INVERTED FLUORESCENCE MICROSCOPE
FINAL DESIGN REPORT

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Statement of Disclaimer

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ABSTRACT

The Inverted Fluorescence Microscope senior project team at Cal Poly, San Luis Obispo designed, assembled, and tested a proof-of-concept inverted fluorescence microscope for the university's Microfabrication Laboratory. Administrators of the laboratory wished to use fluorescence for research and experiments involving cell growth and flow visualization on the micro-scale, and did not have the budget to purchase one of the costly commercially available options. The scope of this design challenge was to produce a low-cost inverted fluorescence microscope employing available optical components and additional readily sourced parts to expand the use of fluorescence microscopy accessible to undergraduate students in the Microfabrication Laboratory.

This document is an account of the final microscope design as well as the engineering design process, project management procedures, and timeline followed to produce a working design verification prototype. The final product successfully resolved images of microfluidic devices in brightfield mode with automated maneuverability in the X-Y plane. It is equipped with fluorescence capabilities, and will serve as a valuable, low-cost research tool and platform for future student projects.

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1 INTRODUCTION

The primary project objective was to create a low-cost inverted fluorescence microscope for use in the Cal Poly Microfabrication Laboratory. Our team was comprised of four undergraduate students studying Mechanical Engineering at Cal Poly San Luis Obispo. This project was brought to the department by Dr. Benjamin Hawkins and Dr. Hans Mayer of Cal Poly San Luis Obispo.

A fluorescence microscope imparts energy to a specimen injected with an indicator dye by illuminating it with a specific wavelength of light. This energy causes the indicator die to fluoresce, emitting light at a longer wavelength than the excitation light used. The fluorescent emission is sent through a filter cube, which blocks all wavelengths of light not desired for the selected dye used. Commercially available models of microscopes capable of fluorescence can cost upwards of $30,000. This high price necessitated the creation of a low-cost version to make fluorescence microscopy accessible to Cal Poly students. This microscope will allow students to perform research projects including the study of how fluids react within microfluidic channels as well as the inspection of cell growth.

The following report captures the results of the design process, detailing the background research conducted, the problem’s scope and requirements, a timeline for efficient project management, and documentation of the final design selected for each microscope subsystem based on extensive iteration, analyses, and testing.

2 BACKGROUND

Our project research was focused predominantly in three main categories: customer research, product research, and technical research. Customer research entailed sponsor meetings, investigation of the available workspace in the Cal Poly Microfabrication laboratory, and discussion of design goals and requirements. Product research involved comparing out-of-budget, commercially available microscopes as well as do-it-yourself style home projects to the desired quality and capabilities of our product. Most of our knowledge on the physics of fluorescence, the anatomy and components of microscopes, and the application of fluorescence microscopy was derived from technical research in the form of journal articles and textbook chapters.

2.1 CUSTOMER RESEARCH

To begin to understand the needs and motives of both our project sponsors and potential users of our product, we conducted interviews, one-on-one with each sponsor and as an entire team. During these meetings, we were able to clarify design goals, discuss some of the available components that would be potentially useful for our setup, and define a budget for the project. Interviewing each sponsor allowed for us to distinguish more individual project motives. Dr. Hawkins, as part of the biomedical engineering department, was primarily interested in observing and imaging cell growth using fluorescence, while Dr. Mayer wished to visualize flow through microfluidic channels. Following these individual interviews, conducting meetings via Zoom conferencing yielded more technical information and helped to define of project constraints. The following is a summary of our customer findings.

- Low cost (achieved by employing available components and making use of rapid prototyping)
    - Utilizes available camera, optical components, and filter set for proof-of-concept testing
    - Makes use of rapid prototyping for appropriate microscope parts
- Modular and adaptable for future projects
    - Changeable objectives
    - Changeable filters (ability to switch cubes as well as individual filters)
    - Modular camera attachment (uses standard camera attachment port)
    - Swappable actuators
- Room for experiments
    - Inverted configuration
- Manual and computer control of stage
- Operable by 400-level student in the Microfabrication class

2.2 PRODUCT RESEARCH

Our goal was to design and create a low-cost microscope, but when searching for similar products, we quickly found that a majority of the commercially available research microscopes cost well over $5,000. However, we were able to collect plenty of valuable information regarding dimensions, tolerances, and specifications for stage and optical components from the online data sheets provided for these high-end microscopes.

2.2.1 Nikon Ti-2E

Figure 2.2.1. Nikon Ti-2E Inverted Fluorescence Microscope [1]

Currently, there are many inverted microscopes on the market developed by various companies. Each of these microscopes have more functions than what we are designing and at a higher cost. For example, Nikon has its Eclipse Ti-2 series inverted microscope [1]. The microscope has capabilities such as a large field of view at 25 mm and a Z-axis stabilizer to reduce vibrations in the system to maintain focus on the specimen. Being one of the more robust inverted microscopes, the Ti-2 series has a "fly-eye" lens within the epi-fluorescence* illuminator to ensure uniform illumination [1]. Impressively, the stage uses motorized and mechanical maneuverability on its main body, condenser, nosepiece and other components. As a final touch, Nikon added sensors to assist users and auto-detect errors to increase user performance.

* "Epi-fluorescence," abbreviation for episcopic fluorescence, refers to microscopic imaging that utilizes reflected light. In this configuration, the objective lens also operates as a condenser.

2.2.2 OPTIKA IM-3

Figure 2.2.2. OPTIKA IM-3 Inverted Fluorescence Microscope [2]

OPTIKA is an Italian company that constructs microscopes in various configurations. Unfortunately, not much could be gathered from their specifications outside of their catalog. From the information found, OPTIKA's IM-3 inverted microscope is capable of two types of fluorescence imaging: mercury unforced cooling luminescence and LED. Along with these fluorescence types, the IM-3 exhibits a fixed 250x160 mm stage which can be fitted to become mechanically maneuvered, if requested [2]. Other features can be added to the microscope such as a UV fluorescence protection plate and replaceable objectives for its 3-lens objective turret. OPTIKA has pushed for longevity with their microscope which is rated with a 65,000-hour lifetime on its X-LED illumination system along with reducing electricity by 90% with its 8W light source [2].

2.2.3 Olympus IXplore Standard

Figure 2.2.3. Olympus IXplore Standard Inverted Microscope [3]

The IXplore Standard by Olympus is another microscope that utilizes blue/green and ultraviolet excitation for fluorescence imaging. Following Nikon's motorization of components in the microscope, Olympus has done similar with the IXplore Standard. The IXplore Standard has 8 position fluorescence mirror turrets and a 6-position nosepiece with the option to be either motorized or encoded. Other motorized components are the microscope's long-distance universal condenser and 114x75 mm stage [3]. The IXplore Standard uses stray light reduction by coating each of its fluorescence mirror units ultimately reducing stray light by 99%.

2.2.4 Motic AE31 and Zeiss Primovert iLED

(a) Motic AE31 [4] (b)Zeiss Primover iLED [5]

Figure 2.2.4. Two inverted fluorescence microscopes (a) by Motic and (b) Zeiss

The AE31 microscope series by Motic is similar to the previous three microscopes in its technological capabilities. The main difference is the fluorescence imaging must be done using one of their attachments and three filter cubes to conduct epi-fluorescence imaging [4]. The AE31 has a large stage (200x260 mm) with the option to add auxiliary extension plates. Encouraging a large workspace, the AE31's standard condenser has a working distance of 72mm which can be increased to 231mm if the condenser body is fully removed. Zeiss produced a similar microscope, in which they reduced the complexity for user to overcome with the Primovert iLED [5]. The Primovert iLED has Epi-Fluorescence capabilities and can use its integrated camera and the Labscope imaging app for the iPad™ to observe a specimen outside of the workspace.

2.2.5 DropletKitchen

A project similar to our potential design is produced by DropletKitchen. Each of the materials required are detailed on their website, downloadable for 3D printing or a listed to be purchased at a store. The light source is small high-powered LED and its structure is made of acrylic. The required optics consist of a lens with a focal length of 50 or 100 mm. The design is adaptable and can allow for a mechanical stage or camera holder [6]. Because large portions of the body are 3D printed, the cost of production is significantly lower.

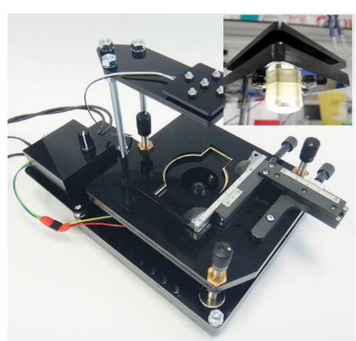

Figure 2.2.5. DropletKitchen's DIY Inverted Microscope. Note that fluorescence is not present. [6]

The key specifications of each of the microscopes have been placed into Table 2.2.1 below. As stated previously, current state-of-the-art microscopes are expensive in comparison to what to be developed by our team. The closest microscope to our potential design is the microscope produced by DropletKitchen regarding cost. Some of the microscopes listed are not initially equipped with fluorescence microscopy capabilities. Because of this complication, the costs listed are the costs listed on each off their respective websites and quotes would be required to find the true price.

Table 2.2.1. Comparison of Products in relation to their specifications

Products	Cost	Specifications
Nikon Ti-2e	$38,995	• LED light source • Fly-eye lens for uniform illumination • Motorized and Manual 114x73 mm stage • Camera Port and motorizing focusing unit
OPTIKA IM-3	$4,706	• X-LED illumination system with a 50k hour lifetime • HBO or LED fluorescence • Camera port with multiple adapters • 250x160 mm fixed stage
Olympus IXPlore	Unknown.	• Motorized 114x75 mm stage • Motorized long working distance universal condenser • Filter wheels and shutters • 8 position motorized or encoded fluorescence mirror turrets
Motic AE31	$3,395	• 200 x 260mm Stage • Centering Telescope • Condenser with a working distance of 72mm • Possible Epi-fluorescence with 3 filter cubes with attachment
Ziess Primovert	$5,190	• Epi-fluorescence • Transmitted light brightfield • UV protection plate
DropletKitchen	Varies	• Interchangeable objectives • Not capable of fluorescence microscopy • 3D printed main structure

2.3 PATENT SEARCH

Developing an acceptable baseline for the design process required investigating existing patents. Understanding what is patented and the ideas that have generated them is very important, especially when designing commercial products. While our team was not creating a product for market, researching patents related to fluorescence microscopy still very useful information. Patents contain important general ideas and communication strategies despite the lack of technical information. Descriptions of a patent are supposed to be general to provide less of a barrier of entry to understanding thus providing succinct descriptions, and useful graphical communication tools. Table 2.3.1 summarizes the results of our patent search. All the documents found related to high end, commercial, research grade instruments. The products that these patents described fall outside of the scope of this project, but the communication tools and general ideas were useful.

Table 2.3.1. Patent Search Results

Patent Title	Patent / Application Number	Highlights / Description of Patent
Inverted Microscope Having A Variable Stage Position [7]	US 6160662 A	Patent by Nikon for an inverted microscope, not fluorescence. The figures illustrate microscope component layout well.
Fluorescence Microscope [8]	EP 1666947 B1	This is a fully enclosed and computerizes microscope.
Compact, High-resolution Fluorescence And Brightfield Microscope And Methods Of Use [9]	US 9494783 B2	This patent lays out important component relationships in its list of claims. It also has informative schematics.
Microscope Especially Inverted Microscope [10]	US 2005/0099679 A1	This is a patent application for an inverted microscope. It has exemplary examples of how to graphically communicate microscope form and function.
Inverted Microscope [11]	EP 2003481 B1	This is a fully enclosed and compact microscope design. It has a removable cover that allows for stage access.
Inverted-design Optical Microscope [12]	US 4210384A	This patent is for a compact inverted microscope with a deployable light source.

Figure 2.3.1, shown below, exemplifies how one patent applicant clearly communicated the design of their product [10]. The image is clean and simple, and it clearly shows critical component relationships with optical pathway lines. In design presentations, we used this image format, with drawn out light paths, to clearly illustrate the function of our final design.

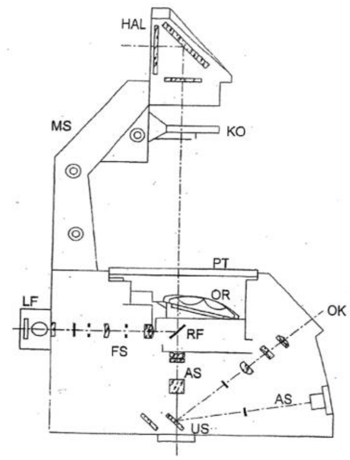

Figure 2.3.1. Example of clear graphical communication of a microscope's light pathway. This sketch was taken from US Patent Application Publication: US 2005/0099679 A1, "Microscope Especially Inverted Microscope" [10].

United States Patent, US 9494783 B2, for a "Compact, High-resolution Fluorescence and Brightfield Microscope And Methods Of Use" provides an extremely comprehensive list of 18 claims that overview every microscope component. This list also highlights critical relationships between components. For example, the patent describes its illumination and detection system as being "fixedly mounted relative to one another" [9]. The patent then continues to state that this relationship allows the whole system to move together. Details like this helped us highlight important features to include in our final design.

2.4 Technical Research

Peer-reviewed journal articles, textbooks and chapters, and websites for various biomedical organizations provided substantial sources of technical knowledge when building familiarity with the principles of fluorescence microscopy. An understanding of how optical components work together to produce an image was paramount in the construction of a microscope, especially considering the available parts were not necessarily designed for fluorescence applications.

2.4.1 Books and Book Chapters

Fluorescence Microscopy: From Principles to Biological Applications, by Kubitscheck and Dobrucki, details the typical components and anatomy of standard, commercially available inverted fluorescence microscopes [13]. The fundamental challenge of fluorescence microscopy revolves around the fact that emitted fluorescence is much weaker than bright excitation light. The "epi-illuminator," a series of optical components in series, greatly simplifies separation between excitation and emission wavelengths. Kubitscheck and Dobrucki delve into the available options for major components in the optical path, advantages and disadvantages of each, and how the optimal components may be different for varying applications of fluorescence. For example, the light sources at our disposal was a Xenon-arc lamp, a broadband fiber-optic illuminator, and various single-wavelength LEDs. We determined that the fiber-optic illuminator was a better option for brightfield than the arc lamp due to its higher intensity, and for fluorescence, we chose a single-wavelength LED to eliminate the need for an excitation filter. After future testing, we may decide to add additional optics to correct for intensity, such as a neutral-density filter.

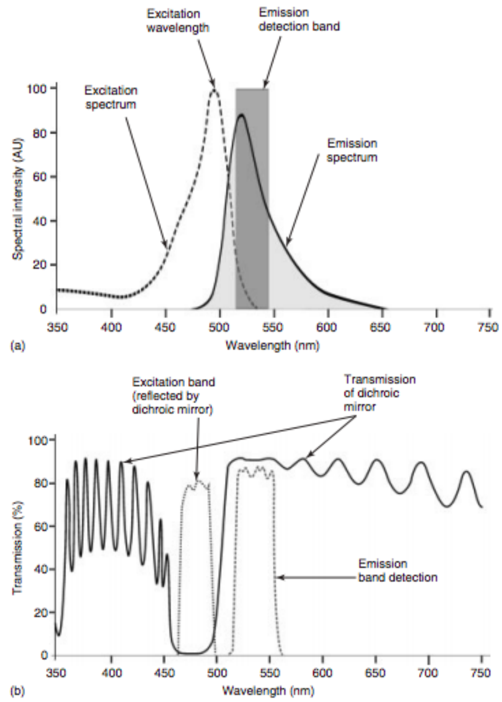

Figure 2.4.1. (a) Spectral properties of fluorescein, denoting excitation and emission spectral maxima, compared to (b) its particular corresponding filter set. [13].

In *Fundamentals of Light Microscopy and Electronic Imaging (Chapter 11)*, Murphy and Davidson explore the characteristic quantities of fluorescence and specifications that determine the choice of fluorophore for a specific application, including spectral region, Stokes' shift, quantum efficiency, photostability, and spectral profile of the illuminator [14]. Included is a table of the properties of commonly used fluorescent dyes, noting spectral excitation and emission maxima, the color of fluorescence, and the corresponding standard filter set. For our microscope, proof-of-concept testing is designed for a single filter set and complementary fluorophore, so the choice of dye or filter combination is not necessarily a heavy design consideration. However, understanding the main principles of how a fluorescence microscope operates to produce fluorescent images was knowledge that we deemed essential for creating a satisfactory product.

2.4.2 Journal Articles

In *"An Inexpensive and Simple-to-Use Inverted Fluorescence Microscope: A New Tool for Cellular Analysis,"* Kahle accounts her fluorescence microscopy design challenge; it had similar goals and constraints to ours, including an emphasis on minimizing cost [15]. Kahle notes some of her discoveries:

- A light source must be of adequate intensity and spatial uniformity; an LED satisfies this requirement.
- LED light sources can have high emission angles, so it is important to collimate the beams before passage through the excitation filter.
- CCD imagers are the most commonly used in epi-fluorescence microscopy, but CMOS may be better with respect to cost, sensitivity, and power consumption. Many are also USB compatible.
- (However, our design will be restricted to the imager in the microscope camera at our disposal.)

Kahle's microscope used a 40× objective lens at 1.8 fps (0.5 second exposure). She obtained images of appreciable quality; in the primary stages of conceptual design, we needed to determine our own specifications in relation to the 4× objective lens we purchased.

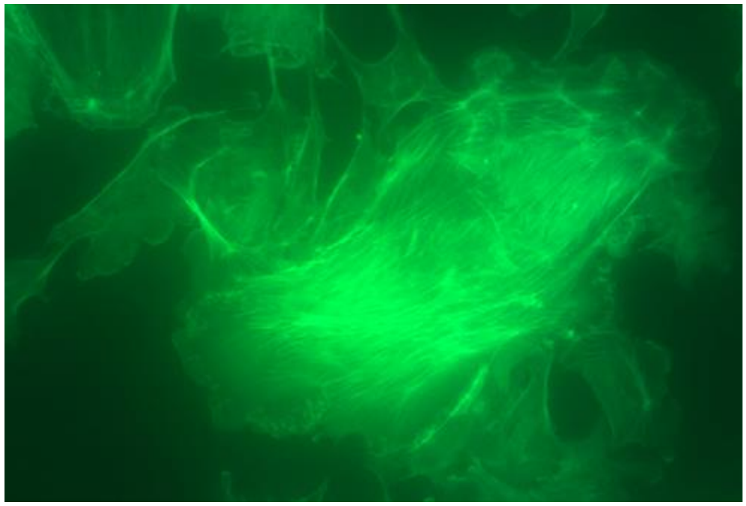

Figure 2.4.2. Bovine pulmonary artery endothelial cell images tagged with its appropriate fluorophore (MitoTracker Red CMXRos, Alexafluor 488 phalloidin) collected with Kahle's inverted fluorescence microscope setup. [15].

"Portable, Battery-Operated Fluorescence Field Microscope for the Developing World," recounts Miller and his team's approach to a design challenge of devising and fabricating a simplified portable fluorescence microscope, implementing components and techniques, such as rapid prototyping, that minimize cost for more accessible use [16].

- Optical illuminating components held in alignment by a single housing component, which was SLA printed in ABS plastic.
- High magnification (up to 1000×) and resolution were achieved in brightfield mode by use of a macro lens and a doublet (achromat) lens onto monochrome CCD imagers.
- Final product cost $480 USD, and reproduction is estimated at $230 USD.

Improvements to resolution can be made by the addition of a condenser lens and higher quality objectives, we considered the factor during our own design process.

In "*Advances in the Design of the Inverted Prismatic Microscope*", MacArthur details components of one of the first well-received inverted microscopes in 1933 [17]. Advantages of the inverted slide on this microscope include auto-focusing capability, simplified adjustment of objectives and optical components, and simpler centering of samples. This senior design project's primary rationale for having an inverted configuration was to allow for space for tubing for microfluidic devices. MacArthur's microscope did not incorporate filter cubes for fluorescence imaging but is a good example of a simplified design of an inverted configuration.

2.4.3 Web Resources

"Molecular Expressions – Fluorescence Microscopy" is a website administered by Michael Davidson through a variety of links, images, articles, and applets, details processes of choosing light sources, aligning optics, troubleshooting, and optimizing microscope configurations [18].
Davidson details means through which to optimize microscope image quality and specifies causes for diminished performance.

- To achieve uniform brightness, known as Kohler illumination, proper alignment of arc lamps is crucial. We may need to follow this procedure if we decide to use the Xenon-arc lamp as excitation illumination.
- Tungsten-halogen lamps are useful for traditional brightfield illumination, while Xenon-arc lamps are better for fluorescence applications.
- Even the slightest overlap in spectral profiles of excitation and emission filters can reduce fluorescence.
- Objective lenses of lower magnification (longer focal length) produce brighter images.

"Infinity Corrected Optics," an article featured on Microscope World organization's website, examines the benefits of the advancements made in infinity-corrected objective lenses [19]. Purchasing such a lens was a heavy consideration when designing an optical path and eventually ordering parts; infinity correction allows for auxiliary components to be placed in the optical path without significantly compromising focus. Making this specification, however, increased the difficulty of sourcing an objective lens of appreciable quality while minimizing cost.

2.5 Standards and Regulations

Standards and regulations were considered to make sure that the new product was made properly and operates safely during the design process. These standards provide a designer with insight when creating a new design. Without standards and regulations, proper design base points are not apparent and prevent the assurance of design results are sound.

ASTM Standard E883-11 "Standard Guide for Reflected-Light Photomicrography" explains the requirements and possible routes for constructing a reflected light microscope. This standard lists the common methods and ratios used in microscopes of this nature, such as:

- Preferred magnifications
- How photomicrographs should be reproduced, so that someone viewing the photograph is able to tell what the photograph is and its magnification
- Optical systems, and how the components must interact to get the desired image
- Light sources and filters, as well as how to illuminate the sample
- Focusing
- Film processing techniques for microphotographs,

The standard above provided a suitable cornerstone that we used when creating our design. This allowed us to create a product that functions similarly to models on the market today.

2.6 3D PRINTING

Throughout the course of our project, we utilized 3D printing as a method of fabricating and iterating components. Specifically, we used this method of rapid prototyping to create concept models used in the concept prototype [22]. In addition, we created low-cost versions of mounting brackets used in various locations, such as the stage and overhead beam. Industry is widely using additive manufacturing techniques for rapid prototyping, with a variety of materials. Some 3D printers are capable of fabricating metal and ceramic parts, which proves to be unnecessary for our project because the material can cost a significant amount. Also, the components we used for our project did not require the properties that these materials offer. The 3D printer used for our project is a Fused Deposition Modeling (FDM) unit where the material is pushed through an electrically heated nozzle [21]. Typically, the material is a form of plastic such as Polylactic Acid and Acrylonitrile butadiene styrene, or commonly referred to as PLA and ABS respectively. Our project utilizes PLA, as it is widely available, offers adequate properties, and does not require a lot of post processing.

Figure 2.6.1. Example of a 3D printer similar to the one in the Micro-fabrication lab [23]

The 3D printer displayed in Figure 2.6.1 is similar to the 3D printer we have in possession. Stepper motors are used move the nozzle in two axes and the table in one independently. The software required to operate the 3D printer is a CAD software, like SolidWorks®, and 3D printer software to communicate between the computer and the printer itself. Another way to load a model into the printer is to upload the CAD file onto an SD card and load the SD card into the 3D printer. Through this simple process, fixtures and other components can be made with plastic.

3 OBJECTIVES

The Microfabrication Lab needed a microscope to view specimen such thin channels on a wafer. Our goal was to produce a low-cost inverted microscope that can perform fluorescence microscopy. The lab supervisors had some parts required for the microscope already in their possession and wanted to incorporate them into the final design. The boundary diagram shown in Figure 3.0.1 presents a visual representation of the scope of this project. Components within the red dotted line account for all the design parameters that were within our control, such as the objective lens and optical components we purchased, as well as the configuration of the stage and overall microscope frame. Outside the dotted line are the project framework that we needed to cater to: our customer(s), the components already available to us, and the end goal of the project.

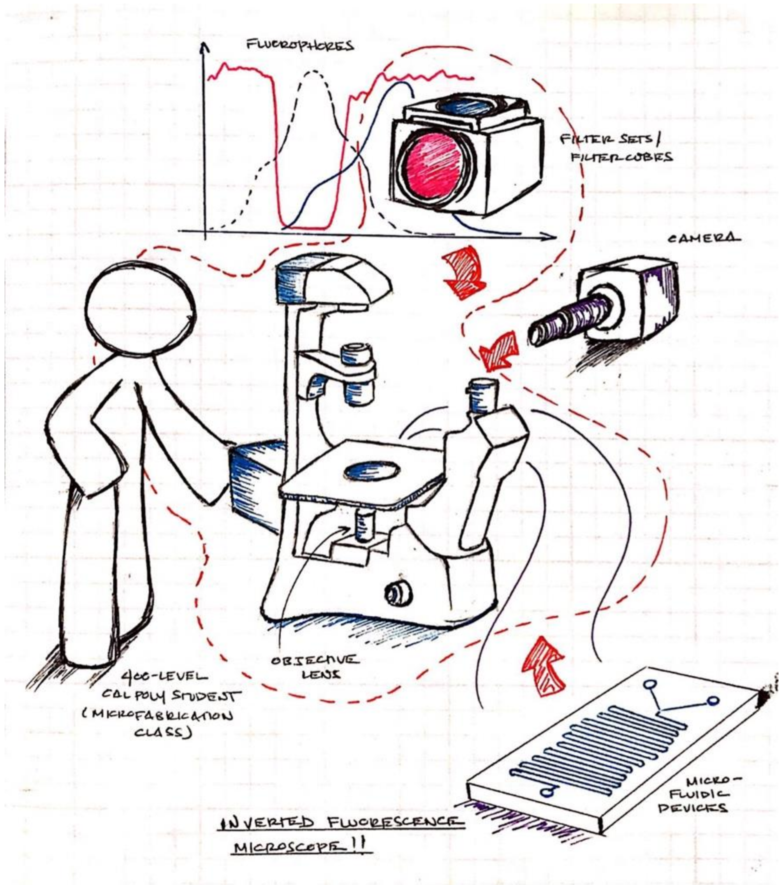

Figure 3.0.1. Boundary sketch of our project solution space.

3.1 DESIGN CONSIDERATIONS

According to Dr. Mayer and Dr. Hawkins, the following considerations should be employed when designing the microscope:

- Low cost construction through rapid prototyping or using already acquired materials.
- Open and Modular for interchangeability of parts such as optical filters and objectives.
- Infinity corrected optical components. In this case, infinity corrected means that image distance is set to infinity to create a parallel optical path to the camera without needing to account the objective’s optical distance.
- Modular camera attachment so multiple cameras can be interchanged with one another.
- Large open space on stage for future experiments and to prevent obstructions.
- Consistency of focus while the stage travels.
- Computer driven actuators will work in tandem with the manual controlled portion of the stage. These actuators must also be able to be replaced.
- Operable by 400 level students with a low learning curve.

3.2 Quality Function Deployment

To ensure that we provided the best solution to the problem, we employed the use of the quality function deployment. The House of Quality contains sections for each of the questions asked pertaining to the project. The House of Quality has each section organized to display how each item influences one another. As shown in Appendix A, the "who" section details the customers of our project which are Professor Mayer, Professor Hawkins and 400 level students. Their wants and needs are detailed adjacent to the "who" section, with how each want ranks with each customer in terms of importance, in the "how" and "what" sections. These sections can be seen interacting through assigning correlations. Leading competitors are placed in the "now" section with their ranking determined on how well each satisfies customer needs. The competition is also compared to engineering specifications in the "how much" section through target values. The House of Quality allowed for a better view of what was asked by our sponsors and was used to determine the final specifications discussed in the next section.

