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Fiber Optic Tweezers for Cell Studies

A Major Qualifying Project

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By

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William Beatty

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Professor Qi Wen

Abstract

This report outlines the implementation and proof of concept testing of dual-optical-fiber optical tweezers in studying cell properties. Customized equipment was created to enable testing. This equipment included an aluminum platform to stage equipment that otherwise could not align with the microscope and a 3D stage assemble to allow precise positioning of optical fibers and other equipment near the target sample cells. A method for applying 5 μm diameter silica beads to live 3T3 fibroblast cells was developed using a 20 μm diameter tip micropipette. These microbeads were manipulated on the cells using the fiber optic tweezers to demonstrate the feasibility of using such a setup to measure cell mechanical stiffness.

Table of Contents

List of Figures

List of Equations

1. Background

The first optical tweezer setup was made in 1969 by Arthur Ashkin. It was a single-laser design that formed the basis for today's varied constructions.[1] They are used to manipulate, and investigate the properties of, things like DNA[2], cell membranes,[3] motor proteins,[4] and microtubules,[5] either through directly manipulating the molecules or through dielectric microbeads.[6] Here, fiber optical tweezers are employed to manipulate silica microbeads 5 μm in diameter to determine lateral cell stiffness.

Cell forces are linked to cell growth and division,[7] and these forces are commonly measured using Atomic Force Microscopy (AFM) or Traction Force Microscopy (TFM). TFM measures cell forces based on the amount they deform a polymer substrate, relying on special surfaces and cell adhesion to make measurements of lateral cell forces.[8] AFM measures the deflection of a micrometer scale cantilever of known stiffness to, among other uses such as topographical measurements, determine the stiffness of cells the cantilever is pressed against.[9] Optical tweezers are able to determine lateral or vertical forces, without an atypical substrate, as is needed in TFM, and without a solid connection to a measurement device, as needed in AFM.[10]

1.1 Mechanism of Optical Trapping

The particle being targeted is exposed to gradient forces, generated by the laser light, that push it towards the center of the trap. This restorative force is approximated as a spring force, which becomes relevant to calculating the forces on the trapped particle layer.[11] For this to happen, the particle must have a greater refractive index than the surrounding fluid.[12] When the target particle is much larger than the wavelength of light, the force is generated when the light from the trap laser is refracted out of the beam by the target as shown in Figs. 1A, 1B, and 2. This creates a momentum change in the light that is counteracted by the momentum of the target shifting toward the center of the trap. For targets much larger than the laser's wavelength, the force is derived from the target approximating a perfect dipole in the intensity gradient inherent in the laser as shown in Figure 1C. This force, F, which typically falls around 20 pN, usually follows the form:

$$< \boldsymbol{F} >= \left(\frac{\alpha}{2}\right) \nabla < \boldsymbol{E^2} > \qquad \text{(eq. 1)}$$

Here, α is the polarizability of the target and E is the electric field.[1] Equation 1 shows the formulation for the time-averaged force. Several factors influence the efficiency and power of optical traps; including size, shape, and laser power. A trap's size is inversely proportional to its efficiency, the more circular the trap is the more efficient it theoretically is, though this often involves fiber coupling and the associated losses, and a higher power laser yields greater trap strengths.[4]

1.2 Objective Lens Based Optical Trapping

Most optical traps today rely on a laser passed through a single microscope objective lens, which focuses the laser beam to create optical trap as illustrated in Figure 1A. While being extensively applied in many biophysical studies of single molecules, this method is severely limiting in application. Traps relying on microscope objectives cannot change where the trap is, and so must target objects at the objective length of the lens, usually around 0.5 mm. Due to the sensitive nature of objective lenses they are also unable to be inserted into a sample, such as cell media, further limiting their use.

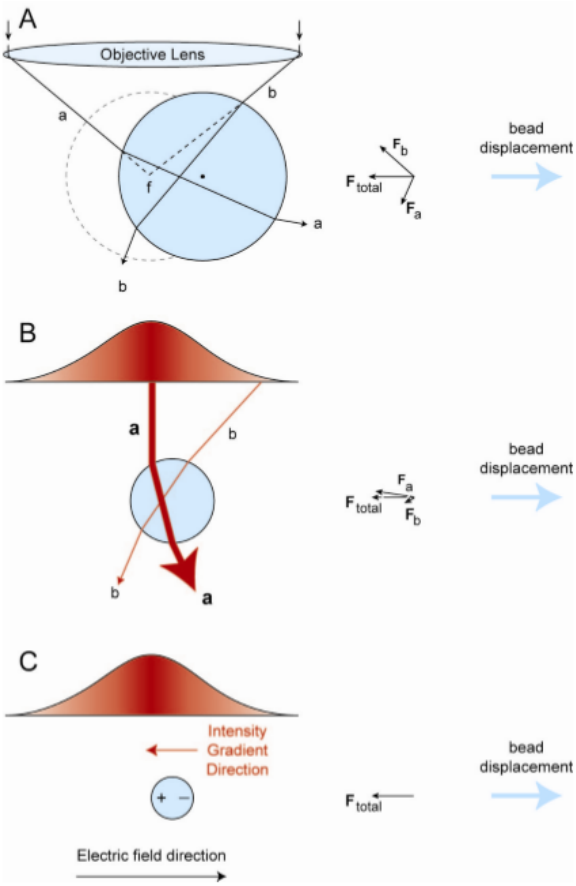

Figure 1: Origin of lateral trapping force.[6] A) Lateral trapping force by a focused beam of light. Dotted lines indicate where the edges of the laser would focus in the absence of the bead (represented as the blue circle). Solid lines indicate the path light takes with the bead's refraction, shifting the focus to the right and bringing the path of beam a up and to the right, and beam b down and to the right. These changes in the light's path result in the forces diagramed to the right, with a total force bringing the bead left, to the trap focus. B) Lateral trapping force by a single beam of light with Gaussian intensity profile. Forces on a bead due to light gradient. Two beams, representing different originating points in the gradient are represented, with a being thicker than b to represent higher intensity. Beam a exerts a greater force on the beam than b, and the resultant total force is again pointing left. C) Lateral trapping of particles whose size are smaller than the wavelength of light. Under this situation, the dielectric particles can be considered to be dipoles in a Gaussian laser intensity gradient. As the laser intensity increases towards the middle the electric field points out, creating a force towards the center of the beam on the particle.

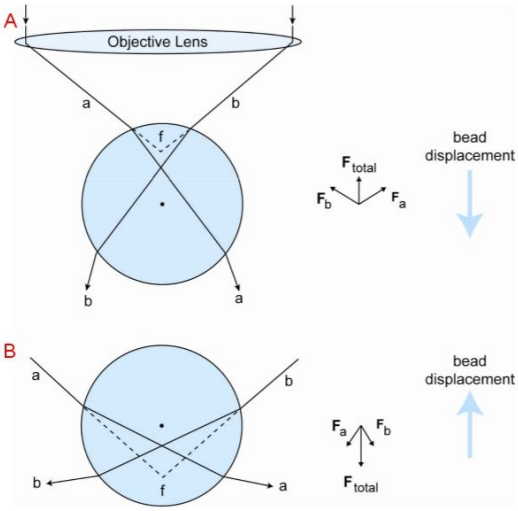

Figure 2: Origin of axial trapping force [6] Dotted lines indicate where the edges of the laser would focus in the absence of the bead (represented as the blue circle). Solid lines indicate the path light takes with the bead's refraction, shifting the focus down in the upper image and up in the lower image. A) Path a is refracted down and left of its original trajectory, and path b is refracted down and right. The resulting forces are diagramed at the right, with a total force bringing the bead up towards the trap focus. B) Path a is refracted up and right of its original trajectory, and path b is refracted up and left. The resulting forces are diagramed at the right, with a total force bringing the bead down towards the trap focus.

