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. You can also use Wolfram Alpha for free online. Don't forget to label all your axes. ***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?

Both inelastic scattering events and certain types of elastic scattering events provide amplitude contrast by creating differences in percent transmission of electrons through different parts of the sample. Inelastic scattering events provide amplitude contrast because in-elastically scattered electrons are removed from the image primarily by the energy filter. Electrons that are backscattered or elastically scattered at extremely high angles will also not make it to the detector (they will not be able to go through the objective aperture). All of the scattering types mentioned here create regions of low electron density in parts of the image corresponding to strong scattering centers (higher density objects) in the sample.

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

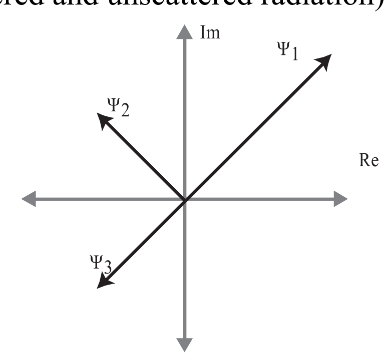
- Elastic scattering events provide phase contrast because the scattered electron beam interferes with the unscattered beam (constructively or destructively, depending on the phase of the scattered electron) to influence the final image. Note: the phase of elastically scattered electrons can be changed relative to that of un-scattered electrons via two sources of phase shift:
    - Scattering delays the wave by 90 degrees.
    - An elastically scattered electron will be scattered at a high angle, which means its path length will be different than that of the unscattered electrons. Therefore, the phase may be shifted further relative to the unscattered beam.
- Inelastic scattering events do not create phase contrast because inelastically scattered electrons are removed from the image by the energy filter, since their energy is reduced during scattering.

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)

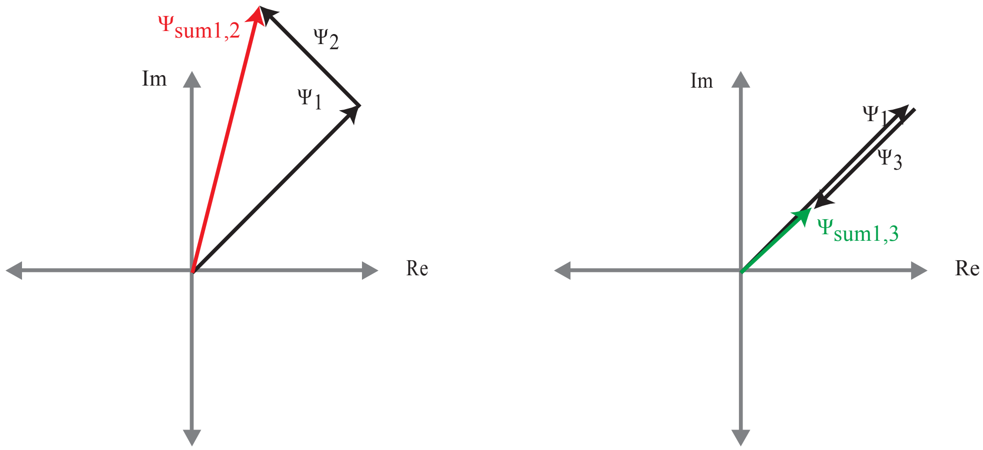
1. unscattered, ΔL = 0, amplitude of 5
2. scattered, ΔL = 0, amplitude of 3
3. scattered, ΔL = λ/4*, amplitude of 3

(be sure to label your axes correctly)



*Assume this electron's phase is further delayed by λ/4.

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 5.83. The amplitude of $\Psi sum_{1,3}$ is 2. How much more probable is $\Psi sum_{1,2}$ to be detected than the $\Psi sum_{1,3}$?
Probability of detection is proportional to amplitude^2. Therefore, it is $(5.83^2/2^2)$ = 8.5 times more likely to be detected.

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?
Get the radial average of intensities from the Thon rings in the power spectrum of your image (power spectrum = FT^2). Plot radial average versus spatial frequency, and perform a nonlinear regression to solve for the unknown variable, defocus.

