DESIGN AND DEVELOPMENT OF AN INSTRUMENT TO MEASURE PICONEWTON FORCE

Mitchell Klosowski

Bachelor of Engineering
Mechanical Engineering

Department of Electronic Engineering
Macquarie University

November 7, 2016

Supervisor: Dr. Shaokoon Cheng

ACKNOWLEDGMENTS

I would like to acknowledge my supervisor Dr. Shaokoon Cheng for his guidance and assistance with this project when required and pushing for the approval of laboratory access and other safety requirements in order to complete my thesis project.

STATEMENT OF CANDIDATE

I, Mitchell Klosowski, declare that this report, submitted as part of the requirement for the award of Bachelor of Engineering in the Department of Electronic Engineering, Macquarie University, is entirely my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualification or assessment an any academic institution.

Student's Name: Mitchell Klosowski

Student's Signature: M.Klosowski

Date: November 7, 2016

ABSTRACT

The aim of this project is to design and develop the framework of a Piconewton Force Transducer (PFT) to be experimentally used at Macquarie University. More specifically a unique and specifically tailored Optical Tweezer device is designed and developed to allow for measuring the stiffness of cells and other micrometre sized object or biological specimens. In order to achieve this, all components and subsystems of the framework had to be very accurately modeled in CAD throughout all stages of the design process to ensure the correct layout and alignment of the device. To show the full functionality of the device an alignment calibration procedure is completed to validate that the device is ready to be experimentally used. The main problem faced within this project was the lack of funding to create a framework that could be entirely designed and constructed using purchased optical mounts. Due to this lack of funding an alternate design was developed and generated through extensive research and a thorough understanding of the requirements of each individual component. This process determined which components of the framework could be CAD modeled and 3D printed as a replacement to using purchased optical mounts, without sacrificing any functionality or accuracy of the device.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

There is a significant lack of knowledge in regards to the effects of different diseases on cell stiffness and other changes due to cellular biomechanical events. The knowledge gaps in literature are slowly being filled through the use of Piconewton Force Transducers (PFT). A PFT is a very useful scientific instrument that allows that allows for a wide range of biological systems to be studied. These devices are now considered to be indispensable tools for measuring forces and displacements associated with cellular and molecular biomechanical events.

One specific application of a Piconewton Force Transducer (PFT), is to measure the stiffness of different diseased cells' to gain further understanding into how the cells' deal with disease as well as looking at the direct effect the disease has on the properties of cells'. By researching these effects on diseased cells, the mechanisms associated with disease can be further understood, and therefore, potentially provide methods to prevent or cure diseases [10]. Consequently, any improvements on the literature already available in this field could have tremendous health benefits in todays society.

Besides from this there are many other vital applications for the use of a PFT device such as the effects that mechanotransduction has on cell properties and stiffness. Mechanotransduction is the process of mechanical forces being converted into chemical or electrical signals. These chemical and electrical signals cause the cells to transform and adapt to suit the surrounding environment [9].

Due to the importance of Piconewton Force Transducers (PFTs) in today's biomedical industry, this project is aimed to design and develop a PFT that allows for the measuring of cell stiffness and other biomechanical events. The device to be designed and constructed is that of an optical trap device (Optical Tweezer) which can be used to measure forces smaller than 200 pN and displacements smaller than 150um. This device is used as an attachment to a high-resolution microscope, to measure forces and displacements on cells, viruses, bacteria, organelles and even DNA as discussed in [4].

1.1 Project Goal

- Understand the operation of an OT based on it's individual optical components.
- Use this understanding to manipulate the optics to allow for the OT to be designed for the Nikon Ti-U microscope.
- Use CAD modeling to set the location required for each individual optics component of the OT.
- Use CAD modeling to design the framework allowing for the construction of a unique working OT.
- Calibrate the optical alignment of the device to ensure correct operation of the OT.

1.2 Costs

The budget allocated to this project as per the standard at Macquarie University is approximately $300, this depends loosely on the project itself and the specific requirements as well as the costs associated with other projects. For this project the standard budget of $300 was exceeded in the purchasing of vital optical components and therefore, some adjustments had to be made to accommodate for the lack of additional funding. The details on the purchases and the costs that they incurred can be found in Section 5.4.5.

Chapter 2

Background and Other Related Work

This section of the document contains the background research relating to the Optical Tweezer (OT). This is focused on the background behind the OT and what it is used for and why a Piconewton Force Transducer (PFT) is so important in biomedical research. Research specifically based on OTs and their operation and use can be found further on in Chapter 3.

2.1 Mechanical properties of cells

Human organs are constantly subjected to physiological forces and this is necessary to regulate their biological function. The process is complex and knowledge on this remains fragmentary. Physiological forces such as the pulsation of the heart and mechanical shear forces at the walls of blood vessels regulate cells' responses and their function such as cell-cell and cell-matrix interaction [5].

2.2 Mechanotransduction

The cells' response to these forces is Mechanotransduction, which is described as the ability of cells to convert mechanical forces to electrical or chemical signals as demonstrated in [9] and [10]. Due to this process, cells are able to transform and adapt into different cell types with specific stiffness, size and structure, and this is largely dependent on their environment and the magnitude of mechanical forces they are subjected to as discussed in [10]. Cells which are subjected to more physiological forces, alter their structure and stiffness to become more compliant (less stiffness) - this adaptation to the environment is vital as emphasised in [17]. Journal article [5] addresses how the integrated forces affect the cell behaviour itself which in turn creates its own mechanical tension throughout the cell. This leads to the underpinning hypothesis as stated in [5] that the shape of the cell is controlled by its location, which allows for changes in the stiffness and tension in the cell, thus regulating the cell's behaviour.

2.2.1 Pathways of Mechanotransduction

The extent of the effect that mechanical input has on the development and migration of cells and how this process works is still poorly understood as emphasised in [32]. However, it is known that mechanotransduction operates in pathways depending on the location within the body and the mechanical forces applied to it. As analysed in [32] tension, exerted either by muscles or externally, promotes the migration of cells in that specific pathway to resist mechanical stress and helps to coordinate the morphogenesis of muscle and epidermal tissue. Although it is not fully understood how this process occurs, it is understood that the mechanotransduction follows a pathway that allows for all the cells under those conditions to adapt to suit. This then suggests that similar pathways can promote other cell adaptations, not just the stiffness and structure, such as adhesion to promote the healing of wounds or other tissue damage as hypothesised in [32].

2.2.2 Effect of Disease on Cell Stiffness and Mechanotransduction

Cell stiffness is likely to change in diseases and understanding how cell stiffness can change according to health and disease is important to elucidate the disease mechanisms as addressed in [10]. This article [10] also addresses that although many diseases have very few similarities, they still show similar signs of defective mechanotransduction in the cells. When analysing [10] and [32] a better understanding is developed in regards to the resulting defective mechanotransduction in cells due to disease affecting the pathways. This reinforces that although a lot of research has gone into understanding mechanotransduction and cell stiffness there are immense holes in current research and information and entire components that are still a mystery.

2.3 Devices Allowing for the Measurement of Piconewton Forces

2.3.1 Optical Tweezer

Optical tweezers (OTs) use a laser beam in tight focus which allows it to use optical gradient forces to manipulate micrometre-sized particles such as cells as discussed in [7]. This manipulation allows for the analysis and measuring of minute forces in the piconewton range as well as displacements within the nanometre range. These devices also allow for the assembly of components such as cells into groups and structures. As portrayed in [7] the device works by the particle being attracted to the gradient force of the laser's focal point. It is this mild attraction that allows for the application and

measurement of the minute forces and displacements.
As mentioned earlier in Chapter 2 further information and research on the OT device itself can be found in Chapter 3.

2.3.2 Dynamic Holographic Optical Tweezers

Dynamic holographic optical tweezers are and work similar to a standard OT as portrayed in [7]. However, the single laser beam is transformed into multiple separate beams, each of which can be focused into an OT as described in [6]. This is highly beneficial where applicable as it allows for the controlling of multiple independent OTs through a computer while only requiring a single initial laser beam. As addressed in [6] dynamic holographic optical tweezers are unable to reach their full potential due to the complexity of calculating the phase hologram required for each tweezer.

2.3.3 Micro-manipulation using magnetic particles

The force transducer portrayed in [8] and [12] is a flexible alternative to the optical tweezer (OT) that also allows the measurement of forces in the piconewton range. It works using the original technique of single magnetic beads attached to leukocytes combined with new micro-manipulation techniques as portrayed in [8] and [12]. This allows for the application and measurement of a well-controlled piconewton force on micro particles, cells and small tissue samples.

Although all of these device are usable PFT devices, this project is focused specifically on the design and construction of an Optical Tweezer as the optics required for such a device are already available for use in this project, this was mentioned previously and is discussed throughout this thesis.

Chapter 3

Research on Optical Tweezers and their operation

The main focus of the specified OT research was on Block's method for constructing an optical tweezer as shown in [3], as Block's article was used by a previous academic to set up this project to be previously completed, including the purchase of the optics components specific to the presented optics layout. In some instances in this section other literature has been used and listed for the more generalised areas of research, those aspects less dependent on a specific optics layout and setup.

As previously mentioned this project is focused on the design and development of a device that can measure piconewton forces, a piconewton force transducer (PFT). More specifically an Optical tweezer (OT) device which was briefly outlined and explained in 2.3. The requirement is to design a unique framework for its specific application at Macquarie University for use with the Nikon Ti-U microscope. To successfully design and produce a unique framework for an OT, it is imperative that the device itself is extensively researched and all aspects and components of the device are entirely understood.

This section of the thesis focuses on the research of existing literature on the OT device and how it operates. It is vital to have a complete understanding of the operation of the device at a system, subsystem and component level. This allows the system requirements to be defined and followed to produce the specific device for its application and use with the Nikon Ti-U microscope. The research done on OTs and discussed in this section is vital in understanding how the device will operate and which components of the OT need more focus, such as greater accuracy and/or independent calibration.

As mentioned the layout and design of the OT created are based loosely on the layout as discussed and analysed in [3]. The OT layout that has been previously developed is shown to be easily adapted and used with inverted Nikon microscopes such as the Nikon Ti-U that is accessible for this project, making it an ideal foundation for the OT layout.

3.1 Optical tweezer (OT) system Analysis

The general use for optical tweezers was briefly discussed and mentioned earlier in section 2.3 when looking into what a working OT can do in terms of experimental research and why it is vital in the biomedical field. This section focuses on how the device itself actually works as a system and the sections following will break this down into the sub systems and components that allow for the general device to meet its experimental requirements. As previously mentioned an optical tweezer (OT) is a device that is able to use focused laser light to manipulate and attract microscopic specimens and objects. This is possible because a laser has a trapping capability through the gradient of light intensity that is generated at the laser's focal point. This means that in order to have a fully functioning OT device there must be a unified laser beam that can be manipulated to form a focal point with the steepest gradient possible and to three-dimensionally locate that focal point directly next to a specimen under the microscope as found in [3].
In order to allow for this there are a combination of requirements that all optical tweezers must have and these can be considered to be the overall general OT system requirements, as follows.

3.1.1 General Optical Tweezer Requirements

1. An infrared laser source with a wavelength between 700-1300nm, ideally with an adjustable attenuator to allow for testing with the laser set at different powers to allow for an adjustable force output from the laser as listed in [3].

2. Using external optics (separate from the microscope) the optical trap must be able to be steered in the x-y (specimen) plane as well as the z (axial) direction. This allows for complete manipulation and control of the location of the trapping force and laser's location, before entering the microscope at all, as researched and discussed in [3].
   This allows for a completely independent device to be designed and set up ready for use with any compatible microscope in any situation. It is this requirement that this design thesis is aimed to uniquely achieve and meet.

3. A microscope equipped for epi-fluorescence that has a x-y-z translatable specimen plane. This allows for the positioning of the specimen to be adjusted if required to meet the laser's gradient perfectly without having to rely entirely on the external system components for fine tuning. The microscope also requires a camera port to be connected to a computer to allow for the viewing of the trapped specimen.

It is these basic requirements that are needed from any optical trap as a complete device, in order to successfully generate an optical trap that can be used experimentally to test micrometre sized objects and cells.

3.2 OT subsystems

The operation and functionality can be very easily separated into two major sub systems with independent roles to generate the optical trap. These subsystems are the external optics and the internal optics. The internal optics refers to the microscope itself and the optics and the optical path used to generate the optical tweezer within it. Whereas the external optics refers to all the optical equipment and optics that are separate from the microscope, generating and manipulating the laser beam before entering the internal microscope subsystem.
The external optics are the focus of this thesis as this subsystem requires the design and creation of the framework and layout. This subsystem can actually be split again into two separate subsections as they have independent tasks which will be analysed and discussed below. It is this focus and understanding of each subsection that enables for an ideal, unique design to begin formation through research.

