Evaluating Drag on a Sphere and other Optical Trapping Experiments

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Abstract

A three part experiment using the undergraduate optical trap is used to expose students at the advanced high school or undergraduate level to basic instrument techniques and physics principals.

Students are given a hands-on opportunity to use cutting edge optical trapping instrumentation to manipulate samples and make precision force and position measurements. The first step is calibration of the instrument accomplished by trapping a small bead and measuring the nm scale position displacements caused by the thermal energy in the system. The equipartition theorem is used to connect the variance in these positions to the stiffness of the optical trap.

The drag coefficient for a sphere is determined in the second portion of the experiment. Students use integrated motion control on the instrument to monitor how the velocity of the fluid flow around the bead affects the displacement of a spherical bead out of the optical trap beam. Experimentally determined drag coefficients are compared to theoretical values.

Stall torque of a bacterial flagellar motor is measured in the final experimental segment. The rotation of a genetically modified strain of e.Coli, which sticks to the surface of a slide and spins, is stalled out using the trapping laser. Students are able to accurately estimate the stall torque of the molecular motor using knowledge of the optical trap calibration and the characteristics of e.Coli.

Materials

- Optical trap
- 3.12 μm diameter silica microspheres (Bangs Laboratories SS05N)
- 1M NaCl
- KAF95 E.coli
- Growth media for ecoli (LB/Bactotryptone, 100μg/mL Ampicilin)

Laboratory Notes

This laboratory was run for the WTP program on July 2, 2007 for a pair of pre-senior year high school students. Two teaching assistants were present to guide the students through the lab module, as the primary focus was understanding the physical principals being discussed rather than becoming intimately familiar with the operation of the optical trap. Three hours were budgeted for the laboratory, including calculations and time to make a short presentation on results, which was sufficient to complete all segments. A

pre-laboratory worksheet with background information for the students was prepared by Dr. Hughey and is available from www.openwetware.org/optical_trap. The optical traps used were part of the BE.309 undergraduate instrumentation laboratory and are described fully in (Appleyard et. al, Amer. J. Phys. **75**(1), 5-14 (2007)). Additional information is available in the AJP article concerning construction, alignment, and use of the optical traps as well as relevant background techniques and details on bacterial culture.

Preparations

In order to prepare this laboratory for students E.coli samples need to be started 18-24 hours in advance of the planned lab time. Frozen KAF95 is inoculated into a liquid growth medium with 100µg/ml ampicilin and grown at 37°C.

In addition to starting the bacterial culture, instrument calibration and familiarization is recommended. Two bead solutions need to be made for the experiment.

1. 1:10000 dilution of the 3.12µm beads in H_2O
2. 1:1000 dilution of the 3.12µm beads in 1M NaCl

These solutions should be stored at 4°C and should last indefinitely.

For this module, a position calibration is provided to the students to keep within the allotted time. Details on the specific position calibration procedure can be found in the AJP paper, however, in brief they consist of:

1. Adjusting the laser power to 20-30 mW (low stiffness)
2. Loading a sample slide with the 1:10K bead dilution in water.
3. Trapping a bead and zeroing the detector using the `QPD Alignment Tester.vi`
4. Run the `DragCoefficient.vi` and test to see that adequate displacement is detected during the drag runs. Power is adjusted to either increase displacement or to prevent beads from falling out of the trap.
5. Loading a sample slide with the 1:1K dilution of beads in NaCl
6. Centering a bead stuck on the surface in the trap using the `QPD Alignment Tester.vi`. A centered bead should be at ~0V in both X & Y
7. Using the `Positon Calibration.vi` with a setting of ~300 steps (9µm of travel) to scan the bead through the trap beam in one axis.
8. Repeating the process for the other axis.
9. Fitting the linear portion of the acquired data and extracting the slope to get a nm/V calibration parameter for each axis.
10. Optionally, take multiple calibrations and generate an average value.

This calibration value will differ from instrument to instrument and with the trapping laser power, so once taken, the laser power should not be further adjusted. For the instrument used the X calibration was 154 nm/V and the Y calibration was 112 nm/V.

At this point, it is simple to collect the data for the Stokes drag portion of the experiment to familiarize oneself with the vi operation and calculations.

