Advanced Dual Beam Optical Tweezers for Undergraduate Biophysics Research

Brian Daudelin

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Advanced Dual Beam Optical Tweezers for Undergraduate Biophysics Research

Brian Daudelin

Mentor
Dr. Thayaparan Paramanathan

Submitted in Partial Completion of the Requirements for Departmental Honors in Physics

Bridgewater State University

December 22, 2016
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Dr. Thayaparan Paramanathan, Thesis Director

Dr. Jeff Williams, Committee Member

Dr. Edward Deveney, Committee Member
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-Brian Daudelin
Abstract

Optical tweezing is a modern physics technique which allows us to use the radiation pressure provided from laser beams to trap very small microscopic particles. In the last two decades optical tweezers have been used extensively in biophysics and atomic physics to study the building blocks of our world on the cellular and quantum levels. Our goal is to construct a dual beam optical tweezers for future undergraduate biophysical research. In this thesis we discuss how the construction and assembly of the dual beam optical tweezers is done from start to finish. Construction consisted of assembling a polarization maintaining laser. This was then split into two to create a dual beam effect. Directing both beams in symmetrical and equidistant paths with the help of optical elements, the beams were overlapped in a counter propagating orientation. Microscopes were then used to focus the lasers into the target flow cell. Another set of optics were used to image the inside of the flow cell so we could visualize the laser trapping. It was necessary to construct a custom compressed air system to isolate the optical table from surrounding vibration so that we can accurately measure the pico-newton scale forces that are observed in biological systems. In addition, the biomaterial flow system was designed to supply the flow cell with biological solutions essential for experimentation. Future plans for this project include developing the software in order to collect experimental data and run biophysics experiments. This optical tweezers apparatus will allow us to study potential cancer drug interactions with DNA at the single molecule level and be a powerful tool in promoting interdisciplinary research at the undergraduate level.
Introduction

Optical Tweezers use the radiation pressure created by finely focused laser beams to trap very small particles. Optical Tweezers are used in many different fields and focus on trapping various different particles, which range from the size of atoms to biological macromolecules.[1, 2] Currently, Optical tweezers are used extensively in biophysics to study biological systems at a single molecule level.[3-9] The goal of our project is to create an optical tweezers set up to be used for biophysics research at Bridgewater State University. The current plan is to study interactions of cancer drugs with DNA. In this introduction, we will be going over the physical principles and specific applications pertaining to the optical tweezers in a historic perspective.

The First Optical Trap

The first known trapping of micron sized particles using the potential created from a laser was accomplished by Arthur Ashkin in 1970 at the Bell labs located in New Jersey.[10] This was the first instance of lasers being used to create an optical potential which could trap a particle in three dimensions.

He accomplished this by directing two counter-propagating laser beams through a liquid medium to trap an object with diameter around 2.6 µm. He found that the speed of light through the tapping objects and trapping medium play a huge role in trapping. Comparison of the speed of

Figure 1: Arthur Ashkin in his lab at Bell Labs (photo courtesy of Laser Fest)
light through a medium is often referred based upon the index of refraction, higher the index of refraction, lower the speed of light. Objects made with a material of greater refractive index would be pulled into the center beam waist at an equilibrium point between the counter-propagating lasers.[10] The technique would later be coined as optical tweezers, and has since contributed to both the fields of atomic physics and biophysics.

**Trapping Atoms with Modified Optical Molasses**

Optical tweezers come in a variety of different forms, which aid in the specific trapping needs of an experiment, with all forms relying on the pressures of light radiation. Similar to how Ashkin was the first to have observed the trapping of dielectric particles by an optical trap, Steven Chu was the first to observe the trapping of single atoms coincidentally also at the Bell Labs sixteen years later. Chu was awarded the 1997 Nobel Prize in physics for his ground breaking developments on the trapping and cooling of neutral atoms using a single beam optical trap in 1986.[2] Most atoms are roughly a half nanometer in width, whilst the dielectric beads which Ashkin trapped were on a scale of microns. This difference in size and mass further complicated the trapping process. The smallest of thermal forces would compromise the trap, “boiling”, as Chu called it, an atom out of the equilibrium created by an optical tweezers. Chu was able to trap atoms using a single beam optical tweezer in conjunction with a
modulated Optical Molasses (OM) to stabilize the atoms during the trapping in order to limit any thermal or spatial obscurities.[2] OM is created purely of photons induced by three pairs of counter propagating laser beams. Essentially making OM creates a three dimensional trapping potential designed to dampen the translational and more importantly the angular velocities of atoms which enter it. OM reduces the speeds of atoms to tens of meters per second resulting in the rapid cooling to temperatures a fraction of a single Kelvin. Due to OM’s ability to dampen motion, atoms are able to be targeted individually by conventional optical traps.

**Trapping Biological Molecules**

The first biological application using optical tweezers occurred in 1987, where Arthur Ashkin and Joseph Dziedzic directed their interests onto manipulating viruses and bacteria with light.[1] Ashkin believed that due to his previous experimental results trapping micron scale dielectric beads it would be feasible to trap microscopic biological particles as well. The pair referred to the trapping technique now as an “optical tweezers” for how it easily manipulated clusters of live bacteria without damage.[1] This was only after using laser wavelengths in the near infrared spectrum as the radiation was not so easily absorbed by the water medium.[1] Experiments were also able to be visualized through a high resolution microscope as well by electronic detection from scattering light rays. Experiments
were done on TMV (Tobacco Mosaic Virus) and E Coli bacteria. They found TMV was a more difficult object to capture possibly do to its cylindrical geometry. As Ashkin explained in his earlier paper, object geometry can play a crucial role in trap effectiveness; spherical and ellipsoid shapes are favored as they induce a greater attractive force onto the object.[10] The bacteria on the other hand was easily captured by the optical tweezers at powers around 10 mW allowing for full manipulation of the bacterial cell.[1] Ashkin and Dziedzic’s study pioneered the use of optical trapping in the field of biology, revealing a wide range of innovations to be made onto their platform.

Following in the footsteps of Ashkin, physicists like Steven Block began to experiment on biological objects using optical tweezer as they allowed for safer and precise manipulation for accurate measurements. In 1992, Steven Block, at Rowland Institute, Harvard University measured the membrane elasticity of bacteria and red blood cells using optical tweezers.[11] This allowed Block to test the technique on various different biological particle types throughout the 1990’s.[5, 11]
Trapping Single DNA Molecules

Life on this planet are composed of a combination of individual cells. All information that pertains to the cell such as what to do, when to divide and make a daughter cell etc. are stored as codes in DNA (Deoxyribo Nucleic Acid). Thus every cell contains a DNA molecule that carries the organisms’ genetic code. The DNA molecule structure is a double helix which has to twist and unwind in order to read the information encoded. In addition, since DNA is a polymer chain it is elastic. It wasn’t very long until optical tweezers were being used to trap a single DNA molecule to stretch them and study the elastic properties of DNA. The first DNA stretching experiments with optical tweezers were done by Steven Block when he was at Princeton University. [5] DNA molecules were chemically attached to dielectric beads that were trapped with an optical tweezers. The other end of the DNA was directly tethered to the slide which experiments were done on; stretching the DNA molecule as the slide moved. As the DNA is stretched, the tension on DNA was measured based off the deflection of the laser as the bead was pulled out of the trap by the DNA molecule.

