# **Trapping Polystyrene Beads with Optical Tweezers**

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### Introduction

Optical tweezers are an important tool in the field of biophysics due to their ability to precisely manipulate micron-sized particles without causing damage to the sample (Ashkin et. al., 1987). The first optical traps relied on the use of either two laser beams (Ashkin, 1970) or the combination of one laser beam with an external force such as gravity or an electric field for stabilization (Ashkin et. al., 1971). The next important advance occurred when the single beam optical trap was developed (Ashkin et al., 1986). The single beam optical trap is much preferred due to its simple design as well as its ability to use a single microscope to trap and view the particle simultaneously.

The goal of this project was to build a single beam optical trap capable of trapping micron-sized beads. We investigated the range of bead sizes we could trap with our setup, and then attempted to further optimize our setup to increase this range.

# Theory

The trapping force  $(F_T)$  experienced by an object is a combination of two forces: a gradient force  $(F_G)$  and a scattering force  $(F_s)$  and determined by the relation:

$$F_T = F_G + F_S$$

The gradient force arises from the photon pressure of the incident laser beam against the surface of the trapped object and as such points in the direction of photon propagation. The scattering force is a result of the change in velocity that occurs when light travels between medias of two different indices of refraction (Figure 1a,b). Since momentum is velocity dependent and always conserved, the trapped object moves with equal but opposite momentum of the photons i.e. in the direction of the incident light (Figure 1c).

# Methods

#### General Setup

We designed an optical trap based on simple setups found in the literature (Smith et al., 1999). The general purpose of the design is to fill the back of a high NA objective with collimated laser light, which focuses the beam onto a sample of beads that can be imaged with a CCD camera. A diagram of the setup is shown in Figure 2. The entire setup was built on an anti-vibration optical table. A 1064nm, 200mW laser was used. To align the optics, we used an IR viewer to allow us to see the beam. The laser was sent through two consecutive beam expanders to expand the beam to fill the back of the objective. The first beam expander consists of two lenses with 38.1mm and 400mm focal lengths (Figure 2b,c). The second beam expander is made up of two lenses with 76.2mm and 150mm focal lengths (Figure 2d,e). This gives total beam expansion by a factor of 20.7. In principle, we could have constructed a single beam expander to do this. However, with the lenses available to us, it was necessary to construct two to get a large enough expansion. The 76.2mm focal length lens (Figure 2d) was placed on a micrometer to allow fine adjustments to its position along the optical path. This gave us control over the divergence of the laser light as it entered the objective and allowed us to fine-tune the position of the focus in the z-direction.

After going through the beam expander, the laser light hit a mirror angled at approximately 45 degrees to redirect the beam due to space constraints. Next, the beam hit a dichroic mirror that allows IR light to pass through. Another mirror angled vertically at 45 degrees directed the light up to the back of the objective, which then focused the beam onto a slide of beads. To image the beads, a light bulb and a 100mm lens to collimate the light were placed above the slide (Figure 2k,l). This visible light passed back through the objective, hit the mirror to redirect it horizontally, and reflected off the dichroic mirror to the CCD camera (Figure 2g-l). This was hooked up to a monitor, where we could view the image of the beads. We used two different objectives in this experiment: a 40x, 1.3NA, infinity corrected, oil objective; and a 63x, 1.4NA, infinity corrected, oil objective. The back of the 40x objective was larger than the back of the 63x objective, but under-filled the back of the 40x objective. This meant that the NA that we actually got from the 40x objective was less than 1.3.

### Sample Preparation

Number 1 coverslips were painted around the edges with a thick layer of nail polish to create a well for a polystyrene bead solution. A microscope slide was then attached and nail polish was used to seal the slide to the coverslip. It was necessary to create the well for the beads so that they would remain free to move in the solution rather than getting pressed between the slide and coverslip. Solutions of high and low concentrations of polystyrene beads of a range of diameters  $(2\mu m, 4.5\mu m, 6\mu m, and 15\mu m)$  were created.

### Three Dimensional Trapping

To prove that the beads were actually trapped, we used the laser to manipulate the position of the bead. To do this, we changed the angles at which the laser light entered the objective. By making fine changes to the angling of the second mirror (Figure 2h), we were able to change the position of the focus of the laser in the x-y plane. To prove the bead was also trapped in the z-direction, we moved the 76.2mm focal length lens (Figure 2d), which adjusted the position of the focus of the laser to be higher above the objective. This brought the trapped bead out of focus. Turning off the laser (or blocking it) after this step, allowed us to see the bead fall back into focus.

