Quantifying Trapping Forces in a Simplified Optical Tweezers Setup

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Abstract

Optical tweezers utilize the radiation pressure exerted by a single tightly-focused beam of laser light to trap microscopic particles in three dimensions. Such devices are particularly useful in biological applications such as cell manipulation and studying molecular motors as a means of noninvasive manipulation. However, optical tweezers are generally considered to be complex and costly. The purpose of this research was to construct an inexpensive optical tweezers setup that could stably trap particles in all three dimensions. A new method for quantifying transverse trapping forces with the drag force method was implemented, using a gradually accelerating stage driven by a motor and video analysis of the trapped particle. This project demonstrates that simple tweezers setups can be implemented in undergraduate or even high school laboratories and still achieve valid quantitative results. Inexpensive optical tweezers designs are more accessible for undergraduate and even high school research in biology and other fields, and their simple design provides a unique opportunity for academic learning about optical trapping. Our setup was also used to study the effect of altering the trapping beam’s intensity distribution on trapping efficiency, particularly by overfilling the aperture and creating annular beams of orders $\ell=1-8$. 
1 Introduction

It is well known that light carries energy, but less well known that light also carries momentum. When light experiences a change in momentum, like any material particle, it exerts a force. This "radiation pressure" was predicted by Maxwell’s equations in the late 1800s [1], and the first precise measurements of the radiation pressure of light were taken in the Nichols-Hull experiment (1900-1903) [2]. The practical applications of radiation pressure increased greatly after the invention and development of the laser in the 1960s, and soon after, Ashkin and coworkers at Bell Laboratories demonstrated that a continuous beam of laser light could accelerate and trap micron-sized particles [3]. Their first optical trap used two laser beams pointing directly at each other: this geometry allowed the forward scattering forces of the two beams to cancel and the transverse gradient force to attract the particle to the center of the coincident beams, where intensity is highest.

Two-dimensional trapping can be achieved with a single beam by balancing the scattering force against gravity or by trapping the specimen against the sample chamber wall. However, in 1986 Ashkin and coworkers demonstrated that a single tightly-focused beam of laser light could trap stably particles in three dimensions by utilizing the backwards gradient force to oppose the forwards scattering force [4]. Such a device is called an optical tweezers. The ability to create optical tweezers with commercial laboratory microscopes has revolutionized optical trapping, especially in biological applications. In this field, optimizing trapping efficiency (force per unit power) is especially important; high-power lasers have high trapping forces but damage biological specimens [5].

Optical tweezers are generally considered to be complex and costly. For example, a standard Thorlabs Modular Optical Tweezers kit would cost over $20,000 [6]. The purpose of this research was to construct an inexpensive optical tweezers setup that could stably trap particles in all three dimensions. A new method for quan-
tifying transverse trapping forces was implemented, using a gradually accelerating stage driven by a motor and video analysis of the trapped particle. This project demonstrates that simple tweezers setups can be implemented in undergraduate or even high school laboratories and still achieve valid quantitative results. Our setup was used to study the effect of altering the trapping beam’s intensity distribution on trapping efficiency. However, as discussed in this report, optimizing trapping efficiency has many complexities.

2 Trapping Theory

There are two distinct models to predict the forces of optical tweezers; which model is applicable depends on the size of the trapped particle and the wavelength of trapping light. For particles significantly smaller than the wavelength of light \( d << \lambda \) the dipole approach is used, while for particles significantly larger than the wavelength of light \( d >> \lambda \) the geometric ray-optics model is used. When particle size is comparable to the optical wavelength, the much more complex Lorenz-Mie theory must be applied [7].

This paper discusses the ray-optics model: particles are in the geometric regime, which means their diameter is at least 20 times the optical wavelength [8]. Transparent particles with a higher index of refraction (IOR) than the surrounding medium are trapped due to the absorption, reflection, and refraction of light that interacts with the particle. When light changes direction due to an interaction with the particle, the light has a change in momentum. Due to the conservation of momentum, the particle experiences a reactionary change in momentum.

