Fourier Transforms and Image Formation

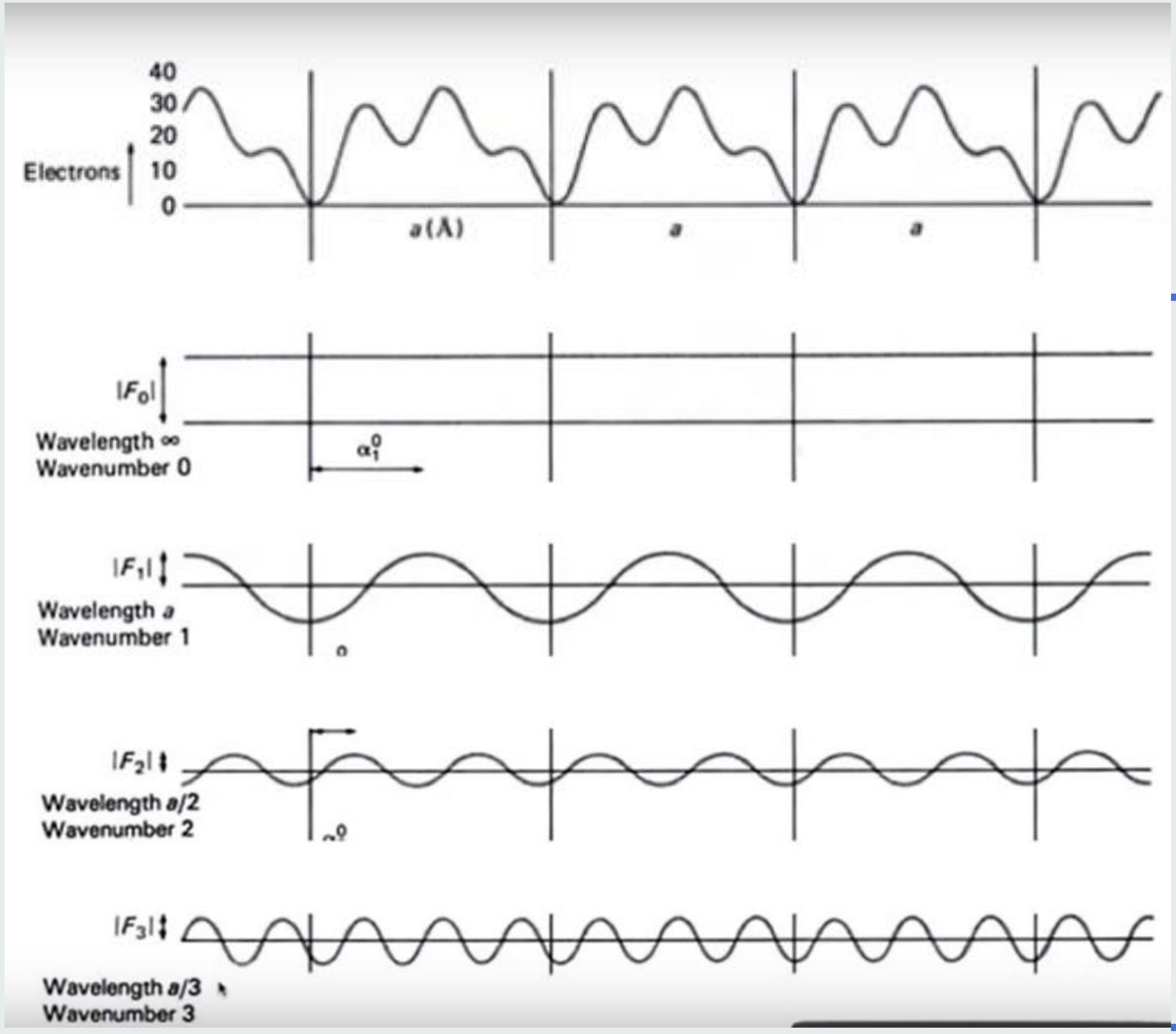
SEMC Winter EM Course

January 27th, 2020

Overview

- Review of Fourier Transforms
- Electron scattering
- Amplitude and phase contrast
- Contrast Transfer Function (CTF)
- CTF estimation & correction
- Examples in EM