For the e.coli segment, a stiffness vs. power calibration is required for a 1μm diameter silica sphere. This can be completed as described in the AJP article, or any prior calibration can be used. This calibration does not need to be exact as it will be used as part of the stall torque estimation.

On the day of the laboratory a 1:10K bead in water dilution slide can be loaded on the instrument and the detector zeroed for a trapped bead.

Experiment

Equipartition

The goal of this segment is to expose students to the basic operation of the optical trap, gain some familiarity with the optical setup and computer interface, and determine the stiffness of the trap using the equipartition theorem. A short discussion on the basics of optical trapping is very useful, building on and refreshing the information presented in the pre lab. Potential points of discussion include: conservation of photon momentum used to form the trap, Hookean spring like behavior of the trap, potential energy well, equipartition theorem, thermal energy, position and force resolution of an optical trap, connection between measuring position and measuring force. This is best coupled with a tour of an optical trap with the cover off detailing the parts of the instrument highlighting components that form a basic microscope, focus an direct the optical trap, and detect the position of trapped beads.

A slide with the 1:10K dilution of 3.12μm beads in water is loaded into the trap. The instructor can load and focus the slide. Students are given the opportunity to trap and move beads around with the instrument, adjust focus, and drive the stage using the picomotors. When they are comfortable with this very basic operation they are instructed to trap a bead and the instructor will zero the detector signal out (this can be done ahead of time if the slide is pre-loaded on the instrument). A trace of the X&Y voltage signals is taken using `Cont Acq to Spreadsheet File.vi`, acquiring ~5 seconds of data at 100kHz. The measurement is repeated at least 3 times.

The variance of each data trace is calculated using the matlab script `Variance.m`. This function is called as "Variance('filename.txt')", it plots the raw voltage data from the detector, adjusts the average to zero, plots the adjusted data, and calculates the variance for both the X & Y channels. In order to get accurate variance values in nm^2, the position calibration values must be updated in the script to reflect those just measured.