3.2.1 External Optics

The external optics subsystem of the OT device is all the components that require a unique framework design to suit the specific needs of the OT being developed. This subsystem involves having the laser source and then manipulating the laser beam in a way that allows for it to be steered and generate a par-focal laser spot [3]. This means that the beam will maintain focus after magnification has been changed inside the microscope.

In order to best understand and analyse the external subsystem it is best to break it into two separate sections so more appropriate requirements can be understood and set later. These subsections as found below are the laser beam initialisation and the lens telescope.

3.2.2 Laser setup

The laser system refers to the use and operation of an infrared or near-infrared laser to manipulate the beam, generating a gradient that applies the force to the micrometre sized particle and "traps" it [4]. The laser beam is expanded using a beam expander where it is then guided by the rest of the device's optics and framework into the epi-fluorescence port of the microscope [3]. This is often done in most standard designs by using two or more angled mirrors that can be adjusted and moved to allow for the laser beam to be guided, as required directly into the centre of the microscope's input port.

3.2.3 Lens Telescope

The lens telescope subsystem of this style of optical tweezer consists of two identical planoconvex lenses L1 and L2. These lenses are set up to be the sum of their focal lengths away from each other, with the flat faces facing in towards the other lens [3]. The

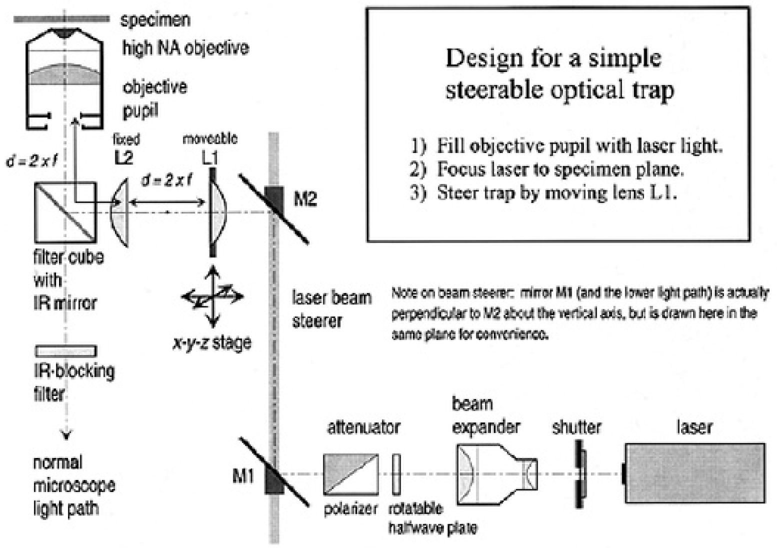

Figure 3.1: The layout of optical components in OT [3]

distance between the two lenses being double their focal length means that the parallel laser light entering the first lens will produce parallel light of the same beam size leaving the second lens. Demonstration of this can be seen in reference to Figure 3.1 of the overall system layout and Figure 3.2. By having the flat lens faces facing towards one another the spherical aberration (loss of image definition due to surface of spherical lens) can be reduced without having to use aplanatic lenses, which are very expensive, to avoid distortion [3].
The lens telescope component is a vital component in this design and layout of an OT device as it controls the majority of the beam steering which allows for the development of an external fully steerable optical trap to be created. This beam steering is made available through the manipulation and adjustment of lens L1 seen in Figure 3.1 which allows for the generation of three dimensional movement of the laser trap [3]. How this beam steering takes affect will be discussed below in the individual component section for each lens.

3.2.4 Internal Optics (Microscope)

The subsystem of the internal optics in the microscope has a vital role in the generation of an optical trap. Although the microscope itself is what actually generates the optical trap to be used, it is not the focus of this thesis as it does not require any additional design or development. In order to set up the optics to be placed inside the microscope,

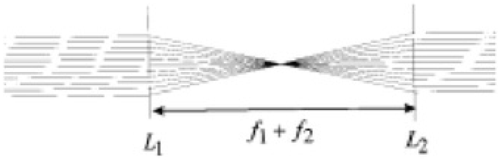

Figure 3.2: Interaction of laser light with lens telescope

all that is needed is to purchase the correct optics components and place them into the pre-existing fixed mounts within the microscope.

Role of the Internal Optics The microscope itself has many major aspects that are vital in the successful generation of an optical trap. The laser beam must be guided and directed through the optical path before being reflected to allow for the collimated beam to be focused onto the sample slide [1].
This operation is what causes the laser beam to have a focal point centered next to the specimen being tested and generating the gradient that allows for the generation of the optical trap [3]. In this instance the optical trap is then observed through the computer via a mounted camera that is attached to the existing microscope. The microscope's internal components, when looking at a microscope attachable OT, is the subsystem that allows for the focusing of the laser beam, and therefore the generation of the optical trap itself. This process and the beam steering within the microscope itself can be seen in Figure 3.3.

3.3 OT individual components

This section looks at the roles and requirements found for the overall system and each subsystem, before allocating them to individual components of the overall OT device. This is a vital step in understanding what is required of each component individually and how to generate a unique design using this understanding.

3.3.1 Diode laser

A variety of different lasers can be used without impacting the optical trap too greatly. The critical factors of the operating laser are that the wavelength is between the range of 700nm and 1300nm and that it has a variable attenuator attached to it [3]. This attenuator is vital as it allows for the laser power itself to be changed and reduced for the purposes of alignment as well as for certain experiments and optical traps to be generated. The trapping laser itself can focus up to 1W of light into the microscope depending on the laser. The large fluxes at the specimen plane can cause optical damage and ruin the

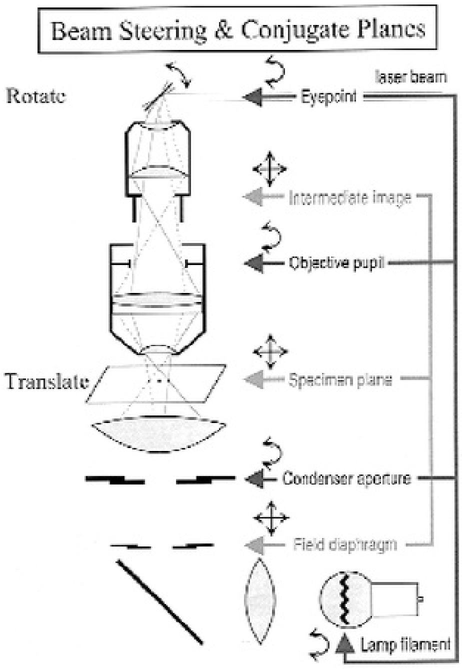

Figure 3.3: How beam steering is achieved in a microscope [3]

specimen and the experiment [3]. For this reason a laser with a wavelength that lies close to the infrared region is best suited, such as a 1064nm or 1047nm laser [3].

3.3.2 Beam expander

The Beam expander is integrated for a fairly simple reason and that is to magnify the laser so that it approximately fills or slightly over-fills the objective pupil of the microscope objective being used. This process allows for the objective to properly focus the entire laser beam for maximal optical trapping to occur [3]. Beam expanders come in fixed sizes such as 3x, 5x, 7x magnification and are designed to suit specific wavelengths. It is vital that depending on the laser wavelength, the correct beam expander is obtained as it will significantly reduce wavefront distortion at that wavelength over the entire opening of the device [3]. By minimizing the wavefront distortion spherical aberration can be further reduced, which will lead to improved focusing in the specimen plane and therefore improved potential trapping [3].

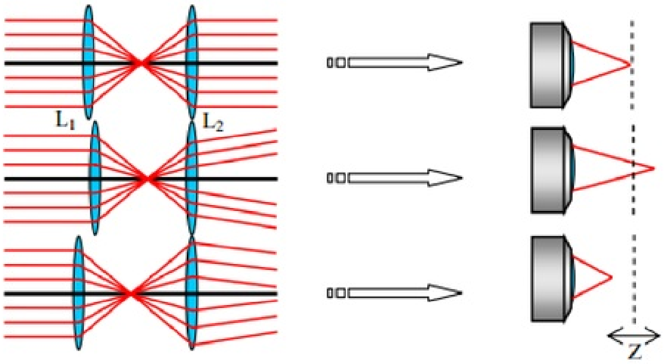

Figure 3.4: The effect of changing the distance between L1 and L2 as shown in [2]

3.3.3 Lens L1 of the lens telescope

Lens L1, the lens located immediately after the beam expander as seen in Figure 3.1 is the vital component in enabling the manipulation and steering of the laser trap. This lens needs to have x-y-z translation in order to allow for the OT device to have a steerable focal point. The way this works is for example movement in the axial (z) direction such as L1 being moved closer to L2 will force the parallel beam entering the telescope to become slightly divergent as it leaves L2. By having this exiting light being divergent the focal point will be pushed away from the objective and further into the test specimen as discussed by Block [3].
The opposite occurs when L1 is moved further away from L2 making the distance between them greater than 2f where f is the focal length of the lens. This will cause the light from the telescope to become slightly convergent, which will bring the focal point closer to the microscope objective, a visual example of this can be seen in Figure 3.4.

Moving lens L1 in the x-y plane, the plane perpendicular to the laser light and the optical axis, causes the deflection of the light leaving the lens. This essentially is rotating the beam, where the rotated light is then imaged through the back of the microscopes objective pupil by lens L2. As the rotation of the beam is occurring in the objective pupil's conjugate plane, this results in the translation and movement of the laser spot and the optical trap itself [3] as can be seen in Figure 3.5.
The x-y-z fine translation of L1 is vital in the design for this optical tweezer as movement of this lens in all three dimensions results in the corresponding translation of the optical trap in the same three dimensions [3].

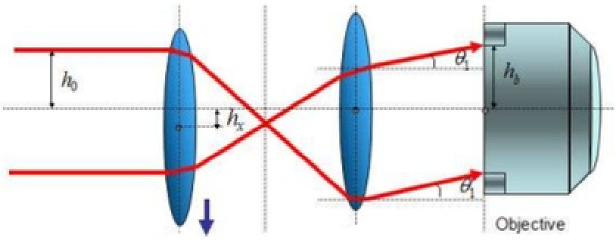

Figure 3.5: The effect of x-y plane translation of L1 [2]

3.3.4 Lens L2 of the lens telescope

Lens L2 is the second lens that forms the vital subsystem of the lens telescope as discussed earlier. While lens L1 must be translatable to allow for the beam steering, L2 must be very accurately and securely fixed into position to convert the movements of the beam generated from L1 into movements of the optical trap itself. As L2 is fixed and mounted parallel to L1 with its flat face facing inwards, the light that is being received from L1 is converted back into parallel light upon exiting its spherical (rear) face [3]. It is due to this layout that any parallel light entering the lens telescope will, after any translation or rotation, result in parallel light exiting the telescope via lens L2.

3.3.5 Dichroic mirror in microscope filter cube

The dichroic mirror or as it is sometimes called "hot mirror" is used to reflect the laser light into the microscope objective. This dichroic mirror should reflect the wavelength of the laser, while still transmitting visible light below 650nm [3]. The mirror needs to fit into the epi-fluorescence cube for the microscope being used as it is an existing mount specifically for use with the microscope, and that will provide a guaranteed 45 degree angle for the mirror. The mirror itself should reflect almost all laser light from the front surface of the glass while also having an anti-reflection coating for 1064nm wavelength on the rear surface [3].

3.3.6 Microscope Objective

The microscope objective is what actually generates the gradient at the focal point, therefore, generating the optical trap to be used. This is a standard microscope component and there are many different objectives that can be purchased for different reasons. In this instance a high NA objective is required to generate the steepest force gradient, for which in this case, the ideal range is between 1.25 and 1.40. The objective's magnification ideally is 100X and because of this high magnification, oil-immersion is best suited.
Once purchasing an objective it is simply used by screwing it into the port or rotator of the microscope for the objectives.

Chapter 4

System design methodology

This chapter focuses on the preliminary design stages and aspects in order to set a foundation for the final design. For this project this chapter of the design process was very important as it sets the unique design dependent parameters and requirements. Focus in this chapter is drawn to the many different factors that affect the system being designed. These factors can then be used to generate a unique set of system requirements for the Optical Tweezer being designed in this project for operation at Macquarie University.
This project is about designing an OT device ideal for the use and application with the specific set up at Macquarie University. In order to achieve this, not only must the device itself be understood as shown in Chapter 3 but also the specific layout, requirements and possibilities available must be considered and that is the basis of this chapter.