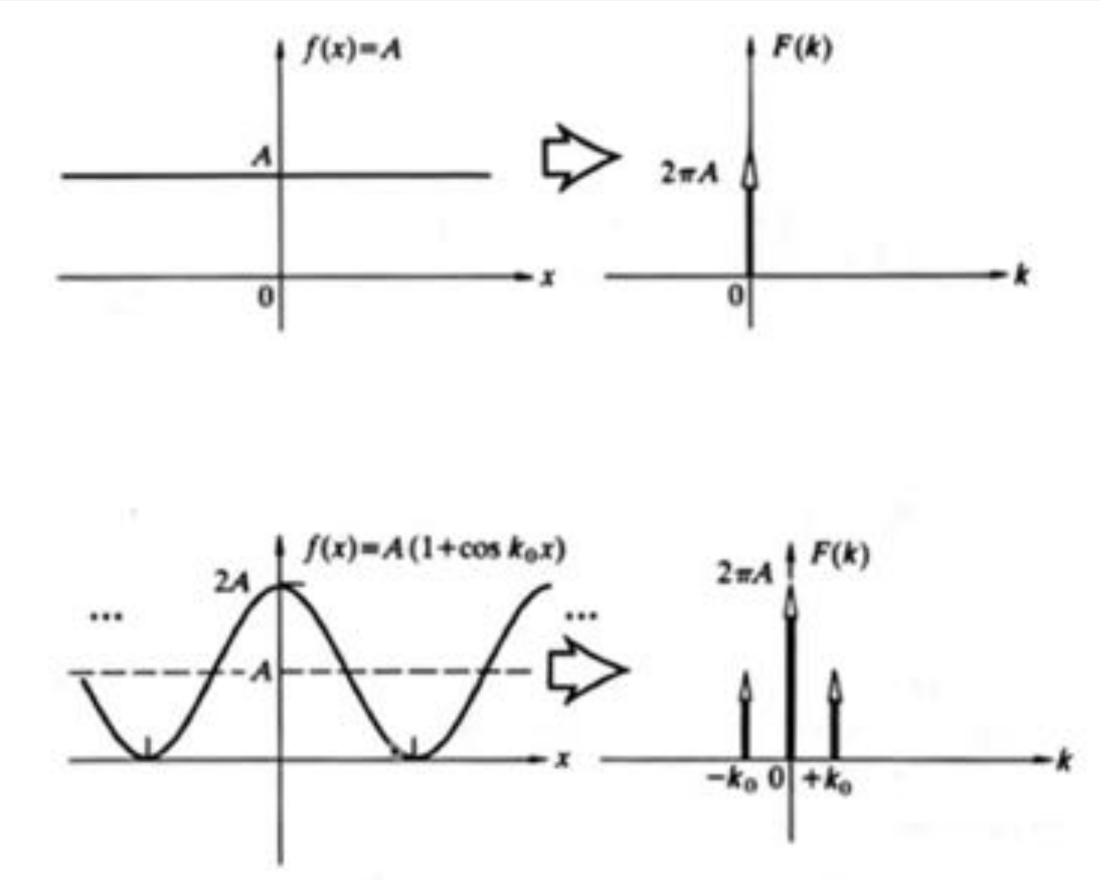
1D Fourier Transforms

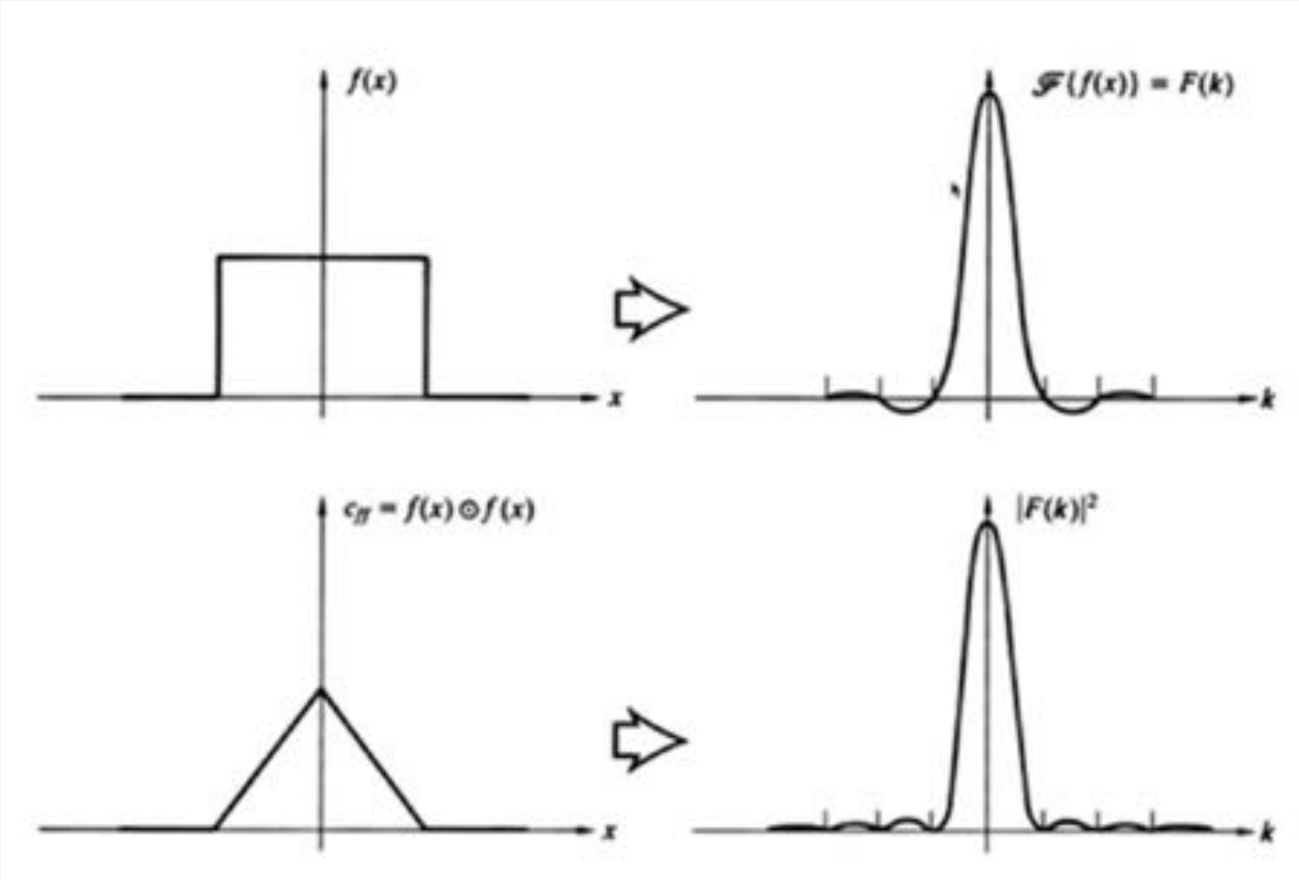


Complex wave

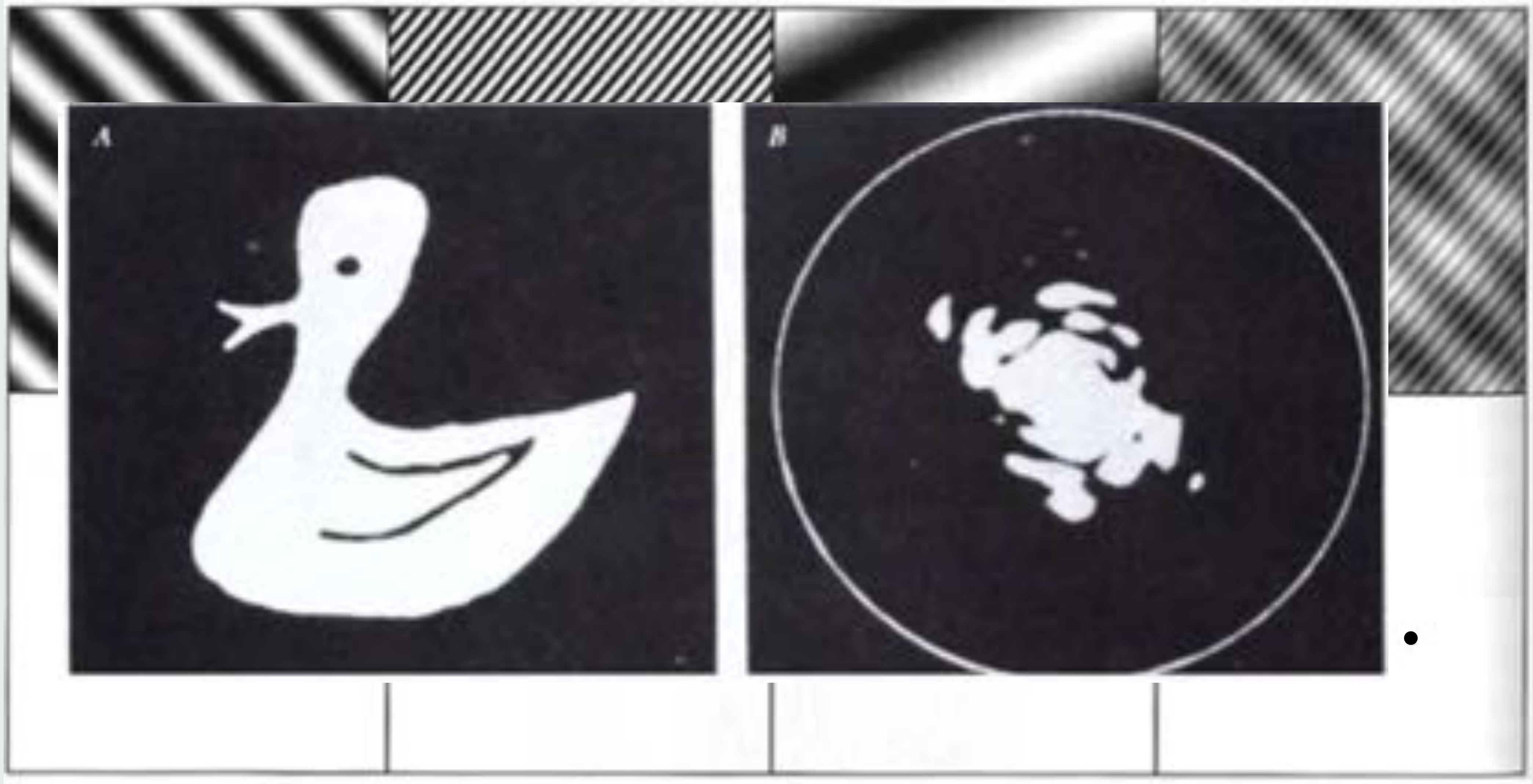
Fourier components

1D Fourier Transforms – Reciprocal space

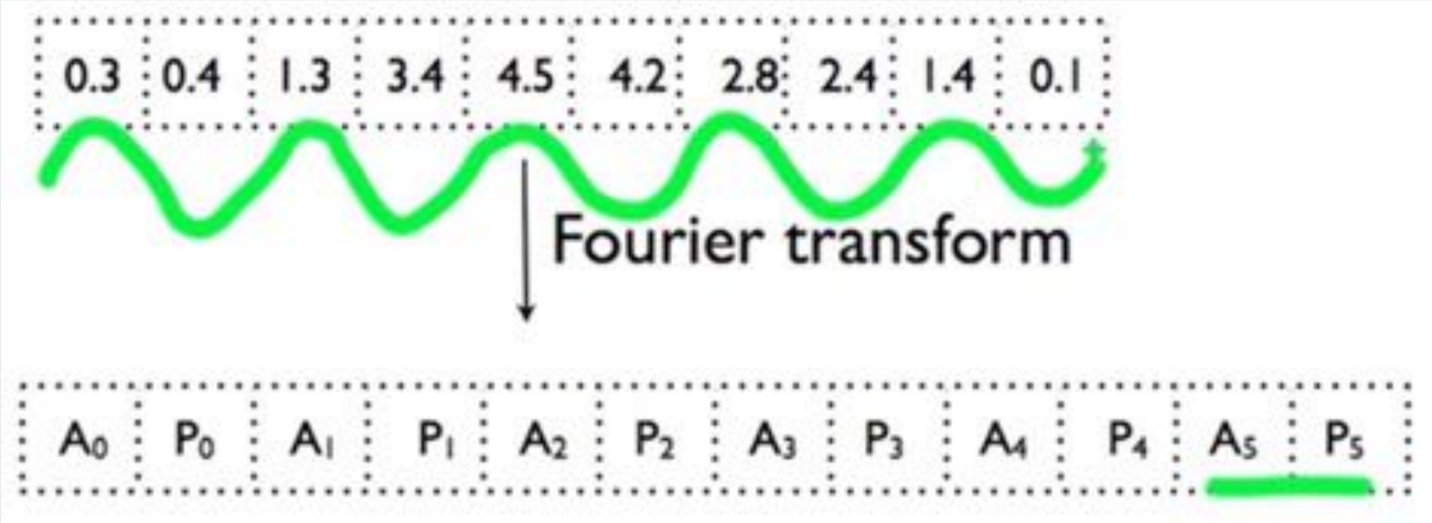




2D Fourier Transforms – reciprocal space

