Calibration of Optical Tweezers

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A highly-focused laser beam can provide an attractive force (on the order of piconewtons) capable of trapping small, dielectric objects. Optical tweezers make use of this phenomenon to manipulate and study microscopic objects, especially in biology. Much like a spring, the approximate force that the laser beam applies to an object is proportional to the object's displacement from the center of the beam. A known trap stiffness is often required to make reliable force measurements. The purpose of the report is to test different methods that experimentally measure the trap stiffness for our optical tweezers set-up.

Introduction

Optical tweezers (also known as an optical trap, laser trap, or gradient force trap) was first reported by Arthur Ashkin of Bell Labs in 1970. Optical tweezers played a major role in the current United States Secretary of Energy's winning of the Nobel Prize in Physics in 1997 for laser cooling. The most recent applications for the optical tweezers have been in the field of biophysics where optical tweezers can be used to measure the piconewton forces exerted by molecular motors such as those found in DNA polymerase or used in RNA transcription.

In order to accurately measure these molecular motor forces we need to have a well-calibrated instrument. Calibration for optical tweezers means knowing what the trap stiffness and trap force for a laser beam are at certain intensities. In this report we aim to explain the methods for calibrating a typical optical trap that could be built in a university laboratory. The methods attempted here are just a few of many, and we give suggestions and overviews of other methods at the end of this report.

Theory

The goal of our optical tweezers is to trap a transparent silicon microsphere at a very tight laser beam waist. In order to understand why this phenomenon happens we need to know the beam profile of a laser, Snell's Law, and the quantum mechanical postulate that photons have momenta.
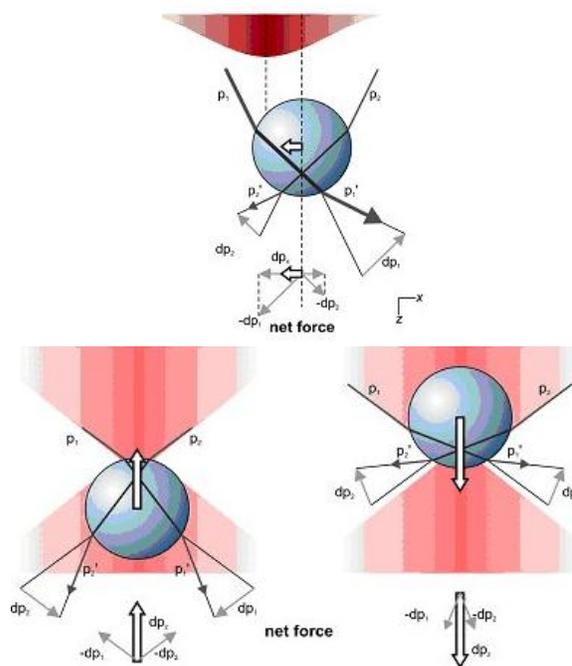

Fig. 1

As we can see in Fig. 1 the Gaussian beam profile of a laser has a much higher intensity at the center of the beam than at the edges. Thus, according to Snell's law, the majority of the light is bent away from the beam's center. This means that the direction of the photon flux changes, where the momentum of a photon is given by:

$$p = \hbar k$$

By conservation of momentum we would expect the microsphere to recoil towards the center of the beam, which it does. Similarly we can see from Fig. 1 that the bending of light forces the microsphere towards the center of the beam in all three directions.

It is important to note that if the reflectivity of the microsphere is too great, then the radiation pressure will actually push the microsphere out of the trap.

Experimental Set-up

Our optical tweezers set-up consists of: a source laser, optical components to guide and refocus the beam, a sample slide with translational stages, a white light source, and a CCD camera and computer for acquiring data. The set-up can be seen in figure 2 at the bottom of the page.

A 637nm, 70mW laser is guided through a fiber and passed through a linear polarizer that allows us to vary the light intensity, and thus the trap strength. Next, the beam passes through a bi-convex lens of focal length 30cm. The position of this lens allows us to focus the laser beam. The light is then reflected off a dichroic mirror and passed through a microscope (100X, NA=1.3). The focused beam is projected onto a microscope slide that contains silica microspheres in deionized water. A white light source is used to illuminate the sample and a CCD camera on the opposite side of the dichroic mirror is used to view the optical trap.

The sample slide is mounted onto X-Y-Z stages that allow us to bring the sample into focus and drag the trapped particles through the medium.