1.3 Optical Fiber Based Optical Trapping

The optical tweezers used are multi-fiber traps instead of single-laser aperture traps. This method was developed by Professor Yuxiang Liu's group in the WPI Mechanical Engineering department. Fiber optic based traps use two fibers with polished ends in order to achieve the necessary 3D trapping. The numerical aperture of a single fiber is not enough to sustain a 3D trap, only a 2D trap, so two crossing lasers are employed to achieve full trapping.[13] Both fibers exert forces similar to those in single-source traps, resulting in four optical forces that balance each other and external forces.[10]

These systems address the issues that objective lens based systems encounter. The distance between trap and fiber tips can be slightly adjusted by changing the angle of the fibers and, more significantly, the fibers can be inserted into samples, such as cell media, allowing closer inspection of cell cultures.

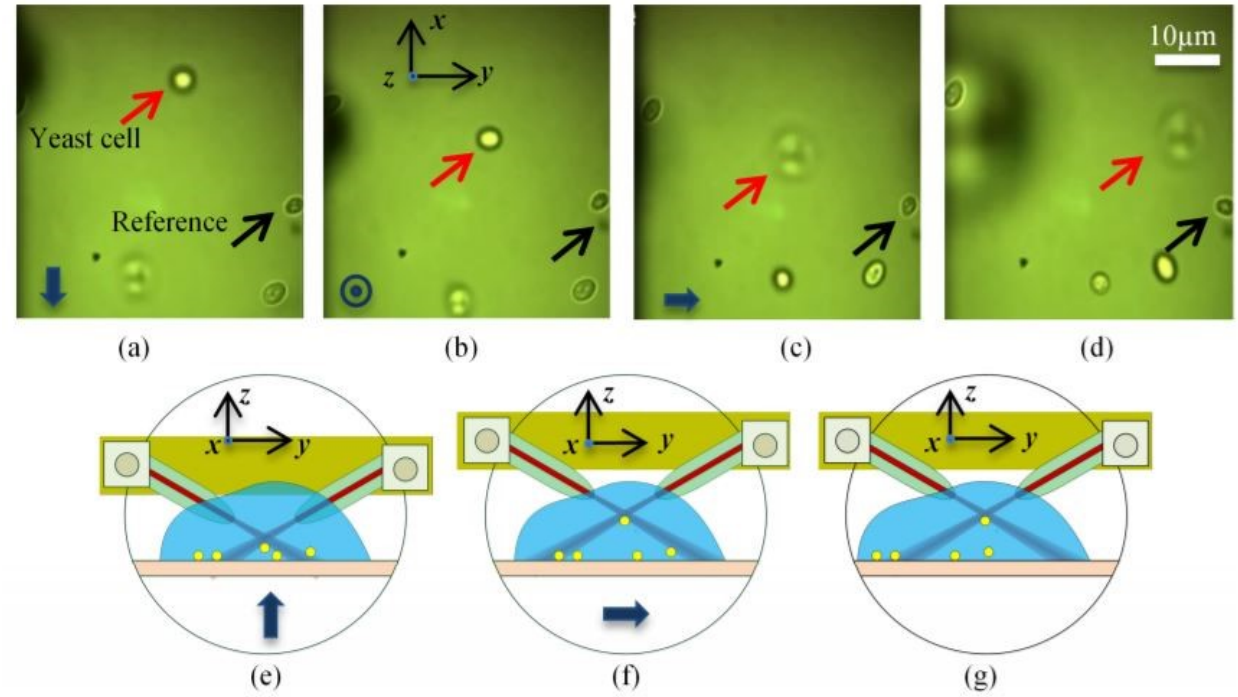

Figure 3: Demonstration of trapping living yeast cell, and graphical representations of a side-view[10] (a-d): Consecutive images from a trapping event using optical fiber based trapping. The red arrow follows a trapped yeast cell, the black arrow points to a free reference cell. The trapped cell was moved in the -x direction in b), then in the +z direction in c), and the +y direction in d). (e-f): Positions of the fibers and cells in (b-d) are represented in (e-f) respectively.

In order to accurately measure forces with optical tweezers it is important to measure the spring constant of the trap. The spring constant is determined using the system's power spectrum of the backscatter signal collected from the target, described by:[10]

$$S(f) = K_B T/\pi^2 \gamma({f_0}^2 + f^2) \quad \text{(eq. 2)}$$

Where k_B is Boltzmann's constant, T is absolute temperature, γ is the hydrodynamic drag coefficient of the object given by:

$$\gamma = 6\pi\eta a \qquad \text{(eq.3)}$$

For drag on a sphere with radius a in fluid of viscosity η. f_o is the corner, or roll-off frequency that provides the spring constant k as:

$$k = 2\pi\gamma f_0 \qquad \text{(eq. 4)}$$

Fourier analysis is used to obtain the power spectra from collected photodiode data.

The spring constant of the optical trap can be related to the properties of the cell to be analyzed. As a microbead is moved against a cell, either through the cell's movement or through

movement of the trap, the force exerted by the cell on the microbead is approximately equal to the force exerted on the microbead by the optical trap. This can be expressed as:

$$k\Delta x_{trap} = F_{trap} = F_{cell} = k_{cell}\Delta x_{cell} \quad \text{(eq. 5)}$$

Where Δx_{trap} is the distance between the microbead and the center of the optical trap, F_{trap} is the force exerted on the microbead by the optical trap, k_{cell} is the spring constant of the cell, and Δx_{cell} is the distance the microbead moved against the cell. This allows for the analysis of the stiffness properties of target cells through known properties of the optical trap.

2. Implementation of Dual Fiber Optical Tweezers System

Implementation of optical tweezer technology in the lab required the use and creation of special platform to stabilize and hold the optical fibers, as well as to have a reliable protocol for depositing microbeads onto cells.

2.1 Design of a Customized Stage to Mount the Fiber Optical Tweezers to an Olympus IX83 Microscope

To produce as stable a system as possible, relative to target samples, it was determined that the stage the optical tweezer system rested on should be connected to the optical microscope the sample is viewed on. This reduces relative shifting between the optical tweezers and the sample. An aluminum stage was designed in Solidworks, consisting of four legs, four connecting joints, and the primary stage surface. A system of 3D stages serve to hold the optical fibers for experimentation, which is attached to the aluminum stage during testing. This system makes it possible to perform optical tweezer tests on the Olympus IX 83 microscope in Professor Wen's Gateway Park lab.

2.1.1 Aluminum Stage Construction

The primary surface and legs were manufactured out of ⅜ inch thick aluminum using CNC milling. This material was chosen due to ready availability, as well as being a rigid material that would not put more weight than necessary on the system. Excess weight would put stress on the microscope that it was not designed to handle, and could compromise the structure. Connecting joints were cut from L bar aluminum and drilled using a drill press. The legs are bolted directly onto the microscope, and to the connecting joints and primary surface. All holes for bolts on the stage and legs are threadless, as the holes they correspond to on the microscope are threaded and aligning connections has greater freedom without threaded holes. The points of connection between the legs and the primary stage have four holes each, evenly spaced at the corners of a 1 inch square. This provided a design that was easy to build connecting parts for, as well as multiple points to place bolts at each joint, should unforeseen issues cause preference of certain bolt arrangements. The primary surface has six threadless holes, 7 mm in diameter, positioned such that a variety of optical stages and 3D stages can be mounted directly onto the surface and the attached instrumentation will reach samples that are in the microscope's focus.

All holes on the stage assembly, except two on the front-right leg, are 7 mm in diameter to accept standard optical bolts in both metric and imperial sizes. The two smaller holes on the front-right leg are 4 mm in diameter to match the small bolt holes on that side of the microscope.

The odd shape of the stage was designed to reach four points of contact on the microscope while leaving the sample area as unobstructed as possible. To keep the system as stable as possible all possible points of contact, those with bolt holes, were utilized. Connections to the back and to the right side of the microscope provide excellent stability as well as acting as a partial counterbalance to instrumentation that must be placed to the left of the left side connections, causing lever action. The central region is 9 ¾ inches by 8 inches. This area allows the microscope platform to move for experiments, and ensures that the stage does not need to be removed for other kinds of testing, such as traction force testing or use of an incubation chamber, which are commonly performed in Professor Wen's lab. The points of contact are located on the back of the microscope, on the right side, and two on the left of the microscope. Because none of the contact points are evenly spaced, the legs at the back and back-left of the assembly utilize slots 7 mm wide by 15 mm long to allow for imperfection in measuring around the body of the microscope during the design of the stage.