### Lowering the Power of the Laser

To test the effect of lowering the power of the laser on our ability to trap  $2\mu$ m beads, we inserted neutral density (ND) filters in the path of the laser between the first mirror (Figure 2f) and the dichroic mirror (Figure 2g). We used ND filters of four different optical densities (d): 0.10, 0.50, 0.70, and 0.80. The ratio between the intensity after the filter (I) and the incident intensity (I<sub>0</sub>), the fractional transmittance, is given by the following relation: (I/I<sub>0</sub>)=10<sup>-d</sup>. The filters that we used corresponded to the following fractional transmittances (optical density in parentheses): 79% (0.10), 32% (0.50), 20% (0.70), 16% (0.80).

### Results

### Trapping Beads

Using the 63x objective, we were able to trap 4.5µm beads (Table 1). To prove that we had trapping in the x-y plane, we moved the position of the focus of the laser in the x-y plane and watched the bead move with it (Figure 3, see attached movie). We did not need to position the

laser on top of the bead in order for it to be trapped. Rather we observed beads near the focus of the laser being sucked into the focus where they became trapped. We also observed that beads that were being sucked in could push out a trapped bead. To prove that we had trapping in the z-direction as well, we pulled the bead to a higher plane where it was no longer in focus in the image, then turned the laser off and watched the bead fall back into focus.

We were not able to trap  $2\mu m$ ,  $6\mu m$  or  $15\mu m$  beads (Table 1). The laser did not have a noticeable effect on the  $6\mu m$  or  $15\mu m$  beads. However, the laser did have a very noticeable effect on the  $2\mu m$  beads. The beads were rapidly sucked into the focus of the laser, but rather than getting trapped, they continued along their path through the focus of the laser and out the other side.

#### Lowering the Power of the Laser

We lowered the power of the laser and tried again to trap  $2\mu$ m beads with the 63x objective. As we decreased the power, we saw a decreasing effect of the laser on the beads. Without any power reduction, we observed the  $2\mu$ m beads being sucked rapidly through the focus of the laser in the x-y plane. With 79% fractional transmittance (d=0.10), we did not observe any visible change in the effect. With 32%, 20% and 16% fractional transmittance (d=0.50, 0.70, 0.80, respectively), we observed the beads being sucked through the focus with successively less speed. We did not observe trapping of the  $2\mu$ m beads with any of the four reduced power setups.

#### Effect of Bead Concentration

Testing the  $2\mu m$  beads, we observed a difference in the effect of the laser on samples of low bead concentration versus high bead concentration. With high bead concentration, we observed the effect noted in the previous results sections. However, with low bead concentration, we observed little to no effect of the laser on the beads.

#### Effect of NA

We tried trapping each bead size with the 40x objective as well as the 63x objective. We did not observe trapping of any bead size with the 40x objective. However, we did see an effect of the laser on the  $2\mu$ m and  $4.5\mu$ m beads with the 40x objective. When the laser was placed in an area with a high concentration of beads that were in focus, these beads were slowly pushed out of focus. We concluded that the NA was not sufficient with the 40x objective, given the diameter of our beam, to be able to trap a bead.

### Discussion

#### Optimizing the Setup

Using the setup described in this report, we were able to trap  $4.5\mu m$  beads. Despite lowering the power of the laser with ND filters, we were not able to trap  $2\mu m$  beads. Additionally, we were not able to lower the power enough to stop seeing an effect of the laser on the beads. Therefore, in the future, we would like to further lower the power until we either get trapping or stop seeing an effect on the beads. This would allow us to conclude whether the  $2\mu m$  beads are too small to be trapped with this setup or if a low powered beam is required to trap them.

We also noticed an interesting effect of the bead concentration. With a high concentration of beads, we saw a very strong effect of the laser, but with a low concentration of beads we saw little to no effect of the laser on the beads. In the future, it would be interesting to quantitatively

test this effect and determine an optimal bead concentration to best observe the effect of the laser on the beads. We hypothesize that this effect may arise because in the low concentration samples there is a lower probability of encountering beads in the same plane as the focus of the laser. Additionally, with the high concentration of beads, as one bead was sucked into the laser, several beads around it were often perturbed and subsequently sucked into the laser as well. This also may have played a role in the effect that we observed.