4.1 External framework temporary layout

Early on in the development of this project it was decided that in order to gain some insight into the requirements of each component of the external framework, by attempting to set it up using temporary framework. This layout of the external optics was attempted using temporary framework, such as cut outs, without using the laser itself due to having poorly mounted optics. Setting up this layout using temporary cut outs and other forms of framework did not actually work properly due to the drastic inaccuracy of the two lenses making up the lens telescope. This was not possible as with the temporary framework the correct alignment and vertical positioning of the lenses could not be achieved.

Although the proper layout of the external optics could not be achieved this process did generate some valuable insight into the requirements of the framework. This included how the framework could be manipulated to ensure a safer, simpler and improved design. The main aspect in regards to this was that the laser and beam expander could be considered as one complete subsystem (similar to the lens telescope) and that the height and alignment of this subsystem can be quite easily fixed and set as required.

This understanding led to the incorporation of this idea in the design, as by fixing the beam expander and laser relative to each others position, the rest of the design can be modeled in CAD around the required and set location of this subsystem.

4.2 University safety guidelines

There are many risks associated with the use of a class 4 laser such as the one required and used in the Optical Tweezer device (OT). The OT being designed in this project is for operation and use at Macquarie University. Due to this it is vital to analyse the university's laser safety guides and procedures to help determine the system requirements, thus producing a device that meets the universities safety requirements.
Safety in university's is very important and must be taken into consideration in order to prevent injuries and reduce possible risks. In order to gain approval of such an OT device to be used, all aspects of university regulations and safe practices must be followed. This section looks at all the potential aspects of good practice that may relate either directly or indirectly to the operation of an OT. The important aspects of Macquarie University's policies have been split into sub sections below.

4.2.1 Horizontal single plane setup

According to the university policies defined at [13], all optical devices are best contained to one single horizontal plane, maintaining a parallel line from the optical table. Periscope designs using mirrors and other reflectors must be completely avoided as this form of multi plane layout with vertical beams has a very high risk for laser operators. Having any vertical laser beams poses a very high risk of the laser light reflecting up into an operator's eyes. The use of vertical beams also puts others using the laboratory at risk as they may not realise a laser is in operation and take the necessary precautions when approaching the device.
This risk is further increased through the operation of a class 4 infrared laser as there will be no visible vertical beam. This leaves the operator and other individuals at serious risk of placing themselves above the mirror and in the beam's path. The beam may also reflect slightly unintentionally and could be directed at an individual, putting them at risk of serious permanent damage being done to their eyes.

4.2.2 Reflective Surfaces

When using class 4 lasers or other infrared lasers any reflective surfaces or materials presents a risk of unwanted reflection. This is made very clear in the university's good practices documentation [13] to prevent any unnecessary risk of lasers reflecting or refracting without the operator being aware of it. Due to working with infrared laser light, unless an IR detector card or filter is used to show the light, reflected beams will not be visible, making them extremely dangerous. As the OT device uses a class 4 IR laser, even slight or partial reflections of the laser into an eye will cause permanent damage. Due to this it is imperative that when operating a device at university any potential surface reflections are reduced to a minimum if not entirely at all times.
Another potential risk associated with reflections is those relating to the operator and

not the device itself. This refers to all non-essential reflective materials, such as jewelery, watches and belt buckles. All objects and materials such as these must be removed before working in the area of the laser and operating it.

4.2.3 Beam steering optics

As per the University safety policy [13], any optics involved in beam steering must be securely mounted with proper optical mounts. This secure mounting is vital because any accidental misalignment or the fall of an optic component out of position may cause some reflection of the laser and expose an individual to a high intensity, dangerous beam. For this reason rotating mounts that move easily must not be used for optics involving beam steering. This is mainly applicable when using mirrors to steer the beam and they are able to rotate the mirror because it may rotate during operation and reflect the beam into someone's eyes.

4.2.4 Beam Enclosures

Where possible, university advises that all beams be fully contained within beam tubes or a complete enclosure for the device. While not always applicable, this is advised in order to remove any potential risk of reflecting IR beams. This is mandatory in the case of the laser being operated outside of a laser laboratory equipped with interlocking and with no windows. In these circumstances the laser must be contained and enclosed at all times to prevent the beam from being reflected out of windows or doors and putting other individuals at risk of exposure.

4.3 System Requirements of the Optical Tweezer

From a combination of the literature reviewed in Chapters 2 and 3, the author's knowledge and personal judgment and the experience gained from attempting initial set up discussed in Section 4.1, the system's unique requirements can be set. These aspects combined with Macquarie University's laser safety guidelines, regulations and procedures [13] as analysed above in 4.2 make for an integrated framework from which to develop the requirements. The system requirements can be split into multiple different sections as found below, which allows for both a holistic and compartmentalized understanding.

4.3.1 Overall Optical Tweezer (OT) device requirements

1. An infrared laser with a wavelength of 1064nm and an adjustable attenuator to change the power output of the laser for alignment purposes and adjustable testing as discussed previously. This laser beam must be at a height of 188.5mm off the optical board and running in a parallel (horizontal) plane for its entire path through the external optics. This will input the laser directly into the center of the epi-fluorescence port of the microscope.

2. The laser beam must be steerable in the external optics using the lens telescope to manipulate the location of the optical trap within the microscope. This requirement will be discussed in detail below in the lens telescope requirements.

3. The external device must be easily removed and stored from the optical board as permanent fixture is not available due to the microscope being used for other experiments and tasks. This removal of the OT's external framework must be made possible in a way that does not effect the layout or positioning of the overall optics and framework. This is a vital requirement as this will prevent having to perform the entire alignment procedure every time the device is to be installed and used.

4. A complete enclosure for the external OT framework will be required as the location of the microscope is not in one of the University laser laboratories. This also means that there are windows, doors and other individuals in the room so the laser beam must be contained at all times.

5. As the entire framework must be enclosed when the laser operation is in use, an alternative method for an initial alignment check before each use must be used. This alternate method must allow the alignment to be completed before each separate session using the OT without the enclosure secured in place so any minor alignment adjustments can be made to the external optics while using the microscope. Without this the device would have to be aligned with the high intensity diode laser in a separate laser laboratory where the microscope can not be accessed and used. During transportation and re-installing the device with the microscope the results of the fine tuning in the optical alignment may have changed, thus making calibration of the device inside the microscope more complex.

6. The OT must be used with the Nikon Ti-U microscope as set up on the optical table at Macquarie University. The microscope can and must provide x-y-z translation of the specimen plane to allow for the specimen to be easily located next to the laser beam focal point.

4.3.2 Laser framework requirements

These requirements correspond to the framework for the diode laser itself and the beam expander as one subsystem.

1. The laser must be positioned in a way that makes its beam parallel with the horizontal optical table while being precisely 188.5 millimetres above it. Due to the positioning of the microscope port and therefore the laser, the beam expander must also be positioned to allow for the beam to travel directly through its centre. This is crucial as if the beam for any reason does not travel straight through the beam expander the beam may become slightly distorted or expanded to the incorrect beam diameter. In order to remove any need for mirrors to change the height or location

of the beam it is very important to ensure the laser and beam expander are correctly positioned.

2. By considering the beam expander and laser as one subsystem emphasis on the initial alignment to ensure the laser and beam expander are perfectly aligned as discussed above is simplified. This subsystem must generate the laser and expand it out to a diameter equal to or greater than the diameter of the objective pupil of the microscope objective used for testing.

3. The beam expander must be suited to the specific output wavelength of the laser, for example if the laser outputs a beam at a wavelength of 1064 nm, then the beam expander must be specifically designed to take light of 1064 nm wavelength. This is so that the lenses used in the beam expander are specifically suited to the correct wavelength and will therefore minimise distortions and spherical aberrations [3] .

4. This subsystem should also incorporate in the design a system that will help to simplify the alignment check of the optics before each separate session using the OT device. As this procedure may need to be done without being completely enclosed by a casing while outside the laser laboratory, it must present far fewer and less hazardous risks. An example of this is using a filter to make the IR light visible and using the laser on the lowest power setting, however, this is still not safe to use in the environment it would be operated in. A better alternative would be using a laser pointer or other less dangerous laser for the alignment check, although this would have to be incorporated in a way that ensures the beam paths of the two lasers are identical and that it is tested.

4.3.3 Lens Telescope Requirements

1. The lens telescope is required to be a 1:1 design, which means that as the beam expander increases the beam diameter enough on its own the lens telescope is not required to do any further beam expanding. This means that the purpose of the telescope is to allow for the manipulation and translation of the laser's focal point without further impacting the beam's properties. As discussed previously this requires the two lenses L1 and L2 making up the telescope to have their flat surfaces facing each other to minimise spherical aberration and any other image distortion.

2. Any parallel light being received by the lens telescope must also allow for the exiting light to be parallel in the same plane. This is essential in order to allow a direct and precisely aligned beam along the optical path that, if required, can be finely translated to adjust the location of the laser's gradient without taking it out of the horizontal optical path entirely. This requirement can be seen in Figures 3.4 and 3.5.

3. The distance between L1 and L2 must be accurately adjustable to allow for any need to change the location of the laser's focal point relative to the microscope

specimen plane. However, this distance is generally set and equal to the sum of the lense's focal points, or as the lenses are identical in this system, equal to 2f (double the focal length of the lens). In order to accurately accommodate this subsystem the distance between L1 and L2 (z-axis) should be easily determined and accurately set. As knowing the distance between the lenses is very important this must be incorporated in some way into the design to ensure the correct set up of the external optics.

4. Lens L1 must be fine-tunable in the x-y plane to allow for the translation of the focal point's force gradient as located in the microscope. This must be fine-tunable to allow for the precise manipulation of the beam within the microscope, so that the micrometre sized test specimen can have the trapping force applied, as required by the overall system. For translation in the x and y directions, the relative distance to the original point is not necessarily required as it is entirely dependent on the location of the test specimen and the application of a force under the microscope.

4.3.4 Internal microscope requirements

1. The laser beam directed into the microscope must be reflected into the objective with a dichroic mirror. This dichroic mirror must be able to reflect light above a certain wavelength that ensures the laser beam is reflected. Visible light must still be transmitted by the mirror, an example of this is if the mirror transmits all light with a wavelength below 800 nm but reflects all light above 800 nm. By only allowing the laser beam we want reflected, the intensity of the light is increased and interference due to all other light reflecting is not an issue, as it would be if a normal mirror was used.

2. The mirror itself must be mounted perfectly at a 45 degree angle to ensure the precise reflection of the beam through the specimen plane. This mirror is located within the fluorescence filter cube rotating turret of the Nikon Ti-U that has 6 mounting slots for filter cubes. Due to these very strict requirements the dichroic mirror should be mounted inside a filter cube specifically for the Nikon Ti-U turret. This mounting requirement ensures pinpoint precision due to the use of Nikon Ti-U microscope specific components.

3. The microscope objective required for the OT is a 100x oil objective lens that is compatible with the Nikon Ti-U microscope. It is the requirement of this objective to focus the laser into the specimen plane in a way that generates a focal point with the steepest possible force gradient. It is this generated force gradient that is used to trap the micrometre specimen under the microscope. Where the stiffness of the object can be found by analysing the force applied by the laser and the corresponding displacement of the object.

Chapter 5

Optical Tweezer Framework Design

5.1 Optics components List

All of the optics to be used for this project including the laser itself were purchased over a year ago based on Block's design [3] which is why the research in Chapter 3 was based on the same piece of literature. Below is the full list of equipment previously purchased and obtained by a previous academic for the purpose of creating an OT. This equipment was not selected or purchased by the author for this project specifically and rather the equipment was purchased first. Consequently this project is focused on the design and development of the framework for the OT and its layout, with reference to a generalised fully functioning and previously tested OT system.

5.1.1 Diode laser

The full laser data report for the laser obtained can be found in Appendix A, however some specifications of the laser will be included here.
A diode laser purchased from Civil Laser that has a wavelength of 1064 nm and a maximum power output of 62 mW. This laser was also purchased with a lab adjustable power supply which as discussed in Section 4.3 allows for the manipulation of the power output for experimental and alignment purposes. The beam diameter (1/e2) of the laser is 1.5mm and it is a continuous wave (CW), collimated straight laser beam.

5.1.2 Beam expander

The beam expander available for this project is a 5x collimated lens for YAG (Yttrium aluminium garnet)/diode lasers with a wavelength of 1064 nm. This beam expander is manufactured using lenses specifically for use with beams of 1064 nm wavelength in order to reduce image distortion or spherical aberration when using light of that wavelength.

