

Polystyrene Bead Size Behavior in Optical Trapping

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Introduction:

Although optical trapping came about in the early 1980's the theory behind it has been around since the days of Kepler. His theories suggested a comet's tail was always pointed away from the sun because of some pressure caused by solar radiation. Newton and Bartoli also theorized that light exerted a kind of pressure on surfaces it interacted with. These ideas were better understood when Maxwell's electromagnetic theory placed a quantitative view on the magnitude of this radiation pressure and associated force which was followed by actual experimentation done by Bevedev and later Nichol and Hull in 1900 and 1901, respectively.

There were a couple of goals for this experiment. The first was to build a functional optical trap and become familiar with the settings and maneuvering of it. Next was to vary polystyrene sphere diameter sizes to better understand the consequences of Van der Waal forces, gravity effects, and sphere-sphere interactions when attempting to trap a particle. The main question that motivated this experiment's design was: How does beam and bead size affect trapping abilities? Modifications of the design occurred in accordance with testing the lasers holding capabilities. The third lens in the design was switched out to see if any trapping response was observed based on changes in the size of the beam.

Theory:

Particle trapping by means of light can be thought of as a cancelling out of 2 forces that arise from certain phenomena in light and matter. Equations 1 and 2 describe gradient

forces, \mathbf{F}_{grad} which are the forces generated by the pressure of light on a surface and the scattering force, \mathbf{F}_{scat} brought on by the transferring of momentum when passing through different mediums.

$$\mathbf{F}_{\text{Grad}} = -(\mathbf{n}_b/2)\alpha \{\nabla (\mathbf{E}^2)\} = -(\mathbf{n}_b^3 \mathbf{r}^3/2) (\mathbf{m}^2-1/\mathbf{m}^2-2)^2 \{\nabla (\mathbf{E}^2)\} \quad (1)$$

where n_b is the medium's index, m is the effective index and α represents the polarizability for a spherical particle.

$$\mathbf{F}_{\text{Scat}} = \mathbf{I}_0/c (128\pi^5 \mathbf{r}^6/3\lambda^4)(\mathbf{m}^2-1/\mathbf{m}^2+2)^2 \mathbf{n}_b \quad (2)$$

Here, I_0 is the intensity of the beam.

There are two ways to describe the relationship between a laser beam and a particle when trapping optically. If a particle has some diameter, d , where $d \ll \lambda$ (λ is the wavelength of the electromagnetic radiation) then related forces are better described in terms of magnetic and electric fields, for example when trapping electrons. If $d \gg \lambda$, a more practical approach can be used to discuss the interaction, radiation pressure. This pressure is responsible for generating a force onto the particle of interest.

Photons carry some momentum as described by $p=h/\lambda$ where h =Planck's constant. When a photon is emitted or absorbed by an atom, there is some change in momentum, and thus momentum transfer. The same occurs when electromagnetic radiation hits a dielectric sphere. The reflection and refraction of the light will exert a force on the sphere. This can be better understood through the following. Recall that acceleration a , is defined as dv/dt and momentum is defined as $p=mv$ but $F=ma$ so, $F=m(dv/dt)$. Here we can agree that when there is some momentum change, Δp in the light and therefore an equal but

opposite change, $-\Delta p$ must occur in the object to satisfy conservation of momentum. A change in velocity occurs when light passes from one type of medium to another. Each medium has an index of refraction, which is indicative of how fast photons can move through it. Momentum, $p = mv$, so when this change in velocity, Δv takes effect, momentum also experiences a change Δp . This change, dv over some time correlates to an acceleration which can then be linked to a force as seen in the equation mentioned previously: $F = m(dv/dt)$ Newton's 3rd law of motion says there must be a corresponding force that the particle experiences. This is known as the scattering force.

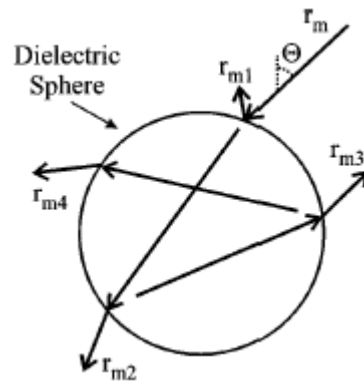


Figure 1. Ray tracing diagram. Smith (1998)

Figure 1 provides a visualization of how the rays of light interfere with a polystyrene sphere. r_m represents an incident ray on a polystyrene bead. r_{m1} and r_{m2} are reflected and refracted rays. A total force exerted on the bead can be calculated by summing all individual forces produced by their corresponding rays.

Methods:

The apparatus used for the experiment was adapted from similar experimental designs used in previous attempts at optical trapping (Smith et al, 1999, Appleyard et al, 2007).

The set-up was constructed based on what tools and optical devices were available in the laboratory (Fig 2).

