Optical Tweezers (Phys498: EBP, Spring 2020)

Introduction

Light or electromagnetic wave is a fascinating natural phenomenon. It allows us to see things all the way from distant galaxies down to single molecules. It also heats up our food and aids us in communication and almost instant transfer (at the speed of light, or 3 x 10^8 m/s) of information. Light also carries momentum and thus exerts force on objects. If you shine a flashlight at a coin, the coin is feeling the force from that light. However, that force is very small, of the order of piconewtons (pN). Hence the coin does not move. But what if the object is also very small, of the order of micrometers? That is exactly what Arthur Ashkin was trying to study at of Bell Laboratory in the 1970s. He found that a tightly focused light beam can actually attract particles from nearby lower intensity areas. The trapping force is proportional to the intensity of the light, and after calibration, you can watch an object move and eventually "stall". He thus created the first optical tweezer (or trap) and won the 2018 Nobel Prize in Physics for it (at the age of 96!). Now it is used extensively in many physics and biophysics labs, for trapping anything from atoms to biological cells (See references, at end of this report).

Objectives

1. **Understand the basic working of an optical tweezer**
2. **Learn to trap beads and calibrate the trap stiffness**
3. **Use the trap to study the flagellar dynamics of E. Coli**
4. **Use the trap to study internal transport in onion cells**

Timeline:

Week 1: Getting familiar with the trap and stiffness calibration

You will be able to see the optical trap in action and learn to trap some beads. You will also learn how to measure forces with the optical trap and two methods of calculating the force constant.

Week 2: E. Coli Flagellar Dynamics

Using the optical tweezers, you will be able to study motion of E. coli (a type of bacteria), which use corkscrew motion of their flagella to move around. By trapping them, you can look at their rotation frequency as a function of the retarding force exerted by the tweezers.

Week 3: Internal Transport in Onion cells (Bring your own onion!)

Using the optical tweezers, you will be able to study internal transport of vesicles by Myosin motor proteins in onion epithelial cells. (Myosin is one of three types of protein-sized "motors" or engines, which

use the gasoline of the cell, called Adenosine Triphosphate (ATP), to carry a vesicle to the place where the vesicle is needed to do its action. When the vesicle is at the place for it do its action, it is released from the myosin.) You will be able to calculate the speed of myosin and the force exerted by them to move the vesicles.

Recommended Reading:

1. Make sure to read the handout before starting the experiment.
2. https://phet.colorado.edu/en/simulation/optical-tweezers
   This is a cool Java applet that lets you visualize the motion of a bead in the optical trap. Try playing with the fluid properties (changing fluid speed, viscosity and temperature) to see what effect it has on the stability of the trap.
3. J. Shaevitz, A Practical Guide to Optical Trapping (http://genomics.princeton.edu/shaevitzlab/OT_Practicle_Guide.pdf)
4. K. C. Neuman and S. M. Block, "Optical Trapping," Rev. Sci. Instrum. 75, 2787 (2004). Pay particular attention to the overview and theory of optical trapping and the sections on calibration techniques.

Working Principle

The optical tweezer works by focusing a (strong) laser beam on the sample. The cross-section is generally Gaussian shaped, i.e. strongest at the middle, trailing off to zero far from the middle (fig 1). The force of an object scattering the light, which from simple optics (or from more sophisticated E & M), is largest at the beam center compared to the edges. Thus, a spherical particle with a refractive index higher than that of the medium surrounding it experiences a force towards the center of the light beam. This is because anytime light traverses an interface between two media of different indices of refraction, its path is deviated as described by Snell's law. In this way the beads function like *spherical* lenses in the path of the laser beam. Snell's law is

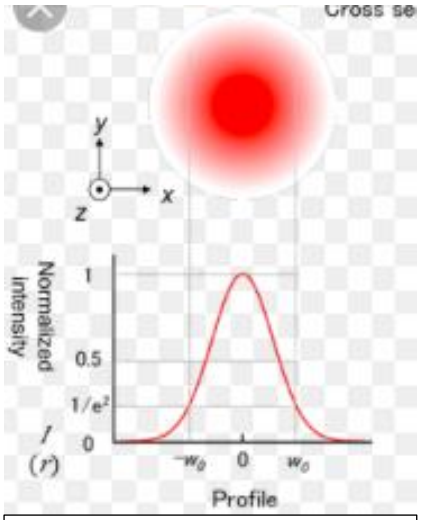


Fig. 1: The waist of a Gaussian beam is defined as the location where the irradiance is $1/e^2$ of its maximum value.

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \qquad (1)$$

Light rays exit the trapped particle with a different momentum vector than that which it entered with; hence the particle had transferred momentum onto the light. By Newton's third law, this means the passing light had also transferred momentum to the particle. This is illustrated in figure 2.

In case (a), with the particle slightly to the left of center, the two rays refract through the particle and bend inwards. The reactionary force vectors, F1 and F2, of each ray on the bead are shown. Because ray

2 is more intense (and thus carries more momentum) than ray 1, the net force on the bead is to the right. Thus, a perturbation to the left causes a rightward-directed force back towards the trap's center. In case (b) the particle is centered laterally in the beam and will not be pushed left or right. The net gradient force is downward, which is balanced by an upward scattering force (not shown) due to reflection of some of the light. This is the trap center or the stable position of the trap laterally and axially.

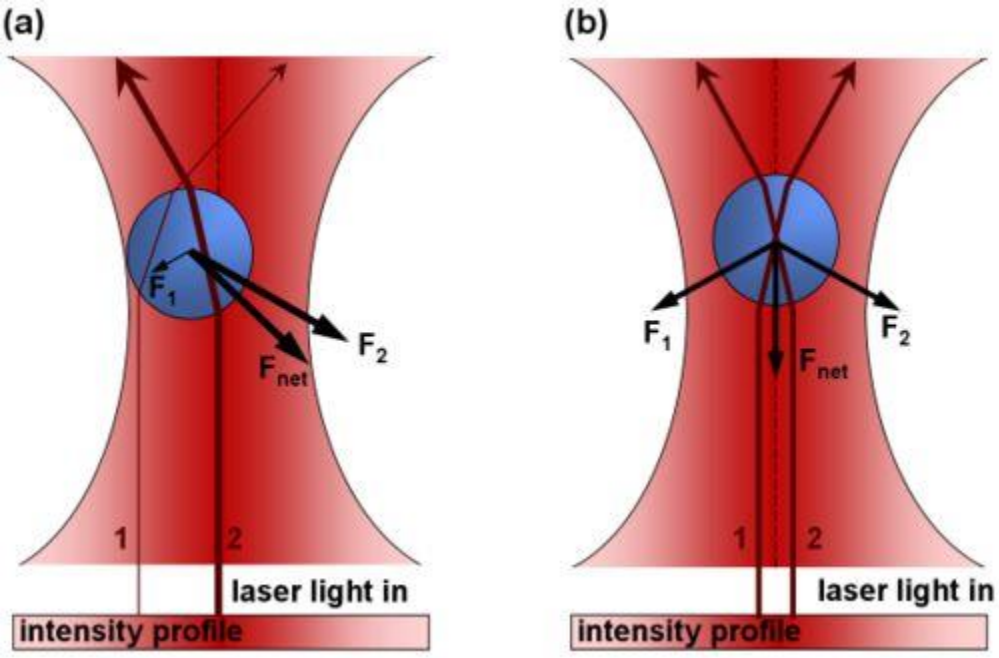


Fig. 2: A ray diagram showing how the gradient foce stabilizes the trap. Red area is the light beam and darker the red, higher the intensity.

You can think of the focused laser beam as creating a potential well, located just slightly downstream of the laser focus. It is customary to think of this potential well as that of a spring, which is equivalent to Taylor series expanding the functional form of the potential to second order. Then the optical tweezer has a spring constant which relates how far an object is from the tweezer with how much restoring force it feels. This force is linear with the distance and dependent in some way on the intensity of the laser. Scattering forces such as reflection and absorption also play a minor role, pushing the bead away from the laser focus. This is why the stable position of the trapped particle is slightly above the laser focus in the direction of the beam.

Setup

At minimum, an optical trap requires a strong laser, an objective to focus the light and to actually form the trap, some way to position the trap relative to the sample, and electronics for data collection.

