Part 3 - Image Formation
Three classes of scattering outcomes
Types of electron microscopes
Example SEM image:
fly nose
Example TEM image: muscle

Skeletal muscle.

Cell and Tissue Ultrastructure
Mercer
Amplitude contrast
plane wave
A=1  Θ=0°
Diffraction can be thought about and predicted using Huygen’s construction.

Maxima occur where \( n\lambda = d \sin \theta \)
• Each spot represents a Fourier component (a 3-D sine wave)
• Identified by (h, k, l) indices
• Each has an amplitude and a phase
• Both must be known to recalculate the “real space” object
Amplitude and phase contrast
Concept check questions:

• In what ways are inelastic and elastic scattering different? What causes them?

• What signals emerge from scattering events in the electron microscope that can be measured, and how do they lead to the three main types of electron microscopy?

• How does amplitude contrast arise?

• Why does phase contrast require us to think of imaging electrons as waves?

• What is a “plane wave”? What about a plane wave changes as it travels through a vacuum?

• Explain how/why atoms scatter X-rays.

• Why are there discrete peaks in the scattering from crystals?

• What information is delivered by each peak?
Sample

lens

Back focal plane

Magnified image
\(|\psi_{\text{total}}|^2 = \text{probability of detection in any particular pixel}\)
$4\sin(x) + 1.5\sin(x + \phi)$

- $\phi = 90^\circ$
- $\phi = 180^\circ$
- $\phi = 0^\circ$
Wave propagation and phase shifts
Concept check questions:

• How is the scattering from an object converted into an image in a microscope?

• What is the relationship between the density of the sample and the wavefunction present on the back focal plane of the objective lens? The image plane? Can you draw a picture showing why?

• How are plane waves represented in an “Argand” diagram? What are the axes?

• Why were Argand diagrams introduced (how do they help us understand wave propagation and interference)?

• How does the phase difference between two waves of identical frequency effect their interference?

• What property of an electron wave gives the probability of its detection at each position?
phase contrast transfer function (CTF)

$$CTF = \sin \left[ -\pi \Delta \lambda k^2 + \frac{\pi C_\lambda k^4}{2} \right]$$
The contrast transfer function
Concept check questions:

• What two factors make the phase of a scattered component of a wave different from that of an unscattered component?

• The contrast transfer function is typically plotted as a sinusoidally-varying function of what variable (what is the horizontal axis)? What quantity is plotted on the vertical axis?

• What is the CTF’s domain and range?

• What does a “contrast transfer” of 1.0 mean? -1? 0?

• Why does the CTF oscillate sinusoidally?

• What four variables appear in the argument of the sine function?
Contrast transfer function (CTF):

\[ \text{Contrast} = \frac{\text{transfer function}}{\text{focal plane}} \]

\[ \text{Contrast} = \sin \left[ -\pi \Delta e^2 \lambda k^2 - \frac{\pi C_{\text{s}} \lambda k^2}{2} \right] \]
Thuman-Commike and Chiu, *Micron* 31:687
Defocus and its effects
Concept check questions:

• What is a “Thon” ring?

• How can the defocus of a TEM image be determined?

• Why is defocus part of the argument of the CTF sine function?

• Does increasing the current in the objective lens make the image more or less defocussed?

• What is “over-focus”?

• How do heavily defocussed images look different than “closer-to-focus” images?

• What are the advantages of taking pictures far from focus? close to focus?
Envelopes
Concept check questions:

• What effect does partial spatial coherence have on the CTF? Why?

• What effect does partial temporal coherence have on the CTF? Why?

• What is their combined effect?

• How do these effects depend on defocus?
Point spread function

EM → ○

PSF
PSF = \mathcal{F}\{CTF\}

\text{object} \rightarrow \text{image}

\text{CTF} \quad \text{PSF}

\text{convolution}

I = O \otimes \text{PSF}

\mathcal{F}\{I\} = \mathcal{F}\{O \otimes \text{PSF}\}

\mathcal{F}\{I\} = \mathcal{F}\{O\} \cdot \mathcal{F}\{\text{PSF}\}

\mathcal{F}\{I\} = \mathcal{F}\{O\} \cdot \text{CTF}

I = \mathcal{F}^{-1}\bigg[\mathcal{F}\{O\} \cdot \text{CTF}\bigg]
\[ \mathcal{F}o = \frac{\mathcal{F}I}{CTF} \]

\[ O = \mathcal{F}^{-1} \left[ \frac{\mathcal{F}I}{CTF} \right] \]

CTF correction

\[ I = O \ast PSF \]

\[ \mathcal{F}I = \mathcal{F}o \cdot \mathcal{F}PSF \]

\[ \mathcal{F}I = \mathcal{F}o \cdot CTF \]

\[ I = \mathcal{F}^{-1} \left[ \mathcal{F}o \cdot CTF \right] \]
The diagram illustrates the process of transforming an object into its Fourier transform (FT), then applying a phase correction (CTF), and finally obtaining the Fourier transform of the corrected image (FT(I)). The mathematical expressions are:

- Object: \( A \)
- CTF: \( -0.2 \) and \( -0.3 \)
- FT(I): \( 2 \) and \( 0.2 \)
- CTF-Corrected FT(I): \( -2 - 10 \)

The process includes:
1. Object
2. Fourier transform of the object (FT)
3. Image
4. Fourier transform of the image (FT(I))
5. CTF correction
6. CTF-corrected image

Mathematical calculation:
- \( \frac{15}{0.3} = 5 \)
- \( \frac{-0.6}{-0.3} = 2 \)
CTF correction

Record image

Calculate Fourier transform

Fit power spectrum to theoretical CTF

Divide amplitudes by CTF (taking care not to divide by zero)

Inverse Fourier transform result
CTF correction
Concept check questions:

• What is a “point spread function”?
• How is the point spread function related to the CTF?
• What is the relationship between the wavefunction that exists on the back focal plane of the microscope and the Fourier transform of the recorded image?
• How (conceptually) can EM images be “CTF-corrected”?
• How can the CTF of a TEM image be determined?
• What special issue arises at CTF-zeros? How can it be handled?
• What would it mean if someone said they “CTF-corrected by phase-flipping only”?
• How can the information loss at CTF-zeros be overcome?