Other Single Molecule Applications

The early single molecule experiments done by Block using optical tweezers inspired new innovative techniques to study single molecule systems across the field. Using optical tweezers, Block set the precedent by distinguishing the “hand over hand” motion of the motor protein

Figure 4: Steven Block, currently Professor at Stanford University is the pioneer of DNA stretching using optical tweezers (photo courtesy of Block-Lab Stanford University)
kinesin along a microtubule. [6] Kinesin is a necessary transport protein within a cell, and how it mechanically traveled was up to much debate prior to Blocks results. Recent experiments done by Bustamante’s lab, another leading optical tweezers based lab, showed the characteristics of viral packaging motors adapting as they fill viral cavities with DNA. [3] Other experiments used nonspherical dielectric particles such as cylinders when trapping in order to induce torques on molecules such as DNA. [9] Drug DNA interactions between possible HIV and Cancer fighting compounds are currently studied using optical tweezers and are tested on their ability to inhibit DNA transcription. [8] More recently the combination of optical tweezers and fluorescent imaging technology has been used to study motor protein mechanisms in greater detail than ever before. [7, 12]

**Why Optical Tweezers**

To study biological systems at single molecule level, the question usually comes down to which manipulation method should be used. Optical tweezers, magnetic tweezers or AFM (atomic force microscopy) are the three techniques of choice which allow us to trap and manipulate single biological molecules. Magnetic tweezers trap and manipulate tiny magnetic beads with the help of varying magnetic fields. They use the images of the circular diffraction patterns formed around the magnetic beads to estimate the forces acting on them when they are displaced. AFM uses a
very small cantilever system which physically scans the surfaces of objects which can create a higher resolution image than any light microscope could possibly obtain.

Though each method was able to produce similar results, the optical tweezers method ended up being the preferred technique for experimenting on biological macromolecules as it was the most versatile and had the best force resolution. Magnetic tweezers can suffer from a lack of experimental resolution due the visual data acquisition techniques used during experiments. The change in these patterns are used to distinguish the applied forces on the beads and require the help of very high speed, and likely expensive, cameras to measure the data accurately.[4] AFM cantilevers tend to be pretty large and may not be able to pick up all of the lower bound forces applied to the biological molecules. Force data could also be obscured because the AFM may not be secured to the correct molecule binding site or interacting with a non-target object.[4] Though there are some drawbacks with the optical tweezers technique, as the trap cannot distinguish the difference between a dielectric bead or dielectric debris without visual aid. These drawbacks are easy to manage as long as the sample is visualized.
Theory

Radiation Pressure and Force Exerted by Light

If we were to take a laser and shine it against a mirror, what would we observe? We would see the laser reflect off the mirror and land somewhere else. In essence, is this any different from a ball being bounced off a wall? Both carry with them a linear momentum and interact with the surface in a similar way. The Newtonian definition of a force $F$ onto an object is the change of momentum $p$ in respect to time $t$:

$$F = \frac{dp}{dt} \quad (1)$$

Now both the light and the ball would have to exert a force on the contact object in order to bounce due to Newton’s third law. Say if we took the total area $A$ affected by this impact force $F$, we could say it has a pressure $P$:

$$P = \frac{F}{A} \quad (2)$$

$$P = \frac{1}{A} \frac{dp}{dt} \quad (3)$$

This is true both in the case of the ball and the laser. In the case of the laser this pressure exerted by the electromagnetic radiation on the mirror is called radiation pressure. [10, 13, 14]

Say in both examples you held onto the wall and mirror. What would you feel? You would definitely feel the force of the ball bounce off the wall, but none from the lasers reflection. From quantum mechanics we know that light acts like a wave but could also be quantized as a massless
particle known as photon. A laser beam is made up of a collimated collection of coherent photons. The momentum of a massless particle can be expressed as follows:

\[ p = \frac{U}{c} \]  \hspace{1cm} (4)

Where \( U \) the energy of the photon and \( c \) is the speed of light (a constant). Therefore, the rate of change of momentum can be expressed as:

\[ \frac{dp}{dt} = \frac{1}{c} \frac{dU}{dt} \]  \hspace{1cm} (5)

Combining Equation 4 with Equation 5 we can express the pressure caused by light as

\[ P = \frac{1}{Ac} \frac{dU}{dt} \]  \hspace{1cm} (6)

The change in energy with respect to time indicates a power, and for electromagnetic radiation traveling through a surface it refers to a Poynting flux.[14]

\[ \Phi_p = \frac{dU}{dt} = \oiint SdA \]  \hspace{1cm} (7)

Where \( S \) represents the Poynting vector which represents the power flow per unit area and also indicates the direction of the flux flow through a surface area \( dA \).[14] Based on Equations 6 and 7 we can express the radiation pressure of the light as:

\[ P = \frac{1}{Ac} \oiint SdA \]  \hspace{1cm} (8)

The force due to radiation pressure produced by light on an object can be obtained by multiplying the above pressure by area:
\[ F = PA = \frac{1}{c} \iiint S dA \quad (9) \]

In general, we have to account for the electromagnetic flux entering \((S_{in})\) and leaving \((S_{out})\) the object in order to find the net force acting on it.

\[ F = \frac{1}{c} \iiint (S_{in} - S_{out}) dA \quad (10) \]

When the laser hits a perfect mirror the light is completely reflected meaning none of the radiation is absorbed. If this is true, then \(-S_{in} = -S_{out}\), which yields us \(F = \frac{2}{c} \iint (S_{in}) dA\). Since the entering flux term is from the laser, from the definition of Poynting vector mentioned earlier, the term \(\iint (S_{in}) dA\) is essentially the power of our laser. Therefore, the force on our mirror would be \(F = \frac{2}{c} (P)\). So back to our original question, why can’t we feel the force or can we see the mirror move? Let’s say our lasers power was 500 mW, this would apply a force of only \(9 \times 10^{-7} \text{ N}\) onto the mirror.[14] The force is so minimal that objects that we can see with our naked eye are virtually unaffected by this force.[13, 14]

**Trapping Dielectric Particles with Optical Tweezers**

As we showed in the previous section, on the visible scale, moving objects with radiation pressure is almost impossible because it provides such a weak force. But manipulating objects on the microscopic level using the forces produced by radiation pressure has been shown possible in the studies completed by Arthur Ashkin. He used the radiation pressure from two finely focused lasers to capture micron scale dielectric beads.[10] These dielectric beads are transparent and
allow for light to be absorbed and pass through them. Considering light is passing through a medium other than a vacuum, the speed of light would be less than \( c \). The force on a specific bead would be \( F = \frac{1}{v} \oint (S_{in} - S_{out}) \, dA \) based upon Equation 10, where \( v = \frac{c}{n} \).

\[
F = \frac{n}{c} \oint (S_{in} - S_{out}) \, dA \tag{11}
\]

The direction of the force on the bead would be in the direction of the net electromagnetic flux through its surface.

For example, when the bead size (in our case 5 micron) is much bigger than the wavelength of the light (in our case 785 nm) used to trap, you can qualitatively show the net force on a bead due to radiation pressure using geometric (ray) optics. When light ray is incident upon an interface of two mediums the ray gets bent because the speed of light varies in different medium, which is known as refraction. When the light passes through the bead in an optical tweezer set-up it gets refracted twice, once while entering the bead and then while leaving the bead.

Figure 6 illustrates a light ray travelling through a medium of refractive index \( n_1 \) and incident on another medium of refractive index \( n_2 \). Snell’s law states that \( n_1 \sin \theta_1 = n_2 \sin \theta_2 \) and dictates how light is refracted at an interface of two mediums. The angles \( \theta_1 \) and \( \theta_2 \) are the angles made by the incident beam and refracted beam respectively with the normal (perpendicular line drawn at the point of incidence in the plane created by the incident and refracted beam). As we see in Figure 5, based on Snell’s law, light is either bent toward the normal or away from it. Having a bead of refractive index higher or lower than the mediums index can result in drastic differences in the direction of the light as it’s refracted through the bead. Also because the bead is not
perfectly transparent some of the light is always reflected as it contacts the surface, and as we’ll see later can cause some problems.