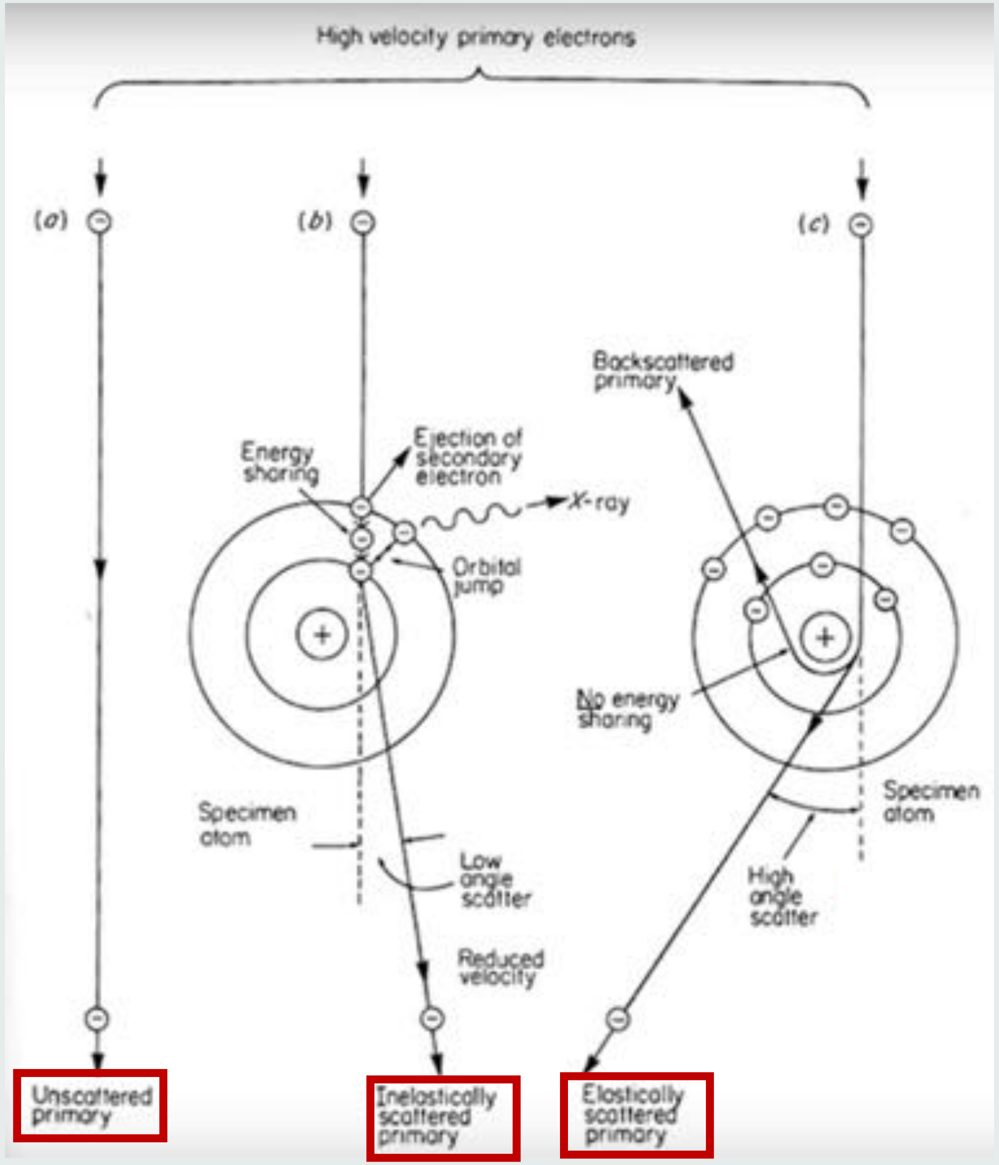


Fourier Transforms – Nyquist frequency

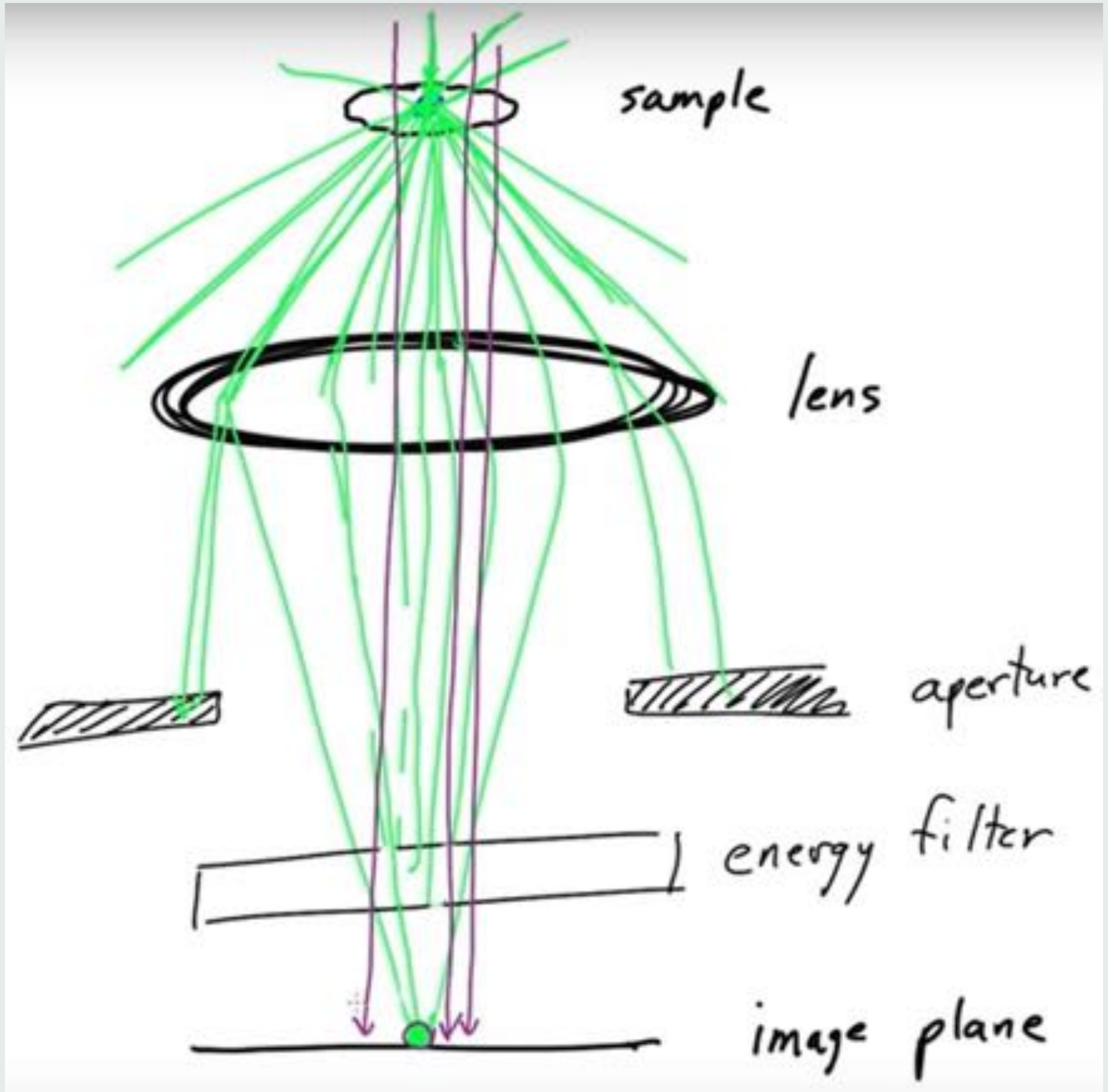


Nyquist frequency = 2 x Pixel Size [Å]

Image formation - scattering



Amplitude Contrast



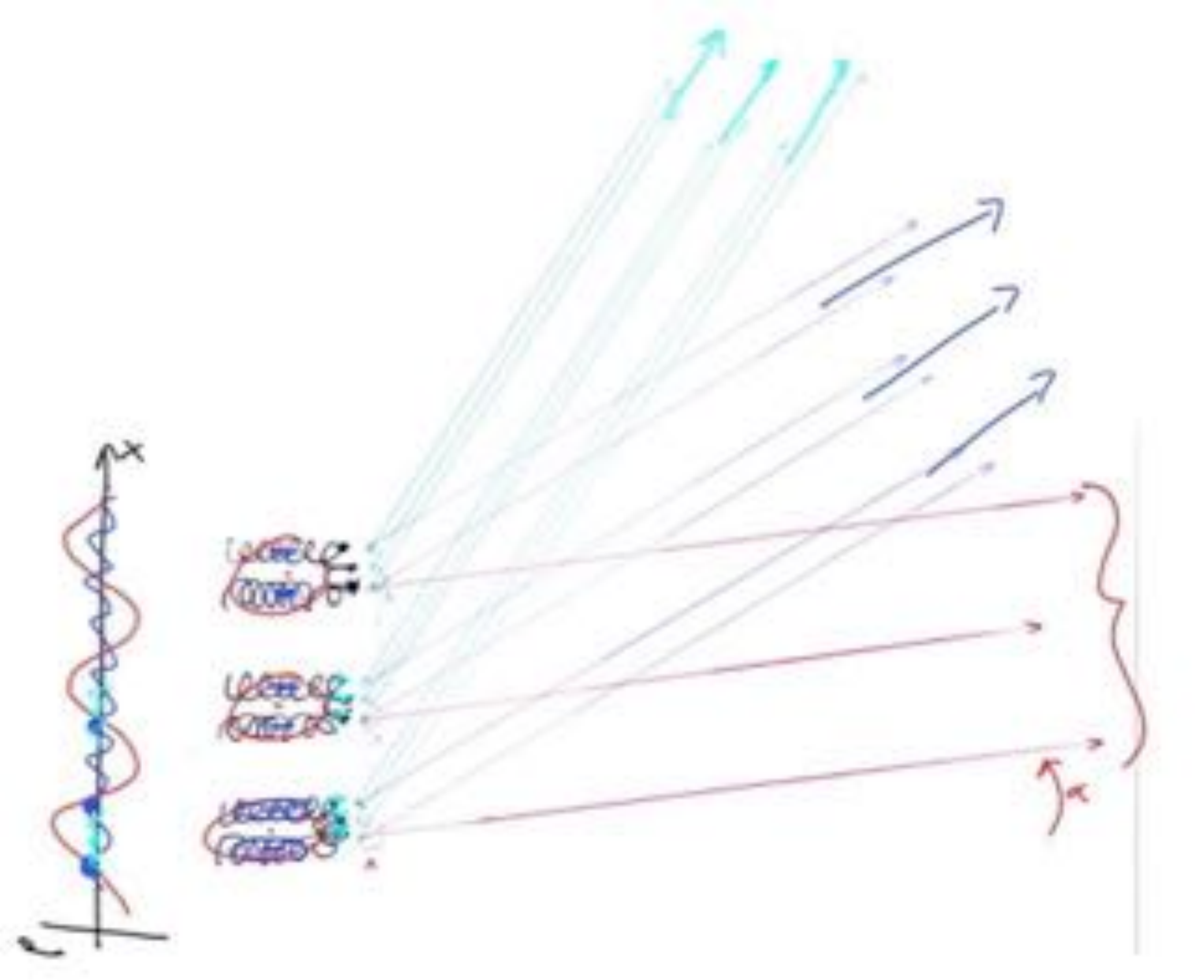
Amplitude contrast = difference between sample & background due to lost electrons*

