Optical Trapping in the Teaching Lab

1 Lab Objective

1. Become familiar with the fundamentals of optical trapping.
2. Learn to calibrate the 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 The Optical Trapping System

2.1 Background

Arthur Ashkin developed the first optical traps in the 1970's working at Bell Laboratories. 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.

If you'd like to learn more about optical trapping fundamentals, Google 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 greater than the diameter of the 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 $\lambda = 975nm$ laser, and the beads and bacteria we'll be trapping are approximately $1 - 2\mu m$ in diameter, our situation is actually at the boundary of these regimes, and we will not concern ourselves with precisely calculating optical forces.

2.2 System Overview

Make sure you are able to identify the major system components (*in italics*). 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 *objective lens* positioned under the sample. The objective focuses the laser to form the trap, and the transmitted and scattered light is captured by the *condenser lens*, and reflected onto the *QPD position detector* (more about this in Section 4.1).

Along nearly the same optical path, but in the reverse direction, a *white light source* is used for optical 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.

The *3-axis positioning stage* that holds the sample slide is controlled along two axes by joystick-driven picomotors. These will be used during position calibration, as well as "driving" the trap around.

Optical path

A schematic of the optical layout is given in Figure 1. The red line gives the path of the trapping laser (from source to QPD), and the blue line 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 light source to the Camera.

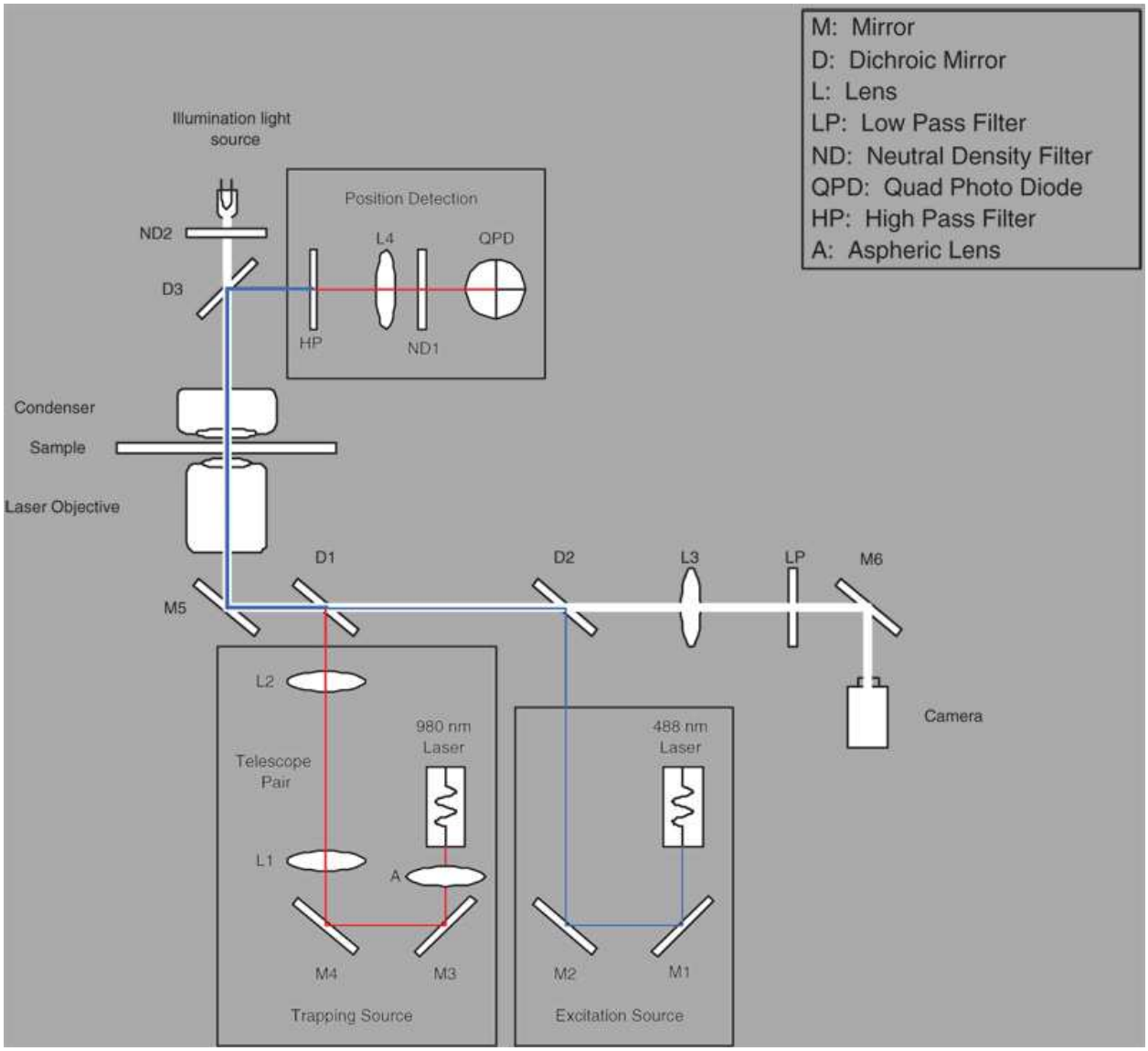

Figure 1: The optical layout of the trapping system (including the optional 488nm fluorescence branch, which is not used in this lab, indicated by the blue line).