Let’s consider the refracted light for when \( n_1 < n_2 \). In the absence of the bead, light rays will be focused by the objective at its focal point (as shown by the dashed line in Figure 7A and 7B). As light enters the bead it is refracted towards the normal and it is refracted away from the normal when it exits the bead. When the bead is along the optical axis of the objective (Figure 7A) we can figure out the momentum change of photons and hence the net force on photons. This will

---

**Figure 6:** The impact of Snell’s law on radiation as it interacts with the interface of a bead. Incoming radiation (blue) partially gets reflected (purple) and partially refracted at the interface of the bead. Refractions toward (red) or away (green) from the normal of the interface are dependent on the indexes of refraction of the medium and bead.
give us the direction of the net force acting on the bead, which will be in the opposite direction of that on the photons and directing towards the focal point of the objective.

![Diagram of Gaussian Laser and Objective](image)

*Figure 7: (A) Two symmetric rays (red and orange) trapping a bead while it is along the optical axis of the objective. (B) Trapping a bead while it is offset from the optical axis, where the force from the center ray (red) is larger than that of the ray from edge (orange). The black arrow identifies the net force felt by the bead due to the trap. The orange and red arrows of the vector diagram correlate to the forces due to momentum change in orange and red rays. The blue dashed lines indicate the normal to the interface at the point of incidence.*

When the bead is away from the axis of objective (Figure 7B), the Gaussian nature of the laser helps to trap the bead. Due to the Gaussian nature of the laser, more intense beams of light are found in the middle (red ray in Figure 7B) and will undergo greater momentum changes than that
of the outside beams (orange ray in Figure 7B). Similar analysis like in Figure-7A can be used to show that the final direction of the net force it applied onto the bead will have a horizontal and vertical component (Black in Figure 7B).

In both of these examples the bead is drawn toward the objectives focal point, which acts as a potential equilibrium for the trap.

Now let’s consider the case when $n_1 > n_2$. As Ashkin found, beads with lower indexes of refraction than the medium surrounding will be rejected from the trap and pushed out of the beams focus.[10] As depicted in Figure 8 the net force on the bead (Black Figure 8) would completely send it the opposite direction of the focal point. [13]

![Figure 8: Bead ejection from the trap when the index of refraction of the bead is less than that of the medium surrounding it. The dashed lines indicate the normal vector of the interface and the solid black arrows indicate the net force on the bead. The orange and red arrows of the vector diagram correlate to the forces due to momentum change in orange and red rays. The blue dashed lines indicate the normal to the interface at the point of incidence.](image)

**Scattering Force and Increasing the Trapping Force**

Because the beads surface will reflect some of the incoming radiation a reflecting force is exerted onto the bead. This force is enough to easily overpower the trapping force and lose the bead. Radiometric forces, the thermal forces caused by temperature fluctuations within the medium,
may also be liable for losing the trap as they also apply their own pressure on the bead. Higher trapping forces are required to fix these problems and can be acquired in multiple ways.

The microscopic objectives used in optical tweezers finely focus the laser light to a desired focal length, but also contribute to most of the trapping potential. Objectives come in many sizes, with different numerical apertures (\(NA\)) for different mediums. The numerical aperture of an objective is defined as;

\[
NA = n \sin \theta
\]  

(12)

Where angle \(\theta\) is the maximum angle which light can be focused by an objective and \(n\) refers to the refractive index of the medium directly after the objective. As angle \(\theta\) increases so do the trapping forces from the perimeter beams of light. Based on the above equation, for maximized trapping forces the NA should be numerically close to the index of the medium to maximize angle \(\theta\). For example, water immersion objectives require a water medium and would require a \(NA \approx 1.33\) for maximum trapping power.

A single beam trap can only be as effective as the objective and laser power allows, and this still may not be enough to keep a bead at equilibrium due to external forces. Two beams are often used in optical tweezers set ups to further increase trapping forces onto a bead during experimentation. Having two counter propagating laser beams doubles the traps effectiveness, greatly reducing the effects of reflection forces (green rays in Figure 9) along with any other external forces on the bead.

Though the dual beam trap is the most stable, it gets increasingly more difficult to use as target size drops. In order for a dual beam optical tweezers to trap a target object its two microscopic
objectives must be focused to a spot on a scale well within the targets diameter. For beads as small as a couple of microns this technique can be used effectively as it is possible to focus these objectives to micron precision. As target size becomes sub-micron the traps effectiveness lacks due to an inability to focus the objectives. Manipulating sub-micron sized objects is mostly done with single beam traps for the sake of practicality. As particle size decreases so do the effects of radiometric forces on an object. This allows single beam traps to be more effective as particles get smaller, but much larger particles above the diffraction limit of visible light may need two laser beams to trap a particle.[2, 13]
Methods

Dual Beam Optical Tweezers Design Overview

Our design consists two counter propagating laser beams focused at the same point inside a flow cell to trap polystyrene beads (the red line path in Figure 10). An LED-camera system is also used to image the flow cell (the blue line path in Figure 10) so we can see the trap. As we have discussed in previous sections, the dual beam design provides greater trapping forces and allows for greater forces applied during experiments.

Figure 10: Dual beam Optical Tweezers schematic
Construction of optical tweezers consisted of the general alignment and positioning of optics in order to trap a bead. Polarized butterfly diode laser (Figure 10 #1) with PM fiber (Figure 10 #2) is coupled to the fiber port (Figure 10 #3) to provide the collimated laser beam with linear vertical polarization. We split the beam into two separate beams with the help of 50/50 beam splitting cube (Figure 10 #4), directing the beams towards the polarizing beam splitting cubes (Figure 10 #5). Then each of the beams pass through a quarter wave plate (Figure 10 #6), which makes the polarization circular before entering the microscopic objectives (Figure 10 #7). The beams are focused into the flow cell to create the trap and emerge through the opposite objective. The emerging beam then hits another quarter wave plate on the opposite side, where the polarization is made to be linear again but rotated 90 degrees with respect to the original polarization of the laser so that the current polarization is horizontal. This helps the beam to be directed up towards the detectors by means of a polarizing cube (Figure 10 #8) to measure the deflection of the laser so that we can estimate the force acting on the trapped object. A lens with the appropriate focal length and an intensity filter were placed in front both detectors to focus the laser while also limiting the laser power entering the sensitive detectors.

The LED-camera system consists of two blue LED’s which are directed through lenses to provide collimated light through the objectives to illuminate the flow cell Figure (Figure 10 #9). After this light passes through the objectives it is reflected with the help of beam a splitting mirror (Figure 10 #10) towards a camera (Figure 10 #11) to capture the image. An achromatic lens is used to focus the image into the camera and a specific bandpass filter is used to filter unwanted light from entering the camera.
All the optical elements mentioned above are mounted to an optical table with the help of a base (BA1S from THORLABS), ½ inch post (TR2 from THORLABS) and post holder (PH2 from THOR Labs), unless otherwise specified. This mounting is referred to as standard mounting method in following sections where we discuss the above overview in detail.

**Compressed Air Systems and Optical Table**

External vibrations will play a disruptive role when collecting data as experimental forces will be on a pico-newton scale, and could easily be skewed by ground vibration. Vibrations will also affect the alignment of the laser over time, which could drastically effect the trapping potential of our instrument. In order for us to negate these possible vibrations we built the entire set up shown in Figure 10 on an optical table (T46HK from THORLABS) that is equipped with heavy duty legs (Figure 11). This table uses four active isolating support legs which raise the Optical table up about 12mm from the top of the leg with the help of compressed air. These legs have both a vertical and horizontal damping system. The vertical dampening system uses three parts: a reservoir, a smaller upper chamber placed above the reservoir and most importantly a laminar flow damper. If the vibration is created by the floor the legs air is pushed into the upper chamber and if the vibrations occur from the table, the air is pushed into the reservoir. As the air travels...
from one chamber to the other, air passes through the laminar flow damper which dampens the system and allows vertical vibration to settle faster.