Most electrons are unscattered

Very low amplitude contrast

*Oversimplification. Electrons are more accurately understood as waves

Phase Contrast

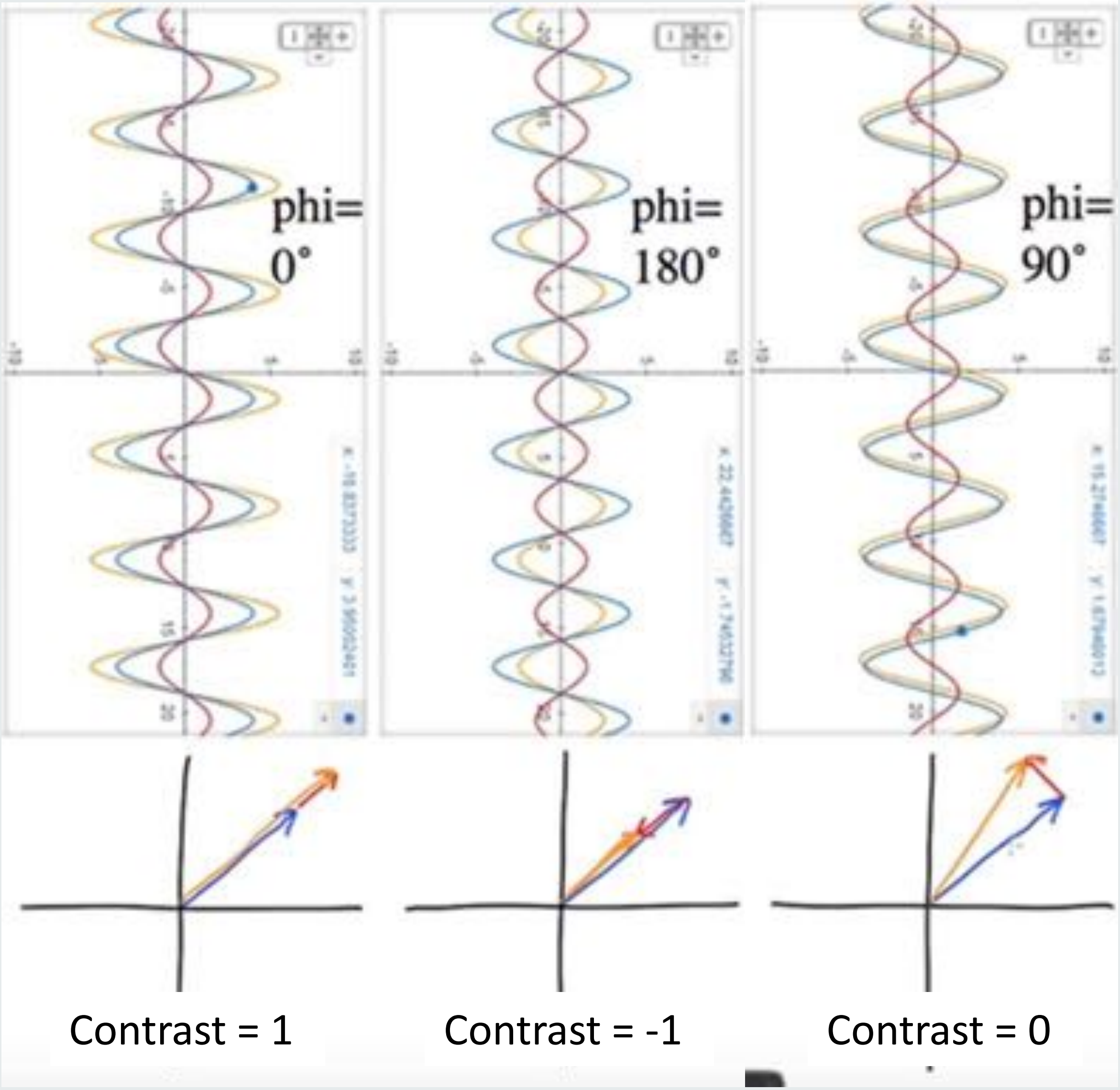


High angle scattering = high resolution info

Moderate angle scattering = moderate resolution info

Low angle scattering contains low spatial frequency Fourier components – this is information about the general shape and spacing of the molecules

Phase shift and interference



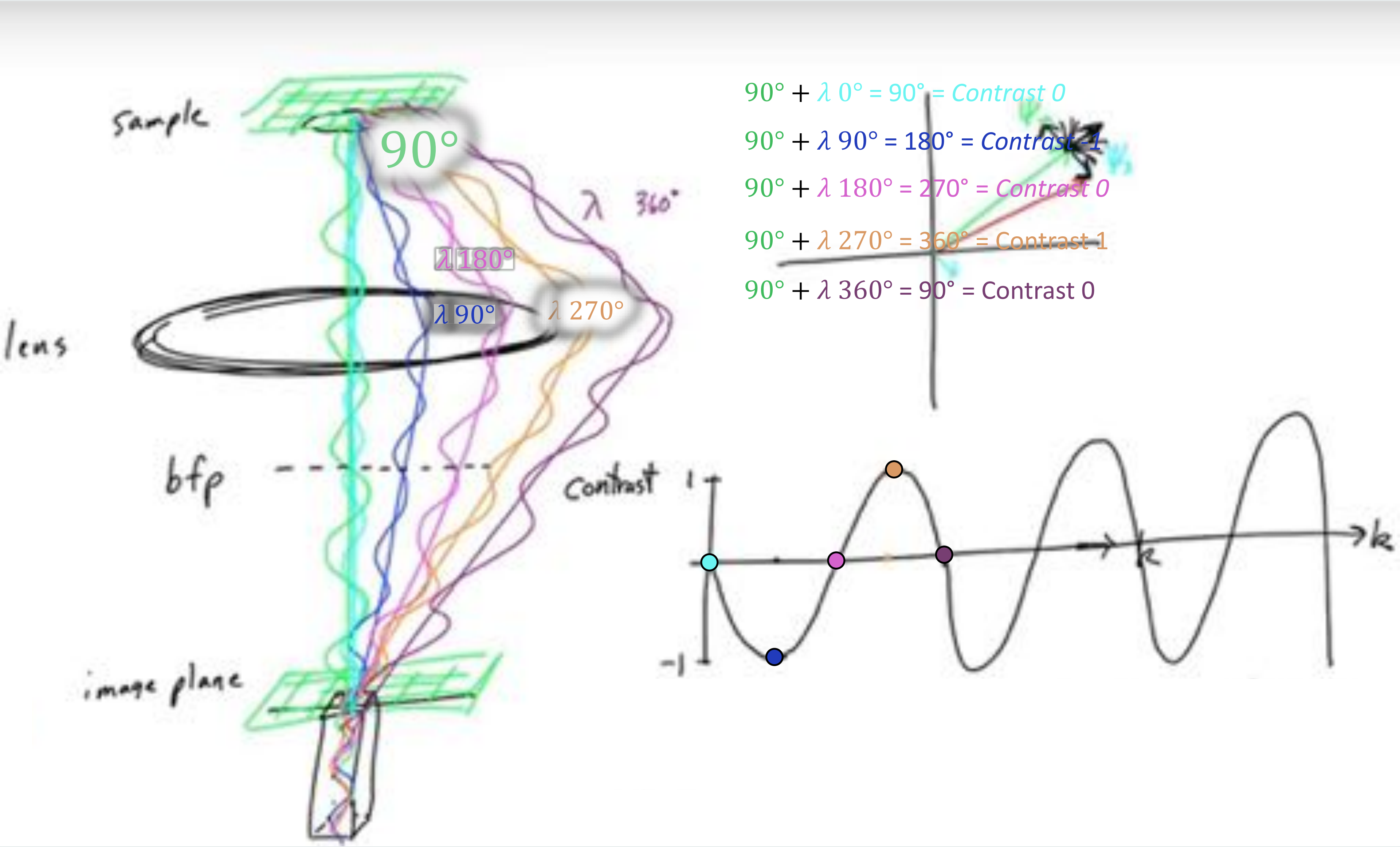
Unscattered wave is high amplitude

Scattered waves are low amplitude

Some will be completely lost!
(if they have a 90° phase shift relative to the unscattered wave)