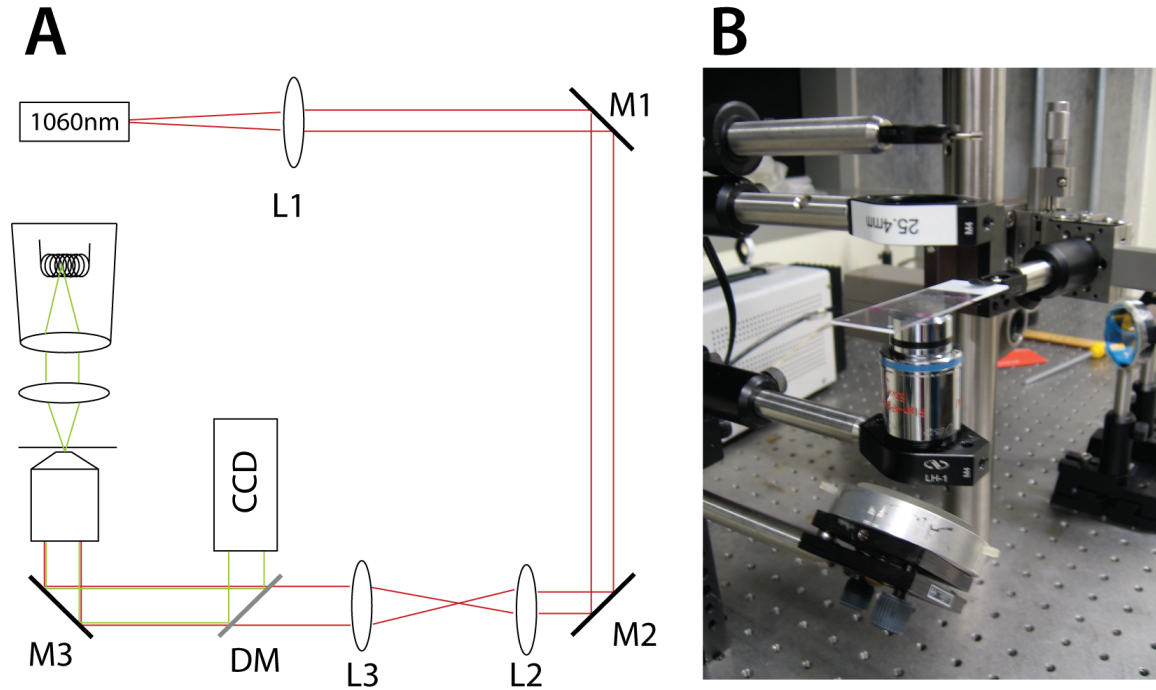


Figure 2. (A) Schematic diagram of apparatus. (B) Photo of apparatus.

Laser path

The entire apparatus was constructed on a large air table. The IR beam (wavelength=1060 nm, power =1065 mW) used first passed through a 500mm focusing lens (L1) and then reflected off the first angled mirror (M1) and again to another angled mirror (M2). Both these mirrors were fine tune adjusting mirrors. The IR beam was then passed through a telescopic pathway. The telescope consisted of a second converging lens (L2) with a magnification of 100mm, a pinhole (P1) and a third lens which was interchanged between

a 200mm converging lens and a 400mm converging lens for experimental purposes. The beam then followed a path through a dichroic mirror (DM), then off to a highly reflective mirror where it was reflected upwards through an infinity corrected oil immersion objective (NA=1.4). A glass slide with a glass cover slip held the sample in place. This was adjusted with a 3D micromanipulator.

Image path

The illuminating light source had a lens built in. We added a second 25.4mm lens (L4) to collimate the visible light incident upon the sample. The image of the sample passed through the objective and was reflected off the reflective mirror (M3), the dichroic mirror (M3) and into the CCD camera. In order to prevent saturation of the CCD by IR light reflected by the dichroic mirror, an IR filter was placed over the CCD while observing trapping.

Collimating the beam

Effective trapping requires that the light incident upon the back of the objective be collimated. As the light from the laser was diverging, we had to first collimate the beam by inserting a focusing lens (L1) proximal to the laser source. This was accomplished by coarsely measuring the beam size at two points along the track with an iris. The two different diameters were used to estimate a “point source” for the diverging laser light. We selected a focusing lens, placing it such that its focal point was at the estimate “point source” of the laser. Its final position was adjusted such that the diameter of the beam

was constant along the track, and M1 and M2 were adjusted to ensure that the laser path was parallel to the track.