A compressed air system was designed and constructed to float the optical table. Components of the compressed air system consisted of an air compressor connected to a 33 gal tank with a maximum operating pressure of 150 psi. This tank was positioned in an adjacent room in order to reduce vibration while compressing air. The air brought in from the other room is split into two lines (Figure 12) so we could supply both the air reservoir attached to the optical tables legs and the biomaterial supply system (more details discussed later in biomaterial supply system). As mentioned above an additional 10 gal compressed air reservoir was placed right next to the legs (Figure 11) so that when we switch the air system to biomaterials while running experiments, the legs still have air to float the table. This secondary air reservoir was set to maintain the air pressure at 50 psi.

Figure 12: Air from compressor (top channel) is split between the optical table (bottom channel) and biomaterial supply (right channel)
The Laser and Laser Controller

At the heart of all optical tweezers designs lies a laser, which the rest of the apparatus can be built around. We ordered a custom designed butterfly laser diode with a polarization maintaining pigtail optical fiber (Figure 13) which has an operating power of 250 mW to produce a 785 nm linearly polarized beam (LU0786M250 from Lumics).

Though all lasers will apply radiation pressure onto objects, not all lasers are ideal to use in every specific research environment. For us we needed to find a laser, which would optimally trap micron size dielectric beads in a liquid water based medium and hold it while we attach a single DNA molecule and manipulate it by exerting forces in the order of pico Newton (pN). If we consider the power of the laser, we need a minimum of 80 mW of power per beam just before reaching the objectives (Figure 10 #7) to trap and manipulate DNA (This is a value obtained from multiple dual beams set-up used in Williams Lab at Northeastern University). As our laser transmits through every optical element along its path, some absorption and partial reflection of the light by most of the elements will take place. The choice of a 250 mW laser, when split into two beams each beam will have 125 mw per beam, which was to ensure that we would have enough power when reaching the target.

The beam wavelength is also very important to us as it has two functional purposes. Light in the visible to near infrared spectrum is not heavily absorbed by liquid water. As liquid water is the base of our trapping medium, laser light will be able to transmit through with little to no
absorption by the water. Secondly, lack of absorption means less heating of the trapping medium. During experiments with fragile biological material, any increase in temperature can change or destroy the properties of these materials.

The laser diode is very sensitive to the slightest electrical surge from a static discharge, so the person handling the laser should always ground themselves prior to working with the diode. A grounding wire is attached to the ground on an electrical power strip located on the ceiling rack above the optical table and coupled to both the rack and optical table, so that a user can easily discharge themselves with the table.

The butterfly laser diode is housed in a compact LD and temperature controller (TEC) (CLD1015 from THOR Labs Figure 14), which controls operational current of the laser diode and the temperature. The first step is to lift the levers on the TEC controller so that we can place the laser diode on the heat sink of the controller. Then the heatsink was covered with thermal paste (Arctic Silver 5), and the thermal pad provided with controller was attached to the laser base for optimal heat transfer between the heatsink and the diode. Installing the laser diode into the TEC is a very sensitive operation, as discussed earlier slightest electrical surge from static charge can destroy the diode.
For safe installation the installer must be grounded through the TEC’s ground or by other means. Optimally two persons should be used, one to install and other to maintain a constant ground

The butterfly diode has a 14-pin orientation, which was aligned properly and fitted firmly into the TEC’s conducting clips (Figure 14). The pins can be easily bent; therefore, extra care should be taken while fitting them into the grooves of the conducting clips. The housing was secured by locking the lever back (a clicking sound ensures the locking). Finally, the four locking screws located on the corners of the diode body are tightened to further secure the diode to the TEC controller

In order to maintain the specified power, maintain polarization and keep the diode from possibly overheating, the diode is maintained at a constant temperature and operated at an optimal operating current. For our laser to operate at 250 mW these optimal settings were provided by the manufacturer as 25°C is 343 mA.

A very crucial property of the laser to our design, other than its power and wavelength, is its polarization. The polarization of the beam can be used to aid beam steering when other conventional optical elements like mirrors and beam splitters cannot send our laser to an operation specific location. The manufacturer directly couples the butterfly laser diode to a polarization maintaining (PM) fiber optic cable to produce linearly polarized laser. The PM fiber allows us to maintain the polarization of our laser beam with a single optical element rather than clutter our beam path with multiple bulky and likely expensive polarizing optics. This fiber tail of the laser is then coupled to a fiber port carefully in order to produce collimated laser beam.
Coupling the Laser to Fiber Port

In order to create a finely collimated beam, the PM fiber was coupled to an aspheric fiber port (PAF-X-15-B from THOR Labs Figure 15). As explained earlier, trapping objects is most effective when using a collimated beam because all parallel beams will travel through the focal point of the microscope. A diverging or converging beam will not focus every part of the beam to the focal point and will result in an unstable trap.

The PM fiber and fiber port join using an FC/APC connection. The FC (Fixed Connection) fiber connection protects the end of the delicate PM fiber from damage, while the APC (Angled Physical Contact) portion of the connection allows for a high return loss in laser power. This type of connection protects both the PM fiber and laser diode from damage, which could compromise proper function. Installation of the FC/APC ferrule of the PM fiber into the fiber connector is relatively simple as the orientation of the APC can be distinguished with the naked eye (Figure 16). The fiber insertion point on the port is also visibly angled and will only allow the fiber to be seated once the ferrule is in the correct orientation.

Fiber cable insertion into the fiber port should only be done when the port is safely secured within a desired optical mount to ensure correct alignment of the ferrule. In order to control the angle
at which the beam leaves the port we needed a 6-axis optical mount (K6XS from THOR Labs—Figure 17). The fiber port was first connected to a specialized fiber port adapter (CP08FP from THOR Labs).

To finalize the coupling of the fiber to the port you must tighten the threaded collar of the fiber around the bulkhead depicted in Figure 18.

As the laser enters the fiber port, it is directed through an aspheric lens with focal length 15.4mm to create a collimated beam with diameter of around 3.3mm. The aspheric lens eliminates the effects of spherical aberration disturbing the focus of the lens. Spherical aberration is the deviation of a spherical lens’s focal point due to lens thickness. Eliminating spherical aberration allows us to produce an almost ideal stable trapping potential
like the one shown in our theory section. This lens is also anti-reflection coated with material rated for wavelengths of 600-1050nm to ensure most of the laser radiation transmits through.

**Testing and Adjusting the Polarization of the Laser**

To diagnose if polarization is maintained by the laser a polarizing beam splitting cube (PBS122 from THORSLAB Figure 19 top) was placed in the path of the laser emerging from the fiber port. The polarizing beam splitting cube is designed to split an un-polarized laser into two perpendicular linearly polarized beams when its reflecting plane is placed 45° to the incident beam (Figure 19 bottom). The reflected laser will be polarized horizontally and the transmitted laser will be polarized vertically given the orientation seen in Figure 19 (bottom). Having two radiation power monitors positioned to intercept both the reflected and transmitted beams allows us to see the laser power of both polarization states in real time.

If the laser is linearly polarized, then by rotating the fiber port using the kinematic mount (shown in Figure 17) we should be able to achieve a situation where we can have the laser vertically polarized. At this state, the transmitted laser power should be close to original laser power and the reflected laser power should be almost zero. Laser polarization is maintained when the power
shown in each monitor is stable. If the power fluctuates back and forth between both beams the laser is most likely mode hopping. Unfortunately, there is not much which can be done by us at this point as it comes down to manufacturer error. The periodic mode coupling between both polarization states of the laser can be caused in a PM fiber when improper coupling to the either fiber ferrule or to the laser diode is done. Mode hopping can also be caused by external mechanical stress put on the PM fiber, which negates the desired phase difference. One of the two lasers we bought had this issue and we had to return it to the manufacturer to fix this problem.

Using our replacement laser diode, we were able to rotate the fiber port and obtain a stable condition by having all the laser power transmitted through the polarizing beam splitting cube. This indicates that laser emerging from the fiber port is linearly polarized in a vertical orientation. After this is done we can remove the polarizing beam splitting cube and begin alignment.