National Instrument's Vision Assistant software is used to record and analyze live image-feeds obtained from the CCD camera.

Brownian Motion Trap Stiffness Measurement

Particles suspended in liquid exhibit random movement due to collisions with the moving molecules that make up the liquid. This phenomenon is known as Brownian motion. The equipartition theorem states that a molecule in thermal equilibrium has an average kinetic energy for each degree of freedom given
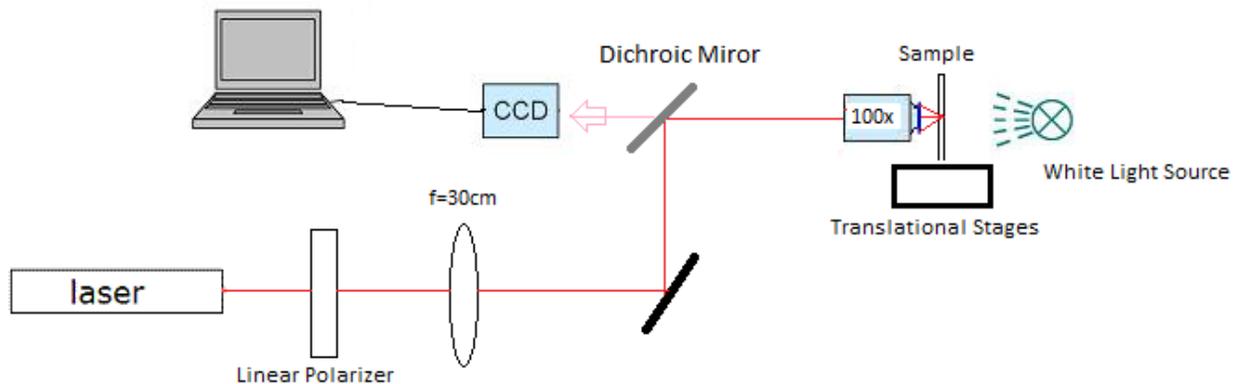

Fig 2. - Experimental Setup

by:

$$\langle H \rangle = \frac{1}{2}k_{\mathrm{B}}T$$

Assuming that the movement of the trapped bead is due only to thermal fluctuations, we can set the kinetic energy equal to the potential energy of the trap:

$$\frac{1}{2}k_b T = \frac{1}{2}k < x^2 >$$

Where $<x^2>$ is the time-averaged square of the bead's horizontal displacement from the center of the trap.

By measuring the Brownian motion of a trapped bead, we can experimentally determine the stiffness of our optical trap without having to know any information about the fluid viscosity or geometry of the trapped particle.

Position data was taken for .56μm beads trapped under various intensities.

$<x^2>$ (m^2)	Beam Power (mW)	Trap Stiffness, k (pN/ μm)
8.43 * 10^{-15}	4.5	.48
4.3 * 10^{-15}	6.2	.95
2.09 * 10^{-15}	10.1	1.96
1.37 * 10^{-15}	14.0	3.06
9.5 * 10^{-16}	18.0	4.28

Table 1

The bead's displacement from the center of the beam was first measured in pixels, and then converted into micrometers using a conversion ratio of 15.625 pixels per micron. This ratio was obtained by measuring the pixel diameter of a 2.56μm bead.
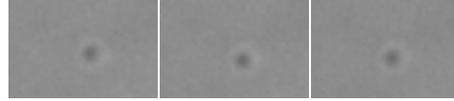

Fig. 3 (Brownian motion for a .56μm bead)

A plot of trap stiffness as a function of beam power suggests a linear relationship which can be used to predict the stiffness for a trap at maximum power.
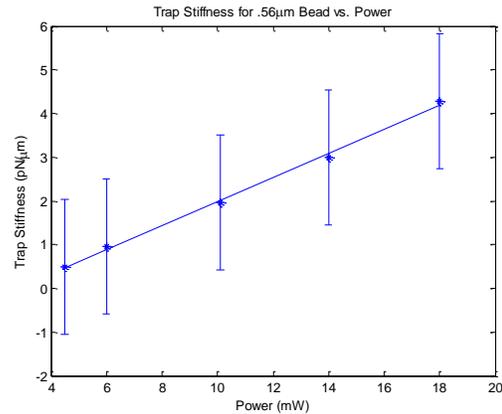

Fig. 4

We extrapolate a maximum trap stiffness (P=70mW) of 17 pN/μm for a .56μm silica bead submersed in deionized water.

