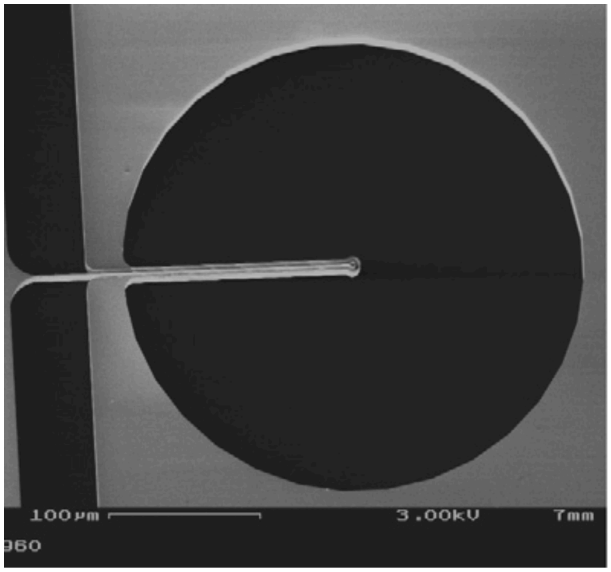






TEM imaging modes AND Various PP Designs

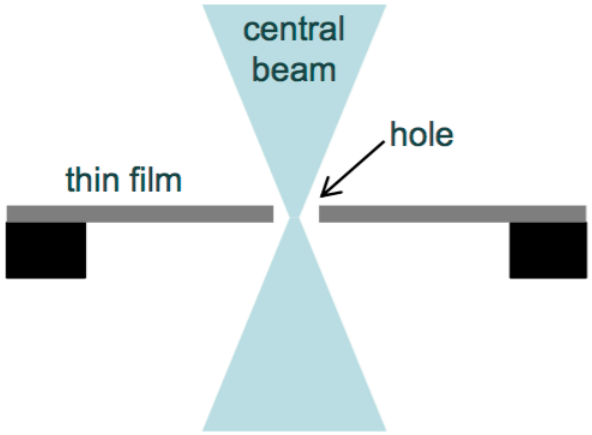
Electrostatic Phase Plate



R. Glaeser

Zernike Phase Plate

ZPP

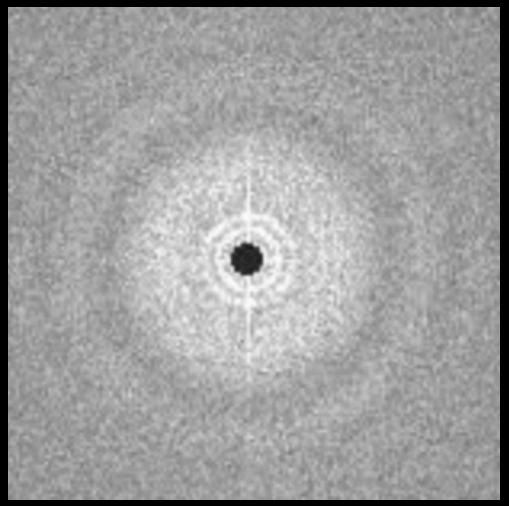


K. Nagayama

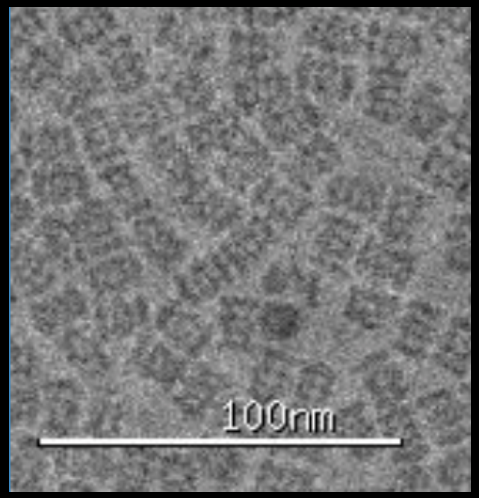
Modified from slides by Radostin Danev

Contrast development

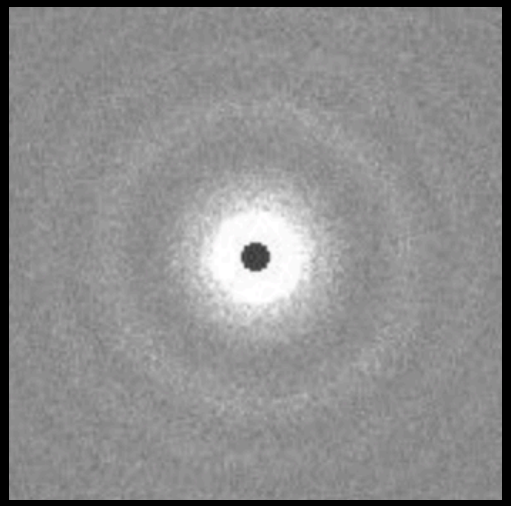
Δz = 0.5 µm (underfocus)