5.1.3 Plano-convex lenses

Based on the OT layout design from Block in [3], two plano-convex lenses had been previously purchased for this device. These lenses were purchased from Stock Optics [16] and are made from N-BK7 SCHOTT glass, with a focal length of 50 mm, a centre thickness of 2.61 mm and a diameter of 15 mm. The for SCHOTT N-BK7 lenses over 99% of light with a wavelength of 1064 nm is transmitted through the glass [16].

5.1.4 Dichroic mirror

The dichroic mirror was purchased from Lighting Images Technology and is 150 mm in diameter and 3 mm thick, made of Borofloat material. The purchased parts list as given from the previous academic who bought them says that the mirror reflects light with a wavelength above 800 nm and transmits light with a wavelength less than 800 nm as shown in Appendix B. However, no materials specifications can be found for this mirror stating the qualities of the glass and what wavelengths are transmitted. This dichroic mirror is not compatible with a microscope filter cube and is made for other uses such as in cinema. The inability to use this mirror in the OT and the requirement of a different mirror will be discussed further in Chapter 6.

5.1.5 Microscope objective

A Nikon 100x Oil E Plan Achromat Microscope Objective was previously purchased for mounting in the Nikon Ti-U microscope. This objective has a Numerical Aperture (NA) of 1.25 and a 25mm thread pattern to allow for mounting on Nikon Eclipse microscopes such as the one available at Macquarie University. This previously purchased piece of equipment for the microscopes meets the requirements as specified earlier in Section 4.3.

Macquarie University is already in possession of one of these objectives with the same specification but that specifically came with the Nikon Eclipse Ti-U microscope. This objective is already mounted in one of the rotating objective slots and is suggested that it can also be used in the operation and use of the OT device.

5.2 External framework design process

This section focuses on the actual design of the framework and the process that was taken to develop the design.Again the framework was split into two separate sections to allow for a better understanding of the design process and thought process behind each decision. These sections are the lens telescope subsystem and the laser initialisation subsystem involving the laser and beam expander. This designing of the framework involved extensive Computer-Aided Design (CAD) work for every possible design or design alternative as discussed below.
These alternate designs looked into frameworks that would be cheap and cost effective

while not losing too much efficiency or accuracy so the OT is still able to function correctly, this is due to the limited budget for this project. Designing the OT was an iterative process that was occurring in direct relation to the research and initial set up components of this project. For this reason many design ideas began to form and be modeled in CAD before fully understanding the requirements of the system, causing the design to either change entirely or be adapted and improved to meet the requirements. This process allowed for lots of design ideas to be created which helped to further improve understanding and efficiency of the design process and designing using CAD modeling.

In order for the final design to form and take shape the requirements had to be completely set and the operation of the OT entirely understood. However, it was designing alternatives throughout the lifetime of the project that led to improved knowledge and skills which in turn lead to an improved final design. Throughout this section the decisions made and the processes taken will be discussed and justified, based on the extensive research on the construction of an OT in Chapter 3 and the set system requirements found in Chapter 4.

5.2.1 CAD modeling of design

CAD is a very large factor of the design process, especially in this instance when the framework for a biomedical device must be designed and produced with precision to ensure that all the optics components in the device are perfectly aligned. At each stage of development all aspects of the design were modeled in CAD to ensure the components were compatible and the system requirements were being met at each stage and for each subsystem and individual component. The CAD models themselves that were generated for each component can be found below in their relevant sections of this report.

The CAD process varied quite drastically for this project as what was decided on and required for each component required very different approaches. This can be seen in the design analysis when purchased optical mounts for some components of the framework required the CAD models already generated to be downloaded and adjusted in order to simulate the alignment of optics and ensure the system would work. In contrast, other sections of the framework had to be modeled using CAD from scratch to be 3D printed and had to be designed to be compatible with the purchased components.

This process led to extensive time being allocated to reviewing designs and altering the entire framework frequently to ensure the best possible framework could be made with the available resources and funding. Some of the design alternatives and revisions are included in their unique sections to provide some insight into the process of how the final design formed and also to demonstrate the process required to design a similarly unique framework for an optical tweezer.

5.3 Lens telescope framework design

There were many different approaches that were taken to develop the framework of the lens telescope. Due to the system requirements being set as shown in Chapter 4 the design was able to evolve through the use of CAD modeling and became distinguished from the other design alternatives that will be discussed briefly. This process of designing the ideal framework for this specific subsystem of the lens telescope is achieved through the understanding of the OT device's operation due to the extensive research and initial set up, that allowed for the specific system requirements to be set and understood at a component level.
The lens telescope framework was identified as the subsystem that needed to be designed first as the set up and positioning of the lens telescope would change the framework that would be required for the laser and beam expander. There were many alternate designs for the subsystem that were modeled in CAD and assessed to see if they would meet the requirements. Of these alternatives, two have been included and discussed below to allow for the understanding of why some frameworks would not work and the process involved to create the final design for the framework of the lens telescope.

5.3.1 Design Alternative A

Design alternative A was a very early design idea that was very quickly decided against due to its inaccuracy and inability to meet the system requirements as the understanding of the project developed. This design came to fruition as it was initially advised and suggested to design and manufacture the entire framework using the existing 3D printer at Macquarie University to heavily save on costs and remain within the budget. This idea was very quickly understood to be unfeasible as the accuracy that could be achieved with this method of design would not be adequate for the mounting of the plano-convex lenses. This was because, in order for the lens telescope to provide some beam steering through translation of Lens L1, 3D printing could not be used as the printing accuracy is too poor to allow for fine translation. Any designed slot or gear mechanism to be 3D printed would only be able to translate fixed distances greater than 1 mm when the lens would require free translation in less than 0.5 mm increments at the maximum.

In order to consider this option and heavily save on costing, the construction of an OT that was not capable of being steered was considered. This would mean that the trapping force being generated by the laser would be fixed and could not be manipulated to properly focus next to the test specimen. Even if designing the device in this way was applicable, the 3D printed components would have to be manufactured with precision and accuracy to ensure the perfect alignment of all optics as they can not be moved if required. This level of accuracy is not achievable through this prototyping method with the printer available and therefore, the use of 3D printing for the lens telescope framework is not possible.

Figure 5.1: Lens telescope framework for design alternative B

5.3.2 Design Alternative B

Another design alternative also focused on reducing expenditure on the lens telescope framework by having an OT device that is not steerable, as considered for design alternative A. This design alternative looked to use purchased optical posts and mounts to ensure the accuracy of the framework was as required, thus allowing for the correct alignment of the lens telescope. Although the purchasing of optical mounts is required, the costs associated with it are well within the set budget of approximately $300, due to not having to purchase components that allow the telescope to steer the beam.

The fixed optical mounts used in this design alternative can be seen in Figure 5.1 which shows two optical posts [30] of 30 mm height and 12.7 mm diameter, with two lens mounts [20] mounted on top. The lens mounts are for 15mm optics such as the two plano-convex lenses available for this project and have an optical centre height of 17.8 mm. Extensive analysis of the system led to the understanding that an independent breadboard to mount the optics on is ideal for set up and alignment purposes, as discussed in full in Section 5.7. Consequently, the design for the lens telscope uses two short optical posts mounted on a raised breadboard (see Figure5.2).

This design of the lens telescope being mounted on an optical breadboard [21] can be afforded within the budget funding due to saving money by not accommodating for translation of lens L1. To allow for this framework to be used the optical breadboard is mounted on top of a variable height, translating post [18] that is secured to the optical table with a clamping fork [22] as can be seen in Figure 5.2. As labeled Figure 5.2 shows the entire layout for setting up the lens telescope for this design, the green line represents the laser beam and the alignment of the lens telescope, while the two blue objects are the laser and the beam expander showing their required location and layout.

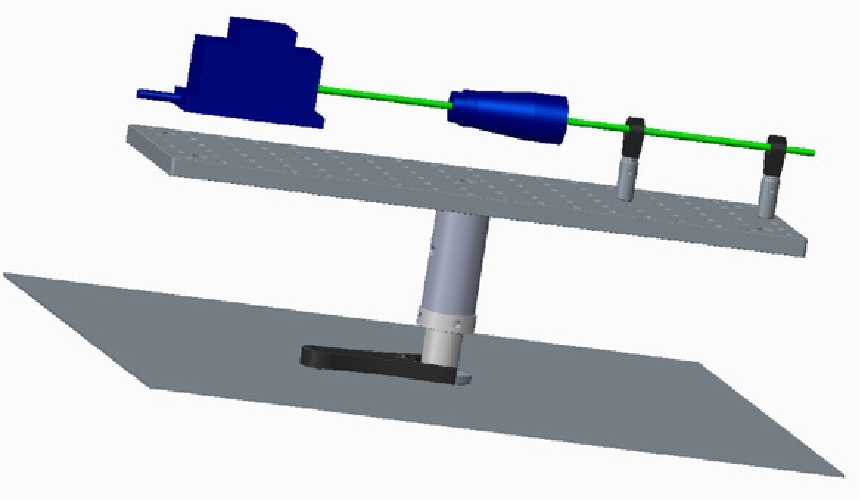

Figure 5.2: System set up and layout for telescope design alternative B

Layout and mounting specifications for Lens Telescope Alternate Design B

The translating post itself has a variable height between 108mm and 146mm, and the breadboard is 12.7 mm thick. This means that the centre of the lenses, L1 and L2, of the lens telescope can be adjusted to a height of anywhere between 168.5mm and 206.5 mm. This was designed to allow for the lenses to be fixed at 188.5 mm off the optical table to ensure they are in a direct line between the laser and centre of the microscope's input.

The mounting of this telescope design is very straightforward as during the design process, when looking for parts to purchase and use from Thorlabs, it is ensured that all components are compatible and can be mounted together. An M4 setscrew [28] is included and located at the top of the post, which is directly screwed into the M4 tapped mounting hole in the base to secure the lenses to the posts. The base of the optical post has an M6 tapped mounting hole which, using M6 setscrews [29], can be screwed into the M6 threaded holes on the optical breadboard. The threaded holes on the breadboard are spaced 25 mm from each other's centre. This means that if translation is not required, the posts can be screwed directly into the breadboard at 100 mm apart, as per the required distance if fixed as discussed in Chapter 3 and Section 4.3. The variable height post is also fixed to the breadboard using an M6 setscrew, where the post is mounted to the optical table using the clamping fork which is secured with an M6 screw [27].

This setup would allow for the generation of an optical trap within the microscope that can be used for testing and the framework is within the budget dedicated for this project. However, after extensive research and many discussions with other academics at Macquarie University, it was deemed that an optical trap that could not be steered using a lens telescope was not ideal or viable. This meant that the design for the lens telescope framework had to be adapted to allow for the translation of L1 in all three dimensions to ensure the OT produced a steerable optical trap to allow for more accurate experimental results and an overall fully functioning OT device [3].

5.4 Lens telescope final design

Since it was established that design alternative B was not applicable due to its lack of being able to steer the trap, more expenditure had to be used on the framework for the lens telescope and budgeting cuts had to be made in other sections of the framework as discussed further in Section 5.5 and 5.7. As per system and subsystem specific requirements as outlined and discussed in Section 4.3, the fine tuning of Lens L1 is vital in the steering and manipulation of the laser beam and therefore, the optical trap itself. As a logical result of this, the initial stages of designing the final layout and framework of the telescope subsystem were focused on providing the required translation and using the best equipment to ensure the specific requirements for translation in each dimension were met. For the sake of the understanding and repeatability of this device the subsystem is split into sections below with reference to the dimension in which translation is achieved and how this is achieved.

5.4.1 Axial (z) axis translation of lens L1

Allowing for the axial translation of lens L1 is vital as this changes the distance from the specimen plane to where the focal point of the laser beam is located, thus changing where the optical trapping force is being applied relative to the specimen plane. There are many different ways that this translation can be provided using various optical mounts and pieces of equipment. The requirements for the translation of L1 in the z direction, along the optical path, are as was stated previously in Section 4.3.1. One of these conditions of translation was for fine-tuning and detailed accuracy. The fine-tuning in this direction could be done using a range of mounts and translation stages such as the translation stage from Thorlabs [19] which provides smooth translation of 0.35mm per full revolution of the precision leadscrew. Although this stage has superb accuracy in translation, it does not allow for the easy, reliable measurement of the distance between the two lenses L1 and L2 making up the lens telescope.

