Phase-contrast imaging in the EM

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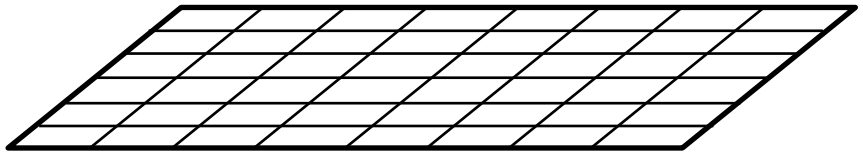
Outline

- Amplitude and Phase Contrast
- The Contrast Transfer Function
- Defocus and its Effects
- Envelopes
- 2D CTF
- Aberration
- CTF Estimation

1 Amplitude and Phase Contrast

Plane Wave

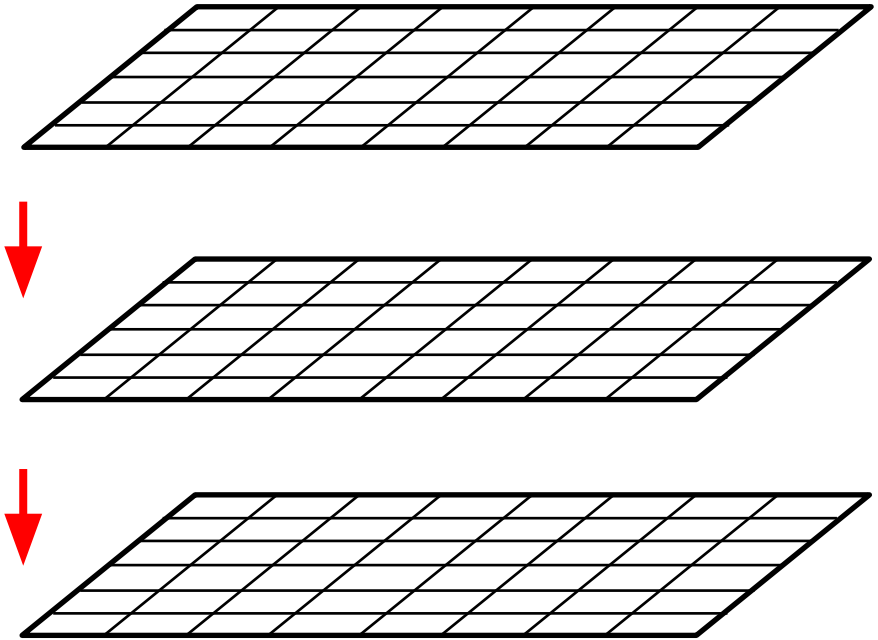
Thinking the Electron as a wave, instead of thinking it as a particle