Calculating the laser diameter

In order to ensure maximal trapping force, it is important to transfer an adequate amount of power through the objective. This requires that the diameter of the incident beam match the diameter of the back of the objective (7.5mm). This is adjusted by carefully selecting the focal lengths for the telescope portion of the laser path (lenses L2 & L3) and requires specific knowledge of the size of the beam incident upon the telescope. We used the “knife-edge” method to calculate the laser diameter. By progressing a razor blade across the path of the beam in small increments and calculating the amount of power in the portion of the beam which is passed, one can estimate the profile of the beam. By assuming a Gaussian profile, we were able to fit the power transfer curve to an error function and estimate the Gaussian power profile. Telescoping lenses were selected to evaluate the efficacy of two different final diameters on trapping success.

Finding the laser

We were able to take advantage of the suboptimal filtering of the dichroic mirror to locate the focal point of the laser on the slide. By loading a slide with dense polystyrene beads and lowering the slide to the objective, the refraction of the laser on the beads caused a circular interference pattern which could be visualized on the CCD (Fig 3). The camera was adjusted such that the laser focus was centrally located on the monitor. The focal point of the laser was not in the same plane as the image focus, indicating that the

laser was not collimated. Attempts to adjust the second lens in the telescope (L3) were not sufficient. Still, by noting the level of the stage where we began to observe the interference pattern, we rationalized that the laser was focusing closer to the objective than the image.

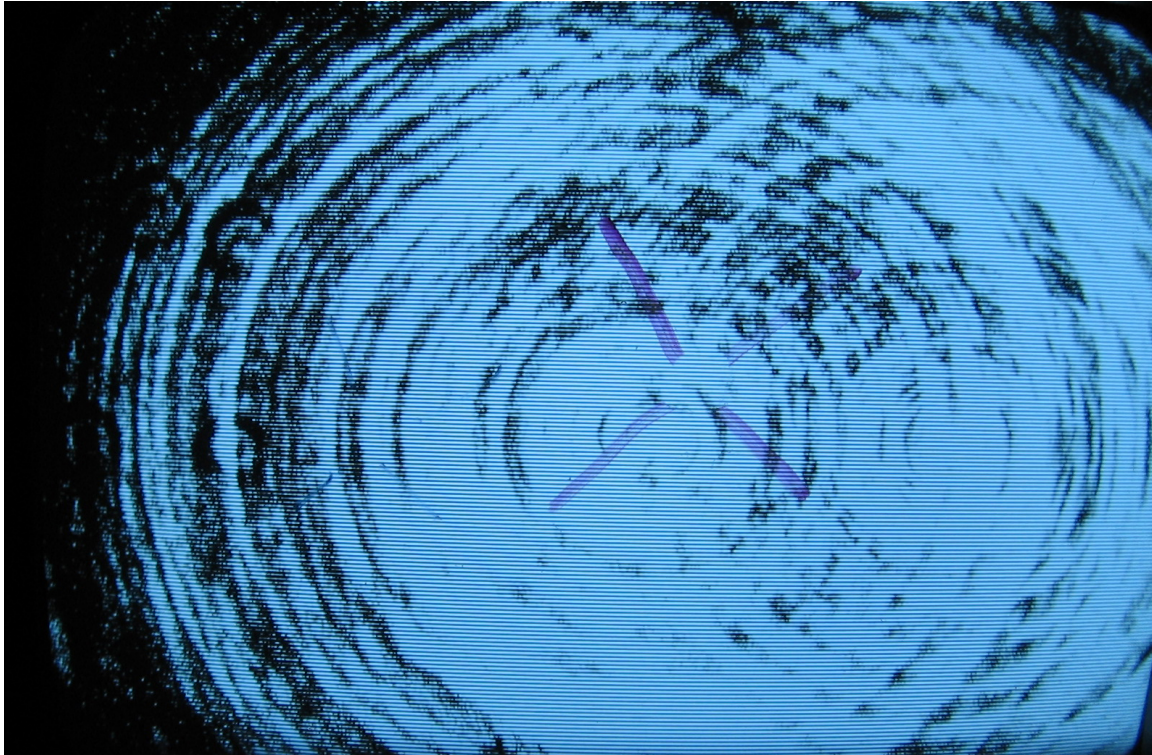


Figure 3. Interference pattern indicating location of laser center. The second pattern seen to the right is a reflection from the dichroic mirror.

Trapping

By carefully moving the slide back and forth while increasing the trap depth, we were able to trap poorly focused beads well enough to confirm that they had been trapped. In order to judge the efficacy of the laser at trapping various bead sizes and determine the efficacy of different beam sizes, we attempted to trap beads of $2.0\mu\text{m}$, $4.5\mu\text{m}$, and $6.0\mu\text{m}$

diameters with beam diameters (full width half max, FWHM) of 7.6mm and 3.8mm. The ratios of concentration solutions consisted of 1:2, 1:3, 1:5, 1:10, and 1:20 (bead solution : DI water).