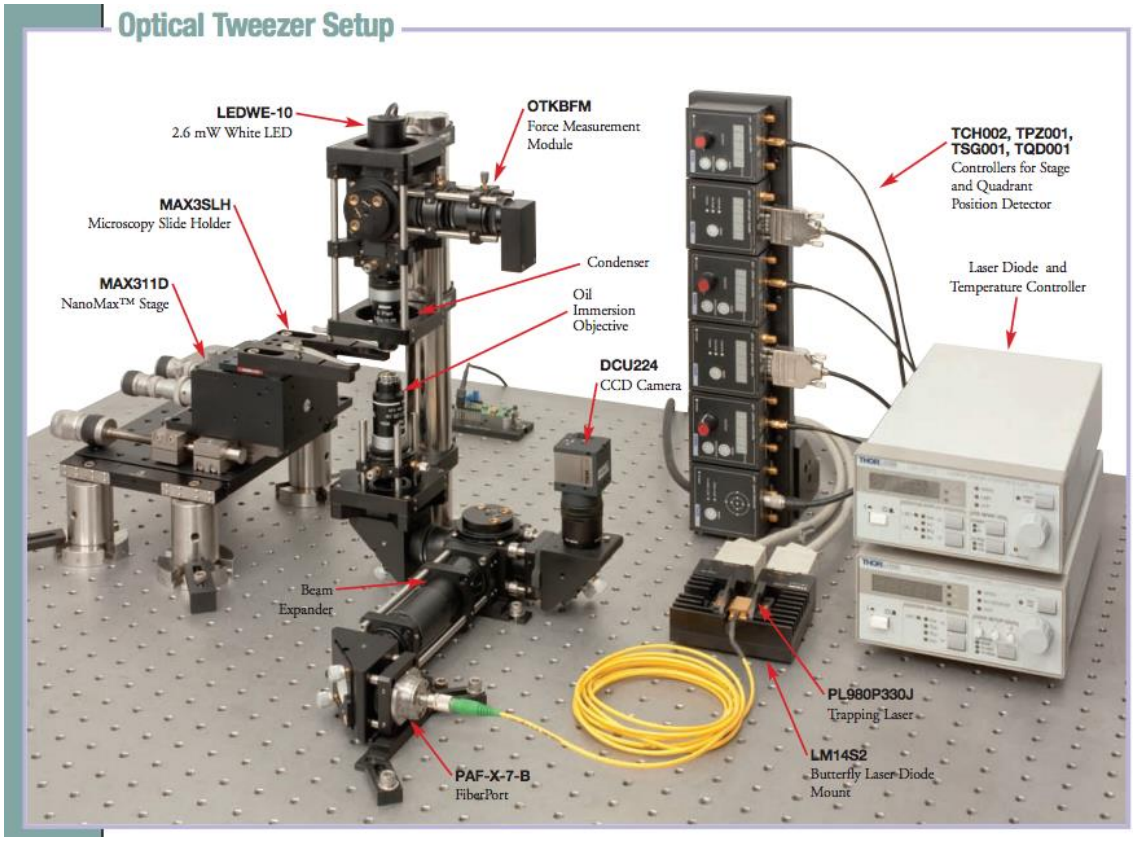


Fig.3: Optical Tweezer Setup

In particular, you will be working with:

- Laser: 350mW laser at peak emission 975nm (Class 3b IR)
  The 975nm wavelength is far enough from the resonant frequency of water to prevent much heating of live cells and is less likely to affect the behavior of cells than if visible wavelengths.
- Objective: 100x oil immersion 1.25 NA
  The size of the focal spot (d) at a particular wavelength (λ) depends on the Numerical Aperture (NA) of the objective as follows:

$$d = \lambda/2NA \quad (2)$$

- Positioning: XYZ manual translational stage with XY piezo for motorized high precision movement
- Data Collection: CCD camera (low precision tracking)
- Data Collection: Quadrant photodiode (QPD) for high precision back focal plane interferometry position detection

The quadrant photodiode (QPD) is our principal way of collecting data on the position of the trapped particle relative to the center of the trap. This distance also tells us the force the trap exerts on the particle since force increases linearly with distance from the trap's center. Some simpler optical traps use the video camera image to provide position information, but a photodiode allows finer spatial resolution and much higher frequencies (we'll be collecting data at 12 kHz for power spectrum analysis – compared with 30-500 Hz for video cameras). The QPD consists of 4 photodiodes in a quadrant formation to allow X and Y position calculation. Within a certain range of light intensities, the output voltage of a photodiode scales linearly with the intensity of light incident upon the diode. The light incident upon each quadrant in the QPD generates a voltage. The analog circuitry then outputs a voltage Vx and Vy which are proportional to the actual X and Y position of the incident beam. As the light scatters in a predictable way off of the spherical beads, this information can be used to recover actual bead position within a narrow range around the center of the trap. With nothing in the trap (or a trapped bead exactly centered in the trap), the laser beam is tightly focused on the center of the QPD, giving Vx and Vy signals of zero. When a trapped bead moves slightly away from the center of the trap, the laser spot moves on the QPD, causing V_x and V_y to vary accordingly.

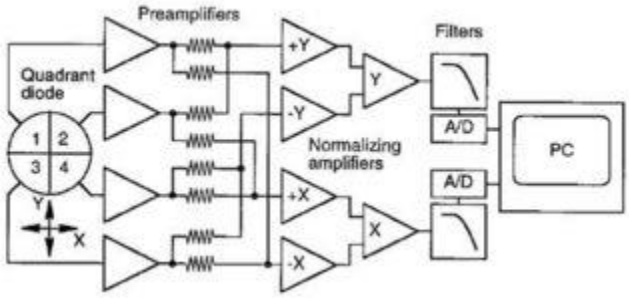


Fig.4: Schematic of the Quadrant Photo Diode position detection system. The signal from the diodes are amplified by low-noise preamplifiers and then networked to calculate the X and Y position of the incident light beam. The figure above, depicting the block diagram, is taken from Allersma et al.

Precautions:

1. Make sure that you are always wearing gloves, especially while working with E. Coli.
2. The 975 nm laser is in the near infra-red (NIR) range and so is invisible. That makes it even more dangerous to work with. At its peak power, it can cause serious irreversible damage to the retina even if it falls on the eye for a short moment and without you realizing it. Always make sure that you are wearing eye protection when the laser is on, **do *not* look directly in the laser path! Never put your eye at the height of the laser beam!** and check for stray laser near the setup before beginning of every experiment using an IR viewer. Keep the laser turned off when not in use or while putting oil on the objective.

3. The objective lens is a very expensive optical component (worth a few thousand dollars) and is very sensitive. Make sure never to touch its tip where the optical glass is, and always have oil on objective before placing it on the sample.
4. The numerous mirrors and lenses that steer and shape the beam to this end require delicate alignment by a trained staff member with safety goggles, an IR viewer, and special tools. Do not tamper with anything in the laser beam path unless specifically instructed! If you are curious about the alignment, TAs will be happy to answer all your questions.

Pre-Experiment Questions:

(Answer the following questions and make sure to get them checked by the TA before beginning the experiment)

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?

Sample chamber preparation:

For the following experiments, you will need to prepare microfluidic chambers for imaging and trapping beads and E. Coli.

1. Take a new glass slide and lay it on the table.
2. Make strips of double-sided tape and affix them on the glass slide.
3. Take a glass coverslip and place it on top of the glass slide such that the tape strips are sandwiched between them.
4. Press firmly and make sure that there are no air bubbles left or else the liquid will leak out.

1. Stiffness Calibration using Equipartition Theorem (Week 1)

The optical trap can be assumed to be a spring, obeying Hooke's law:

$$F = -kx \qquad (3)$$

Where F is the force applied, k is the spring constant or stiffness and x is the displacement of the trapped particle from the mean position (standard deviation). Thus, to calculate the force exerted on the trapped particle, we need to know the stiffness (k) of the trap. One simple way to do this is to use the equipartition theorem:

$$\frac{1}{2}k_x x^2 = \frac{1}{2}k_B T \quad (4)$$

$$\frac{1}{2}k_y y^2 = \frac{1}{2}k_B T \quad (5)$$

Where k_B is the Boltzmann constant (1.38 x 10^{-23} kg.m^2.K^{-1}.s^{-2}) and T is the temperature.

Data Collection:

1. Dilute 1 micron polystyrene beads in water by 10,000 times.
2. Mix well and transfer 5 microliters (uL) of this bead solution into one of the channels of your sample chamber.
3. Using a little epoxy resin, seal the ends of the chamber. You can use a plastic dish and a pipette tip to mix the epoxy. After sealing, wait at least 10 minutes and make sure that the epoxy is completely solid before proceeding to the next step (even a little epoxy on the objective lens will ruin it).
4. Place the chamber (coverslip side down) on to microscope stage (make sure that there is oil on the objective first!)
5. Adjust the x-y stage position such that the correct channel is above the objective and then adjust the z-position to focus such that you can see the beads.
6. Turn on the IR laser, set it at 100 mA and try to trap one of the free-floating beads (you will know a bead is trapped when it moves as you move the stage). The bead will be released when you turn off the laser.
7. Practice trapping until you are comfortable with trapping, moving, and releasing beads.
    - TIP: The potential well created by the trap is not symmetric along the z axis, and consequently beads get pushed into the trap from one direction but away from it from the opposite direction – when not in the immediate vicinity of the trap. When a bead appears black as opposed to white on the screen, it is below the trap focus and that is the correct side to be on.