Amplitude = 1
Phase = 0°

Travel through the vacuum

Plane Wave

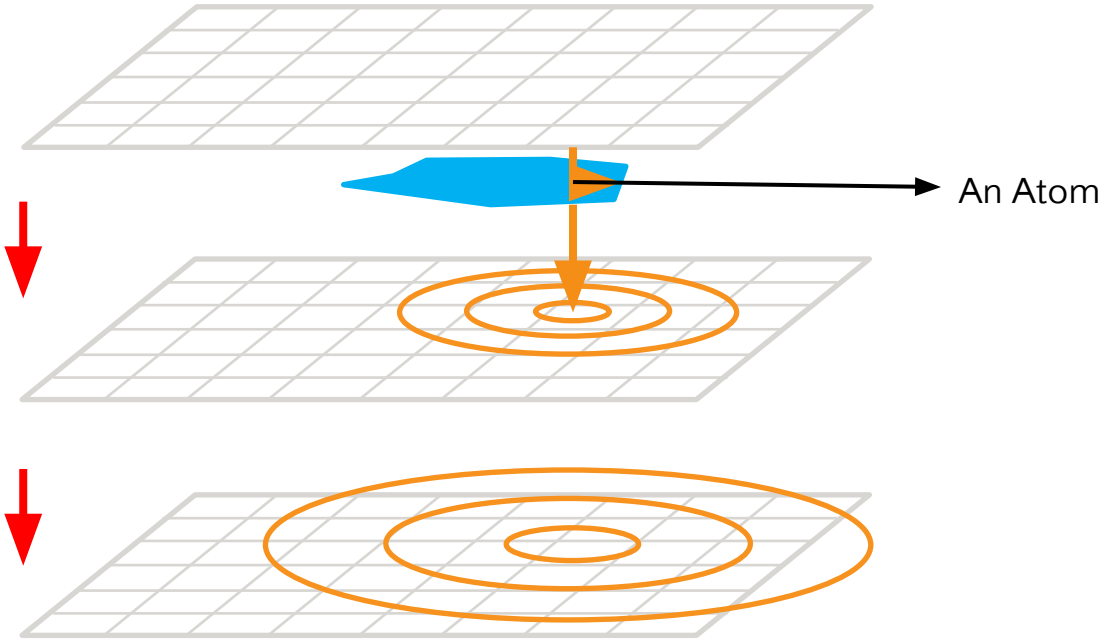


$A = 1$
$\theta = 0°$

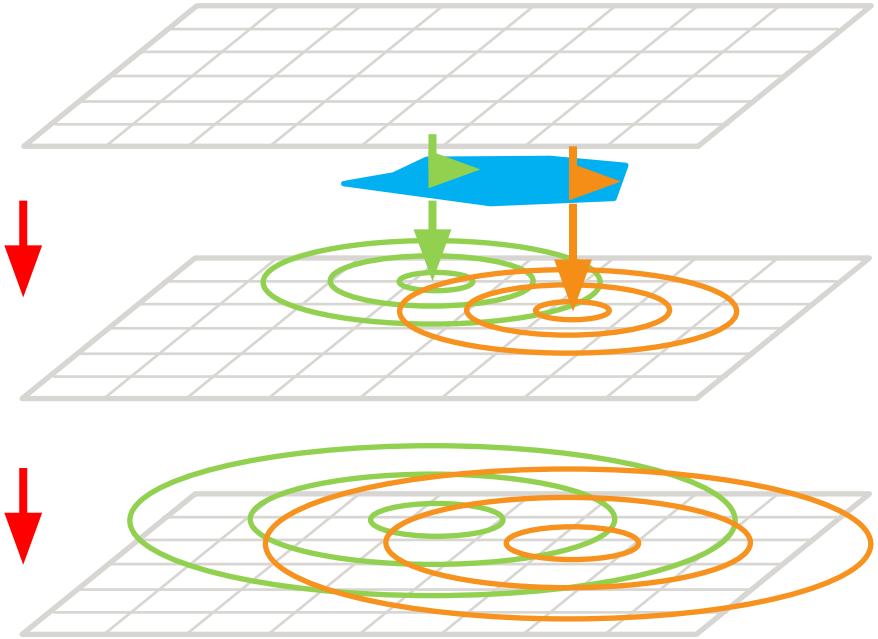
$A = 1$
$\theta = 90°$

$A = 1$
$\theta = 180°$

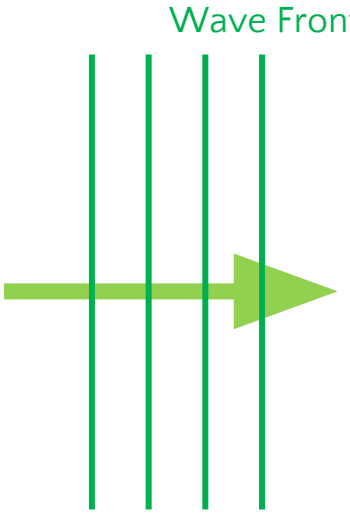
Scattering

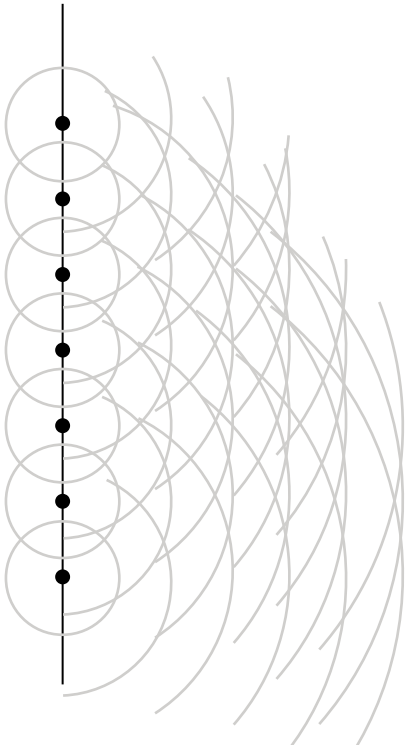


Scattering

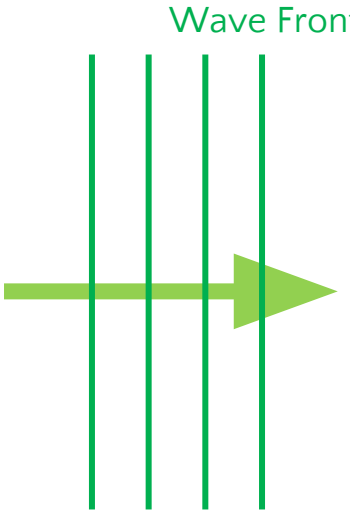