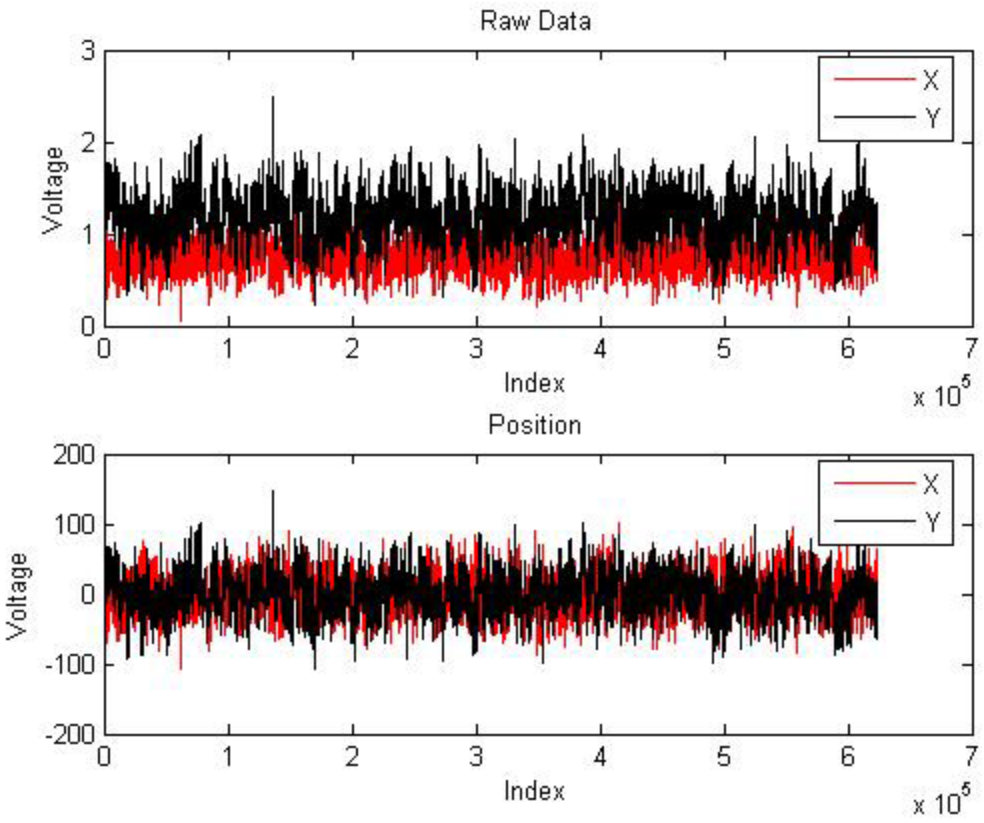

Figure 1. Raw and adjusted voltage traces from the Matlab script for variance calculations.

Stiffness is calculated from the variance values using the equipartition theorem.

Equipartition Calculation

$$k_B := 1.38 \cdot 10^{-23} \frac{J}{K} \qquad T := 298K \qquad var := 5.865 \cdot 10^{-16} m^2$$

$$\frac{1}{2} k_B \cdot T = \frac{1}{2} \alpha \cdot var$$

$$\alpha := \frac{k_B \cdot T}{var} \qquad \alpha = 7.012 \times 10^{-3} \frac{N \cdot 10^{-12}}{nm}$$

Trap stiffness between 0.03 pN/nm and 0.005 pN/nm are reasonable for the 3.12μm beads. Discussion of the stiffness value can help connect the common units of optical trapping (pN/nm) to more physically relevant forces such as intermolecular bonds or protein interactions. This also can lead to a discussion of the force applied vs.

displacement from the center of the trap as well as the maximum displacement attainable in the optical trap (~100nm) due to the size of the beam waist.

Stokes Drag

Usually, the Stokes drag method is used to calibrate the stiffness of an optical trap, and this value is compared to stiffness calibrations from the two alternate methods, equipartition and roll off (see AJP article). Here, we are going to use the calibration from the equipartition experiment just completed to back out the drag coefficient for a sphere rather than use a theoretical expression for the drag on a sphere to extract the stiffness of the trap.

Stokes drag is based on a basic force balance, and is easy to visualize. The drag force from a fluid flowing around the bead is balanced with the restoring force from the optical trap (Figure 2).

$$\alpha x = \beta v$$

Here, α refers to the stiffness of the trap, x, the displacement from the center of the trap, v, the velocity of the fluid and β is the drag coefficient. The theoretical expression for the drag coefficient for a sphere is $\beta = 6\pi\eta r$, where r is the radius of the trapped bead, and η the viscosity of the medium (water). The drag coefficient for a sphere is Fluid velocities are created by holding the bead in the trap and moving the fluid around it by translating the stage at a set speed with the picomotors.

Students will measure the displacement of a bead at a variety of velocities and plot the results. From the slope of this plot an experimental value of the drag coefficient for a sphere can be found, which is then compared to the theoretical values.