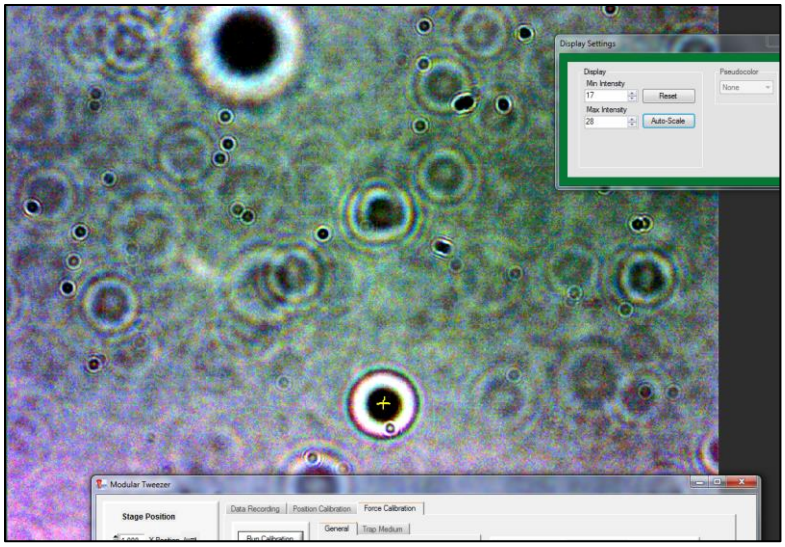


Fig.5: Trapping beads in solution

8. Mark the position of the trap on the screen using the small pencil icon on the left hand side.
9. Crop a small (~256 x 256) area around it.
10. Adjust the camera settings such that the exposure time is 40 ms (25 FPS).
11. Record 1500 frames (and don't forget to save!). Make sure that no other beads fall into the trap whilst you record.

Data Analysis:

Here, we will use the ThunderSTORM plugin in FIJI to localize the bead precisely and track its movement in every frame.

1. Open the image files using FIJI.
2. Select Image→Color→RGB to Luminance
3. Go to Plugins→ThunderSTORM→Run Analysis
4. Go to Camera setup and input pixel size as 53 nm, count to A/D as 11.72 and Base level count as 50. Click OK.

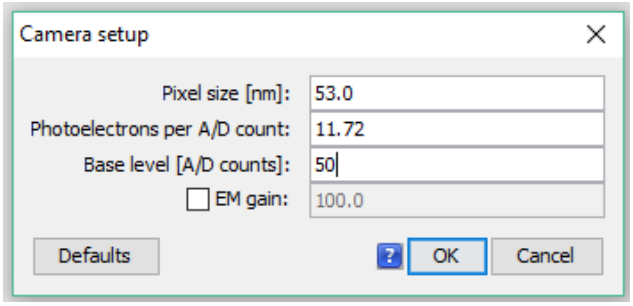


Fig.6: Camera Setup

5. Input rest of the parameters as follows and click Preview.

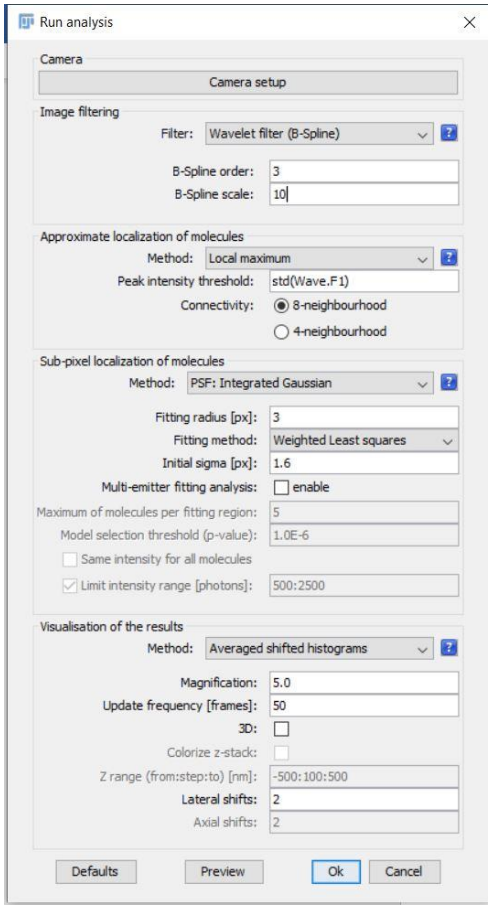
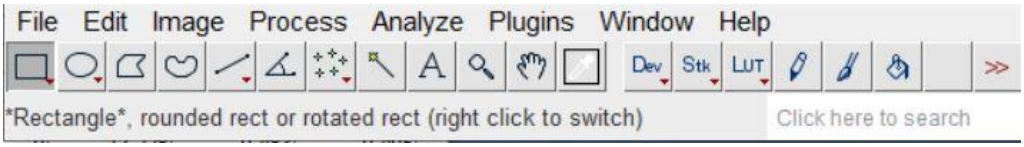
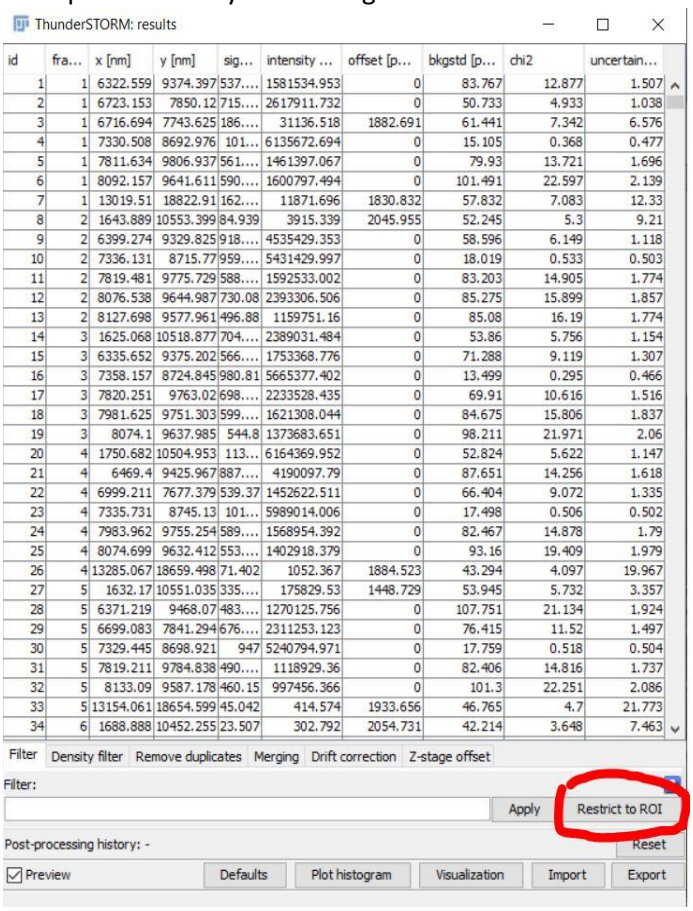


Fig.7: ThunderSTORM setup

6. You should see a single red crosshair at the center of your bead. If you do, click Ok and proceed to step 10. If you do not, then click Ok and proceed to next step.
7. You should be able to see the reconstructed image in the window titled “average shifted histograms”. Notice the central spot denoting the bead center surrounded by some other spots.
8. Select the rectangle selection tool in FIJI (under the “file” tab) and draw a rectangle around the central spot.



9. Click “Restrict to ROI” button in the ThunderSTORM:Result window. This will select only the data from points inside your rectangular selection.



id	fra...	x [nm]	y [nm]	sig...	intensity ...	offset [p...	bkgstd [p...	chi2	uncertain...
1	1	6322.559	9374.397	537....	1581534.953	0	83.767	12.877	1.507
2	1	6723.153	7850.12	715....	2617911.732	0	50.733	4.933	1.038
3	1	6716.694	7743.625	186....	31136.518	1882.691	61.441	7.342	6.576
4	1	7330.508	8692.976	101...	6135672.694	0	15.105	0.368	0.477
5	1	7811.634	9806.937	561....	1461397.067	0	79.93	13.721	1.696
6	1	8092.157	9641.611	590....	1600797.494	0	101.491	22.597	2.139
7	1	13019.51	18822.91	162....	11871.696	1830.832	57.832	7.083	12.33
8	2	1643.889	10553.399	84.939	3915.339	2045.955	52.245	5.3	9.21
9	2	6399.274	9329.825	918....	4535429.353	0	58.596	6.149	1.118
10	2	7336.131	8715.77	959....	5431429.997	0	18.019	0.533	0.503
11	2	7819.481	9775.729	588....	1592533.002	0	83.203	14.905	1.774
12	2	8076.538	9644.987	730.08	2393306.506	0	85.275	15.899	1.857
13	2	8127.698	9577.961	496.88	1159751.16	0	85.08	16.19	1.774
14	3	1625.068	10518.877	704....	2389031.484	0	53.86	5.756	1.154
15	3	6335.652	9375.202	566....	1753368.776	0	71.288	9.119	1.307
16	3	7358.157	8724.845	980.81	5665377.402	0	13.499	0.295	0.466
17	3	7820.251	9763.02	698....	2233528.435	0	69.91	10.616	1.516
18	3	7981.625	9751.303	599....	1621308.044	0	84.675	15.806	1.837
19	3	8074.1	9637.985	544.8	1373683.651	0	98.211	21.971	2.06
20	4	1750.682	10504.953	113...	6164369.952	0	52.824	5.622	1.147
21	4	6469.4	9425.967	887....	4190097.79	0	87.651	14.256	1.618
22	4	6999.211	7677.379	539.37	1452622.511	0	66.404	9.072	1.335
23	4	7335.731	8745.13	101...	5989014.006	0	17.498	0.506	0.502
24	4	7983.962	9755.254	589....	1568954.392	0	82.467	14.878	1.79
25	4	8074.699	9632.412	553....	1402918.379	0	93.16	19.409	1.979
26	4	13285.067	18659.498	71.402	1052.367	1884.523	43.294	4.097	19.967
27	5	1632.17	10551.035	335....	175829.53	1448.729	53.945	5.732	3.357
28	5	6371.219	9468.07	483....	1270125.756	0	107.751	21.134	1.924
29	5	6699.083	7841.294	676....	2311253.123	0	76.415	11.52	1.497
30	5	7329.445	8698.921	947	5240794.971	0	17.759	0.518	0.504
31	5	7819.211	9784.838	490....	1118929.36	0	82.406	14.816	1.737
32	5	8133.09	9587.178	460.15	997456.366	0	101.3	22.251	2.086
33	5	13154.061	18654.599	45.042	414.574	1933.656	46.765	4.7	21.773
34	6	1688.888	10452.255	23.507	302.792	2054.731	42.214	3.648	7.463

10. Select Export to save the analyzed data as a spreadsheet.
11. Open it in excel. Calculate the standard deviation of x and y and plug it in Eq. 4 and 5 to get the trap stiffness.