For a trap to be stable in three dimensions there must be both axial and lateral (transverse) trapping. Transverse trapping (Fig.1) results from the trapping light’s intensity gradient. Refracted light from regions of higher intensity will have a larger change in momentum than refracted light from regions of lower intensity; the net force on the particle is therefore towards the regions of highest intensity.
Figure 1: Transverse forces on a particle in a Gaussian beam. The particle on the beam axis (left) has no net lateral force because Ray 1 and Ray 2 are equal in magnitude. The particle to the left of the beam axis (right) has a net force to the right since Ray 2 has a greater change in momentum than less intense Ray 1. [10]

Figure 2: Axial forces on a trapped particle in a tightly-focused beam. Rays refracted through the particle on the left produce a net forwards scattering force, while refracted rays through the particle to the right, which is past the beam waist, produce a net backwards gradient force. [10]

Axial trapping (Fig.2) occurs in the direction of beam propagation and has two primary forces: scattering force and gradient force. The scattering force is in the direction of beam propagation, caused by reflected rays or rays refracted away from the beam axis, since the forwards component of their momentum is reduced. The gradient force is caused by rays striking the particle at steep angles and refracting towards the beam axis; their forward momentum increases and thus exert a backwards force on the particle. The backwards gradient force counters the forwards scattering force; if $F_{\text{gradient}} > F_{\text{scattering}}$ then axial trapping is stable.
2.1 Trapping Efficiency

To optimize an optical trap, one must consider not only the magnitude of the force on the particle but also trapping efficiency: force relative to trapping beam power. The incident momentum flux from a laser of power $P$ in a medium with an IOR $n$ is known to be $nP/c$, in which $c$ is the speed of light. The force on a particle in an optical trap is therefore defined as $F = QnP/c$, in which $Q$ is the parameter that defines trapping efficiency and $P$ is the beam power at the trapping plane [9]. It follows that a higher $Q$ value corresponds to higher trapping efficiency.

Trapping efficiency depends on the intensity distribution of the light entering the microscope objective. Ray-optics theory predicts that efficiency can increase with overfilling the microscope objective, in which the laser beam is expanded to a diameter larger than the objective opening, truncating the outer portion of the beam [9]. The outermost rays entering the objective, which are focused at the steepest angles, increase in intensity relative to the central on-axis beams. Overfilling therefore increases the ratio of backwards gradient force to scattering force and increases axial trapping efficiency. The effect of overfilling on lateral trapping, however, is less clear. Factors such as particle size have produced conflicting results [11].

![Figure 3: An optical vortex. The wavefront (left) is helical, which creates a phase singularity in the center. When projected onto a flat surface (right) an optical vortex appears as a ring with a dark hole in the center. [12]](image)

It has also been suggested that trapping efficiency can be increased by using annular beams. Annular beams (optical vortices) are helical modes of light that are defined by their topological charge (the number of $2\pi$ phases in one wavelength);
at the beam center these phases interfere to create a point of zero intensity (Fig.3). This central region of low intensity increases the ratio of the gradient force to the scattering force and therefore increases axial trapping efficiency.

3 Optical Tweezers Setup and Construction

The optical tweezers setup (Fig.4) was assembled on a 36”x48” optical breadboard. All mirrors and lenses were mounted low on the optical breadboard in order to minimize vibrations. Standard Thorlabs posts and post holders were used, along with other mechanical components such as adjustable mirror mounts, translation stages, and two optical rails.

3.1 Trapping Laser

A surplus 632.8 nm SpectroPhysics 127 HeNe laser (38 mW) was the source of the trapping beam; this is a relatively low power laser for particle trapping. A laser mount was constructed for vertical adjustment. The laser was mounted on two supports each made of two threaded metal rods screwed into the optical breadboard with a wooden crossbeam on top of which the laser was placed, and a second wooden beam was secured on top of the laser to prevent vibrations. The wooden beams could be moved vertically by screwing the supporting nuts up and down the threaded metal rod. The laser was checked for horizontal alignment with a level.