**Collimating the Beam**

After the confirmation of the vertical polarization of the beam, the beam should be collimated by adjusting the lens inside the fiber port. To achieve this a mirror was placed in the path of the laser, directing the beam to a non-reflecting spot somewhere in the room, say a wall, a distance greater than our designed optical beam path. The idea was to check the beam diameter remains constant over a large distance to ensure beam collimation. This max testing distance is arbitrary; longer distances allow for a more precise collimation.
The aspheric lens adheres to an adjustable tilt plate which can be adjusted by turning three screws marked Z1, Z2 and Z3 (Figure 20). These screws were systematically adjusted and after each adjustment the size of the beam diameter was measured at various distances to verify that the collimation. This allows us to judge if the lens is causing the beam to diverge or converge before hitting the max testing distance. Though this is simple in theory, making any adjustment to the port in order to effect the focus is extremely tedious. Because adjustments in the Z-direction are made using three individual screws, three individual adjustments of the same distance must be made to change the displacement of the lens. Adjusting one screw even by the smallest amount causes the lens to tilt, moving the beam in the X/Y-direction on the wall and changing its focus. This makes it very difficult to know how much the lens needs to move to improve beam collimation. From experience this calibration was the second most tedious and time consuming part whilst constructing the optical tweezers; taking hours to slightly improve collimation. Once Laser beam collimation is achieved the fiber port shouldn’t need any further calibration and should be left alone.

Figure 20: Fiber Port Adjustment Screws

(Pictures Courtesy of Thor Labs)
**Tilt Adjustment of the Laser Beam**

After collimating the laser beam, the next step is to ensure that the laser path always stays parallel to the optical table. This was achieved by setting the laser height at 10 cm after each new optical component. The choice to make the beam path 10 cm from the optical table was done given the translational limitations of stages which hold the microscopic objectives and flow cell later in the optical path. To keep our alignment consistent, we used a magnetic beam height measuring tool (BHM1 from THOR Labs). By placing BHM1 near the fiber port right after the emergence of the laser and along the straight line at the edge of the table, the kinematic mount K6XS was adjusted to provide the same height of the laser beam at the same spot on the BHM1 post. This will assure that the beam emerges from the laser port parallel with the table. To keep the beam parallel with the table after the laser encounters a new optical component, tilt adjustments on the components optical mount are used to set the laser height back at 10cm after it passes through or reflects off.

**Creating and Directing Two Beams**

Our design requires two finely collimated counter-propagating laser beams in order to trap particles effectively. This was achieved by splitting the laser into two equal strength beams and directing their paths with optical elements. In order to split the laser beam into two we use a laser line 50/50 beam splitting cube (**05BC16NP.6** from Newport Optics). This cube allows us to create two evenly intense beams with orthogonal paths (Figure 10 #4).
The cube is centered at 10 cm above the optical table in a kinematic prism mount (KM100PM from THORLABS) with the help of small adjustable clamp (PM3 from THORLABS). The assembly is mounted to the optical table using the standard mounting method described in the overview of the design. The kinematic mount allows us to adjust the tilt of the cube to maintain the beam at the same height before and after the cube. As mentioned in the previous section, the beam height was set to be 10 cm emerging from the laser port (Figure 21 Top). Tilt adjustments of KM100PM were made to ensure that the beam heights for both the transmitted (Figure 21 Bottom) and reflected beams after passing through the beam splitting cube remain the same as before entering the cube.

We use three dielectric broadband mirrors (BB1-E03 from THOR Labs) to direct the beams to reach the polarizing beam splitting cubes (Figure 10 to positon #5). These mirrors are coated to provide maximum reflection of radiation from 750-1100 nm, reducing possible radiation absorption by the mirror. The mirrors are housed in kinematic mirror mounts (KS1 from THOR Labs) mounted to the table using standard mounting method described in the design overview. Again, the tilts of the kinematic mounts were adjusted to maintain a height of 10 cm from the table after each reflection and checked whether it was maintained at constant height until the
laser reaches the polarizing beam splitting cubes. The laser trace after reflecting off the beam splitting cube and after transmission through the beam splitting cube are shown in Figure 22.

Figure 22: Dielectric mirrors directing the laser to the left and right polarizing beam splitting cubes. The red line is to help visualize the direction of the beam path from the fiber port

Overlapping the Beams

Position #5 in Figure 10 marks the position of the polarizing beam splitting cubes (PBS122 from THORLABS). One of these cubes was used by us for testing beam polarization earlier. For laser beams which are planar polarized, like ours is, these cubes will act like one-way mirrors. We use these cubes to direct the lasers instead of conventional mirrors because for imaging purposes we need an un-polarized light source to transmit through the cubes uninhibited. Our design will use two of these cubes to direct both beams at each other so that we can create a fine overlap.

Overlapping the beams begin once we have the both polarizing beam splitting cubes in place, using two of the same prism mounts (KM100PM and PM3 from THORLABS) used for the 50/50 beam splitting cube. These mounts allow us to adjust cube tilt not their vertical and horizontal
translation. This overlap should be simple if we were able to maintain the 10cm beam height so that there are no tilted beams. Incident diagonal beams into the cubes will make it impossible for us to create a fine overlap of the lasers.

![Figure 23: Checking beam overlap. The beam height measuring tool (BHM1) is placed on the left side arm of the front beam between the beam splitting cube and objective stage (left top and left bottom). Viewing this from the cube side (top left) and from stage side (bottom left) allow us to confirm if the beams overlap. BMH1 is placed on the right arm of the beam in a similar spot and viewed from the cube side (top right) and from stage side (bottom right) to make sure they overlap.](image)

Figures 23 shows the process of creating the overlap. To overlap the beams, measurements of the beam height must be made similarly for the beams emerging from the opposite sides of the polarizing cubes. Adjusting the tilts of the prism mounts holding the polarizing cubes allowed us to level the beams. The Beam alignment tool (BHM1) was placed on both ends of the table between the polarizing cubes on both ends of the optical table and were checked for the height and horizontal tilt of the laser beam using the hole located on the tool at 10 cm. The adjustments
were repeated until the measurements were consistent from one cube to the other. Once this is achieved, the beams are overlapped and ready for the next phase of alignment.

As an addition to this, we placed the stages on the optical table, which will be used to mount the microscopic objectives later. This is to acquire a more general alignment for the objectives before we install them. Three stages were installed onto the table, two of them used to house the objectives (MAX302 and PT3 from THORLABS) and one to hold the flow cell (MAX313D from THORLABS). Kinematic objective mounts (KM100R from THORLABS) are directly coupled to the two objective stages, and centered along the beam path as seen in Figure 23 (bottom right and left). All three stages have a maximum travel length in all three directions of an inch, which limits our ability to position them correctly. A half inch aluminum spacer (MB6U from THORLABS) is used as a spacer underneath the right hand stage seen in Figure 25, while a quarter inch aluminum plate machined by us is used under the target stage to elevate it.

**The Objectives**

The microscopic objectives (Figure 24) which we use to focus the laser beams onto the target are two Nikon 60X magnification objectives with a working distance of around 2 mm and numerical aperture of 1.2 (MRF07620 from MVI Inc.). As mentioned before, closer the numerical apertures of objectives to their respective immersion mediums index of refraction ($n$)
will allow for a tighter grip on dielectric beads. Considering our objectives use water immersion, N.A = 1.2 is very close to the $n = 1.33$ of water, the microscopic objectives should provide a sharp enough focus angle to trap effectively.

In order to finely focus the laser beams onto the flow cell through means of microscopic objectives we need to make sure the objectives are positioned correctly with respect to the flow cell and beam path. This is achieved with use of two stages that have three dimensional control to manipulate the objectives. These stages are placed such that the flow cell can be accommodated in between the objectives. Figure 25 shows these two stages with respect to the flow cell stage (the details of this stage will be discussed later in the flow cell section). One of the objectives is housed on a stage that is mechanically controlled (PT3 from THORLABS) and the other is housed on a stage (MAX302 from THORLABS) controlled by a Piezo Controller (MDT630B from THORLABS). The later stage (Figure 26) has the ability to make larger adjustments mechanically too.