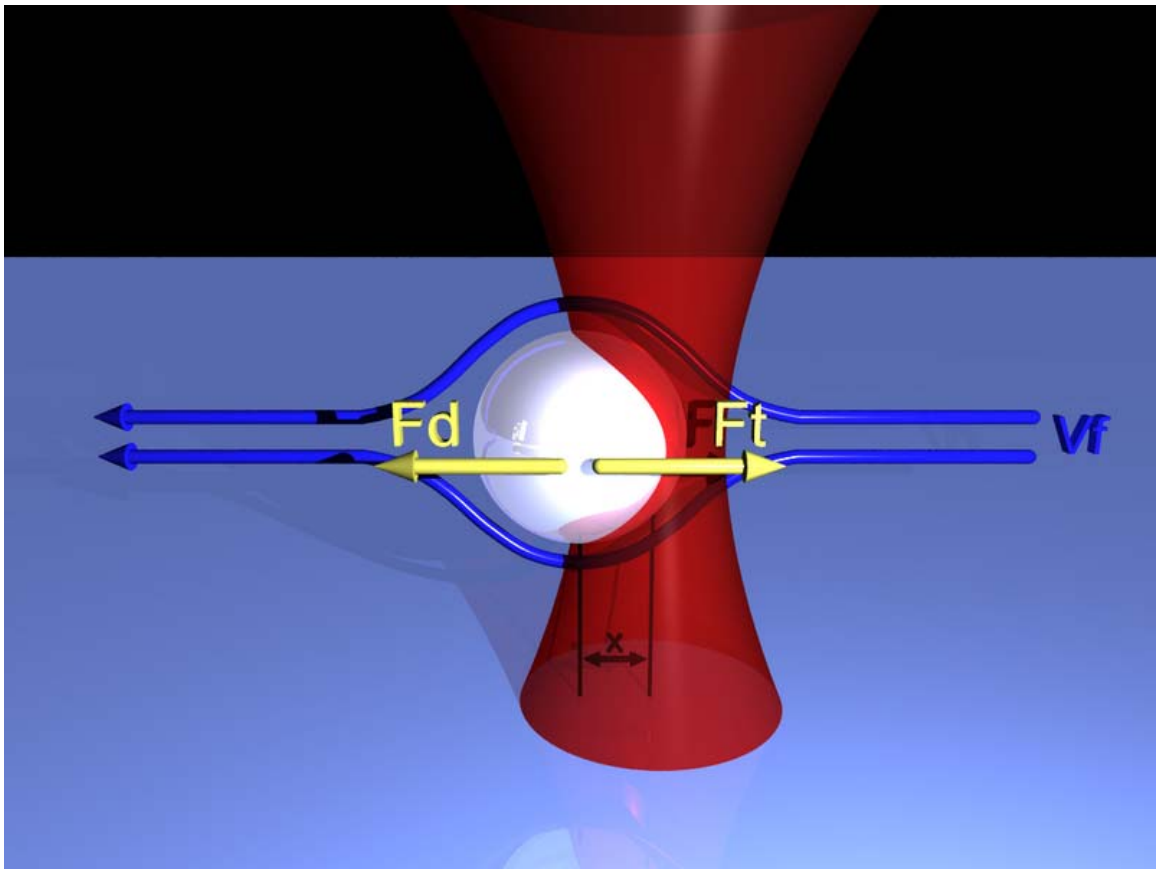

Figure 2. Cartoon of Stokes drag using an optical trap. The drag force, Fd, on the sphere from a fluid flow of velocity, Vf, causes a displacement, x, out of the center of the trap, resulting in a restoring force from the trap, Ft.

The same slide used for the equipartition measurement can be used for the Stokes drag experiment. A low bead concentration is necessary to prevent other beads in solution from interfering with the trap during a measurement. Students find and trap a bead, then run the `DragCoefficient.vi` program. This program will run the picomotors at a series of increasing speeds (defaults are 7 speeds of: 200, 400, 600, 800, 1200, 1600, 2000 Hz). The students should be able to see increasing movement of the bead as the speeds increase. The `DragCoefficient.vi` will prompt to save a data file for the bead displacements. Students can repeat the measurement for the other axis, or take a replicate on the same axis if they desire.

The data file contains 14 columns with 500 data points in each column. The odd numbered columns contain the voltage from the detector during the pause, and the even columns contain the voltage data for the movement portions (column 2 at the first speed, column 14 at the last speed). A Matlab script is provided to convert this file format into a 2 column arrangement with the first column containing a index number (1:7000) and the second column a concatenation of the voltage data (columns 1:14 of the original). This converted file will have "_linear" in the name and can be loaded easily by logger pro or excel. Additionally, the Matlab script plots the index vs. voltage for the experiment (blue) and the average for each segment (red). The average values for each segment are

also printed on the plot window, the top row has the value for the pause segments, the bottom row the movement segments, and the middle row the difference between the movement and the preceding pause segment. The pause segments serve as a baseline, and should be near 0 V if the detector was recently zeroed.

Students can either use the plot from the matlab script to get the voltage differences for each movement segment or extract them using excel or logger pro and the "_linear" data file.

