PHYS498:EBP Optical Trap Module
Remote Work Supplement

Please read the lab manual carefully. For some of the experiments, data has been uploaded into a box folder and you should have access to it. Refer below for further instructions. The lab report questions in the manual have been modified and given below. You should answer these questions instead of the lab report questions.

Data Analysis:

1. Stiffness calibration using equipartition theorem
   Movies of bead trapped at different laser powers are uploaded. Please refer to section 1 in the lab manual for instructions on how to analyze them. You will need FIJI software, which can be downloaded here: https://imagej.net/Fiji

2. E. Coli Flagellar Dynamics
   Tracking data (text files) and corresponding movies of many rotating E. Coli at different laser powers are uploaded. Every person from a group should analyze a separate E. Coli. You can refer to data analysis part in section 4 of the manual.

3. Internal transport in onion cells
   You should refer to data analysis part in section 5 of the manual for analyzing this data.
   For speed calculation, tracking data and corresponding movies of vesicles moving under directional transport inside onion cells are uploaded. Assuming the trap is roughly at the center of the field of view of the movie, you will have to correspond passing vesicles with blips in the tracking data.
   For force calculation, movies are uploaded where a moving vesicle is stalled by slowly ramping up the laser power. The stiffness coefficient in cytosol at the stalling laser powers are provided.

Questions:

1. Consider two identical particles trapped in two separate optical tweezers generated by lasers of equal intensities but different wavelengths (1000 nm (IR range) and 200 nm (UV range) respectively). Will both the particles experience the same amount of force? Why?

2. Consider two identical particles trapped in two optical tweezers generated with identical laser power and wavelength. First particle is suspended in pure water while the other is in water which has 20% (w/v) sugar dissolved in it. Will both the particles experience the same amount of force? Why?

3. Let us try to estimate the trapping force as a function of laser power with some simple calculations without knowing much about the system. Write down energy in terms of momentum and velocity of the photon travelling in a medium of refractive index n. Also, $F = \Delta p/\Delta t = Qp_{photon}/\Delta t$. Q is the factor by which momentum of photon is changing (e.g., for pure reflection, Q is 2). From these two equations, try to find the force as a function of power. For a spherical particle with radius close to the wavelength of the photon, Q is 0.1. If this particle is suspended in water, what would be the force per unit power (pN/mW) exerted upon it by the trap?

4. Fig. 1 in the lab manual shows how the trap is stabilized in the lateral (x-y) direction. Draw a similar ray diagram to show how the bead will be stably trapped if it is above or below the focus.

5. What will happen if the refractive index of the material of the bead is less than that of the solvent? Will the trap still work?

6. In the stiffness calculation, we separately calculate k_x and k_y . Why can we not assume them to be same?

7. How do the assumptions for equipartition method and PSD method for calculating stiffness differ?

8. Plot stiffness vs laser power for equipartition method and interpret your results. How do you expect these values to change if we used 2 micron beads instead of 1 micron?

9. Compare the power spectrum of E. Coli for different trapping powers.
   A. The Fourier Transform of f(x) = constant is a delta function. We also see a large spike at f=0Hz. What does this tell you about the QPD measurements?
   B. If we see a peak in the power spectrum at f = a, where a is a constant, why do we also see peaks at f = n*a, where n = 2, 3, 4?
   C. How do the rotation frequencies and stall lengths change as a function of laser power? (Show your plots and interpret your results.)

10.
   A. Why is directed transport faster than simple diffusion over longer distances? (Hint: Compare displacement vs time relationship in both cases. As an additional clue, look up mean-squared displacement (MSD) for a freely diffusing particle.)

B. The longest neuron in our body goes from the base of the spine to the foot and is about 1 m long. (In some adult whales, it is about 32 m!) Let us assume a mitochondrion is to be transported across that length. In our body, it will be hauled by kinesin motors running at 1 μm/s. How much time does it take to transport this mitochondrion? How much time would it take if simple diffusion was used instead for the transport? Is it similar or appreciably slower? (Consider the diffusion coefficient of mitochondria to be 0.25 $\mu m^2/s$.)
[As an aside, disruption of this active transport in neurons is currently considered a leading cause of neurodegeneration in dementia related diseases, such as Alzheimer's disease and Parkinson's disease.)

11. How do vesicles in active transport respond to manipulation by the trap? Does stopping and releasing a vesicle result in resumed motion, motion in the opposite direction, or ceasing of motion? What effect does trapping a vesicle have on other vesicles travelling the same route?

12. How fast are the myosin motors moving in your onion? How much force are they exerting? Myosin-XI is a subclass of Myosin family of motors, and it is the primary motor found in plant cells. The stalling force of **one** Myosin-XI has been measured in-vitro to be about 0.5 pN. Based on this information, can you estimate on average how many motors are actively driving the vesicles that you observed?