Stokes' Drag Force

Our next method uses the viscosity of the liquid itself to test both the trap stiffness and the trap strength. In this method, the sample is moved at a constant velocity. We know that the force exhibited on the microsphere at a given velocity is:

$$F_d = 6\pi\,\mu\,R\,v_s$$

Where μ is the dynamic viscosity (8.90×10^{-4} Pa·s), R is the radius of the spherical object and v_s is the particle's velocity. This force causes the displacement of the bead from the laser and this displacement, x, should be measured. If we model our trap as a spring like we did before, our trap stiffness is given by:

$$k = {F_d}/{x}$$

Since Stokes' drag force is more apparent for microspheres of larger radii we chose 2.56 μm beads for this method.

If we slowly accelerate a particle until it breaks loose from its trap, we can calculate the trap force. The trap force is simply equal to the stokes drag force and the force caused by the acceleration.

It is easy to show that the accelerations we are dealing with create minimal forces compared to the drag force. Thus the trap force is equal to the drag force at which the bead breaks from the trap.

$$k = F_d$$

Drag Force Trap Stiffness Measurement

To measure the displacement and velocity we relied heavily on our CCD camera. With the knowledge that the camera took one picture every 2.44 ms we were able to both calculate the velocity and ensure that the object was not accelerating over the desired frames.
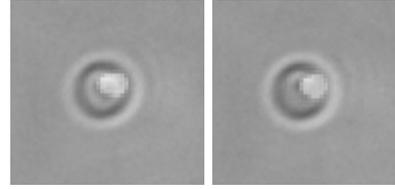

Fig. 5a   *Fig. 5b*

Fig. 5a is an image of the bead looks when still, and Fig 5b shows the particle undergoing a velocity. The change in position is measured by using the computer software to calculate the displacement in pixels, which can be converted to microns.

To measure velocity, we needed a reference point on the slide. This was usually a microsphere that was stuck to the cover slip. Over ten frames we measured how far the reference point moved, and then divided it by the time that elapsed over ten frames to get the velocity. To ensure that the velocity was constant we checked to see that the number of pixels moved every frame stayed constant. We calculated the average displacement over the ten frames and then calculated the trap stiffnesses.

Velocity/ Displacement (ms^{-1})	Beam Power (mW)	Trap Stiffness (pn/ μm)
.3767	7.9	.8090
.7265	12.4	1.560
.6580	22.3	1.413
.09530	42.0	2.0465
1.5725	50.7	3.3767

Table 2

While this method obviously shows the expected trend, we did not find it to be very precise or reproducible for given intensities. This is even more evident from Fig. 6:
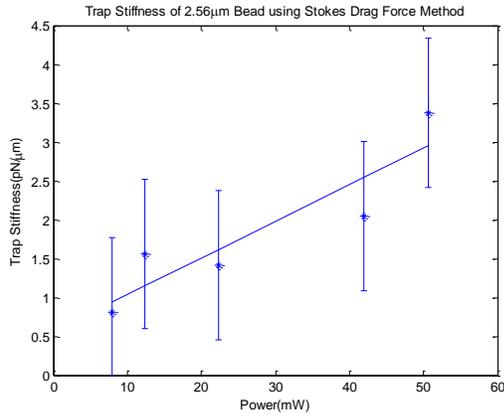

Fig. 6

Drag Force Trap Force Measurement

We next attempted to calculate the trap force of our microsphere at a maximum intensity of 67.8mW. We did a similar data processing technique as in the previous section, but this time only took the velocity over four frames to gain a more precise velocity for the time in question. As can be seen in Fig.7 we measured the velocity using a reference point as the light and bead began to separate.
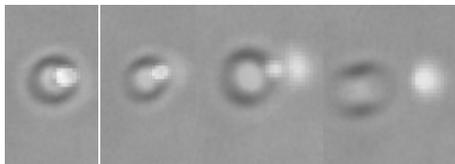

Fig. 7

The data for four attempts at attaining the trap force at 67.8mW are:

Trial Number	Escape Velocity (mm/s)	Trap Force (pN)
1	.616	13.28
2	.372	7.99
3	.455	9.57
4	.367	7.88

*Which gives an average trap force of **9.68 pN***
Table 3

These are all reasonable numbers, but as before there is work to be done in getting them to be more precise.