II. How can you use the CTF to improve the contrast in an image?
You can perform CTF correction, which means that you can divide pixel values in the FT of your image by the corresponding value of the CTF at that spatial frequency, and then take the inverse FT to reproduce the image.

III. Why is CTF correction not perfect?
Because we can't divide by zero. The spatial frequencies where the contrast transfer = 0 will therefore remain missing from your final image.

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

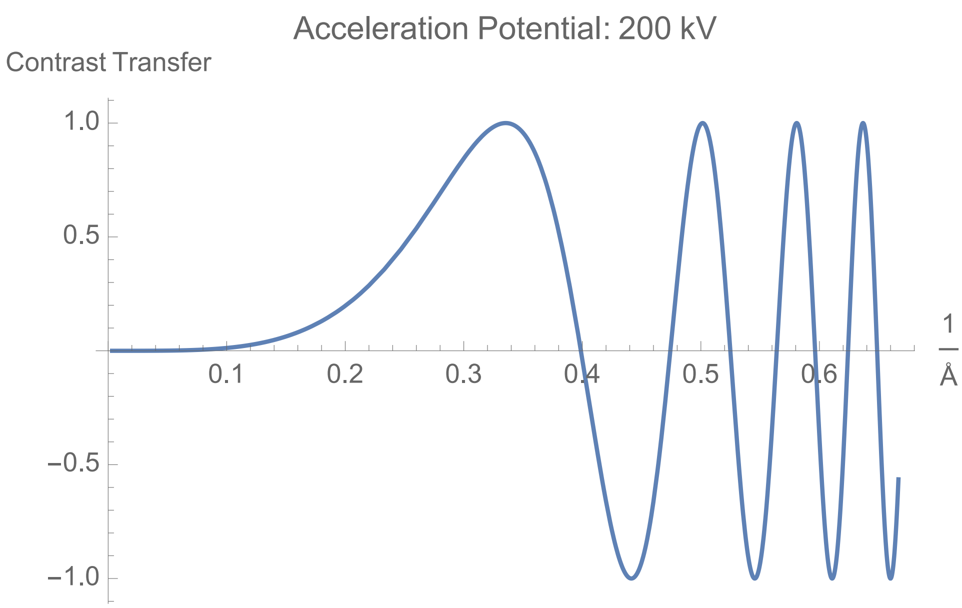
- C_s is spherical aberration. Assume C_s is 0.5 mm.
- Δz is defocus.
- λ is the relativistic wavelength of the electron.
- k is spatial frequency.

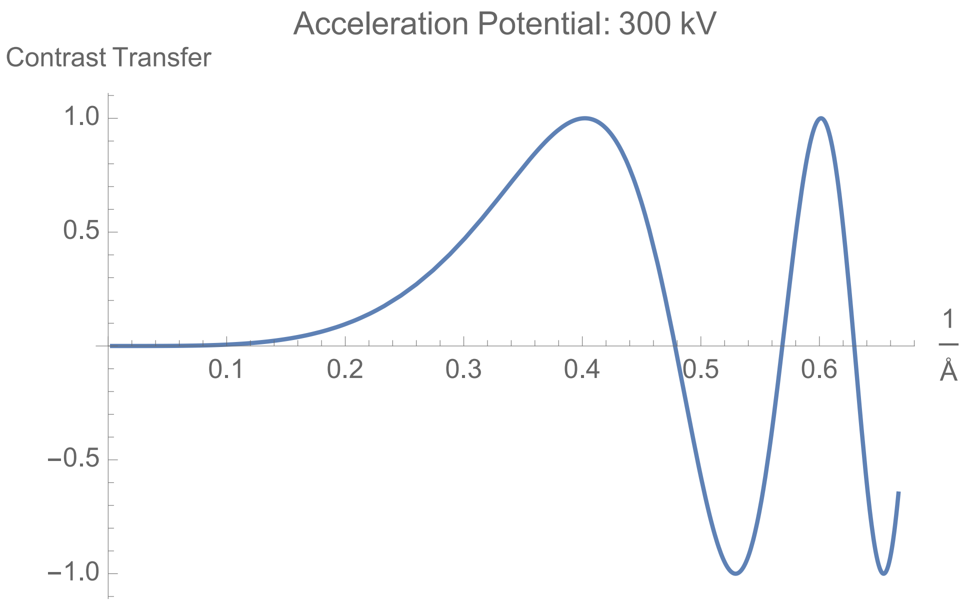
IV. Separately plot the CTFs for a 200 kV electron microscope and a 300 kV electron microscope. The relativistic electron wavelength for each instrument is provided in the table below. **Assume the detector is conjugate to the image plane**. Plot for the following range of spatial frequencies: $\frac{1}{300}\text{Å}^{-1}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