Phase shift comes from scattering (90°) and from the extra distance traveled thanks to scattering (varies)

Contrast Transfer Function (CTF)



Contrast Transfer Function (CTF)

$$CTF(\nu) = \sin\left[\frac{2\pi}{\lambda}\left(\frac{Cs\cdot\lambda^4\cdot\nu^4}{4} - \frac{\Delta f\cdot\lambda^2\cdot\nu^2}{2}\right)\right]$$

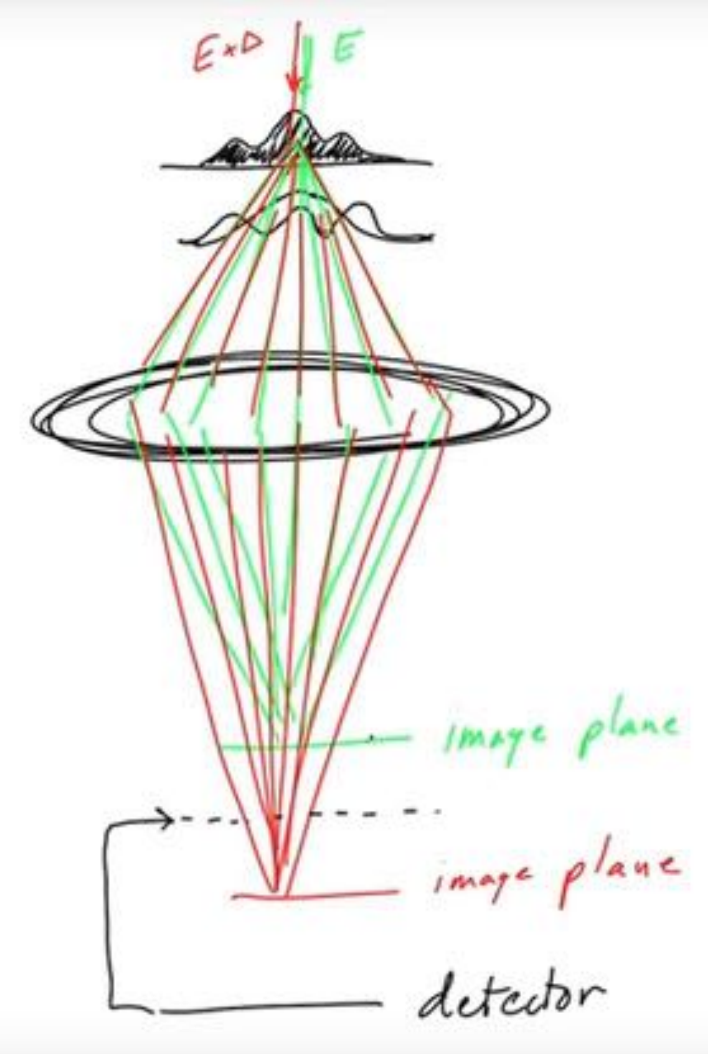
ν: Spatial frequency

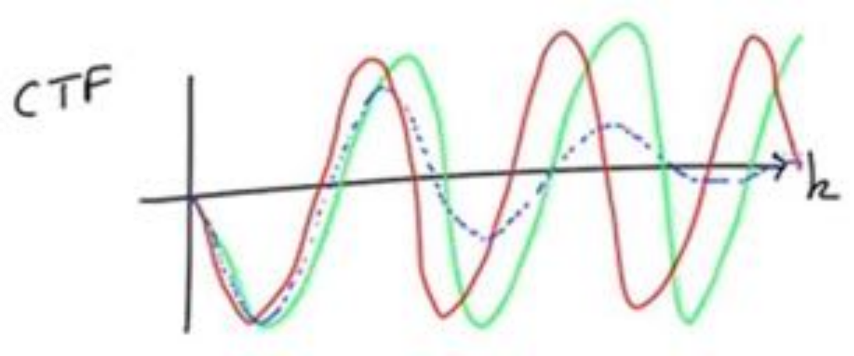
Cs: Spherical aberration coefficient

λ: Electron wavelength

Δf: Defocus

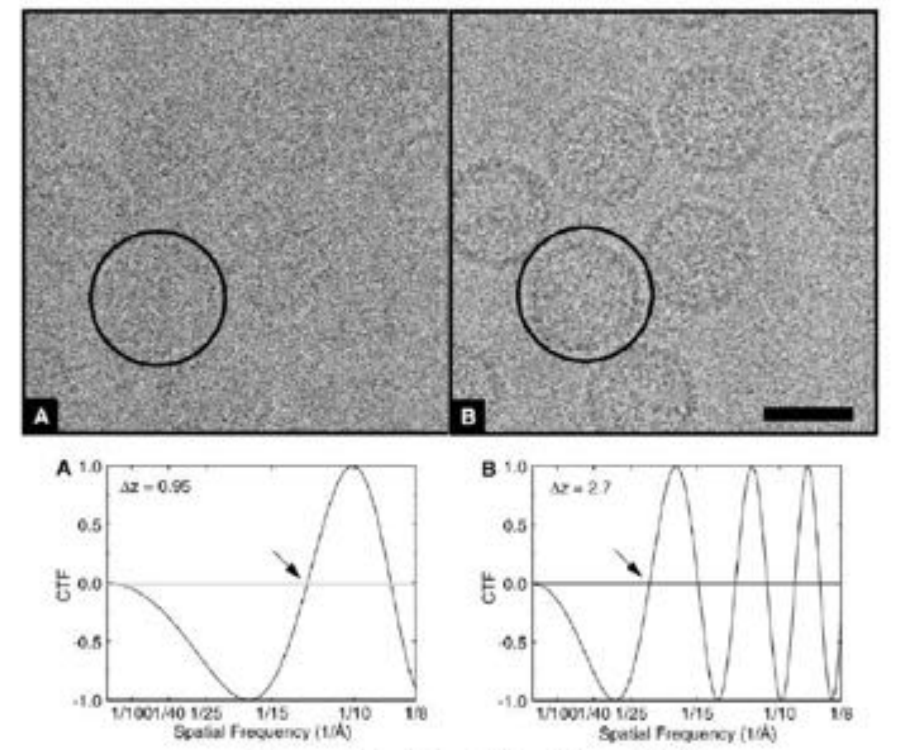
Envelopes – Temporal coherence



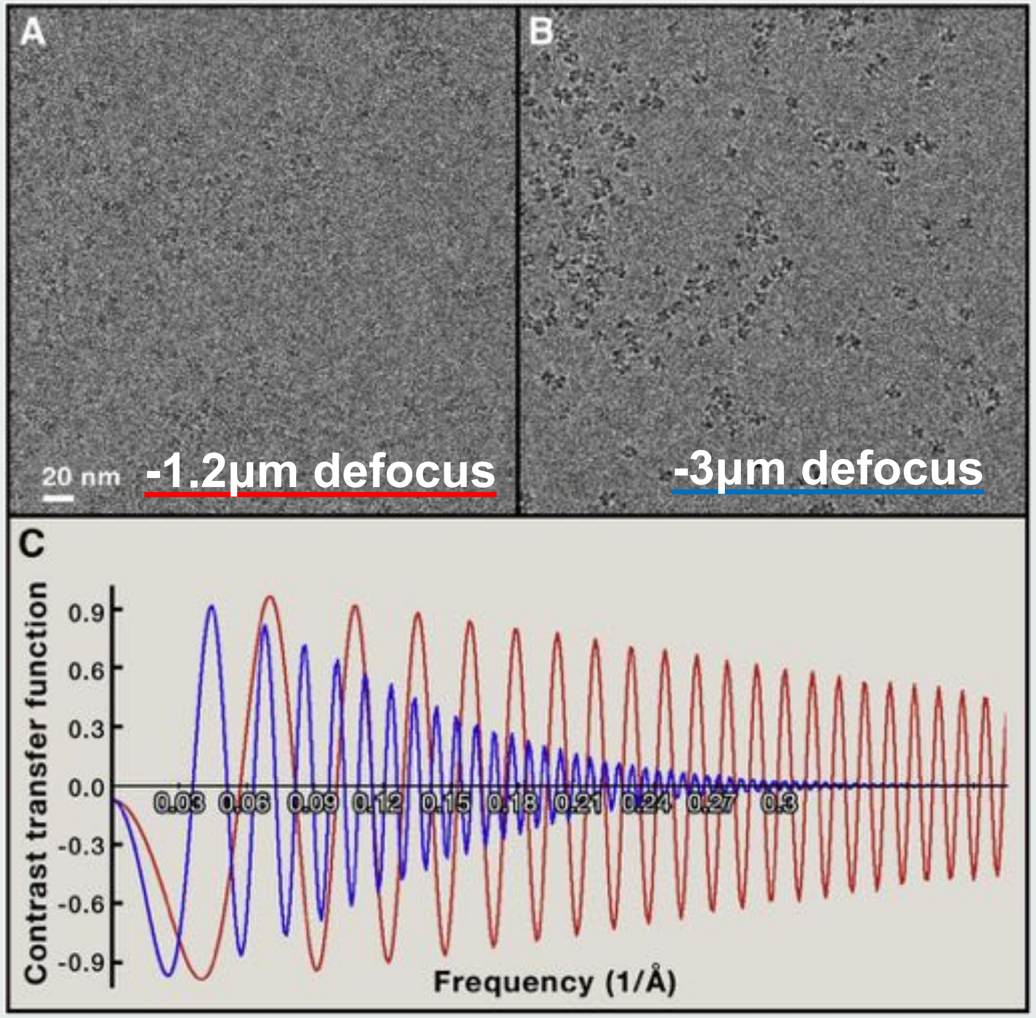


Many other factors (imperfect detectors, defocus itself, etc.) also attenuate high spatial frequencies

