Measurement of Onion Myosin Protein Force with Optical Trapping

Keywords: Optical Trap; Myosin; Motor Protein; Biophysics; Protein Force.

In a living cell, everything from disposing of waste to combatting viruses requires organelles to be transported to different locations. This transport is controlled by motor proteins that travel along cytoskeleton fibers. In onion cells, vesicles are commonly transported by the myosin protein. Using optical trapping, we can measure the speed of the myosin protein as well as the force it exerts on a vesicle it is pulling. We found that myosin travels at a speed of $(0.89 \pm 0.36_{\text{stat}} \pm 0.27_{\text{sys}})$ μm/s and exerts a force of $(5.52 \pm 0.57_{\text{stat}} \pm 0.71_{\text{sys}})$ pN on the vesicles it moves.

I. INTRODUCTION

Efficient transport of food, waste, and other material is essential to cell survival, so there are many proteins dedicated to intracellular transport. The myosin motor protein steps along a cytoskeletal fiber to move vesicles to different locations in the cell [1]. The goal of this experiment is to use optical trapping to determine the force myosin exerts on vesicles in onion cells.

Optical trapping is an experimental technique that allows measurements of forces on the scale of piconewtons, making optical traps ideal for studying biological systems. At this scale, hydrodynamic forces such as drag and diffusion are significant, allowing us to study the theory of random Brownian motion and use it to calculate the forces exerted by the trap [2].

An optical trap consists of a highly focused laser hitting an object. The photons in the laser have momentum, so when they deflect off an object, their momentum changes, and they impart a force onto the object. While this force is small for large objects, it is significant for micron-sized objects in a high-powered laser. When an object is centered in the trap, the net lateral force is zero. When the object is off-center, the gradient of the laser intensity results in a net force towards the center of the laser. Thus, an object can get trapped in the laser's potential. Further discussion of the physics of optical traps can be found in [1].

The potential describing these forces is quite complicated, but it may be approximated as a harmonic oscillator for small deviations from equilibrium [1]. From this potential, we can calculate the force exerted on an object by the trap; we will use this to find the force with which onion myosin proteins pull vesicles.

II. EXPERIMENTAL SET-UP

A diagram of the experimental set-up is shown in Figure 1. We used a Thorlabs' OTKB(/M) Modular Optical Tweezers kit, set up on a breadboard with active leveling. Information on the models and brands of each component, as well as details about the set-up beyond the discussion here can be found in [3].

We used a 975 nm 330 mW diode laser. Its power was adjusted by changing the current supplied to it. The

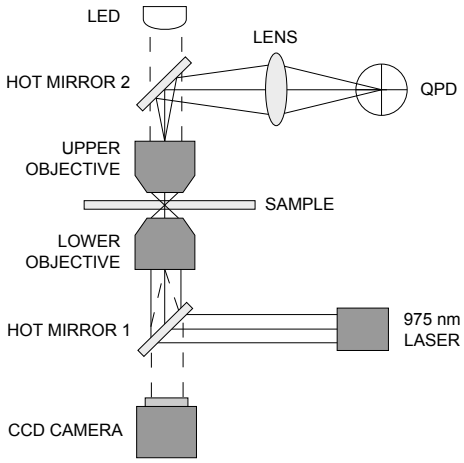

FIG. 1. This is a simplified diagram of the laser and LED light paths. Some mirrors and lenses have been left out for clarity. The laser beam, represented by three solid lines, is reflected by the hot mirrors such that it is focused onto the sample and lands on the QPD. The LED light, represented by two dashed lines, passes through the hot mirrors and lands the CCD camera for visual observation.

laser beam is reflected by "hot mirror" 1, which only reflects infrared light, into the lower objective lens of the microscope. The beam is focused by the lens and further focused by an oil droplet between the lens and the sample to 1.1 μm. The beam then passes through the upper objective lens, is reflected by hot mirror 2, and is focused onto the quadrant photodetector (QPD).