The stage controlled by a piezo controller uses a piezoelectric material, a crystal or ceramic which expands or contracts due to applied electrical current, in order to make very fine micrometer...
adjustment based on input voltages. As mentioned in the previous section, once the beam overlap alignment was made, the objectives were mounted to the stages with the help of thread adapter (SM1A12 form THORLABS).

The Flow Cell

Once the objectives are mounted properly we need to check whether the laser light entering one of the objectives is still coming out from the other objective at the same height and is still overlapped. To do this we need a flow cell in between the objectives so that we can focus the laser inside the objective and re-collimate.

The flow cell is where we trap all the biological material and conduct single molecule experiments. The flow cell allows for the controlled introduction of experimental materials. The main frame (spacer) of the flow cell is created and supplied by our collaborators, Williams Lab at Northeastern University.

The flow cell spacer is a Plexiglas slide (30 mm x 22 mm) about 2mm thick with a channel cut out along the center with holes drilled at each end to access the middle channel. The holes drilled into the sides of the cell are used to insert delivery hoses on one end and a waste hose on the other. There is another hole drilled into the top of the spacer leading into the main channel to insert a micropipette. The micropipette is used during experiments to grab dielectric beads with suction and pull them out of the trap. First the main channel is sealed off using microscope cover glass (#1, 30 mm X 22 mm, 12-545A from Fisher Scientific) on each side of the spacer to create the flow cell. All the gluing is done with bio safe UV adhesive (NOA68 from Norland Products).
Then the 4 inlet tubes (64-0751 PE-10/100 from Warner Instruments) and an outlet tube (06418-02 from Cole Parmer) are attached to the ends. Finally, the micropipette tip (TIP1TW1 from WPI) is inserted through the top hole with the help of a microscope such that the tip lies in the middle of the main channel. The end of the micropipette tip is roughly a micron in diameter, allowing it to grasp the five micron dielectric beads at the tip by suction. Figure 27 depicts the completed flow cell and the magnified image of the micropipette tip.

**Biomaterial Reservoir System**

In order for us to have biomaterials ready to enter the flow cell, a reservoir system is necessary. This reservoir system consists of five 15ml centrifuge tubes (20050 from Syringa Lab Supplies), four of which hold either DNA, beads, drugs, or buffer. The fifth tube collects the waste material. The caps of these tubes are connected to a compressed air system with the help of Masterflex plastic tubing (HV-06509-16 from Cole-Palmer). The bottoms of these centrifuge tubes were drilled to accommodate the thin tubing (06418-05 from Cole Parmer) which the materials exit from. The tubing is fed through Castaloy hose clamps (05-848Q from Fischer Scientific) to manually switch the flow on or off. These hose clamps were assembled on a machined aluminum
block which is attached to the optical table. This reservoir allows us to individually manage the biomaterials being fed to the optical tweezers, as well as allowing for proper waste collection.

In order to properly couple the compressed air supply to the biomaterial supply system we had to first drop pressure from 100 psi down to less than 1 psi by using a series of regulators. This reduced air pressure is applied to a series of solenoids (Blue circle in Figure 28) which is controlled by an electrical control box (Red circle in Figure 28). The control box has switches that open and close the solenoids. When we switch on a specific solenoid the air pressure is applied to the biomaterial reservoir connected to it. This creates pressure inside the reservoir causing the material inside to flow into the flow cell.

**Manipulating the Flow Cell**

To manipulate the flow cell, we use the stage circled in blue in Figure 25. This is a mechanical Nanomax three dimensional stage ([MAX313D](http://www.thorlabs.com)) from THORLABS which is fitted with a single piezo driver with digital feedback ([DRV120](http://www.thorlabs.com)) with [MCA1](http://www.thorlabs.com) and [RBA1](http://www.thorlabs.com) from THORLABS) to control the movement in X-direction (Figure 29).
The displacement of the flow cell in the X-direction can also be controlled mechanically, but during experiments it must be controlled through the piezo driver (BPC301 from THORLABS). The piezo driver allows the stage holding the flow cell to move distances as small as 5nm over a total range of 20 microns and controlled by the software developed for data acquisition.

The ability to resolve nanometer motion is crucial to accurately testing the stretching regimes of the biological molecules like DNA we intend on studying with the optical tweezers. To allow the flow cell to position itself between the two objectives, a 6-inch dovetail optical rail (RLA0600 from THORLABS) elevated by an additional stage adapter plate (RB13P1 from THORLABS). The rail has tapped holes along its length which allow us to fix a filter holder (115-1310 from OptoSigma) to the end of it so that it can hold the flow cell (Figure 30).
Visualizing the Target

If we look back at the dual beam optical tweezers schematic (Figure 10) the blue lines indicate the path of illumination and imaging of the flow cell.

In order to illuminate the flow cell between the objectives, we use two blue LED's (M470L3 from THORLABS) positioned symmetrically (blue arrow Figure 31) on each side of the table along with a set of 50 mm focal length plano convex lenses (LA1131-A-ML mounted to LMR1 from THORLABS) to collimate the light (violet arrow Figure 31). Each LED is adjusted with individual variable current controllers (LEDD1B with supply TPS001 from THORLABS) to adjust the brightness of the image (red arrow Figure 31). A 50/50 economy beam splitter (EBS1 from THORLABS) is used (green arrow Figure 31) in order to let LED light pass through from this side and reflect the LED light coming from the opposite side. A similar beam splitter was placed on the other side in a similar spot. These beam splitters are coupled to KM100 mirror mounts in order to adjust their tilt to direct LED light.

As the LED light enters the first objective and emerges from the second objective the 50/50 economy beam splitter on the other side was used to direct the light towards a CMOS (complementary metal-oxide semiconductor) camera (DCC1545M from THORLABS) (red circle in Figure 32).
The light is focused onto the camera sensor using an achromatic doublet (AC254-200-A-ML from THORLABS) of focal length 200 mm and antireflection coated for 400-700 nm (Blue circle in Figure 32). This achromatic doublet is coupled to the camera by means of a lens tube (SM1V05 and SM1E60 from THORLABS) mounted with the help of adjustable lens mount (LH1 from THORLABS) to avoid any ambient light entering the camera. A band pass filter (FSR-BG40 from Newport Optics) is added in front of the camera in order to protect the camera from excess laser exposure. To process the camera information, we will use a CPU dedicated to imaging the flow cell. The CPU is located with a monitor just above the optical table on a ceiling rack for easy access by the user.

**Detection**

Once the laser passes through both objectives it is re-collimated on the opposite side. This emerging beam can be used to find the applied force on a trapped bead. When a force is applied on the trapped bead, the bead is displaced out of the trap and as a result the laser beam gets deflected. These deflections can be measured by directing the laser into a position sensing photodiode as shown in Figure 33. The detector system is directed upward so that it does not interfere with the trapping or imaging optics. This is achieved by rotating the polarization of the laser from linearly vertical to linearly horizontal with help of two quarter wave plates. A polarizing beam splitting cube is used to direct the deflected laser light vertically upward towards the

![Figure 32: Reciprocating imaging components from the right side of table shows the achromatic doublet (blue circle), lens tube, filter and camera (red circle).](image)
detector. The entire detector system is mounted to a 1.5-inch diameter, 12-inch-long post (P12 from THORLABS), anchored using a post table clamp (CL8 from THORLABS).

Two zero order quarter wave-plates (WPQ10ME-780 from THORLABS) were positioned symmetrically, with one on each side of the objectives (see the schematic Figure 10). The quarter wave plates housed in 1-inch rotational mounts (RSP1 from THORLABS) so that they can be rotated to convert the polarization from linear to circular and vise-versa. When the vertically polarized laser hits the first quarter wave plate it is made into circularly polarized light by setting the birefringent axes at $45^\circ$ from vertical (Figure 34).