3.2 Inverted Microscope

A surplus Nikon inverted microscope with an overhead illumination system and manually controlled stage was used for particle trapping. An inverted microscope has the objective below the trapping plane, so that the trapping beam is sent upwards through the objective. This technique was first used in early trapping
Figure 4: The optical tweezers setup. The L2 f=150 mm beam expander is in place; a Gaussian beam is used. The dichroic mirror reflects vertically into the inverted microscope.

experiments using optical levitation [13], in which the downwards gravitational force countered the upwards scattering force. While regular optical tweezers have only the backwards gradient force to balance the scattering force, inverted tweezers take advantage of gravity to enhance axial trapping strength.

3.3 Optical Components

All mirrors were first-surface; M1 (Mirror 1) was silver, while M2 and M3 were gold. The dichroic mirror reflected laser light optimally at 45° (99% reflectivity). Lenses were 1-inch circular plano-convex lenses. A vortex phase plate (VPP)(RPC Photonics) was later inserted between M1 and L1 for optical vortex traps.
3.3.1 Microscope Objective

The microscope objective had a back aperture opening of 6.0 mm and was a generic 50X NA=0.85 objective. Numerical aperture is defined as $NA = n \sin \theta$, in which $n$ is the IOR of the medium in which the objective operates, and $\theta$ is the half-angle of the cone of light that exits the lens (Fig.5). A higher NA corresponds to steeper rays of light and therefore a stronger axial trap. This objective has a very low NA for trapping; in most optical tweezers, more advanced and expensive oil-immersion objectives with $NA > 1$ are used [14].

![Microscope Objective](image)

Figure 5: Numerical aperture is defined by the half-angle $\theta$ of the cone of light that exits its focusing lens.

3.3.2 Alignment

The laser beam was carefully aligned to ensure the beam was straight and centered entering the microscope objective. Vertical alignment was achieved by measuring the height of the beam center a short distance from the laser, and then centering the beam at this height after each optical component using micrometer adjustment knobs.

Horizontal alignment was achieved similarly; micrometer adjustment knobs were used to adjust mirrors to ensure the beam traveled parallel to the row of holes on the optical breadboard on which the lenses were mounted. After each lens, the beam’s reflection was aligned to shine straight back into the laser.

3.3.3 Optical Component Efficiencies

Every optical component at which the light is reflected, refracted, or otherwise redirected results in a loss of beam power. The final setup efficiency was 26%, with
a trapping power of 10 mW. The greatest power losses occurred in the objective; the component efficiency of the objective is not included in Fig.6 because the beam partly lost power due to truncation. The VPP when inserted also resulted in significant power losses. There was no correlation between power loss in the VPP and beam order; the efficiencies of individual orders ranged from 91-93%.

![Optical Tweezers Setup Power Losses](image)

Figure 6: Power losses at each optical component. The initial laser power is 38 mW. Percentages displayed above each bar are the individual efficiencies of the respective optical components.

### 3.4 Imaging System

In order to view the trapped particle in focus, there must be conjugate image planes of the objective so that the viewing camera is focused in the same plane as the trapped particle. The objective was not infinity corrected: the image plane was at 160 mm. To create these conjugate image planes at the objective’s optimal distance, the camera would be at 160 mm from the objective, and a 3rd lens (L3) would focus the laser light 160 mm before the objective. However, the closest the camera could get to the objective due to physical limitations of the setup was 230 mm. To create conjugate image planes, L3 (f=200 mm) was placed 430 mm from the objective to focus the light 230 mm away from the objective.

The trapped particle was viewed with a CMOS camera at 14 fps. The light
was focused directly onto the CMOS element without a lens. The camera was used with a laptop computer to view and record particle motion. The magnification of the imaging system was determined to be 3050X by comparing the size of the computer images of 9.964 ± 0.058 µm latex spheres to their actual size.

