

Module 4: Optical Trapping

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1 Objectives and Learning Goals

1. Become familiar with the fundamentals of optical trapping.
2. Learn to calibrate the 20.309 optical traps for position detection and force measurement.
3. Use calibration information to observe the rotation of *E. coli* bacteria, and determine the forces required to stop this rotation.

2 Roadmap

1. Familiarize yourself with the optical trapping system and the controls for it.
2. Load a sample slide into the trap for calibrating the position detector, and find a bead attached to the coverglass surface. Run the LabVIEW position calibration VIs to relate the voltage output of the position detector to bead displacement in nanometers.
3. On the same slide, find a bead that is not attached to the surface and trap it. Use three different methods to calculate the trap stiffness and compare their results.
4. Load a second sample slide, containing a suspension of spinning *E. coli* bacteria. Using the position and force calibrations that you have done, measure their rotation rates and calculate the forces required to stop the *E. coli* from spinning.

3 The Optical Trapping System

3.1 Background and theory

Arthur Ashkin developed the first optical traps in the 1970s working at Bell Laboratories.^{1,2} Since the discovery of this technology, optical traps have been applied to pure physics and biological applications from atomic cooling to DNA unzipping. State of the art instruments used for biological applications can apply pico-Newton forces and provide sub-nanometer position resolution.

Optical forces are generated by a laser beam that is focused using a high numerical aperture (NA) objective. These forces come from the conservation of momentum of photons refracting through the trapped object, and will work for any object whose index of refraction is greater than the surrounding medium. A gradient force component draws an object into the center of the trap and a scattering force component pushes the object along the direction of light propagation. Unless there is a steep gradient of light intensity, the scattering force will push the object out of the trap; however, when using a high numerical aperture objective, the gradient of light near the focal point is large enough to balance the scattering force and trap the object. The trap location ends up slightly beyond the focal point.

The slides from Prof. Matt Lang's Nov. 28 lecture on are key material to review. If you'd like to learn more about optical trapping fundamentals, Googling yields excellent results, and you can also skim any of the papers referenced in the footnotes of this document.

Theoretical calculations of the forces exerted by an optical trap on the trapped object generally fall into two regimes: (1) when the trapping wavelength is much larger than the diameter of the

¹A. Ashkin and J. M. Dziedzic, "Optical Levitation by Radiation Pressure," *Appl. Phys. Lett.* **19**(8), pp. 283-85 (1971).

²A. Ashkin, "Optical trapping and manipulation of neutral particles using lasers," *PNAS* **94**, pp. 4853-4860 (1997).

trapped object $\lambda > d$ — called the Rayleigh scattering treatment, and (2) when the wavelength is much less than the diameter of the trapped object $\lambda < d$ — the Mie scattering treatment. Since we are using a $\lambda = 975 \text{ nm}$ laser, and the beads and bacteria we'll be trapping are approximately $1 - 2 \mu\text{m}$ in diameter, our situation is actually at the boundary of these regimes, and we will not concern ourselves with precisely calculating optical forces.

3.2 Safety

3.2.1 Laser radiation

The trapping diode laser has a maximum operating power of 175 mW, placing it in the Class IIIB category. It is important that you familiarize yourself with the beam path and avoid interrupting the path with your hands, any other body parts, or reflective items like rings, watches or other jewelry. There should be no need for you to put your hand in the beam at any time. The black plastic safety cover makes it unlikely that you can do this, but it's important to be aware of the safety concerns.

What is potentially dangerous about this laser is its high power output, combined with the fact that the IR light is invisible. It's absolutely imperative that you ***do not look directly at the beam or any reflection of it.*** Appropriate safety goggles will be made available to you.

3.2.2 Biological materials

Most of the trapping experiments will be run using small diameter glass and polystyrene beads. ***Please use the available nitrile gloves*** both for cleanliness, personal safety and to minimize sample contamination. A genetically altered version of *E. coli* will be used for the experiments in Section 5. These *E. coli* are live and infectious, so glove use is absolutely mandatory. After the experiment is finished, discard the slide containing the *E. coli* sample into the Biohazard Sharps container as directed by the laboratory instructor. As always, wash your hands with soap after completing the laboratory, and do not bring food or drink into the lab.