	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	5 x 10^{6}
λ_{rel} (200 kV)	2.51 x 10^{-2}
λ_{rel} (300 kV)	1.97 x 10^{-2}
Δz	0





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

Recall that there is a relationship between the wavelength, the angle of scattering, and the path length (the number of wavelengths traveled between the sample and the detector) of a scattered electron. Therefore, the path length difference (ΔL) between a scattered electron and unscattered electrons depends on the electron wavelength. The contrast you see for a given spatial frequency depends on this path length difference, as we have seen in our Argand diagrams.

More detailed answer (not required for full credit): the angle of scattering by objects of a given spatial frequency increases with wavelength; thus, a higher energy electron (shorter wavelength) would be scattered at a smaller angle than would a lower energy electron (longer wavelength) by objects of the same spatial frequency. A difference in scattering angle produces a large difference in the path length of the electron, and therefore changes the path length difference (ΔL) between the scattered and unscattered beam. This will shift the CTF and alter the range of spatial frequencies between "zero" points on the CTF.

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

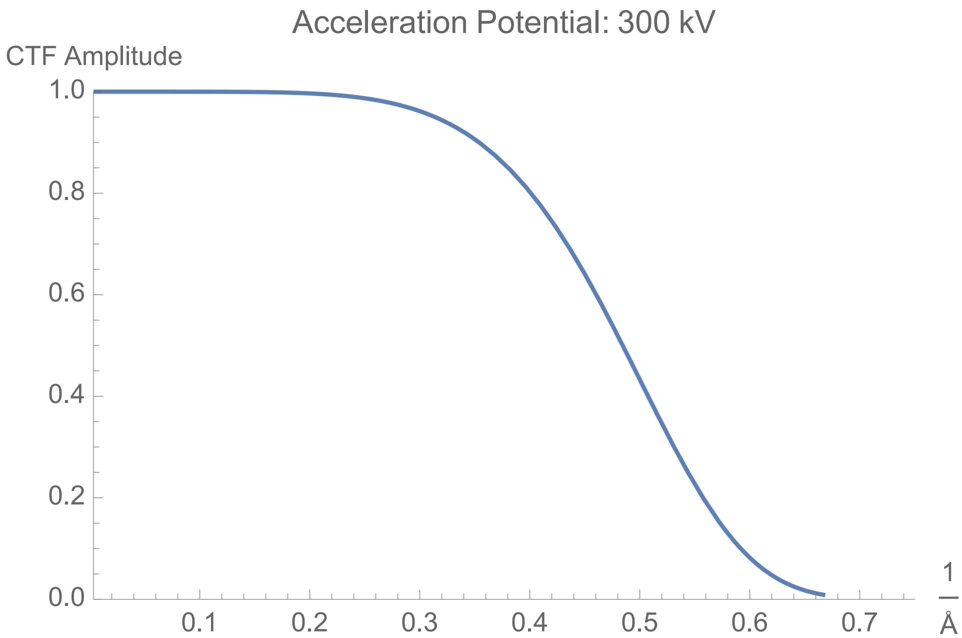
$$Env_{spatial\ coherence}(k) = e^{-\left(\frac{(\pi C_s \lambda^2 k^3 - \pi \Delta z\, k)^2 \alpha_1^{\,2}}{\ln 2}\right)}$$