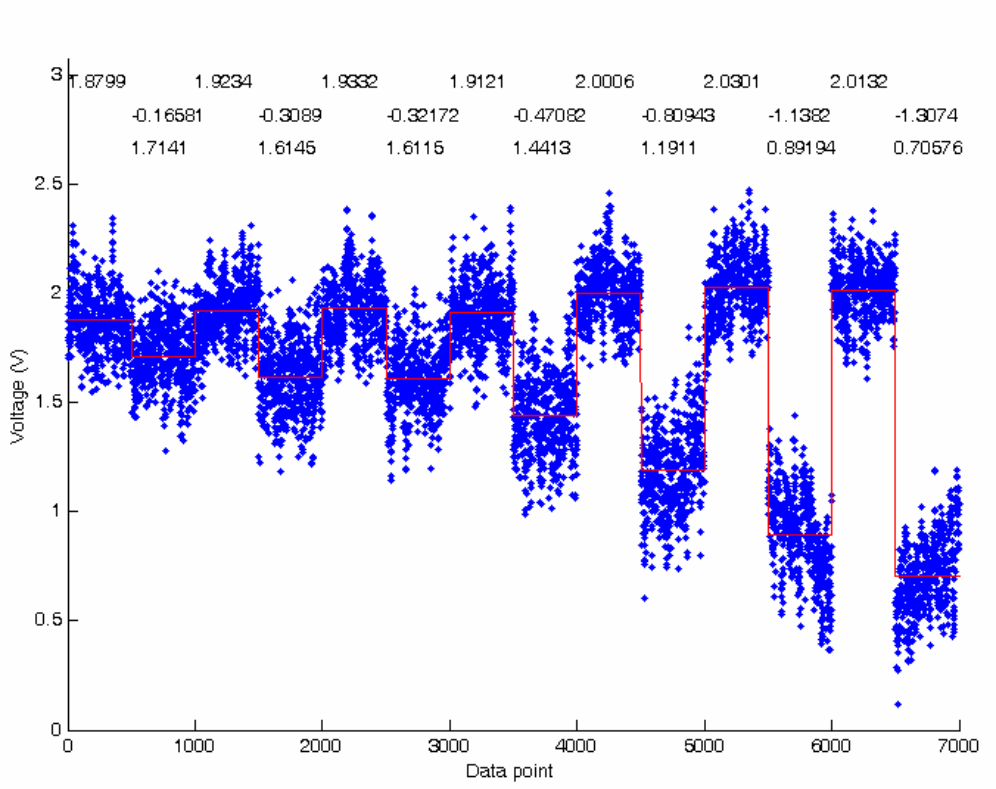

Figure 3. Output from `plotStokes.m` indicating raw data (blue dots), average value (red line), average baseline voltage (top row), average movement voltage (bottom row), and voltage difference (middle row).

The students need to plot displacement (in nm) vs. velocity (nm/s). The slope of this plot will be β/α. Displacements are calculated by multiplying the voltage differences by the position calibration value. The velocity values are taken from the motor speeds set in the `DragCoefficient.vi`. The motor speeds in the .vi are in Hz, and they should be converted to nm/s. For the New Focus picomotors used in the undergrad trap article the following conversion can be applied:

$$\mathrm{vel}\left(\frac{\mathrm{nm}}{\mathrm{s}}\right) := 35.9 \cdot \mathrm{Rotation(Hz)} + 85$$

The plot should be nearly linear, and a fit, forced through zero, is used to extract the slope. Students can use logger pro or excel to fit the data.