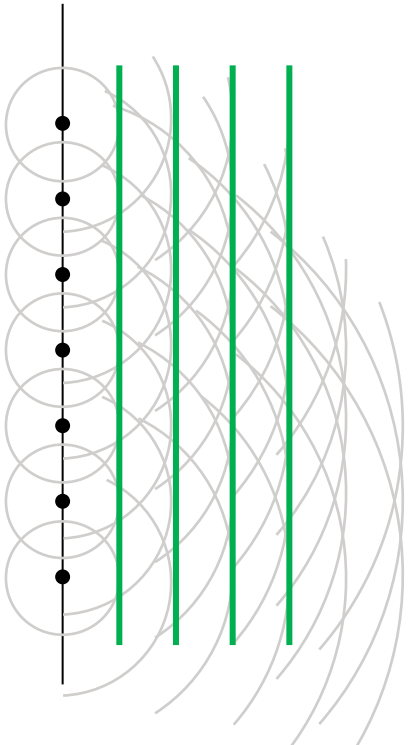
Interference



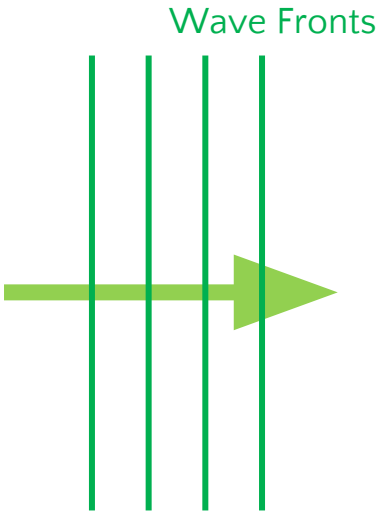


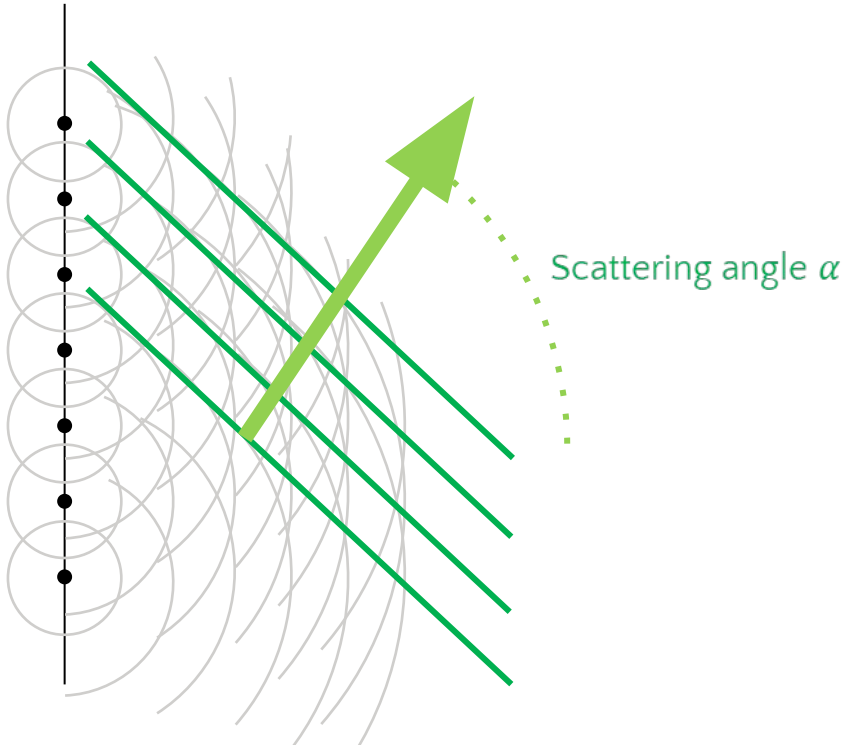
Interference



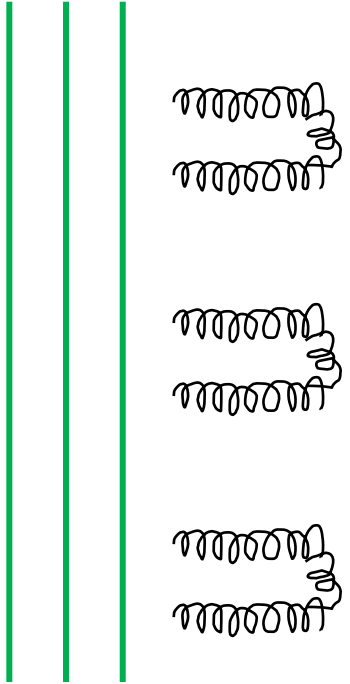


Interference

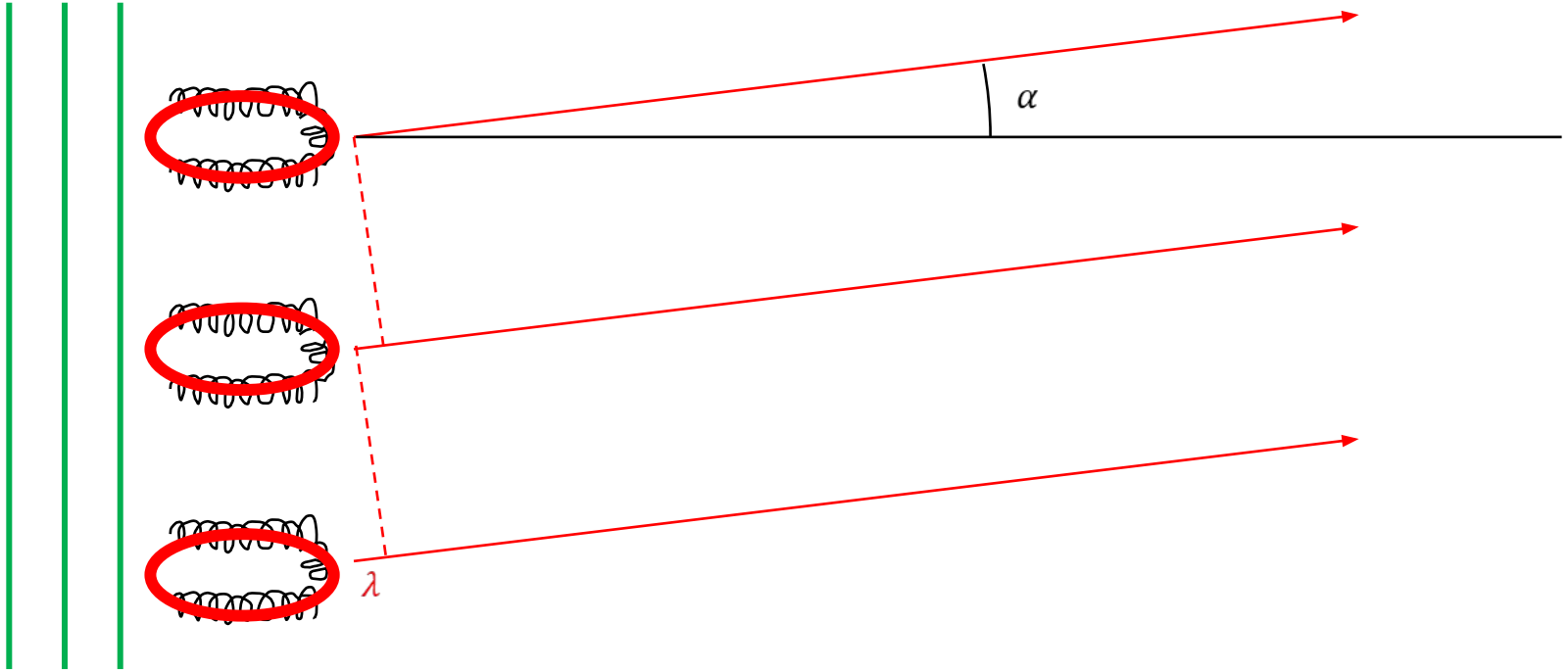




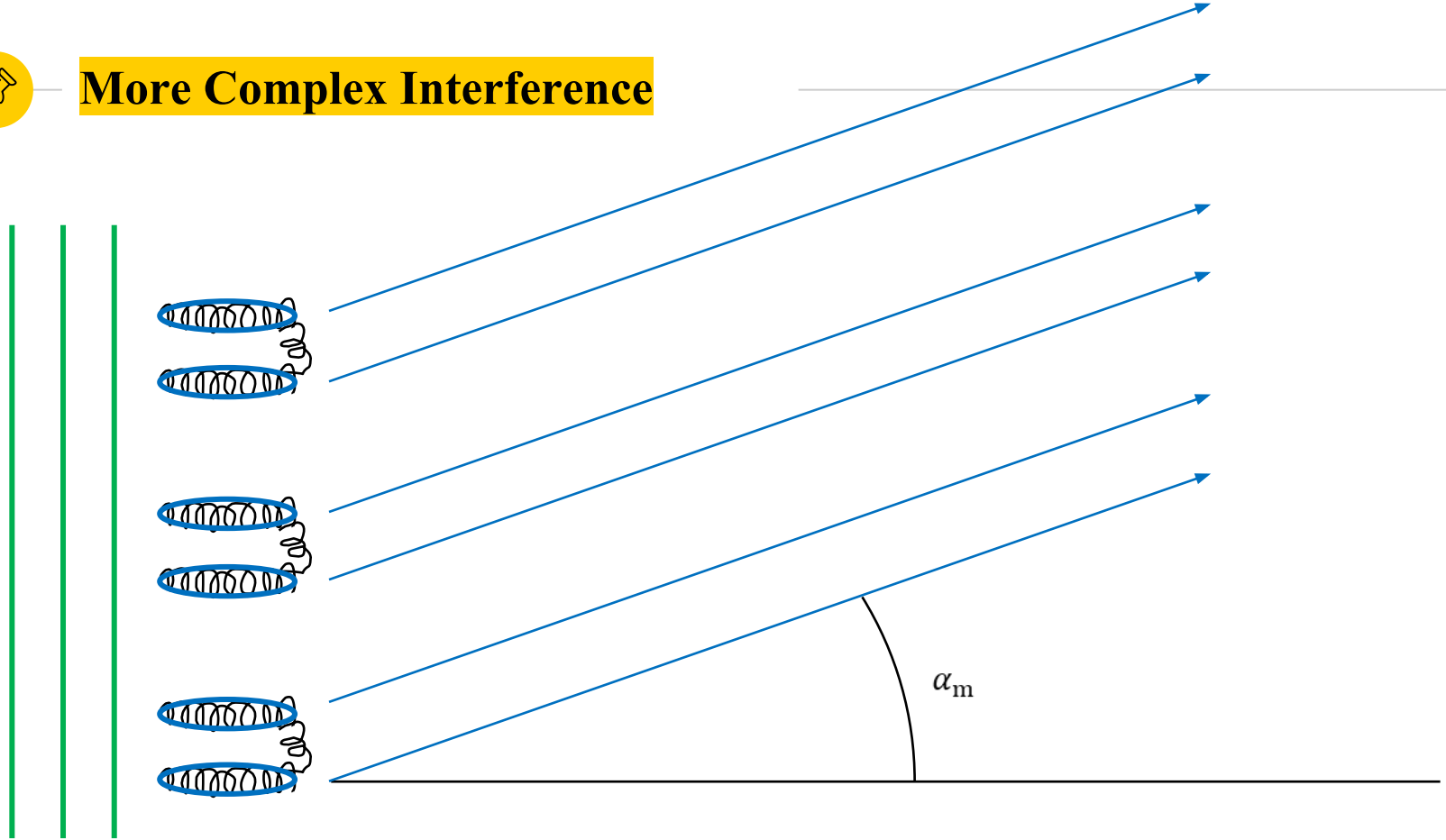
More Complex Interference



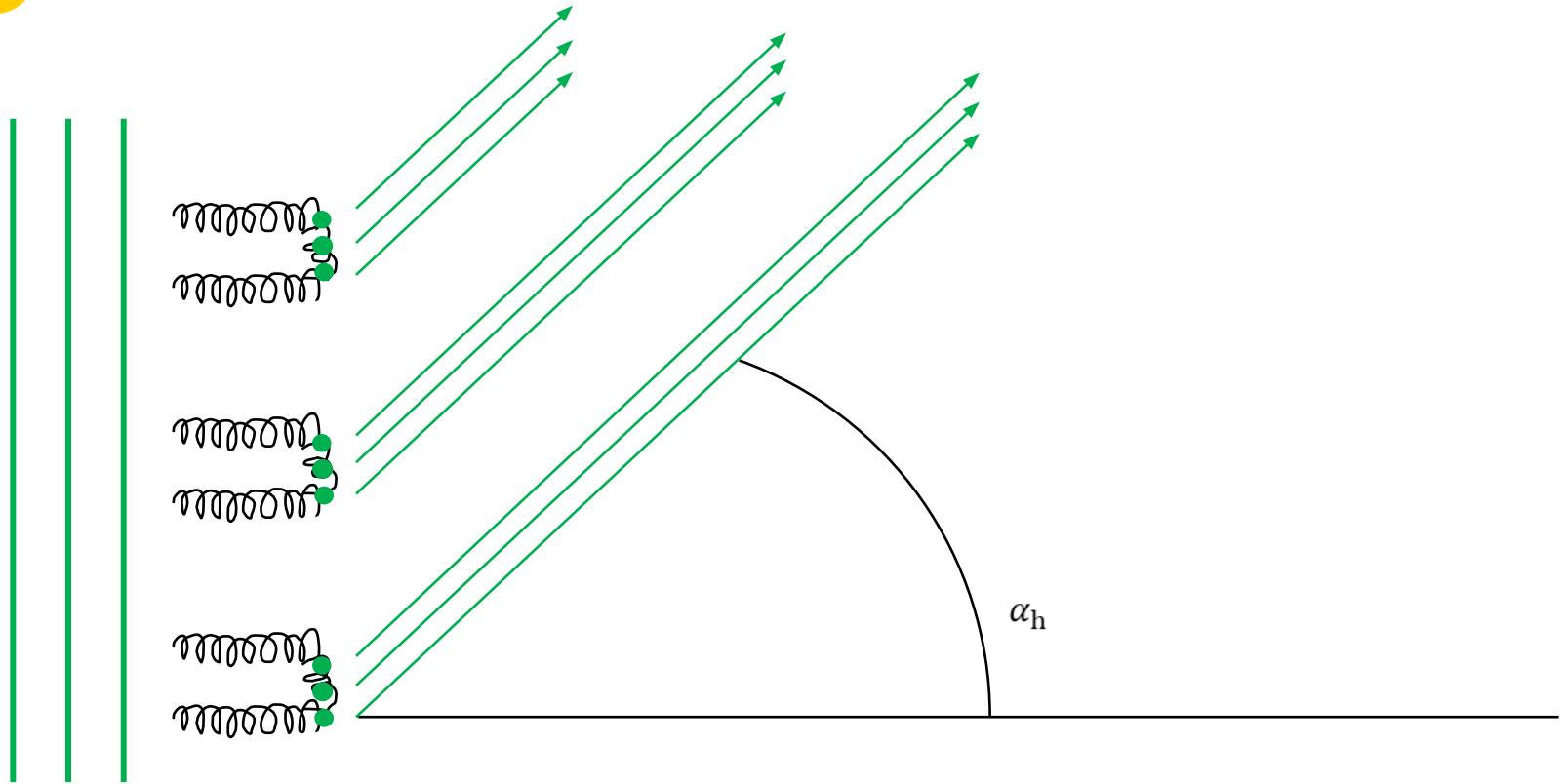
More Complex Interference