3.2.3 Operating precautions

In order for the optical trap to work well, a very precise laser alignment is required. Any slight bump of mirrors or lenses can significantly shift this and render an instrument unusable. The laser diode is coupled to an optical fiber, which is sensitive to being crimped, kinked, or otherwise stressed and can be broken if not handled carefully. Be gentle with the optics on the microscope and check with a laboratory instructor before adjusting knobs not explicitly specified in the directions. It is much easier to realign the trap if only one optic has been moved, so if there is a problem, please contact the lab instructor before attempting to solve the problem yourself.

3.3 System overview

Make sure you are able to identify the major system components (*in italics* below). Many of these remain covered by the black plastic safety cover during operation, but you may remove this cover to look over the system as you get started. ***DO NOT run the trap with the safety cover removed.***

The light source used for trapping in our instruments is a *975nm diode laser*. Its beam is steered through optics that expand the beam and direct it into the high-NA $100 \times$ *objective lens* positioned under the sample. The objective focuses the laser to form the trap. The transmitted and scattered light is captured by the *condenser lens*, and reflected onto the *QPD position detector* (more about

this in Section 3.6.1). (*Please do not touch the condenser-height micrometer – all focusing is done by adjusting the objective height position.*)

Along nearly the same optical path, but in the reverse direction, a *white light source* (we use a gooseneck FiberLite) illuminates the sample for visual observation. Its light passes down through the condenser, trap, and objective, and is reflected into a *CCD camera*. This is a simple white-light microscope, very similar to what you built in the previous lab module.

The *3-axis positioning stage* that holds the sample slide is controlled for the x- and y-axes by joystick-driven picomotors. You should not need to adjust the stage z-axis. The stage motors are used during position and force calibration, as well as “driving” the trap around.