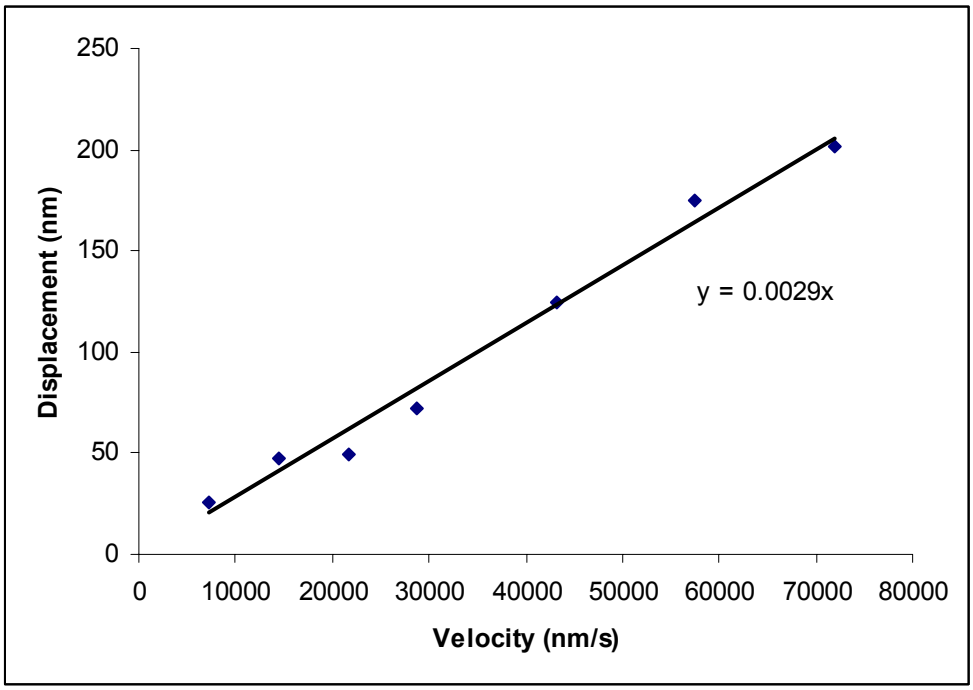

Figure 4. Displacement vs. Velocity plot for determining an experimental value for the drag coefficient. The slope from a linear fit forced through zero is used in the following calculation.

The slope is then divided by the stiffness to get a value for the drag coefficient (units of Ns/m are easy to work with).

Experimental Drag Coefficient

$$slope_m := 0.0029\frac{s}{m} \qquad \alpha := 7.012\,10^{-3}\cdot\frac{N\cdot 10^{-12}}{nm}$$

$$\beta_{exp} := slope_m\cdot\alpha$$

$$\beta_{exp} = 2.033\times 10^{-8}\,\frac{N\cdot s}{m}$$

The theoretical calculation of the drag coefficient can be completed using the formula noted above, assuming the viscosity of water is ~ 0.00089 Pa*s.

Drag Force Calculation

Viscosity of water at room temperature

$\eta := 0.0008\text{Pa}\cdot\text{s}$

Radius of beads used (3.21 μm diameter)

$r := \frac{3.21\cdot 10^{-6}\text{m}}{2}$

$\beta := 6\cdot\pi\cdot\eta\cdot r \qquad \beta = 2.693\times 10^{-8}\,\frac{\text{N}\cdot\text{s}}{\text{m}}$

Although this portion of the experiment has only be run a few times, the results seem to agree within a factor of 3 or much better, which is excellent agreement considering that surface effects are not taken into account.

E.coli

The spinning E.coli are the final segment the students run. This gives the students the opportunity to estimate the stall torque of the bacterial flagellar motor on a single molecule scale. It is a fantastic demonstration of the power of the optical trap as a monitoring device, for determining the rotation speed of the bacteria, and as a force probe. Extensive experimental details can be found in the AJP article, and a summary is provided here.

E.coli should be loaded into a flow cell about 10 minutes prior to use, giving the bacteria time to settle to the surface and attach. The sample is loaded on the optical trap and students can scan around the stage to locate spinning E.coli. Usually a large amount of bacteria is in solution and just clogs up the optical trap, drawing 50μl of water through the sample chamber (using a piece of paper towel to wick the water through) can help eliminate the detritus.

Students are shown how to modulate the output power of the optical trap, and are encouraged to drop the power below the level where it interferes with bacterial rotation. The trap is positioned over the outer radius of rotation and the position detection voltage signal is monitored with `Cont Acq to Spreadsheet File.vi`. A short voltage vs. time plot is saved and the students are encouraged to estimate the rotation speed by looking for periodic structures in the data (this should be very obvious, if not, reposition the trap or try a new bacteria).

The students now position the trap at the outer edge of rotation and increase the laser power until the bacteria just stalls its' rotation. The power of the optical trap can be measured using the `Power Meter.vi` or using a power meter. Power meter measurement necessitates removal of the cover and should be done by an instructor.

Repetition of the stall using the optical trap can be captured on video for presentation purposes using the IC Capture program. A difficulty in codec compatibility came up during the experiment and should be ironed out for future use (Cinepak is too slow, LEAD had compatibility issues).