The second quarter wave plate is set at another $45^\circ$ from vertical which converts the circular polarization back to linear polarization. The combination of the wave-plates will adjust the total polarization a full $90^\circ$, allowing for the laser light to be horizontally polarized.
This horizontally polarized light is then directed towards a polarizing beam splitting cube as shown in Figure 33. This time the plane of the cube was positioned so that horizontally polarized light is directed upward. The cube was inserted in a beam splitter holder (UPA-CH.5 from Newport Optics) and then housed in a ½-inch kinematic mirror mount (KS05K from THORLABS) and coupled to the post using a 3/2-inch post mounting clamp (C1510 from THORLABS). The next optical element up the post is a 100 mm focal length, anti-reflection coated for 650-1050 nm, plano convex lens (LA1509-B-ML from THORLABS) using the same 3/2-inch post mount (C1510 from THORLABS) and a 1-inch lens mount to couple it to the post (LMR1 from THOR Labs). This lens is used to focus the laser into the position sensing diode (PDP90A from THORLABS). The
photodiode is positioned at the top of the post using a two dimensional dovetailed stage (DT12XY from THORLABS) mounted on a custom aluminum mount made by Devon West-Coates here at BSU. To limit the laser power into the photodiode, a neutral density filter is used and mounted using identical parts as the focusing lens.

Final Optical Alignment

During the final alignment of the optical tweezers, we used a flow cell coupled to the biomaterial flow system. The flow cell was placed in the filter holder positioned between the two objectives, and then the space between the flow cell and objectives were filled with water using a squirt bottle. The continuous medium of water is necessary for the objectives to work as specified. The cover slips creating the sides of the flow cell are very thin, causing a negligible effect due to laser refraction through it. The flow system was then used to fill the flow cell with water to create a constant water medium between the two objectives. From here the laser was turned on and the beam shape was observed with a measuring tool.

Three dimensional translations are made with both stages that hold the objectives, while the kinematic mounts holding the objectives were used to change the angle at which the objectives tilt. Once laser light is seen to emerge from either side, minor adjustments were made to recreate the original beam spot. When the objectives are in their ideal alignment (Figure 9), the incoming
light from one side should focus down and start diverging at the focal point of the second objective, re-collimating the laser. We can begin to overlap the beams a second time once the beam spot from both re-collimated beams are close to their original size and shape. The same measurements were taken on both sides, making sure the incoming and exiting beams are overlapped at every distance from the objective to the polarizing cube.

Making sure the beams overlap by eye is good way to start, but unfortunately this does not mean that the objectives are aligned properly. For the dual beam design to work effectively the beams need to be near perfectly aligned. To get the proper alignment we must use the digital imaging optics to see inside the flow cell. Our first job was to find the tip of the micropipette. If we can acquire a clear image of the pipette in both cameras, our objectives should be aligned. This phase of the whole optical tweezers construction was significantly more difficult to accomplish than any prior construction or alignment. Because we are trying to focus on a spot which is only a micron in size with two objectives that have free movement in three dimensions and as well as the ability to tilt, it’s trial and error until the proper alignment is found. With the proper alignment achieved the beam spot should be visible with minimal excess reflection light visible due to the flow cell being at an angle in respect to the objectives. Our camera filters transmit a minimal amount of light at 785 nm which we can see a beam spot on our image with.
diameter about the same size of the micropipette tip. Once this image is acquired we should be able to start trapping particles.

Once the alignment is complete, it should no longer be maintained by hand. The three dimensional piezoelectric stage will be used to maintain the alignment as it drifts overtime.
Now that we have completed the major construction of the optical tweezers (Figure 37), a new set challenges await the next series of students adding to this project. The next direct step in this project would be to write the code in order to connect our software to the position detecting photodiodes. This will allow us to measure the applied forces on a trapped bead when its pulled out of equilibrium. Measuring these forces allows us to acquire the DNA extension curves so crucial in the single molecule biophysics research we plan on doing here at BSU. I realize that the
future of this project would require a completely different set of skills than the ones I’ve displayed while constructing the optical tweezers. The future will cater not only to the physicists but also towards the biologists, chemists and computer scientists. The beauty of this project lies in its interdisciplinary potential to educate and bring students together from generally different fields to apply their knowledge.
# Appendix: Project Expenses

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<tr>
<td>1&quot;Broad Band Dielectric Mirror (750nm -1100 nm)</td>
<td>THORLABS</td>
<td>BB1-E03</td>
<td>3</td>
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<td>Precision Kinematic Mirror Mount (3 Adjusters, for 1&quot; Mirror)</td>
<td>THORLABS</td>
<td>KS1</td>
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<td>Kinematic Prism Mount, Eight 6-32 Taps</td>
<td>THORLABS</td>
<td>KM100PM</td>
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<td>Small Adjustable Clamping arms (6-32 Threaded)</td>
<td>THORLABS</td>
<td>PM3</td>
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<td><strong>Overlapping the Beams</strong></td>
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<td>1/2&quot; Polarizing Beam splitter Cube (620 nm - 1000 nm)</td>
<td>THORLABS</td>
<td>PBS122</td>
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<td>Kinematic Prism Mount, Eight 6-32 Taps</td>
<td>THORLABS</td>
<td>KM100PM</td>
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<tr>
<td><strong>The Objectives</strong></td>
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<tr>
<td>Nikon CFI Plan Fluor Nikon W 60X objective (NA=1, WD=2mm)</td>
<td>MVI, Inc.</td>
<td>MRF07620</td>
<td>2</td>
<td>$5,937.26</td>
</tr>
<tr>
<td>Kinematic Mirror Mount for Microscopic Objectives</td>
<td>THORLABS</td>
<td>KM100R</td>
<td>2</td>
<td>$140.80</td>
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<tr>
<td>Adapter with External SM1 Thread and Internal M25-0.75 Thread</td>
<td>THORLABS</td>
<td>SM1A12</td>
<td>2</td>
<td>$39.50</td>
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<tr>
<td>3-Axis Nano Max Flex Stage with Bundled Controller</td>
<td>THORLABS</td>
<td>MDT630B</td>
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<td>XYZ Translation Stage with Micrometers (25 mm)</td>
<td>THORLABS</td>
<td>PT3</td>
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<td>$1.00</td>
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<td>1/2 inch Aluminum Breadboard</td>
<td>THORLABS</td>
<td>MB6U</td>
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### The Flow Cell

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<thead>
<tr>
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<tr>
<td>1µm prepulled borosilicate glass micropipettes</td>
<td>WPI</td>
<td>TIP1TW1</td>
<td>1</td>
<td>$138.00</td>
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<tr>
<td>Warner PE Tubing (0.61 mm OD, 0.28 mm ID)-10 m</td>
<td>Warner Instruments</td>
<td>64-0751 PE 10</td>
<td>1</td>
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<td>Fisher Brand Microscope Cover Glass (22x30 mm No:1)</td>
<td>Fisher Scientific</td>
<td>12-545A</td>
<td>1</td>
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<td>Norland Optical Adhesive</td>
<td>Norland Products</td>
<td>6801</td>
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<td>$28.00</td>
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<tr>
<td>Tygon Microbore Tubing (0.020&quot; ID, 0.060&quot;OD) 100 ft</td>
<td>Cole-Parmer</td>
<td>06418-02</td>
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### Biomaterial Reservoir System

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>15 mL Centrifuge tube (Sterile) - Case of 500</td>
<td>Syringa Lab Supplies</td>
<td>20050</td>
<td>1</td>
<td>$65.00</td>
</tr>
<tr>
<td>Tygon Microbore Tubing (0.050&quot; ID, 0.090&quot;OD) 100 ft</td>
<td>Cole-Parmer</td>
<td>06418-05</td>
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<tr>
<td>Castaloy Hosecock Clamp with Bracket</td>
<td>Fisher Scientific</td>
<td>05-848Q</td>
<td>5</td>
<td>$196.75</td>
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<tr>
<td>Masterflex Tygon E-Lab (E3603) Pump Tubing, L/S 16, 50 ft</td>
<td>Cole-Parmer</td>
<td>HV-06509-16</td>
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### Manipulating the Flow Cell