3.3 Engineering Specifications and Risk Assessment

Engineering problems can be defined using specifications. They are used to identify and quantify important design considerations. Table 3.3.1 provides a list of the specifications that we believed were critical to defining our problem. Each specification has an associated risk level (high, medium or low). The level relates to the amount of difficulty we believed was present in meeting a specification. Low risk specifications could be met with simple initial design choices. This category is comprised of size and motion requirements. Medium risk specifications required a finer degree of component selection / design. For example, to achieve our target resolvable sample size, we needed to ensure we chose adequate optical components. High risk specifications identify areas that we knew needed special design consideration. These specifications were subject to a high degree of tolerance stack up and were difficult to satisfy without diligent consideration. The table also includes the values we hoped to achieve for each specification, an assessment of risk, and the method we intended to use to verify each specification. Each of the methods include inspection (I) and testing (T).

Table 3.3.1. Inverted Fluorescence Microscope Engineering Specifications

Spec. #	Specification Description	Requirement or Target (units)	Tolerance	Risk	Compliance
1	Resolvable Sample Size	10 µm	Min	M	T
2	Z Travel	64 mm	Min	L	I
3	X-Y Travel	50x50 mm	Min	L	I
4	Repeatability of Actuation	50 µm	Max	H	T
5	Stage Parallelism with Frame	0 µm	±25 µm	H	T
6	Clearance Above Stage	50 mm	Min	L	I
7	Hard Cap Budget	$3,000	Max	M	I
8	Microscope Footprint	2x3 ft	Max	L	I
9	Frame Deflection in Operation	TBD	TBD	M	T
10	Detectable Fluorescence	Any	Min	M	T

A more detailed description of each of our specifications and a discussion of how we intended to measure them is displayed below.

- Resolvable Sample Size — The sample size resolvable by the microscope is determined by the objective used. When using a 4X objective, this microscope should be able to detect a feature 10 μm in diameter.
- Z Travel — This microscope was designed with one objective initially, but it has the travel flexibility to accommodate various objective sizes.
- X-Y Travel — The microscope's X-Y travel had to be large enough to fully utilize the range of our linear actuators (50x50 mm). This provided enough travel fully observe any microfluidics experiment.
- Repeatability of Actuation — The stage is positioned with two 850G Series Linear Actuators, which are repeatable to approximately 40 μm. To verify the final prototype's actuation repeatability, we intended to create a test cycle in which the stage is moved to a variety of points, and then back to the start. The stage should be able to end within 50 μm of its starting position. During our last day of testing we qualitatively checked the system's repeatability. A quantitative test was not possible.
- Stage Parallelism with Frame — It is extremely important for our microscope to maintain focus. To verify that our stage stays within 25 μm of parallel to the base. We hoped to verify that our system stays parallel by using a dial indicator (quantitative), and by observing if our microscope stays focused on a 50 μm deep microfluidic channel over a 50 mm travel. Due to time constraints this was not possible.
- Clearance Above Stage — Ample clearance must be left above the stage to allow for microfluidic tubing, and other experimental equipment. We were careful to design the upper gantry of the microscope with sufficient clearance.
- Hard Cap Budget — Minimizing costs wherever possible allowed us to spend what we have on higher quality optics. We fully utilized rapid prototyping to stay under budget.
- Microscope Footprint — Our microscope had to fit within the 2x3 ft optical bread board that we have been provided for construction.
- Detectable Fluorescence — Fluorescence detection is highly dependent of the camera and light source used. Any level of detectable fluorescence using the equipment currently available in the Microfabrication Laboratory is sufficient to verify that the optics are functional. Unfortunately, we were not able to purchase the components needed to test fluorescence imaging.

We believed that actuation repeatability and parallelism would be our two most challenging specifications to satisfy. Both were highly dependent on the quality of the components we use, and the level of precision with which they align. By identifying these risks early and considering them throughout the design process, we prevented them from presenting a problem.

* The specification for "Frame Deflection in Operation" was removed because it was deemed redundant by our sponsors. They felt that our parallelism specification fully captured our system's rigidity requirement.

4 CONCEPT DESIGN DEVELOPMENT

This section overviews the idea refinement process used to converge upon a single design concept. This development process was applied to our project in three subsystems: optical pathway, microscope stage, and frame. During the ideation and design distillation phase, we quickly realized that due to the nature of the project, the design choices made for each subsystem had minimal interdependence; therefore, ideation for each subsystem was tackled individually and the best alternatives for each were chosen and developed further. Each optimized subsystem was then integrated into the final design. After defining the problem, establishing quantitative specifications, and identifying budget constraints, we were able to begin searching for microscope components and develop potential configurations to maintain progress towards a final design concept.

4.1 PROCESS

To generate as many ideas as possible before analyzing the strengths and pitfalls of each design concept, we followed a standard engineering design process. Functional decomposition, brainstorming and ideation, sketching, modeling, and meeting with sponsors to gain feedback promoted the eventual convergence upon a single concept design to move forward with. Due to the nature of this project, the process was applied to each of three subsystems, and then integrated into one final design.

4.1.1 Functional Decomposition

Prior to brainstorming and generating solutions, it was necessary to first identify the main functions that our final product will accomplish, determine their interdependencies, and define the critical subfunctions that would result in detecting and imaging fluorescence. The functional decomposition of our project is displayed below in Figure 4.1.1.

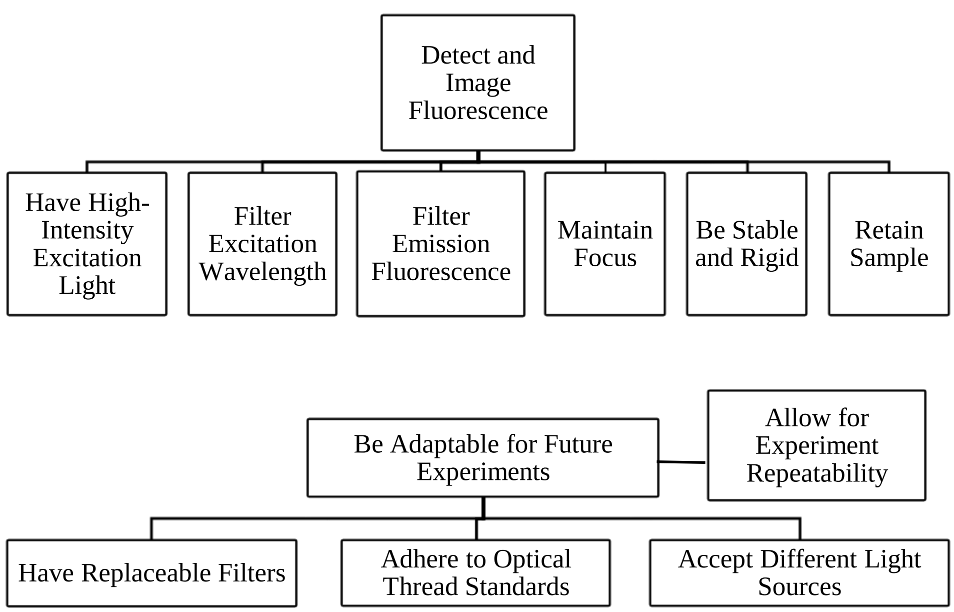

Figure 4.1.1. Decomposition of project scope into general functions, subfunctions, and dependencies.

4.1.2 Concept Sketching

In the context of our project, ideation began with group discussion and individual concept sketches for each microscope subfunction. From the ideation, an early design of the stage can be seen in Figure 4.1.2. Leaders for each respective subsystem illustrated two concepts and presented them to the group in a team meeting. Proposals were also brought to project sponsors for feedback and suggested improvements for design concept refinement. A comprehensive list of all concept sketches is detailed in Appendix [C].

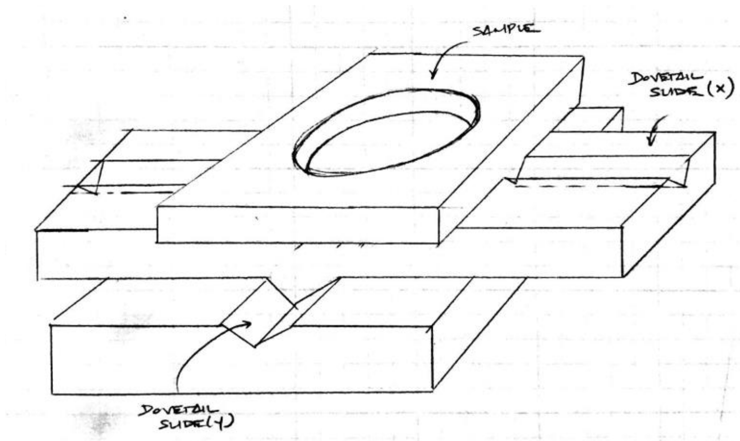

Figure 4.1.2. Sketch of one of our initial X-Y stage concepts implementing slides.
Note: we are aware that the slides pictured are not dovetail slides.

4.1.3 Concept Modeling

Further ideation was stimulated by making models of design concepts to test concepts, generate more potential solutions, communicate ideas, and gain feedback from teammates and peers. Lab time on October 29th, 2019 was spent prototyping using craft materials (foam board, pipe cleaners, cork, hot glue, etc.) and presenting ideas amongst team members. For the optical pathway, concept modeling was primarily useful for communicating ideas and showing a physical representation of the light path, while concept modeling of the stage and the frame was effective at stimulating additional ideation. A concept model of a means of achieving z-axis focus is shown in Figure 4.1.3. Additional photos of some of the prototypes that helped us to broaden our list of potential solutions and eventually converge on a single design are included in Appendix [D].

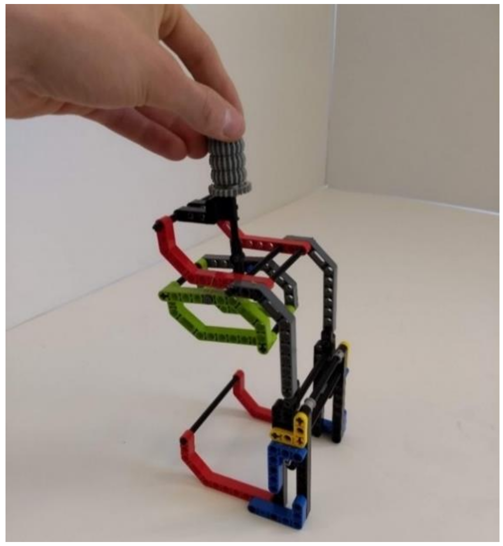

Figure 4.1.3. A “quick and dirty” concept model of a z-axis traversing mechanism for maintaining focus on the sample of interest, constructed with plastic building bricks.

4.1.4 Sponsor Feedback

Ultimately, much of the decision-making process was facilitated by sponsor communication and feedback. We presented initial concepts for the optical pathway, stage, and frame to our sponsors, who would either confirm or suggest modifications based on budget and feasibility. Both the objective lens and the stage, our primary high-cost design components, needed to be found and purchased at a discounted price, prompting us to maintain continuous sponsor contact via email and meetings.

In the following sections, we detail solution concepts for each microscope subsystem, the design factors considered in the ideation process, and the selection of a single concept to proceed with using decision matrix analysis and controlled convergence.

4.2 Optical Pathway

Ideation for the microscope’s optical pathway was rigid since there is little flexibility regarding required optical elements or alignment of these components. However, based on our previously defined engineering specifications and constraints implemented by our project sponsors and the availability of components, some decisions needed to be made concerning the light source, objective lens, filters, and corresponding indicator dyes to have the best chances of creating a final product capable of imaging fluorescence.

Prior to initial ideation and brainstorming, we took inventory of all the optical components available for use in the Microfabrication Laboratory and determined which additional components we needed to purchase and which we could manufacture or print ourselves. A listing the parts at our disposal is detailed in Appendix [E].

Devising a final optical pathway necessitated extensive discussion and deliberation with our project sponsors, from which we were able to narrow down the available parts to use for our design and establish a “test case” excitation wavelength, desired magnification factor, fluorescence dye, and emission filter for proof-of-concept testing. Shown in Figure 4.2.1 is a sketch of the target optical pathway devised from sponsor feedback and suggestion. The following subsections detail selection of specific components.

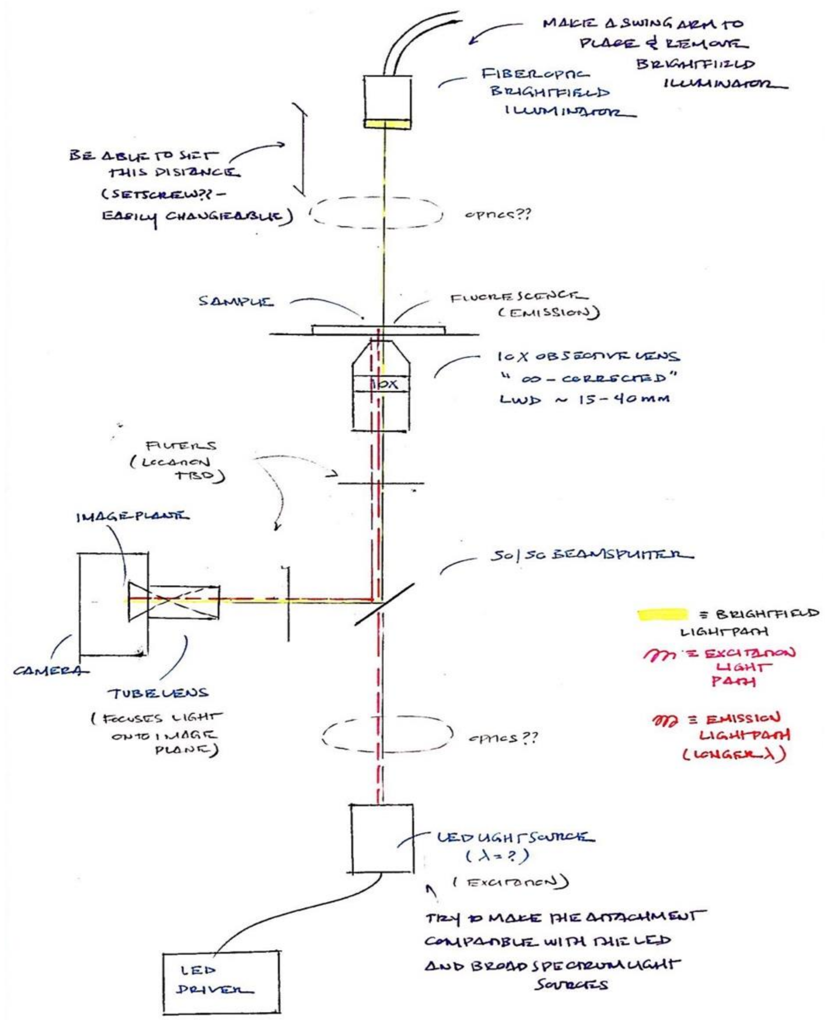

Figure 4.2.1. Sketch of the optical pathway agreed upon by project team and sponsors.

4.2.1 Light Source

We envisioned the final inverted microscope being equipped with both brightfield and fluorescence capabilities; this required two different light sources and two optical pathways that do not interfere with one another. Brightfield illumination is simple – sufficient brightness can be achieved with one of the Fiber-Lite fiber optic illuminators in the lab, and the neck of the cable adapter can be secured with a collar-setscrew assembly for position adjustment.

Two options for fluorescence illumination were at our disposal: a Deuterium-Halogen broad-spectrum lamp and a series of single-wavelength LED light sources. Initially, we opted for the broad-spectrum source under the premise that having access to the full range of the electromagnetic spectrum would simplify the selection of corresponding filters and fluorophores. While this may be true, we quickly discovered that this lamp is likely not capable of the excitation intensity required to produce sufficient fluorescence emission to be captured by the camera. Further research confirmed that upon exciting the specimen, energy losses result in fluorescence with longer wavelength and much weaker intensity than its supplied excitation light.

Our final design employs the use of a single-wavelength LED and driver, which will provide a higher intensity light source than that of the broad-spectrum. The LED will provide only a single specified excitation wavelength. Despite this, the design can be adapted for different fluorophores and filter sets simply by replacing the LED with one of a different wavelength corresponding to that application. Figure 4.2.2 shows the excitation wavelength spectra of both light sources.

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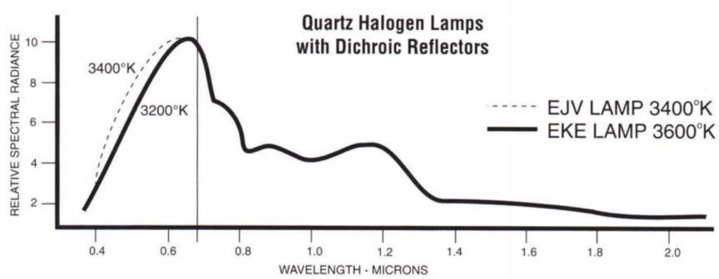
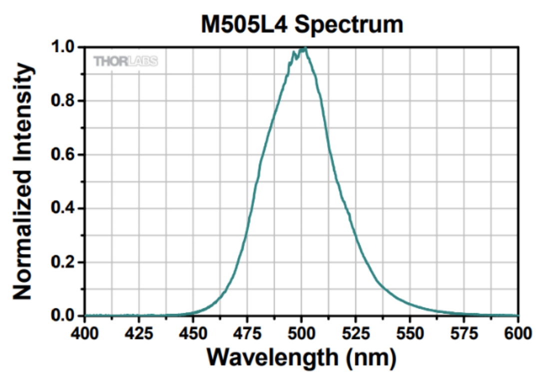


(a)



(b)

Figure 4.2.2. A comparison of (a) the broad-range emission spectrum of the Deuterium-Halogen lamp [24] and (b) the spectrum of a single-wavelength LED [25].

In future iterations of this microscope design, given an larger budget, it would be advantageous to utilize a high-power LED built for microscopy to bombard the specimen with greater intensity excitation light. ThorLabs sells its Solis Series LED's for prices on the order of $1300.00 and accompanying drivers for $525.00. While this extends far outside of this project's specified budget, a more powerful single-wavelength Solis LED or a white light Solis LED coupled with an excitation filter would result in higher intensity and more easily detectable fluorescence.

Once functionality has been confirmed with the LED light source currently available in the Microfabrication Lab, we suggest upgrade it with a more powerful source sold by AmScope. While conducting searches for objective lenses, we found that AmScope has a section of heavily discounted microscopy components, including options for higher-intensity illuminators ranging from $300-$500.

4.2.2 Objective Lens

Following extensive analysis of our budget and inventory of the optical components available in the Microfabrication Lab, it was resolved that a high-quality objective lens would likely be the greatest budget expense. Therefore, compiling a thorough list of viable options was crucial before converging on a final item to purchase.

Searches pursued the following criteria:

- 10X Magnification
- Designed for Epi-Illumination
- Infinity-Corrected
- Long Working Distance
- Less than $1,000
- Common Thread Standard

The Mitutoyo Plan Apo 10X sold by Edmund Optics was one of the first objective lenses found that was in-range with respect to budget and met the desired specifications; this lens became the "datum" to which all other alternatives were compared to. A Pugh Matrix for selecting an optimal objective for imaging fluorescence using these criteria is included in Appendix [F]. The results obtained were acknowledged as a guideline for a final decision.

Ultimately, after discussing the listed options with our project sponsors, we concluded that the most practical step forward was to purchase a cheaper objective lens (under $100) from eBay for proof-of-concept and alignment purposes. We intend to replace it later with a higher quality, more expensive objective lens once the optical pathway and all other components are established.

Following this plan, we purchased the AmScope Fluor Plan 4X, which can later be upgraded to the Edmund Mitutoyo Plan Apo 10X or another AmScope with higher magnification. One caveat we needed to remain aware of was that AmScope objectives follow an older thread standard than the more current products; we chose to find an adapter for a different thread standard. Also, considering the smaller magnification objective had both a shorter length and working distance, we needed to allow for sufficient z-travel to accommodate a much larger, long working distance objective.

4.2.3 Mounting the Optics

Nearing the detail design phase, one of the greatest considerations before proceeding with a final product concept was the means by which the objective, filter cube, and other components of the optical train would be oriented and mounted under the stage.

The first concept was a simple machined aluminum block, with internal RMS threads on one end to mount the objective, external S1 threads on the other to fix the filter cube, and an 8-32 tapped hole on the bottom face to attach the optical assembly to a standard-series post so that it would be further fixed to a slide or to the breadboard table. After careful consideration, this option was ruled out due to the difficulty of manufacturing the fine threads necessary for interfacing to optical components. Cost of materials would be lower than commercially available options, but the cost of tooling would outweigh the alternatives greatly. It was also noted that a block would be not be as elegant as other options.

ThorLabs sells relatively inexpensive thread adapters; an RMS directly to SM1 adapter is not available, but it is possible to use two adapters in tandem. A RMS external to SM05 (or other common intermediate thread standard) internal thread adapter in conjunction with an SM05 external to SM1 external adapter would allow for the objective to be coupled to the filter cube. This assembly also required an RMS threaded collar to mount the objective, by which half of the threads would be occupied by the base of the lens, the other half by the adapter. This method was preliminarily determined to be the most cost-effective since one of the adapters is already available in the clean room; this would bring the expenses for coupling parts down to ~$37. However, upon further consideration, we decided that other options would better prevent any potential shifting of the optics.

Attachment of the objective lens and filter cube assembly by cage optical components ensures structural integrity of this section of the optical train due to multiple points of contact and the ability to fasten each cage rod using small setscrews. Another viable option is to use an RMS threaded cage plate to mount the objective and four 1/4" cage rods to attach this assembly to the filter cube, which is compatible with cage optical parts. A caged optical system is the most structurally sound of the three in a primarily vertical optical train; however, it was determined later in the design process that using an infinity-corrected objective allowed for much more flexibility in the design.

After consultation with Cal Poly Optics professor, Dr. Glen Gillen, we decided to mount only the objective in its vertical position and to fix all other optical components horizontally to the breadboard to minimize the number of components underneath the stage and mitigate the risk of misalignment, using a 45º plane mirror between them. Theoretically, an infinity-corrected objective allows an infinite distance from the shoulder of the objective (the typical location of the lens itself) to the tube lens; in practice, this distance is usually restricted to 100-200mm to minimize aberration, but this was still ample space to extend the optical train in a horizontal configuration.

To provide adequate rigidity and to prevent any rotation of the objective during microscope operation, we designed a 3-D printed bracket to hold an RMS-threaded mount and to interface with the z-axis focusing translation stage. A Pugh Matrix exploring the described design options is in Appendix [F] and specifics of this design are detailed in the Final Design Concept (Chapter 5).

4.2.4 Fluorescence Detection

To observe fluorescence, it was necessary to have a mirror at an oblique angle of incidence (typically 45°) to efficiently reflect light in the excitation band and transmit light in the emission band [26]. In commercially available fluorescence microscopes, a dichroic mirror provides this additional filtering. Upon deliberation with our project sponsors, we decided to instead use a beamsplitter for our application for the following reasons:

(1) A 50:50 beamsplitter differs from a dichroic mirror in that it is not wavelength-selective; it simply splits a beam of light in two, transmitting half and reflecting half. Since beamsplitters are wavelength independent, they have the flexibility of working with any set of filters, improving the adaptability of our microscope for different samples and indicator dyes. A diagram of a 50:50 beamsplitter and differences in the wavelengths transmitted between the dichroic mirror and beamsplitter and can be seen in Figures 4.2.3 and 4.2.4 respectively.

(2) No additional filtering is required to further isolate the excitation light since we are using a single-wavelength LED light source.

(3) A 50:50 beamsplitter is currently available for project use in the Microfabrication Lab. Even in future development of this project, it would likely not be advantageous to purchase a dichroic mirror unless the microscope will only be used for a specific fluorophore.

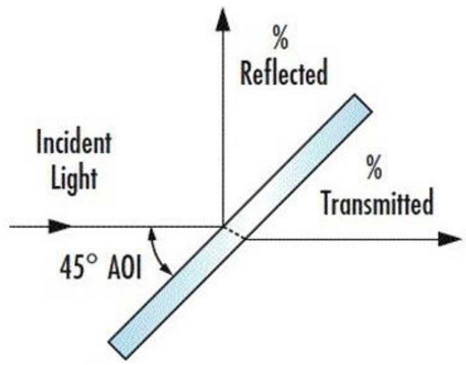
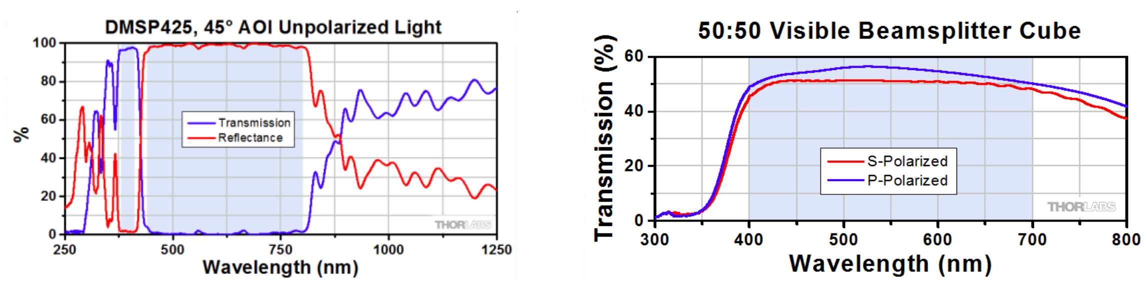


Figure 4.2.3. Schematic diagram of how a 50:50 beamsplitter functions. 50% of the incident light is reflected 90° while 50% is transmitted [27].



(a) Dichroic Mirror

(b) Beamsplitter

Figure 4.2.4. Comparison of the transmission spectra of (a) a short pass dichroic mirror (% transmission denoted by blue line) and (b) a 50:50 beamsplitter cube [25].

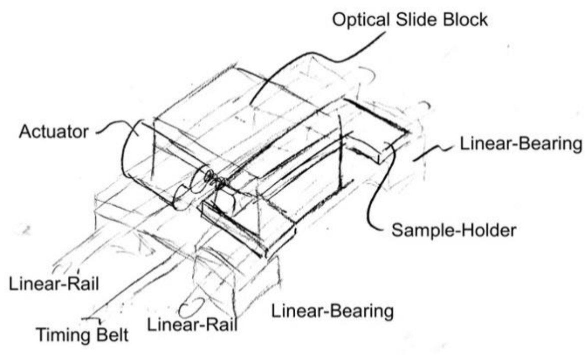
We expected that our final product would be capable of detecting and imaging fluorescence on the basis of a single test case under stagnant conditions. To aid this process, we tested the emission spectral output of the various single-wavelength light sources in the Microfabrication Lab using a spectrometer. These spectra were used to select a number of appropriate indicator dyes and emission filters. Attached in Appendix [G] is a comprehensive list of common fluorophores, peak excitation and emission wavelengths, and complementary filter sets. Under consideration are dyes that are excited by and emit light within the visible spectrum, such as Fluorescein and the AlexaFluor series.