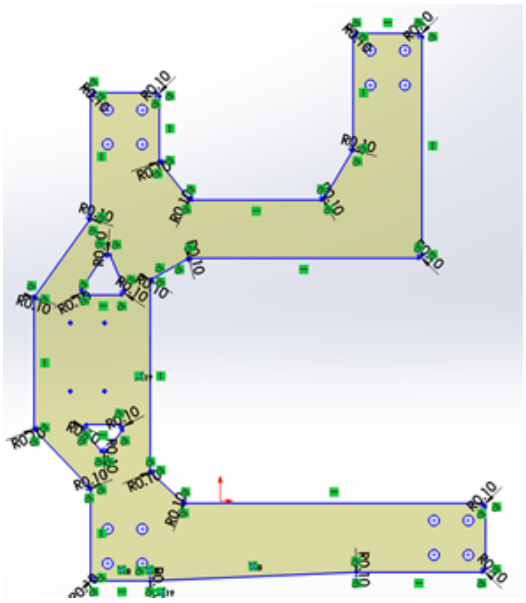

Figure 4: Solidworks drawing of the primary surface.

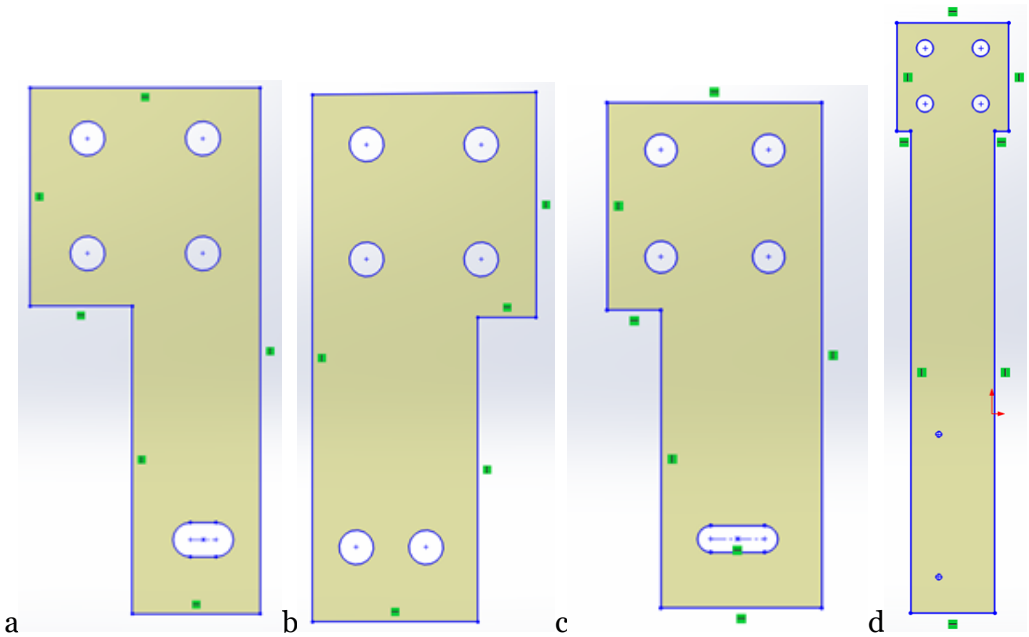

Figure 5: Solidworks drawings of the stage legs (not to relative scale). a) back-left leg (when using the microscope), 4 ¼ inches tall; b) front-left leg, 4 ½ inches tall; c) back-right leg, 4 ¾ inches tall; d) front-right leg, 10 ¼ inches tall

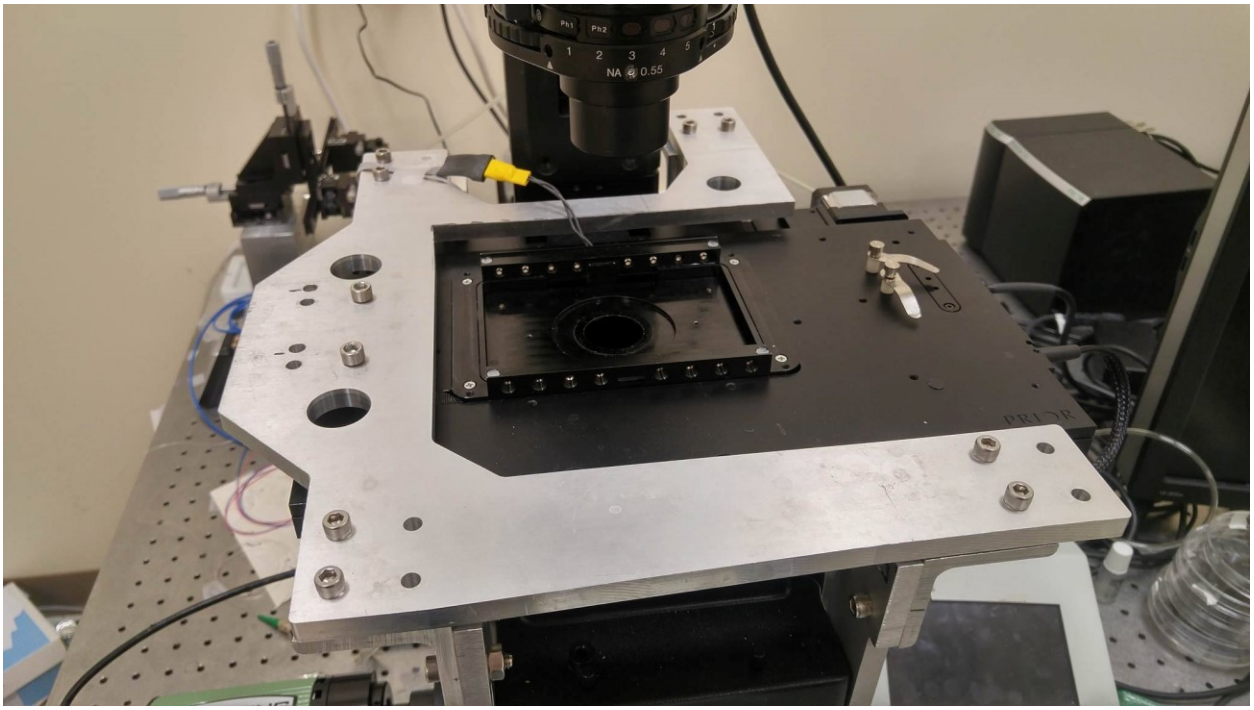

Figure 6: Completed main aluminum stage attached to the microscope.

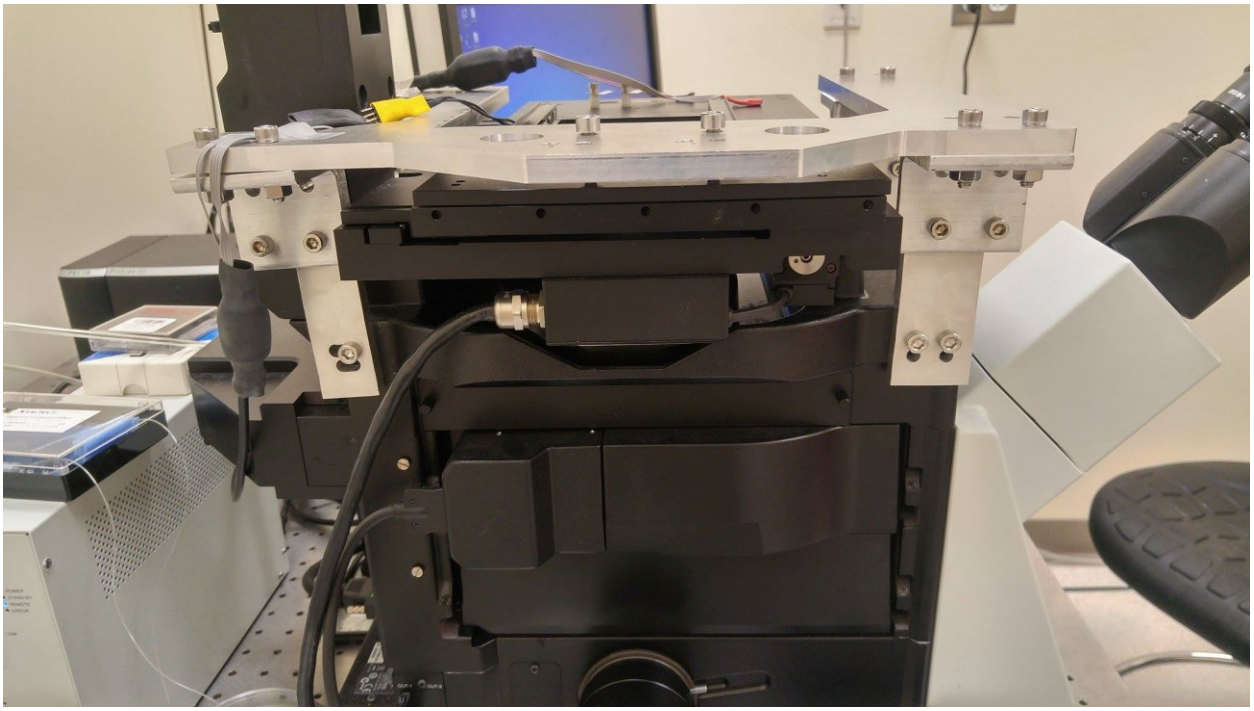

Figure 7: Left-side view of stage legs connected to the microscope.