The detailed and in-depth research into the construction of OT devices and how they work as shown in Chapter 3, allowed for the knowledge to determine the ideal way to accommodate the axial translation requirements of L1. The ideal method for translation in this direction was found to be using a Dovetail optical rail from Thorlabs [26] with two rail carriers [25] to mount the rest of the framework supporting the lenses. The optical rail is laser engraved for precision accuracy with graduation every mm and a distance

Figure 5.3: Optical rail with rail carriers mounted for axial translation

reading every 10 mm. The optical rail with two rail carriers for mounting fixed at 100mm apart can be seen in Figure 5.3.
This mounting method is ideal for the axial translation of lens L1 relative to L2 as it shows a precise distance measurement between the two which can be adjusted and noted in experimental and alignment procedures. By having a straight-forward and precise method of measuring the distance between the lenses, set-up and calibration of the device is made dramatically easier and more accurate. Accuracy of the distance between lenses is not lost when making minor adjustments as the carriers are specifically designed for use with the rail and the locking thumbscrew provides wobble-free translation due to the spring-loaded plunger built into it [25].

Using the optical rail in this way also allows for the recording of the distance between the lenses that causes the focal point of the laser and the force gradient to be located directly on the specimen plane, next to the micrometre test object. This is important as the measured distance between the lenses is directly related to the distance the focal point is from the objective. By knowing and recording this distance between lenses, the required distance that the specimen plane should be from the objective can be known and set prior to calibration, dramatically simplifying the process. This allows for much more accurate repeatability of experiments and therefore more viable and accurate test results.

5.4.2 x axis translation of lens L1

As stated and discussed earlier in the OT research, chapter 3 and the system requirements, Section 4.3.1, the x translation of lens L1 in the telescope is to steer the optical trap through the specimen plane so that it is located next to the test specimen and therefore applying the trapping force to the object. This translation is not relative to the distance from the specimen plane to the microscope objective, unlike the axial translation above. This means that the required positioning of L1 in the x direction will be different and independent for each experiment as it is relative to the exact location of the microscopic specimen that is being trapped on the microscope viewing slide. With this knowledge the ideal component focuses on allowing for fine adjustments to be maintained while having the best possible accuracy and smooth translation.

Figure 5.4: Dovetail translation stage, for x axis translation [19]

The previously mentioned Dovetail translation stage [19] meets these specific requirements perfectly. As discussed, the translation in the x direction is completely independent for each experiment and the exact displacement of lens L1 is not required in terms of distance as it is used to position the optical trap next to the specimen under the microscope through the camera feed displayed on the computer. The translation stage can be seen in Figure 5.4 and has a travel range of 12.7mm and one complete revolution of the precision leadscrew shown equates to 0.35mm of smooth, stable travel. This component is also equipped with a secure locking mechanism using a setscrew located on the side to ensure no unexpected or unplanned movements occur once the correct translation is achieved and set.

5.4.3 y axis translation of lens L1

The x and y direction translation is in the same plane, this means that translation in either direction is done for the same effect as discussed earlier in Chapter 3 in regards to Block's findings in [3]. This means that like x axis translation, translation in the y direction also is used solely to allow for the same translation reflected through the specimen plane. As any translation in the y direction causes direct movement of the optical trap in the relative direction in the specimen plane, precise and accurate translation is required, without the need for a measure of the displacement of L1 relative to its initial position.

As x and y translation therefore have identical requirements, ideally a vertically mounted translation stage identical to the one used for x translation could be used. However, there are some issues associated with mounting this translation stage vertically in this specific design. One issue with the use of this stage is that when mounted vertically it can only support up to 0.25 kgs of load, while the lens mount and optical post combined produce approximately 0.25kg of load. The load applied to the stage being relatively equal to the

Figure 5.5: Thorlabs translating post holder [24]

absolute maximum load that can be supported by the stage, could potentially cause the part to fail or the travel locking mechanism not to work as required, and therefore cause unwanted and potentially hazardous displacement of the lens.
Another major reason why this stage was not used in this instance for translation in the y direction is because the stage is rather expensive and in order to mount it vertically additional mounting plates are required and would further increase the costing associated with this part.

Instead of using a vertically mounted translation stage, Thorlabs offers a translating post holder [24] that provides non-rotational translation of the holder itself of up to 16.5 mm, this can be seen in Figure 5.5. This post holder provides lockable, non-rotating height adjustment that can be used single handedly. A thumbscrew with a spring-loaded plunger provides holding force while height adjustments are made and a square relief cut within the bore of the internal post offers a stable two-line contact [24]. A knurled adjustment ring generates the non-rotating height adjustment as required, which allows for 0.635 mm translational adjustment per complete revolution of the adjustment ring. Once the desired height positioning has been set, the entire mechanism can be locked by tightening the lower thumbscrew securely and then doing the same for the locking hex socket on the upper thumbscrew.

5.4.4 Fixed mounting of lens L2

As defined in the system requirements in Section 4.3.1 the plano-convex lens L2 makes up the rear of the lens telescope, reflects the light being received from L1 and generates the light being output from the lens telescope. This lens is to be fixed at a height equal to that of the microscope's epi-fluorescence input port which as defined earlier is at a height 188.5mm above the optical table. In order to achieve this a simple optical post and post holder set-up is required and used. This will allow the centre of the lens to be fixed to a height of 188.5 mm and in line with the centre of the microscope's input, where it can then be locked in position and left so that no additional adjustments or alignment of that

Figure 5.6: Thorlabs lens mount for 15mm diameter optics [20]

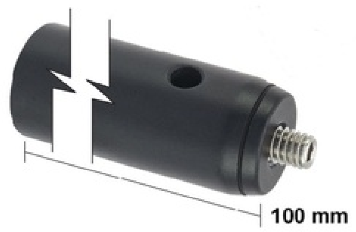

Figure 5.7: Thorlabs 100mm low reflectivity post [31]

Figure 5.8: Thorlabs 100mm post holder [23]

individual lens is required. When looking at the framework for Lens L2 it simply requires the lens mount as previously discussed in alternate design B for the plano-convex lenses (Figure 5.6) [20], a low-reflectivity aluminium post of height 100 mm (Figure 5.7) [31] and a post holder 100 mm in height (Figure 5.8) [23], that allows for the purchased post to be fixed during alignment to ensure L2 is at the correct height.

5.4.5 Combined lens telescope framework

This section looks at the combination and final design of the overall framework for the lens telescope and how it is set up and constructed. The assembled framework as purchased from Thorlabs can be seen as modeled in CAD in Figure 5.9. When designing a framework from parts to be purchased as these were, it is vital that all components are checked thoroughly and modeled in CAD to ensure compatibility with one another to allow for the ease of setting the system up and mounting each component together. A product list

Figure 5.9: CAD model of assembled lens telescope system design

and the cost of each component in the lens telescope framework can be seen in Appendix C.

Assembly of the lens telescope framework

For this framework for the lens telescope, the procedure for assembly is extremely simple due to the extreme caution and careful CAD modeling of each component to ensure compatibility. The process for assembly is listed below.

1. One of the purchased M6 cap screws is placed through the base of each rail carrier from below.
2. The two post holders are secured tightly onto the rail carrier by screwing a screw into the M6 threaded mounting holes in the base of each holder.
3. Then one of the lens mounts is taken and screwed onto an optical post with a M4 setscrew already locked into the top via the threaded mounting hole in its base.

This makes up the entire assembly for lens L2, so the post with the lens mount attached can be placed into the fixed height post holder and secured.

4. The translation stage is then secured to the remaining optical post using the M4 setscrew in its top via the M4 threaded mounting hole in the stage's base.

5. An additional M4 setscrew is now required and is screwed into the base of the lens mount via the threaded mounting hole in its base.

6. The lens mount with the setscrew inserted can now be locked down onto the translation stage by screwing it into the M4 threaded mounting hole on the top of the stage until it locks in place and the lens mount front and rear faces are parallel to the translation stage's sides. This step is important as it ensures the translation of the stage is parallel to the lens itself and thus ensures the translation exactly along the x axis as required.

7. This optical post with the translation stage and the lens mount secured can be placed into the adjustable height post holder and secured via the thumbscrew.

8. The optical carriers with the entire framework for both L1 and L2 can now be placed on the optical rail and locked in place a distance of 100mm apart, with the holder for L2 closest to the microscope input.

9. The leading post (fixed post holder) is then rotated within the holder to ensure that the lens holders are facing the same way. This ensures that, when the lenses are mounted, the distance between the two lenses (from inside the mount) is equal to 100 mm.

10. The lenses can now be secured inside the lens mounts ensuring that the flat faces of each lens is facing inwards of the lens telescope (in towards the flat surface of the other lens) and the assembly process is now finished.

That is the entire assembly process for the lens telescope system that makes up the majority of the external framework for this OT and the most important part to be completed accurately and precisely. This process was used and followed to assemble the lens telescope framework for the device as can be seen fully assembled in Figure 5.10 and Figure 5.11.

5.5 Laser source framework design

The laser source framework refers to the framework that supports the Beam expander, 1064nm diode laser and any other components such as a laser pointer, as will be discussed in this section below. Due to the costs associated with constructing a fully functioning and steerable lens telescope with high precision and accuracy there is not enough money in the budget left to design the laser source framework from purchased optical mounts.

Figure 5.10: Lens telescope mount (front-view)

Figure 5.11: Lens telescope mount (view from L2 to L1)

However, as analysed and understood from the OT research shown in Chapter 3 and then converted into system requirements in Section 4.3.1, the framework does not need to allow for any translation or purchased optical mounts. This framework design is discussed and shown to only require accuracy in height dimensions in order for the OT device to work at and achieve full functionality.

With this knowledge the framework for the rest of the external components could be designed from scratch using CAD modeling and then 3D printed with an accuracy greater than that required for the subsystem. This framework can be split into three separate components and these are: the IR diode laser, beam expander and the laser pointer frameworks which are outlined and discussed below. A 3D printed replacement for a breadboard was also incorporated into the design as discussed later in Section 5.7 and this is 20mm thick which is taken into consideration for all of the framework components for this section in order to maintain the alignment of all components.
This entire section of the framework does not require any complex calculations for the design as until the laser beam enters the lens telescope it is simply a fixed, horizontal beam that only requires a height alignment for the rest of the OT framework to work and generate a fully functioning OT as shown throughout Block's literature as analysed and discussed in Chapter 3.

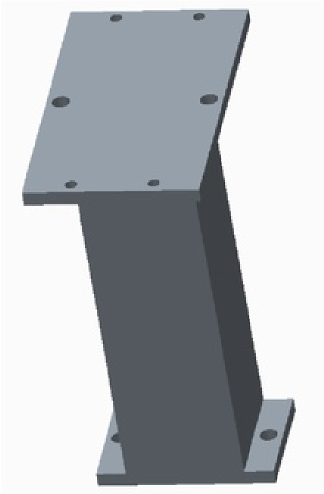

Figure 5.12: 3D printed frame that the laser is secured to

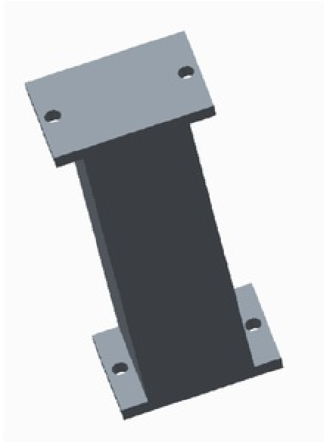

Figure 5.13: 3D printed support post for the laser

5.5.1 IR Laser framework

This section looks at the framework designed specifically for the IR diode laser with a 1064 nm wavelength as previously obtained and listed in Section 5.1. The laser available for this project outputs the beam 25 mm above its base. Therefore, the framework was designed to ensure that the fixed laser would be directed through the centre of the microscope's epi-fluorescent input at a height 188.5 mm above the horizontal optical table. Figure 5.12 shows the framework for the IR laser as a whole, which includes two components that are separately printed due to size constraints on the available printer. These components are a base plate and a supporting post. The laser base plate is 5mm thick and the laser is bolted onto it and secured. This base plate is then bolted to the supporting post which has a total height of 138.5mm. The laser support post on its own is shown in Figure 5.13.

5.5.2 Beam expander Framework

The beam expander framework is similarly designed to the laser framework for simplicity, while allowing for the fixed height of the beam expander to be accurate and in alignment with the rest of the OT external optics. The printed support post is almost identical to the post used for the laser, however the only difference is that the the upper flat surface that the beam expander case is bolted onto is 70 mm in width instead of 60 mm, this can be seen in Figure5.14.