4.3 STAGE

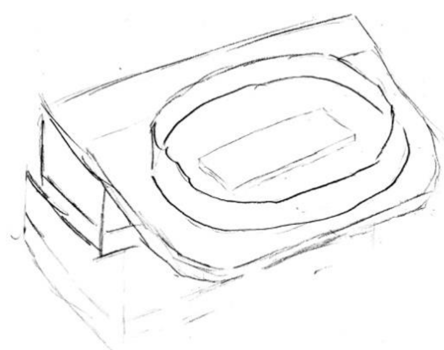
The stage of a microscope is an essential piece of hardware. It rigidly locates the sample and allows the user to finely position it within observation field. Because the stage also serves as an experimental platform, it enables the microscope user to attach other experimental devices that must travel with the sample throughout observation.

4.3.1 Ideation

Our initial Functional Decomposition yielded four functions that related directly to the stage: rigidity, repeatability, motion, and sample retainment. Using these functions to guide the ideation process, we created concept sketches of the stage mechanism. Figure 4.3.1 show two of our earliest sketches.



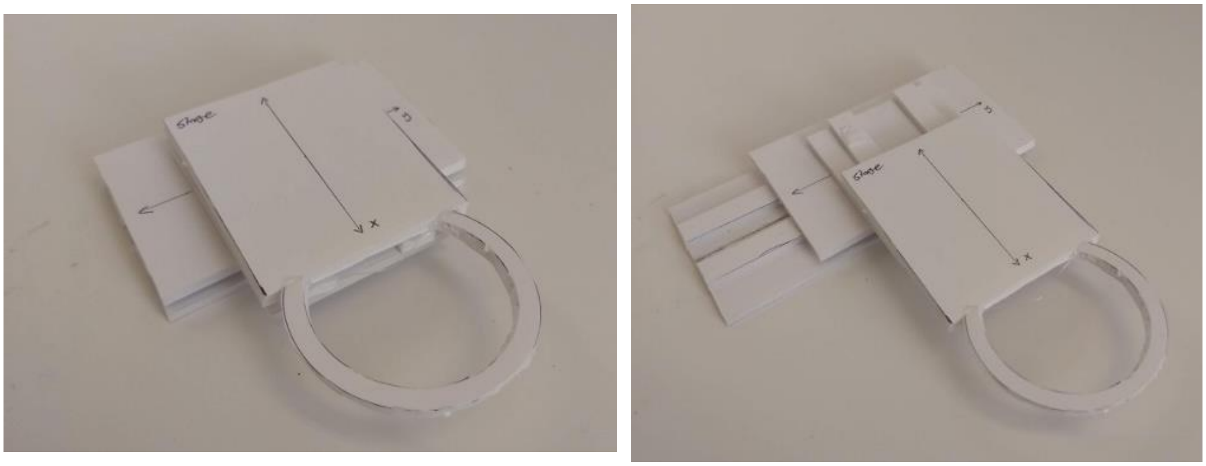
(a) Linear Rail with Optical Slide Block



(b) Dual Optical Slide Block

Figure 4.3.1. Two of the earliest concept drawings of the stage. Figure 4.3.1a shows a stage that rides on linear rails in one axis, and on an optical slide block in the other. The rail axis is driven by a stepper motor / timing belt, while the slide axis is driven by a linear actuator. The sample is held with an over-hung, old fashioned, mechanical stage (not drawn in detail). The stage in Figure 4.3.1b has both axes driven by linear actuators connected to optical slides. The stage is designed to hold a sample in a petri dish cantilever over the microscope optics.

Both concepts were created to maximize the clearance around the sample. We thought that leaving lots of space was the best way to facilitate microfluidics research. After completing the sketches, we created a concept prototype of a potential stage design. It was created to show basic slide actuation and to demonstrate the high clearance over hung sample holding. In Figure 4.3.2, the model can be seen collapsed and actuated.



(a) Collapsed (b) Actuated

Figure 4.3.2. This show two positions of the stage concept model that we created.

Our team utilized Pugh Matrices to settle on a direction forward for the stage. Because the functions have little interdependence, we were able to apply this design tool to stage form and actuation separately (included in Appendix F). They showed us that the best stage form utilized purchased optical slides. The other form options that we considered could not compete with the alignment ease, and axis parallelism that a purchased stage offers. Because a new stage is cost prohibitive, our purchasing options are confined to used equipment. We initially struggled to find viable used stage options, so we began to lean towards completely manufacturing the stage. Our first round of Pugh Matrices did not indicate a clear choice for stage actuation. Ball screws, optical actuators, and micrometers all produced the same score. After some review, we added a category for cost. This addition shifted the results, giving optical actuators the highest score.

We took our preliminary ideation findings to our sponsors for review and asked their thoughts on manufacturing a stage. They emphasized the importance of stage parallelism and raised valid concerns regarding our ability to achieve this specification with the tooling available to us. They also discussed the importance of having a large experimental platform to anchor experimental tools like micro fluidic lines. This led us to question our initial assumption that overhanging the sample was the best way to facilitate microfluidics experimentation. Hanging lines have the potential to catch and dislodge the sample. Without a large support platform, this could lead to damage of the optical components under the stage. Drawing from both our ideation results and sponsor comments, we decided that the best stage design involved retrofitting a used stage to use the linear actuators available in the Microfabrication Laboratory.

4.3.2 Proposed Manufacturing Process

Even though we chose to purchase the stage assembly, we knew manufacturing would be necessary to adapt it for use in our microscope. All the used stages we saw for sale were designed for manual conventional microscopes. This meant that they had limited to no clearance underneath, and, in some cases, restricted actuation capabilities through integral threads. We felt that the retrofitting process would be significantly easier if our stage had decent clearance and is designed for use with linear actuators. Figure 4.3.3 displays the stage that we purchased. We anticipated that the stage may require the following modifications:

- The addition of physical stops or limit sensors on each travel axis.
- The removal of the stage center to allow for inverted observation. We plan to fabricate removable plates for the stage center to allow for the adjustment of the viewing field.
- The attachment of our linear actuators to each stage axis. This may require the fabrication of coupling pieces.
- The creation of threaded mounting holes on the stage surface.

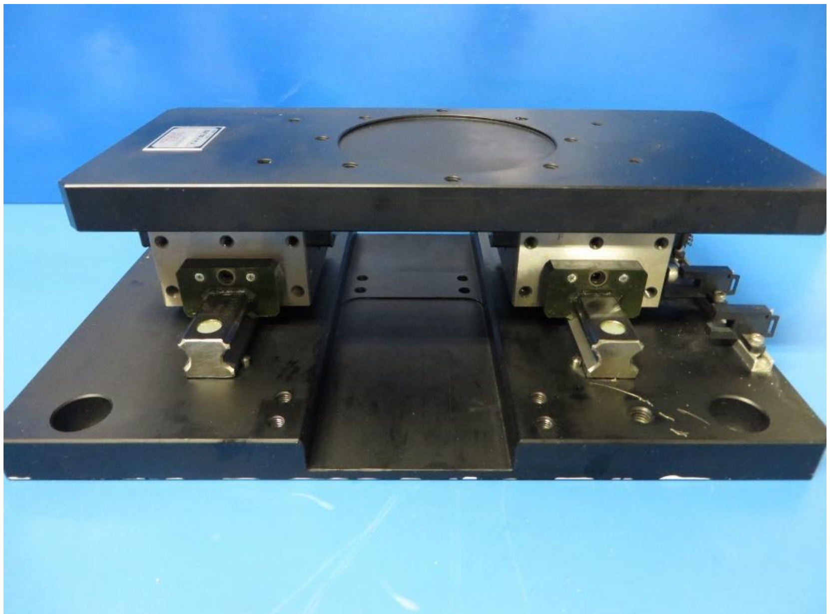


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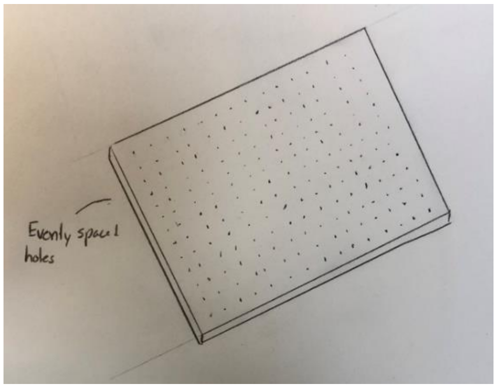
Figure 4.3.3. Image of one of the stages that we purchased. This option has a good deal of room underneath, and pre-aligned attachment points for actuators.

4.4 FRAME

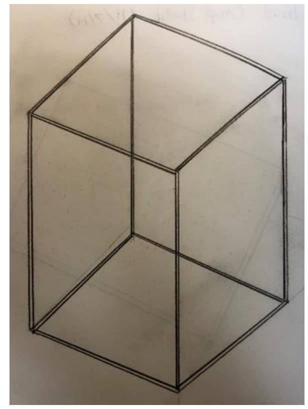
Like the stage, a rigid frame is a necessity in a microscope's reliability. The frame secures the components and allows the microscope to function. Experiments can also be secured onto the frame so the sample may be observed. This is especially important for this type of microscope, as often there are parts of an experiment that do not need to be directly observed through the lens.

4.4.1 Ideation

We followed a similar ideation process for the frame as we did for the stage. The main concerns for the frame are rigidity and the ability to contain the components. We ideated two possible ideas, a simple breadboard that allowed for the positioning of components, and a cage structure that would allow components to be attached to the sides, keeping the base open. Figure 4.4.1 below shows these two concepts as concept sketches.



(a) Breadboard frame



(b) Cage Frame

Figure 4.4.1. (a) Shows an optical breadboard which allows for components to be placed wherever they need to be in premade holes; (b) is a sample cage design for the frame, consisting of a cube-like structure that encases the entirety of the microscope, allowing for components to be attached to the outside. The breadboard is sturdy but has the possibility of being cluttered with components. The cage allows for vertical mounting but will not be as strong as the breadboard.

Following the design and purchase of the stage, we needed to include a way to have Z-Axis travel within our microscope. This can either be done by moving the sample up and down, or by moving the optical components below the stage up and down while maintaining the distances between the optical components.

After the initial ideation process, concept models were created to demonstrate basic ideas and assess functionality. We did not create a concept model of the optical breadboard, since these are readily available for purchase; therefore, we would not need to construct one should we decide on this design. Figure 4.4.2 shows a concept model of the cage-like frame.

Figure 4.4.2. Concept model of cage-like structure for frame design. Shown with stage attached to the bottom rail, and brightfield light hanging from top shining on the stage.

4.4.2 Selection Process

After taking inventory of the components available for our use, we found an optical breadboard, and decided to implement it in our design. We planned to incorporate an overhead frame, consisting of one beam connected to posts on either side and attached to the breadboard. The frame is be made of 80/20 T-slot aluminum and is attached to the base using brackets. We chose to incorporate the Z-Axis travel by moving the optical components beneath the stage. This was decided to alleviate the possibility of stretching or disconnecting the microfluidic lines that will be connected to the sample. This frame design is shown in the concept CAD model.

4.4.3 Possible Risks

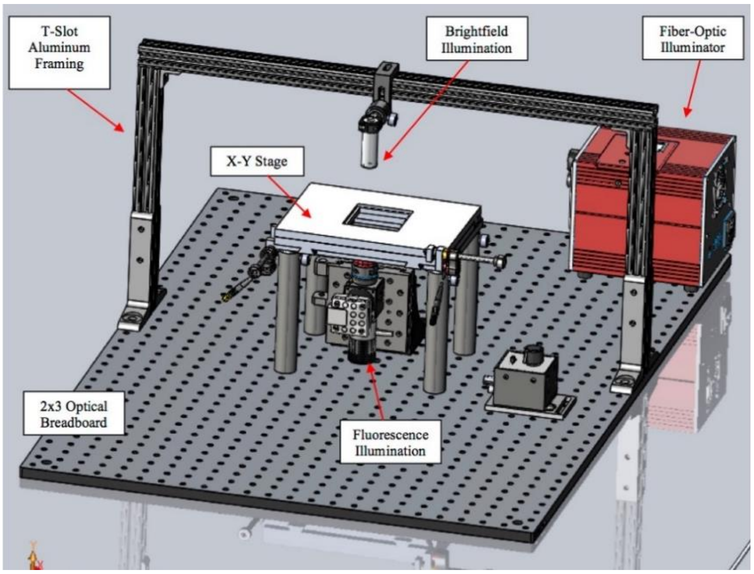
Our frame design is relatively simple, with no moving parts. After analysis of the design, the only apparent risk or hazard associated with the frame is the breadboard. Because the optical breadboard is heavy, it provides a hazard if it were to fall on someone. To eliminate this risk, we thought about the breadboard on either a solid table surface or atop a cart that is weighted down.

4.5 Final System Integration

Following the selection of the best design direction for each subsystem, these solutions were integrated into a final main microscope system. To illustrate our intended path forward, we built a conceptual SolidWorks model and a conceptual prototype from readily purchased materials.

4.5.1 Concept CAD

When devising a conceptual model in SolidWorks, we made extensive use of part files provided by ThorLabs and McMaster-Carr to produce a preliminary concept assembly that closely resembles our intended design direction. Some of these parts will be sourced from other vendors or are already available in the Microfabrication Lab. The model was not a perfect rendering, but it outlined the general form and function of our initial design concept. Screen captures of the preliminary CAD model, as shown in Figure 4.5.1, are labeled in greater detail in Appendix [H], coinciding with part names and letters listed in the Bill of Materials.



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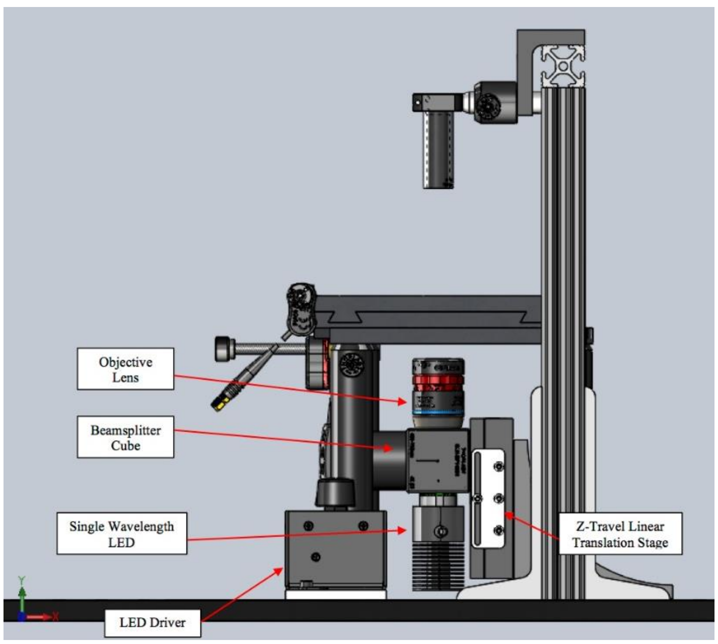
(a)



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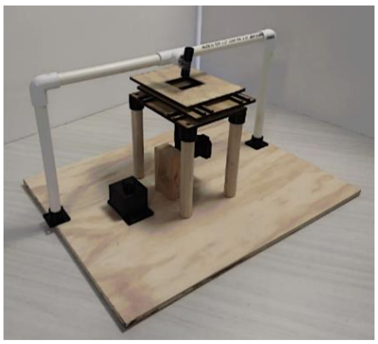
(b)

Figure 4.5.1. (a) Isometric view of Concept CAD model; (b) Side view of concept CAD model, showing details of fluorescence components and optical pathway.

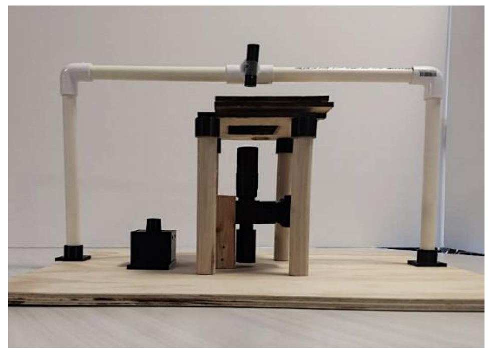
A Bill of Materials listing all "stand-in" components sourced from ThorLabs, McMaster-Carr, and various other vendors is included in Appendix [I]. At this stage in the project, the predicted expenditures for a first-case design using a cheaper objective lens, a stage sourced from eBay, and the already available optical breadboard were roughly $550. With an allotted budget of $2,200, this allowed funds to be allocated to more critical system components.

4.5.2 Concept Prototype

The CAD model proved to be useful starting point, but we understood what we wanted from the project by building a physical concept prototype, as shown in Figure 4.5.2. We built our model out of plywood, PVC, and PLA. The two processes that we used in the prototyping were 3D printing and laser cutting. This gave us an easily modifiable physical way to explore our design space. Right away we saw that we were not spatially confined. We had the room to place our stage slides wherever we needed on the breadboard. We also realized that it will be challenging to ensure that our stage has the clearance to reach full travel with large optical components underneath. We believed making the stage large or locating the actuation system away from the optics will be the best way to ensure sufficient travel.



(a) Isometric View



(b) Front View

Figure 4.5.2. Image of the final Concept Prototype.

4.5.3 Design Hazards / Potential Risks

As we devised new design ideas and began to converge on a final concept, it became necessary to assess our solutions from a safety standpoint. Commercial microscopes are usually contained within a cast body, and so there are very few safety concerns to make note of. However, since the design we have decided to proceed with is more of an open design with the intent to ensure room for experiments, there were a number of potential hazards we needed to keep in consideration when developing our design further.

We decided to affix all our microscope components to a large optical breadboard; the breadboard is extremely robust, weighing in at approximately 250 lbs. While this is advantageous for eliminating optical misalignment due to vibration, it brought about the potential that the breadboard may fall, causing injury. To alleviate this concern, the breadboard was placed on a flat top file cabinet with rectangular edges, and other equipment was placed in the lower cabinets to make the unit bottom-heavy enough to be resistant to tip-over.

When ideating the stage, another hazard that we were aware of is the potential for finger entrapment due to moving slides. However, we did not believe this to be a large concern with the commercial microscope stage, which has likely already accounted for these pinch points. A more extensive list of hazards and considerations is available in Appendix [R].

5 FINAL DESIGN

Following extensive concept iteration, consultation with project sponsors and experts, and analyses and testing for design decision verification, we converged upon a final design and procedure of project completion to move forward with. This chapter details the design direction of each subsystem, including optics, microscope body structure, and translation stage design and actuation, as well as the integration of subsystems into a final product.

Prior to buying components, a purchase list was provided to project sponsors for review and confirmation. Optics and optomechanics were sourced from Thorlabs Inc. and fasteners and raw materials from McMaster-Carr. Total component and manufacturing expenditures were less than $1,600, which falls well within the constraints of the project's $3,000 hard-cap budget. We suggest sponsors use the remaining funds to upgrade to a higher-magnification objective lens with a long working distance. An objective of this quality costs ~$900-$1000 but still fits within the project budget and will result in a higher resolution image.

The detailed final project budget is attached in Appendix [Q]. Note that the expenditures listed equate only to approximately $1,550 as some of the components had already been purchased.

5.1 OPTICAL SYSTEM

The final microscope design is capable of both brightfield and fluorescence microscopy, requiring the use of two different light sources and two optical paths. While both systems will not be in operation concurrently, design choices were made to ensure that neither path interfered with the performance of the other in each mode.

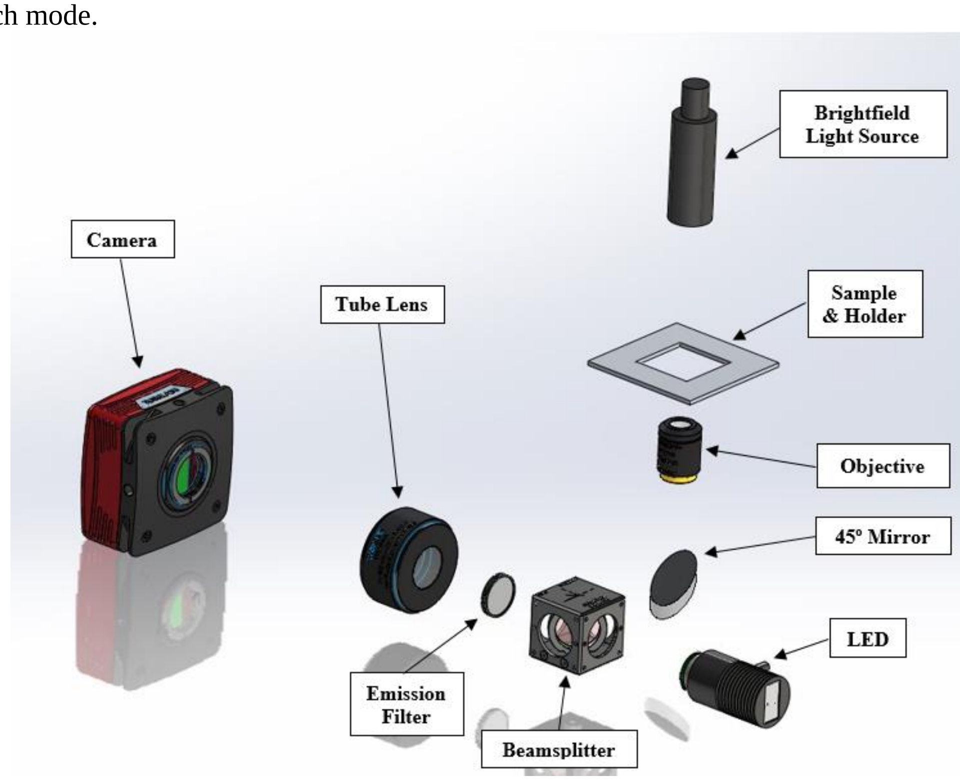


Figure 5.1.1. Exploded view of sample illumination components and microscope optics, including elements for both brightfield and fluorescence mode.

5.1.1 Brightfield Mode

Few changes were made to the brightfield illumination configuration and components since the Preliminary Design Review, other than to modify the path in coordination with the slightly more complicated fluorescence optical system [Figure 5.1.2].

In the final design, the primary brightfield light source is a Dolan-Jenner Fiber-Lite Model 3100 fiber-optic illuminator. Its port is connected to a flexible gooseneck fiber-optic cable, allowing the user to position it at the desired angle of incident light. At the end of the gooseneck, a lens attachment collimates the light into parallel rays, providing uniform illumination of the sample. The collimator is mounted to the top horizontal member of the microscope's 80/20 T-slot aluminum frame gantry with a 3-D printed slim right-angle bracket, Thorlabs post holder and post, and a 1-inch diameter slip ring, allowing the user to adjust the position of the light in two axes to provide the best illumination.

After light passes through the sample, it comes to a focus at the objective lens. For proof-of-concept testing, the microscope utilizes a Plan Fluor 4X Infinity-Corrected objective lens sourced from AmScope Microscope Superstore, but in future iterations of this project will be altered for long-working distance objectives with higher magnification. Light passing through the objective emerges collimated in the "infinity region," and it is reflected by a 45° mirror into a 50:50 beamsplitter. The beamsplitter passes 50% of incident light and reflects the other 50% at a 90° angle, so it is expected that half of the light will be lost to the surroundings. With an adequately high-intensity light source, this will not be detrimental to image quality.

Reflected light from the beamsplitter, still collimated, is focused by a tube lens. The tube lens selected for this design has a focal length of 200mm, a standard specification for tube lenses used in conjunction with infinity-corrected objective lenses. An image is produced on the camera CCD, placed 200mm away (a working distance of 148mm from the back plane of the lens), at the focus of the tube lens. The length over which the light converges is shrouded by a series of lens extension tubes to eliminate transmission losses.

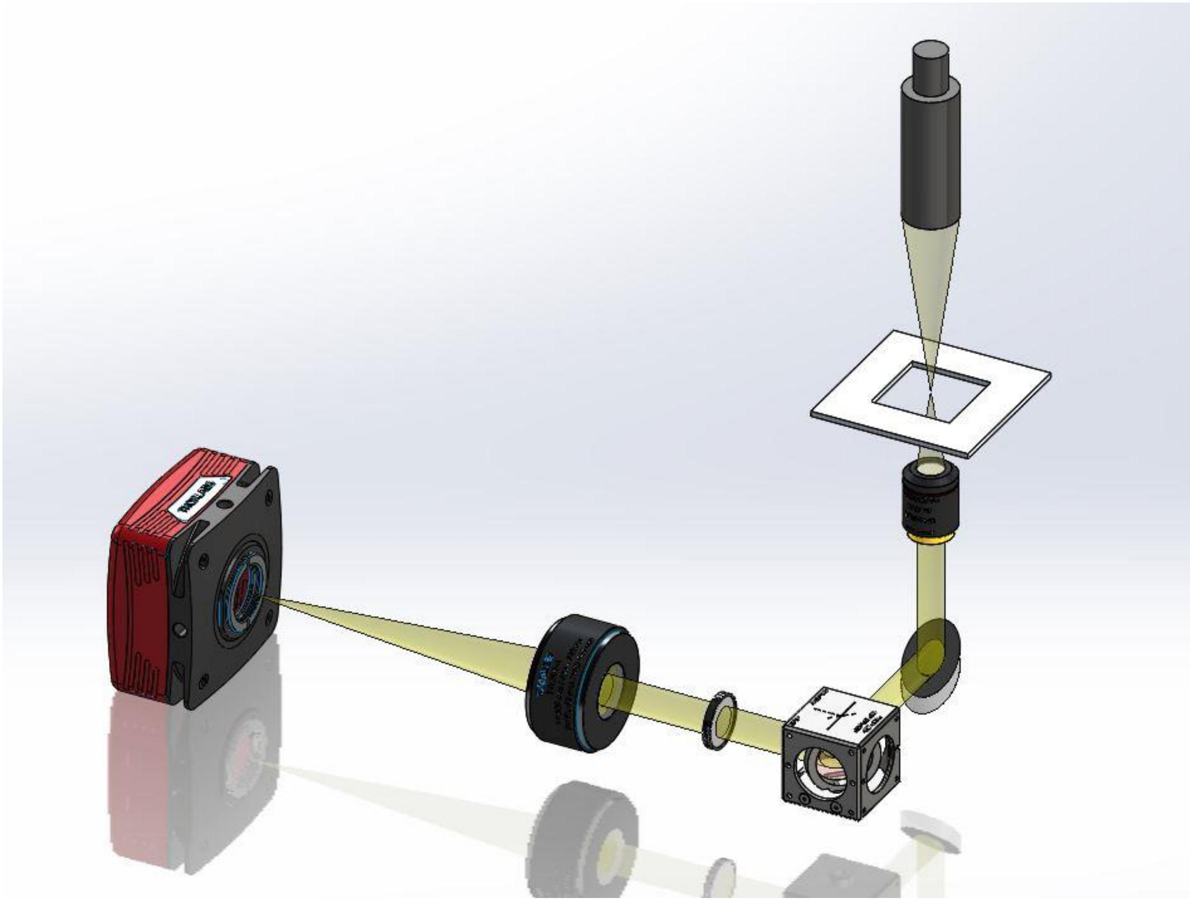


Figure 5.1.2. Exploded view of optical train, demonstrating brightfield mode light path.

5.1.2 Fluorescence Mode

Fluorescence illumination of the sample is accomplished using a single wavelength LED of 490nm, powered by a Thorlabs LED driver connected to an accompanying fiber-optic cable. The microscope optics are selected for one test-case wavelength, indicator dye, and filter combination but the optical train is modular to allow the user to swap out the emission filter and LED for compatibility with a different fluorescent dye. From the LED, light passes through the beamsplitter. Whereas a traditional fluorescence microscope requires an excitation filter to select a wavelength corresponding to that which excites the fluorophore, our final design does not feature one since the chosen light source is already a single wavelength. Reflected light from the beamsplitter is redirected 90º towards the 45º mirror, and reflected upwards towards the objective, where it is focused on the sample at a working distance of 16.3mm. The fluorescence excitation light path, from LED to sample, is pictured in Figure 5.1.3.