The QPD is a semiconductor photodiode which detects the position of the laser beam by reporting the x and y position as a voltage. The QPD can respond to changes in beam position and intensity within less than 100 μs, making it extremely useful for capturing effects too fast for a camera to detect [2].

In addition to the laser, there is an LED light to allow for visual observation. This white light travels through the microscope and into the CCD camera.

The sample is loaded onto a microscope slide with a coverslip, and placed on the microscope stage with the coverslip facing down. The sample's position can be manually adjusted using micrometers with a range of 4 mm. For finer control, piezoelectric actuators move the

stage by micrometers in response to feedback from strain gauges. The strain gauges also output a voltage, which tells us the position of the stage [2].

Our sample for the myosin experiment was a single-cell layer taken from the inner membrane of a yellow onion and placed on the microscope slide with a drop of saline solution. For calibration of the trap, solutions of silica beads of diameter 0.97 ± 0.02 μm in DI water or 0.1M NaCl were loaded onto a thin section of the microscope slide, as described in [2].

To measure the force of a myosin protein on a vesicle, we located a cytoskeletal fiber along which many vesicles were moving and positioned the laser over it. At low laser current, the vesicles moved through the trap without slowing or stopping, allowing us to measure the speed of the vesicles. We then slowly increased the laser current until the vesicles were stopped by the trap. The goal of our analysis is to calculate the maximum force the laser can exert on the vesicle at that cut-off current; that force should be equal to the force of the myosin protein.

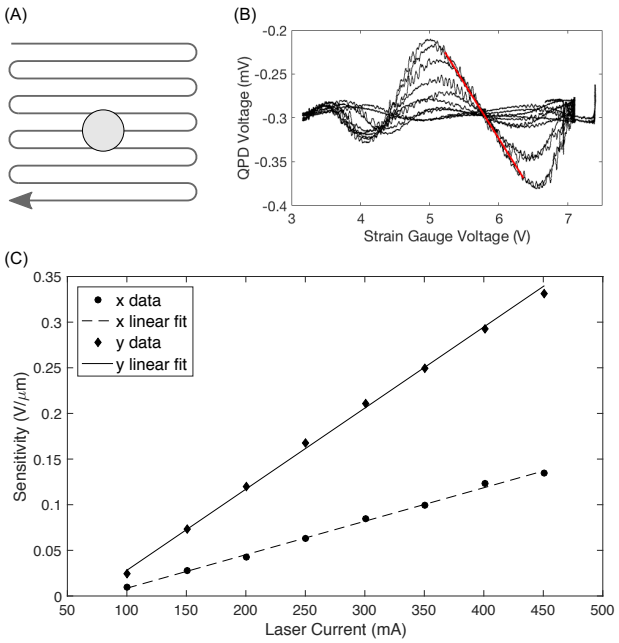

FIG. 2. (A) shows the path of the calibration scan over a fixed bead. (B) is a plot of strain gauge voltage vs. QPD voltage from calibration scan. The laser current was 250 mA, and the fast axis for this scan was the x axis. The slope of the steepest section gives a conversion from QPD to strain gauge voltage. (C) shows that sensitivity, ρ, increases linearly with respect to laser current in each of the x and y directions.

III. ANALYSIS

In order to calculate the force exerted by the trap on an onion cell vesicle, we must determine the "trap stiffness". We define the trap stiffness as α, where the harmonic potential created by the trap is equal to $\frac{1}{2}\alpha x$ for a displacement x. We will determine α by studying the random Brownian motion of a silica bead. First, however, we must calibrate the QPD to find the conversion from voltage to position, in units of μm.

III.1. Calibration: QPD Voltage to Position

Sensitivity, ρ, is the conversion factor that allows us to calculate position from QPD voltage [1]. To find the sensitivity of our set-up, we prepared a slide with 0.97 μm silica beads in a solution of 0.1M NaCl. The ions in solution cause the beads to stick to the slide, resulting in a slide with beads that aren't pulled into the laser trap.