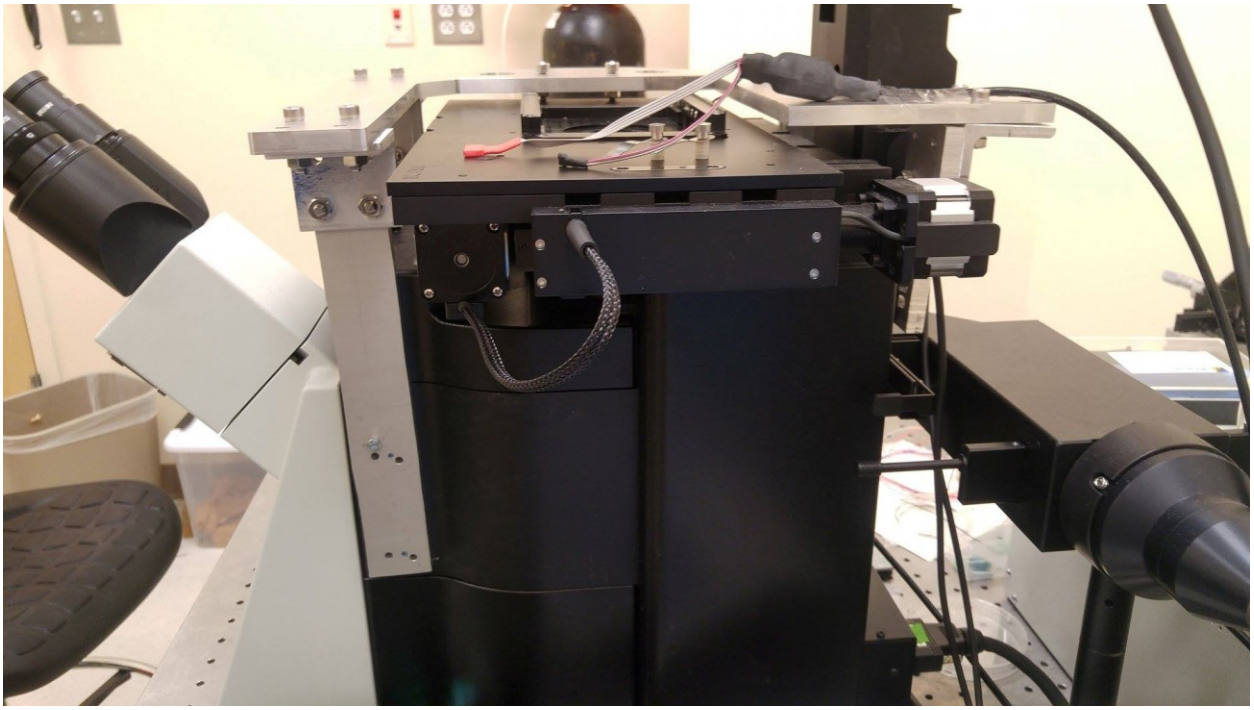

Figure 8: Right-side view of stage leg attached to the microscope.

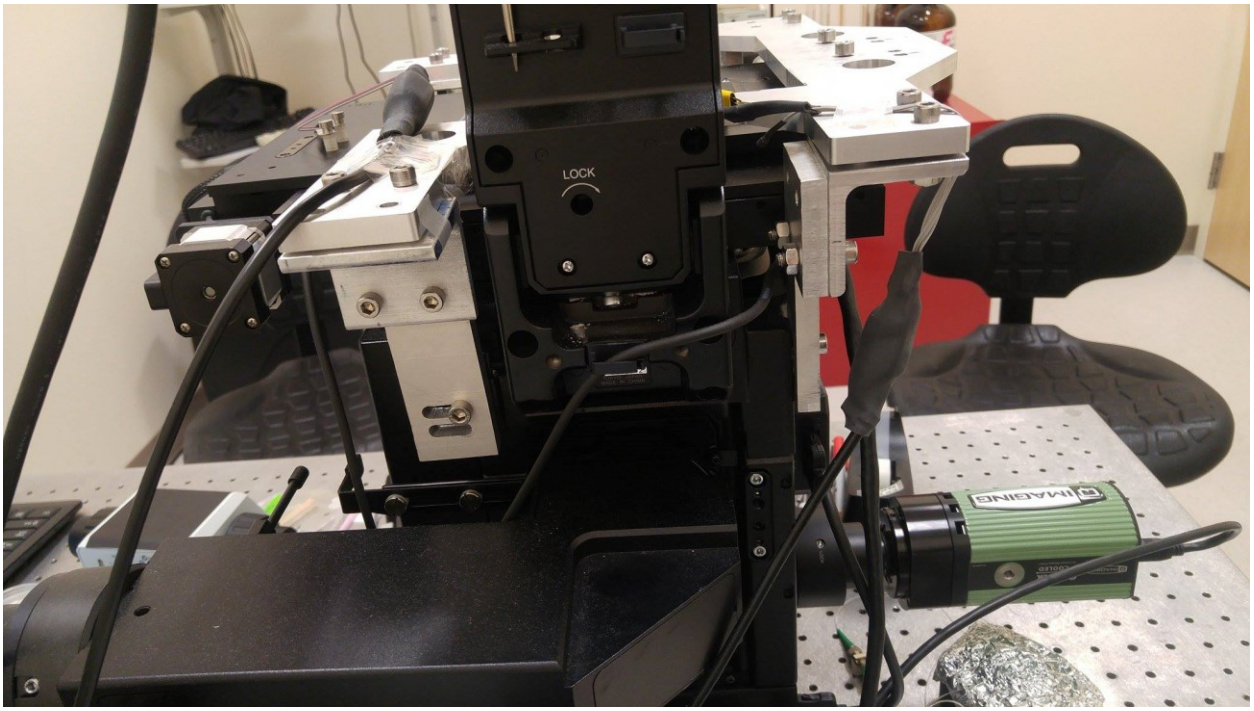

Figure 9: Rear-view of stage leg attached to the microscope.

2.1.2 3D Stage Assembly

To hold the optical fibers in place and allow for ease of alignment as well as movement for testing a 3D stage assembly was constructed. A large, Newport brand, New Focus line 3D stage with a range of 25 mm and measuring resolution of 0.01 mm on all axis is bolted onto the aluminum stage, and a rigid bar is attached horizontally to the large 3D stage. On either end of the rigid bar a small, Standa brand, 3D stage with a range of 5 mm and measuring resolution of 0.01 mm is attached. These smaller 3D stages also have the capacity to adjust the angle of parts connected to them, which is important to aligning the optical fibers. Attached to the smaller 3D stages are rigid plastic arms that have a shallow groove that the optical fibers rest in.