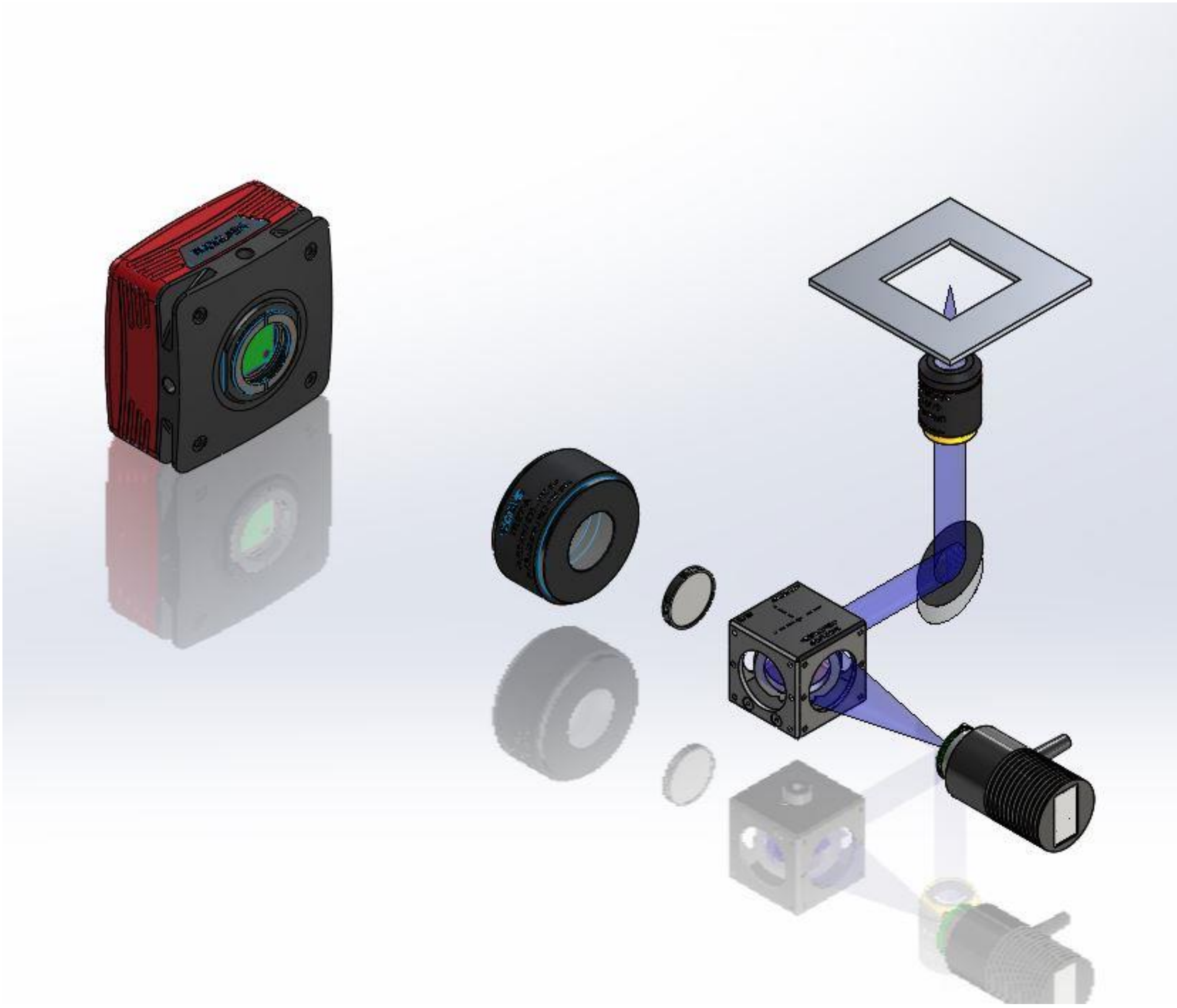


Figure 5.1.3. Exploded view of optical train, demonstrating fluorescence mode excitation light path. Blue color denotes the “short” excitation wavelength from the LED (490nm).

Fluorescent dye excites at a particular wavelength and fluoresces at a longer emission wavelength due to energy losses. A more detailed analysis of the proposed test case is outlined in a later section. The filtered emission light passes through the imaging system described, and fluorescence images are captured by the camera. The light path of fluorescence emitted from the sample is shown in Figure 5.1.4.

In documented applications of fluorescence microscopy, it has been frequently noted that fluoresced light has much lower intensity than its respective excitation photons and can therefore be difficult to image. This is one of this project’s primary concerns; the imaging system has been designed to minimize transmission losses to the camera to mitigate this issue. In a stagnant test case with the camera set to a high exposure time, we expect the system to have capability of resolving an adequate image.

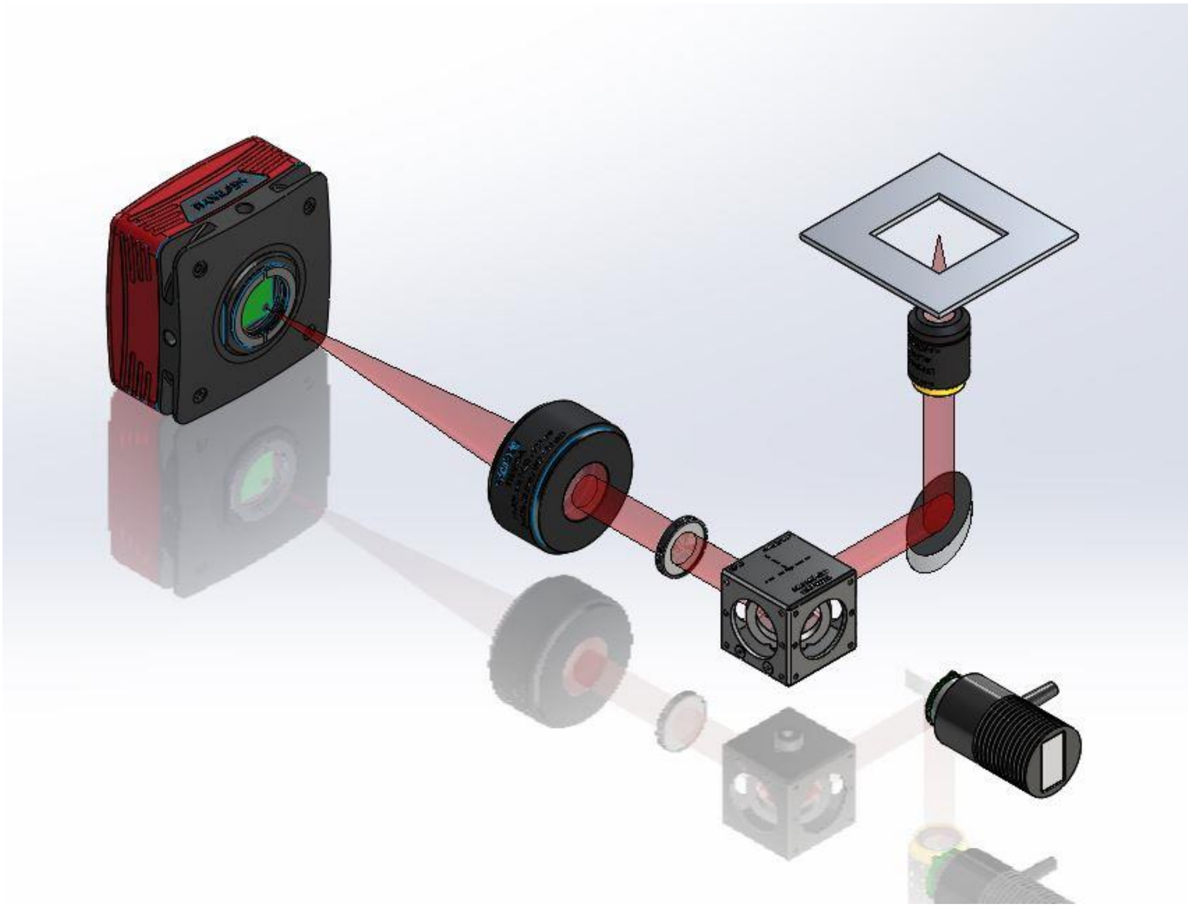


Figure 5.1.4. Exploded view of optical train, demonstrating fluorescence mode emission light path. Red color denotes the “long” emission wavelength following energy losses induced by interaction with the sample (530nm).

5.1.3 Mounting the Optics

Prior to the Preliminary Design Review, the optical pathway was depicted in a form showing only the elementary components with no consideration for mounting, distances between components, or alignment. To attain the primary specification of resolving an image in both brightfield and fluorescence modes, we have both designed and sourced optical mounts with considerations for compactness, rigidity, and modularity for implementation into the final microscope design. We chose to move forward with a principally horizontal optical train for ease of assembly, structural rigidity, space consideration, and alignment precision.

Objective Lens:

Initially selecting an infinity-corrected objective lens introduced a lot of design flexibility into the layout of the rest of the optical components. In the final configuration, the objective is mounted in its upright position on a 3-D printed rigid bracket holding a Thorlabs RMS-threaded objective mount. The RMS mount’s outer plane has a single flat surface with an 8-32 tapped hole, typically used for attachment to optical posts. In our application, this threaded hole will be used for fixture to the bracket, which has a through hole, allowing the mount to be fastened to the bracket using an 8-32 screw. The design features slots compatible with ¼”-20 hole-spacing to secure the bracket to a linear slide for z-axis focus capability.

The focusing slide is fixed to the vertical face of an angle bracket, while the bracket's other face is mounted atop a secondary slide, allowing for y-axis precision positioning of the objective underneath the sample. The objective mounted in the bracket and attached to the z-axis focusing subassembly is pictured in Figure 5.1.5. The components highlighted in gold depict the z-axis focusing assembly, which is composed of two Newport single-axis linear slides and a 90° angle bracket between them. Drawings and details of the objective lens assembly component layout are further specified in Appendix [K].

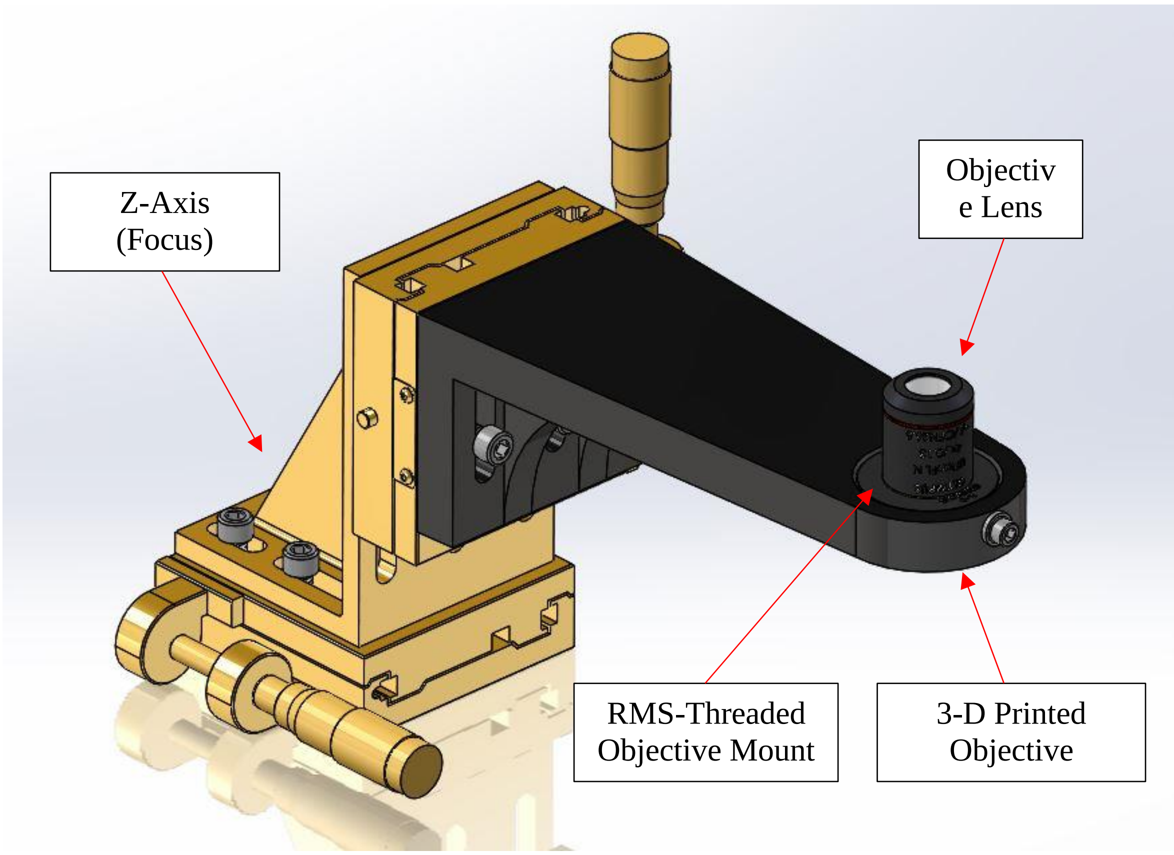


Figure 5.1.5. This figure displays the mounting of the objective to a 3-D printed bracket and fixtured to two linear translation stages for precision adjustment below the sample and focus.

45º Mirror:

Fixing most of the optical train to the breadboard in a horizontal orientation requires the use of a 45º mirror to reflect the beam from the objective to the entrance window of the filter cube. For this application, we chose to use a plane aluminum mirror and a preset mounting assembly provided by Thorlabs for ease of assembly and integration. The package includes optic housing compatible with 1" round economy-level mirrors, a 1.5" post, and a universal slotted base plate. The mounting assembly has a beam height of 1.98"; this needs to be elevated to adjust for the height of the fluorescence illumination components, so an optical post of length 1.5" will be added to the assembly to provide extra height. Product details and drawings outlining the assembly of the optic in its housing are shown in Appendix [K].

Filter Cube:

With regards to filtering components, the final design, like the concept design, implements a 50:50 beamsplitter housed in an 30mm optical filter cube. As previously expressed in Concept Design (Chapter 4), this component was chosen due to its modularity exceeding the typically used, wavelength-selective dichroic mirror. In the final configuration, the cube is secured with a post and post holder to a linear translation stage shared with the LED. It is desirable for the LED and the filter cube to be close together within the light path to reduce the change of aberration and maximize transmission. Since neither component needs to be adjusted horizontally with respect to the other, they can be fixed to the same stage and moved simultaneously. The emission filter is housed in an SM1 lens tube for ease of replacement and connection to the imaging train.

Imaging Components:

Selection of an appropriate tube lens was the driving factor in the design of the optical train's imaging system. For infinity-corrected objective lenses, a tube lens of focal length 180-200mm is standard; our microscope implements a lens of 200mm focal length and a 148mm working distance from the lens shoulder to the camera. The imaging assembly is composed of primarily Thorlabs threaded extension tubes, industry-standard thread adapters, and lens tube mounting components. From connection to the filter cube assembly to the imaging plane, the imaging train is as follows:

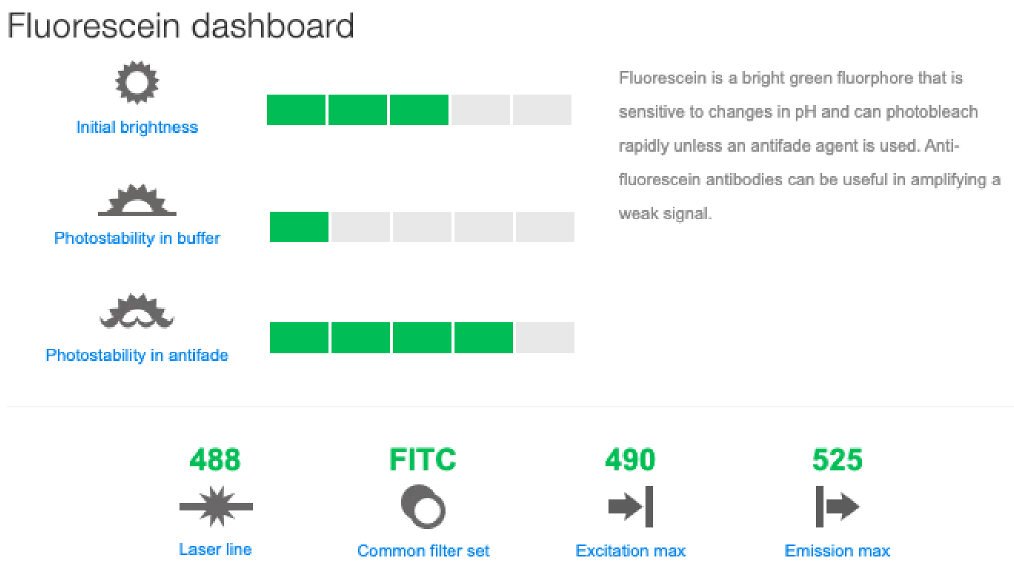
- Tube Lens, f = 200mm
- SM2 Extension Tube, 3” (76.2mm) Thread Depth
- SM2 Extension Tube, 2” (50.8mm) Thread Depth
- SM2 Extension Tube, 0.5” (12.7mm) Thread Depth
- SM2 Extension Tube, 0.3” (7.62mm) Thread Depth
- Thread Adapter, Internal SM2 Threads and External C-Mount Threads
- Evolution LC MegaPixel Firewire Camera

The sequence of extension tubes, summing to the 148mm tube lens working distance, reduces fluorescence transmission losses from the lens to the camera. A slip ring and optical post assembly supports the imaging subsystem near the extension tube-camera junction. Detailed assembly drawings are included in Appendix [K].

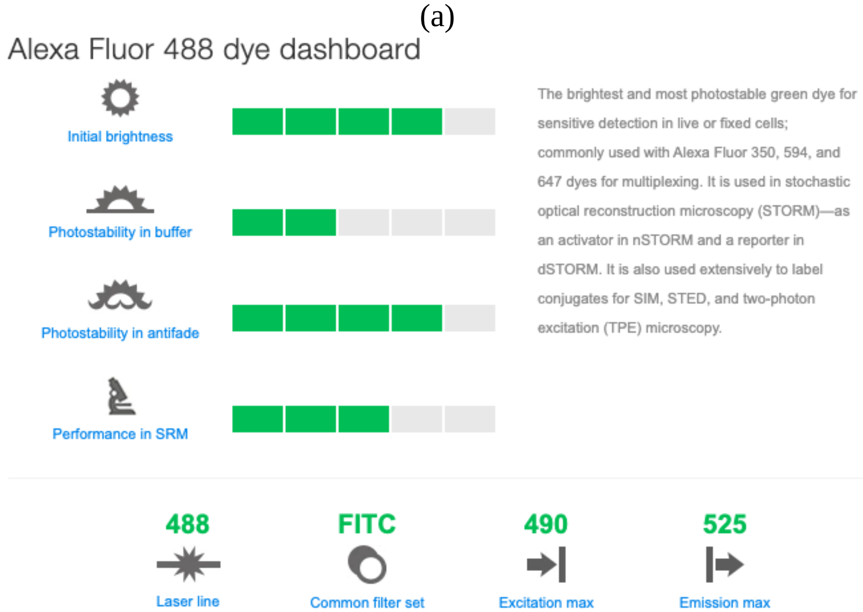
Test Case:

The test-case scenario features fluorescein, a common fluorophore for microscopy applications, that excites at the LED wavelength 488nm and produces a fluorescent signal at 530nm. The commonly known corresponding filter set is FITC; this is the standard our testing will follow to resolve a fluorescent image. The microscope operates using a single-wavelength LED emitting light at 490nm to excite fluorescein dye injected into the sample. The emitted light is filtered through a 530nm bandpass FITC filter. Specification sheets for fluorescein and its analogous filters, including details on spectra and transmission, are included in Appendix [O-8].

A different indicator dye, Alexa Fluor 488, was also considered for the test case. It has excitation and emission spectra nearly identical to that of fluorescein, thereby requiring the same FITC filter set. Alexa Fluor has a greater initial brightness than that of fluorescein, so could be easier for the camera to detect its fluorescence. Both dye options have been suggested, and it is at the discretion of our project sponsors to select one based on cost and preference for continued use in the Microfabrication Laboratory. Light properties of both contender indicator dyes are compared in Figure 5.1.6.



(a)



(b)

Figure 5.1.6. Comparison of the light properties of the two potential fluorescent indicator dyes utilizing the FITC filter set: (a) fluorescein, and (b) Alexa Fluor 488.

5.1.4 Analysis

A design consideration critical to product function and performance is the ability for the microscope to maintain its focus on the sample during use. When selecting mounting components for the objective lens, this was a primary concern; after choosing to implement a 3-D printed mounting bracket in place of rigid, industry-standard optical mounting components, it was necessary to perform some analysis.

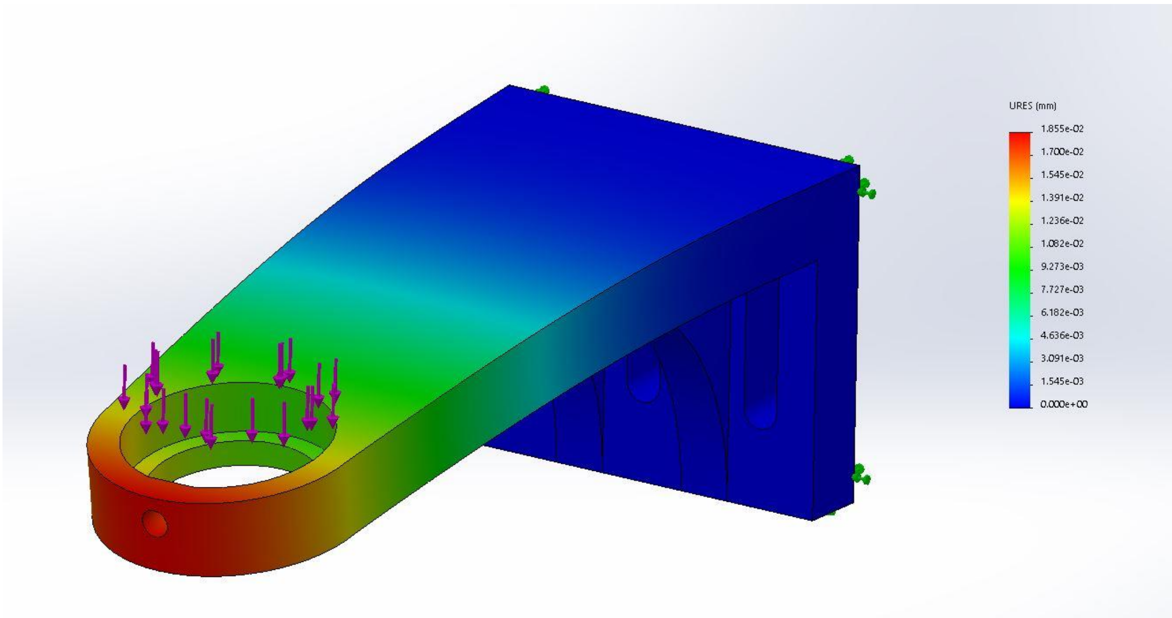


Figure 5.1.7. This figure displays SolidWorks Simulation results of deflection due to loading on the circular face of the objective mounting bracket. The bracket notices a maximum deflection of 18.55μm.

Finite Element Analysis was performed on the mounting bracket design using SolidWorks Simulation and modeling the part as ABS plastic with a fixed back face to simulate attachment to the z-axis translation stage [Figure 5.1.7]. A load of 0.75N representing the sum of the weights of the objective lens (~0.55N) and the RMS-threaded Thorlabs mount (~0.2N), with a built-in factor of safety of 1.5, was applied to the circular planar surface upon which the threaded objective mount sits. The bracket deflects 18.55μm, which does not surpass the 25μm parallelism deviation constraint established in the "Engineering Specifications" table (Chapter X).

Analyses to be performed:

- Following component purchasing, the objective lens will be tested directly with the tube lens and camera to ensure adequate image resolution in the simplest configuration.
- Since the objective mounting bracket was modeled as ABS plastic but will be printed in PLA (not an available material in SolidWorks), we intend to run a load test to confirm result

5.2 STAGE

The stage of the microscope is a platform that allows the user to position and hold the sample under observation. Our stage is electrically actuated, and spring loaded in the X-Y plane. The stage was built using a used microscope stage purchased on eBay. This stage came on pre-aligned THK linear rails with many mounting points already drilled.

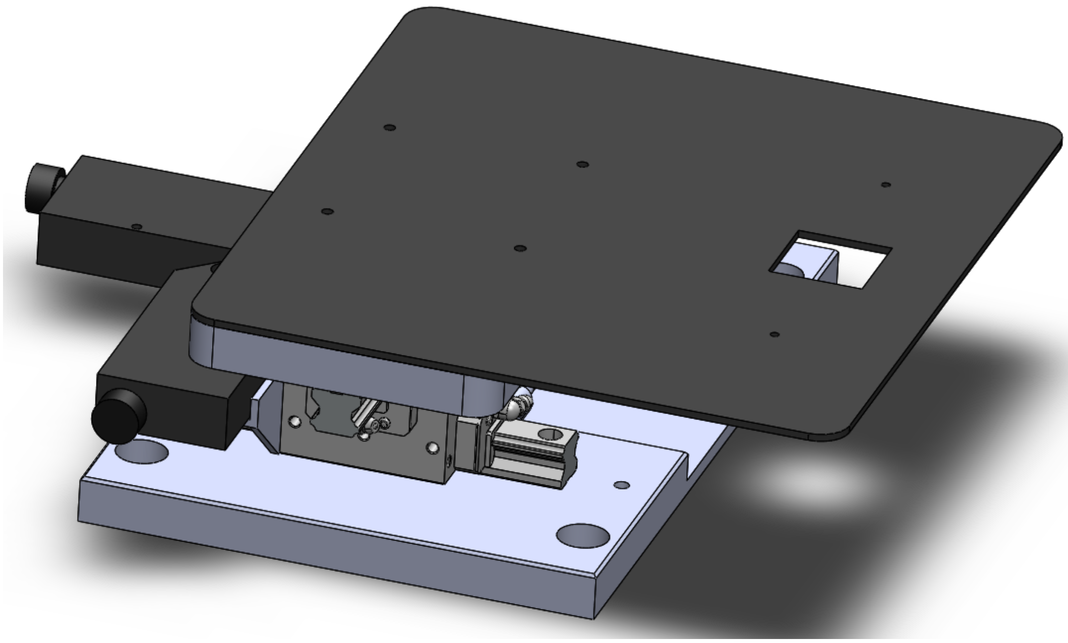
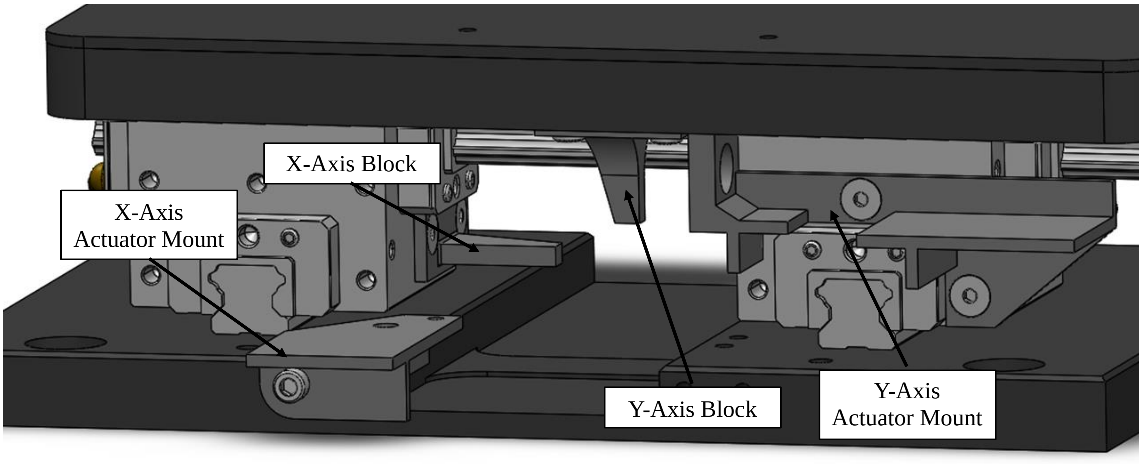


Figure 5.2.1. This figure shows the stage system isolated from the rest of the microscope. The stage base plate is 8.25” x 12” and the entire stage is just under 4” tall.