2. Stiffness calibration using PSD Roll-off method (Week 1)

In this method of force constant calibration, the power spectral density (PSD) of a time series of trapped particle positions (due to Brownian motion) is computed. This is fit to the response of a harmonic oscillator with known damping due to the viscosity of the solvent and has the following Lorentzian profile:

$$S_{vv} = \frac{\rho^2 k_B T}{\pi^2 \beta (f_0^2 + f^2)} \qquad (6)$$

in Volts^2 / Hertz. Where $\beta = 3\pi\eta d$ is the drag, η is the fluid viscosity of water, d is the diameter of the bead, ρ is the sensitivity of the trap, and f is the frequency of bead vibrations. If the fluid is water, then we can take: $\eta = 8.90 \times 10{-}4$ Pa*s. Using this, a curve can be fit to the log of the data sets giving the roll-off frequency f_0. Using this f_0 we can compute the trap stiffness as follows:

$$k_x = 2\pi . f_{0x} . \beta \qquad (7a)$$

$$k_y = 2\pi . f_{0y} . \beta \qquad (7b)$$

1. Flow in 5 μL of 10,000 times diluted bead solution in one of the chambers and seal the chamber with epoxy as before.
2. Put oil on the objective, mount the sample chamber on the microscope stage and focus on a freely diffusing bead.
3. Start the OTKBFM-CAL software.
4. Turn on the IR laser, set it to 100 mA and trap the bead.
5. Go to the force calibration tab, set the calibration length as 0.3 and 10 averages and hit “run calibration”. You will see a power spectrum. (Note: make sure no other particle falls into the trap while the calibration is running. Release the bead once you are done to make sure there was no other bead hiding underneath it.)
6. Note down the force constant value (k_{PSD}) on the right-hand side along with corner or roll-off frequency value.

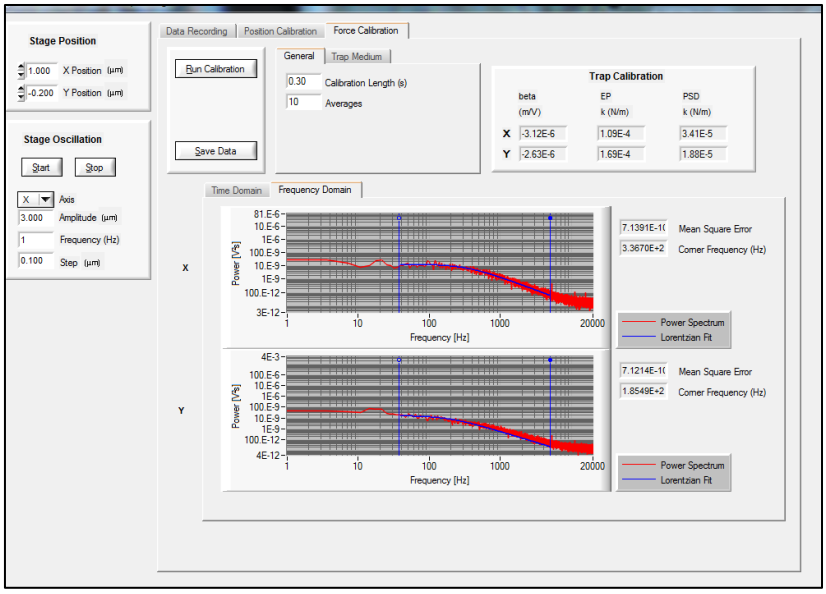


Fig.8: Force Calibration (PSD roll-off method)

7. Repeat steps 5 and 6 for 4 other laser powers.
8. Plot k_{PSD} vs laser power.

3. Position Calibration (Week 1)

The QPD gives out readings in volts. To be able to estimate the position of a particle, we need to convert these reading into a distance unit. That is why we do the position calibration to get the 'sensitivity' of the trap (in V/m). For this, we use a fixed particle and move it through the trap at a controlled speed in x and y directions and record V_x and V_y readings from the QPD. In a small region (100-200 nm) around the center of the trap, voltage depends linearly on the distance. In this region, if we take the slope of the voltage vs distance plot, the inverse of that is the sensitivity.

1. Flow in 5 µL of 10,000 times diluted bead solution in one of the chambers as before.
2. Put oil on the objective, mount the sample chamber on the microscope stage and focus on a freely diffusing bead.
3. Start the OTKBFM-CAL software.
4. Turn on the IR laser, set it to 100 mA and trap the bead.
5. Go to the position calibration tab and input a scan length of 5 micron, number of steps 100 and 1000 averages.

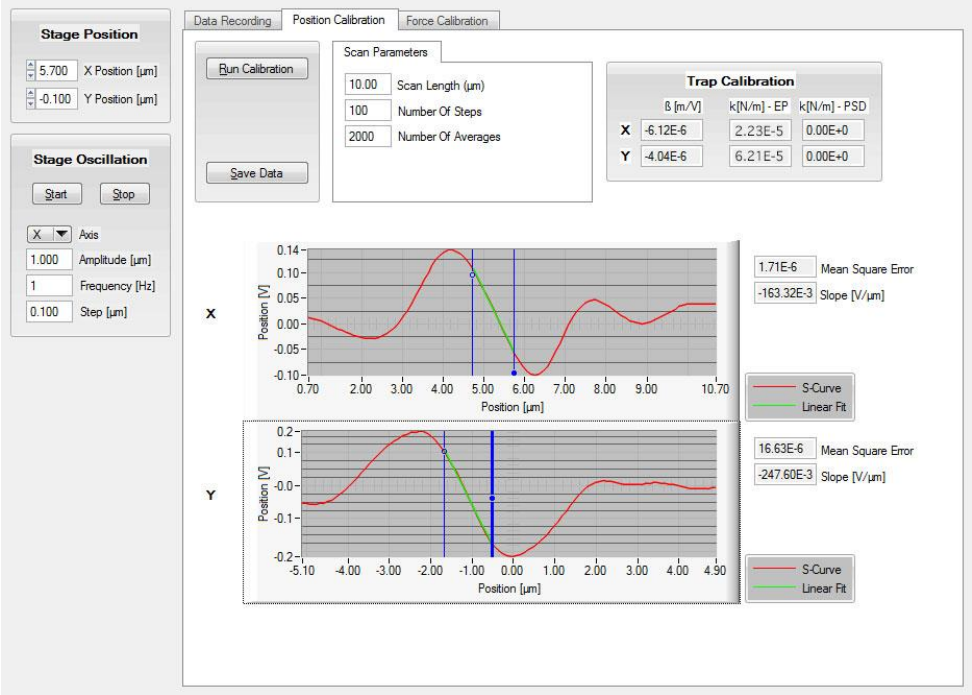


Fig.9: Position Calibration

6. Hit "run calibration". In the position (V) vs position (µm) graph, move the two blue bars so that the linear region is confined within them.
7. The green line indicates the linear fit. Make sure it is fit well and note down the slope. Inverse of that slope is the sensitivity (denoted by β in the software).

Conclusion of Week 1:

In this week we learned basics of optical traps, got familiar with trapping small beads and calibrated the trap stiffness using two different methods. We also calibrated the position for the conversion of QPD values. Now we are ready to use our optical trap in the next two weeks to study some biological systems.

4. E. Coli Flagellar Dynamics (Week 2)

Now that we have characterized the optical trap, we will use it to study the motion of a type of bacteria, E. Coli. Bacterial motility is associated to a wide range of biological processes and it plays a key role in the virulence of many pathogens. They move around using their flagella, which can rotate in a corkscrew fashion to propel the bacteria forward.