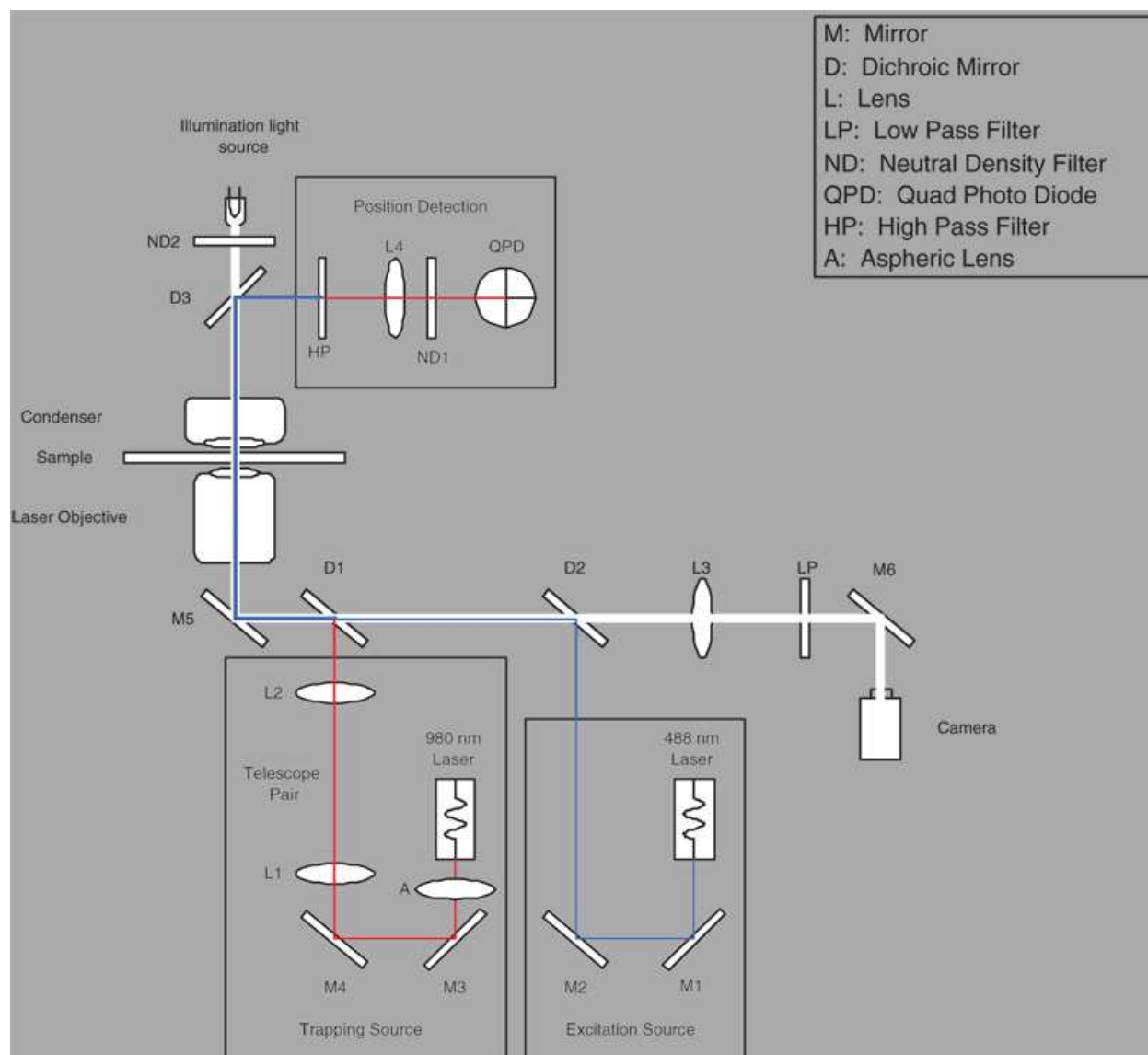


Figure 1: The optical layout of the trapping system (including the optional 488 nm fluorescence branch, which is not used in this lab, indicated by the blue line).

Optical layout

A schematic of the optical layout is shown in Figure 1. The red line gives the path of the trapping laser (from 975 nm source to *QPD*), and the blue line is a second short-wavelength excitation laser (used for fluorescence experiments), which we will not use in this lab. The white light for sample observation follows the broad white line, from *illumination source* to the *camera*.

3.4 Power-on and shutdown

The laser is controlled from a dedicated driver board, mounted on the rack above the trap, shown in Figure 2. One toggle switch, labeled “POWER” turns the driver board on and off, and a second “LASER ENABLE/DISABLE” controls the laser output. We will keep the boards powered up throughout the day, and you can use the second switch enable and disable laser output as needed. If you find your driver powered down, turn it on and allow it to warm up and equilibrate for 5-10 minutes before starting measurements, since the laser power takes some time to stabilize.

When shutting the trap down, simply flip the left switch to LASER DISABLE, and leave the board powered to cool down. The instructors will shut it off at the end of the day.

Direct the FiberLite beam down through the trap’s top port above the condenser and adjust its angle/position for optimal white-light viewing intensity on the CCD camera.

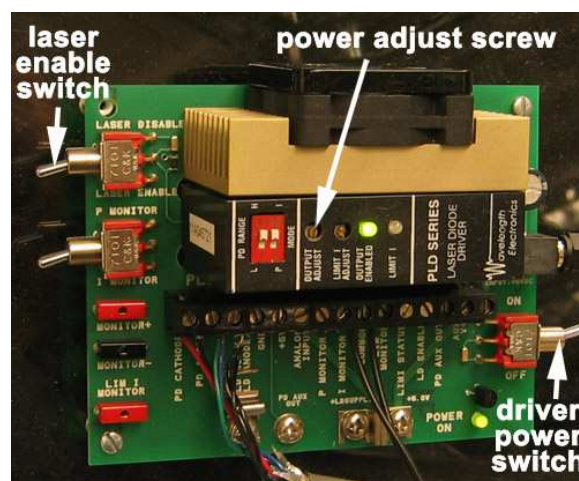


Figure 2: A photo of the laser driver board with the relevant controls labeled. Leave the third switch in the “I MONITOR” position.

3.5 Sample cells

Microscope slides are assembled with coverslips to provide simple fluid chambers for experiments. Wear gloves when making slides, to minimize fingerprints and sample contamination. You will create a flow cell by placing two parallel pieces of double-sided tape across the center of a standard microscope slide about 3–4 mm apart. Place a coverslip over the top of the tape, perpendicular to the microscope slide, and firmly seal the edges (a pen cap or similar plastic object can be used, but avoid pressing too hard and cracking the cover glass). This forms a channel with a volume of $\approx 15\text{ }\mu\text{L}$ as shown in Fig. 3.