Multiple Traps

During our work with optical trapping, we noticed that sometimes the microspheres would get trapped to either side of the beam center as seen in Fig. 8 and Fig. 9.
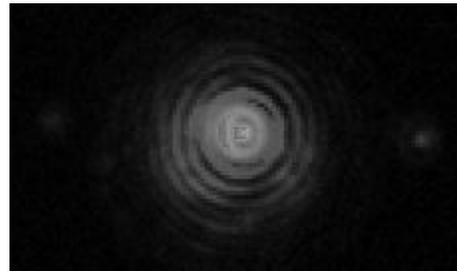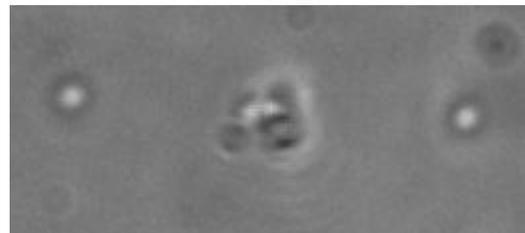

Fig. 8

Fig. 9

We rotated the polarization of the beam with a half-wave plate to see if these "microtraps" rotated as well. They did not. We assume that the smaller traps are due to some sort of reflection or diffraction. With this in mind, we thought it would be neat to deliberately create multiple traps via a diffraction grating. The diffraction grating method produced nice spots of light; however none of them had very much trap strength. Occasionally, beads would hang around at the center of the diffraction maxima (as seen in Fig. 10 where two beads are very weakly trapped), but the trap was too weak to feasibly measure.
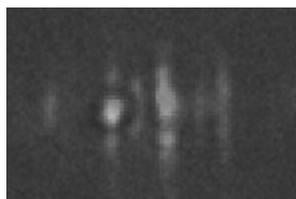

Fig. 10

Future Works

Optical tweezers play an important role in numerous biological studies. Now that the trap strength of our optical tweezers is known, it would be interesting to work with some applications such as measuring the force of different molecular motors.

To do this, one would need to attach a molecular motor (I.E. a single-celled organism) to a microsphere. The intensity of the trap could then be varied until the organism was able to break free. Because the trap force for the given intensity, bead geometry, and medium viscosity are known, so is the force provided by the molecular motor. Someone with knowledge of chemical bonding would need to be consulted to assist in the adhesion of the organism to the microsphere.

When implemented with a spatial light modulator (such as the one recently completed at the University of Oregon's Advanced Projects Lab), optical tweezers can be turned into holographic optical tweezers, capable of trapping and moving many objects simultaneously in three dimensions. Future work with the optical tweezers could explore this possibility, and work to produce multiple traps that could be arranged into different patterns such as the one below:
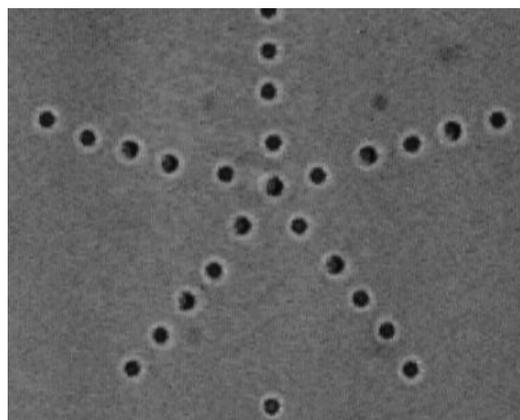

Fig. 11

More precise measurements for the trap stiffness could be done if the proper equipment is obtained. Measuring the displacement of the trapped beads would be more precise with a higher resolution camera. Additionally, having a piezoelectric translation stage to move the sample at a constant velocity (on the order of 10 μm/sec) would greatly improve the trap stiffness measurements via Stokes' drag method.

Another issue that we came across was when the microsphere came in close contact with a surface the viscosity of the medium would change. A possible experiment would be to use this to measure the width of the water sample.

Conclusion

After the successful construction of an optical trap, we were able to measure the trap stiffness to the correct order of magnitude for different bead sizes and laser intensities. The stiffness directly depends on the power and wavelength of the laser, the size and shape of the trapped object, and the refractive indices of the object and surrounding fluid. Ideally, we would be able to take more precise measurements. However, we were restricted by the technology available to us. In the future we would like to see our measurements for the trap stiffness redone with a higher resolution camera and a piezoelectric translation stage to control the movement of the sample slide. We also would like to make force measurements on biological systems and explore the applications of multiple traps.

Acknowledgements

We dedicate this work to our mothers.

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