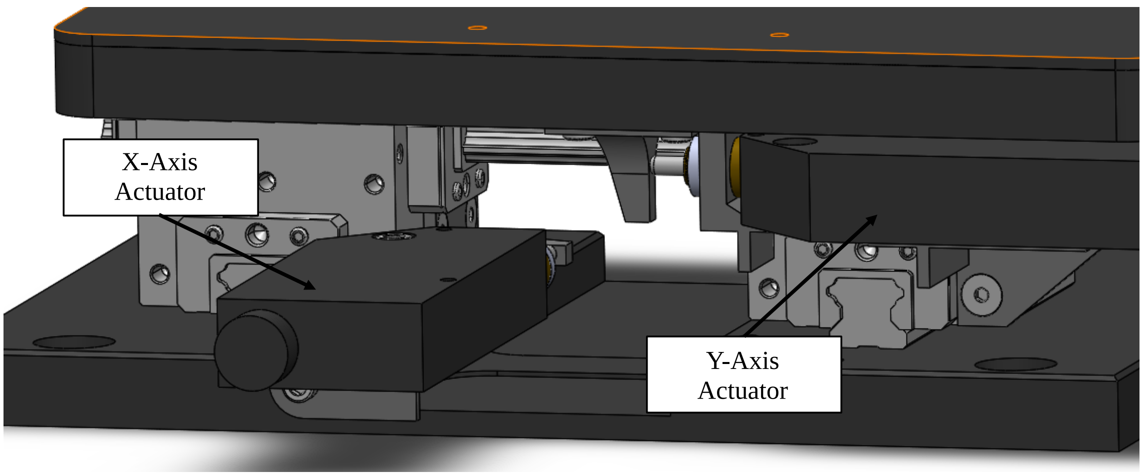
Because the stage we purchased was a complete mechanical system, fabrication was greatly simplified. Since the components were already aligned, staying within our motion tolerances also became far easier. The only drawback of using this stage was that it required us to cantilever the observation region and place the whole stage on posts. This was the only way to achieve the clearance required for our optical components.

5.2.1 Actuator Mounting

The stage is driven with two Newport 850G Linear Actuators. These actuators are mounted onto the stage using custom 3-D printed brackets. The actuators push on 3-D printed blocks that screw onto the stage. The brackets and blocks take advantage of the mounting holes already present on the stage. Figure 5.2.3 shows where our printed parts fit on the stage.



(a) Rear of the stage with actuators removed



(b) Rear of the stage with actuators installed

Figure 5.2.2. The actuators are located at the rear of the stage. Each actuator is held with a custom bracket and applies force to a push block. The spring return is hidden in this view. These paired pictures show how the actuators interface with our brackets.

5.2.2 Spring Return System

The stage actuators that our sponsors provided are only able to apply a force in one direction. This requires that the stage axes be spring loaded to return the stage to its zero-travel position. To achieve this, we created three brackets that hold extension springs. Each axis has one independent bracket; both use a combined bracket connected to one of the bearing blocks. All the spring brackets use 3-D printed bases to hold press fit steel pins. The pins retain the extension springs using machined grooves.

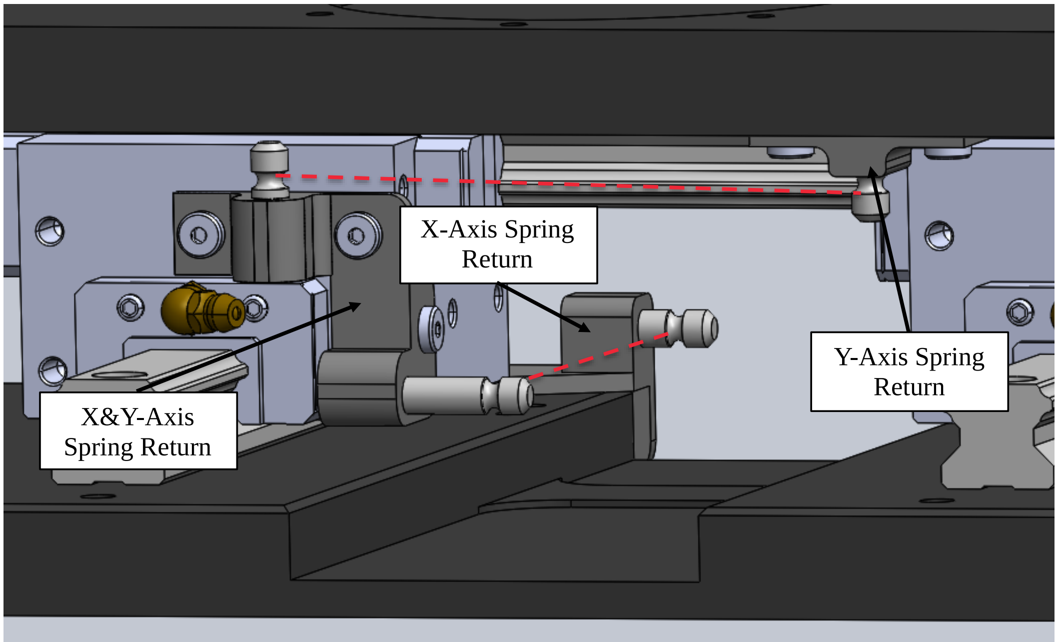


Figure 5.2.3. View from the front of the stage looking at the spring holding brackets. The cantilevered stage plate and actuation systems are hidden to allow for a clearer view. After CDR we split the X&Y spring return bracket (shown above) into two separate parts. Functionally it is the same.

To size the stage springs, we needed to know the system limits. The upper bound was found in the actuator documentation (18 lbf upper capacity). The lower bound was found through testing. We ran a pull test on each axis of our purchased stage to find the minimum force needed to move our axes (setup shown in Figure 5.2.4). The test used a spring scale hoked to the stage using a 3-D printed pull tab. The scale was drawn back using a length of 1/4 -20 all-thread with an eye bent in one end. We found that both the X and Y axes only needed 1.5-2 lbs. to move. Once moving, very little force was needed to maintain motion.

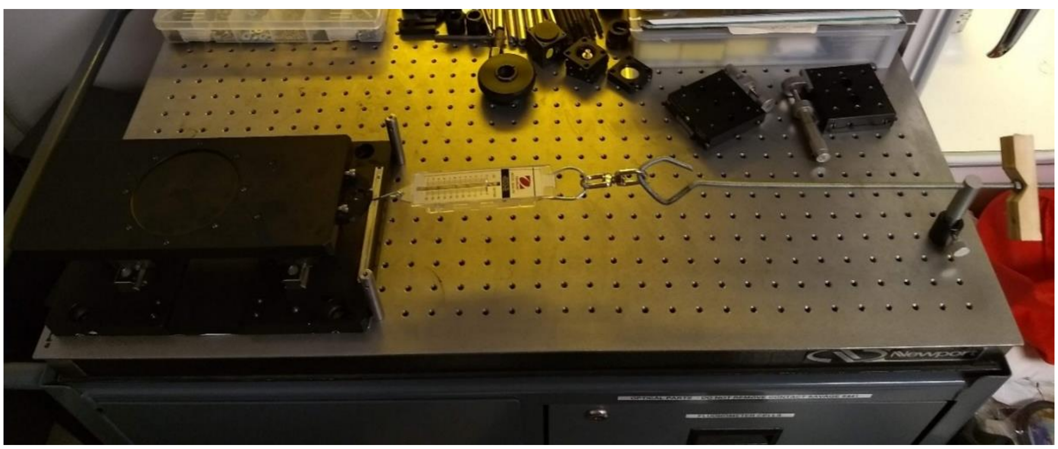


Figure 5.2.4. Experimental setup for stage pull test. This image shows the configuration used to pull the Y-axis. To connect the test device to the stage we 3-D printed two screw on pull tabs.

The actuator documentation and experimental findings allowed us to create force limits for our system. Using these with the travel limits set in our specification (0-2”) we were able to select extension springs that fit our system. We chose 4-inch steel extension spring with a force range of 2.65-12 lbs., and a travel of 2.34 inches. Figure 5.2.5 shows where the springs we selected fit within our system constraints.

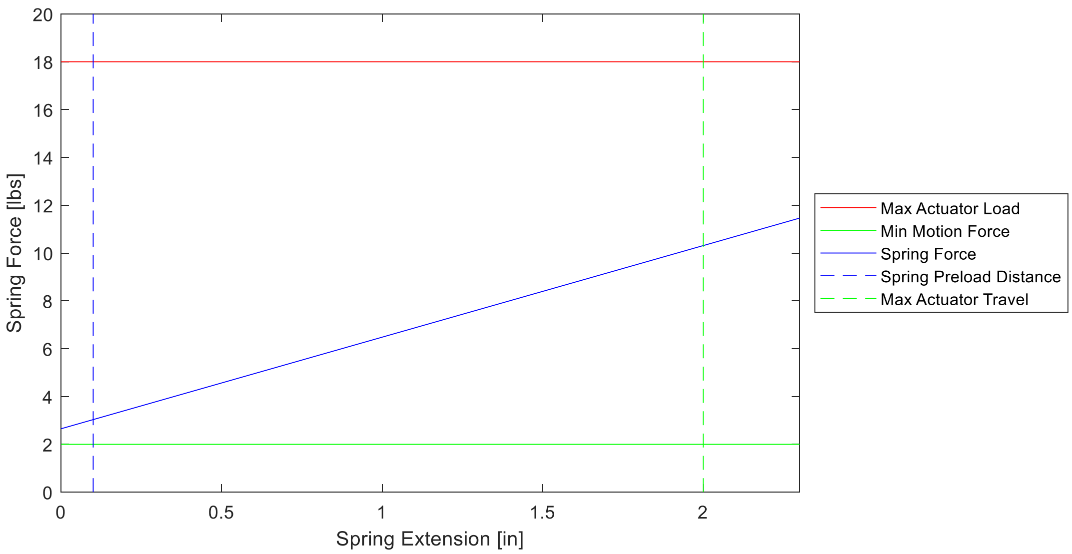


Figure 5.2.5. This plot shows where the extension springs we selected fall within our system constraints. We decided to choose springs with a low spring constant. Even though our system forces are low, it is important to reduce any unnecessary load. We aimed to keep the system as far as we can from the actuator’s limit.

5.2.3 Sample Holding

To hold the sample steady for analysis, we decided to use an overhung plate attached to the top of the stage using pre-existing holes. The plate will be 3mm thick aluminum, that will be cut with a waterjet cutting machine. This plate will feature threaded holes near the sample window where stage clips will be attached to hold the sample in place. To minimize the reflected light off the plate, our sponsor has requested that we anodize the plate black.

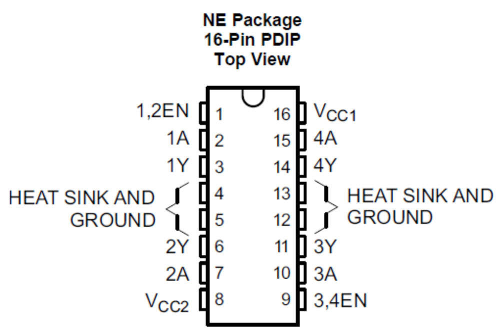
5.3 ELECTRICAL CONTROLS

A significant component to the microscope is its ability to move the stage to see the entirety of the sample from the objective. While using simple stage travel micrometers is traditional in many microscopes, the sponsors wished for more modern maneuverability. From this, we decided that the state will being using actuators with an Arduino® control system. In this control system, the actuators will be able to operate in two modes: Joystick and Programmable Path.

5.3.1 Actuator Hardware

The actuators provide the stage movement required instead of using a traditional micrometer stage travel. The actuators used in this project are both 850 G series actuators produced by Newport. In these actuators there is a DC motor along with an encoder to keep track with said motor's positioning. The encoders have a resolution of 0.6051 µm per encoder reading ensuring precise movement. The 850G series also contains forward and reverse limit switches to prevent over-extrusion and over-retraction of the actuator plunger. The actuator motors can operate between 5V and 12V to directly adjust their speed.

The actuator system uses a standard L293D motor driver by Texas Instruments, which can operate two DC motors simultaneously. As shown in Figure 5.3.1, the L293D has 4 driver inputs, 4 driver outputs, 2 enable pins, a logic input voltage and motor voltage input. Each enable pin dictates the motor speed for their respective motor which is dependent on the voltage delivered to said enable pin.



Pin Functions

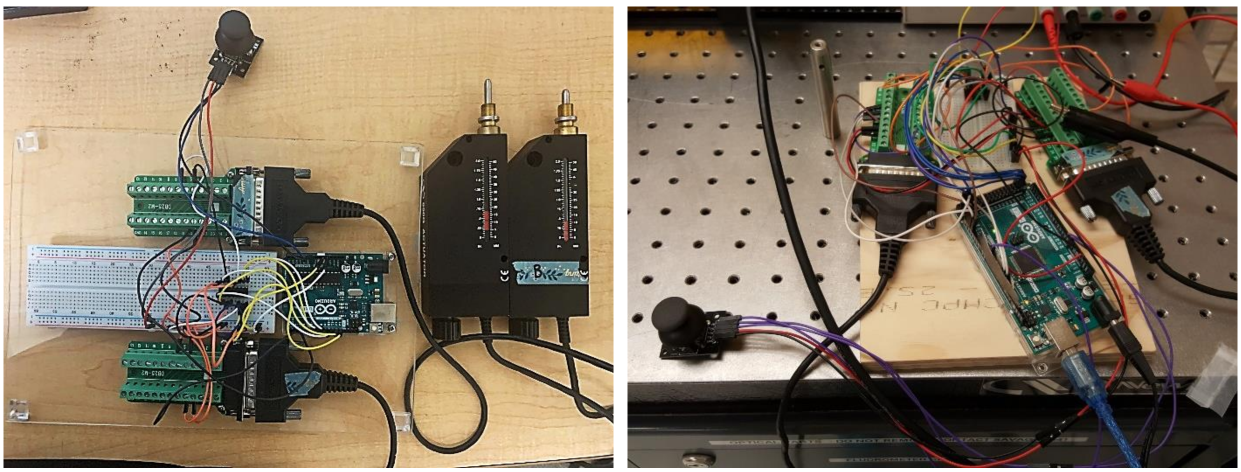
PIN		TYPE	DESCRIPTION
NAME	NO.		
1,2EN	1	I	Enable driver channels 1 and 2 (active high input)
<1:4>A	2, 7, 10, 15	I	Driver inputs, noninverting
<1:4>Y	3, 6, 11, 14	O	Driver outputs
3,4EN	9	I	Enable driver channels 3 and 4 (active high input)
GROUND	4, 5, 12, 13	—	Device ground and heat sink pin. Connect to printed-circuit-board ground plane with multiple solid vias
V_{CC1}	16	—	5-V supply for internal logic translation
V_{CC2}	8	—	Power VCC for drivers 4.5 V to 36 V

Figure 5.3.1. L293D Pinout diagram taken from Texas Instruments Datasheet

The Arduino® Mega acts as the main controller of the system and allows for the user to interface with the actuators. The Arduino Mega contains 54 digital pins, 15 of which can provide Pulse Width Modulation (PWM), and 16 analog input pins. The large number of pins are required for the following functions we wished to use:

- 2 encoder channels to provide the 0.6051 μm resolution
- Forward and reverse limit switches
- Maneuverability with a joystick

Originally, the Arduino® control system, designed by Dr Hawkins, used an Arduino® Uno which contains a smaller number of pins. The small number of pins was suitable for the original design because the actuators were only using 1 of their 2 encoder channels used for position monitoring. As we added the joystick to the control system, we realized the Arduino® Uno did not have enough pins. We exchanged the Arduino® Uno for the Arduino® Mega to ensure the limit switches and other encoder channels can be monitored as well.



(a) Original Design (b) Current Design

Figure 5.3.2. Upgrades to the Arduino® Control system were necessary for full development of the system. (a) displays the original design with the joystick added to the system. Only one of the two encoder channels were used on both actuators which decreased the resolution of the encoder counts. (b) is the current design with the Arduino® Mega instead and a DC Power Supply connected to raise the motor voltage above 5V.

5.3.2 Control System Wiring

With the development of control system, the wiring become more complex with the addition of the joystick, limit switches and encoder channels. For easier visibility, the wiring diagram in Figure 5.3.3, was constructed. The wires are color coded with respect the following:

- Blue - Driver Inputs
- Orange - Driver Outputs
- Brown - Driver Enables
- White - Encoder channel readings
- Grey - Reverse Limit Switch
- Purple - Forward Limit Switch and Joystick Analog Readings
- Red - Positive Terminal
- Black – Negative Terminal

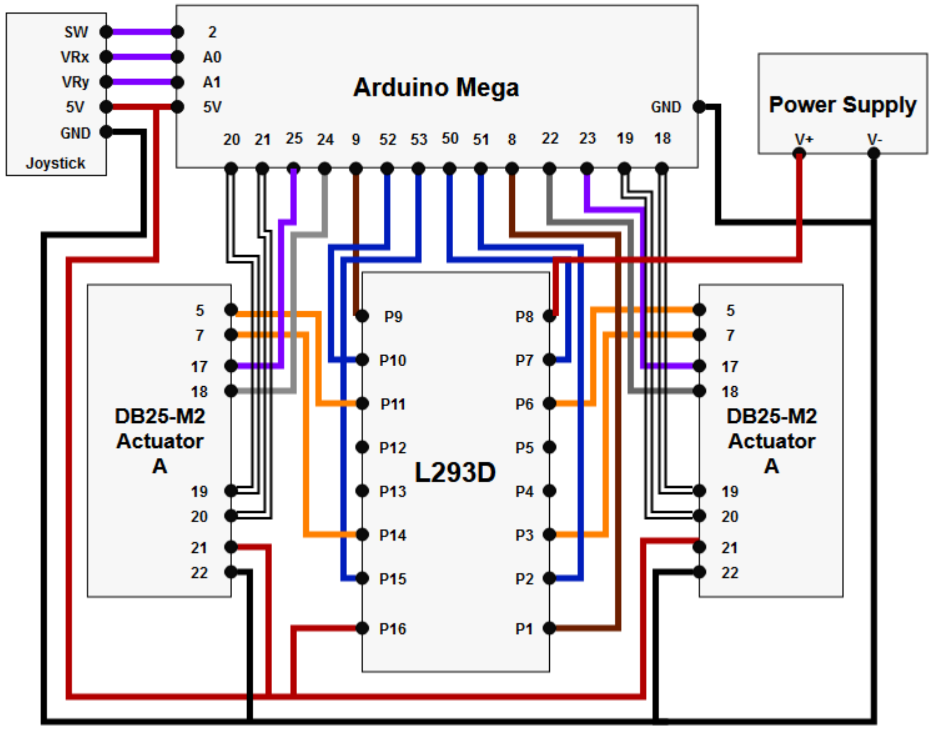


Figure 5.3.3. Wiring diagram of Arduino® Mega Control System to the L293D, DB25-M2 adapters, DC Power Supply and joystick.

The Arduino® Mega interfaces with the L293D motor driver with the most connections between two components in this system. The Arduino® microcontroller dictates the speed of the motors using PWM. The PWM duty cycle determines the duration of a high voltage signal is being sent thus controlling the speed of the actuators. While the PWN signal dictates the motor speed, the driver inputs controlled by the Arduino® Mega determine the direction the motor is rotating by alternating which input receives a high or a low signal. The internal logic of motor driver is powered by the 5V source the Arduino® mega delivers while the motor power is delivered by an external power supply. Table 5.3.1 displays the pin correspondence between the Arduino® Mega and L293D as a supplement for the wiring diagram.

Table 5.3.1: Arduino Mega to L293D Pin Correspondence

Arduino Mega	**L293D**	**Description**
8	1	PWM line to control speed of Actuator A
9	9	PWM line to control speed of Actuator B
51	2	HIGH/LOW Logic for 1st driver input of Actuator A
50	7	HIGH/LOW Logic for 2nd driver input of Actuator A
52	10	HIGH/LOW Logic for 1st driver input of Actuator B
53	15	HIGH/LOW Logic for 2nd driver input of Actuator B
GND	12	Grounds the L293D
5V	16	Supplies the logic of the motor driver. Do not exceed 5V
GND	5	Grounds the L293D
-	8 (PS+)	Power Supply voltage to the motors. Do not exceed 12V

Receiving readings is different with limit switches and encoder channels. Because the voltages are low, setting them Arduino® Mega pins to wait for a reading will be futile. To combat this, internal pull-up resistors are activated in the Arduino® Mega to raise the voltage of the reading, therefore when a limit switch or encoder is activated, the read voltage will be higher. All the pins used are digital pins which determine whether a reading is high or low. Table 5.3.2 displays the pin correspondence between the Arduino® Mega and DB25-M2 adapters as a supplement for the wiring diagram.

Table 5.3.2: Arduino Mega to Actuators Pin Correspondence

Arduino Mega	Actuator A	Actuator B	Description
20		19	Encoder Channel A
21		20	Encoder Channel B
18	19		Encoder Channel A
19	20		Encoder Channel B
25		17	Forward Limit Switch
24		18	Reverse Limit Switch
23	17		Forward Limit Switch
22	18		Reverse Limit Switch

The joystick uses potentiometers for the X and Y axis to determine its position. The analog pins of the Arduino® Mega are used generate an integer from the varying voltages that are created by the potentiometers. The joystick also uses a switch to change states but uses a digital pin because only the voltage variance has two mode: high and low. Table 5.3.3 displays the pin correspondence between the Arduino® Mega and Joystick as a supplement for the wiring diagram.

Table 5.3.3: Arduino Mega to Joystick Pin Correspondence

Arduino Mega	Joystick	Description
2	SW	Reads Switch input for changing states
A0	VRx	Analog reads inverse Y direction
A1	VRy	Analog reads inverse X direction
5V	+5V	Supplies joystick with 5V
GND	GND	Grounds joystick

An issue arose with the joystick because the potentiometers do not follow the Cartesian coordinate system. We solved the issue by changing which pin corresponds to an axis in the code so the orientation could be as shown in Figure 5.3.4.

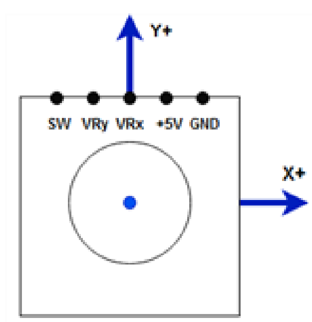


Figure 5.3.4: Joystick Orientation in respect to stage actuation.

The driver outputs deliver a voltage to the actuators that correspond to the PWM signal the enables are given, the power supply voltage and the driver input voltage. For example, if the power supply voltage is 12V but a driver input delivers a low voltage signal, the driver output corresponding to the driver input will be low. If the driver input were high, the drive output voltage will also be dependent on the enable pin voltage due to PWM. If the PWM duty cycle is set to 50%, the driver output voltage would be designated high 50% of the time. Table 5.3.4 displays the pin correspondence between the Arduino® Mega and Joystick as a supplement for the wiring diagram.

Table 5.3.4: L293D to Actuator Pin Correspondence

L293D	**Actuator A**	**Actuator B**	**Description**
3	7		Negative Motor Terminal Input
6	5		Positive Motor Terminal Input
11		7	Negative Motor Terminal Input
14		5	Positive Motor Terminal Input

5.3.3 Software Development

For the user to interface with the system without directly manipulating the code, we have decided to use the Arduino® Serial Monitor to act as the user interface. The monitor can only be used when the Arduino® Mega is plugged into a computer via data cable. Before the user can be granted full control of the system, zeroing the actuators must take place to ensure repeatability. The logic of moving between each state is show in Figure 5.3.5with a finite state diagram.

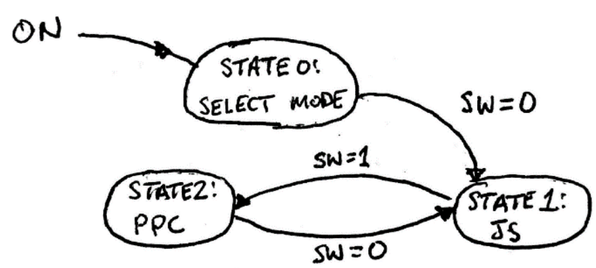


Figure 5.3.5: Arduino® Mega moves through the zero immediately on startup and will perform the zeroing of the actuators. Movement between states 1 and 2 are done through clicking the switch on the joystick.

On startup, we have the Arduino® ask the user to press “Enter” to start the zeroing process. During this process, both actuators retract at a set speed till the reverse limit switches are activated. Afterward, the actuators extrude until they reach the zero indicator on the actuator and automatically the actuator transfers to Joystick mode.

In Joystick mode, the user can move the stage on both axes. The code detects the direction the joystick is pointed through the analog ports and corrects the orientation using the SatBlock function in the code (Appendix N). The SatBlock function relays information the either LimitSwitchA or LimitSwitchB functions to move the appropriate actuator. While the actuator begins to move two things are occurring. First, the LimitSwitch functions are checking if the limit switches have been activated to prevent crashing. Second, the speed is being controlled by the SpeedDifferential function. The SpeedDifferential function determines the speed by checking the current encoder position of the actuator and converts current position

to a speed the actuator can use. This is done in both Joystick and Programmable Path states to maintain consistent travel as the spring forces vary. At any time, the user can press down on the Joystick to switch to the Programmable Path state. After the button is pressed, the user is prompted of the state change.
A “Select Actuator” prompt is displayed on the serial monitor, and the user can select the axis they wish to use by typing either capital “X” or “Y”. After the Enter key is pressed, the recWithEndMarker function. This function grabs each character entered and stores them into an array individually until the end marker is detected. The array is sent to the selectActuator function where the characters are compared to the hexadecimal values. If a match is detected, the appropriate actuator is selected and if not, the user is prompted and returns to the selectActuator prompt. With an actuator selected, another prompt is generated asking for the encoder counts to be entered. Similar to the “Select Actuator” prompt, the recWithEndMarker function grabs the numerical value entered and places it in an array. The showNewNumber function then takes the numbers from the array and converts them from ASCII characters to an integer. The function will detect if a negative sign has been written as well to change the number to a negative integer. The showNewNumber function uses a threshold between 90000 and -90000 to prevent encoder counts larger than full travel, which is roughly 88100. If the threshold is exceeded or an improper character is entered, the user returns to the “Select Actuator” prompt.