The flow chamber contents are exchanged by depositing the new solution on the coverslip on one side of the channel with a pipet tip, then drawing the solution through from the other side. Using the corner of a folded Kimwipe or tissue paper provides suction by capillary action. The sample chamber can be sealed with nail polish or vacuum grease so that the sample doesn’t evaporate during use.

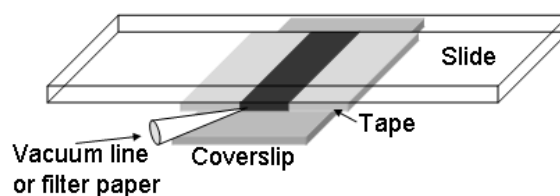


Figure 3: Diagram of a flow channel (black) constructed from a standard microscope slide, two pieces of double sided sticky tape (light grey), and a coverslip (dark grey). The channel is about 3–4 mm wide.

3.5.1 Slide loading

1. Lower the objective lens, so that it will not touch the slide when it is loaded.
2. Place a drop of immersion oil on the microscope objective.
3. Place the new slide in the slide holder with the coverslip down.
4. Raise the objective lens until the oil makes contact with the coverslip.
5. Adjust the position of the slide so that the sample channel is in the center (moving a tape edge into the field of view will make it easier to identify the glass surface).
6. With the white light on, slowly raise the objective until the sample comes into focus.

3.5.2 Unloading and clean-up

1. Lower the objective lens until there is no contact between the oil and the slide.
2. Remove the slide without adjusting the condenser height.
3. Discard the slide in a sharps container if it will not be used again.
4. Remove the oil from the objective lens by placing a folded piece of lens paper on top of the objective, and gently rolling a q-tip over the lens paper to blot the oil.

3.6 Quantitative measurements

3.6.1 Position detection

The laser light scattered from the trapped object is directed onto a Quadrant Photodiode (QPD) to provide a position signal for the bead location. The QPD outputs a voltage signal for the x- and y-axes of bead displacement. These signals must be related to the physical position of a bead, and our goal is to record voltage vs. position data for each axis. More information about this detection method can be found in Gittes and Schmidt.³

3.6.2 Force calibration

For calculating the forces exerted by the trap, the key parameter we need to know is its *stiffness* stiffness, analogous to the spring constant k of a spring-mass system. We will look at three different ways to measure it.

In optical tweezer systems, the usual symbol for stiffness is α . In general, for small displacements x from the equilibrium position, the optical trap is considered to be a harmonic potential, which means that trapped particles experience a Hookian restoring force $F = -\alpha x$, and the potential energy stored due to displacement is $\frac{1}{2}\alpha x^2$.

3.7 Laser power adjustment

For measuring the laser power output, a VI is provided for you – `PowerMeter.vi`. The VI will only work if have the current-monitoring output of the driver board connected to ACH7 of your DAQ board. While running, the VI will give you a laser power readout, and you can adjust it in realtime by turning the OUTPUT ADJUST screw on the driver housing with a small screwdriver (see Fig. 2).

³F. Gittes, C. F. Schmidt, "Interference model for back-focal-plane displacement detection in optical tweezers." *Optics Letters*, **23**(1):7-9, 1998.

4 Experiment 1: Calibration

As is typical for instruments in 20.309, the goal of this section is to relate the detector outputs to physical quantities we would like to measure. However, before you proceed, it's important to consider **laser power dependence**. Both the position calibration, as well as the stiffness of the trap depend greatly on the power output of the 975nm trapping laser. Therefore, you'll want to take data for 3-4 different trap power outputs in the range of 30–150 mW, and determine the (hopefully linear) relationship between power and stiffness, and power and QPD position readout. Keep in mind which of the measurements you make are dependent on accurate position calibration, since you'll need to recalibrate when you change the laser power.

4.1 Position

To calibrate the position detection, a relationship between the QPD output voltages and position data must be determined. In our system, joystick-driven picomotors are installed for x - and y -axis movement. These motors travel 30nm per step. The calibration is performed by finding a 1 μ m bead attached to the glass surface (this sample is deliberately mixed in a high-salt buffer to make the beads stick to the glass by hydrophobic interaction), then scanning it along the x - and y -axis while recording the QPD signal.