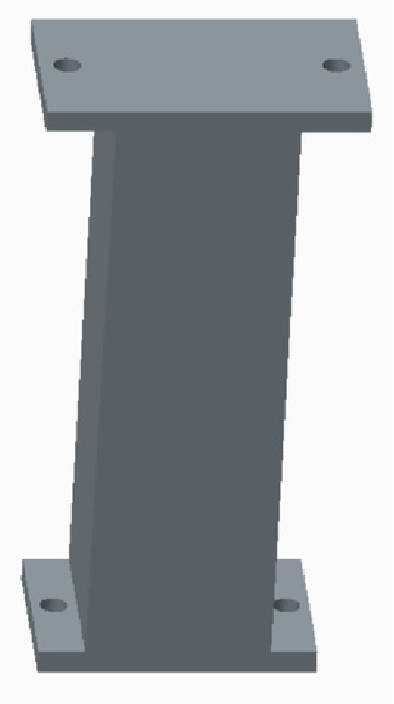

Figure 5.14: CAD model of the support post for the beam expander framework

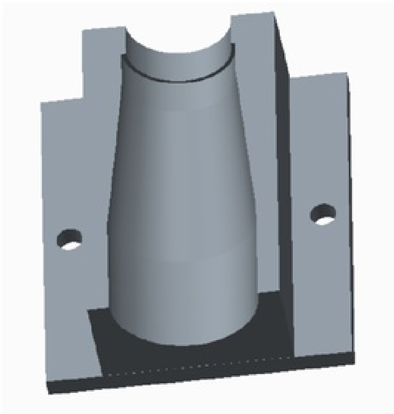

Figure 5.15: CAD model of the frame to secure the beam expander

The beam expander support case is more complicated and is the most important component out of the entire 3D printed framework. This is because the case component has to perfectly hold the beam expander so that it is horizontal and does not cause beam distortion in anyway due to inaccuracy. Designing the beam expander was made much easier due to planning ahead and the prior CAD modeling of all components that make up the OT device. By already using CAD modeling extensively to generate the device layout and then from there the framework for the lens telescope, all the components were already modeled in CAD. Therefore, for the generation of the beam expanders support case a block could be modeled and then using a removal process the exact model of the beam expander itself was removed, this leaves a perfect slot for the beam expander to be securely positioned as can be seen in Figure 5.15.

Since the design itself in CAD is ideal for the support of the beam expander, when

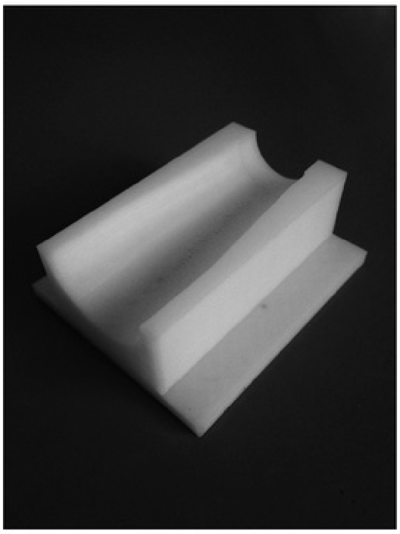

Figure 5.16: 3D printed frame to secure the beam expander

Figure 5.17: Beam expander secured within the 3D printed frame

printing the 3D part the greatest possible accuracy was used to ensure the printed component would be as required and support the beam expander horizontally and securely. This necessity led to the printing of the component set to a resolution of greater than 90 microns and an x-y axis precision position of 1.5 microns, on a 'light print' setting as printed on the Zortax M200 printer that was accessed independently through employment connections for no additional cost. This printed beam expander support case was printed perfectly shown in Figure 5.16 and was able to correctly support the beam expander, being flush to all surfaces as can be seen in Figure 5.17.

5.5.3 Laser pointer framework

The importance of the laser pointer and its use, as well as its specific properties are discussed in detail in Section 5.7, in this component the design of the framework for the laser pointer is discussed. The laser pointer used in this design is a keyring laser pointer from Jaycar Electronics [11] which is 70 mm in length and has a diameter of 13 mm. The framework was designed specifically for this laser pointer by having a 40 mm long block with a 13mm diameter semicircle cut out along its length. This allows for the centre of the laser pointer to be securely held both horizontally and flush while allowing for easy removal and placement of the laser pointer at any time for alignment purposes, as discussed in Section 5.6 and 5.7.

The separate support post that is bolted to the laser pointers support frame is identical

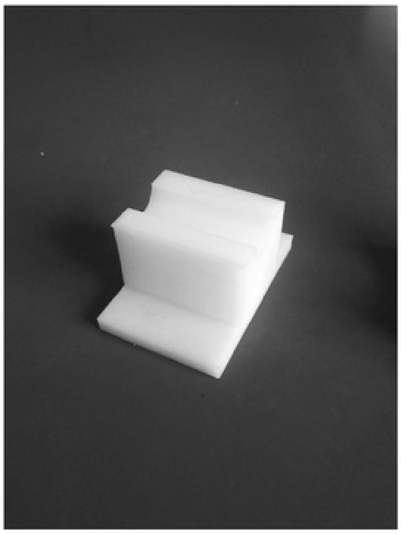

Figure 5.18: 3D printed frame to hold the laser pointer

Figure 5.19: Laer pointer secured within the 3D printed frame

to the laser support post as shown earlier in Figure 5.13, this ensures that the beam from the laser pointer and the beam from the IR laser are in perfect alignment as the 3D printed posts ensuring the correct height are identical. The 3D printed part for the laser pointer was also printed using the Zortax M200 printer on the same settings as shown in Figure 5.18, to ensure the accuracy of the cylindrical support frame supported the laser pointer horizontall for accurate alignment as can be seen in Figure 5.19. The overall framework for the support of the laser pointer can be seen in Figure 5.20.

5.6 Design for Safety

Due to the use of a class 4 diode laser operating at a wavelength of 1064nm, safety must be a major consideration when designing the OT and it's framework as shown in Section 4.2. Because of this the design must include a variety of different safety features to ensure the laser is never exposed to open air when being used and that it can be properly contained as well as no unwanted reflections and other potential hazards occurring. The safety needs and policies at Macquarie University that must be followed and met by this optical tweezer (OT) device have been extensively discussed in Section 4.2 and then adapted and incorporated into the system requirements in Section 4.3.1.

Although it has been mentioned in each stage of the design process for individual component and subsystem throughout this document, this section draws attention to the aspects of design specifically tailored to safety when operating and using the OT device. There

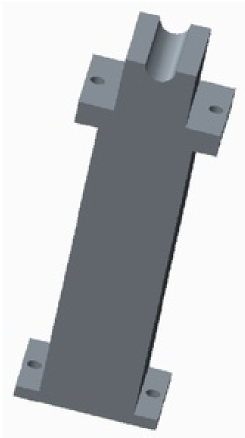

Figure 5.20: CAD model of the entire framework for the laser pointer

are some main areas that required special attention in regards to safety and these are single horizontal plane configuration of laser beams, minimising reflective objects, correct mounting of beam steering optics and having a completely enclosed laser beam as required from Section 4.3.1.

5.6.1 Horizontal single plane layout

The way this OT device has been designed in regards to each individual component and subsystem is very specific in order to maintain the laser beam in a single horizontal plane (albeit having very minor steering using lenses, not mirrors). The incorporation of this layout into the design is vital as has been portrayed throughout this document in each section of the design as shown in Chapter 5, as set in the system requirements in Section 4.3, as determined through the University's Safety policy and good procedures policy as analysed in 4.2.

The fixed mounting of the laser and beam expander at the required location in order to enter the centre of the microscopes input path was a crucial variation to all other designs, allowing for the suitability for operation of the OT within university laboratories. This is because once the laser and beam expander are fixed there is not only no potential for misalignment or unintentional misdirection of the laser itself, but the other optics components can then be adjusted to meet the fixed beam perfectly, thus allowing for the operation of the class 4 laser in a single plane.

A periscope design being used would have the laser mounted directly onto the optical board and then later projected vertically onto a mirror which steers the beam into the microscope port. This vertical projection of the beam means that any minor misalignment of the mirrors, or any unintentional movement to the mirrors either by the operator or due to a faulty mount, could cause IR beam that is invisible to the naked eye to be projected towards the operator or another individual which would cause permanent damage or complete blinding of the eye. It is for these extremely dangerous risks that this design was not deemed viable for operation in a university laboratory and therefore the alternative was made.

5.6.2 Minimise reflective surfaces

To minimise the possibility of unwanted beam reflection or partial reflection, all unfixed (adjustable) optical mounts and objects must have a low-reflectivity coating. This coating is standard on the lens mounts [20] and the translation stage [19]. This is crucial due to the fact that they are on adjustable mounts. Therefore, if an operator error occurred putting either component in the path of the laser beam, the entire IR beam will not be reflected potentially into their eyes or another individuals eyes.

The standard optical posts from Thorlabs are made from stainless steel and have no protective coating on them to reduce reflectivity. As the posts are rounded, any laser beam contact could reflect in any direction and since it is an IR laser this beam would not be visible. Therefore, an unaware individual could have the beam directed into their eye, causing very serious and permanent damage. It is because of this severe risk and danger that extra funding was used to purchase optical posts made of aluminium that has a special low-reflectivity coat on them [31], reducing the risk and avoiding such a situation.

5.6.3 Beam steering mounts

To reduce any possible accidental reflection or refraction of the laser beam, all optics associated with any sort of beam steering must be secured and mounted using the correct optical mounts for that component. Aside from the need for accuracy, translation and stability, safety is another crucial reason why the lens telescope is mounted using the purchased optical mounts from Thorlabs. If a similarly adjustable framework was designed using independently modeled printed parts or another form of prototyping or manufacturing, there would be severe risks. Not only would the inaccuracies of the parts themselves cause unnecessary risks of beam misdirection, but the instability and inaccuracy due to translation would cause further risks too.
It is for these reasons that the 15 mm diameter lenses are firmly secured inside lens mounts specifically designed for 15 mm optics and secured by a retaining ring to keep the lens fixed and parallel to the mount at all times. All other components that make up the adjustable framework for the lens telescope are also specifically designed for securely and accurately mounting optics to ensure precision, as discussed throughout this document.

5.6.4 Contained laser beam

Although all the previous steps have been taken to minimise or remove all the potential foreseen risks it is still best advised that an enclosure that contains the entire external framework within it is incorporated and used in case of any unforeseen risks. This enclosure must be bolted down to either the optical table or the base of the external framework to ensure no stray beams can escape the enclosure. The laser used in this project operates at a fairly low maximum power output of 62 mW and can be reduced using the lab adjustable power supply that it uses (see Section 5.1.1).
While the enclosure is recommended and used in this design, it could be omitted due to all the previous precautions in the design to minimise all possible risks. However, if a more powerful laser was used and in located in a laboratory not specifically meant for the operation of such lasers, then the complete enclosure of the entire laser beam wherever possible is imperative.

5.7 Design for set-up and usability

This section looks at how the framework is designed to accommodate for ease of use and set-up in regards to the storage of the OT, the alignment and the experimental testing of the device. The main component of the framework that allows for ease of use and set-up in general is the inclusion of a base that the entire framework is bolted onto. Other aspects of the design that were incorporated to increase the ease of use and operation as discussed in detail below are, the use of the optical rail for the lens telescope and the incorporation of a laser pointer in the design.

5.7.1 Base for entire framework

This base ideally would be an optical breadboard, as discussed below in Section 5.9, however, due to insufficient budget funds this component was CAD modeled and tailored to the specific layout of the rest of the framework, to be 3D printed and used as a replacement part. The designed framework base can be seen in the CAD in Figure 5.21, as shown it is specifically designed to accommodate and allow for the mounting of the entire external framework for the OT. The entire framework can be independently bolted onto this base through the 10 mm deep centre section being cut out as can be seen in Figure 5.22, this ensures the framework is mounted to the base independently of the optical table. The entire base is then bolted down to the optical table using the fixture holes around the 20 mm thick outside of the base which are specifically spaced at multiples of 25 mm increments to ensure compatibility of the components. The overall dimensions for this component are 450mm x 85mm x 20mm (L x B x H).