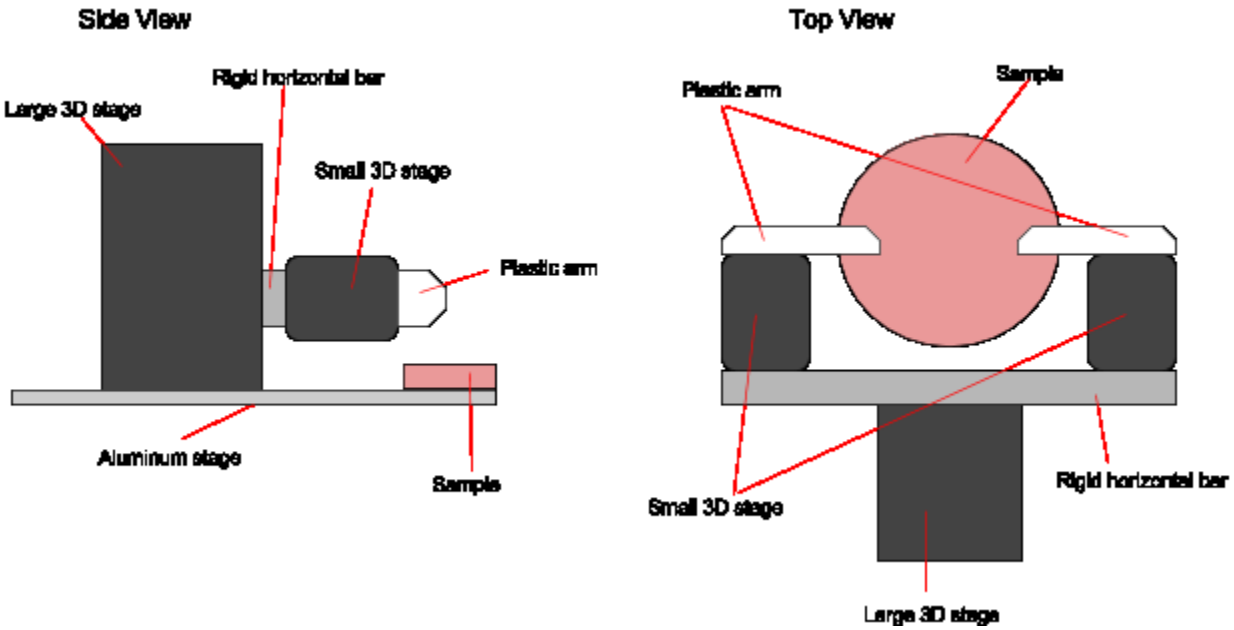

Figure 10: Diagram showing side and top view of the 3D stage assembly construction.

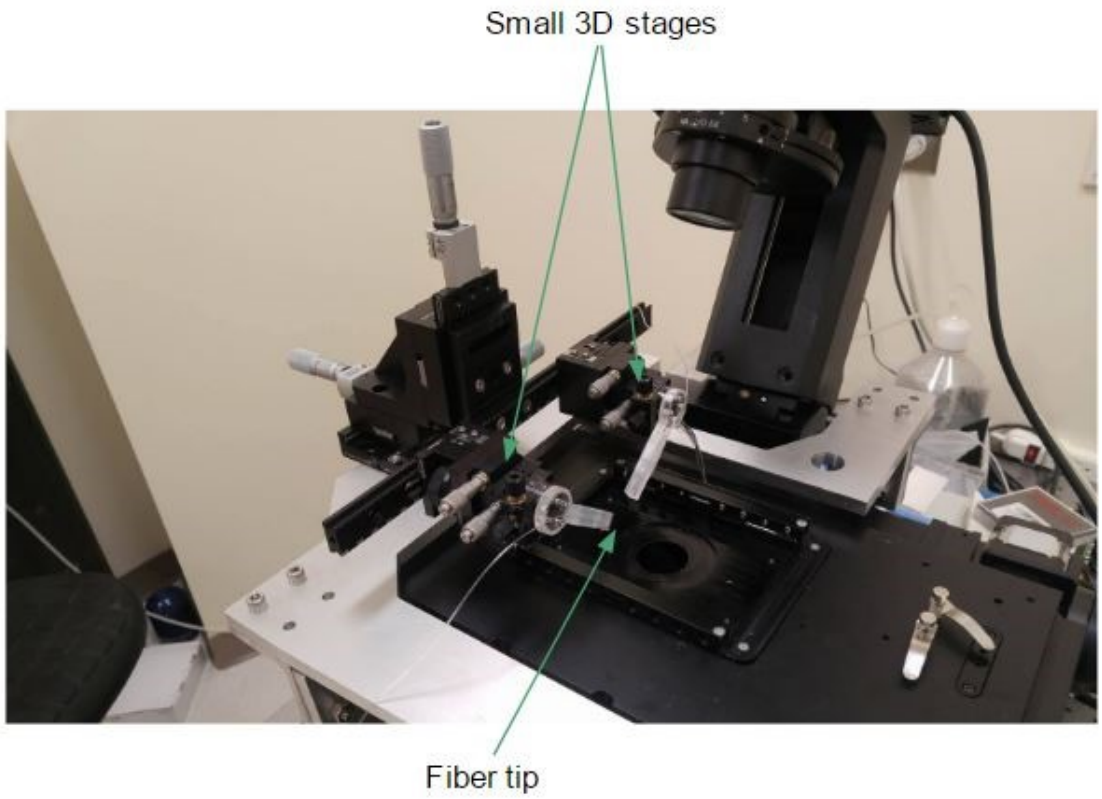

Figure 11: 3D stage assembly mounted to the microscope.

2.2 Implementation of the 3D Stage Assembly on the Olympus IX83 Microscope

2.2.1 Fiber Setup and Alignment

Proper alignment of the optical fibers is imperative to the functionality of the system. The more precisely they are aligned, the smaller the laser spot produced at the beam crossing, and the stronger the trap. What follows is the basic procedure to set up and align the fibers for the Gateway lab setup. At all times be careful not to touch the tips of the fibers to anything to avoid damaging them.

1. Set the fiber-holder arms to the demarcated angles on the angle adjustment portions of the small 3D stages.
2. Tape the optical fibers to the fiber-holder arms, crimping the tape down against the fibers. This helps reduce fiber drift. Be sure to tape in at least two places for each fiber.
3. Using the small 3D stages, move the fiber tips close together by eye. They should be roughly in line in all axis, and no more than 1 mm apart in the y direction at this point.
4. Place a drop of water about 3 mm across on a petri dish and place the petri dish on the microscope under the fibers.
5. Position the petri dish such that the edge of the water drop is visible in the field of view at 10x magnification.
6. Raise the microscope's field of view as high as possible.
7. Using the large 3D stage, lower the fiber tips to the same edge of the water drop.
8. Using the large 3D stage, adjust the x and y positions of the fibers until one is visible in the microscope's field of view.
9. Using the other fiber's small 3D stage, move it into view with the fiber that was found previously.
10. Avoid letting the fiber tips touch while aligning the fibers in all axis under 10x.
11. Repeat this alignment under 40x magnification.
12. Under 60x magnification, turn on the laser and find the laser spot. Adjust the fibers to make this spot as small as possible, with as much overlap from both fibers as possible.
13. Turn off the laser and fully raise the fibers in the z direction using the large 3D stage. The system is now ready for trapping.

For full experimentation including the capture of backscatter data additional equipment is needed. Two additional fiber couplers, a photodiode, and a DAQ system must be connected to the computer, which must have software ready to interpret the data. Testing was done using this equipment, but data was not able to be collected for unclear reasons, most likely due to an internally disconnected fiber going to the photodiode.