3.5 Sample Preparation

Both yeast cells (IOR of 1.49-1.53 and diameter of 3-6 µm) and polystyrene latex spheres (IOR of 1.59 and diameter of 9.964±0.058 µm) were trapped. Particle trapping was achieved in a large droplet of tap water on a No. 0 microscope coverslip (Fig.7), which was suspended over the microscope objective on a piece of card paper with a large hole. This setup was more effective than the traditional Rose chamber (Fig.8), which was very shallow and led to particles sticking to the bottom of the coverslip, increasing the difficulty of trapping. The disadvantage to our setup was that the sample evaporated under the illumination light and had to be replaced periodically.

Figure 7: Trapping sample setup. Figure 8: Traditional Rose Chamber.

4 Optimizing Trapping Efficiency

The trapping beam intensity distribution was altered in order to determine the effect of trapping beam profile on trapping efficiency. This was achieved through both beam expansion and optical vortices.
4.1 Beam Expansion

As discussed earlier in this paper, trapping efficiency can be optimized by overfilling the microscope objective. The Gaussian beam of initial $1/e^2$ width of 1.4 mm was expanded to 7.9 mm, 9.5 mm, and 15.8 mm in order to overfill the 6.0 mm aperture.

Beam expansion was achieved using a basic telescope (Fig.9), in which two lenses with focal lengths $f_1$ and $f_2$ are placed $f_1+f_2$ apart. This yields a recollimated beam, expanded by a factor of $f_2/f_1$. The first lens had a focal length $f_1=25.4$ mm, and the second lens had a focal length of $f_2=125$ mm, 150 mm, or 250 mm depending on the desired final beam size. The expansion factor was limited by the number of available lenses. Additional expansion occurred before the objective due to L3.

4.2 Optical Vortices

Trapping efficiency can also be optimized through the use of vortex beams. The unexpanded beam was sent through a VPP to create optical vortices of orders $\ell=1-8$.

4.3 Measuring the Trapping Beam Profile

The laser beam was profiled for each vortex order and as a Gaussian beam. Measurements of current were taken by connecting a photodiode with a 200 $\mu$m pinhole to a multimeter and translating the photodiode across the beam’s diameter. They were then entered into an Excel spreadsheet and plotted versus position for the
Figure 10: Beam profiles for beams of order $\ell=0,1,3,5,7$ and a Gaussian beam.

beam profile. (Fig.10)

5 Quantifying Forces

There are two primary methods to quantify trapping forces in optical tweezers [16]. The first is by obtaining the power spectrum of a trapped particle’s Brownian motion, which requires a quadrant photo-diode or high frame-rate (kHz) camera. The second is the drag force method, which uses a motorized microscope stage to move the sample and determine the velocity at which the fluid’s drag force overcomes trapping force. However, both of these methods require expensive equipment.

I developed a very inexpensive method to quantify trapping forces using the
drag-force method. This included a custom-made motorized stage driven by a motor, and a video analysis system to find the particle’s maximum velocity.

5.1 Motorized Stage Design

The translation stage of the inverted microscope was initially moved manually with dials. In order to quantify trapping forces, I designed a simple motorized stage with variable speeds.

This design used a pulley with a variable gear ratio so that the speed of the stage would accelerate constantly over a certain distance. A 1 RPM motor was coupled to the translation stage dials using tape (Fig.11). The motor shaft had foam tape layers of varying thickness to change the gear ratio. The diameters of the stage dials were extended using a cardboard wheel in order to decrease the speed of the stage to the $\mu$m/s range. The final stage translation speed was 40-150 $\mu$m/s, which was successfully in the range of fallout velocities for the trapped particle.