Results:

Laser profile

A Gaussian intensity profile was also part of the project. This was taken to determine what lens magnifications were needed for our telescope to obtain the most power available given for our set-up. The graph below is a curve generated for the “knife edge” method used to find beam diameters 7.6 mm and 3.8 mm.

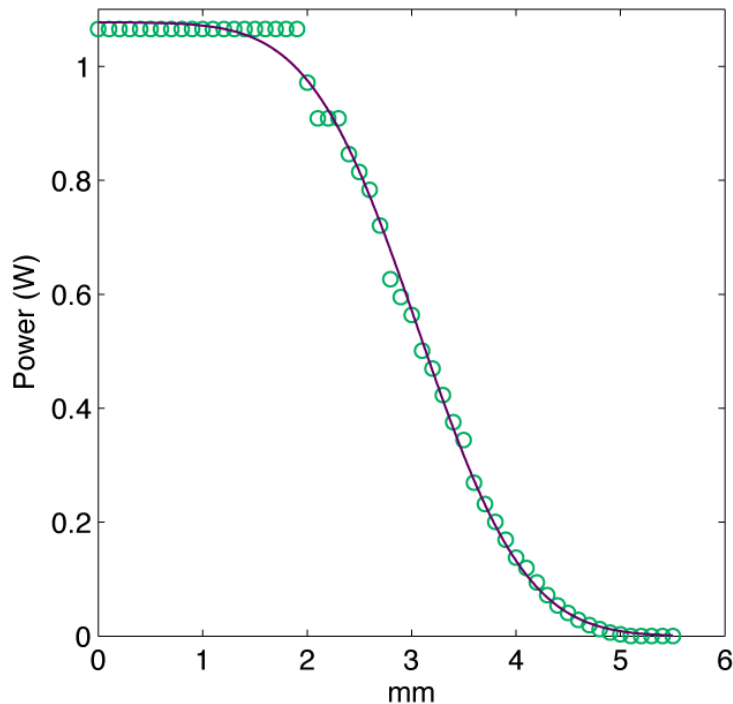


Figure 4. Knife-edge method to determine beam profile. The Gaussian approximation was derived from the fitted Error Function (solid violet line).

Trapping

We were able to successfully trap beads of 2.0 μm and 4.5 μm , but not those 6.0 μm in diameter. Trapping was effective with both beam sizes.

bead >		2.0 μm	4.5 μm	6.0 μm
V beam				
3.6mm		✓	✓	✗
7.6mm		✓	✓	✗

Table 1. Success (green checkmarks) and failures (red X) in trapping beads of different sizes with different sized beams.

Discussion:

Most of the goals set for this experiment were accomplished. We successfully determined the beam profile and captured beads of diameters 2.0 μm and 4.5 μm .

During early attempts at trapping the spheres, a “fountain” effect was observed. It speculated this was caused by the laser beam bringing up beads originally at a lower depth of the slide. These beads were thought to be dwelling in the non-focused area of the laser beam. The imaging depth was believed to be at a different depth than the beams focal point. So when these beads were pushed upwards, the image on the monitor showed many beads coming into the image plane. Once we were able to trap out-of-focus beads,

The size of the beam incident on the back of the objective is a key parameter in properly designing optical tweezers. A beam which is too large will prevent light from entering the objective and will decrease the total power of the trap, whereas a beam that is too small will limit the intensity at the most severe angles of the trap, again limiting the force. The chosen beam widths either matched the full width half max (FWHM) of the beam to the size of the objective or to half the diameter of the objective (allowing more power, but decreasing the intensity at the edges). We confirmed that the 7.6mm FWHM incident beam resulted in less power at the trap (19mW) than the 3.8mm FWHM beam (25mW). However, switching out of the 2nd lens in the telescope (and the subsequent change in beam diameter) showed no major effects on trapping capabilities. Subjectively, when comparing 6.0μm with 2.0μm and 4.5μm spheres, their behavior in aqueous solution was not the same. The beads of diameter 6.0μm gave surprising results even though their capture was not possible with our design. These larger beads displayed no visible motion whatsoever, whereas the smaller beads often flowed across the image field, which seemed abnormal since the other spheres expressed this behavior in all trial slides of varying solution concentrations. There may have been other factors affecting the movement of the 6.0μm beads that prevented their capture.

Since it is possible to trap more than one polystyrene bead at a time using a single beam, the idea of multi-sperm trapping was of interest. If trapping was possible, the next question was whether or not more than one sperm cell could inseminate the oocyte and what kind of response the oocyte would display if any. An early goal for the experiment, which was to trap *C. elegans* sperm cells and artificially inseminate them into an oocyte,

was later discarded as issues with visuals came up. Overall, goals set for this experiment were carried out successfully and offer a proof of concept for future experiments manipulating live *C. elegans* sperm.

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