A more precise method of calibration involves moving the bead in a grid pattern using either the stage, or a separate optical trap, but our stage positioning does not have enough repeatability to enable this, so we will limit ourselves to measurements near the trap axes.

4.1.1 Sample Preparation

To prepare a calibration slide, which will contain beads affixed to the surface of the coverglass as well as free beads in solution:

1. Load a 1M NaCl bead solution containing a 1:1000 dilution of 10 wt% stock beads (1 μ m silica, Bangs SS03N/4669) into the channel (described above) and allow it to incubate for 5-10 min.
2. Flush the channel with 300 μ L of water.
3. Then load suspended beads, a 1:50,000 dilution of the same 10 wt% beads in water (not a high-salt buffer).
4. Finally, seal the channel with nail polish or vacuum grease to prevent evaporation.

4.1.2 Voltage vs. position calibration

1. Run the QPD Alignment VI. Its upper panel displays the raw x and y signals independently, while the bottom panel shows them plotted one vs. the other, giving an indication of position on the QPD.
2. Using the Joystick, maneuver the bead to the center of the trap. To find the center location, scan the stage in one axis while watching the displacement of that axis signal on the QPD Alignment VI. As shown in Figure 4, when the bead is far away on either side of the trap center, the signal should rest at 0 (if not, your lab instructor will help you realign the QPD). As the bead moves through the trap the signal will move to a maximum value X_{max} , then move through zero to a minimum $-X_{max}$, before finally returning to 0 as the bead leaves the other side of the trap. The goal is to place the bead at the point of maximal sensitivity,

which should occur at the 0 point between X_{max} and $-X_{max}$. This will place the bead in the center of the linear portion of the voltage response as shown in Figure 4.

3. Repeat the above to center the other axis. It may take several iterations of both axes to be confident that the trap is correctly centered.
4. Stop the QPD Alignment VI.
5. Open the Position Calibration VI.
6. Select an axis, enter the number of steps (≈ 100 for a $1\mu m$ bead), and the 30 nm/step calibration factor.
7. Run the Position Calibration VI, and SAVE the calibration data that is generated.
8. Repeat these steps to get a calibration curve for the other axis (assume the same nm/step calibration for both motors).

Notice that we're gathering only enough data to cover just the spatial region corresponding to the line segments lying along the axes, rather than the full x-y plane. This limits the accuracy of our position detection for off-axis displacements.

4.1.3 Data Analysis

Fit the linear central portion of the calibration curve for each axis, and use the fit parameters to determine the voltage-to-position relationship. Why might this calibration not yield the most accurate result, and what changes could you make to obtain a more accurate one? Why do these changes improve it?

4.2 Force

As mentioned, we will use three different methods to quantify the stiffness of the traps. Two of the methods will be familiar from the AFM thermomechanical noise lab, and the third is specific to trapping. This is an excellent opportunity to understand the physical limitations and instrument constraints underlying each method, and to choose the one that you find most accurate/useful.

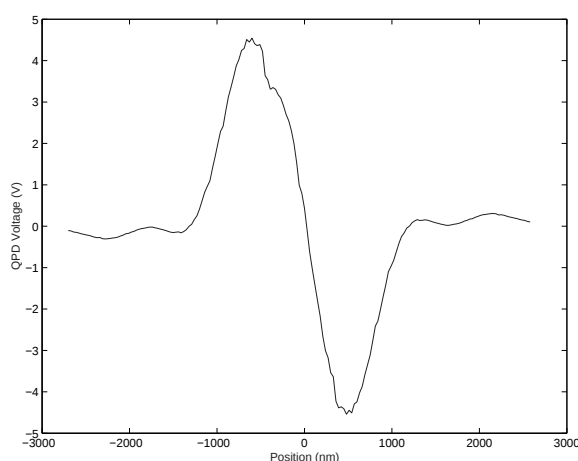


Figure 4: Sample position detection calibration curve for motion along a single axis.