No Phase Plate

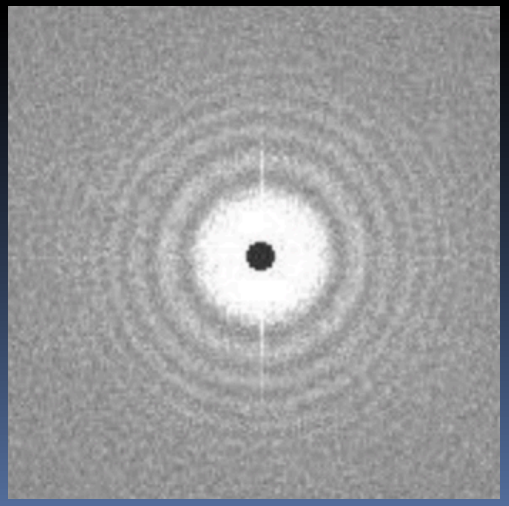


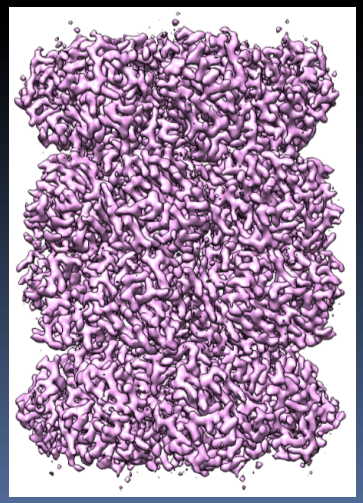
ϕ = 35 °



ϕ = 70 °

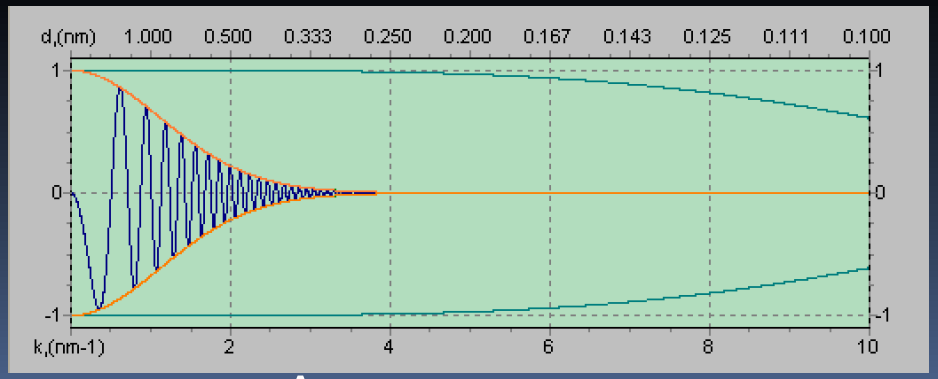
Δz = 2 µm



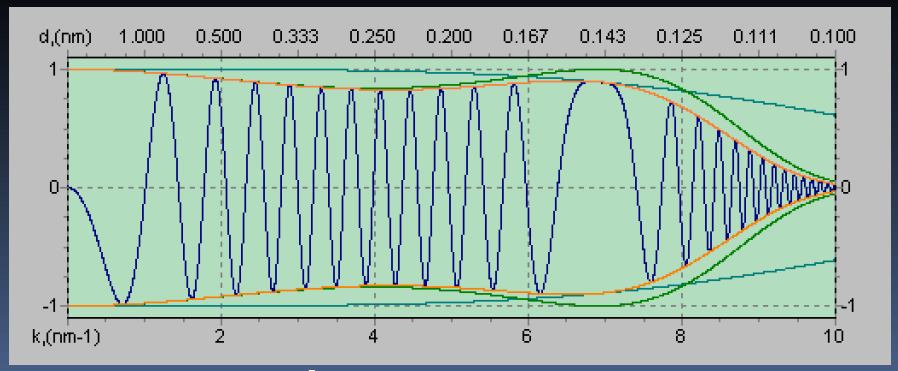


Where will we benefit from phase plate?

- Anything-
  - Pro: Low resolution contrast makes it easier to align particles with different orientation.
  - Con: Slower data acquisition with more uncertainty of device behavior
- Small molecules
  - Allow low defocus to be used that reduces the dampening effect of the envelop function.



Δz = 2 µm



Δz = 0.5 µm

Further readings

- Phase Plate –
  - Radostin Danev et. al. Using the Volta phase plate with defocus for cryo-EM single particle abalysis eLife 2017;10.7554/eLife.23006
- Amplitude contrast coefficient values –
  - K. Yonekura et. al. Electron energy filtering significantly improves amplitude contrast of frozen-hydated protein at 300 kV. J. Struct. Biology 156 (2006) 524-536.