Defocus and its effects

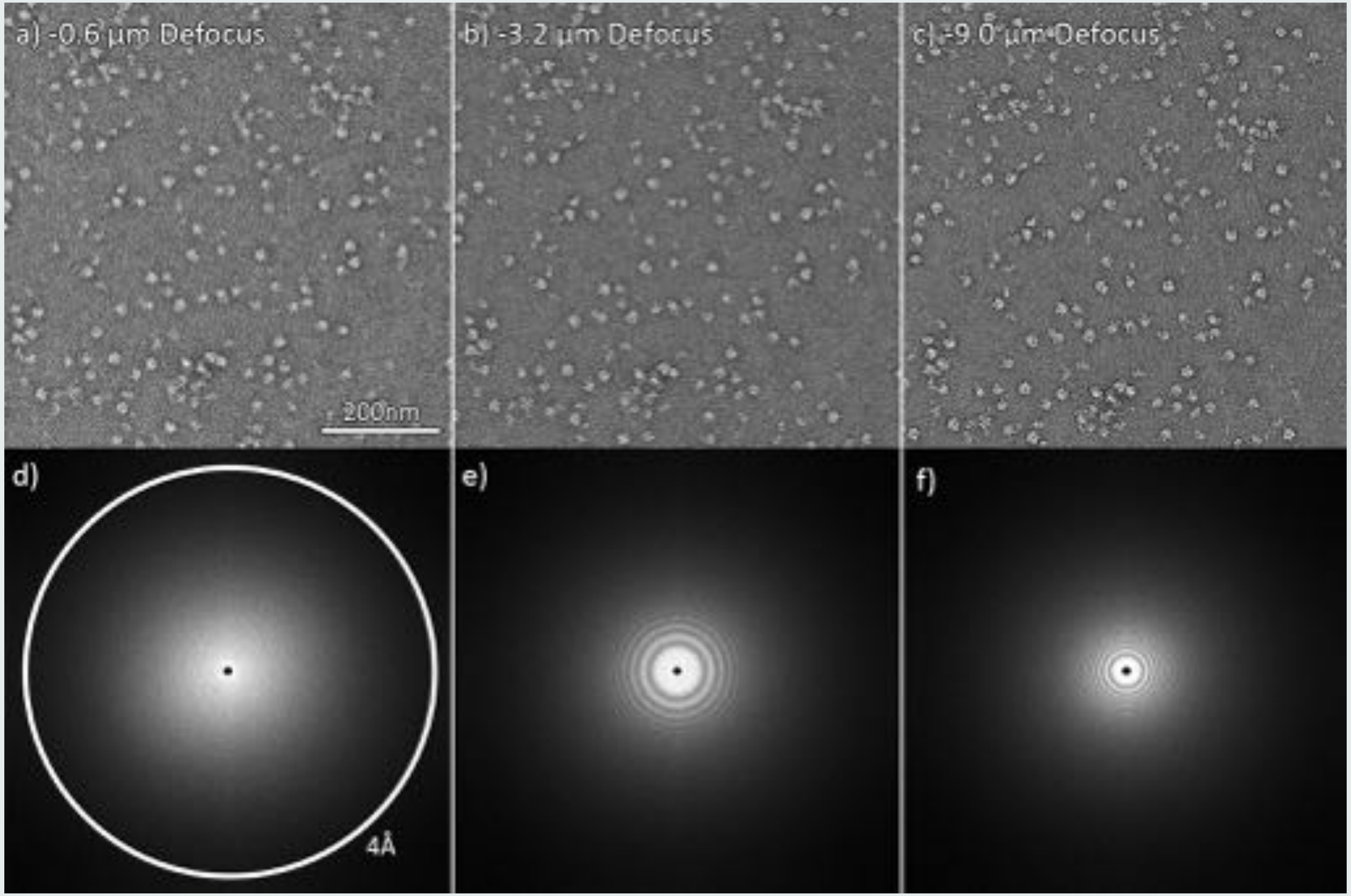


Thuman-Commike and Chiu, *Micron* **31**:687



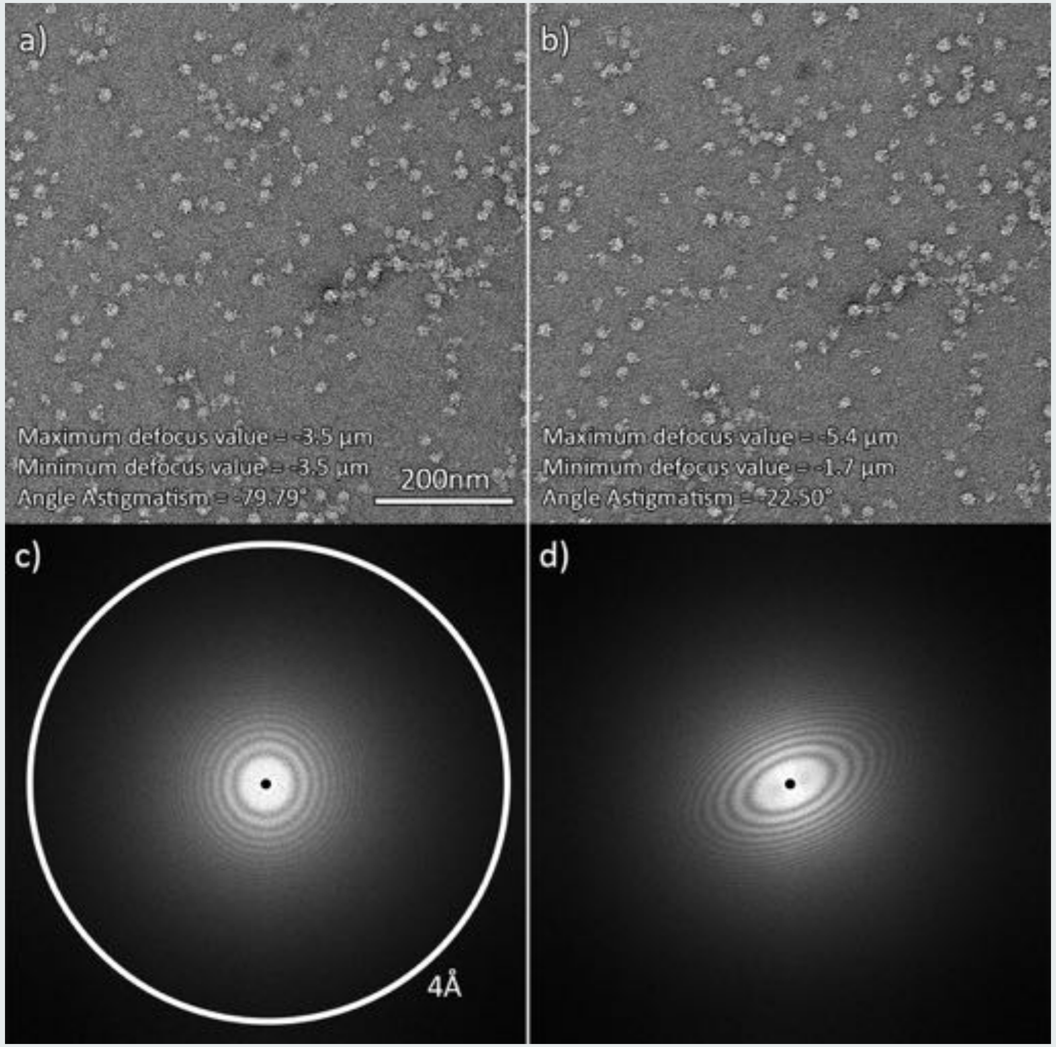
Cheng et al., *Cell* 2015

Defocus and its effects



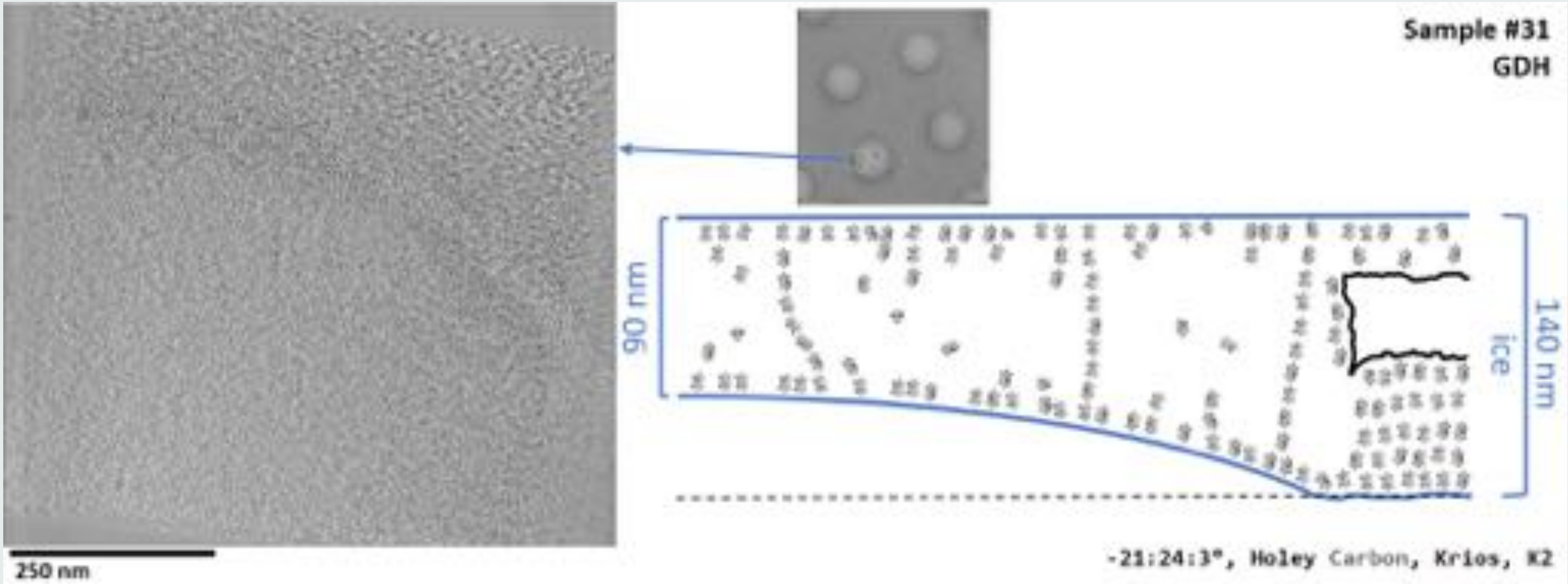
Tan et al., *Journal of Electron Microscopy* 2015