4.2.1 Method 1: equipartition

As you no doubt remember, the Equipartition Theorem states that each degree of freedom in a harmonic potential will contain $\frac{1}{2}k_B T$ of energy, where k_B is Boltzmann's constant, and T is the absolute temperature. Therefore, one method of finding the trap stiffness is by evaluating the variance in position $\langle \Delta x^2 \rangle$ due to thermally induced position fluctuations:

$$\frac{1}{2}k_B T = \frac{1}{2}\alpha \langle \Delta x^2 \rangle \quad (1)$$

This should already be familiar to you from similar measurements done with thermo-mechanical noise in cantilevers. Note that this method requires precise position calibration of the detector, and due to the squared quantity, is sensitive to noise and drift. Further reading on this method can be found in Neuman and Block.⁴

4.2.2 Method 2: noise PSD roll-off

Another way of deriving the trap stiffness is by analyzing the power spectrum of a trapped bead's thermally-induced motions. This power spectrum (in units of [displacement/Hz^{1/2}]) has the form:

$$S_{xx}(f) = \sqrt{\frac{k_B T}{\pi^2 \beta (f_0^2 + f^2)}} \quad (2)$$

where β is the hydrodynamic drag coefficient $\beta = 3\pi\eta d$, in which d is the bead diameter, and η is the viscosity of the medium. Again, similarly to cantilever thermomechanical noise, the roll-off corner frequency can be extracted from a fit of this function to the measured power spectrum. Once known, the corner frequency is related to the trap stiffness as follows:

$$\alpha = 2\pi f_0 \beta \quad (3)$$

For a full derivation and further information about this method, you may want to consult the paper by Allersma and coworkers.⁵

4.2.3 Method 3: stokes drag

A third method of calculating the trap's stiffness is by calculating the drag force $F = -\alpha x$ exerted on a bead as the stage is moved. The most basic formulation of the force exerted on a sphere by fluid flowing past it is

$$\alpha x = \beta v = 3\pi\eta d v \quad (4)$$

where v is the flow velocity. Note that this equation only applies for constant velocity.

4.2.4 Data Collection

The same sample that was used to do the position calibration can be used to do the various stiffness calibrations. However, a bead that is not stuck to the surface must be used for these measurements.

⁴K. C. Neuman, S. M. Block. "Optical trapping." *Review of Scientific Instruments*, **75**(9):2787-2809, 2004.

⁵M. W. Allersma *et al.* "Two-Dimensional Tracking of ncd Motility by Back Focal Plane Interferometry." *Biophysical Journal*, **74**:1074-1085, 1998.

Equipartition and Roll-off – Trap a bead and move the bead away from the surface, using the objective focus adjustment micrometer. Using the stage micrometers, move the bead away from any nearby obstructions (tape, dust, other beads).

Using the SpectrumAnalyzer VI that you are familiar with from the AFM labs, take the power spectrum of a bead's thermal motion at the trap center, recording a separate spectrum for the x- and y-axes. You may also want to record a time-trace of the bead's thermal motion for equipartition method, and compare its results to the power spectrum. These data should enable the two methods of calculating trap stiffness, as described above.

Stokes drag – Open the Stokes VI. Again, trap a bead and move it away from the surface and any obstructions. The Stokes VI will run the bead back and forth at various stage velocities – it's therefore important that the stage micrometers have ample movement left and that there are few other beads in the area.

Run the Stokes VI program, watch the movement on the video capture, if additional beads fall into the trap, discard the data. If this is a consistent problem, ask for help from a lab instructor - a more dilute bead solution may need to be made.

Make sure to run the Stokes program for both the x- and y-axes.

Laser power – You should repeat your force measurements at three or four varying power values to obtain the force-power dependence. Use any of the methods above that you like (preferably all three if you have time), over a range of 25-125 mW laser output. Describe the relationship you see, and explain why you used the force calibration method you chose.

4.2.5 Data analysis

Using the equations described in the theoretical background section, calculate the stiffness of the trap in both X and Y for each of the three methods. Be aware that Equipartition and Stokes measurements use displacement data rather than voltage data for the calculation, so an accurate position calibration is essential. How well do the three stiffness values agree? Which do you trust most?

5 Experiment 2: The *E. coli* Flagellar Motor

5.1 Background

You are working for a nanotechnology startup company that is harnessing the rotational ability of *E. coli* to transport particles in microfluidic channels. A mutated strain of *E. coli* that only spins in one direction has been developed. The company wants you to measure the spinning properties with your photonic force trap microscope.