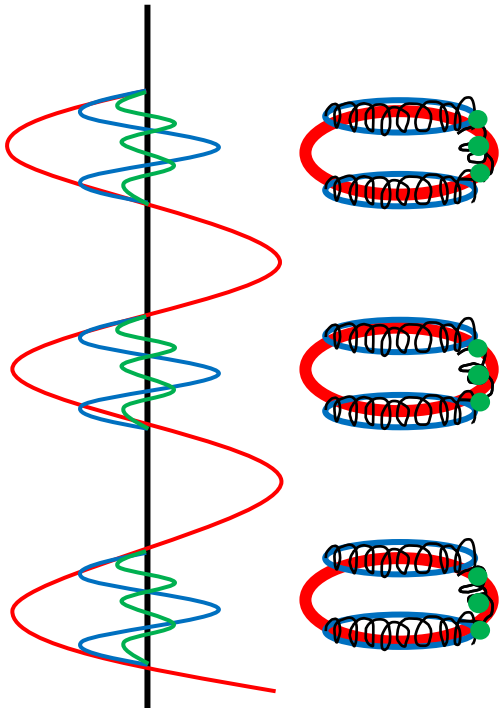
More Complex Interference

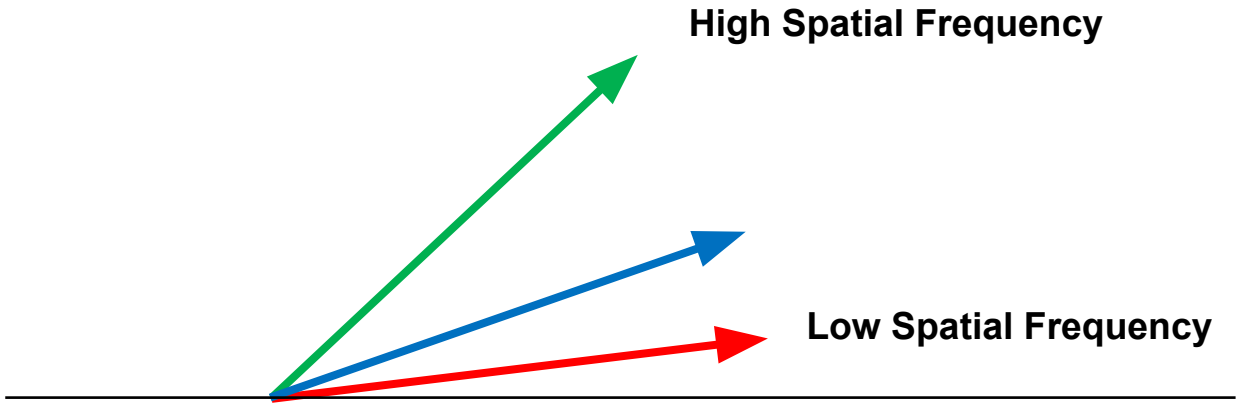


More Complex Interference



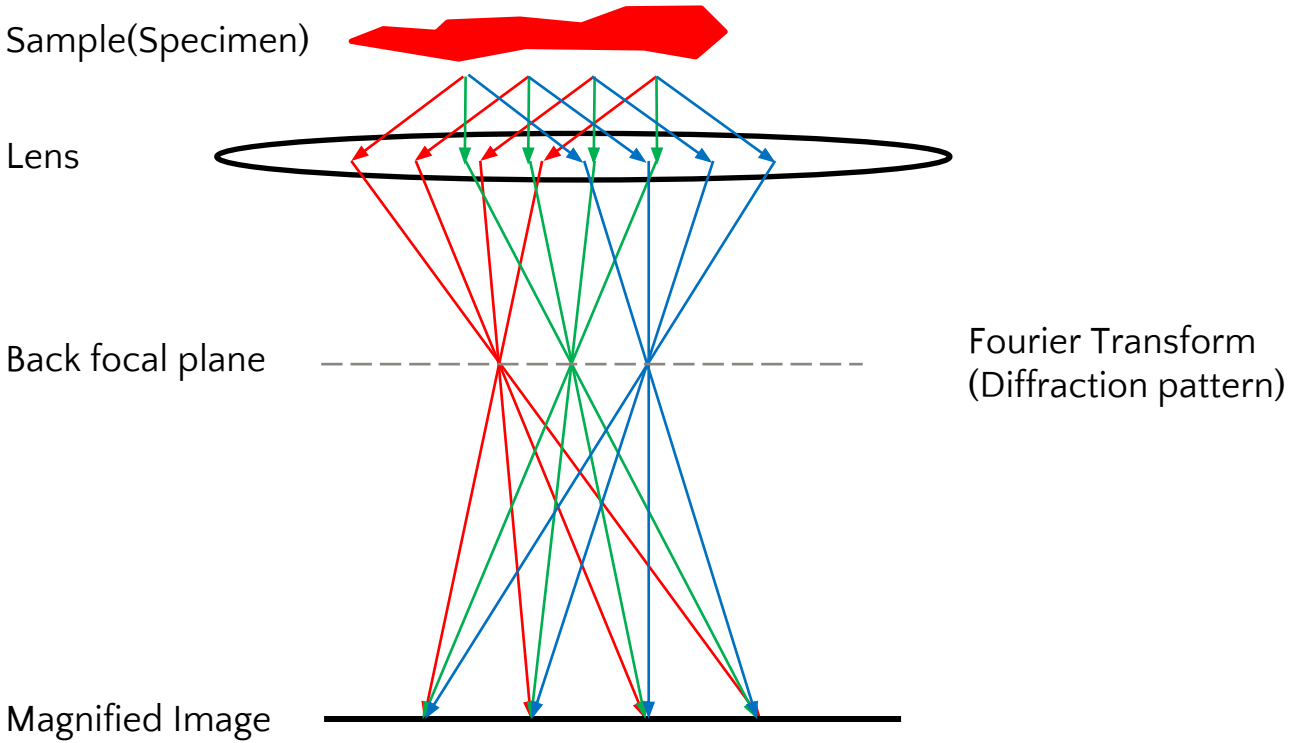
More Complex Interference



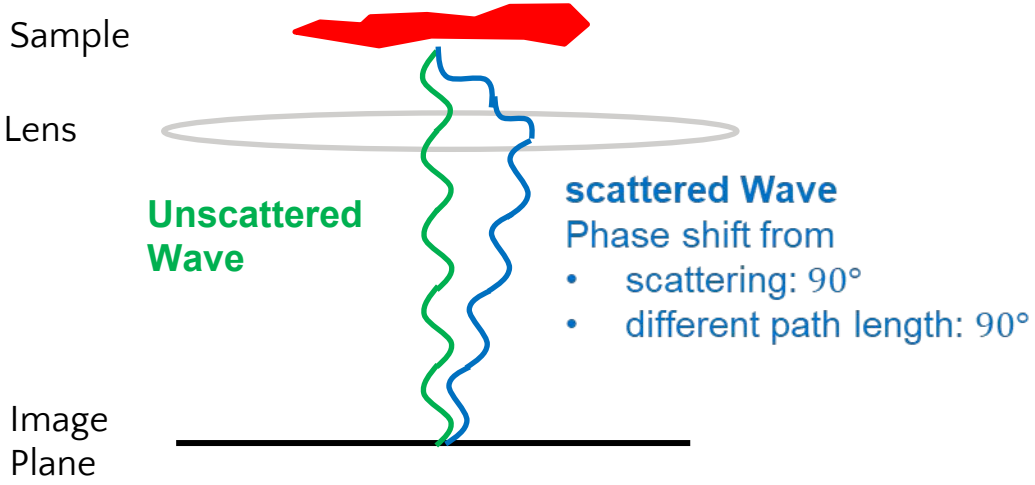


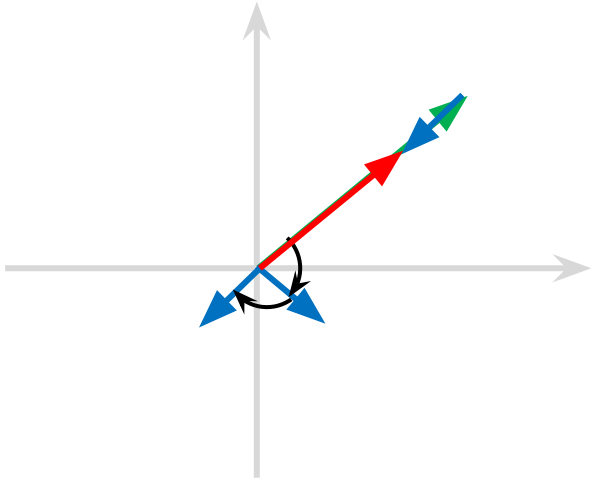
2 The Contrast Transfer Function

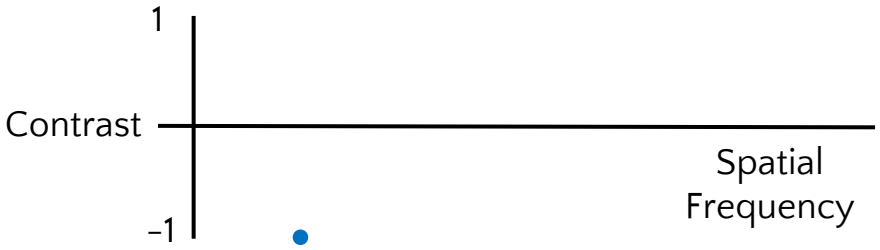
Structure of Cryo-EM



