BMB/Bi/Ch 173 – Winter 2017

Homework Set 2.1 – Assigned 1/17/2017, Due 1/24/17 by 10:30am

TA - Sara Weaver sjweaver [a] Caltech.edu
Office hours in Broad 3rd floor kitchen - Friday 1/20 1:30pm-2:30pm, Monday 1/23 5pm-6pm, or by appointment

Problem 1 – (20 points) Electron scattering in the microscope

Imagine you're trying to measure the total dose your sample experiences during an exposure. Select the most appropriate protocol from the list below. For the inappropriate options, please explain why it is inappropriate and what the measurement would tell you. Would your electron dose reading over- or under-estimate the true dose?

ASSUMPTIONS: All apertures are inserted unless otherwise stated. The sample is inserted unless otherwise stated. The energy filter is inserted and tuned to the zero loss peak with a slit width of 20 eV. With a 300 kV microscope, the energy filter will let electron waves with the energy of 299,980 eV to 300,010 eV pass. Recall that the detector is placed *after* the energy filter.

1.a. (5 points) Protocol A: Remove the sample. Turn off the objective lens system and the projector lens system. Count the electron hits at the detector during your desired exposure time.

1.b. (5 points) Protocol B: Remove the objective aperture, and the selected area aperture, and the energy filter. Drive the microscope to an area of the grid that is empty (like a crack). Make sure that no matter is visible in the image before you measure the dose. Count the electron hits at the detector during your desired exposure time.

1.c. (5 points) Protocol C: Remove the energy filter. Drive the microscope to a focus area that is 2 microns away from the object you're planning to image. Make sure this area has a similar mass composition to the exposure area. Count the electron hits at the detector during your desired exposure time.

1.d. (5 points) Protocol D: Remove the condenser aperture, the objective aperture, the selected area aperture, and the energy filter. Drive the microscope to an area of the grid that is empty (like a crack). Make sure that no matter is visible in the image before you measure the dose. Count the electron hits at the detector during your desired exposure time.

Problem 2 – (30 points) Amplitude and phase contrast in biological cryoTEM

2.a. (5 points) Define amplitude contrast. What types of electron scattering contribute to it?

2.b. (5 points) Define phase contrast. What types of electron scattering contribute to phase contrast?

2.c. (10 points) How does an energy filter influence amplitude contrast? How does an energy filter influence phase contrast? Compare the situation with the energy filter removed and inserted. Discuss the types of electron scattering in your answer

2.d. (5 points) The density of vitreous* ice (0.92 g/mL) is very similar to the density of protein (1.35 g/mL). Explain how this affects contrast in an EM micrograph. **Vitreous (or amorphous) ice lacks a crystalline structure and is used in cryoEM experiments. We will discuss this more next week.*
Densities are from R. Henderson, Image contrast in high-resolution electron microscopy of biological macromolecules: TMV in ice, *Ultramicroscopy* **46**, 118 (1992).

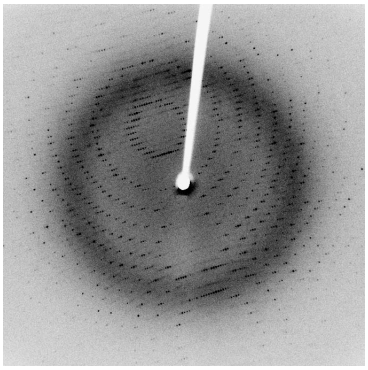
2.e. (5 points) Imagine you are imaging a sample with adeno-associated virus serotype DJ (AAV-DJ). AAV-DJ has a diameter of approximately 25 nm. You've added fiducial markers (here, 10 nm gold beads) to help in further image processing. What types of contrast will dominate your images of the virus? What about the gold beads?

Problem 3 (15 points) – Image formation

3.a. (5 points) What are the major differences between the ideal Fourier transform of an object and the Fourier transform of an electron micrograph image of the object?

3.b. (5 points) What can the relative abundance of different Fourier components tell you about your electron microscope and its alignment?