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? Why does the envelope affect higher resolution information more than lower resolution information?**

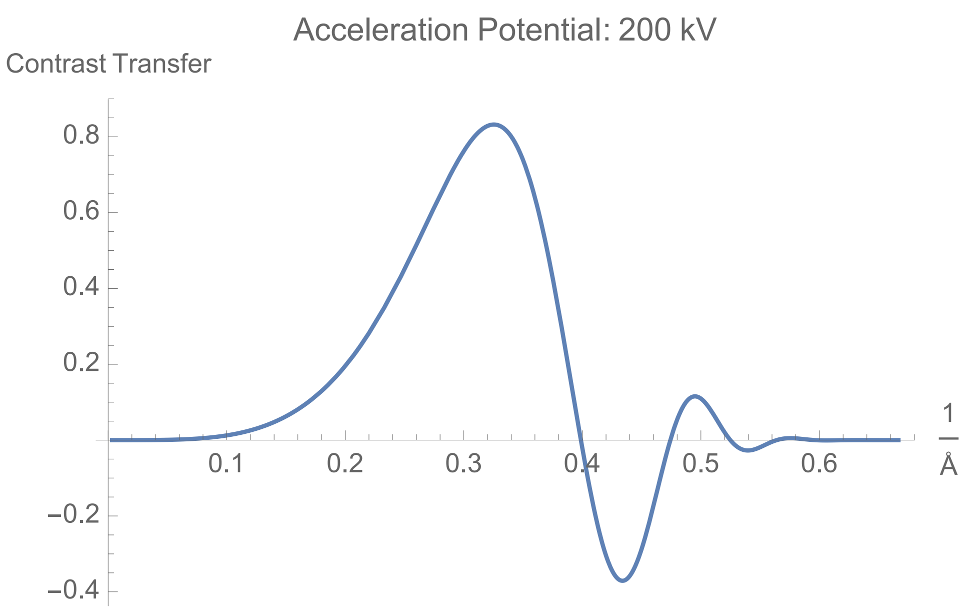
Imperfect coherence affects higher resolution information more than low res info.

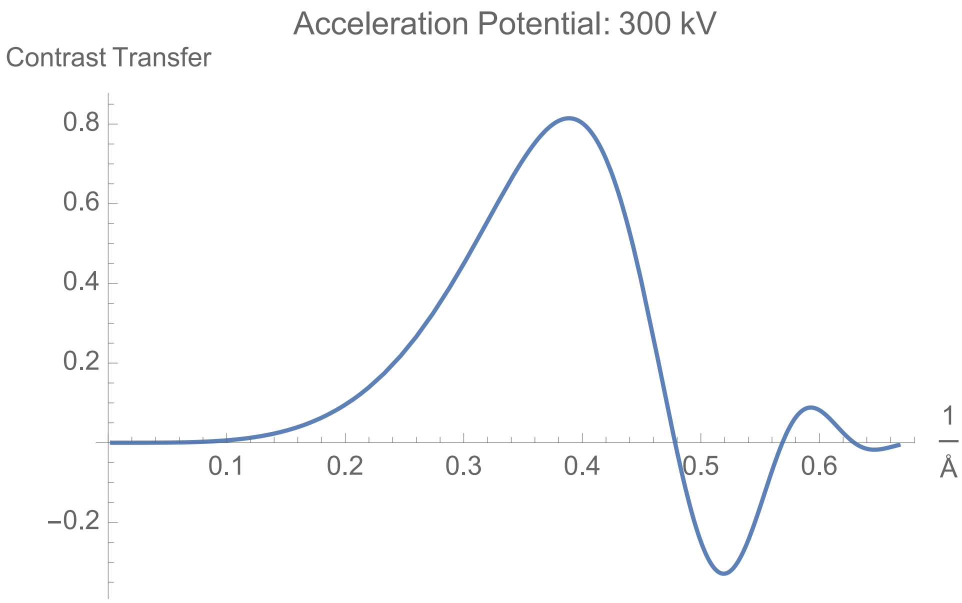
- If you shift images of **large objects** very slightly relative to each other (due to imperfect spatial coherence), the sine waves corresponding to their low spatial frequency will be displaced relative to each other with a **very small phase difference**, and thus they will all still constructively interfere with each other.
- Similarly, if you shift images of **small objects** by the same displacement distance, the sine waves corresponding to their high spatial frequency will also be shifted. However, because their wavelength is small, a small displacement of the images contributed to by different electrons **could result in a very large phase difference** of the component sine