5.2 Sample preparation

Load 15 μ l of the *E. coli* sample into a flow cell (described above). Do NOT seal this slide with nail polish or vacuum grease.

Load this sample onto your microscope and look for rotating bacteria. If you are unable to locate any, ask the lab supervisor to verify that the sample is still viable. Allow 5 to 10 minutes for the bacteria to settle to the surface and attach. Using a piece of Kimwipe or filter paper for suction, flow pure water through the flow cell to remove the dead cells in the sample, leaving the stuck, rotating bacteria. The *E. coli* flow cells are good for approximately 2 hours (unless the sample evaporates).

5.3 Data collection

1. Using the position detection ability of the optical trap, determine (a) the typical rotation velocity of spinning bacteria, and (b) the distribution of rotational speeds.

Use a very weak trap to detect rotation as the bacterium occludes the beam. The trap is set to a laser power just above the lasing threshold (~ 7 mW) so trap forces will not interfere with rotation. The focus of the optical trap is placed at the edge of the zone through which the rotating bacterium sweeps. The quadrant photodiode voltage signal is collected for both the X and Y axes (the Spectrum Analyzer VI will be useful here).

2. Using the force application ability of the optical trap, determine the stall force of the bacteria. As a first approximation, the force exerted on the *E. coli* bacteria can be assumed to be the same as that exerted on a $1\mu\text{m}$ bead.

Set the laser power to approximately 120 mW (forming a relatively stiff ~ 0.08 pN/nm trap) and move a bacterium into the trap such that the tip of it is trapped in three dimensions and rotation is halted. Reduce the laser power until the bacterium escapes the trap and begins rotating. The approximate trap stiffness at the final power is estimated from a linear fit of previous power calibrations. Assume that displacement from the trap center is ≈ 100 nm when the bacteria escapes the trap.

This measurement can similarly be done by starting with a low power laser and increasing the intensity until the bacterial rotation is halted.

6 Report Requirements

6.1 Calibrations

Position measurements – Fit a line to the middle portion of the calibration curve for each axis to get a voltage-displacement relationship. Show a representative plot for each axis of stage motion showing the experimental calibration curve and your fit line. Indicate your fit parameters, and how you derive the volts-to-nanometers relationship from the data. (Please do not include separate plots/fits for all powers and both axis, unless you can come up with an efficient/compact way to display the data)

If you performed position calibrations at different power values, plot an approximate power-calibration dependence for each axis, which you can use in your force measurements.

Force measurements – Use the position calibration values from both axes for your force calibrations. Do each of the following for both the x - and y -axes, at each of the power levels you recorded:

1. Determine the trap stiffness values from the Equipartition and PSD methods. For equipartition you can use either the time-domain variance value $\langle x^2 \rangle$, or the integral of the PSD plot (these should match). For the PSD roll-off method you'll need to fit the spectrum and find the corner frequency (you don't need to worry about magnitude scaling, only to find f_0). Relevant formulas are provided in Section 3.6.2.
2. Make a linear fit to your Stokes displacement values at each power level (Δx vs. velocity, and therefore force) to derive a stiffness value.
3. As before, show representative plots of each type of dataset and relevant fits, but don't show separate plots for each axis at all power levels unless you find a compact representation.
4. Compare the stiffness values obtained by the three methods at each power, and explain which you feel is most likely to be reliable and why.

Finally, for each axis, create a single plot that shows the stiffness values calculated using all three methods as a function of laser power – do you see a linear trend? Does this help determine which force calibration method is most trustworthy?

6.2 The *E. coli* flagellar motor

Rotation speeds – Show a representative PSD and time-trace of a rotating *E. coli* bacterium. Do the PSDs of your X and Y QPD voltages agree?

From recording the rotational speeds of about 20 bacteria, make a histogram of their speeds (use a bin size of 2-3 Hz) – is the distribution of speeds centered around one value, or bi-modal? (Optional: speculate about why this might be the case.)

Stall forces – From the optical power needed to stop the rotation of several bacteria, and your force calibration values, find an approximation to the force exerted by the bacterial flagellum.