2.3 Safety

Laser Radiation

The trapping diode laser has a maximum operating power of 175mW, 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. It is imperative that you ***do not look directly at the beam or any reflection of the beam***. Appropriate safety goggles will be made available to you. There should be no need for you to put your hand in the beam at any time.

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 reduce sample contamination of samples. A genetically altered version of *E. coli* will be used for the last section of the laboratory. 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 as directed by the laboratory instructor. As always, wash hands with soap after completing the laboratory, and do not bring food or drink into the lab.

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 a fiber optic; this fiber 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 Experiments Overview

1. Using a sample slide for calibrating the position detector, find a bead attached to the glass surface. Run the LabVIEW position calibration VIs to relate the voltage output of the position detector to bead displacement in nanometers.

2. Using the same slide, up to three different methods can be used to calculate the trap stiffness and compare their results.

3. A different sample slide contains a suspension of *E. coli* bacteria. Focus on the sample and find spinning 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.

4 Lab Procedures

4.1 Position Detection

For detection, the laser light scattered from the trapped object is captured with 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 the goal of this part is to record voltage vs. position data for each axis. More information about this detection method can be found in Gittes and Schmidt[1].

In order to calibrate the position detection, a relationship between the QPD output voltages and position data must be determined. On our system, joystick-driven picomotors are installed for x- and y-axis movement. These motors have been pre-calibrated and travel $30nm$ per step. The calibration is performed by finding a $1\mu m$ bead attached to the glass surface (the high-salt buffer of this sample makes the bead stick to the glass by hydrophobic interaction), and then scanning it along the x- and y-axis while monitoring 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.

Voltage vs. position measurement

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.

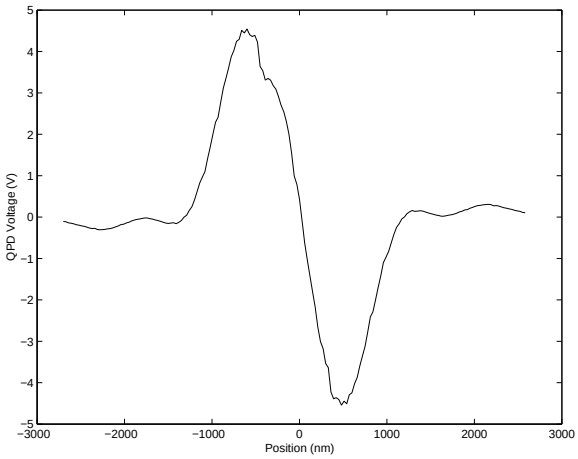

Figure 2: Sample position detection calibration curve for motion along a single axis.

2. Using the Joystick, maneuver the bead to the center of the trap. To find the centering location, scan the stage in one axis while watching the displacement of that axis signal on the QPD Alignment VI. As shown in Figure 2, 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 realign the QPD). As the bead moves through the trap the signal will move to some 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

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

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 2.

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).

4.2 Force Calibration

For calculating the forces exerted by the trap, the key parameter we need to know is its stiffness. We will look at three different ways to measure it.

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 $\frac{1}{2}\alpha x^2$. Here α is called the *stiffness* of the trap, and is analogous to the spring constant k of a mass-spring system.

Equipartition

As you no doubt remember, the Equipartition Theorem states that each degree of freedom in a harmonic potential will contain $\frac{1}{2}k_BT$ of energy. 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:

$$\frac{1}{2}k_BT = \frac{1}{2}\alpha \left\langle \Delta x^2 \right\rangle \tag{1}$$

This should already be familiar to you from similar measurements done with thermomechanical noise in cantilevers. Note, however, 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[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/$\text{Hz}^{1/2}$] has the form:

$$S_{xx}(f) = \sqrt{\frac{k_BT}{\pi^2\beta(f_0^2 + f^2)}}\,, \tag{2}$$

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:

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

$$\alpha = f_0 2\pi\beta \ . \tag{3}$$

For a full derivation and further information about this method, you may want to consult the references in the footnotes[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 is

$$\alpha x = \beta v = 3\pi\eta d v \ , \tag{4}$$

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

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. If the measurements you make are dependent on accurate position calibration, you'll need to recalibrate when you change the laser power.

Data Collection

Equipartition and Roll-off – Open the Equipartition VI. Trap a bead and, using the objective focus adjustment micrometer, move the bead away from the surface. 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. This data will yield two ways 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 – thus it's important that the stage micrometers have ample movement left and that there are few beads in the area.

Run the Stokes VI program, watch the movement on the monitor, if additional beads fall into the trap, discard the data. If this is a consistent problem, ask for help from a lab instructor.

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 prefer, over a range of 25-125mW laser output. Describe the relationship you see, and explain why you used the force calibration method you chose.

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

4.3 *E. coli* Rotation

Goals

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.

2. Using the force application ability of the optical trap, determine the stall force of the bacteria. Assume that displacement from the trap center is $100nm$ when enough force is applied to hold the bacteria.

Data Analysis

1. Rotation: Take the power spectrum of your trace to determine the rotational frequency. Plot both the PSD and the trace. Does the PSD of your X and Y QPD voltages agree?

   Describe the distribution of rotational speeds. Why do you think this is?

2. Stall Force: For a first approximation, the force exerted on the bacteria can be assumed to be that exerted on a $1\mu m$ bead.