waves. Therefore, the sine waves representing these small objects may interfere constructively, destructively, or anywhere in-between, thereby averaging out (flattening) the sine waves and erasing contrast.



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?**
To get the dampened function, multiply the CTF by the envelope function. The 300 kV potential yields greater contrast at high-resolution spatial frequencies.





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?

It will be hard. The CTF has very low values in the region around 40 Å -> k = 0.025 $Å^{-1}$. Low CTF values mean poor contrast in your image, due to very little difference in amplitude between elastically scattered electrons and unscattered electrons. Therefore, objects at spatial frequencies with low CTF values will not show up well in the image.

Side note: In practice, CTF correction can't be used to recover true amplitudes when contrast is very close to zero, because the signal-to-noise ratio is just too low.

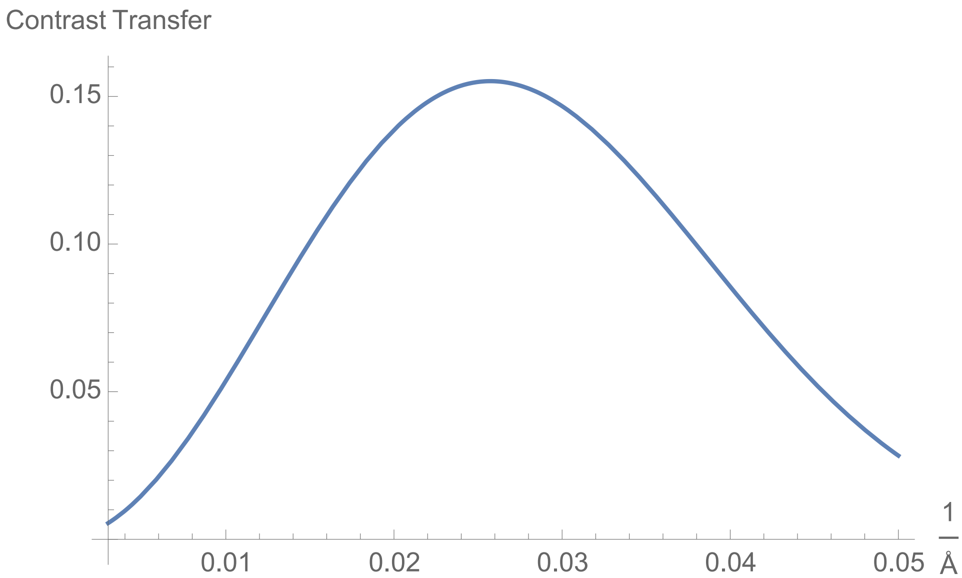
IX. You are determined to get a good image of these cytoskeletal filaments.

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

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). More specifically, shift the curve so there is a CTF maximum around the spatial frequency of these filaments. Assume you have access to a 300 kV instrument.
Zoom in to the region of interest by plotting for $\frac{1}{300}\,Å^{-1} < k < \frac{1}{20}\,Å^{-1}$.
Helpful hints:

- Use a negative value.
- 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.

The value should be around -10,000 Å, or -1 μm.



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

You need to change the distance between the detector and the image plane. You can do this by changing the strength of the objective lens, which shifts the image plane relative to the detector.