BMB/Bi/Ch 173 – Winter 2018

Homework Set 2 (200 Points) – Assigned 1-17-18, due 1-23-18 by 10:30 a.m.

TA: Rachael Kuintzle. Office hours: SFL 229, Friday 1/19 4:00-5:00pm and SFL 220, Monday 1/22 4:00-5:30pm.

For the problems that involve plotting, feel free to use your favorite plotting program (Matlab, Excel, Mathematica, etc.). These programs and more are provided to Caltech students for free by the institution. ***If you need help with plotting functions, come to office hours on Friday.***

1. Electron Scattering (20 Points)

I. Define amplitude contrast. Which kind of electron scattering (elastic or inelastic) provides amplitude contrast, and why?

II. Define phase contrast. Which kind of electron scattering (elastic or inelastic) provides phase contrast, and why?

2. Argand Diagrams (30 Points)

I. On an Argand diagram, draw each of the following electrons with correct relative phases: (ΔL = difference in path length between scattered and unscattered radiation)

1. unscattered, $\Delta L = 0$, amplitude of 5
2. scattered, $\Delta L = 0$, amplitude of 3
3. scattered, $\Delta L = \lambda/4$, amplitude of 3

(be sure to label your axes correctly)

II. Draw the sum of electron (1) and electron (2). Call this $\Psi sum_{1,2}$. Draw the sum of electron (1) and electron (3). Call this $\Psi sum_{1,3}$.

III. The amplitude of $\Psi sum_{1,2}$ is about 5.78. The amplitude of $\Psi sum_{1,3}$ is 2. How much more probable is $\Psi sum_{1,2}$ to be detected than the $\Psi sum_{1,3}$?

3. Putting the "Fun" back in Contrast Transfer Function (150 Points)

I. How can you figure out what the contrast transfer function (CTF) looks like for a particular image?

II. How can you use the CTF to improve the quality of your image?

III. Why is CTF correction not perfect?

The contrast transfer function can be written as follows:

$$CTF = \sin\left[-\pi\,\Delta z\,\lambda\,k^2 + \frac{\pi\,C_s\lambda^3 k^4}{2}\right]$$

where

- C_s is spherical aberration. Assume C_s is 0.5 mm.
- Δz is defocus.
- λ is the relativistic wavelength of the electron.
- k is spatial frequency.

IV. Separately plot the CTFs for a 200 kV electron microscope and a 300 kV electron microscope. The relativistic electron wavelength for each instrument is provided in the table below. **Assume the detector is conjugate to the image plane**. Plot for the following range of spatial frequencies: $\frac{1}{300}\text{Å}^{-1} < k < \frac{1}{1.5}\text{ Å}^{-1}$.

	Relativistic wavelength (pm)
200 kV potential	2.51
300 kV potential	1.97

It may be useful to first convert all variables with distance units to Angstroms (Å) using this fancy table (1 Å = 1 x 10^{-10} m):

Variable	Value (Å)
C_s	
λ_{rel} (200 kV)	
λ_{rel} (300 kV)	
Δz	

V. Based on your understanding of why the CTF oscillates, explain how the electron wavelength affects the CTF shape.

VI. Your CTF function is dampened by the following envelope function:

$$Env_{spatial\ coherence}(k) = e^{-\left(\frac{\left(\pi\,C_s\,\lambda^2\,k^3 - \pi\,\Delta z\,k\right)^2 \alpha_1{}^2}{\ln 2}\right)}$$

Plot the envelope function, assuming an acceleration potential of 300 kV. For the parameter "source size" (α_1) , use a value of 1 mrad = 1 x 10^{-3} radians. For the other parameters, use the same constants as in part (IV). **What is the cause of this dampening?**

VII. The main advantage of buying a microscope with a higher acceleration potential (e.g. 300 kV instead of 200 kV) is that it has a more generous envelope function. Re-plot the two CTF curves you generated in part (IV), but now plot them dampened by the envelope function above. Which acceleration potential yields greater contrast at high-resolution spatial frequencies: 200 kV or 300 kV?

VIII. Let's suppose that you are interested in imaging some cytoskeletal filaments inside a cell. These filaments, called MreB and FtsZ, have a 4 nm (40 Å) diameter. Based on the CTFs you plotted in part (VII), do you expect it will be easy or hard to see the filaments? Why?

IX. You are determined to get a good image of these cytoskeletal filaments.

a. Which parameter in the CTF equation can you tune in order to see them better?

b. Plot a new CTF with the relevant parameter changed so you can see the microtubules better, and write the new value that you used (with units). Zoom in to the region of interest by plotting for $\frac{1}{300}\text{Å}^{-1} < k < \frac{1}{20}\ \text{Å}^{-1}$.
Helpful hints:

- First try changing the parameter in the un-dampened CTF, in order to more clearly see the peaks. You'll get a ballpark parameter value based on this.
- Try to get a final contrast value above 0.1.

c. What do you need to physically change within the EM column in order to change the value of this parameter?