The entire framework was designed and modeled in CAD base on the specific requirements of the OT for operation at Macquarie University with the existing set up (see

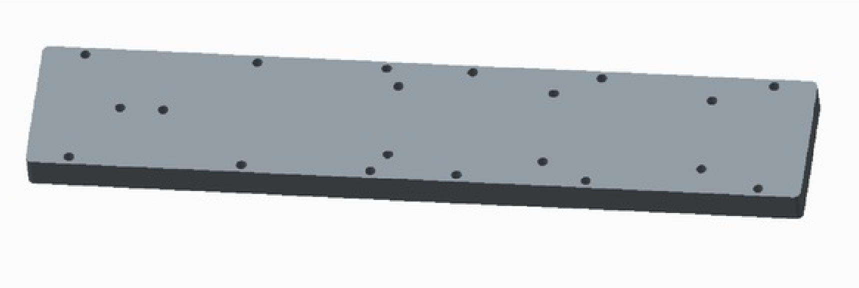

Figure 5.21: CAD model of the framework base (view from above)

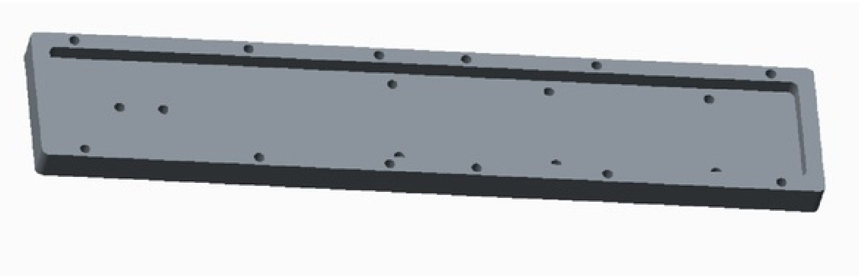

Figure 5.22: CAD model of the framework base (view from below

Section 4.3 for details). This means that the designed base incorporates the specific location required of each component of the framework into the design, to allow for ease of constructing the overall framework by bolting the components down through the holes included in the design. By doing this the direct optical path can be set and alignment in the axial direction of the framework itself guaranteed, thus simplifying the alignment process to be completed as only the fine tuning of the lens telescope components is required.

The workplace area and optical table that is used by this OT is very cluttered and is often used for many other experiments. This is another major benefit to using a framework base independent to the optical table as the entire external framework of the system can be removed from the optical table and stored elsewhere without having to make any adjustments to any of the framework itself. By using this framework, the entire alignment procedure of the external optics is not required between every experimental session as no adjustments are required. This means that a simple alignment check using the laser pointer as discussed below in Section 5.7.3 to ensure nothing has been tampered with or mistakenly adjusted, will suffice before commencing the experimental procedure.

5.7.2 Optical rail

The use of the optical rail for the base mount of the lens telescope subsystem provides enormous benefits in relation to setting up the device as well as completing any alignment or calibration processes. This is because each of the rail carriers can be removed and snapped back into place on the rail without having to make any adjustments to the entire supporting assembly for each of lenses in the telescope. By accommodating this the optical rail ensures that entire alignment process can be carried out for each lens as well as the entire telescope without having to make any adjustments that could potentially cause misalignment of the lenses.

5.7.3 Laser pointer

The laser pointer is included in this device purely for the sake of making the entire alignment process not only easier to do, but also safer as well. This refers to the use of the keyring laser pointer from Jaycar Electronics [11] that was already available for use at Macquarie University. This laser pointer is a class IIA laser product with a wavelength of 670 nm (visible/red) and a power output of less than 1 mW. As this laser pointer is a class IIA laser instead of a class 4 it can be used with significantly fewer safety restrictions.

By incorporating the laser pointer in the CAD modeling of the entire framework layout as discussed earlier in Section 5.5 the beam location of the laser pointer is on the exact same path as the beam that would be emitted by the IR laser. This is checked using a very simple calibration, which is done inside a laser laboratory, by ensuring that at the input of the microscope the two beams were in exact alignment. One very important step is to ensure that the IR laser is never switched on during alignment, while the laser pointer is in place or being used.

Due to the laser pointer being on the same beam path as the IR laser the laser pointer can be used as an alternate beam for the entire alignment process. Since only a class IIA laser product is now being used the alignment process can be done while located as required at the optical board in alignment with the input port, and without having to use the entire enclosure for the device. If the IR laser was used for the alignment process, the procedure would have to be carried out and completed away from the microscope itself in a laser laboratory as the room with the microscope has uncovered doors and windows and is not safe for the operation of an un-contained class 4 laser (see Section 4.2).

Note: some mild image distortion of the laser pointer may occur due the beam expander lenses being designed specifically for light of 1064 nm wavelength. However, the laser pointer beam is still transmitted through the beam expander and is still able to be used for alignment purposes.

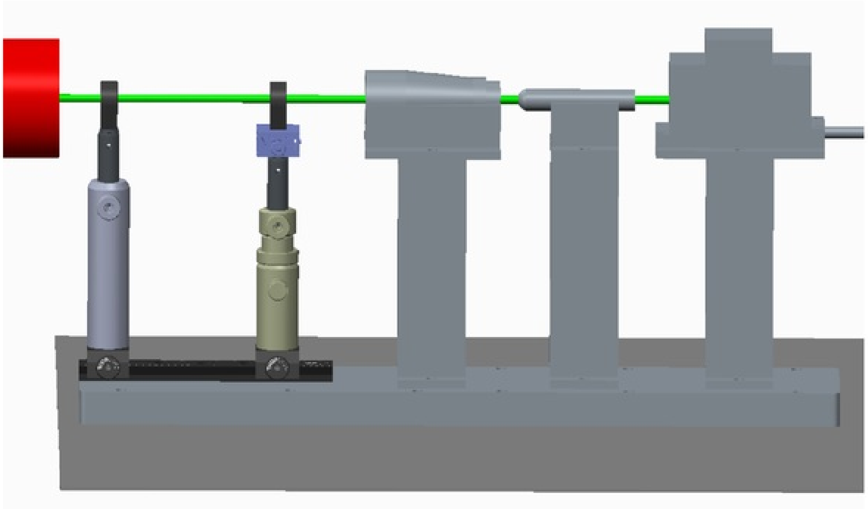

Figure 5.23: CAD model of the entire support framework for the external components of the designed OT

5.8 Complete framework design layout

The framework for each subsystem and individual component has already been discussed an analysed throughout this document, this section just corresponds to the entire external framework of the designed OT. This complete design can be seen in Figure 5.23 and is displayed with a green beam through the entire system, including the laser pointer, to show the system requirement of the beam location relative to the epi-fluorescence input as displayed at the end of the external framework, shown as red in colour.
Nikon was contacted in the hope of receiving a 3D CAD model to include in the final design model for this thesis document. However, it was explicitly stated that there is no possible way to share any form of CAD 3D model of the microscope due to confidentiality clauses.

The assembly of the framework has already been entirely covered in previous sections, in Section 5.4.5 and Section 5.5 for the assembly of the 3D printed framework. The assembly of the external framework to the base and then the fastening of the base to the optical table is included in Section 5.7.

5.9 Improvements on design

All improvements on the design are directly related to the lack of budget funding to allow for the purchasing of certain components as replacements to the 3D printed parts used. If the required funding was available for this project these design improvements would have been implemented and used in the OT framework designed and constructed in this thesis. The 3D printed components have been designed specifically to allow for the incorporation and replacement of parts such as the breadboard to ensure compatibility of the framework with future improvements made to this work as suggested.

The main design improvement that would be made with the allocation of funding would be the purchase and use of the optical breadboard as mentioned earlier and available at [21]. This improvement would ideally be made as such a large 3D printed component may experience warping and other structural damage over time that would severely impact the alignment and location of the laser and beam expander as well as the rest of the external framework. By using the Thorlabs breadboard which is directly compatible with the rest of the external framework it would guarantee lifetime accuracy and a perfectly precise level base that can be similarly removed and stored away from the OT whenever necessary.

To further this approach, with adequate funding all the 3D printed components of the external framework would be replaced for fixed optical mounts that are directly compatible with the obtained breadboard. This is not as vital, however, these 3D printed components (especially the large mounting posts) or also susceptible to warping and damaging over time that could also impact the alignment of the overall device. By replacing all the 3D printed components that were used due to a lack of funding, the precision and accuracy of the device will be increased, as well as the lifetime of the entire framework.

Chapter 6

Alignment calibration of external framework and optics

This chapter is focused on the alignment procedure as adapted and manipulated from Block's literature [3] to be suited for the specific OT device designed and discussed throughout this document. In this section the procedure itself and the results that were achieved by following the procedure will be portrayed and discussed. The importance of the alignment procedure and why the results gathered from this process verify the successful operation and validate the OT device through it successful framework. This is vital as discussed earlier in Section 5.1, due to the incorrect dichroic mirror being purchased and a lack of available funding to buy the correct mirror. An example of the correct dichroic mirror is available from Omega Optical as found at [15] which is approximately 1 mm thick and is a rectangle with dimensions 25.7mm x 36mm which can be purchased for approximately USD$200. These dimensions for the mirror are as required for the specifications of the filter cubes compatible with the Nikon Ti-U microscope [14]. This filter cube as per its specifications, transmits over 80% of light with a wavelength of less that 650 nm and reflects over 90% of light between the wavelength range of 700-1200nm [14].

6.1 Alignment Procedure

The alignment procedure is used to ensure that all optics are aligned and with the correct dichroic mirror and access to microbeads for testing the force calibration for the device and further experimental research can be simply achieved. As the laser pointers beam was designed to be on the same laser path as the IR mirror this procedure is simplified immensely and adapted accordingly to allow for it to be done in the fixed position of where the device will be operated from. This means an IR detector card or filter is not required for the process. The steps are as follows:

1. Remove lenses L1 and L2 (the lens telescope) from the optical path. This is done by without making any adjustments to the rest of the framework, unscrewing the

thumbscrew locking the rail carrier to the rail, and removing the entire rail carrier and support framework for each lens.

2. Turn on the laser pointer and ensure the beam is located at the exact centre of the epi-fluorescence port input for the microscope, this is known to be 188.5 mm above the optical table. The fixed positioning of the laser ensures that alignment is maintained in the x direction relative to microscope input due to the incorporation of this in the entire framework design as discussed in Chapter 5 and the framework being bolted down to the optical table.

3. Insert lens L2 (only) and ensure the lens is positioned in a way that still allows for the beam to enter the optical input at its centre. This is done by taking the rail carrier with Lens L2 mounted on it and slotting it back onto the rail before fixing it at a distance between 5 and 20 mm from the start of the rail, this ensures there is enough distance available to mount L1 in the correct position later.
   Through the design method used in this project, and discussed throughout this document, this will be achieved by setting the centre of the lens to a height of 188.5 mm above the optical board and with the face of the lens mount with the Thorlabs engraving facing the input of the microscope, to ensure the flat face of the lens is facing towards laser. Any fine adjustments being required should be made before fixing the optical post to that height within the post mount.

4. Next Lens L1 is placed onto the optical rail, using the same method as for the rail carrier supporting L2, and set to a distance of 2f as discussed earlier in Section 4.3.1 where f is the focal length of the plano-convex lenses. This lens is then crudely adjusted by eye in a way that causes the beam from the laser pointer to hit L1 exactly in its centre. This will also mean that the beam will still be located in the centre of the microscopes input (the end of the external subsystem of the OT device).
   This crude alignment in regards to the adjustable positioning of Lens L1 in the x-y directions is completely acceptable and permitted. This is because fine tuning of this lens to steer the beam will be required for every individual experiment in order to search for the test specimen on the computer feed via the camera. This is because the locational requirements of the optical trap in the x-y direction will be different in regards to every experiment depending on the location of the micrometre sized objects location on the microscope slide.

5. If the previous steps were completed accordingly there will be a uniform disc of light located at the centre of the input to the epi-fluorescence port. If not, then some adjustments to the x-y location of lens L1 should be made until a uniform disc is achieved.

Figure 6.1: Complete device as used for the alignment procedure for the external framework

6.2 Alignment Results

The alignment procedure as listed above, was carried out at Macquarie university using the OT framework designed and constructed for this project as seen in Figure 5.23 earlier. This procedure was carried out using the test rig as shown in Figure 6.1, to ensure the device is aligned in regards to the optics of the external device as well as the location of the input for the epi-fluorescence, thus ensuring the required alignment of the internal optics as well.

The completion of this process confirmed that the entire OT device was accurately and acceptably aligned. This was found by running through the alignment procedure very carefully and ensuring that at all stages the beam generated from the laser point, as an accurate and aligned representation of the IR laser, was meeting the requirements exactly. This was shown by at all stages of the procedure the beam was displayed at the centre of the microscopes input as a uniform disc, located horizontally 188.5 mm above the optical table for the entire length of the laser path through the device.