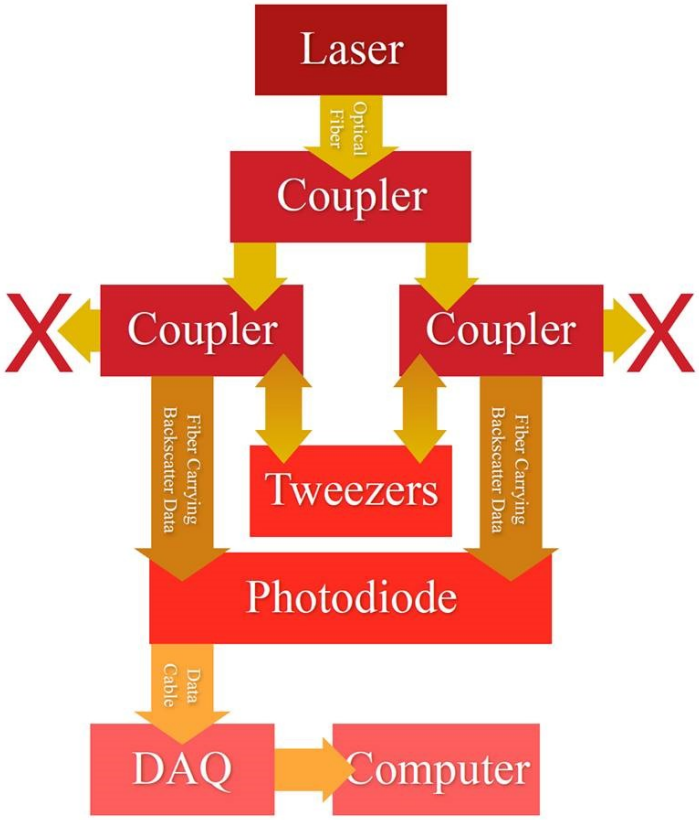

Figure 12: Flow Chart showing the progression of light information.

2.2.2 Bead Trapping

Once the optical fibers are properly aligned, trapping of free microbeads is straightforward. It is important to note that microbeads that have settled on the base of a petri dish in cell media will likely not be able to be moved using the laser trap, but those in DI water will be able to be moved. Microbeads resting on cells are most often unable to be lifted off the cell by the trap, but can be manipulated enough for the purposes of studying cell mechanics.

1. Under 10x magnification find a microbead of interest. This is usually one alone on a cell.
2. Bring the microbead to the center of view, then move the fibers such that they are slightly above the microbead and equidistant from it using first the large 3D stage, then the small 3D stages for fine adjustment. The fiber tips should be roughly 10 to 15 μm away from the microbead in the y direction, and in line with it in the x direction (knowing that the microbead is 5 μm in diameter can help make this judgement quickly).
3. Bring the magnification to 40x and re-center the system.

Turn on the laser. The microbead should visibly refract light and will jump to align with the trap.

3. Application of Dual Fiber Optical Tweezers on Cell Mechanical Studies

Performing stiffness tests on cells using optical trapping of silica microbeads has several overarching steps: microbead solution prep, cell culturing, microbead deposition onto cells, optically trapping beads. The following chapter explains the methods developed and used for each overarching step, and intermittent steps, as well as any other methods attempted in finalizing the methods.

3.1 Cell Culture Protocol

In order to test the stiffness of cells using optical trapping, live cells are needed. The cell culturing procedure allows for a cell line to be continued and grown such that cell samples and more seeding cultures are ready for tests within a day of culturing. This procedure is standard in Professor Wen's lab.

It is important to note that these cells, 3T3 fibroblast cells, can only survive for 30-45 minutes outside a controlled environment such as an incubator. Cells will double every 24 hours in an incubator or other similarly controlled environment.

1. Place DPBS and DMEM (10% BCS, 2mML-Glutamine, 1x Pen/Strep) solutions in 37°C water bath and 0.05% Trypsin solution at room temperature for ≥ 20 minutes.
2. Bring DMEM, DPBS, Trypsin to hood.
3. Check cell confluency of old sample dish to be split from.
4. Bring old dish to hood, vacuum out the cell media.
5. Add 3 mL DPBS to old dish, use pipette to rinse the surface of the petri dish.
6. Add 200 µL Trypsin to old dish, ensure it spreads to cover the entire surface.
7. Incubate the old dish with Trypsin still in it for 5 minutes.
8. Add 3 mL DMEM to old dish and agitate using the pipette.
9. Based on current and target cell confluences, add an appropriate ratio of DMEM from the old dish and fresh DMEM to a 1 mL eppendorf tube (eg. if target confluency is ½ current, add 500 µL old DMEM and 500 µL fresh DMEM). Prepare one eppendorf tube for each new cell dish planned.

10. Centrifuge the eppendorf tube(s) for five minutes at 1000 rpm. Use this time to prep new cell dishes.
    a. Ensure that the centrifuge is balanced!
11. Add 2 mL of fresh DMEM to every new cell dish.
12. Bring the eppendorf tube(s) back to the hood and vacuum out the media, being careful not to touch the pellet of cells that has collected at the bottom.
13. Add 1 mL fresh DMEM to the eppendorf tube(s) and mix using a pipette.
14. Add the media from each eppendorf tube to the corresponding petri dish. Agitate gently for 30 seconds.
15. Place the new dishes in the incubator, leave for ≥ 12 hours.

3.2 Development of a Methodology to Attach Single Beads to Cells

Attaching single microbeads to cells of interest is vital to being able to perform experiments on cell properties using optical tweezers. The microbead acts as both a source of force and measurement tool. The following procedures outline methods used to produce the microbead solutions used in testing and methods tested and developed for attaching single microbeads to cells.

3.2.1 Producing Bead Solution

The 5 μm diameter silica microbeads used for experimentation is originally purchased in 10.2% silica by volume solution with DI water. This is far more microbeads per volume than is needed, so more dilute solutions must be made. 1000x dilution is easy to produce and dilute enough to be generally useful. Limited application of 1000x diluted solution will not overload the sample, but will apply enough microbeads to be experimentally useful.

3.2.2 Pipette and Optical Trap Bead Application

This method was the first attempted by previous groups for applying individual microbeads to cells. The goal is to trap a microbead either in suspension or from the base of the petri dish and move it onto a cell in a favorable position. This level of control would also allow microbeads to be placed on specific regions of cells to investigate potential differences in stiffness across cell regions. This method would grant complete control over which cells have

microbeads applied to them, but even under ideal conditions would be slow and allow for only a few cells to be tested in each petri dish. The following is a list of steps using this method:

1. Align optical fiber tips under 10x magnification in DI water, then under 40x magnification.
2. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
3. Prepare microbead solution (1000x dilution standard).
4. Place cell sample under fibers, focus on the cell layer.
5. Pipet in ≥20 μL of microbead solution at fiber tip location (be careful not to touch fiber tips with the pipet).
6. Wait for 5 minutes for microbeads to settle, or turn on the laser and attempt to trap a microbead while it moves through the cell media (this second option is much more difficult).
7. Capture a microbead on the base of the petri dish using the optical trap and move it to the target cell. This movement should be executed slowly and using the large 3D stage to maintain the trap as much as possible.

Testing this method found that the microbeads adhere to the base of the petri dish under cell media too strongly for the trap to remove them. The alternative, capturing microbeads as they descend through the media, proved too difficult to perform in the lab. Because of these issues, this method is not recommended for use in the future.

3.2.3 Pipette Straightforward Bead Application

The next method attempted was to use a pipette to apply microbead solution to an area of a petri dish with gentle but steady flow. A similar method had been attempted previously, and was adapted for this project[12]. This method relies on the statistical likelihood of a microbead landing on a cell, given cell area, microbead size, microbead dilution, and application area. It has the potential to quickly apply microbeads to many cells in a general area, but would lack the precision needed to choose which cells can be tested. The process of this method is briefly listed as the following:

1. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
2. Prepare microbead solution.
3. Place cell sample on microscope. Use the microscope to observe progress.

4. Draw 50 µL of microbead solution into the pipette and apply it to the sample. The application should take between 1 and 2 seconds.

Testing this method found that microbeads did not tend to stay on cells when applied in this manner. Only clusters of cells would maintain microbeads, and microbeads landing on cells then rolling off was observed under the microscope. Because of this issue, this method is not recommended for use in the future.

3.2.4 Pipette Droplet Bead Application

Droplet application was developed from the straightforward method for this project due to the observed flow in the cell media and its lack of success. Droplet application utilizes the pipette to apply as small a droplet as possible (<10 µL). This method should limit flow, allowing microbeads to drop directly onto cells, while maintaining most of the high volume of application afforded by the straightforward method. A brief list of steps for this process follows:

1. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
2. Prepare microbead solution.
3. Place cell sample on microscope. Use the microscope to observe progress.
4. Draw 20 µL of microbead solution into the pipette and apply it to the sample as one or two droplets. The droplets should fall no more than 1 mm to the cell media surface.