Students now take the power required to stall the bacteria and are led through a back of the envelope estimation of the stall torque.

Torque is force x lever arm. To eliminate the vector cross product, we will assume the trap is applying a force perpendicular to motion, a very reasonable assumption considering the geometry of the system.

Force for the optical trap is $\alpha * x$, and we don't have a value for either of these. First, α varies with the laser power, since the forces are due to conservation of photon momentum. We can use a prior calibration of the instrument to get this relationship.

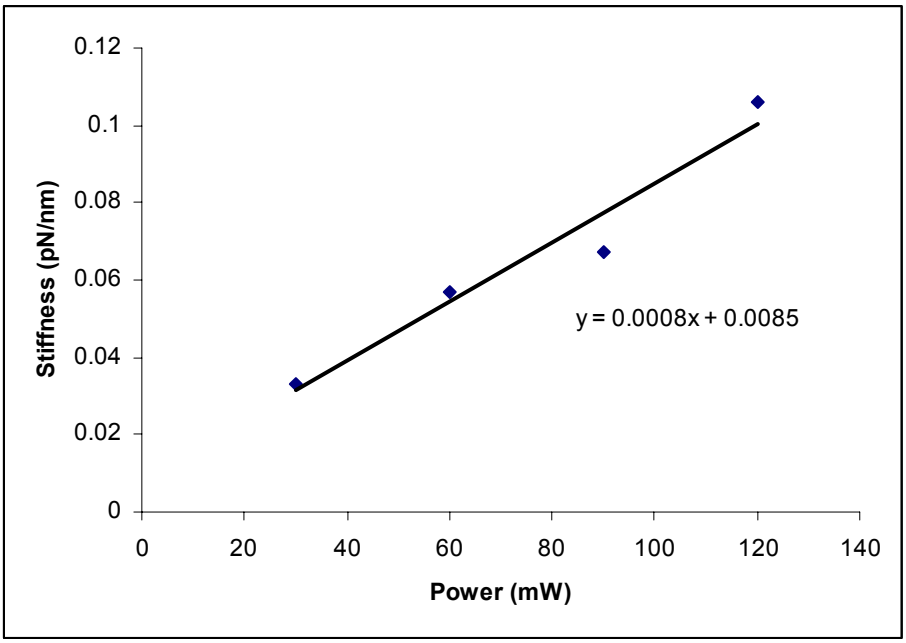

Figure 5. Force calibration for a 1µm silica sphere. This calibration can be used to roughly estimate the stiffness of the trap during the bacterial stall measurement.

The displacement, x, is taken as 100nm-150nm and reflects the maximum distance the bead can get out of the center of the optical trap before falling out, and thus reflects the maximum force that could be exerted on the bacteria. In practice, this distance is around 100nm. This distance could be measured by running a stokes drag measurement and finding the velocity which causes the bead to fall from the trap and solving for the displacement.

The last piece of data necessary is the lever arm length, which is the distance between the center of rotation on the bacteria and the point where the trap was acting on the bacteria.

Students are asked to estimate this distance by comparing the image of the bacteria with the size of the beads in the equipartition and stokes portion of the experiment (the 3.12μm beads are approximately the size of a dime on the screen). Additionally, they are told that the "average" ecoli is about 3μm long by 1μm wide. Usually the lever arm is 1.5-3μm, however, there have been bacteria outside that range.

Stall Torque Calculation

$Laser_{power} := 10mW$ $\alpha_{10mW} := 0.009 \frac{N \cdot 10^{-12}}{nm}$

$x := 100nm$ $d := 3000nm$

$\tau := \alpha_{10mW} \cdot x \cdot d$ $\tau = 2.7 \times 10^{3}\, 10^{-12} \cdot N \cdot nm$

The rigorously experimentally determined average stall torque for the KAF95 E.coli is ~4000 pN*nm. Class values, like the calculated value above of 2700pN*nm, are usually within a factor of 2 of the 4000pN*nm value. A brief discussion of the accuracy of the estimations is beneficial. One interesting question is how well a stiffness calibration for a glass bead would compare to e.coli, and how one could either calibrate the e.coli or rework the experiment to use beads.