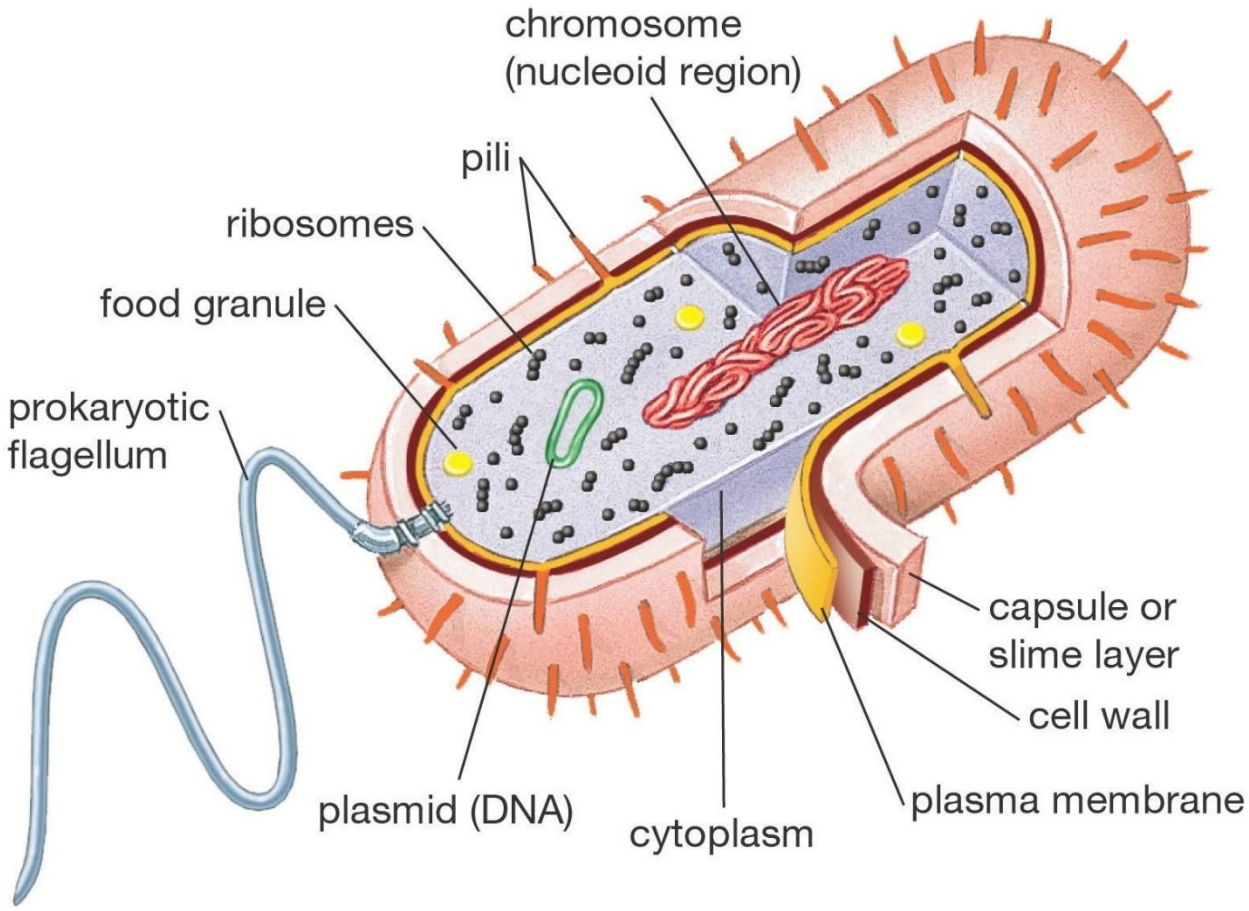


Fig.10: Illustration of an E. Coli cell

In this experiment, we will use a mutant of E. Coli, KAF95, which is specially modified so that their flagella are sticky. They can stick to the surface of a glass coverslip and instead of moving forward, the bacteria will rotate. This motion can be captured and quantified by video, but for high precision measurements you will need the optical trap. The goal is to study the rotation frequency as a function of retarding force applied by the trap; in this way, you can tell us how fast the flagella can rotate and how much force it can apply. To do this you will find the frequency peak in the scattered light. At a high enough force (torque) the rotary motor can be overpowered, and the cell will stop. You will be applying forces in the piconewton range. As with any biological system, you will find that the bacteria are individuals, and the results you get will depend a bit on the exact cells you choose. This is part of the fun.

Data Collection:

1. Using the pipette set to 5 μL, fill one chamber with the *E. coli* culture your TA has grown for you. Give the bacteria about 10 min to settle down.
2. Using the microscope, check if you can spot enough E. Coli on the surface of the coverslip. They will look like this.

Fig. 11: E. coli stuck to the surface

3. Scan around to see how many *E. coli* are rotating. They should not be too difficult to find.

4. Once you have made sure that there are enough rotating E. Coli on the surface, flush away the floating cells by flowing in 5 μL of the Tryptone Buffer provided by the TA (Use a Kimwipe to absorb the liquid from the other end as you pipette in from one end).

5. Turn on the IR laser and set it to 40 mA.

6. Translate the sample until the trap is just inside the sweep of the cell body, as in the picture above.

7. To capture a video, click the record button on the upper left of the camera software. Take long enough to capture the motion, you usually won't need longer than a minute.

8. Gradually ramp up the trap current until the motion completely stops. Take a video and a photodiode readout for 5 different laser powers between the initial and the stalling power. To capture a photodiode readout, go back to the software that you did the calibration with. Go to the "Data Recording" tab, and simply click "start recording". A three-column readout, corresponding to X, Y, and SUM, will be saved as a .dat file in the documents folder. Make sure you rename the newest files in that folder before you walk away, because the default name is simply a timestamp.

9. Collect data from multiple cells and try to analyze simultaneously to ensure that your data is quantitatively good.

Data Analysis:

1. Open RotFreq.m in Matlab.
2. Drag your .dat file from the QPD data into the workspace. A pop up window will open as shown in the figure below. Note that the first row in your data (#Samples…) tells you the sampling frequency you used while collecting the data. Note this down as it will be required later. Then above the tab that says imported data, select Numeric Matrix. On the left, name the data "Data". Then click the green check that says import selection.

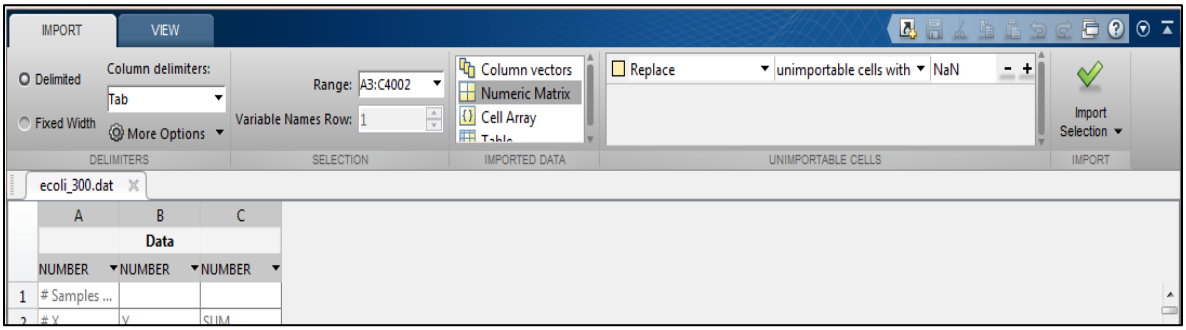


Fig. 12: Importing data for frequency analysis

3. In the editor tab in Matlab, line 1 has sampling frequency Fs. Make sure to change this value according to the sampling frequency of your data.
4. In the editor tab in Matlab, click on the cell that contains lines 1-13. Press control+Enter. A trace of X-position vs Y-position vs time should pop up.

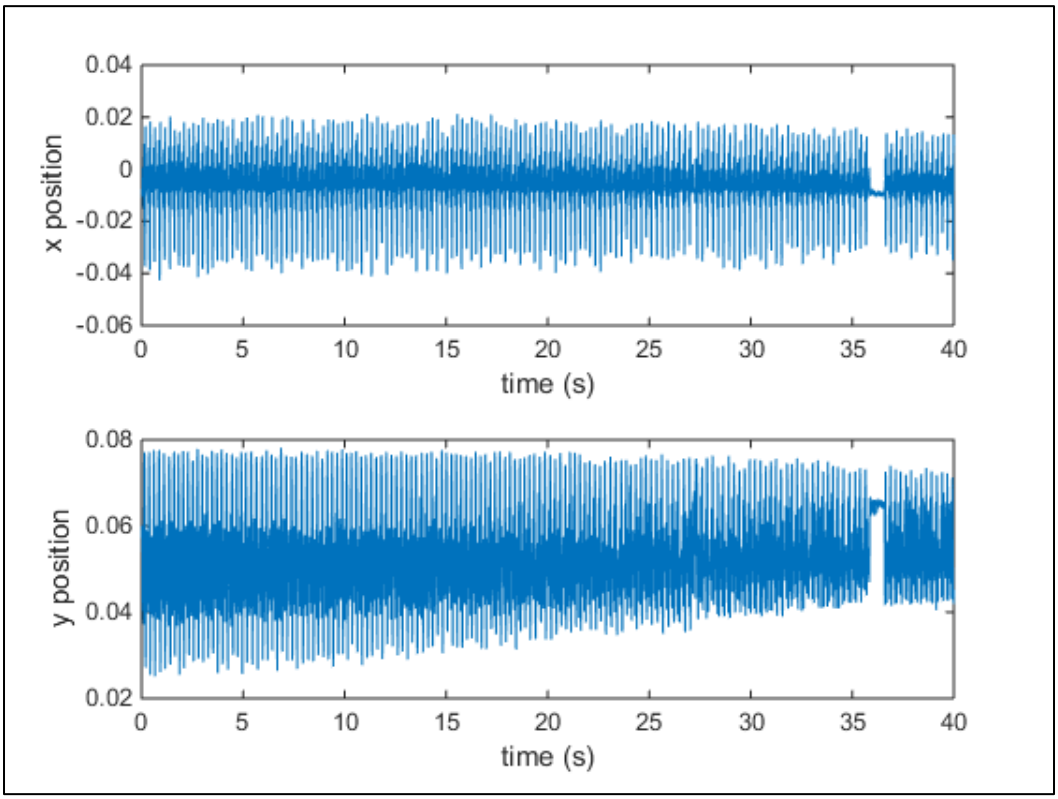


Fig. 13: Position vs time

5. Next we will compute the Fourier transform and power spectrum. Click on the next cell (lines 14-34) and press Control+Enter. As can be seen the workspace values FX and FY, the Fourier transform is a set of complex numbers. By taking the power spectrum we can see which frequency components make up our signal.