We were only able to observe trapping using the 63x objective. However, we hypothesize that it is also possible to achieve trapping with the 40x objective if its maximum NA was utilized. To do this, the beam would need to be expanded further so that it would actually fill the entire back of the objective. This could be done either by modifying the lenses in the current beam expanders, or by adding a third beam expander. Due to the space constraints of our setup, both of these options would have required significant rearrangements.

Finally, for future experiments we would need to be able to move the slide relative to the trapped bead, rather than moving the trapped bead relative to the slide. This would allow the position of the second mirror (Figure 2) to remain fixed and thus, the force exerted on the trapped bead to be more constant. To do this, the slide should be mounted on a micrometer to allow fine adjustments of its position.

### Future Application – Stretching of DNA

A future biological application of our single beam optical tweezers setup would be to study the mechanical force-extension relationship of single DNA molecules. To do this, we would attach one end of a DNA molecule to a coverslip and the other to a 4.5µm bead (Figure 4) using the methods described by Wang et. Al (1997). The bead would be trapped by the methods discussed in this report, while the coverslip would be pulled relative to the trap. The distance the DNA molecule stretches before it breaks would then be measured.

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# **Figures**



**Figure 1.** An Illustration of the Physics Behind the Optical Trap. a) The component of  $F_s$  perpendicular to the beam axis. This component is canceled out by the beam coming in from the

opposite side of the objective. b) The component of  $F_s$  parallel to the beam axis. This is the component that contributes to  $F_T$  c)  $F_G$ . Source: Dholakia, et al. 2008.



**Figure 2.** Optical trapping setup. (a) 1064nm, 200mW laser. (b) 38.1mm focal length lens. (c) 400mm focal length lens. (d) 76.2mm focal length lens. (e) 150mm focal length lens. (f) Mirror angled at approximately 45 degrees. (g) A dichroic mirror that passes IR light and reflects visible light. (h) A mirror angled vertically at approximately 45 degrees. (i) Either a 40x, 1.3NA, infinity corrected, oil objective, or a 63x, 1.4NA, infinity corrected, oil objective. (j) Slide containing the sample of interest. (k) A 100mm focal length lens to collimate the light from the light bulb above it. (l) A light bulb used to illuminate the slide for imaging. Parts labeled (h-l) are on a vertical rail.



**Figure 3.** Trapped Bead. From left to right: (a) Bead moving into the laser trap. (b) Laser with the bead trapped. (c) Bead moved up and to the right by the laser. (d) Trapped bead with the laser blocked.



**Figure 4.** Stretching of DNA. One end of the DNA molecule would be attached to the coverslip by a RNA polymerase complex, while the other end would be attached to a trapped polystyrene

bead at a height h and making an angle  $\theta$  with the coverslip. The coverslip would then be pulled and the distance the DNA molecule stretches would be measured. Source: Wang, et al. 1997.

# Tables

	2μm	4.5µm	6µm	15µm
40x	No*	No*	No	No
63x	No*	Yes	No	No
63x+d=0.10	No*			
filter				
63x+d=0.50	No*			
filter				
63x+d=0.70	No*			
filter				
63x+d=0.80	No*			
filter				

**Table 1.** Trapping beads of various diameters. "Yes" indicates that the beads were trapped successfully. "No" indicates that the beads were not trapped. A \* indicates that the laser affected the beads, but did not trap them.

# References

- Ashkin, A. (1970) "Applications of laser radiation pressure." Science, New Series, 210:1081-1088
- Ashkin, A., and Dziedzic, J.M. (1971) "Optical levitation by radiation pressure." Appl. Phys. Lett., 19:283-285.
- Ashkin, A., Dziedzic, J.M., Bjorkholm, J.E., and Chu, S. (1986) "Observation of a single-beam gradient force optical trap for dielectric particles." Optics Letters, 11, 288-290.
- Ashkin, A., Dziedzic, J.M., Yamane, T. (1987) "Optical trapping and manipulation of single cells using infrared laser beams." Naure, 330, 769-771.
- Dholakia, K., Reece, P., Gu, M. (2008) "Optical micromanipulation." Chem. Soc. Rev., 37(1), 42-55.
- Smith, S., Bhalotra, S., Brody, A., Brown, B., Boyda, E., Prentiss, M. (1999) "Inexpensive tweezers for undergraduate laboratories." Am. J. Phys., 67, 26-35.
- Wang, M., Hong, Y. Landick, R., Gelles, J., Block, S.M. (1997) "Stretching DNA with Optical Tweezers." Biophys. J., 72(3), 1335-1346.