![Figure 11: The motorized stage system. The motor with a variable gear ratio (right) is attached to the extended stage dials (left).](image)
5.2 Drag Force Method

The drag force method was used to determine the strength of the trap. Trapped particles can be dragged through a fluid at varying speeds until the particle reaches a maximum velocity and falls out of the trap. This maximum velocity can be used in Stokes’ law, $F = 6\pi \eta rv$, to find the viscous drag force $F$ exerted on a particle of radius $r$ traveling at velocity $v$ in a fluid of viscosity $\eta$. To avoid optical inconsistencies, the actual trap is not translated: the translation stage is moved.

To find the particle fallout velocity, videos of the trapped particle were recorded while the stage moved (Fig.12). Particles were trapped at a constant distance from the coverslip for each trial; this ensured they were not too close to the coverslip, which would cause inaccurate drag force measurements due to turbulence, or too far, which would result in a weak trap due to spherical aberrations and increased vibrations. Forces were measured in both the x and y transverse directions as they were significantly different. Three trials were recorded for beams of orders $\ell=0-8$ in the x and y transverse directions; this was repeated for beams 7.9 mm, 9.5 mm, and 15.8 mm in width.

![Figure 12: A sequence of video frames taken with the stage moving. The bead remains trapped (top), while the sphere on the coverslip translates with the stage.](image)

5.2.1 Video Analysis and Calculations

The speed at which the particle fell from the trap was calculated by analyzing the captured videos in ImageJ as individual frames. The particle’s velocities through
the medium immediately before and after the particle fell out were averaged for the instantaneous velocity.

The velocity of the translation stage was found by using the imaging system magnification to determine how far particles on the coverslip surface moved between frames. However, the coverslip surface was out of focus because it was not in the trapping plane. The coverslip moved faster across the imaging plane than the trapped particle, so correction factor was needed. I found this correction by calculating the ratio of the apparent sizes of a trapped latex sphere and an identical latex sphere on the slide bottom, which was a 6% difference.

The temperature of the water under the illumination light was also taken into account: the viscosity of water increases as its temperature decreases. The water was measured to be 71.5°F, and the viscosity of water was calculated to be 934 μPa·s (opposed to 890 μPa·s at room temperature).

6 Results

This tweezers setup was successfully able to trap particles in the ray-optics regime both axially and laterally for multiple hours at a time. Experimental data was acquired using the 9.964±0.058 μm polystyrene latex spheres. They were used due to their spherical shape, as opposed to the irregular shape of yeast cells that caused inconsistencies in the quantified forces using the drag force method.

6.1 Lateral Trapping

The custom motorized stage design and video analysis system successfully allowed for acquisition of trapping force measurements using the drag force method. Fall-out velocities ranged from 40-150 μm/s. Trapping forces were measured for beams of diameters 7.9 mm, 9.5 mm, and 15.8 mm, and for annular beams of orders ℓ=1-8. Forces were in the 2-8 pN range.

Trapping efficiency (Q) was determined by the parameter \( Q = Fc/nP \). The
power of the trapping beam was measured at the trapping plane for each beam order and beam width.

6.1.1 Trapping Force vs. Annular Beam Order

Figure 13: Trapping forces for two beam widths as measured using the drag force method. The trapping forces reduce with increasing beam order, which means annular beams decreased trap strength. Blue data points are in the x-direction; red data points are in the y-direction; both are in the transverse trapping plane.

6.1.2 Trapping Force vs. Beam Width

Figure 14: Average trapping forces for beams of orders $\ell=0$-8 versus trapping beam width. Trapping forces decrease for larger beam widths, which means that overfilling the objective decreased trap strength. Blue data points are in the x-direction; red data points are in the y-direction.
6.1.3 Trapping Efficiency vs. Beam Order

Optical vortices have a region of low intensity in the center, which means that their power in concentrated towards the outside of the beam. Therefore, when the beam was truncated by the microscope objective, high-order annular beams had larger losses in power than low-order annular or Gaussian beams. For example, for the 9.5 mm beam, the $\ell=8$ optical vortex had a final trapping power of 3.6 mW, while the lower-order $\ell=1$ optical vortex had a final trapping power of 7.5 mW.