We located an isolated bead and measured the QPD and strain gauge voltages while moving the stage in a grid pattern along the x and y axes as shown in Figure 2(A). For each scan, one axis was increased by increments while we scanned back and forth along the full range of the other "fast axis".

We made 10 scans of the fast axis and covered a 9 $\times$ 9 μm^2 area. We repeated this once for each axis at eight different laser currents, from 100 mA to 450 mA, increasing by 50 mA for each trial. A plot of the strain gauge voltage vs. QPD voltage for 250 mA along the x axis can be seen in Figure 2(B).

The steepest slope corresponds to the scan during which the laser moves across the diameter of the bead. The slope of a linear fit to that section of the plot will give the conversion between QPD voltage and strain gauge voltage for that axis and laser current.

With the slope of this line, which we will call c_1, we are very close to calculating the sensitivity. From changing the display mode on the strain gauge voltage, we find that the conversion from strain gauge voltage to displacement is $c_2 = 2.000 \pm 0.001$ μm/V. We can now find ρ, the conversion from QPD voltage to distance to be $\rho = c_1/c_2$. Finally, we calculate displacement, d, from a QPD voltage, V, to be $d = V/\rho$.

Calculating ρ along both axes for all eight laser currents, we see that the relationship between laser current and sensitivity is linear, as shown in Figure 2(C). From this, we interpolate the sensitivity for laser currents between the eight we measured, allowing us to convert QPD voltage to distance at any laser current.

III.2. Calibration: Trap Stiffness

There are two ways to calculate the trap stiffness, α. The first method uses the equipartition theorem, which states that each degree of freedom of a system at equilibrium will have an energy equal to $\frac{1}{2}k_BT$, where T is temperature and k_B is Boltzmann's constant. For an object in an optical trap, the energy can also be described by the harmonic potential. Since this system has one

degree of freedom along each axis, we can write

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

where $\langle x^2\rangle$ is the variance of the object's position along one axis.

We prepared a slide with silica beads floating freely in water. These beads experience random Brownian motion as particles in the liquid bump into them. We collected QPD data of a bead's position over six seconds with 5000 samples per second. This was repeated at each of the eight laser currents used in the calibration of QPD voltage to displacement. We calculated the variance in position separately for the x and y axes and used equation (1) to find α along each axis.

We expect α to increase linearly with laser current [2]. Our calculated values of α, along with a linear fit, are shown in Figure 3. From these fits we can calculate α for any laser current between 100 mA and 450 mA.

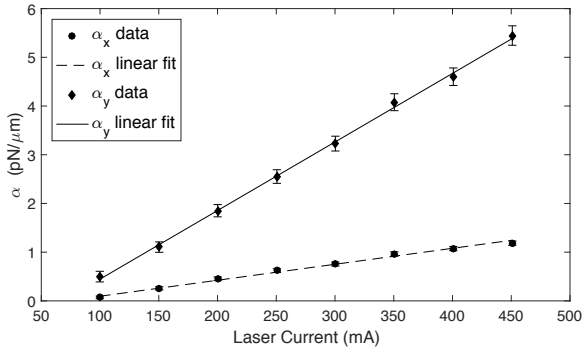

FIG. 3. This plots the α values calculated from the equipartition theorem as a function of laser current, with the x and y dimensions treated separately. The reduced χ^2 values for these fits are 0.868 and 0.201 for x and y, respectively. The difference in magnitude between α for x and y suggest that the laser has an elliptical shape.

We repeated this experiment in onion cells, trapping a vesicle in the cytoplasm instead of a bead in water, to see how the trap stiffness would change in a cell. Unfortunately, the data was too messy to arrive at a linear trend between values of α because other objects besides the target vesicle were pulled into the trap. Visual observation suggested the trap stiffness was on the same order of magnitude in cytoplasm and water, so we will approximate it as equal and account for this in our analysis of systematic error.