Defocus is not always the same in X and Y



Tan et al., *Journal of Electron Microscopy* 2015

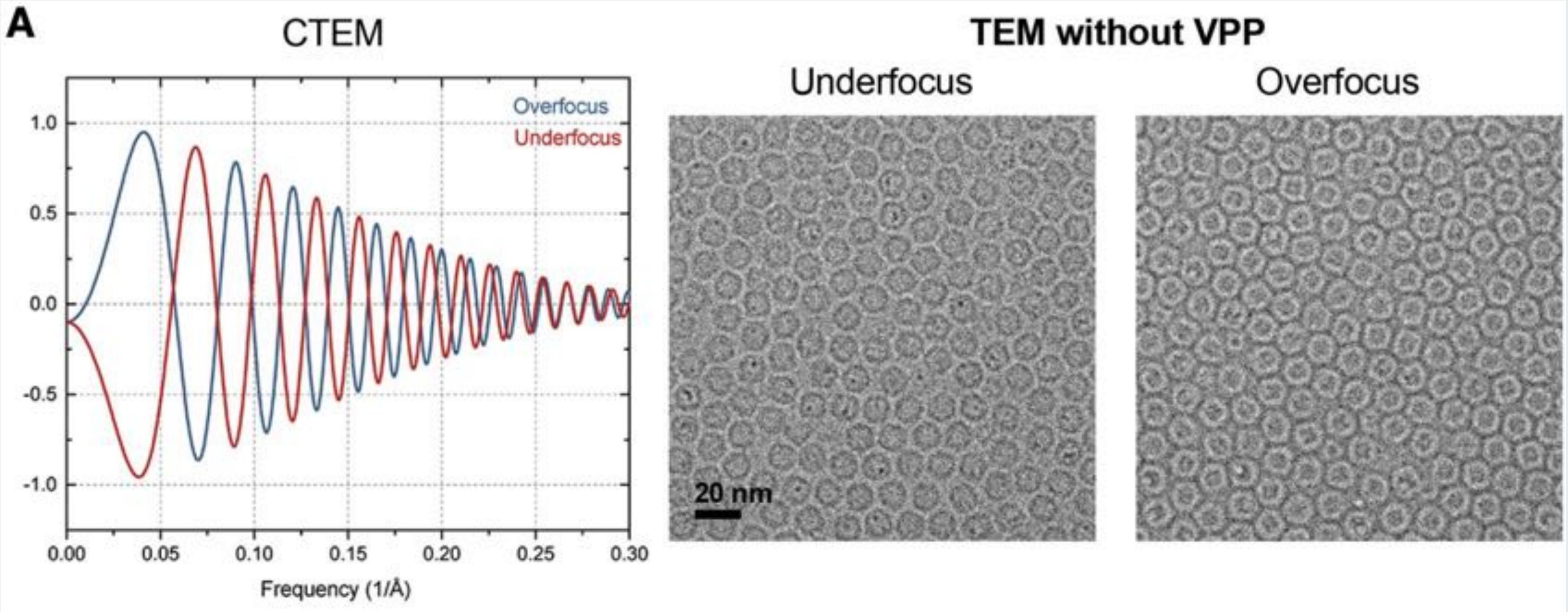
The defocus of each particle is different



Nobel et al., *Journal of Electron Microscopy* 2015

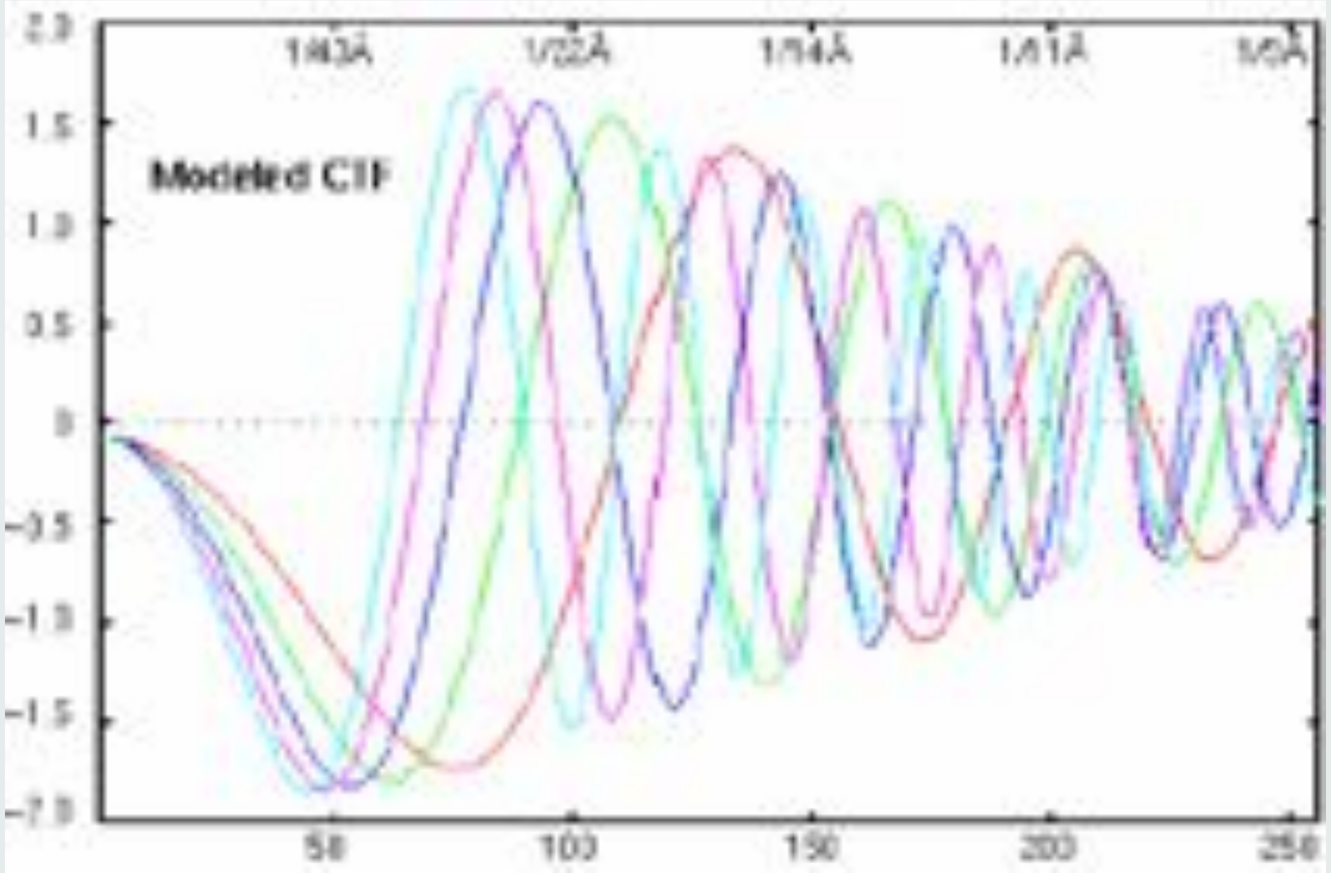
Why defocused?