6.3 Alignment procedure importance

The importance of this alignment procedure as discussed throughout this chapter can be seen through understanding what the output of the process represents and signifies to for the overall OT device. Having and experimentally demonstrating a perfectly aligned OT device in regards to the external optics and the input of the device to the microscope is imperative in proving the usefulness and validation of the OT device designed throughout this document. This can be stated as due to the optics components of the device being directly based off Block's literature of a fully operational and functioning OT device in [3]. By achieving alignment of the device, it can be considered that with the correct dichroic mirror available for use, the beam would be perfectly reflected up through the specimen plane, being immediately ready for the force calibration and further experimental testing. With this information and knowledge, the OT device and its framework that has been designed, constructed and aligned, can be justified as a validated OT device. This device, when using the correct dichroic mirror, it is a fully functioning optical tweezer that after completing force calibration, is ready to be used experimentally to research the effects of mechanotransduction on cell stiffness.

Chapter 7

Conclusions and Future Work

7.1 Conclusions

The goal of this project was to design and develop the framework of a Piconewton Force Transducer (PFT) device that will allow for the stiffness of micrometre sized particles to be tested under a microscope. The device that was designed and constructed for this project was an Optical Tweezer (OT), because the optics components were already available for use due to a previous academic purchasing them with the intention of completing this project.

Since this is a design project heavy emphasis throughout this thesis has been on the actual design process used and followed to allow for the actual design and construction of the required device. Background research was done on the effects of mechanotransduction on cells and other PFT devices to set up a base understanding for why the use of being able to use an OT to test cell stiffness is so important for biomedical advancements.

Research on the OT device itself was then extensively conducted to allow for a complete understanding of the operation of a similar OT device. This section focused on how the overall system, the subsystems, and each component function and the actual requirement of each aspect in regards to generating a fully functional OT device. Due to the in depth understanding of the device's operation from this research, a unique set of system requirements could be formed for the specific use of the OT device to be set up at Macquarie University with the Nikon Eclipse Ti-U microscope.

From these requirements the design as shown in this thesis, was able to be formed through the extensive design process to ensure each component and subsystem meets the specific requirements and is compatible with the rest of the device and its framework. This allowed for the construction and creation of the framework for the OT device to perfectly meet the requirements of the system and thus creating a functioning OT device. This functionality of the device was then validated through the alignment calibration process to ensure and experimentally prove that all requirements for the device have been met and a fully functioning OT device produced.

7.2 Future work

Aside from the incorporation of the improvements on the design as discussed in Section 5.9 all future work is associated with the calibration and experimental use of the developed OT device.

This future work refers to doing a force calibration of the OT to determine its available output trapping force which can be done once the correct dichroic mirror is purchased and mounted in one of the microscope filter cubes available. Once this calibration is completed and the force of the optical trap experimentally tested and verified, the device can be used to test the stress of different cells to gain further understanding into what causes these differences and what the effects could be. This would allow for further publications to be written in regards to the research done using the OT designed in this project.

Chapter 8

Abbreviations

CAD	Computer-Aided Design
IR	Infrared
mW	milliwatt
nm	nanometre
OT	Optical Tweezer
pN	piconewton
PFT	Piconewton Force Transducer
um	micrometre

Appendix A

Appendix A

CivilLaser

www.CivilLaser.com

HangZhou NaKu Technology Co., Ltd

激光器检测报告

Laser data report

序号(SN #)：#15101701F 日期 Date：2015.10.17

型号 Model No.	LSR1064NL-50	
阈值电流(A) Threshold Current	0.2	
工作电流(A) Current	0.35	
输出功率(mW) Output Power	62	
	水平方向 Horizontal	竖直方向 vertical
出口处光斑大小(mm) Beam size at aperture	1.96	
束腰位置(m) Beam waist position	0	
束腰光斑大小(mm) Beam waist size	1.96	
4 米处光斑大小(mm) Beam size at 4 meters	5.01	
14 米处光斑大小(mm) Beam size at 14 meters	12.73	
光束发散角（全角）(mRad) Divergence	0.772	
功率稳定度 Power stablility	5%	
调制频率 (Khz) Modulation frequency	Analogue	
电源 Power supply	LSR－PS－II	

电流功率

工作电流(A) Current	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.35
激光功率(mW) Output Power	0	1	12	19	28	40	48	62

Appendix B

Appendix B

Part	QTY	Spec	Brand	Price per unit	Total price	Postage	Total price	Link	Shipped from
ojective	1	1.25NA objective	Nikon - MRP71900	379	379	0	379	http://www.seoenterprises.com/shop/pages.php?pageid=9	Australia
dichroic mirror	3	reflects 800+ nm and is transparent to 800-nm light	Lighting images technology	60	180	70	250	http://www.ebay.com.au/itm/DICHROIC-HEAT-FILTER-HOT-MIRROR-/170278253333?hash=item27a55ff315	Riverside, California, U States
IR Filter	1		SCOTT glasses	14		35		http://www.ebay.com.au/itm/Schott-Glass-KG3-IR-Blocking-Filter-Heat-Absorbing-Filter-/131510265641?hash=item1e9e9f6729	Westerville, Ohio, Unite States
Plano convex lens	2	50mm focal length	STOCK OPTICS	33	66	19	85	http://www.ebay.com.au/itm/Plano-Convex-Spherical-Lenses-NBK7-15mm-Diameter-/121383540932?var=&hash=item1c4305d8c4	Wetherby, West Yorks United Kingdom
Beam expander	1			138	138	53	191	http://www.ebay.com.au/itm/1064nm-collimated-beam-expanders-4x-5x-6x-YAG-diode-laser-marker-welder-machines-/321093390482?hash=item4ac2a85092	Shenzhen, China
Laser diode	1		Civil Laser	667	667	18	667	http://www.civillaser.com/1064nm-50mw1500mw-ir-dpss-laser-invisible-laser-source-with-power-supply-p-99.html	China

Appendix C

Appendix C

Thorlabs	Part Number	Part Description	Cost (USD)	Quantity purchased	Total cost (USD)
	RLA150/M	Optical Rail	$41.20	1	$41.20
	RC1	Rail Carrier	$24.90	2	$49.80
	DT12/M	Translation Stage	$76.20	1	$76.20
	PH100/M	Post Holder	$9.17	1	$9.17
	PH3T/M	Translating Postholder	$57.70	1	$57.70
	TRA100/M	Optical post	$7.46	2	$14.92
	LMR15/M	Lens Mount	$21.00	2	$42.00
	SH6MS12	M6 Cap Screw (25 pack)	$7.75	1	$7.75
	SS6MS12	M6 Set Screw (25 pack)	$5.50	1	$5.50
	SS4MS12	M4 Set Screw (50 pack)	$5.50	1	$5.50
	W25S050	Waser (100 pack)	$4.50	1	$4.50
Total					$314.24

Bibliography

[1] R. S. Afzal and E. B. Treacy, "Optical tweezers using a diode laser," *Review of scientific instruments*, vol. 63, no. 4, pp. 2157–2163, 1992.

[2] M. Andersson, "Construction of force measuring optical tweezers instrumentation and investigations of biophysical properties of bacterial adhesion organelles," Ph.D. dissertation, Umea University, 2007.

[3] S. M. Block, "Construction of optical tweezers," *Cells: A laboratory Manual*, vol. 2, pp. 81–, 1998.

[4] S. Block. (2014) An introduction to optical tweezers. Online. Block Lab at Stanford University. [Online]. Available: https://blocklab.stanford.edu/optical_tweezers.html

[5] C. S. Chen, J. Tan, and J. Tien, "Mechanotransduction at cell-matrix and cell-cell contacts," *Annual Review of Biomedical Engineering*, vol. 6, pp. 275–302, 2004.

[6] J. E. Curtis and et al., "Dynamic holographic optical tweezers," *Optics Communications*, vol. 207, no. 1, pp. 169–175, 2002.

[7] E. R. Dufresne and D. G. Grier, "Optical tweezer arrays and optical substrates created with diffractive optics," *Review of scientific instruments*, vol. 69, no. 5, pp. 1974–1977, 1998.

[8] V. Heinrich and R. E. Waugh, "A piconewton force transducer and its application to measurement of the bending stiffness of phospholipid membranes," *Annals of Biomedical Engineering*, vol. 24, no. 5, pp. 595–605, 1996.

[9] D. E. Ingeber, "Cellular mechanotransduction: putting all the pieces together again," *The FASEB Journal*, vol. 20, no. 7, pp. 811–827, 2006.

[10] D. E. Jaalouk and J. Lammerding, "Mechanotransduction gone awry," *Nature reviews Molecular cell biology*, vol. 10, no. 1, pp. 63–73, 2009.

[11] Jaycar Electronics, "Keyring laser pointer." [Online]. Available: https://www.jaycar.com.au/keyring-laser-pointer/p/ST3102

[12] S. J. Koch and et al., "Micromachined piconewton force sensor for biophysics investigations," *Applied Physics Letters*, vol. 89, no. 17, p. 173901, Nov. 2006.

[13] Macquarie University: Faculty of Science, "Faculty of science work health & safety - lasers." [Online]. Available: http://web.science.mq.edu.au/intranet/ohs/lasers/index.htm

[14] Nikon, "Fluorescent filter cubes: product brochure." [Online]. Available: https://www.nikoninstruments.com/Products/Accessories/Fluorescent-Filter-Cubes/Literature

[15] Omega Optical, "675dcspxr dichroic mirror." [Online]. Available: http://www.omegafilters.com/products/filters/dichroic/675dcspxr.html

[16] Stock Optics, "Plano convex spherical lenses - nbk7 - 15mm diameter." [Online]. Available: http://www.ebay.com.au/itm/Plano-Convex-Spherical-Lenses-NBK7-15mm-Diameter-/121383540932?var=&hash=item1c4305d8c4

[17] S.-Y. Tee and et al., "Cell shape and substrate rigidity both regulate cell stiffness," *Biophysical Journal*, vol. 100, no. 5, pp. L25–L27, 2011.

[18] Thorlabs, "Blp01/m - variable height p-post." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=BLP01/M

[19] Thorlabs, "Dt12/m - 12.7 mm dovetail translation stage, m4 taps." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=DT12/M

[20] Thorlabs, "Lmr15/m - lens mount with retaining ring for 15 mm optics, m4 tap." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=LMR15/M

[21] Thorlabs, "Mb1545/m - aluminum breadboard, 150 mm x 450 mm x 12.7 mm, m6 taps." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=MB1545/M

[22] Thorlabs, "Pf175 - clamping fork for 1.5" pedestal post or post pedestal base adapter, universal." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=PF175

[23] ——, "Ph100/m - 12.7 mm post holder, spring-loaded hex-locking thumbscrew, l=100 mm." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=PH100/M#ad-image-0

[24] ——, "Ph3t/m - 12.7 mm translating post holder, min height l1=75.2 mm, max height l2=91.6 mm." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=PH3T/M

[25] ——, "Rc1 - dovetail rail carrier, 1.00" x 1.00" (25.4 mm x 25.4 mm)." [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=RC1

[26] Thorlabs, “Rla150/m - dovetail optical rail, 150 mm, metric.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=RLA150/M

[27] Thorlabs, “Sh6ms12 - m6 x 1.0 stainless steel cap screw, 12 mm long.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=SH6MS12

[28] Thorlabs, “Ss4ms12 - m4 x 0.7 stainless steel setscrew, 12 mm long.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=SS4MS12

[29] Thorlabs, “Ss6ms12 - m6 x 1.0 stainless steel setscrew, 12 mm long.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=SS6MS12

[30] Thorlabs, “Tr30/m - 12.7 mm optical post, ss, m4 setscrew, m6 tap, l = 30 mm.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=TR30/M

[31] ——, “Tra100/m - 12.7 mm aluminum post, m4 setscrew, m6 tap, l = 100 mm.” [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=TRA100/M

[32] H. Zhang and et al., “A tension-induced mechanotransduction pathway promotes epithelial morphogenesis,” *Nature*, vol. 471, no. 7336, pp. 99–103, 2011.

Consultation Meetings Attendance Form

Week	Date	Comments (if applicable)	Student's Signature	Supervisor's Signature
1	4/8/16		[illegible]	[illegible]
2	9/8/16		[illegible]	[illegible]
3	16/8/16.		[illegible]	[illegible]
4	24/8/16.	Regards to after hours access.	[illegible]	[illegible]
5	29/8/16.	prep for Progress Report + discuss microscope use.	[illegible]	[illegible]
6	5/9/16.		[illegible]	[illegible]
7	13/9/16.		[illegible]	[illegible]
8	5/10/16.		[illegible]	[illegible]
9	11/10/16.		[illegible]	[illegible]
10	17/10/16.		[illegible]	[illegible]
11	24/10/16.		[illegible]	[illegible]
12.	2/11/16		[illegible]	[illegible]