Typical values for trap stiffness are around 10 pN/μm [2]. These values for α are on the small side, but are a reasonable magnitude. We decided to confirm our values for α by calculating it with another method.

One can find α by using the power spectral distribution (PSD) function. The PSD function describes Brownian motion in further detail than the equipartition theorem, giving the spectrum of the variations in position [2]. We took the p-Welch transform of our position data and fit it to the PSD function, given by

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

where β is the hydrodynamic drag coefficient, f is the p-Welch transform of our data, and $f_0 = \alpha/(2\pi\beta)$ is the quantity we are interested in finding.

When we performed this fit treating Boltzmann's constant as a known quantity, the fits matched the data very poorly. We attempted to vary β, but that did not help the shape match the data either. It appeared that we needed to vary k_BT to make this function fit our data.

From these fits of the PSD function, the values for α we found were about two orders of magnitude larger than what we calculated using the equipartition theorem. When plotted against laser current, these α values fit very poorly to a linear function, with reduced χ^2 values of 149 and 6.58 for x and y, respectively. Finally, the average calculated value of k_B was 2 orders of magnitude bigger than the accepted value. A possible explanation for this is that the bead wasn't exhibiting true Brownian motion; if it was too close to the coverslip, for example, it would not be expected to fit well to the PSD function.

With all this in mind, we will use only the values for trap stiffness found with the equipartition function for the rest of our calculations. However, this discrepancy between the two methods of calculating α tells us we should be somewhat suspicious of our final results.

III.3. Myosin Protein Measurements

Now that we have values for the trap stiffness, we can measure the speed and force of myosin. To measure the speed, we kept the laser at low power and measured the QPD voltage as several vesicles crossed the path of the laser. Most of the time, there was a reasonably clear "blip" in the voltage signal to show how long the vesicle spent crossing the laser, as shown in Figure 4(A). The average time spent in the laser for the 14 vesicles we had clean data for was $(0.75 \pm 0.30_{\text{stat}} \pm 0.20_{\text{sys}})$ seconds. The systematic error was added to account for measurement uncertainty, as some signals was quite messy.

To find the speed of the vesicle, we divide the diameter of the vesicle by this time. We calculated diameter by comparing photos of vesicles with photos of silica beads of known diameter; by measuring the diameter of the silica bead in pixels, we were able to come up with a pixel-to-micron conversion factor, which we used to find the diameter of vesicles. An example photo of some vesicles can be seen in Figure 4(B). We measured the diameter of 8 different vesicles and found it to be $(0.673 \pm 0.058_{\text{stat}} \pm 0.071_{\text{sys}})$ μm. From this diameter, we calculated the speed of vesicles to be $(0.89 \pm 0.36_{\text{stat}} \pm 0.27_{\text{sys}})$ μm/s.

After taking data for the vesicle speed, we increased the laser current until moving vesicles were stopped by