$$CTF(\nu) = \sin\left[\frac{2\pi}{\lambda}\left(\frac{Cs \cdot \lambda^4 \cdot \nu^4}{4} - \frac{\Delta f \cdot \lambda^2 \cdot \nu^2}{2}\right)\right]$$



Fan et al., *Structure* 2017

Collect a range of defocus values to fill in zeros



Willy Wriggers, Image Formation in the Electron Microscope

CTF Correction

$$I = O \otimes PSF$$

$$FT\{PSF\} = CTF$$

$$FT\{I\} = FT\{O \otimes PSF\}$$

$$FT\{I\} = FT\{O\}\ x\ CTF$$

$$FT\{O\} = \frac{FT\{I\}}{CTF}$$

$$O = FT^{-1}\left\{\frac{FT\{I\}}{CTF}\right\}$$

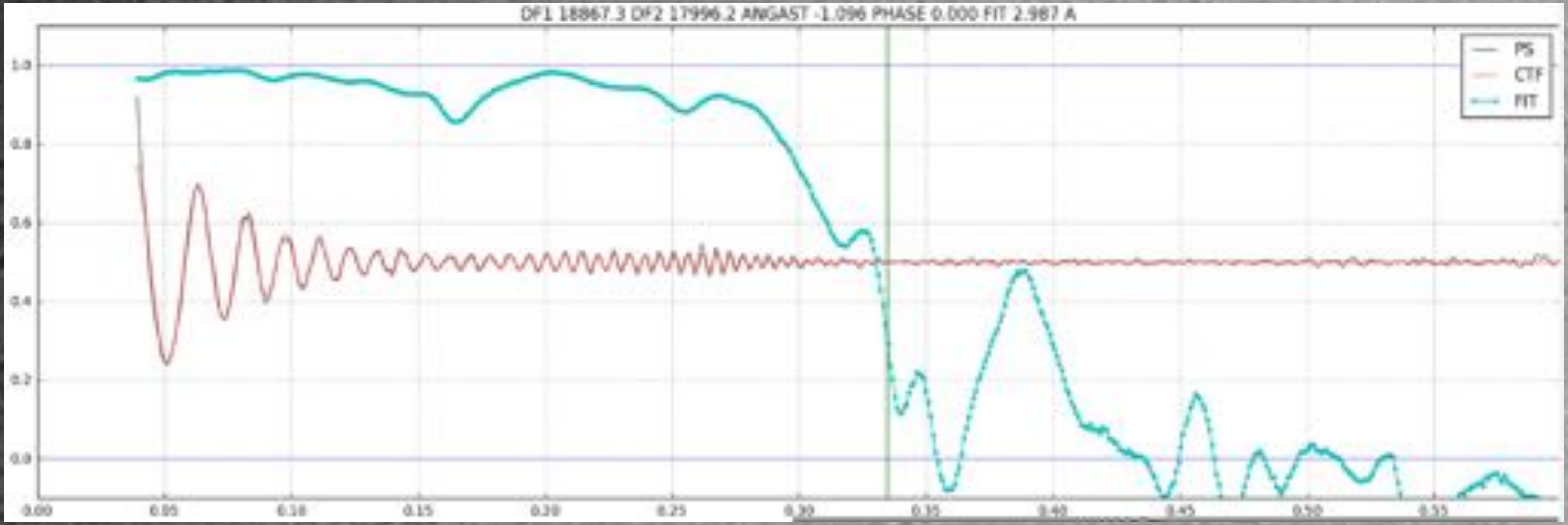
Can't divide by zero!
Those spatial
frequencies are lost

CTF Correction

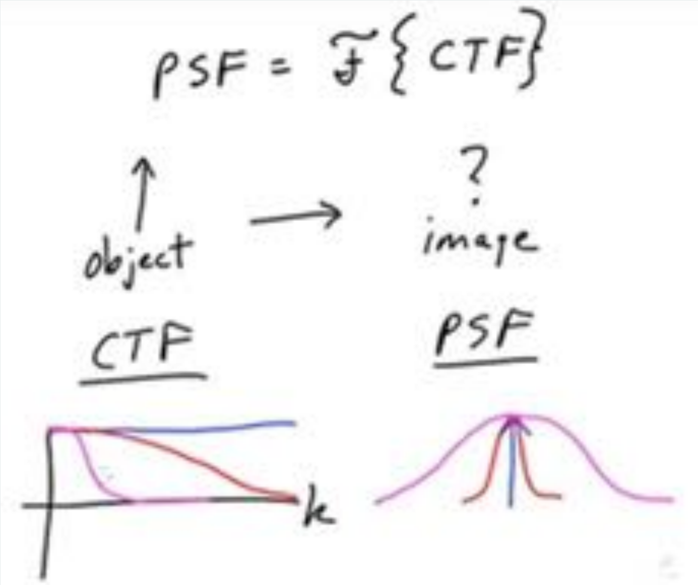
$$O = FT^{-1}\left\{\frac{FT\{I\}}{CTF}\right\}$$

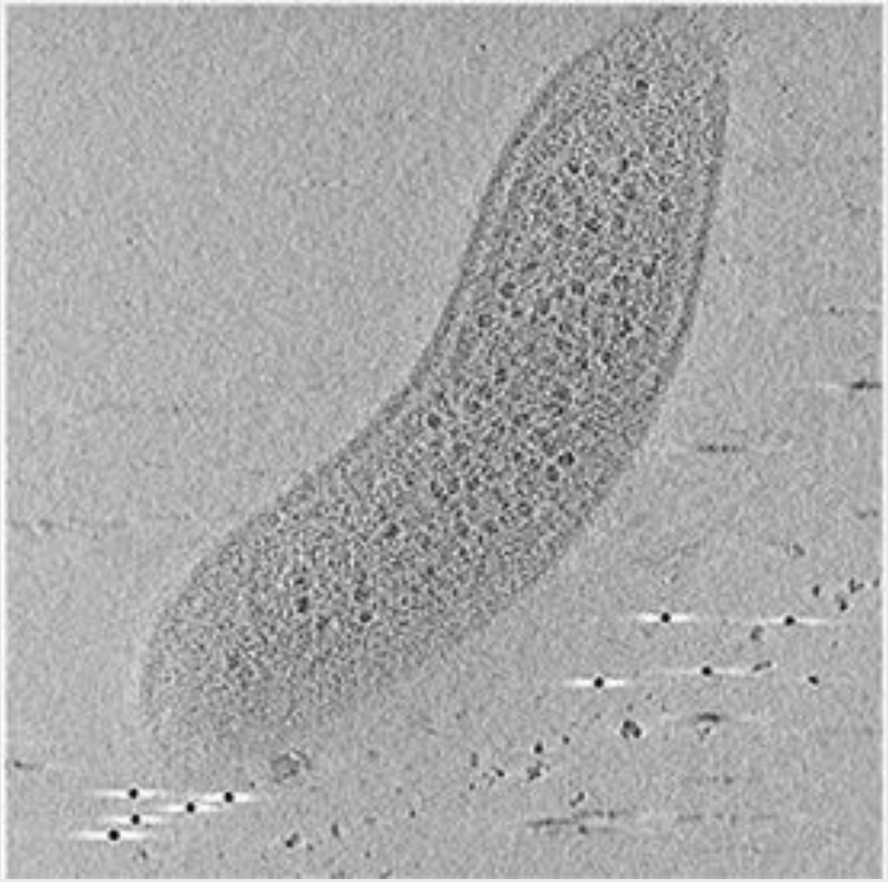
Object	CTF	FT(I)	CTF-Corrected
10	-0.2	-2	-2 / -0.2 = 10
5	0.3	1.5	1.5 / 0.3 = 5
2	-0.3	-0.6	-0.6 / -0.3 = 2

CTF Estimation



Information is delocalized thanks to PSF





Clipping the PSF (cropping your particles too tight in real space) removes CTF information!
At 300 keV & 1.5 µm defocus, a 1Å pixel spreads over 256x256 pixels
Delocalization increases with defocus – box size should take defocus into account
Rule of thumb : max_defocus[Å] = 25 * box_size[Å] * resolution[Å]

Any questions?

Twitter
@RadoDanev

@Marin_van_Heel

?