If an acceptable number is entered, the integer is sent to the proper function, either MoveXEncoder and MoveYEncoder. The integer is then added to the current encoder count and the MoveXEncoder or MoveYEncoder functions moves to the new location at a speed regulated by the SpeedDifferential function. Once the movement is complete, the current encoder count will be displayed and the “Select Actuator” prompt will return. If a limit switch is activated during motion, the move functions are terminated, the current encoder count is displayed and the “Select Actuator” prompt is returned. During any limit switch activation, in Joystick or Programmable Path states, a prompt stating which limit switch is activated will be displayed as well. If the user wishes to return to the Joystick state, a button press on the joystick will allow it only when the Programmable Path state is displaying the “Select Actuator” prompt. All of the functions and their respective descriptions can be found in Appendix N.

5.3.4 Preliminary Testing

Tests have been administered to see the probability of the joystick being utilized. The first test required is testing the sensitivity of the joystick. A separate Arduino® Uno was connected to the joystick and had the values generated onto a monitor. The result displayed the signal read produced a 10-bit reading and that the joystick would reach numerical maximum before reaching physical end range. This alone required the 10-bit readings to be altered to prevent sudden movement when the actuators where implemented. Once the values were corrected, a test to move one of the actuators was conducted. Rudimentary code was implemented with minimal optimization and the test proved to be successful. The pseudo-code for this operation can be found in Appendix [L].

The probability of the programmable path code being utilized was tested by using the Arduino® serial monitor. The test was to determine whether characters could be entered into the serial monitor as data inputs. The recWithEndMarker and showNewNumber functions were created to convert the inputted integers into values the microcontroller code process. Once these two became functional, after testing if the proper integers will be printed, the two functions were integrated into the main code. Once integrated, the integers were sent to the respective move to target functions which resulted in a success with a precision threshold of ±25 encoder counts.

5.4 STRUCTURAL PROTOTYPE

To construct a Structural Prototype, we decided to focus on the motion of the stage. This required brackets for the actuators to be mounted, blocks for the actuators to push on, and brackets for the spring return system. As mentioned in the previous section, the actuator mounts and spring return brackets are 3-D printed parts. We used this manufacturing method because of its flexibility and quick turn-around time. The brackets make use of holes that were already in the stage. 3-D printing made the manufacturing of brackets sized to the stage's existing mounting holes easier. The material that we are using is PLA, printed at 20% infill density. PLA printed parts can withstand a significant amount of load, larger than will be exerted on them for this project. To confirm this, we plan to test the failure load of each of our brackets after CDR.

We aimed to achieve full X-Y actuation in our prototype. During structural prototype final assembly, we realized that the brackets we designed did not perfectly align the stage and actuators. The X actuator was too far forward, and there was not enough clearance to fit the Y actuator. This caused the X spring pins to fall too far apart, raising the spring pretension too high. The springs we selected have a limited travel length, so because they were too extended initially our overall X range was reduced. We decided not to mount the Y spring pin until we could achieve a successful actuator fit.

After CDR we intend to make some slight sizing adjustments to our brackets. Because they are 3-D printed parts, we will be able to quickly converge on final bracket shapes that correctly position our components. The spring pins are the only traditionally machined parts in the actuation assembly. Because they are press fit into place, they could be reused in our new brackets.

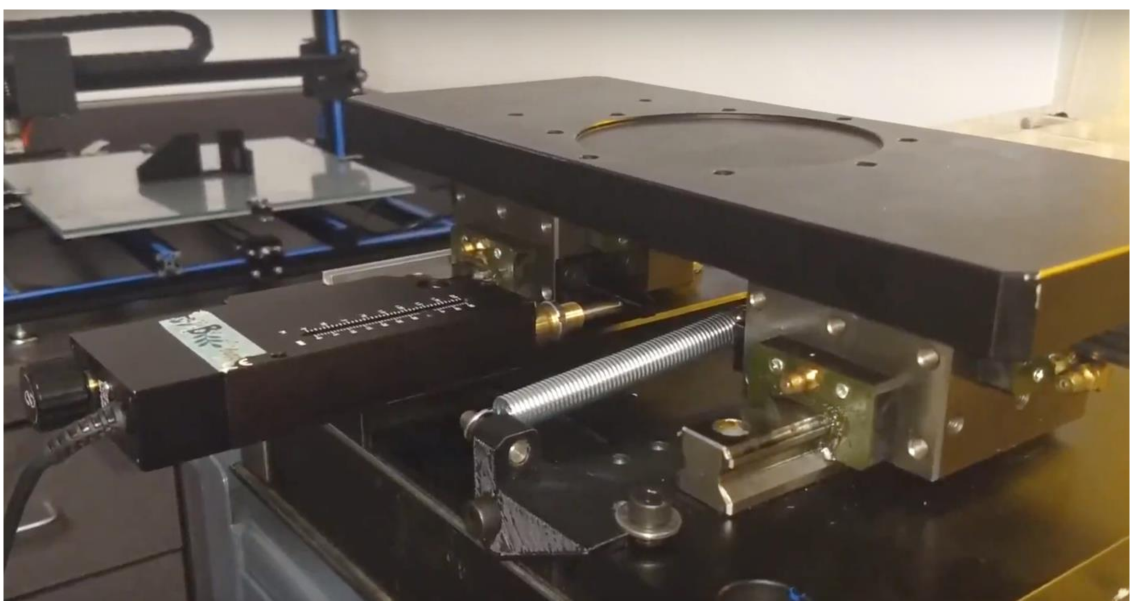


Figure 5.4.1. Photo of our structural prototype. We initially intended to have two functional axes. Due to bracket fit difficulties and actuation uncertainty, we were only able to actuate the X-Axis.

5.5 DESIGN CHANGES POST – CDR

After CDR, we implemented a small number of design changes to facilitate the final build. The imaging system was mounted on a slide to facilitate design flexibility. We cosmetically updated the stage brackets and added some more material after testing. We also finalized the design of the sample plate.

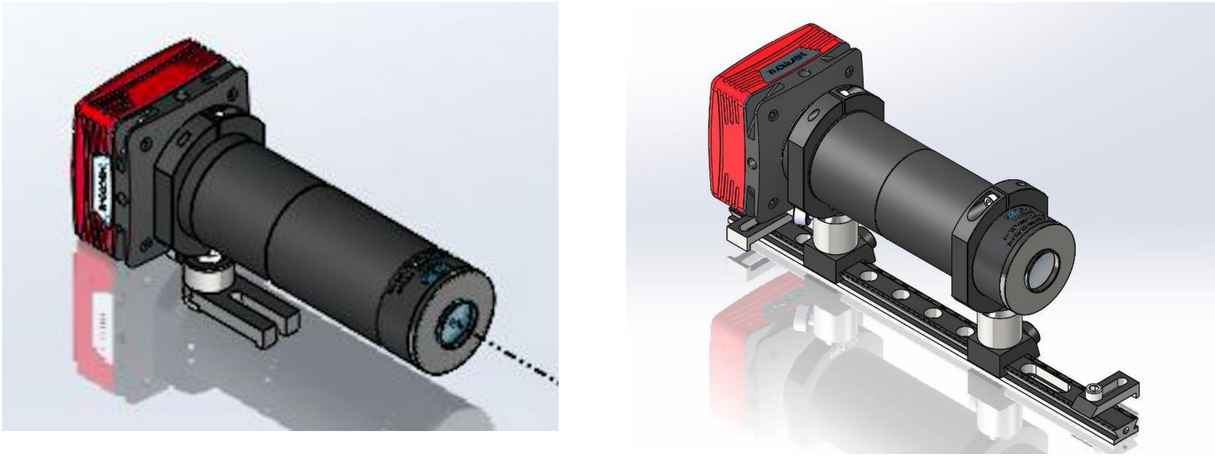
5.5.1 Modified Imaging System

To switch the microscope from brightfield to fluorescence mode, the user must be able to easily insert a fluorescence emission filter into the light path without disturbing component alignment. In the design presented at CDR, the series of tubes and adapters from the exit window of the filter cube to the camera was fixed. Hence, to install a filter, all components in this series would need to be individually detached from the breadboard and then reattached, risking misalignment, and increasing the time and complicacy of switching microscope modes. The concept for the imaging system prior to modification, as presented in CDR, is shown in Figure 5.5.1.

In the modified design, the imaging components, including the camera, lens tubes, adapters, and support structure, are treated as a single optical subsystem. This subsystem is mounted on a dovetail optical rail, so that all imaging components can be moved out of the way when installing an emission filter. Sliding these components to the side is also ergonomically advantageous when changing the objective lens, as it reduces obstruction of the lens and consequently the risk of damage caused by bumping it into the bottom of the stage or other components. Table 5.5.1 lists the additional components required for the improved imaging system.

Table 5.5.1. Dovetail Imaging System Components

PART NO.	PART	QTY.	VENDOR
RLA1200	Dovetail Optical Rail, 12" Imperial	1	Thorlabs Inc.
CL6	Table Clamp, RLA Series Optical Rails	2	
RC1	Dovetail Rail Carrier, 1" x 1"	2	
SM2RC	Slip Ring for SM2 Lens Tubes	1	
TR075	Ø1/2" Post, L = 0.75"	2	



(a) Original Imaging System (CDR) (b) Modified Imaging System

Figure 5.5.1. Changes were made to the imaging system following CDR to increase design flexibility, making it quicker and easier to switch between brightfield and fluorescence modes. (a) Shows the imaging system presented for CDR, prior to modification. The camera is fixed, as are the lens tubes and adapters connecting it to the filter cube; (b) shows the modified imaging system adapted after CDR to meet sponsor design recommendations and improve system modularity and ease of usability. The lens tubes, adapters, and camera are fixed on a single dovetail rail, clamped down to the table, so that the imaging system can be easily moved out of the way when installing the emission filter.

5.5.2 Stage Updates

The sample holding plate shown in our CDR CAD model was a place holder with general geometry. We had not yet received the final specification form our sponsor regarding mounting features. When spring quarter began, we started discussing options with our sponsor. Since we decided to outsource fabrication, we chose not to add features on the underside of the plate. This cut down on the number of machining set ups, keeping our cost low.

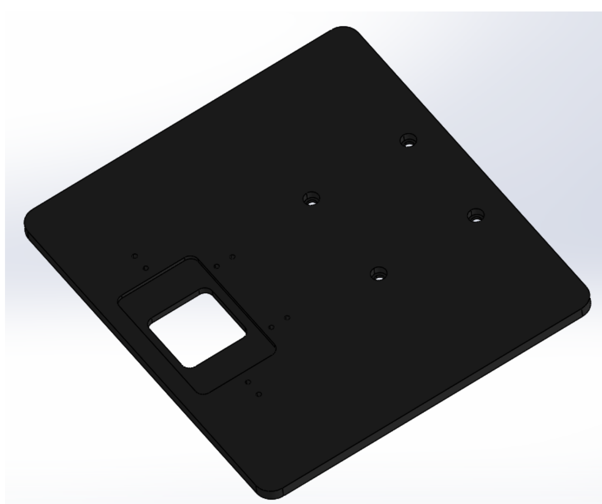


Figure 5.5.2. Final version of the stage plate. It has four counterbored mounting holes for attaching the plate to the stage. The viewing window has a recessed lip to allow for custom inserts. There is a total of eight threaded holes around the viewing window. These attach the microscope sample clips. In the CAD image it is shown as anodized aluminum, but we were unable to complete the plate coating four our verification prototype.

Following CDR, we also modified the stage brackets. These changes were primarily cosmetic, including fillets and other curvature. These helped us to take full advantage of the flexibility of rapid prototyping. The functional changes to our brackets are summarized below:

Y-Axis Actuator Mount	The actuator mounting area was lowered to provide more clearance under the top plate of the stage. An additional mounting point was included for attaching the bracket to the stage. More material was added under the actuator to allow for two additional connection points.
X-Axis Spring Mount	The pin holding feature was extended to add stiffness. The pin hole stayed the same depth, but a relief hole was made through the extension to allow for an easy press fit.
Merged Bracket	This bracket was broken into two separate components. This simplified both mounting and printing. The two new brackets that this separation created were slightly modified to increase spring preload and bracket stiffness.

5.6 SATISFACTION OF SPECIFICATIONS

Our engineering specifications have been a very important guide during the design process. Below is a brief discussion of our specifications and how we believe our final design will satisfy them.

• Resolvable Sample Size (10 μm)	Our optical pathway was verified by Cal Poly physics faculty. We are using a 4x objective for our initial build. With a properly aligned light path this will be just enough to resolve a 10 μm feature. To make sure the objective remains in line with the light path, we will make the objective mounting bracket is rigid enough to maintain alignment. With a higher magnification objective, a 10 μm feature will be easier to see.
• Z Travel (64 mm)	Our objective will be mounted on a vertical 50 mm optical slide. This slide will be mounted on an angled bracket, and can be fixed anywhere along a 50 mm slot. These two together give us approximately 75 mm of Z Travel.
• X-Y Travel (50x50 mm)	The actuators we are using have a 50 mm travel distance. The stage spring return mechanism will be preloaded so that the stage can return to a full zero position.
• Repeatability of Actuation (50 μm)	The overall drivetrain backlash within our actuators is only 15 μm. By providing a spring return mechanism we keep our system backlash restricted to the actuators.

• Stage Parallelism with Frame (0 μm)	We are using a pre-aligned set of linear axes. The entire stage will be set on precisely manufactured optical posts to ensure motion is parallel with the breadboard.
• Clearance Above Stage (50 mm)	Since the upper gantry will be built from 80-20 rail, the height above the stage is easily adjustable.
• Hard Cap Budget ($3000)	As of CDR the total cost of our microscope is around $1700. This leaves us room to purchase expensive, higher quality optical components in the future.
• Microscope Footprint (2x3 ft)	The current footprint of our system is under 2x2 ft. We likely will be able to purchase a smaller breadboard that will be dedicated to our system.
• Detectable Fluorescence	Our optical pathway was verified by Cal Poly physics faculty. We also have no limit on exposure time. By anodizing our stage and allowing a significant time to register a signal we will be able to detect fluorescence.

5.7 SAFETY CONSIDERATIONS

The hazards associated with our system are not severe, but they still merit careful consideration and mitigation. The most hazardous piece of our microscope is the stage. As we assemble our final prototype, we intend to work with our sponsors to design guards for the system, and to integrate user interface components into an ergonomic workstation.

Stage:

The motion of each axis creates potential pinch points. Each axis is also returned using an extension spring. At max travel, the springs only exert 12 lbs. of force, but this still will necessitate a physical barrier. The system is extremely heavy, but all components will be bolted securely to the optical breadboard. The microscope is controlled with a joystick, and the sample is viewed through a computer. The user will not need to have direct access to the microscope once it is loaded with a sample.

Actuators:

There are no apparent ways where a user can become injured from using this system, but the user can damage the system easily in two ways: liquid damage to board, moving actuator past its physical limits. Liquids should be carefully handled near the board to prevent destroying the electrical components and creating the possibility of electric shock. As a precaution, better housing for the electrical components will be constructed once we have more information from our sponsors. As for the actuator limits, if the actuator plunger is pushed or pulled past its mechanical distances, damage to the motor is possible due to an overloading torque. Luckily, there are mechanical limit switches within the actuator housing and steps to have these activated when a limit is reached.

Maintenance & Repair:

The parts that we have chosen & created for our design are easily replaced. Our hardware was chosen from stock sizes, and our custom brackets were all 3-D printed. The pieces that are machined can be turned quickly by hand or cut on a waterjet. Because our system does not generate high levels of force or speed, we do not anticipate much required maintenance. Our stage bearings came with grease fitting that can be used to re-lubricate our linear rails as needed.

6 MANUFACTURING

Many of the large components that we needed to build our microscope were already available in the Microfabrication Laboratory. One of our most important tasks was to inventory what we had to work with. Knowing what we have allowed us to keep out costs low, freeing funds to purchase better components. Some of the important components that we already owned are listed below.

- Optical Breadboard
- LED Light Source
- Linear Actuators
- Optical Slides
- Optical Posts / Holders
- Brightfield Illuminator
- 50:50 Beamsplitter
- 45 Degree Mirror

Final total project expenditures were $1,542.59, including all purchase orders for commercial components through the Mechanical Engineering Department and outsourced manufacturing. This was substantially below both the project's hard cap budget of $3,000 as well as the $2,200 soft cap.

6.1 PROCUREMENT

The optical components that were not available in the lab were purchased from Thorlabs, an online experimental equipment supplier with a large inventory of affordable yet high-quality optical equipment. Some of the critical optics and optics mounting components that were sourced from Thorlabs are listed below.

- Aluminum Mirror
- Tube Lens
- Optical Posts / Holders
- Mounting Bases
- Dovetail Rail (for Imaging System)
- Lens Extension Tubes

We purchased additional project materials (raw stock, fasteners) from local suppliers such as Miners Ace Hardware and reputable online retailers like McMaster-Carr.
Initially, we had planned to utilize the Cal Poly Machine Shop's Contact Fabrication services to face the aluminum stage plate to adequate parallelism and CNC mill the sample-retaining recess geometry. However, following Cal Polys Spring 2020 closure, we outsourced the plate for machining to a local company, JPT Labs, owned by a Cal Poly alumna.

6.2 MANUFACTURING

Many of the custom parts that we designed for the microscope were fabricated using Cal Poly's additive manufacturing resources. We were able to use rapid prototyping processes for our final design because the loads generated by the microscope are small, and the precision alignment of our components relies instead on the tight tolerances of purchased equipment. Listed below are the components that were manufactured on-site at Cal Poly, as well as a few that were outsourced.

- Stage Mounting Bushings
    - 3D printed flanged bushings, secured with screws and washers, mount the stage to optical posts. The non-standard hole dimensions of the purchased X-Y stage posed unique limitations to our ability to purchase off-the-shelf components, and the holes were too large to interface easily with standard ThorLabs optical posts. The PLA bushings accommodate both the large diameter holes in the stage and the small No. 8 screws attaching it to the mounting posts.

- Brightfield Slip Ring Adapter
    - During remote assembly and optical alignment, we found that the brightfield fiber-optic focusing attachment was too small a diameter for the purchased slip ring to clamp and fix into the proper position. To quickly troubleshoot this problem and conserve our limited meeting time, we created an adapter in SolidWorks, sent G-code to our sponsor, and 3-D printed the part on the printer in the Clean Room.

- Cantilever Plate

    As mentioned above, the cantilever plate was outsourced to John Gerrity for manufacturing, due to our inability to manufacture the plate ourselves in the Cal Poly Machine Shop. An aluminum plate was procured and delivered to Mr. Gerrity for manufacturing. In the future, at the discretion of Dr. Mayer and Dr. Hawkins, the plate should be anodized black to reduce the light that reflects off the plate into the optical train.

- Actuator Fixture Block
    - The actuator fixtures are 3D printed in PLA. Additive manufacturing reduces overall manufacturing costs and facilitates iteration, especially since the holes needed to be fitted precisely. The higher load-bearing features were printed on the 3D printer's XY plane.

- Spring Return Assembly
    - See Actuator fixture block

6.3 ASSEMBLY

Microscope assembly was conducted remotely during Spring Quarter. The importance of proper optical alignment drove the order of assembly; we began with components that demanded less precision and moved forward from there. Since the stage did not need to be attached to the optical breadboard for push blocks and spring return brackets to be installed, it was assembled first. We then attached the actuators to the stage and integrated the control system. Then, assembly of the frame allowed a location to be chosen for the brightfield light source, and optical alignment to this beam followed. Further details for microscope subsystem assembly and integration are specified in the following sections.

6.3.1 Stage Mounting

Microscope subassemblies were primarily integrated using 3D printed mounts and screws compatible with the optical breadboard, the centerpiece of the final integration. The stage is mounted onto 3-inch optical posts and post holders that are compatible with the breadboard, using 3D printed bushings to couple it to the posts. In the current design, all posts are set to their lowest setting. However, if the use of a longer working distance objective lens necessitates additional space beneath the stage, these posts can be either raised up or swapped out for longer posts. The actuators are mounted to the stage using 3D printed brackets and several cap screws, many of which are low profile to prevent interference during motion. One of the actuators is secured with a socket head screw to the base of the microscope since it already has a hole with this specification. Spring return brackets are mounted adjacent to the actuators to maintain force while preventing the application of a moment.

6.3.2 Frame Assembly

The aluminum gantry was assembled prior to optical alignment since it is the attachment point for the brightfield light source. Two 1-ft lengths of 80/20 aluminum rail support a 2-ft crossbar. The rails are attached using t-slot-compatible, right-angle corner brackets, and the entire subassembly is fixed to the optical breadboard with slotted 3D printed mounting feet. The mounting feet and brackets are attached with end-feed ¼”-20 rail fasteners.

6.3.3 Control System Integration

The actuators are equipped with long cables, so the Arduino® control system is set a distance away so it does not interfere with the movement of the stage. Additionally, the control system employs longer wires for the joystick to allow the operator to stand closer to the stage during operation. A full SolidWorks model of all integrated subsystems is shown in Figure 6.3.1.

6.3.4 Optical Alignment

Precise optical alignment was a primary concern when assembling the optics to ensure adequate image resolution. To accomplish this, components were first assembled within their subsystems and then fixed to the breadboard and frame for final system integration.

In brightfield mode, alignment of optics beneath the stage proved to be less critical than originally anticipated, since the brightfield beam illuminates a relatively large area. Hence, only coarse adjustments using caliper measurements were necessary.

However, a future fluorescence test will require a more careful alignment procedure since the illuminator is a point source LED. For the more critical elements (objective lens, plane mirror, beam splitter and filters, and tube lens), the optical train should be tested for alignment errors following the addition of each component. Beginning with installation of the camera, the microscope user should shine the LED light source through each subsequent part addition to qualitatively determine system alignment.

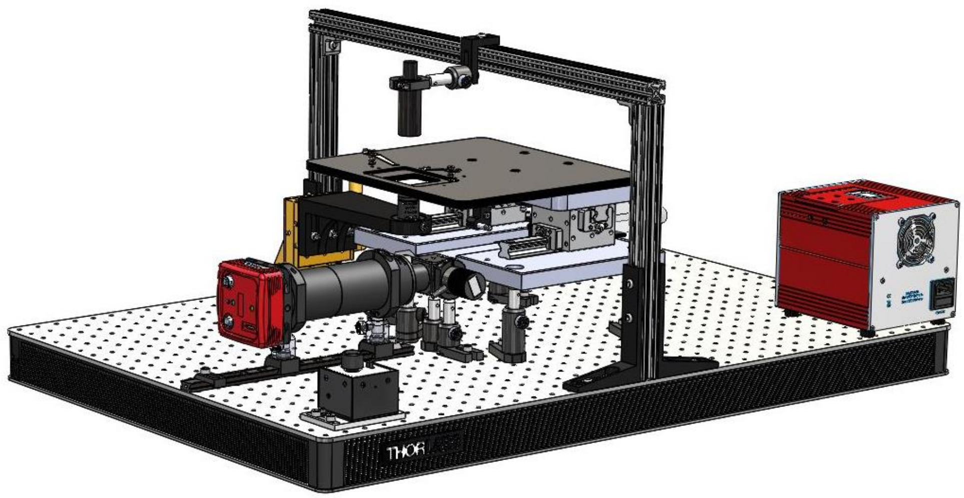


Figure 6.3.1. CAD model of fully integrated inverted fluorescence microscope, including all subsystems and appropriate fasteners.

6.3.4 Remote Assembly

For product assembly, due to restricted laboratory access for students, our team needed to devise an alternative plan to complete our verification prototype. In response to the changing conditions, we scheduled weekly remote meetings with our project sponsor to conduct remaining assembly and testing of the microscope's brightfield capabilities. During these sessions, the CAD model in Figure 6.3.1 served as a helpful visual representation of the desired product outcome to supplement team verbal instruction. Figure 6.3.2 shows the format for these assembly meetings.

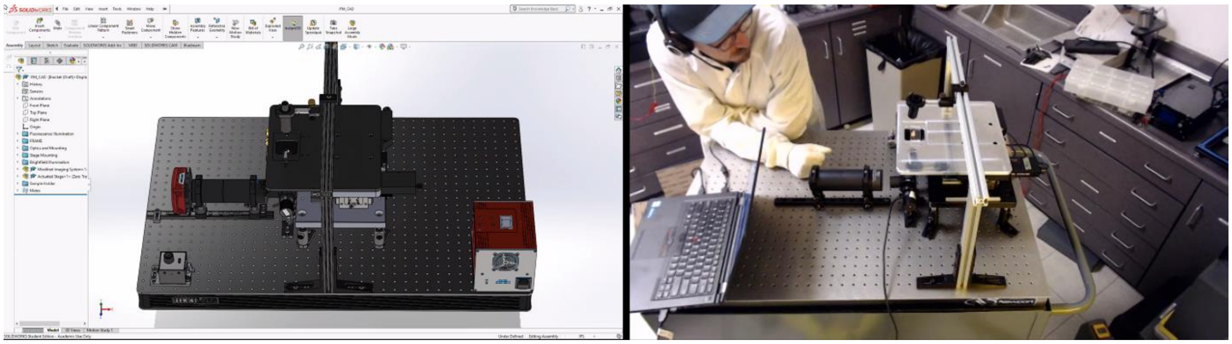


Figure 6.3.2. Photo from a Spring Quarter virtual assembly meeting to finish the design verification prototype. Team members shared a screen with the full CAD model (left) to provide visual and verbal instruction to our project sponsor, who built the prototype (right).

In the final project package delivered to our sponsors, we have provided a full CAD model of the system as well as assembly time lapse video documentation. The microscope has already been fully assembled, and for its lifetime in the Microfabrication Laboratory, we do not anticipate disassembly. The only occasion for which disassembly and reassembly would be necessary is if the department wished to move the microscope to a smaller breadboard.

7 Design Verification

The following section outlines the agenda and methods we followed during Spring Quarter to verify the functionality of our final design. It focuses on the specifications outlined in Chapter 3, and the testing procedures used to verify that each of the specifications meets the requirements.

7.1 Prototype

As a result of considerable effort on the part of the team and our project sponsor, we were able to deliver a working verification prototype despite limitations to laboratory access. Figure 7.1.1 is an image of our inverted fluorescence microscope in the Microfabrication Laboratory, labeled to show the location of microscope subassemblies. While some of the component locations differ slightly from the CAD model, functionality is identical.