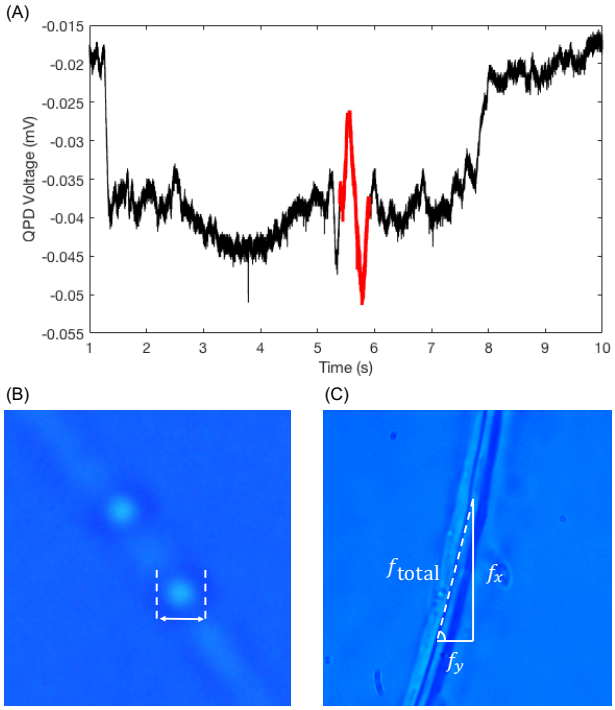

FIG. 4. (A) is an example of the QPD signal from a vesicle passing over the optical trap. The red region indicates the time interval we measured. (B) shows two vesicles in the onion cell; this photo and others like it was used to find the average vesicle diameter. (C) shows the location we took data. Vesicles moved along the dotted line. We used this photo to correctly combine the x and y components of our force vector.

the trap. This laser power was $415 \pm 5_{\text{sys}}$ mA, with uncertainty added to account for the fact that we increased the laser current stepwise by approximately 10 mA at a time. The laser's force on the vesicle at this power is linear with respect to position: $F = -\alpha x$. To get the maximum force, we set x to be the width of the laser.

The effective width of the laser is the region that has a linear relationship between QPD voltage and position. The size of this region in x and y for each laser power was estimated by looking at the plots from the first calibration step, like the one in Figure 2. Now, with both α and the laser width in the x and y directions, we multiply them to arrive at the x and y component vectors of the force, f_x and f_y.

Finally, we must determine the direction of the total force and add f_x and f_y with the correct prefactors. Figure 4(C) shows a photo of where in the cell we took data. Measuring the angle, θ, indicated in this photo allows us to calculate the total force: $f_{\text{total}} = f_x sin\theta + f_y cos\theta$. This force, which is equal to the force exerted by the myosin protein, was found to be $(5.52 \pm 0.57_{\text{stat}} \pm 0.71_{\text{sys}})$ pN.

There were several sources of systematic error in this experiment. Table 1 shows the sources accounted for in calculating the myosin protein force and speed. For calculation of the myosin speed, the largest sources of error were measurement uncertainty. There was lots of visual measurement, such as measuring the diameter of a vesicle from a microscope image. Developing more rigorous and formal methods for taking those measurements might help reduce error in future experiments.

TABLE I. Here are all the sources of systematic error accounted for in the calculation of myosin protein speed, followed by those for the force it exerts. Sources of error are listed along with the size of the error they contributed, expressed as a percent of the final value.

Source of error	**Percent**
Myosin protein speed:	
Measurement uncertainty of time for vesicle to pass through trap	17.3%
Measurement uncertainty of vesicle diameter	11.7%
Manufacturer's error for silica bead diameter	1.4%
Myosin protein force:	
Measurement uncertainty of trap width	5.6%
Difference in α between water and cytoplasm	3.4%
Uncertainty in cut-off laser current	1.1%
Measurement uncertainty of vesicle motion angle	1.2%
Measurement uncertainty of temperature	0.4%

In addition to measurement uncertainty, one of the largest contributions to error for the myosin force was accounting for the difference in trap stiffness between water and cytoplasm. Finding a good way to measure α in cytoplasm would reduce this error.

IV. RESULTS

Using the optical trap, we found that myosin motor proteins in onion cells have an a speed of $(0.89 \pm 0.36_{\text{stat}} \pm 0.27_{\text{sys}})$ μm/s and exert a force of $(5.52 \pm 0.57_{\text{stat}} \pm 0.71_{\text{sys}})$ pN on the vesicles they carry. Accepted speeds for translational motor proteins are between tenths and tens of microns per second [4], and the force exerted by a myosin protein is known to be between 1 and 5 pN [5]. Both our findings appear to match the literature values for motor protein speed and force.

We did have a large discrepancy between the values of trap stiffness calculated with the equipartition theorem and the PSD function. This should prompt us to be suspicious of our values for trap stiffness, and thus of our results. Nevertheless, we have measured the force exerted by a single myosin protein on a vesicle. Optical trapping makes this and many other experiments on macro-molecules possible, allowing us to study many important biological mechanisms and phenomena.

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