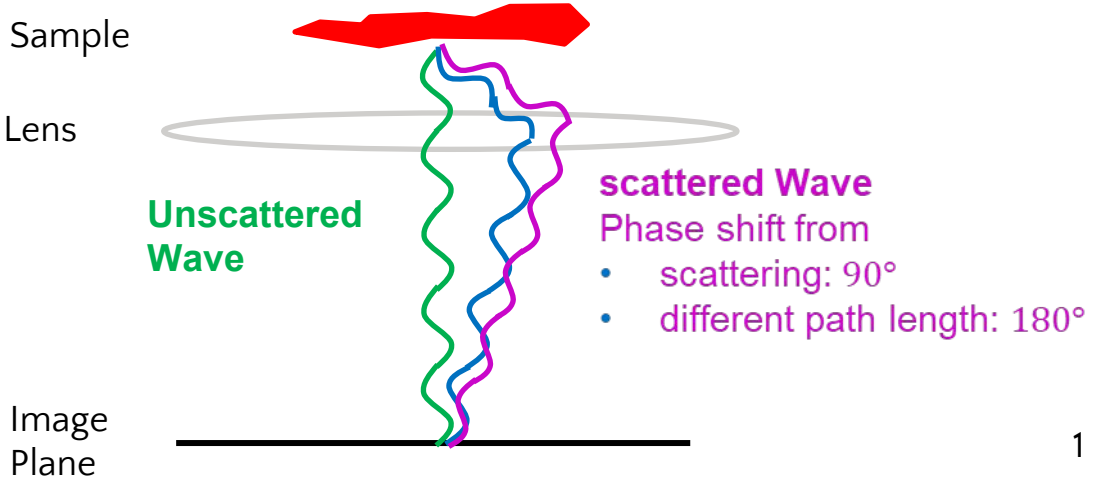
The Contrast Transfer Function

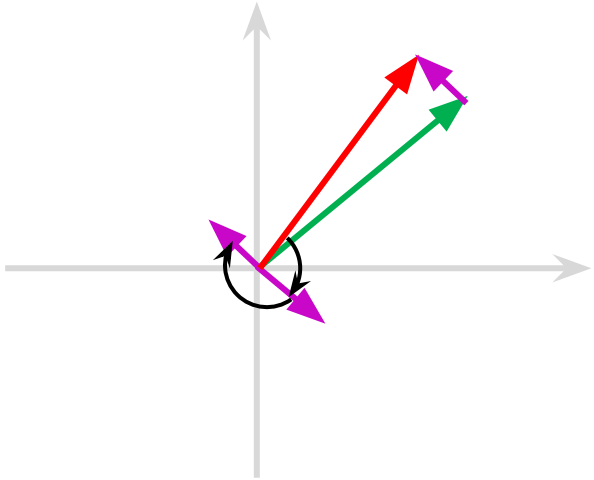


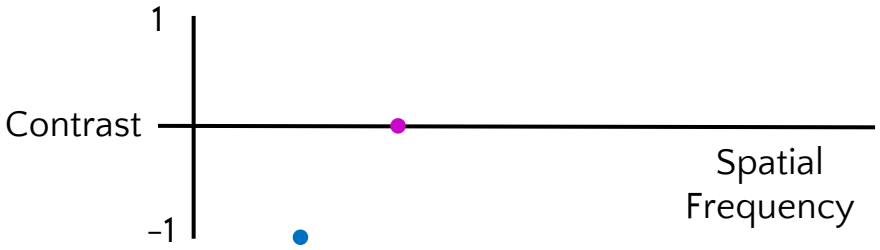




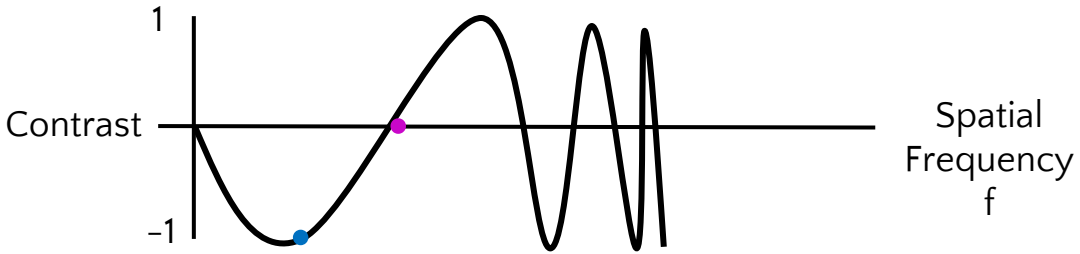
The Contrast Transfer Function







The Contrast Transfer Function



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

λ: wave length

Δz: defocus

f: spatial frequency (1/d)