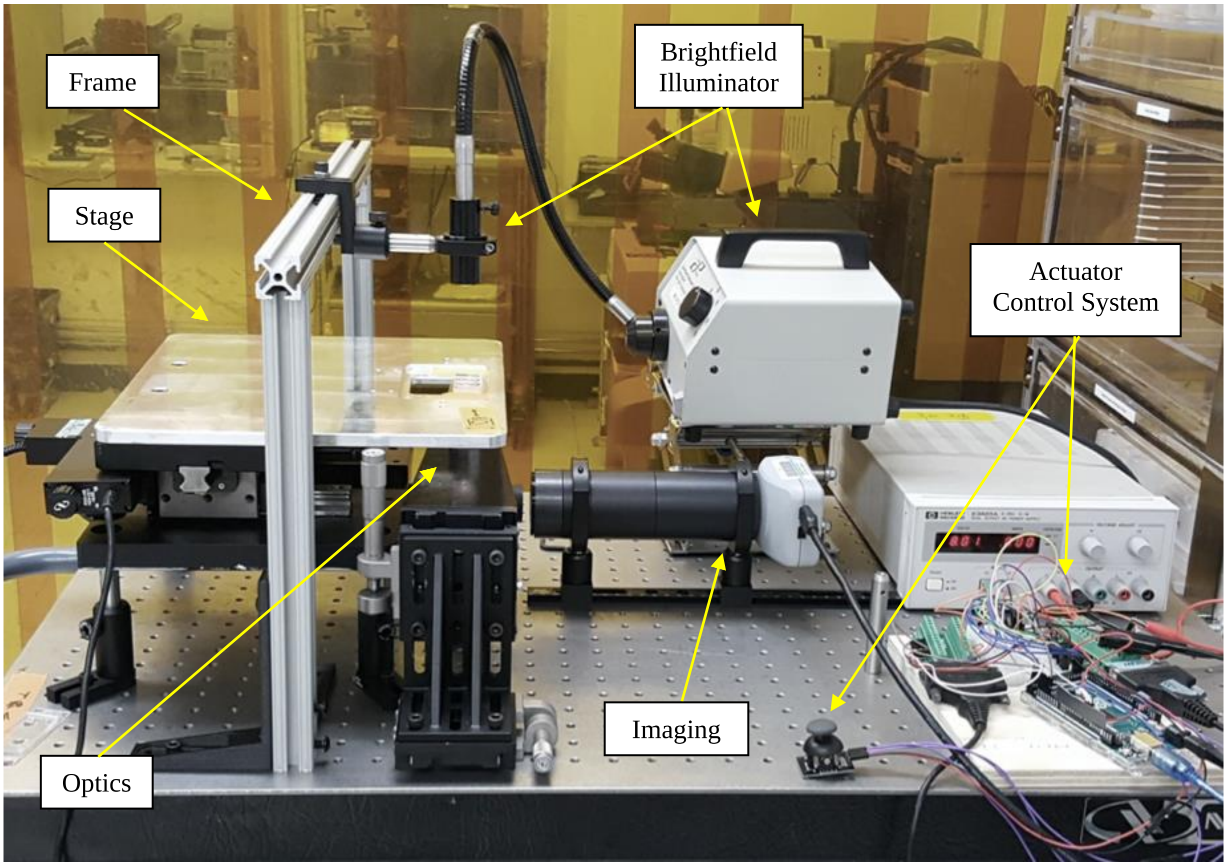


Figure 7.1.1. Final inverted fluorescence microscope verification prototype in the Cal Poly Microfabrication Lab.

The microscope is capable of brightfield illumination, repeatable electronic actuation of the X-Y stage, and imaging of a sample under stagnant conditions.

7.2 TESTING

Each of the specifications mentioned above has a test that must be performed to verify that the prototype meets the requirements. A list of the planned tests is below. Due to the restriction of access to materials, some of the tests were not able to be performed as originally planned. The testing setup shown in Figure 7.1.2 shows how we worked with our project sponsor to remotely verify some of our design specifications.

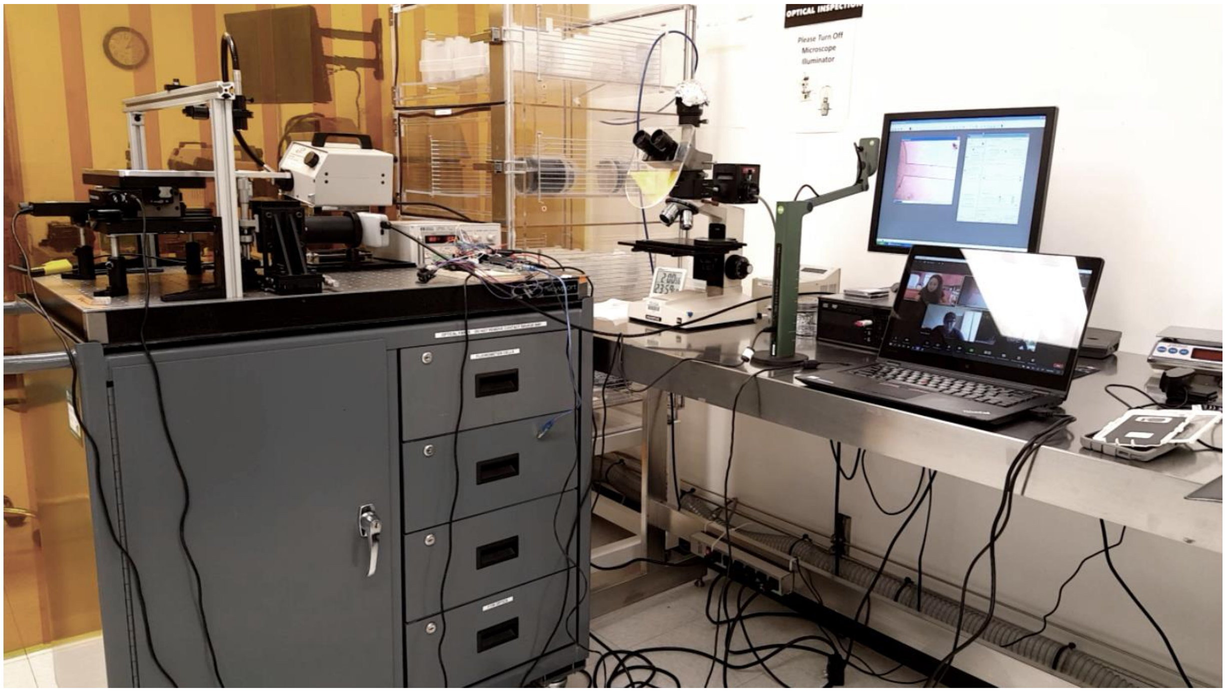


Figure 7.1.2. Design verification testing setup in the Microfabrication Lab. Following prototype assembly, we verified the functionality of the actuators in Joystick Mode and Programmable Path Mode and confirmed the microscope could resolve a brightfield image.

- Resolvable Sample Size
    - Due to the closure of labs, we were unable to create a sample to test. Therefore, we do not have a resolvable sample size measurement. However, we are able to resolve an image using brightfield. The slide that we observed had features around 75 micron and was clearly resolved.

- Z Travel
    - This test was performed qualitatively, instead of quantitatively, due to time restraints. The Objective is mounted to a micrometer-pushed optical slide, so z axis travel is available for focusing the image.

- X-Y Travel
    - This test was completed by reading the distance markers on the actuators. The actuators zero themselves on startup. The final distance moved is represented by how far the markers move.

- Repeatability of Actuation
    - This test was performed by marking a location on the output screen of the camera. The stage was then moved by the programable path code a distance, and then returned. The X axis returned to approximately the same location, but the Y axis had a noticeable offset. We did not have enough time to fully explore this issue.
- Stage Parallelism with Frame
    - Due to time constraints, we were unable to perform this test.
- Clearance above Stage
    - Due to time constraints, we were unable to perform this test.
- Hard Cap Budget
    - This specification does not require testing. The project remained under budget.
- Microscope Footprint
    - This specification does not require testing. All components, aside from the computer to run the camera and the DC power source fit within the footprint of the breadboard. We consider this specification met.
- Detectable Fluorescence
    - Due to an inability to procure the necessary filter and dye, the fluorescence portion of the microscope is non-functional. We are confident that the fluorescence capabilities will be operational once the necessary components are purchased.

We also performed some additional testing that does not directly address our engineering specifications. These include the following:

- Stage Bracket Failure
    - We performed strength tests on the PLA printed brackets that are used for the stage actuation. All the brackets tested passed, but material was added where deflection was observed.
- Image Resolution
    - After the full system was assembled, we were able to test the image resolution of our system. In brightfield mode, an image can be resolved by focusing the objective using the Z-axis travel. Test samples had a feature size of about 75 microns. Microscope images from our brightfield verification test are shown in Figure 7.1.2.

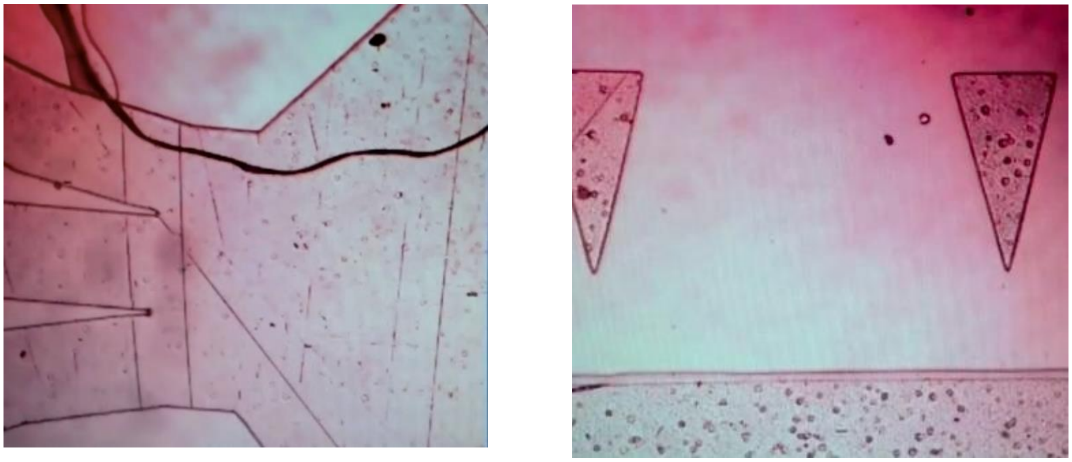


Figure 7.1.3. Successful brightfield images of two different PDMS (Poly-Di-Methyl-Siloxane) microfluidic devices, both with ~75-micron features.

Additional documentation of our design verification test results is included in Appendix [V]. It includes the tests we had planned to perform prior to the lab closure and a revised list of the tests we were able to perform. Due to the lack of proper testing equipment, these were primarily qualitative results.

8 PROJECT MANAGEMENT

Over this academic year (2019-2020), our design process followed a standard product development cycle. Cal Poly's quarter system conveniently segmented our anticipated design stages (outlined in Figure 8.0.1). Table 8.0.1 shows the breakdown of major project milestones by quarter. For a more detailed project schedule, see our Project Gantt Chart (Appendix B). Fall Quarter centered around defining the problem, conceptualizing solutions, and evaluating ideas. During Winter Quarter, we broke our design into subsystems, and began specification through analysis, detailed design, and structural prototyping. Spring Quarter we completed final construction and testing. This process followed the course of the academic year, but its progression was non-linear. The arrows in Figure 8.0.1 highlight the iterative nature of the design process.

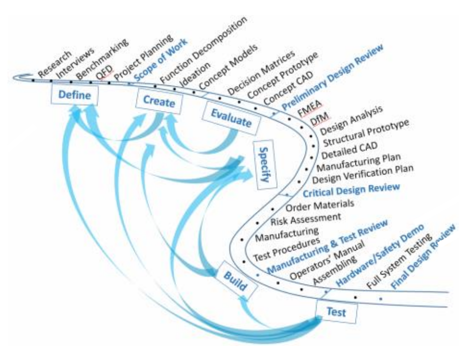


Figure 8.0.1. Graphical senior project timeline. This shows some of the key milestones in blue with important intermediate deliverables in black. For specific dates and additional milestones, see Table 3. The arrows highlight the importance of iteration at every stage of the process [20].

Table 8.0.1. Senior Project Milestones

Academic Quarter	Project Milestones	Date of Completion
Fall Quarter	Scope of Work	10-18-2019
	Concept CAD	11-08-2019
	Concept Prototype	11-11-2019
	Preliminary Design Review (PDR)	11-15-2019
Winter Quarter	Interim Design Review (IDR)	01-16-2020
	Detailed CAD / Manufacturing Plan	01-30-2020
	Structural Prototype	01-30-2020
	Critical Design Review (CDR)	02-07-2020
	Manufacturing & Test Review	03-12-2020
Spring Quarter	Verification Prototype Sign-Off / Delivery	05-28-2020
	Testing Sign-Off	05-28-2020
	Final Design Review	05-28-2020
	Senior Project Expo Website Launch	06-02-2020

Two of the largest challenges that we faced were our low budget and complicated integration requirements. Optical components were very expensive and highly reliant on each other to function. It was challenging for us create informative prototypes without the actual optics in hand. Because of this, it was critical to begin researching and purchasing components early. We needed as much development time with our actual parts as possible to have a chance to effectively iterate. 3-D printing was a key part of our development process. Its speed, flexibility, and cost effectiveness enabled us to spatially test components with one another.

We began the problem definition phase at the beginning of October 2019. Effective problem definition was the first step towards a successful solution. True understanding of the customer needs facilitated the generation of a solution worth pursuing. The Scope of Work represented the end of our formal problem definition phase.

The remainder of Fall Quarter focused on creative ideation and critical evaluation of ideas. First, all team members participated in a functional decomposition, where we broke down the function of an inverted florescence microscope into its basic elements. Using this process as inspiration, we began building concept models. Once we established a base of ideas to draw from, we came together as a team and evaluated the results. From there we took the best pieces of our ideas and designed / built a concept prototype. With a design chosen, we divided the project into subsystems (optics, stage, frame). Each team member led the project in one area, but everyone worked on all aspects of the design. We presented our design concept to our sponsors and peers in a Preliminary Design Review (PDR).

Next, we began the detailed design phase of the process. This primarily involved the creation of CAD models, engineering drawings, and manufacturing plans for each subsystem. We completed the final assembly model just after our Interim Design Review (IDR). In parallel with this, we continued to purchase the components for our confirmation prototype. Between IDR and our Critical Design Review (CDR) we developed our engineering drawings and manufacturing plans.

After our final design was accepted by our sponsors, we purchased almost all of our parts and began manufacturing. Many of our components were 3-D printed and required some iteration to create a satisfactory final model. At the end of Winter Quarter, we had successfully converged on final brackets designs. We intended to continue construction in the spring, but the COVID-19 pandemic forced Cal Poly to transition to online learning. Since we already had all our parts on campus, we were able to continue with very little change. The electrical system was sent to Eduardo at home for completion, and we used virtual zoom assembly sessions to guide our sponsor Dr. Mayer through the build. As we assembled the microscope virtually, we also produced supporting documentation like the operator's manual. We also worked with a local machine shop to outsource the fabrication of the stage plate. Since we had not purchased an emission filter before Spring Quarter, and the Micro Fabrication Lab machinery was shut down, we were not able to complete the fluorescence imaging sub system. However, we did successfully align the full system and achieve brightfield imaging.

9 RECOMMENDATIONS

The scope of work of this design project culminates in the verification of a proof-of-concept test scenario: brightfield illumination, repeatable electronic actuation of the microscope stage, and imaging of a sample under stagnant conditions. It is suggested that in future iterations and developments to the progress we have made, efforts be made to enhance system modularity, sample magnification, and image resolution. A few recommended improvements include:

1. The microscope was designed for fluorescence imaging capabilities but testing of the system in fluorescence mode was not completed. In the future, indicator dyes should be purchased in conjunction with compatible emission filters to utilize this design feature. Since fluorescence imaging required a wavelength-specific light source for each different indicator dye, purchasing a broadband LED and introducing excitation filters into the light path may be advantageous.

2. Develop a caged optic system or 3-D printed enclosure for the optical train to maximize light transmission from source to image.

3. Purchase an objective of higher magnification. The original stated goal of this project was to employ a 10X objective with a long working distance (>30mm) for ease of studying microfluidic devices without interference with microfluidic lines. Ensure that the objective purchased is also infinity corrected.

4. Add an objective turret (ThorLabs Part No. OT1) for microscope use with objective lenses of different magnifications. Designer will need to consider the different working distances of each objective lens; in the current configuration, the height of the stage above the lens would need to

be adjusted with each turn of the turret. Add-on objective lenses must be RMS-threaded or coupled with an adapter of a different industry standard.

5. Anodize the stage plate to reduce reflectivity, thereby increasing the light transmitted to the camera.

6. Program a third actuation mode to execute a sequence of commands.

10 CONCLUSIONS

This document details the inverted fluorescence microscope design that the team designed, assembled, and conducted partial verification testing of during the Academic Year of 2019-2020. Design decisions were made based on extensive concept iteration, engineering analyses and tests, and collaboration with project sponsors. Ease of manufacturability and assembly as well as the project timeline and budget were also significant factors. During Spring Quarter, the team had to pivot quickly to adapt to the challenges of remote assembly. By May 2020, the Inverted Fluorescence Microscope Senior Project Team delivered a valuable cost-reduced research instrument capable of resolving an image of a specified test case in brightfield mode to project sponsors for use in the Microfabrication Laboratory.

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APPENDIX A

QFD HOUSE OF QUALITY

Correlations	
Positive	+
Negative	−
No Correlation	

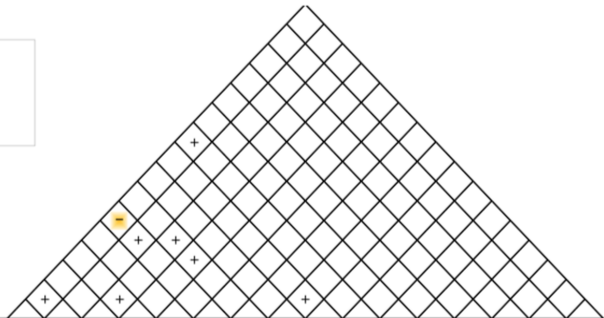
Relationships	
Strong	●
Moderate	○
Weak	▽

Direction of Improvement	
Maximize	▲
Target	◇
Minimize	▼

QFD House of Quality

Project: Inverted Fluorescence Microscope

Revision Date: ____10/10/19____________



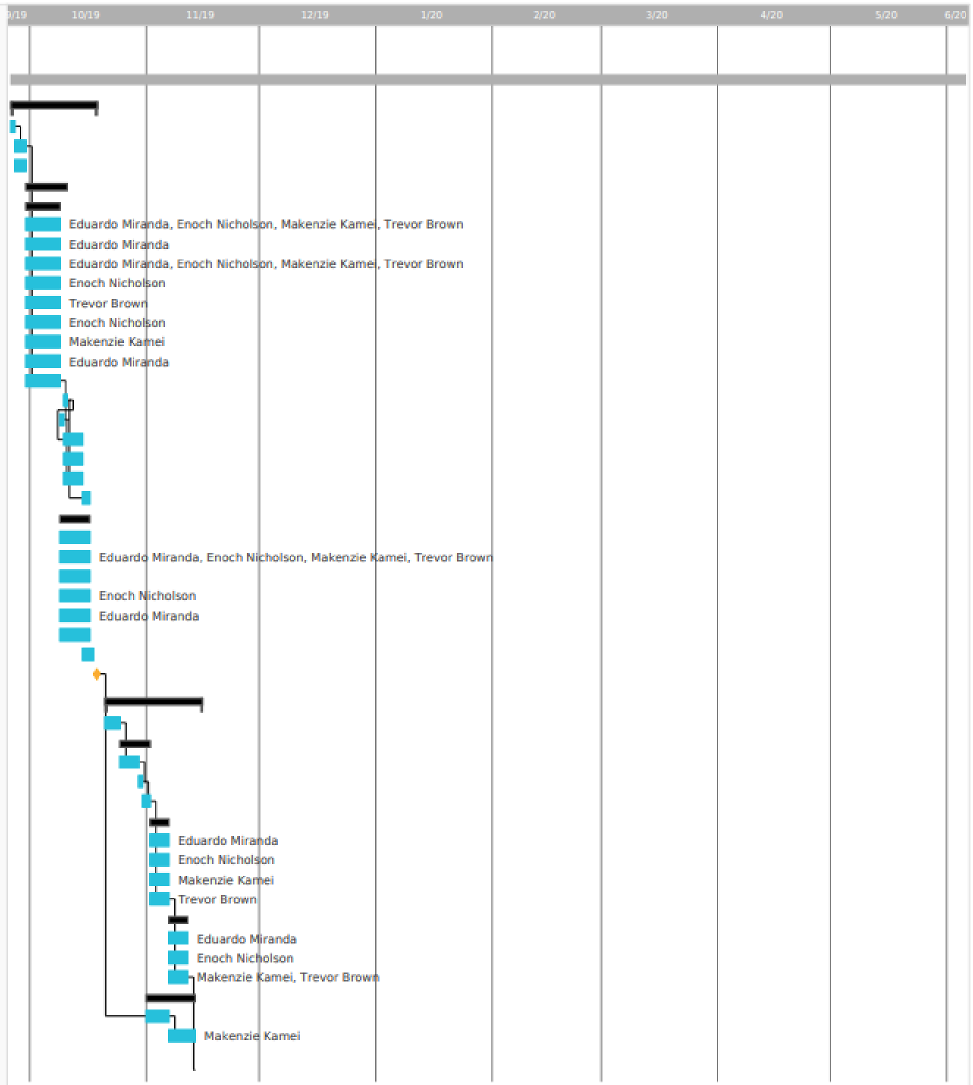
							Column #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						
	WHO: Customers						Direction of Improvement	▲	◇	◇	▲	◇	▲	◇	◇	▼	▲	▲						NOW: Curr. Products					
Row #	Weight Chart	Relative Weight	Dr. Hawkins	Dr. Mayer	Students	Maximum Relationship	HOW: Engineering Specifications (Tests) / WHAT: Customer Requirements (Needs/Wants)	Resolvable Sample Size	Z Travel	X-Y Travel	Repeatability of Actuation	Stage Parallelism with Frame	Clearance Above Stage	Hard Cap Budget	Objective Thread Standard	Microscope Footprint	Frame Deflection in Operation	Detectable Fluorescence						Our Current Product	Olympus IXplore Standard	Nikon Eclipse Ti2	DropletKitchen		Row #
1		9%	10	10	3	9	Low Cost	○			○	○		●	○	●	●	●							0	0	5		1
2		8%	7	8	4	9	Employs Available Components	○			●	●		●	▽	○	○								0	0	4		2
3		7%	3	6	5	9	Use Rapid Prototyping			▽			▽	●		●									0	0	5		3
4		5%	5	5	2	9	Swappable Actuators			●	●														1	1	0		4
5		4%	5	5	1	9	Changeable Objectives		●				○		●			○							5	5	5		5
6		5%	6	6	1	9	Changeable Filters					●						●							5	5	0		6
7		7%	8	8	2	3	Modular Camera Attachment		▽					○		○		○							5	5	2		7
8		11%	9	7	7	9	Room for Experiments	●	○	○	▽		●			●									5	5	5		8
9		14%	10	10	10	9	Operable by 400 Level Students	○			●	○													3	4	2		9
10		11%	10	8	6	9	Maintains Focus Along Travel		▽	●	●			▽											5	5	3		10
11		3%	3	2	2	9	Computer Controlled			●	○						●								2	5	0		11
12		10%	7	7	7	9	Manual Control/Adjustment		●	●	●						●								5	5	5		12
13		6%	6	6	2	9	Infinity-Corrected Optics							●	○														13
14		0%																											14
15		0%																											15
16		0%																											16

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	HOW MUCH: Target Values	1	64mm	50x50mm	1mm	1	50mm	3	$2,200	$3,000	?	?					
	Max Relationship	9	9	9	9	9	9	9	9	9	9	9					
	Technical Importance Rating	189.4	177	295.5	476.7	190.6	114.4	303.2	93.66	285.7	226.1	166.7	0	0	0	0	0
	Relative Weight	8%	7%	12%	19%	8%	5%	12%	4%	11%	9%	7%	0%	0%	0%	0%	0%
Curr. Products	Our Current Product	0	0	0	0	0	0	0	0	0	0	0					
	Olympus IXplore Standard	5	0	5	5	5	5	5	0	0	5	5					
	Nikon Eclipse Ti2	5	0	5	5	5	4	5	0	0	5	5					
	DropletKitchen	5	0	4	3	0	5	5	5	5	0	3					
	0																
	Column #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Appendix B

Gantt Chart – Project Overview

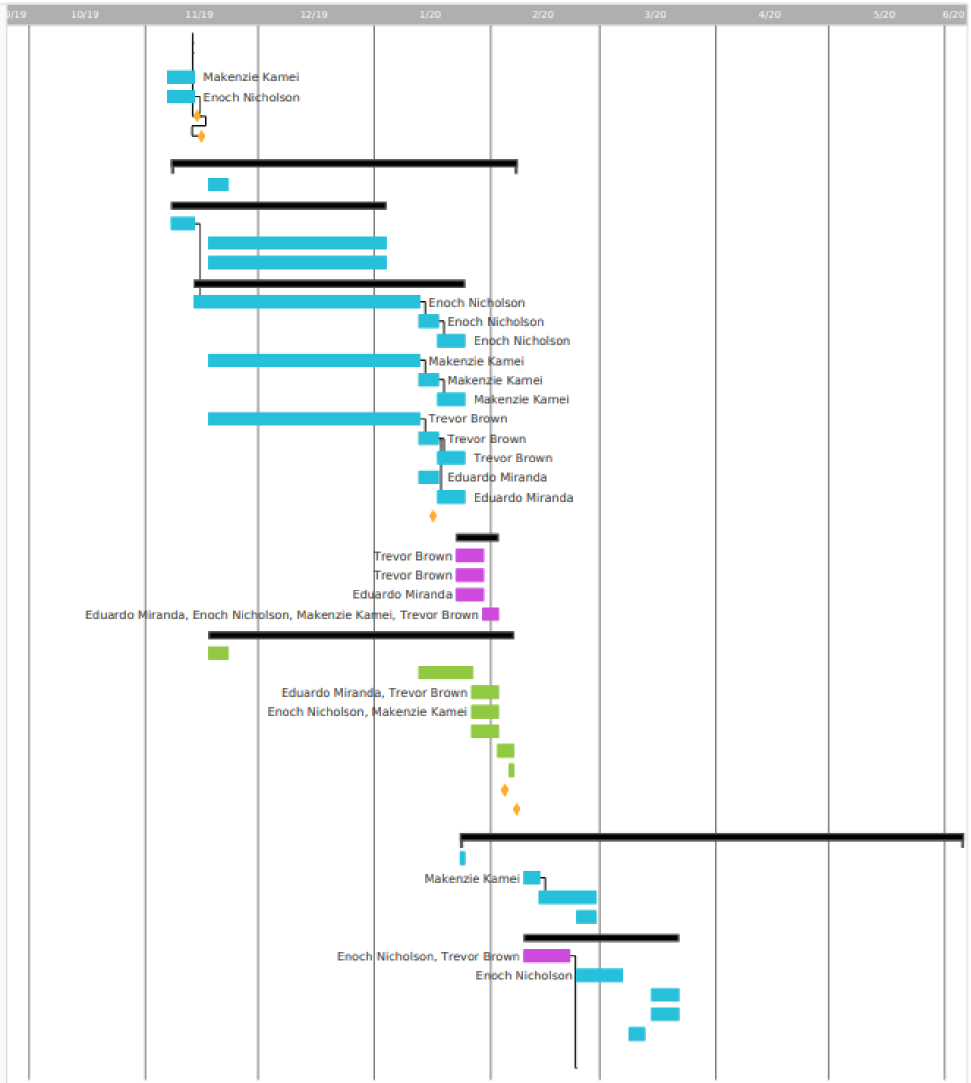
	start	end	0h	100%
F11 - Microscope				
Problem Definition	**09/26/19**	**10/18/19**	**0h**	**100%**
Choose Project	09/26	09/26	0	100%
Meet Team	09/27	09/29	0	100%
Introduce Team to Sponsors	09/27	09/29	0	100%
Customer/Need Research	**09/30/19**	**10/10/19**	**0h**	**100%**
Research Plan	**09/30/19**	**10/08/19**	**0h**	**100%**
Find and document existing prod...	09/30	10/08	0	100%
Document companies	09/30	10/08	0	100%
Find 5-10 journal articles	09/30	10/08	0	100%
Find 5-10 patents	09/30	10/08	0	100%
Investigate profesional standards	09/30	10/08	0	100%
Find government reports	09/30	10/08	0	100%
Identify related books / textbooks	09/30	10/08	0	100%
Find previous senior projects	09/30	10/08	0	100%
Interview Sponsor	09/30	10/08	0	100%
Capture Customer Needs/Wants	10/10	10/10	0	100%
Write Problem Statement	10/09	10/09	0	100%
Perform QFD	10/10	10/14	0	100%
Create Specification Table	10/10	10/14	0	100%
Write Specification Descriptions	10/10	10/14	0	100%
Create Initial Project Plan	10/15	10/16	0	100%
Write Scope of Work	**10/09/19**	**10/16/19**	**0h**	**100%**
Write Introduction	10/09	10/16	0	100%
Write Background	10/09	10/16	0	100%
Write Objectives	10/09	10/16	0	100%
Write Proj Mgt	10/09	10/16	0	100%
Write Conclusion	10/09	10/16	0	100%
Write Abstract	10/09	10/16	0	100%
Develop Initial Gantt Chart	10/15	10/17	0	100%
Scope of Work Milestone	10/18	10/18	0	100%
Concept Generation / Selection	**10/21/19**	**11/15/19**	**0h**	**100%**
Functional Decomposition	10/21	10/24	0	100%
Ideation & Evaluation	**10/25/19**	**11/01/19**	**0h**	**100%**
Develop & sketch two concept idea...	10/25	10/29	0	100%
Create two concept models (each)	10/30	10/30	0	100%
Decision Matrices / Idea Evaluation	10/31	11/01	0	100%
Complete Concept CAD	**11/02/19**	**11/06/19**	**0h**	**100%**
Optical Pathway	11/02	11/06	0	100%
Stage	11/02	11/06	0	100%
Fixtures / Frame	11/02	11/06	0	100%
Control System Block Diagram	11/02	11/06	0	100%
Build Concept Prototype	**11/07/19**	**11/11/19**	**0h**	**100%**
Optics Moc-Up	11/07	11/11	0	100%
Stage Moc-Up	11/07	11/11	0	100%
Frame Moc-Up	11/07	11/11	0	100%
Search for components	**11/01/19**	**11/13/19**	**0h**	**100%**
Establish Conceptual BoM	11/01	11/06	0	100%
Evaluate Objective Options	11/07	11/13	0	100%