Testing of this method showed similar issues to the straightforward application method. Microbeads were observed landing on cells then rolling off. Because of this issue, this method is not recommended for use in the future.

3.2.5 Pipette Overload-Flush Bead Application

This method functions on the opposite extreme of droplet application, covering a larger area in a dense microbead solution then removing excess. It was developed for this project in response to the lack of success with droplet application. Application is similar to the straightforward procedure, but uses intentionally very low dilution microbead solution, which is then washed away to leave microbeads that have attached to cells. This method covers a sizeable area, and should result in a larger number of microbeads stuck to cells, but takes longer than straightforward procedure, due to the added step of washing looser excess microbeads away,

and has the potential to wash away desired microbeads and even cells if the flush is applied too forcefully. Steps for this process are listed below:

1. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
2. Prepare microbead solution at 250x dilution. This lower dilution ensures a large number of microbeads in the application area.
3. Place cell sample on microscope. Use the microscope to observe progress.
4. Draw 50 µL of microbead solution into the pipette and apply it to the target area as droplets. The droplets should fall no more than 1 mm to the cell media surface.
5. Wait for 5 minutes to allow the microbeads to settle.
6. Draw in 50 µL of cell media to the pipette.
7. Placing the tip of the pipette < 1 mm under the surface of the media, apply the media in the pipette to the target area in a steady stream. This should wash away most excess microbeads on the cells in the area and on the petri dish surface.

Testing of this method found that the flush would wash away all the microbeads in an area, as well as cells if the experimenter was not gentle enough. It was also found that microbeads behaved similarly to other methods, rolling off of cells even before the flush step. Because of these issues, this method is not recommended for use in the future.

3.2.6 Capillary Glass Bead Application

The intent of this method, developed for this project in response to previous lack of success, is to bring the applicating device closer to the target cells than is possible with a pipette. A capillary glass tube is connected to a syringe by a flexible tube, and is stabilized by being attached to a fiber holder. The capillary glass was created by Dr. Jiaxin Gong for this project. By bringing the application closer to cells, targeting of cells should be more accurate and microbeads should fall more gently onto cells, mitigating motion that could cause them to fall off. This method covers a smaller area than those involving pipette application, but several areas can be targeted during the application process. Steps for this process follow:

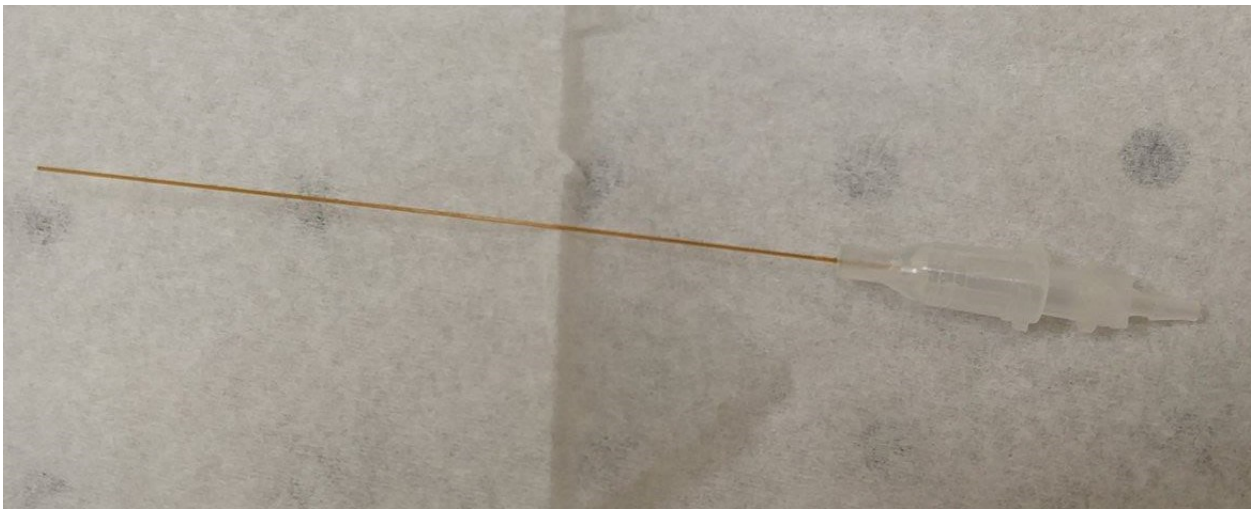

Figure 13: Capillary glass tube.

1. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
2. Prepare microbead solution.
3. Draw .2 mL of microbead solution into the capillary tube.
4. Attach capillary tube to fiber holder and position over microscope objective.
5. Place cell sample on microscope. Use the microscope to observe progress and monitor capillary tube position. It will appear roughly the width of the view under 10x magnification, so it is necessary to align the tube to the edge of the view then move the sample to a desired target location.
6. Apply low pressure to the syringe plunger until a slow stream of microbeads is observed exiting the capillary tube. It is possible to slow microbead motion by applying low negative pressure with the syringe.

Testing found that this method did produce greater control over microbead motion and deposition location, but did not improve on the issue of microbeads rolling off of cells. Because of this issue, this method is not recommended for use in the future.

3.2.7 Micropipette Bead Application

This method was developed for this project as an extension of the capillary glass approach. It utilizes the approach of capillary glass application while replacing the capillary glass with a micropipette. This allows even greater control over the flow and positioning of the microbeads during application. Three sizes of micropipette tips were tested: 30 μm diameter, 20

μm diameter, and 15 μm diameter. Micropipettes of 10 and 6 μm diameter were created, but not tested. The micropipettes used were created by Dr. Jiaxin Gong for this project. This method only allows a few cells to be targeted at a time, but they can be chosen with great precision. The experimenter must be careful not to touch the end of the micropipette to anything solid, as it is extremely fragile. The steps for this process are listed here:

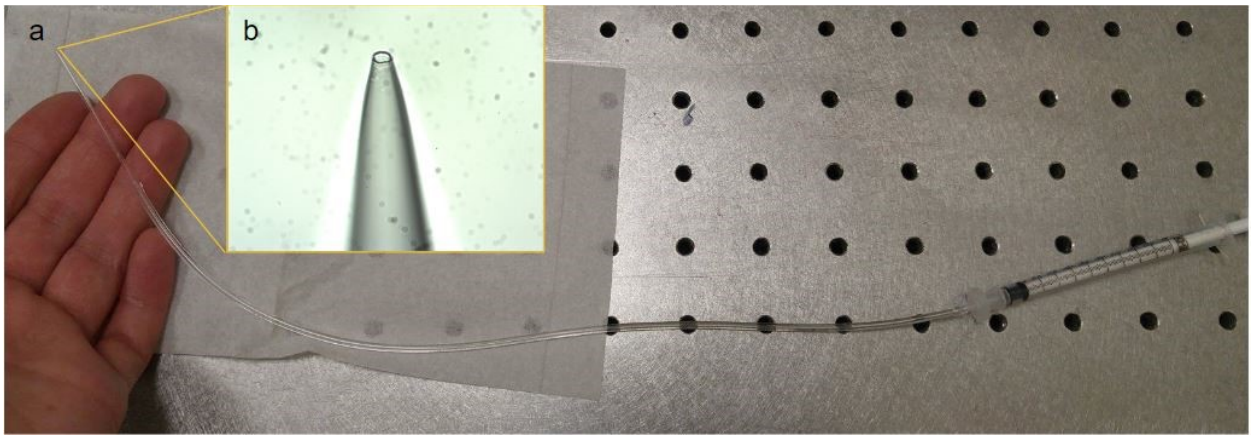

Figure 14: Micropipette tip attached to syringe as used in experimentation. a) The micropipette attached to a rubber tube and syringe. b) a 20 μm diameter micropipette tip under 10x magnification.