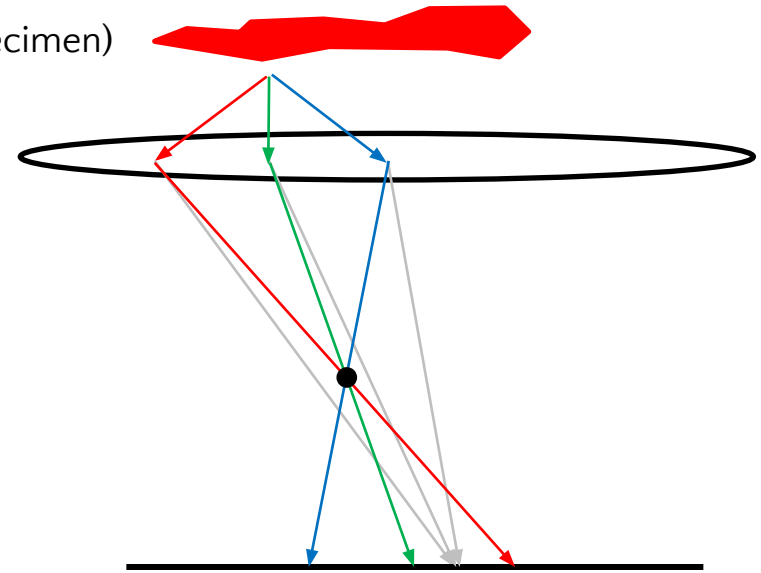
C_s: spherical aberration coefficient

3 Defocus and its Effects

Defocus and Overfocus

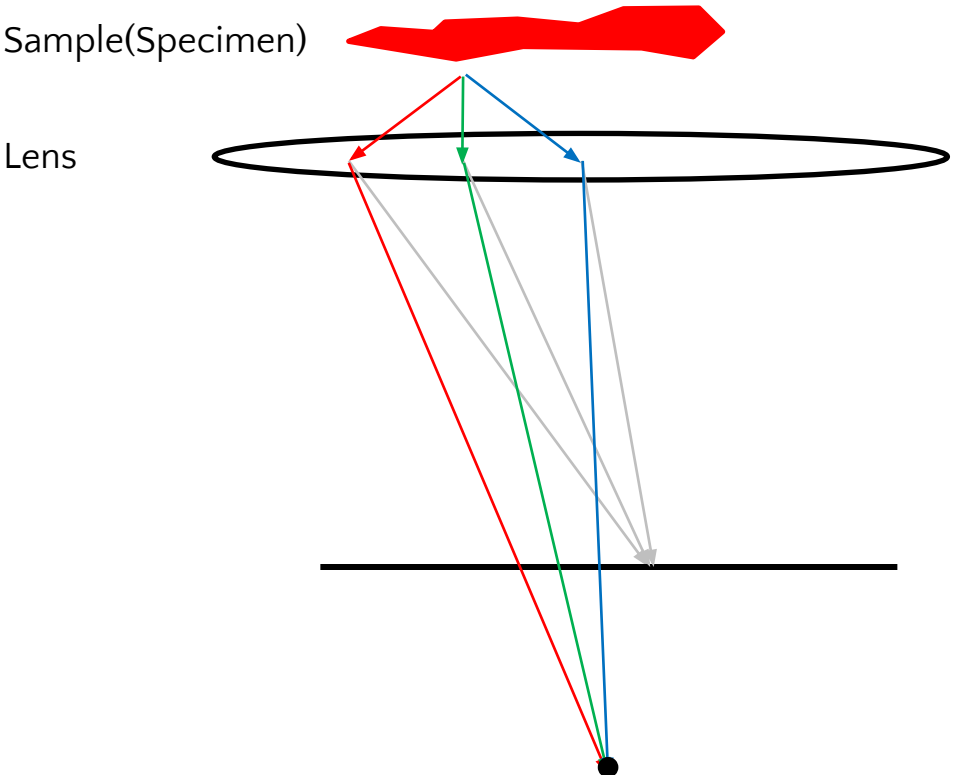
Sample(Specimen)

Lens



Overfocus Image

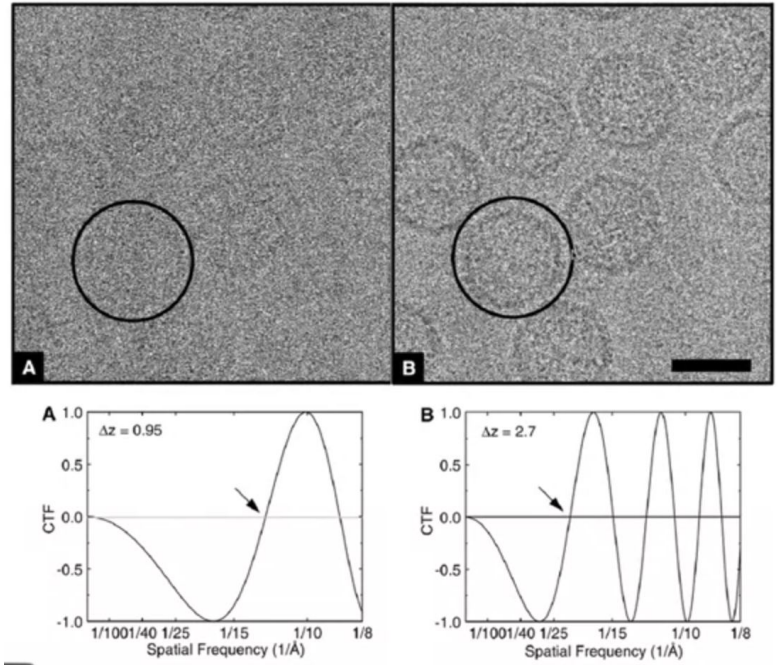
Defocus and Overfocus



Defocus Image

Defocus Effect

Weak low-resolution features
Strong high-resolution features

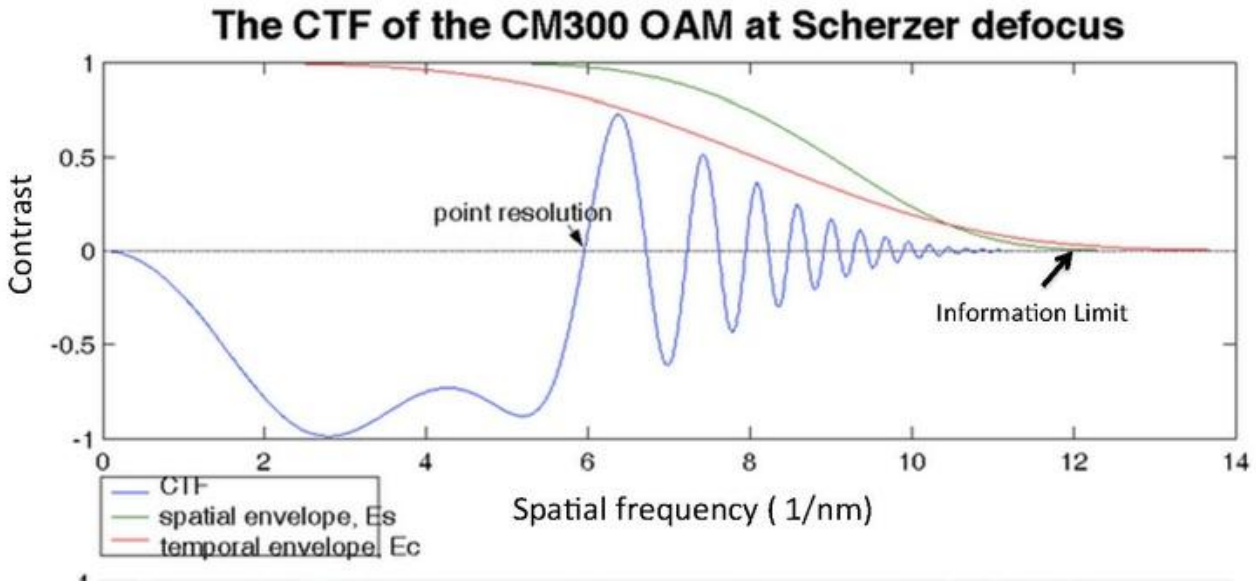


Strong low-resolution features
Weak high-resolution features

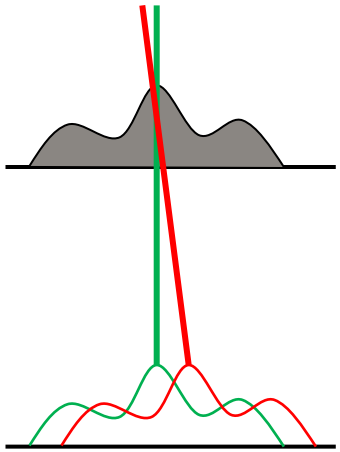
Figure from Thuman-Commike and Chiu, *Micron* **31**:687