6. Finally, in the final cell (lines 35-51) we will plot the power spectrum.

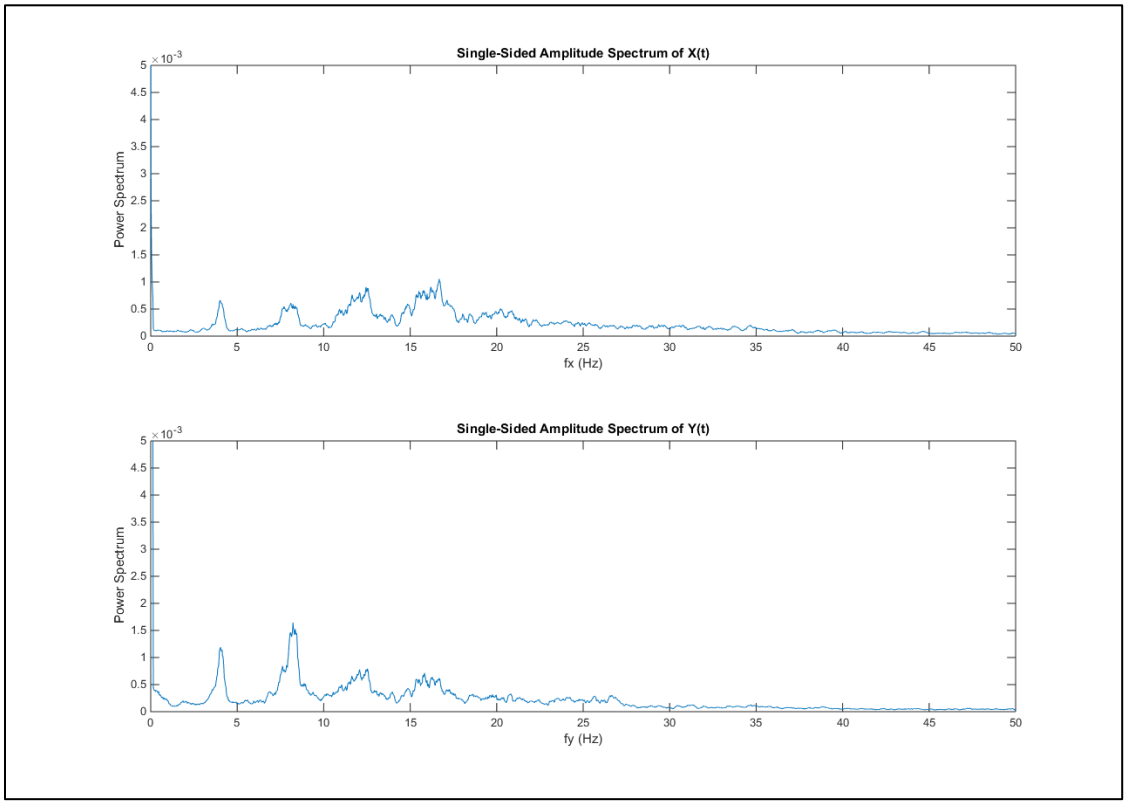
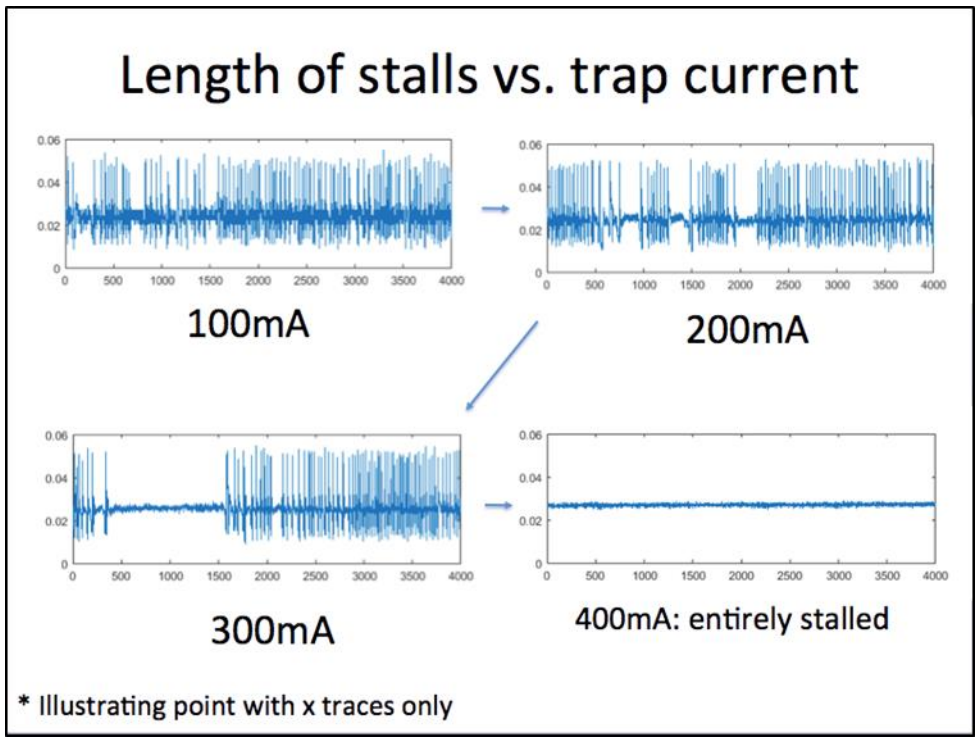


Fig. 14: Power Spectrum

7. For later analyses, you can directly hit the green “play” button in the top panel to produce both the plots together.
8. Following is a sample data. Note that these results are for reference only. Some E. Coli show different behavior than these types.



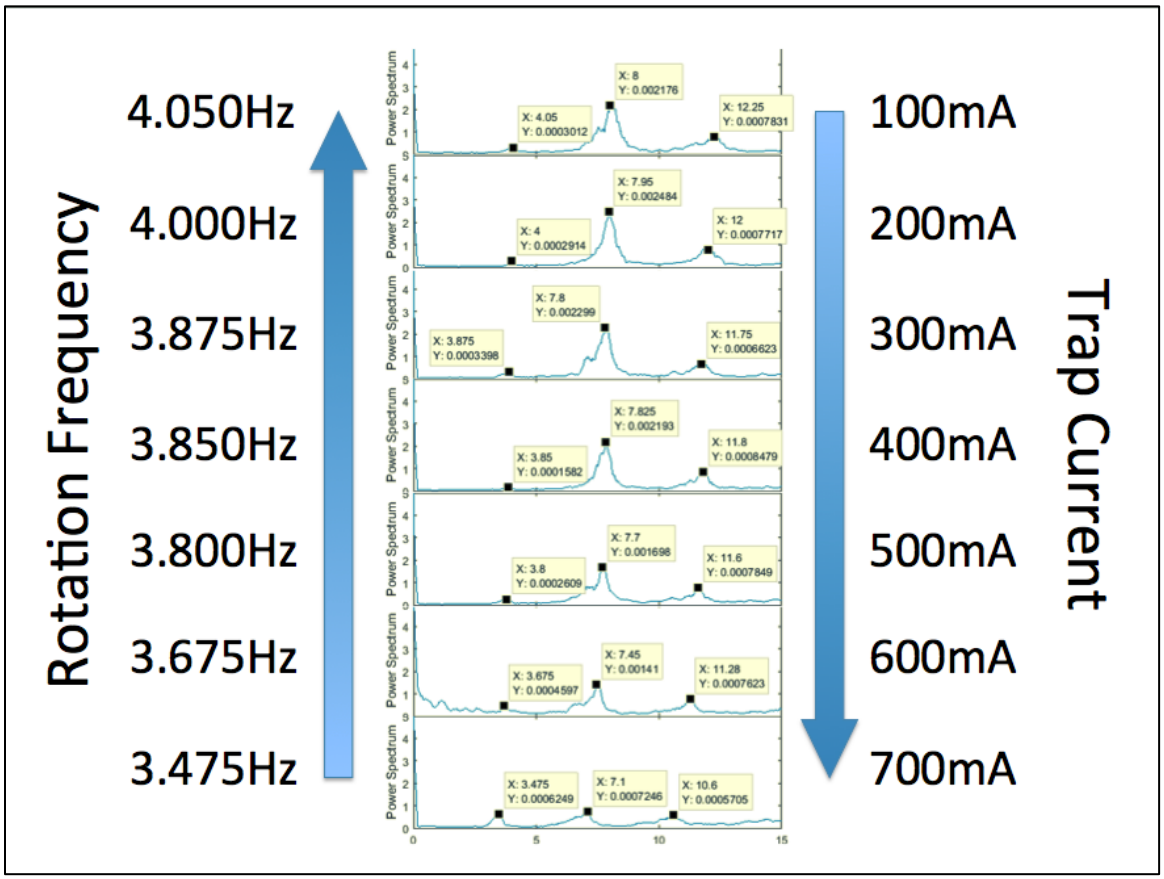


Fig. 15: Sample E. coli rotation frequency analysis data.