Figure 15: Trapping efficiencies for two beam widths, as defined by the parameter $Q = Fc/nP$. The Q parameter increases with increasing beam order, which means annular beams increased trap efficiency. This suggests that although annular beams decreased trap strength, this was due to power losses in the trapping beam. Blue data points are in the x-direction; red data points are in the y-direction.
6.1.4 Trapping Efficiency vs. Beam Width

Figure 16: Average trapping efficiencies (Q) for annular beam orders $\ell=0$-8, versus beam widths of 7.9 mm, 9.5 mm, and 15.8 mm. The largest beam has the highest Q value; the smaller two beams have comparable Q values. The correlation between trapping efficiency and beam width is unclear due to few data points. Blue data points are in the x-direction; red data points are in the y-direction.

6.2 Axial Trapping

Axial trapping forces were not determined quantitatively. The motorized stage system was limited to transverse trapping due to its physical design. Furthermore, the refraction of light from the objective to the trapping medium meant that the position of the beam focus is not fixed when the stage moves. Translating the stage axially at a certain speed doesn’t move the particle at that same speed through the fluid. This made determining trap velocity relative to the fluid impossible without knowing the precise trapping depth.

7 Conclusions

An inverted optical tweezers setup was successfully constructed in an undergraduate laboratory setting. With careful alignment the beam retained up to 26% of its original power at the trapping plane. This tweezers setup was successfully able to trap particles in the ray-optics regime both axially and laterally; stable three dimensional trapping was therefore achieved with a single beam of tightly-focused
light. Trapping forces and efficiencies were quantified using the drag-force method. This was achieved by using a custom motorized stage designed to accelerate the microscope stage, and video analysis to determine the trapped particle’s fallout velocity.

The few undergraduate tweezers setups that do exist are complex and use expensive equipment such as quadrant photodiodes for force calibrations and piezoelectric picomotors for stage translation [17]. This project, however, has demonstrated that by using standard optical components, a low-power laser, a low NA microscope objective, and a manual microscope, we were able to construct a functional optical tweezers setup. Furthermore, our method for quantifying trapping forces allowed us to explore the effect of altering the trapping beam profile on trapping efficiency.

There are disadvantages to simplified tweezers designs. Axial trapping forces are difficult to quantify, and there is limited precision in drag force measurements. However, inexpensive optical tweezers designs are more accessible for undergraduate and even high school research in biology and other fields, and their simple design provides a unique opportunity for academic learning about optical trapping.

Optical trapping is a promising field for the future, as it provides a means of noninvasive particle manipulation. Recent advances include integrating spatial light modulators into tweezers, which can create arrays of computer-controlled optical traps, and the use of orbital angular momentum in optical vortices to rotate trapped particles. However, there are still many nuances to optimizing optical tweezers, particularly regarding trapping efficiency, that are yet to be explored and understood.
Appendix

The idea of optimizing trapping efficiency by increasing intensity of outside rays relative to on-axis rays is based on the idea that steep rays will produce a greater net change in momentum than on-axis rays. I created a simple mathematical model to confirm this concept in Matlab. The model assumed that the laser focus was small relative to the particle and that a beam can be treated as a large number of ray pairs; for every ray at a particular angle to the direction of beam propagation, there is another equal ray at the opposite angle to the normal.

Snell’s law and the Fresnel equations were used to calculate the unit change in momentum for ray pairs striking the trapped particle at different positions along the sphere (defined by the ratio $r/d$, in which $r$ is the sphere radius and $d$ is the distance from the particle axis) as the rays refracted through or reflected off the particle. The ray angles went up to 40° since this is the maximum angle using a NA=0.85 objective. The graphed results confirmed that ray pairs with larger angles to the direction of beam propagation do result in a higher change in momentum and, therefore, exert a stronger force on the particle. This increase is more pronounced in the axial direction than in the lateral direction.

Figure 17: Theoretical changes in momentum versus position of incidence on particle for ray pairs of varying incident angles, based on a simplified Matlab model.
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