4 Envelopes

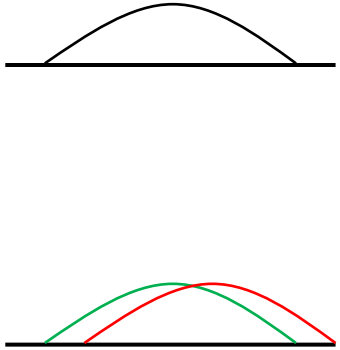
Envelope Function of CTF



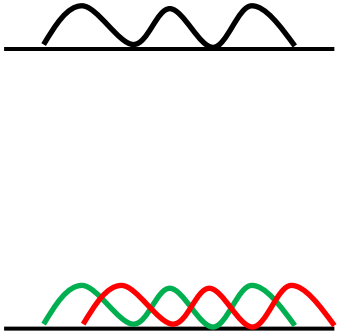
Envelope Function Caused by Different Direction



Low Frequency

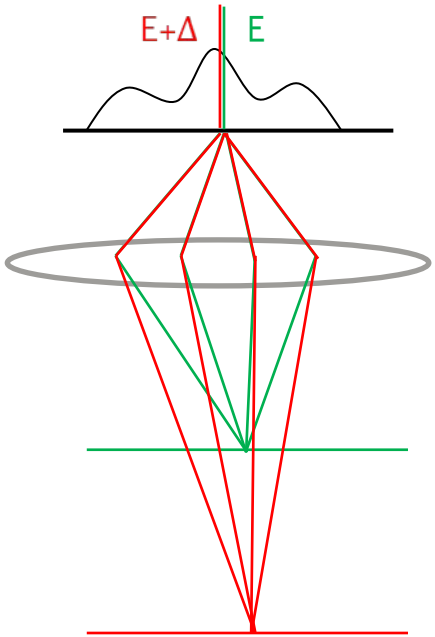


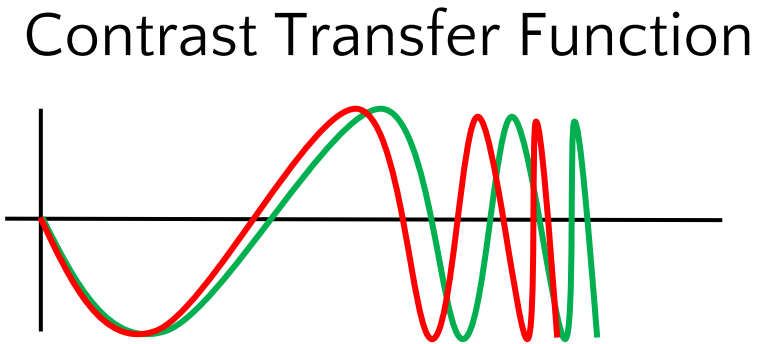
High Frequency



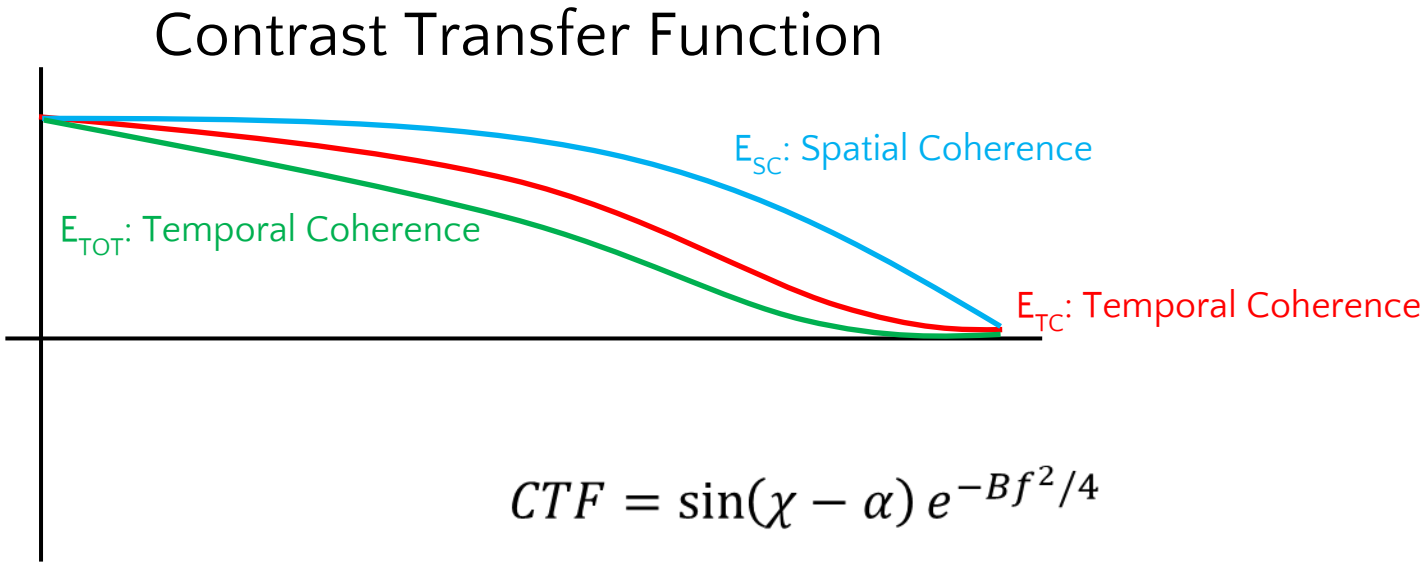
Blurred!

Envelope Function Caused by Different Energy





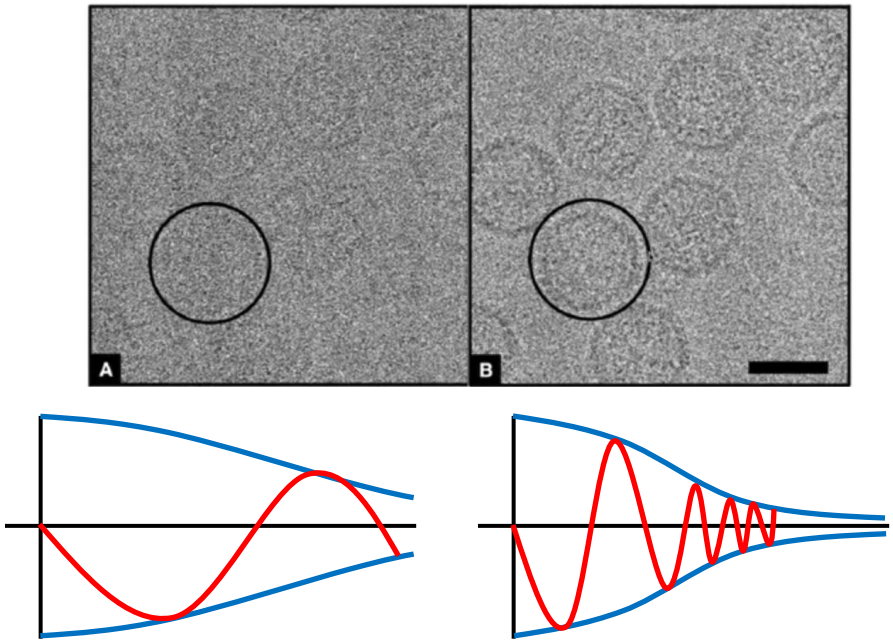
Envelope Function Caused by Different Energy