Conclusion:

In this week we used the optical trap to study the flagellar dynamics of E. Coli. We calculated the rotation frequency and stall lengths of the bacteria as a function of laser power. Next week, we will use the optical trap to study the transport inside live Onion cells.

5. Internal Transport in Onion Cells

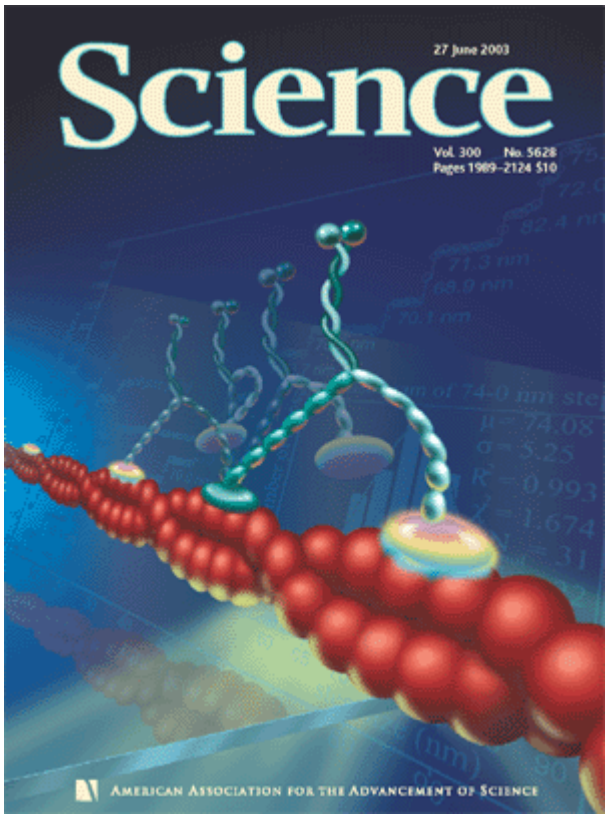


Fig. 16: Myosin V (green), a cellular motor protein, carries cargo within cells by moving along actin filaments (red). It takes 37-nanometer steps by placing one "foot" over the other, as revealed by a fluorophore tag (rainbow-colored oval). Taken from Yildiz et al., Science, 2003.

The fundamental unit of life is a cell. It is a dynamic and complex biochemical assembly, consuming energy to carry out its multitude of functions. Logistics play a crucial role in the health of any cell, as different chemicals and organelles produced in one part of a cell need to be moved for use in a different part. In prokaryotic cells (defined as those cells without a nucleus, such as a bacteria), movement happens by simple diffusion. This is because the typical size is ~ 1 μm. However, in eukaryotic cells which are generally larger in size (~10-40 μm), diffusion itself cannot suffice. This is because diffusion is very slow over longer distances (see lab report question 7). To overcome this problem, eukaryotic cells evolved motor proteins such as kinesin, myosin and dynein which can carry cargo throughout the cytoplasm and move pretty fast (~ 1 μm/sec) on fibers such as microtubules and actin that act as cellular highways. They consume ATP, which is the energy currency of the cell, and mostly walk in a hand-over-hand fashion. (Dynein acts as a walker and caterpillar.) Their motion is directional, with different motor proteins moving in a particular direction on the cytoskeleton. For example, kinesin and dynein both walk on microtubules but in opposite directions, with kinesin carrying the cargo away from nucleus while dynein carrying towards the nucleus. Check out these cool animations from Vale lab at UC San Francisco to understand the walking motion: https://valelab.ucsf.edu/molecular-animations/

In this experiment, we will look at this cargo transport inside onion cells. Typical cargo that is moved is mitochondria (used in the cell to generate ATP), spherosomes (used for synthesizing and storing lipids) and peroxisomes (used for digesting fats and detoxifying ethanol by oxidizing it to acetaldehyde), with the cargo size varying from 0.5 to 3 microns. Myosin is the primary motor protein that moves this cargo in

plant cells and walks on the cytoskeletal fiber called actin which is about 4-7 nm in diameter and many microns in length. The outer layer of these plant cells is composed of a cell wall and most of the interior is filled with vacuole that stores nutrients and maintains hydrostatic pressure (Fig 17). The rest of the components are all found in the cytosol which lies in a thin layer between the vacuole and cell membrane and this is where you will be able to see directed transport carried out by different classes of myosin motors walking on actin fibers. Under the microscope, you will see moving vesicles along the edges and top and bottom surface of the cell if you focus up and down. The cytosol is also found in thin extensions through the vacuole called transvacuolar strands and this could actually be a good place to observe directed transport as it occurs along a narrow line in the center of the cell, providing a good opportunity to trap single vesicles/cargoes.

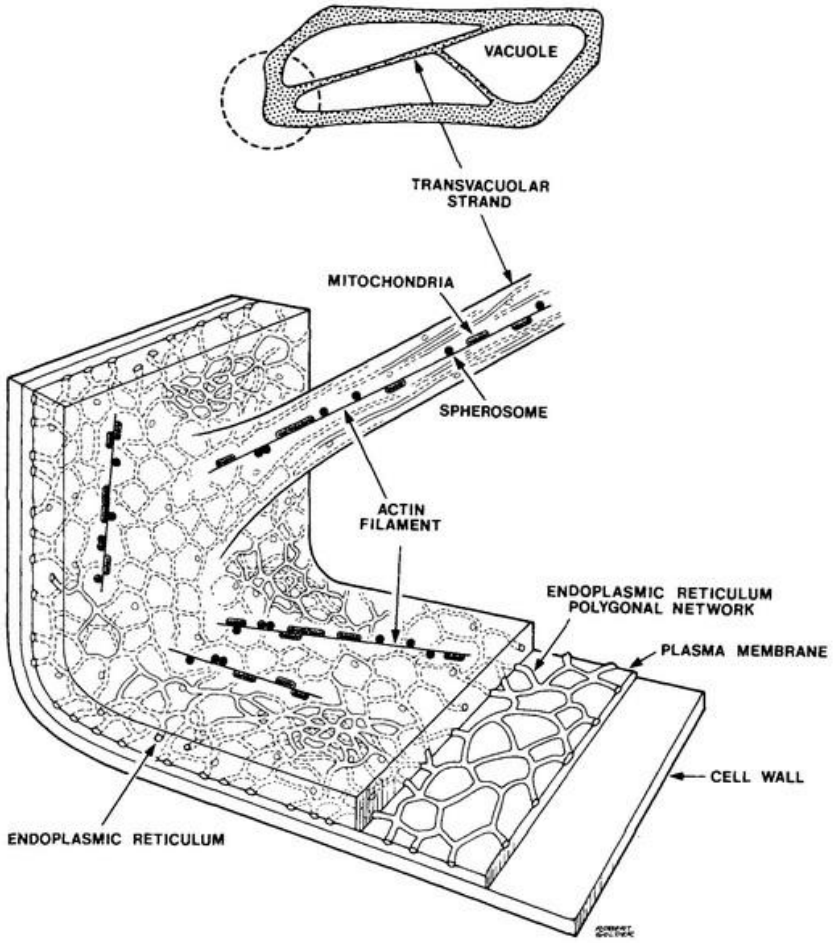


Fig. 17: A 3D cross-section model of an onion epidermal cell, showing actin filaments and vesicles in the narrow bands of cytoplasm within the cell. (Source: N. S. Allen and D. T. Brown, 1988. Dynamics of the Endoplasmic Reticulum in living onion epidermal cells in relation to microtubules, microfilaments, and intracellular particle movement. Cell Motility and the Cytoskeleton 10:153-163 – Wiley-Liss Inc.)

We will calculate the speed of the myosin motors and the force generated by them using our optical trap. For calculating speed, we will measure the QPD voltage as several vesicles pass through the trap. The laser

power should be kept low so that their motion is not hindered considerably. By dividing the vesicle diameter with the time it took to cross the trap, we will get the speed. To calculate the force, we find the laser power at which the vesicle is completely stalled. If we know the stiffness of the trap at this power, we can find the maximum force applied by the trap using Hooke's law, with the maximum displacement being the length of the trap.