3.c. (5 points) In x-ray diffraction experiments, x-rays that are not diffracted by the sample are blocked by a beam stop and therefore excluded from the diffraction pattern. This beam stop (shown in white on the diffraction pattern) is required to prevent the strong x-ray beam from destroying the **detector**. (Note an earlier version of the PS mistakenly said sample here.)



https://en.wikipedia.org/wiki/X-ray_crystallography#/media/File:X-ray_diffraction_pattern_3clpro.jpg

Explain why the beam stop strategy is not typically used in biological electron cryomicroscopy experiments to remove the unscattered beam from the final image. What would happen if you placed the beam stop at the back focal plane? What about at the image plane?

Problem 4 – (35 points) Argand diagrams

4.a. (9 points) On an Argand diagram draw each of the following electrons with correct relative phases:

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)

4.b. (6 points) Draw the sum of electron (1) and electron (2). Call this $\Psi_{sum1,2}$. Draw the sum of electron (1) and electron (3). Call this $\Psi_{sum1,3}$.

4.c. (4 points) The amplitude of $\Psi_{sum1,2}$ is about 5.78. The amplitude of $\Psi_{sum1,3}$ is 2. How much more probable is $\Psi_{sum1,2}$ to be detected than the $\Psi_{sum1,3}$?

4.d. (6 points) Phase plates have been implemented in some electron microscopes to increase phase contrast in electron micrographs. The phase plate advances scattered electron waves by 90˚ while leaving unscattered electron wave phases unchanged. To explore this idea, apply the phase plate to the waves in part 4.a. and draw a new Argand diagram.

4.d. (6 points) Sum the phase electron waves in 4.d. as you did in 4.b, yielding $\Psi_{sum1p,3p}$ and $\Psi_{sum1p,2p}$.

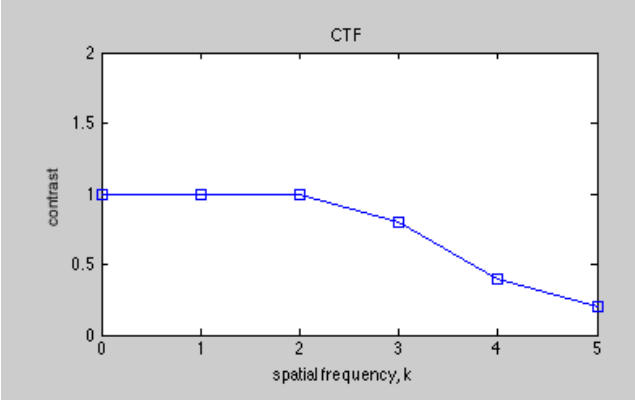
4.e. (4 points) How does detection of $\Psi_{sum1p,3p}$ and $\Psi_{sum1p,2p}$ change when you add the phase plate? How would you decide if a phase plate is appropriate for your experiment?

Problem 5 - (50 points) Fourier Synthesis, Contrast Transfer Function

Given below are amplitudes and phases describing the first five terms of a Fourier Series.

DC component		Fundamental		1st Harmonic		2nd Harmonic		3rd Harmonic		4th Harmonic	
A0	P0	A1	P1	A2	P2	A3	P3	A4	P4	A5	P5
1	∞	1	0	2	0	1	0	5	0	5	0

5.a. (18 points) Plot the individual waves
5.b. (6 points) Plot the sum of the individual waves.
5.c. (6 points) Plot the Fourier Transform of the final summed wave.
Now lets assume that this summed wave is our **object** and we wish to detect its **image** through a microscope. The microscope has a Contrast Transfer Function described by the table below.



Contrast Transfer Function						
Spatial frequency, k	k=0	k=1	k=2	k=3	k=4	k=5
Contrast	1	1	1	0.8	0.4	0.2

5.d. (6 points) Plot the Fourier Transform of the image.
5.e. (6 points) Plot the image.
5.f. (4 points) Which frequencies in the image are affected most by this CTF?
5.g. (4 points) Explain conceptually how CTF correction works to alleviate the result in 5.f.

Hints:

- *F{I} = F{O}*CTF*
- *It may be helpful to plot these using a computer. Useful programs for plotting are Grapher (on macs), Wolfram Alpha (on internet), and Google Search (on internet). Please contact sjweaver [a] Caltech.edu if you need help graphing your functions*