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<tr>
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<tr>
<td>NanoMax300 with Differential Drives (closed loop)</td>
<td>THORLABS</td>
<td>MAX313D</td>
<td>1</td>
<td>$1,600.00</td>
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<tr>
<td>1-Channel 150 V Benchtop Piezo Controller with USB</td>
<td>THORLABS</td>
<td>BPC301</td>
<td>1</td>
<td>$2,050.00</td>
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<tr>
<td>Modular NanoMax 20 µm Piezo Drive with Feedback</td>
<td>THORLABS</td>
<td>DRV120</td>
<td>1</td>
<td>$1,030.00</td>
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<tr>
<td>Manual Drives, Mounting Barrel Adapter</td>
<td>THORLABS</td>
<td>RBA1</td>
<td>1</td>
<td>$8.90</td>
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<tr>
<td>Manual Drives, Modular Quick-Connect Adapter</td>
<td>THORLABS</td>
<td>MCA1</td>
<td>1</td>
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<tr>
<td>Adapter Plate with 1/4&quot;-20 and 8-32 Taps</td>
<td>THORLABS</td>
<td>RB13P1</td>
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<td>$49.90</td>
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<td>Dovetail Optical Rail, 6&quot;, Imperial</td>
<td>THORLABS</td>
<td>RLA0600</td>
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<td>Filter holder</td>
<td>Optosigma</td>
<td>115-1310</td>
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### Visualizing the Target

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<tr>
<th>Item</th>
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<tr>
<td>Mounted High-Power LED (Green, 530 nm)</td>
<td>THORLABS</td>
<td>M470L3</td>
<td>2</td>
<td>$536.00</td>
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<td>T-Cube LED Driver, 1200 mA Max Drive Current</td>
<td>THORLABS</td>
<td>LEDD1B</td>
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<tr>
<td>15 V Power Supply Unit for a Single T-Cube</td>
<td>THORLABS</td>
<td>TP5001</td>
<td>2</td>
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<tr>
<td>1&quot; Plano-Convex Lens (f = 50.0 mm, ARC: 650-1050 nm)</td>
<td>THORLABS</td>
<td>LA1131-B-ML</td>
<td>2</td>
<td>$88.20</td>
</tr>
<tr>
<td>50:50 (R:T) Economy Beam splitter (1&quot;, AOI = 45°)</td>
<td>THORLABS</td>
<td>EBS1</td>
<td>2</td>
<td>$62.80</td>
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<tr>
<td>Kinematic Mount for 1&quot; Optics</td>
<td>THORLABS</td>
<td>KM100</td>
<td>2</td>
<td>$77.40</td>
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<tr>
<td>1&quot; Achromatic Doublet (ARC: 400-700 nm, f=200 mm)</td>
<td>THORLABS</td>
<td>AC254-200-A-ML</td>
<td>2</td>
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<tr>
<td>lens tubes (SM1V05)</td>
<td>THORLABS</td>
<td>SM1V05</td>
<td>2</td>
<td>$59.20</td>
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<tr>
<td>25 mm BG40 Colored Glass Bandpass Filter</td>
<td>Newport</td>
<td>FSR-BG40</td>
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<td>$94.00</td>
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<tr>
<td>Adapter with External C-Mount Threads &amp; Internal SM1 Threads</td>
<td>THORLABS</td>
<td>SM1A9</td>
<td>2</td>
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<tr>
<td>High Resolution USB2.0 CMOS Camera, Monochrome Sensor</td>
<td>THORLABS</td>
<td>DCC1545M</td>
<td>2</td>
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<tr>
<td>6-inch lens tube SM1 thread</td>
<td>THORLABS</td>
<td>SM1E60</td>
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<tr>
<td>Adjustable Lens Mount</td>
<td>THORLABS</td>
<td>LH1</td>
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<td>$79.00</td>
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<tr>
<td>Lens Mount for 1&quot; Optics with SM1 thread</td>
<td>THORLABS</td>
<td>LMR1</td>
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### Detection

<table>
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<tr>
<th>Item</th>
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<th>Code</th>
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<th>Price</th>
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<tbody>
<tr>
<td>1.5&quot; Mounting Post, L = 12&quot;</td>
<td>THORLABS</td>
<td>P12</td>
<td>2</td>
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<td>Studded Pedestal Base Adapter (2.40&quot; Diameter X 0.50&quot; High)</td>
<td>THORLABS</td>
<td>PB4</td>
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<td>Pedestel Post Table Clamp</td>
<td>THORLABS</td>
<td>CL8</td>
<td>4</td>
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<tr>
<td>1/2&quot; Polarizing Beam splitter Cube (620 nm - 1000 nm)</td>
<td>THORLABS</td>
<td>PBS122</td>
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<td>$374.00</td>
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<td>1/2&quot; Precision Kinematic Mirror Mount</td>
<td>THORLABS</td>
<td>KS05K</td>
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<tr>
<td>Description</td>
<td>Supplier</td>
<td>Model/Code</td>
<td>Quantity</td>
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<td>----------------------------------------------------------------------------</td>
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<tr>
<td>Cube Beam Splitter Holder (0.5 inch, For U50-A Mount)</td>
<td>Newport</td>
<td>UPA-CH.5</td>
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<tr>
<td>1&quot;Zero-Order Quarter-Wave Plate, SM1-Thrd Mount, 780 nm</td>
<td>THORLABS</td>
<td>WPQ10M-780</td>
<td>2</td>
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<td>Rotation Mount with Retaining Ring (For 1&quot; Optics)</td>
<td>THORLABS</td>
<td>RSP1</td>
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<tr>
<td>Compact 1.5&quot; Post Mounting Clamps</td>
<td>THORLABS</td>
<td>C1500</td>
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<td>$273.20</td>
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<tr>
<td>Lens Mount for 1&quot; Optics with SM1 thread</td>
<td>THORLABS</td>
<td>LMR1</td>
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<td>$30.46</td>
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<td>1&quot; Plano-Convex Lens (f = 100.0 mm, ARC: 650-1050 nm)</td>
<td>THORLABS</td>
<td>LA1509-B-ML</td>
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<td>2D Lateral Effect Position-Sensor</td>
<td>THORLABS</td>
<td>PDP90A</td>
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<td>PSD Controller</td>
<td>THORLABS</td>
<td>TPA101</td>
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<td>THORLABS</td>
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<td>XY Dovetail Translation Stage with Baseplate Included-1/2&quot;</td>
<td>THORLABS</td>
<td>DT12XY</td>
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<td><strong>Miscellaneous Items</strong></td>
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<td>Digital Power &amp; Energy Meter with Slim Si Sensor</td>
<td>THORLABS</td>
<td>PM130D</td>
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<td>THORLABS</td>
<td>BHM1</td>
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<tr>
<td>3/2&quot; Optical Post, 8-32 Setscrew, 1/4&quot;-20 Tap, L = 2&quot;, 5 Pack</td>
<td>THORLABS</td>
<td>TR2-P5</td>
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<tr>
<td>3/2&quot; Post Holder, Spring-Loaded Hex-Locking Thumbscrew, L = 2&quot;</td>
<td>THORLABS</td>
<td>PH2</td>
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<td>Mounting Base, 1&quot; x 2.3&quot; x 3/8&quot;</td>
<td>THORLABS</td>
<td>BA15</td>
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<tr>
<td>3/4&quot;-20 Cap Screw and Hardware Kit</td>
<td>THORLABS</td>
<td>HW-KIT2</td>
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**Grand Total**            $34,499.36

*This grand total does not include the software and other equipment used in the lab but not directly related to this project.*
References