Data Collection:

1. Remember to have one person from the team bring an onion for this experiment!
2. Take off the skin and cut out a cube half an inch thick.
3. From the inner side of the cube, peel off the translucent layer carefully. It should come off like a sticker. This is a single layer of cells, so make sure it is peeled off clean to avoid damage to the cells.
4. Place it on a glass coverslip. Make sure to lay it down as flat as possible and avoid any folds and wrinkles for obtaining good quality image.
5. Put a drop of Phosphate Buffered Saline (PBS) solution on it and put another coverslip on top. Blot off excess liquid using a Kimwipe.
6. Place the rest of the onion in a plastic bag and keep it in the refrigerator. Make sure to take it back with you when you leave for the sake of other users of the refrigerator.
7. Put oil on the objective, mount the sample on the stage and focus in to see if you can see individual cells and their internal transport. Once you have spotted it, take some time to appreciate all these dynamics going on in the humble everyday onion.
8. Observe that along with directed motion, there will be some Brownian motion going on as well. Identify a less crowded area of vesicles under directed transport. Try trapping a single vesicle, move it around and release it. There will be a question about this step at the end of this handout, so try to play around and investigate freely as much as you can.
9. Now make the laser power low enough that the motion of the vesicles is not hindered considerably. Let a few (at least 5) vesicles pass through the trap and simultaneously record a movie on the camera (for size estimation) and the QPD data.

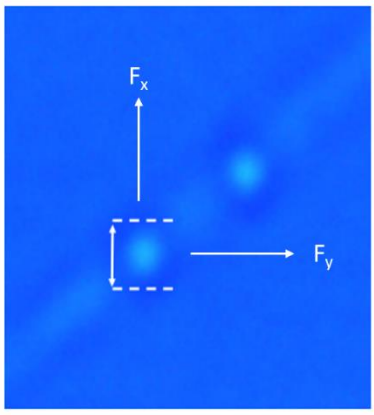


Fig. 18: Example of a good area to study directed transport

10. In a clean dataset, individual passing vesicle should give a "blip" in the QPD signal.

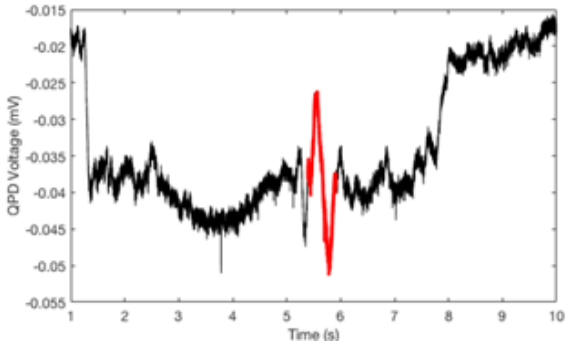


Fig. 19: "Blip" of a single particle passing through the trap

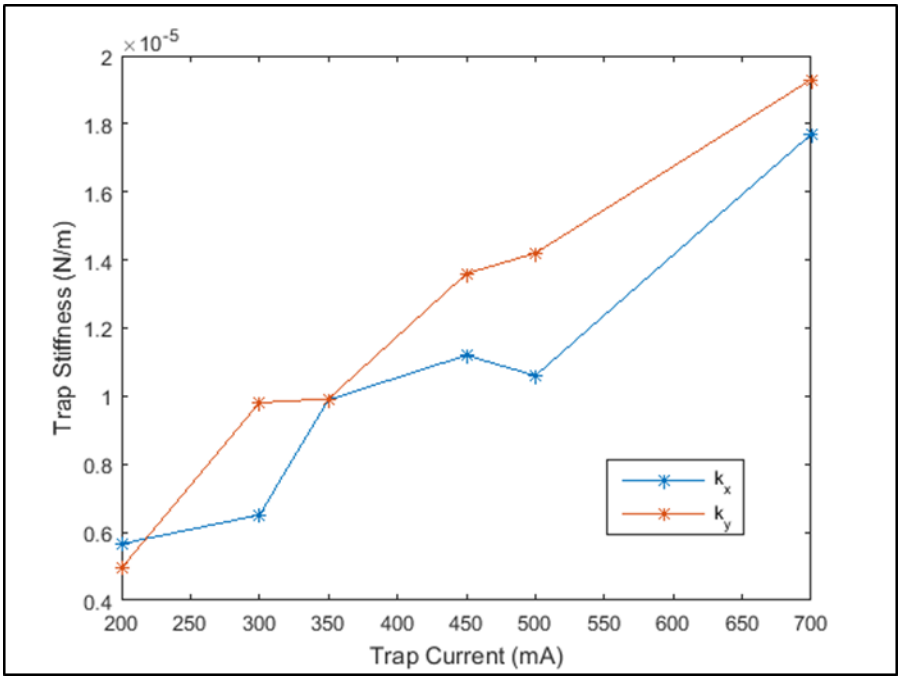
11. For measuring the force, slowly ramp up the laser current (10 mA at a time) until the point where a vesicle under directed motion is completely stopped. Record a movie as you do this to know the direction of motion of the vesicle. Note down the stalling power.
12. Now find a freely diffusing bead (under Brownian motion) and trap it at that same laser power and perform a stiffness calibration using PSD roll-off method to get the force constant at this laser power. Note down the value.
13. Repeat steps 11 and 12 for 4 more vesicles for better statistics.

Data Analysis:

1. For speed calculation, we need to first estimate the vesicle diameter. Open the movie in FIJI software, and by selecting line from the wand-tracing tool, draw a line across the bead.
2. Press "Ctrl+m" to get the length of this line in pixels.
3. Size of one pixel is 52 nm, so you can calculate the actual diameter in microns.
4. Now by looking at the QPD signal, see how much time it took for the particle to pass through the trap by looking at individual blip duration. (You can use excel or the first cell of the rot.m matlab code to plot the data. Make sure to consider the sampling frequency used.)
5. Divide the diameter by this time to get the speed.
6. For force calculation, we will use Hooke's law (Eq. 3). Let us assume the maximum possible displacement to be the radius of the focal spot. Calculate this using Eq. 2. (Note that this equation gives the diameter, so you will have to divide it by 2 to get the radius.)
7. Use Hooke's law to calculate the maximum force exerted by the trap in x and y direction by multiplying the maximum displacement with the force constants that you measured.
8. From the movie, figure out the direction of motion of the vesicle. Referring to fig. 18, if the angle it makes with the y-axis is Θ, then total force $f_{total} = f_x * \sin\Theta + f_y * \cos\Theta$

Lab Report Questions:

1. Fig. 1 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.
2. 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?
3. In the stiffness calculation, we separately calculate k_x and k_y . Why can we not assume them to be same?
4.
   A. How do the assumptions for equipartition method and PSD method for calculating stiffness differ?
   B. Compare the stiffness values at 100 mA laser power for both the methods. Are they the same or different? If different, why?
5. Plot stiffness vs laser power for the PSD roll-off method. You should get a plot as shown below. Interpret your results. How do you expect these values to change if we used 2 micron beads instead of 1 micron?



6. 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.)

7.

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.)

8. 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?

9. 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[14]. Based on this information, can you estimate on average how many motors are actively driving the vesicles that you observed?

Resources and Further Reading

Optical Trapping

1. Arthur Ashkin, "Acceleration and trapping of particles by radiation pressure", Phys. Rev. Let. 24(4), 156 (1970). **This is the original paper debuting the practice of optical trapping using two opposing lasers.**
2. https://en.wikipedia.org/wiki/Arthur_Ashkin
3. Moffitt JR, Chemla YR, Smith SB, & Bustamante C (2008) Recent advances in optical tweezers. *Annu. Rev. Biochem. 77*:205-228
4. Neumann KC et. al. (1999) Characterization of Photodamage to Escherichia coli in Optical Traps *Biophys J.* Nov;77(5):2856-63.
5. K. Berg-Sørensen, H. Flyvbjerg, Power spectrum analysis for optical tweezers. *Rev Sci Instrum* **75**, 594-612 (2004).

Bacterial motility

6. Berg, Motile Behavior of Bacteria. Physics Today (2000) p. 24-29
7. H. Berg, The Rotary Motor of Bacterial Flagella (2003) Annu. Rev. biochem. 72:19-54.
8. Sourjik, V.; Wingreen, N. S., Responding to chemical gradients: bacterial chemotaxis. Current opinion in cell biology (2011) 24*: p.* 262–268.
9. Alon et al., Robustness in bacterial chemotaxis Nature (1999) 397: p. 168-171 chemotaxis.
10. Min, Mears et al., High-resolution, long term characterization of bacterial motility using optical tweezers. Nature Methods (2009) 6: p. 831-835

Internal transport in cells

11. Optical Trapping, UC Berkeley Department of Physics, description of experiment and theory for the Optical Trapping lab.
12. Yildiz et al., Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization, Science (2003) Vol. 300, Issue 5628, pp. 2061-2065
13. Yildiz et al., Kinesin Walks Hand-Over-Hand, Science (2004) Vol. 303, Issue 5658, pp. 676-678
14. Tominaga et al., Higher plant myosin XI moves processively on actin with 35 nm steps at high velocity, EMBO J. 2003 Mar 17; 22(6): 1263–1272.