$$CTF = \sin(\chi - \alpha)\, e^{-Bf^2/4}$$

Envelope Example

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



5 2D CTF

2D CTF

CTF can be formulated as follow:

$$CTF = -w_1 \sin(\chi_\phi(g)) - w_2 \cos(\chi_\phi(g))$$

Or

Called Weak Phase Approximation

$$CTF = -\sin\left(\chi_\phi(g)\right)$$

$$\chi_\phi(g) = \pi\lambda|g|^2\Delta f - \frac{\pi}{2}\lambda^3|g|^4 C_s + \Delta\varphi \ ,$$

$$\Delta f = \frac{1}{2}(\Delta f_1 + \Delta f_2 + (\Delta f_1 - \Delta f_2)\cos(2(\alpha - \alpha_{ast})))$$

Where w_2 depends on the specimen characteristics and microscope properties

2D CTF

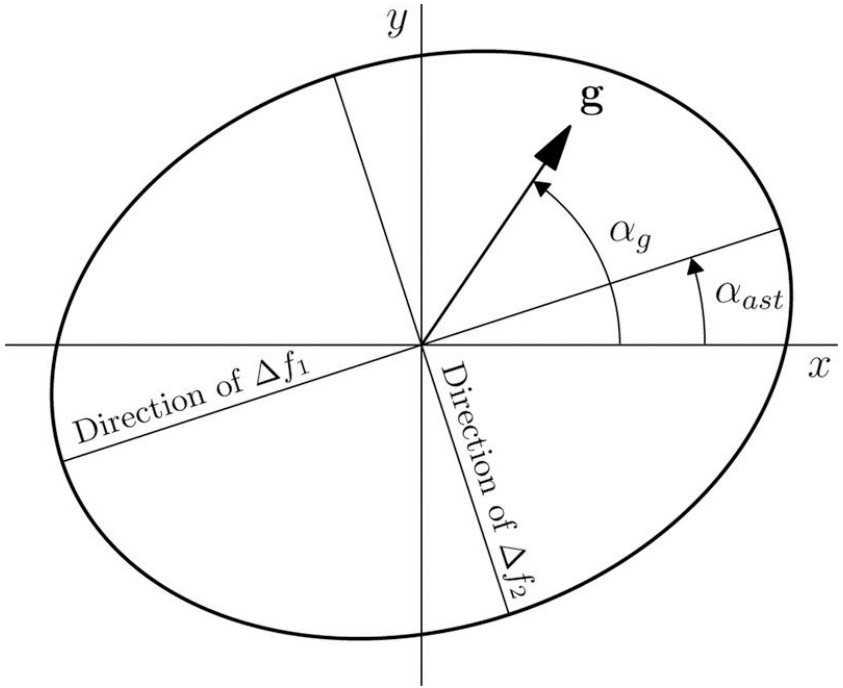
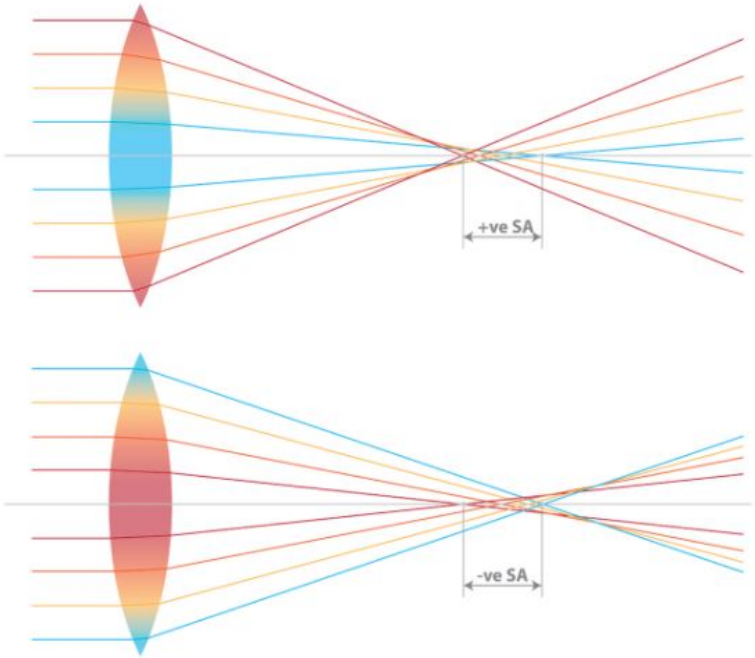


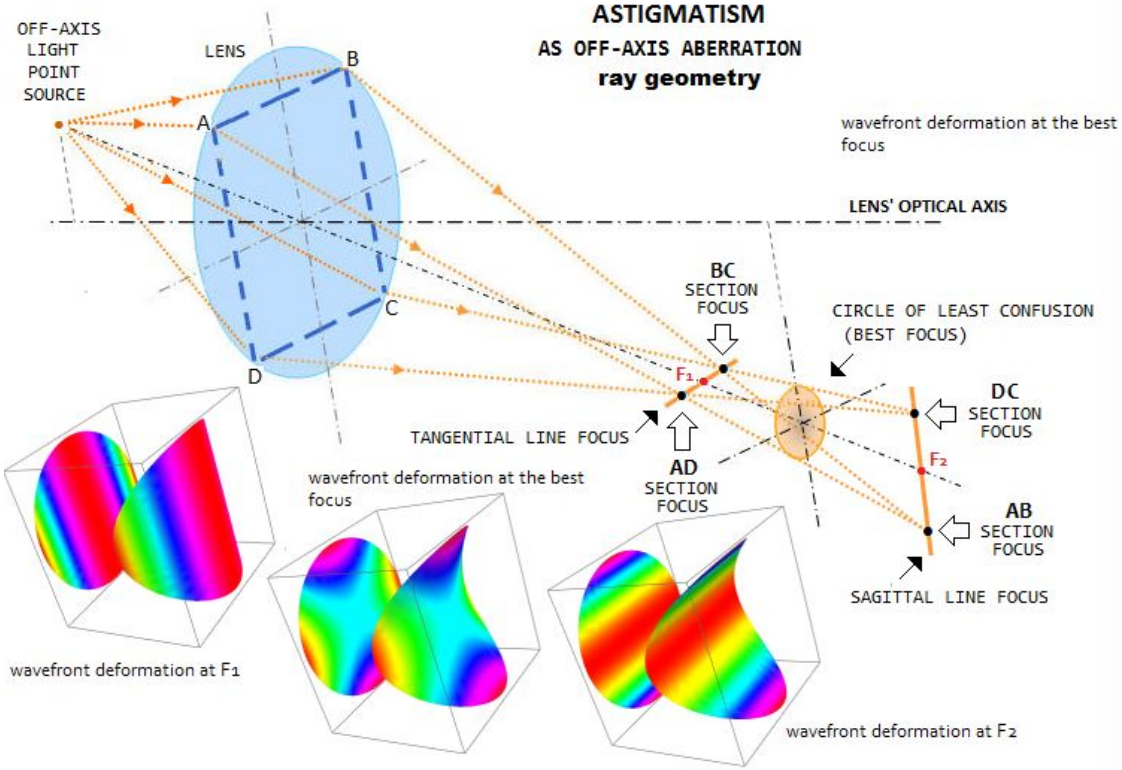
Figure from Alexis Rohou and Nikolaus Grigorieff, Journal of Structural Biology

6 Aberration

Spherical Aberration



Astigmatism Aberration



6 CTF Estimation

CTF Estimation