1. Prepare cell sample at ~25% confluency to enable targeting of individual cells.
2. Prepare microbead solution.
3. Draw < 0.1 mL of microbead solution into the 20 μm diameter micropipette. ~0.05 mL as marked by air in the syringe should be enough, the microbead solution need not fill past the end of the glass of the micropipette.
4. Attach micropipette to fiber holder and position over microscope objective.
5. Place cell sample on microscope. Use the microscope to observe progress and monitor micropipette position.
6. Position the tip of the micropipette over target cell. Move it as close vertically to the cell as possible without touching it.
7. Apply a small amount of pressure to the syringe plunger until a slow stream of 1 to 3 microbeads per second is observed exiting the tip. If these microbeads miss the target cell, adjust the sample position accordingly. It is possible to slow microbead motion by applying low negative pressure with the syringe.
8. After three or four applications the experimenter should rinse out the micropipette by expelling remaining microbead solution and drawing in 0.1 mL of DI water and expelling that. This is to prevent microbeads building up at the micropipette tip and clogging it.

Testing of this method found positive results. It uses very little microbead solution, has a high rate of success >80% with 5 out of 5 and 9 out of 11 cells successfully targeted in initial tests, and is more precise than all other tested methods. It was found that a tip diameter of 20 μm is ideal, as larger loses control over the placement of microbeads, and smaller causes microbeads to become clogged at the opening. The ability to move the micropipette to close proximity of cells and target individual cells prevented many of the problems seen in other methods, including microbeads rolling off of cells. While cells must be targeted individually with this method, it is possible to place microbeads on several cells in under 30 minutes.

3.3 Preliminary Experiments to Measure Cell Mechanical Stiffness

The system as a whole, cells, micropipette microbead deposition, microscope, and trap, was put through preliminary tests to examine its effectiveness. Tests were performed in both cell media and DPBS buffer solution. The system acted as expected, and the methods developed proved effective in placing microbeads onto individual cells and trapping microbeads that were on cells.

Microbeads were deposited on cells and trapped using the methods described in section 3.2.7. They were then manipulated using the optical trap to apply force to cells and measure cell mechanical properties. A preliminary test was performed. Results are shown in Figure. 15. To measure the cell stiffness, the trap center will be moved by a given distance Δx_{trap} . Due to the optical force, the bead tends to move with the trap and causes the deformation of the cell. The viscoelastic response of cell cause a resistance force opposite to the direction of the optical force. When the bead reaches its new equilibrium position, the optical force and cell resistance force are equal in magnitude. The cell deformation, i.e. the displacement of the bead Δx_{bead} can be measured. The trapping stiffness, k_{trap}, can be calibrated onsite. Therefore, the force exerted on the cell can be calculated as $F_{trap} = k_{trap}(\Delta x_{trap} - \Delta x_{bead})$. The effective spring constant of the cell, which is a measure of the cell stiffness, can be calculated as $k_{cell} = \frac{F_{trap}}{\Delta x_{bead}}$. However, due to the breakdown of the laser coupler, we were not able to extract the cell stiffness from this experiment.

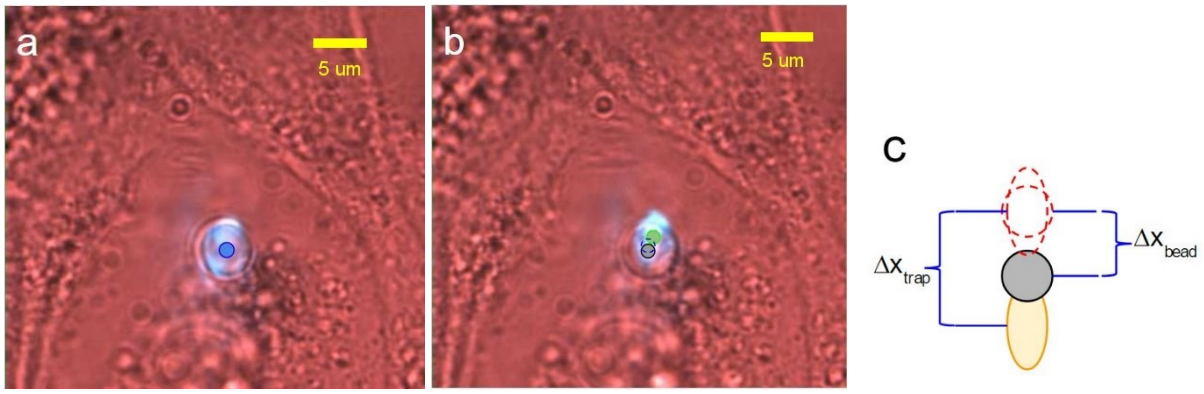

Figure 15: A microbead trapped on top of a cell under 60x magnification and red filtering to show laser spot. a) Shows the microbead centered in the optical trap on a cell (microbead center and trap center both indicated with a blue dot). b) Shows the microbead has shifted in relation to the trap and cell (original positions of trap and microbead indicated with a blue dotted circle, new trap center indicated by a green circle, and new microbead center indicated by black circle). c) A schematic illustration of movement of a microbead relative to trap center. Original positions of the trap and bead against a cell are indicated by red dotted outlines, and new positions of the microbead against a cell and trap are represented by the grey circle and yellow oval respectively.

4. Summary and Future Work

The results of this project are: 1) designed and created a stable testing stage to attach instrumentation to an Olympus IX83 2) developed a method for consistently and accurately placing single silica microbeads on cells 3) performed preliminary testing showing the viability of fiber optical tweezers in studying cell mechanics. The system and methods developed here show promise in testing cell properties, particularly lateral cell stiffness. Preliminary testing indicates that with implementation of accurate measuring systems optical tweezers will be highly effective at determining cell stiffnesses and exploring other cell properties. The stage created provided a stable platform that eliminated cross-platform vibration for testing, micropipette bead application works consistently and accurately, and preliminary testing shows that these systems work well with the optical tweezers to study cells.

Challenges to overcome in future work includes consistently obtaining data from the backscatter light of the microbeads and ensuring consistently known conditions. Consistent knowledge of conditions may be addressed by finding the spring constant of the optical trap before every test. This data can be processed after testing, meaning that little extra time is needed during experiments to do so. A possible improvement to the system may be incorporating precise motors to the 3D stage adjustments, allowing experimenters to exert fine control over the movement of the fibers, and eliminating jostling and vibrations caused by human interaction with the components.

With full data collection in place it will be possible to examine the polarization of cell forces, lending insight to the alignment of internal cellular structures. This would be achieved by measuring cellular forces along multiple axis, namely the x and y axis with relation to the trapping fibers. Once cell forces in two axis have been determined the information can be combined to determine the direction in which the cell is stiffest, and the properties associated with this can be examined, such as if there is correlation between a cell's long axis and the alignment of its stiffness.

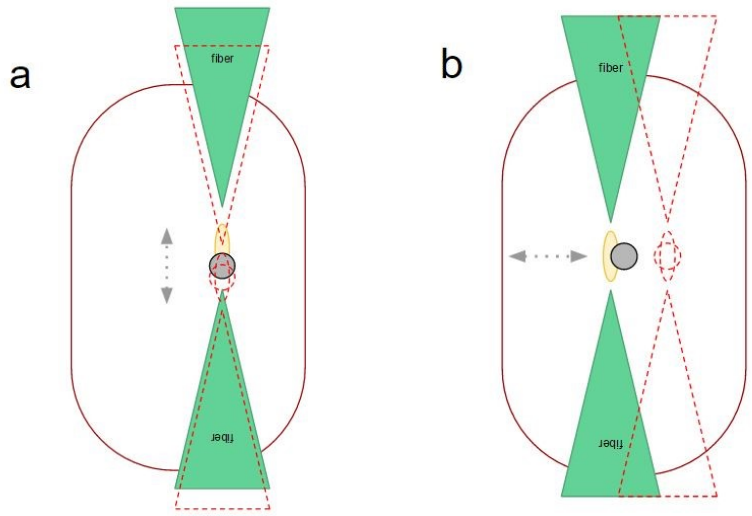

Figure 16: Examination of mechanical anisotropy of polarized cells. a) Measuring the cell stiffness along its long axis. The trap, yellow oval, is moved along the long axis to the cell, brown outline, displacing the microbead, grey circle, as discussed previously to determine cell stiffness. b) Measuring the cell stiffness along its short axis. The trap is moved along the short axis of the cell to characterize the stiffness along the short axis.

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This project would not have been possible without the work of Professor Liu's group in creating the optical tweezers system. Chaoyang Ti's assistance and past experience with the system helped to ensure that tests ran as smoothly as possible and the right equipment was present when not available in the Gateway lab.

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