APPENDIX B

INITIAL GANTT CHART – CONTINUED

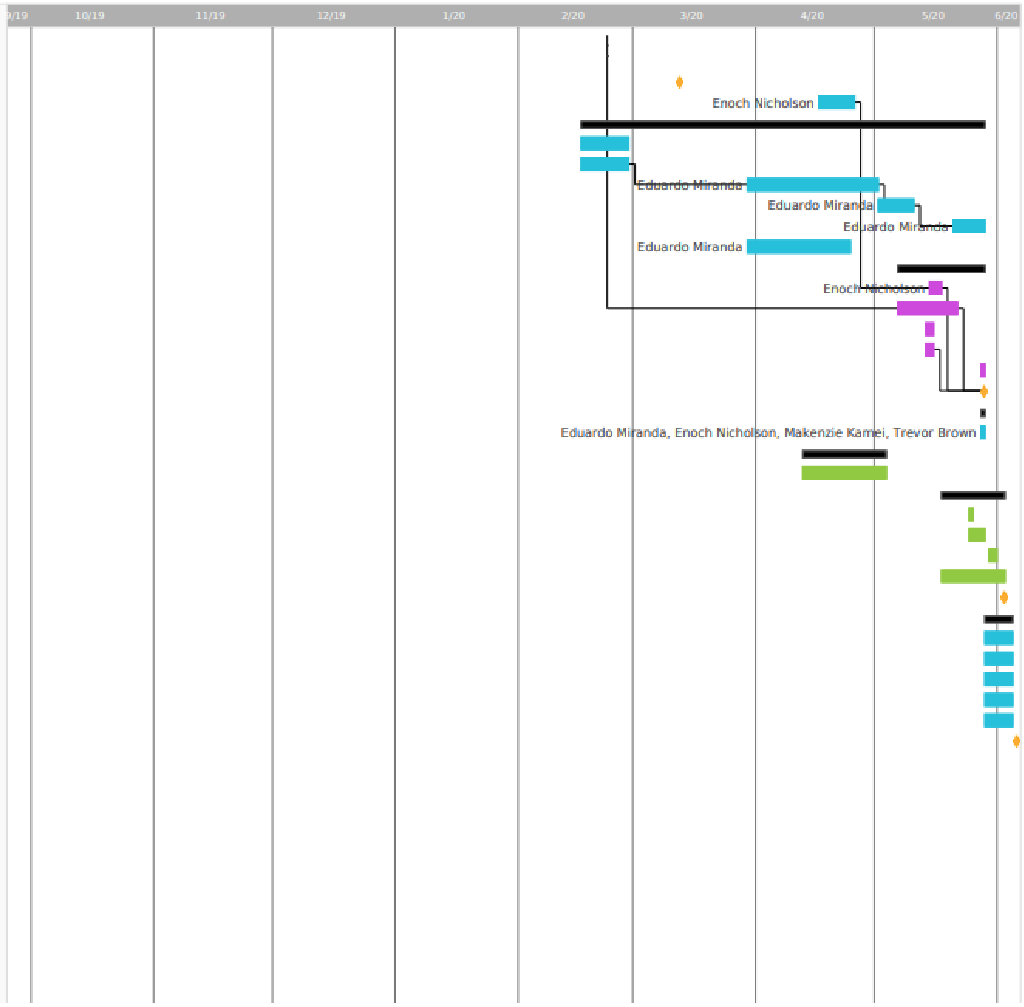
Search for necessary optical comp...	11/07	11/13	0	100%
Search for stage	11/07	11/13	0	100%
Preliminary Design Review (PDR) Pre...	11/14	11/14	0	100%
Submit PDR	11/15	11/15	0	100%
Detailed Design	**11/08/19**	**02/07/20**	**0h**	**100%**
FMEA	11/18	11/22	0	100%
Initial Component Purchasing	**11/08/19**	**01/03/20**	**0h**	**100%**
Purchase a Stage	11/08	11/13	0	100%
Buy initial optical components	11/18	01/03	0	100%
Buy frame and Z-Axis Components	11/18	01/03	0	100%
Detail CAD & Manufacturing Plan	**11/14/19**	**01/24/20**	**0h**	**100%**
CAD Purchased Stage & Implement...	11/14	01/12	0	100%
Make Stage Drawings with Modifica...	01/13	01/17	0	100%
Develop Stage Manufacturing Plan	01/18	01/24	0	100%
Model Actual Optical Components, ...	11/18	01/12	0	100%
Create Detail Drawings for optics	01/13	01/17	0	100%
Develop Optics Manufacturing Plan	01/18	01/24	0	100%
CAD Frame & Z-Axis	11/18	01/12	0	100%
Detail Drawings for Frame and Z-Ax...	01/13	01/17	0	100%
Develop Frame & Z-Axis Manufactu...	01/18	01/24	0	100%
Create Overall Assembly With Views	01/13	01/17	0	100%
Detailed Layout Drawing	01/18	01/24	0	100%
Interim Design Review	01/16	01/16	0	100%
Build Structural Prototype	**01/23/20**	**02/02/20**	**0h**	**100%**
Print Mounting Brackets for Actuati...	01/23	01/29	0	100%
Print Mounting Brackets for Optics	01/23	01/29	0	100%
Write Script to move stage	01/23	01/29	0	100%
Assemble	01/30	02/02	0	100%
CDR Report & Presentation	**11/18/19**	**02/06/20**	**0h**	**100%**
Implement corrections to PDR Rep...	11/18	11/22	0	100%
Update Sections for CDR	01/13	01/26	0	100%
Write Design Verrification plan	01/27	02/02	0	100%
Write Final Design Chapter	01/27	02/02	0	100%
Write Manufacturing Plan	01/27	02/02	0	100%
Create Presentation	02/03	02/06	0	100%
Practice Presentation	02/06	02/06	0	100%
Critical Design Review (CDR) Present...	02/04	02/04	0	100%
Submit CDR	02/07	02/07	0	100%
Manufacturing & Testing	**01/24/20**	**06/05/20**	**0h**	**100%**
Stage Pull Test	01/24	01/24	0	100%
Final Component Purchasing (Optics...	02/10	02/13	0	100%
Waiting for shipping	02/14	02/28	0	100%
Troubleshooting Cleanroom Printer	02/24	02/28	0	100%
Stage	**02/10/20**	**03/21/20**	**0h**	**100%**
Stage Bracket Fit Iteration	02/10	02/21	0	100%
Bracket Failure Test	02/24	03/06	0	100%
Y-Axis Bracket: Add extra mounting	03/15	03/21	0	100%
Adjust Spring Lengths	03/15	03/21	0	100%
Prepare MFG & test review	03/09	03/12	0	100%



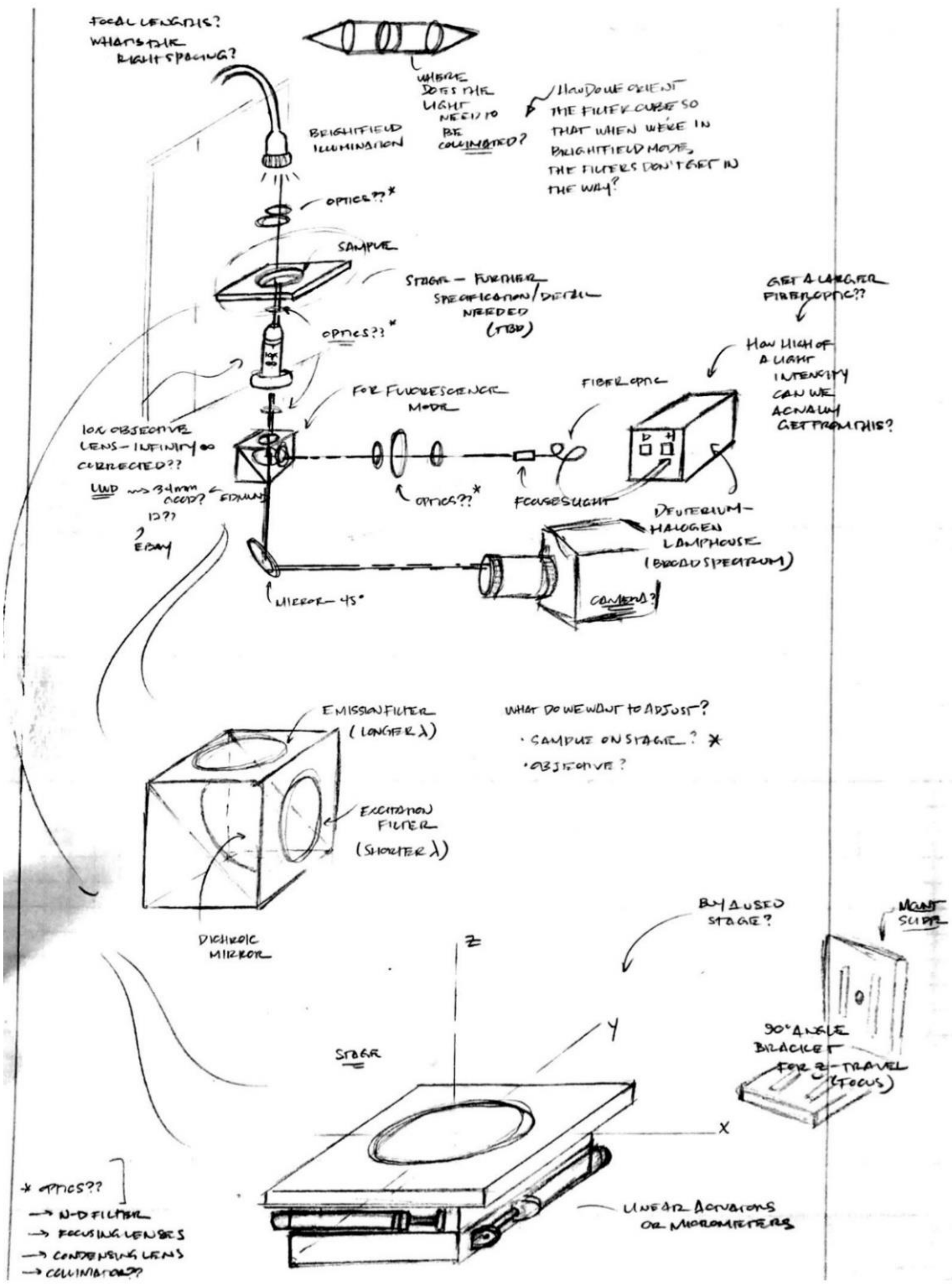
Appendix B

Initial Gantt Chart – Continued

Manufacturing & Test Review	03/12	03/12	0	100%
Finalize Stage Plate Model	04/17	04/25	0	100%
Electrical Controls	**02/17/20**	**05/28/20**	**0h**	**100%**
Limit Switch Testing	02/17	02/28	0	100%
Arduino Mega Setup	02/17	02/28	0	100%
Programable Path Code Outline	03/30	05/01	0	100%
Programable Path Code Creation	05/02	05/10	0	100%
Programable Path Code Testing	05/21	05/28	0	100%
Electronics Enclosure Selection	03/30	04/24	0	100%
Remote Assembly / Outsourcing	**05/07/20**	**05/28/20**	**0h**	**100%**
Outsource Fabrication of Stage Pla...	05/15	05/17	0	100%
Mount Stage on Breadboard	05/07	05/21	0	100%
Assemble 80-20 Frame	05/14	05/15	0	100%
Attach Bright-field Illuminator	05/14	05/15	0	100%
Attach / Align Optics	05/28	05/28	0	100%
Confirmation Prototype	05/28	05/28	0	100%
Functional Verrification	**05/28/20**	**05/28/20**	**0h**	**100%**
Bright field System Testing	05/28	05/28	0	100%
Operator's Manual	**04/13/20**	**05/03/20**	**0h**	**100%**
Operator's Manual	04/13	05/03	0	100%
Create Project Poster	**05/18/20**	**06/02/20**	**0h**	**100%**
Record Audio	05/25	05/25	0	100%
Edit Engineering Video	05/25	05/28	0	100%
Edit General Video	05/30	05/31	0	100%
Create Project Website	05/18	06/02	0	100%
Senior Project Expo	06/02	06/02	0	100%
Prepare FDR Report	**05/29/20**	**06/04/20**	**0h**	**100%**
Rewrite Manufacturing chapter	05/29	06/04	0	100%
Rewrite Testing chapter	05/29	06/04	0	100%
Revise Abstract	05/29	06/04	0	100%
Revise Introduction	05/29	06/04	0	100%
Revise Conclusion	05/29	06/04	0	100%
Submit FDR Report	06/05	06/05	0	100%

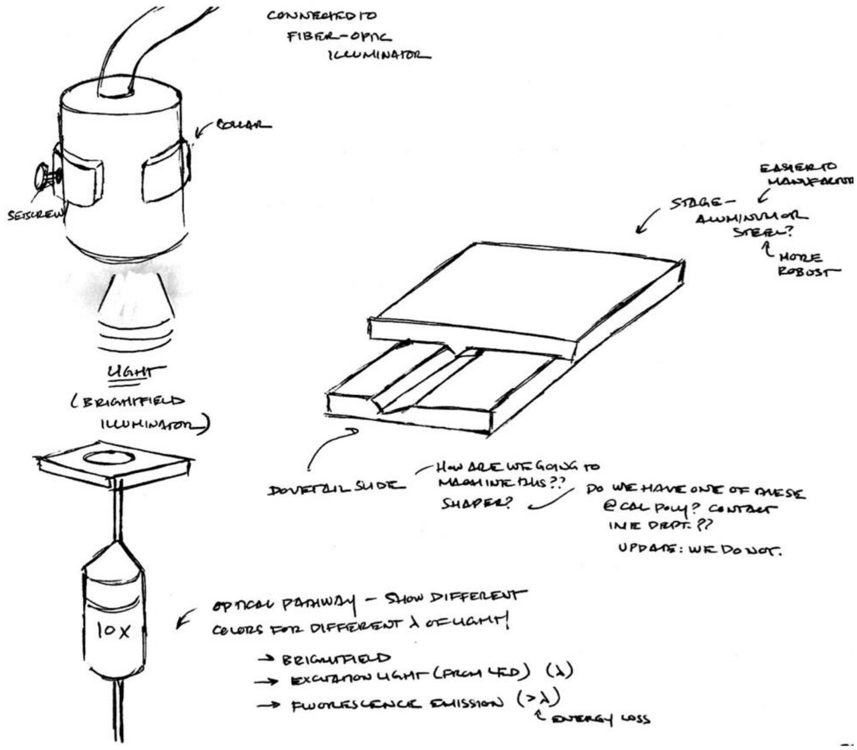


APPENDIX C
CONCEPT SKETCHES

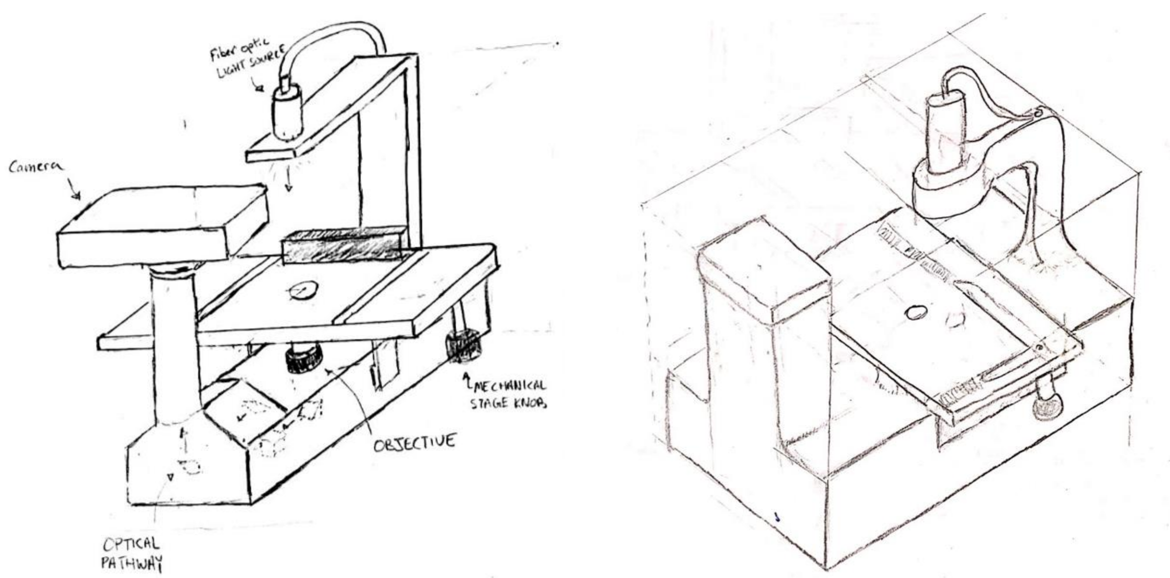


(a) Preliminary concept sketches: optical pathway, X-Y stage, and Z focus.

APPENDIX C
CONCEPT SKETCHES (CONTINUED)

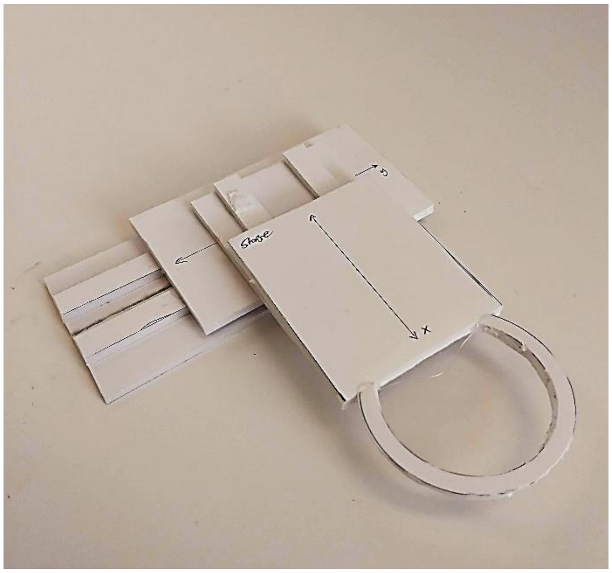


(b) Preliminary concept sketches: collar for brightfield collimator, dovetail slide stage.



(c) Two preliminary concept sketches of the microscope frame and integration of stage and optical components into a single unit.

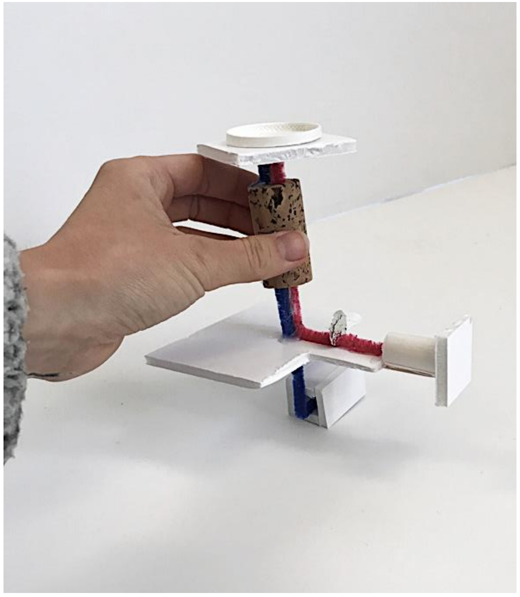
APPENDIX D
CONCEPT MODELS



(a) Concept Stage

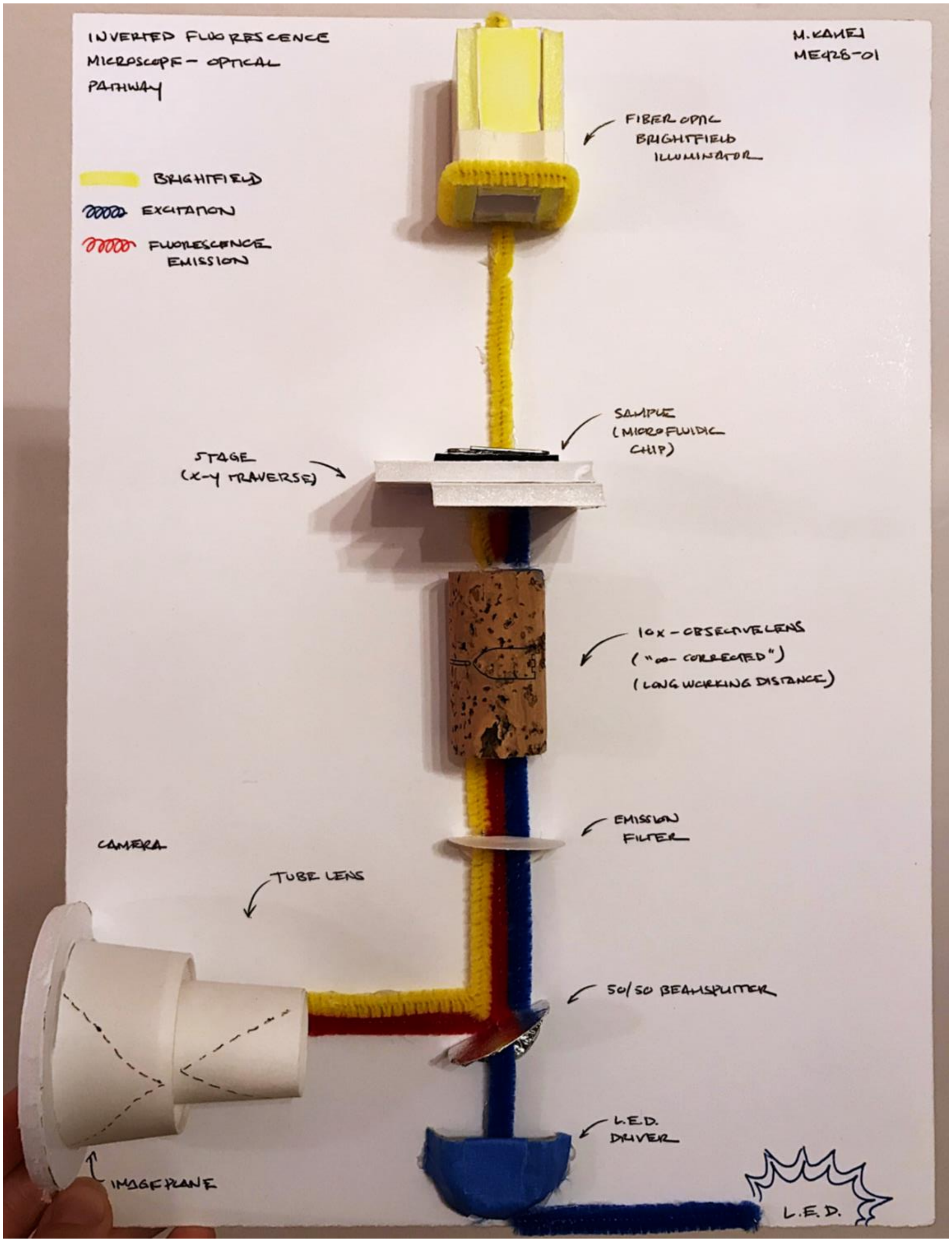
(b) Concept Frame

(c) Z-Axis Focus



(d) Illumination Pathway

APPENDIX D
CONCEPT MODELS (CONTINUED)



(e) Full Optical Pathway

APPENDIX E
INVENTORY: MICROFABRICATION LABORATORY

PART NO.	NAME	VENDOR	DIMENSIONS (if applicable)	#
FB650-10	Bandpass Filter 600-690nm	ThorLabs	Ø1"	?
FB550-10	Bandpass Filter 500-590nm	ThorLabs	Ø1"	?
FB450-10	Bandpass Filter 400-490nm	ThorLabs	Ø1"	?
NE40A	Absorptive ND Filter	ThorLabs	Ø25 mm	3
SM2	Plastic Filters (R,G, B) SM Series	Edmund Optics	Ø25 mm	11R 9G 11B
JF2	Plastic Filters (R,G, B) JF Series	Edmund Optics	Ø25 mm	2
JF3	Plastic Filters (R,G, B) JF Series	Edmund Optics	Ø25 mm	3
CM1-BP150	Cube-Mounted Pellicle Beamsplitter (50.50)	ThorLabs	30mm	1
CM1-BP108	Cube-Mounted Pellicle Beamsplitter (8.92)	ThorLabs	30mm	1
DH-2000-BAL	Deuterium-Halogen Light Source	Ocean Optics		
	USB4000 Spectrometer	Ocean Optics	89.1 mm x 63.3 mm x 34.4 mm	1
3943 (Family #)	TECHSPEC Linear Translation Stages	Edmund Optics		10
	LS-1 Tungsten Halogen Light Source	Ocean Optics		2
3100 002831000000	Fiber-Lite 3100 Illuminator	Dolan-Jenner	25 mm filter port	2
	Fiber Optic Attachment			
GBE10M-A	10X Achromatic Galilean Beam Expander	ThorLabs	Max. input beam diamter 3.5mm	1
PL-A662	Evolution LC Megapixel Firewire Camera Kit	Media Cybernetics		1

APPENDIX F
PUGH MATRICES

(1) Objective Lens

	PRODUCT ALTERNATIVES				
CRITERIA	[1] Mitutoyo Plan Apo 10X	[2] EO M Plan Apo 10X	[3] EO High Resolution 10X	[4] Nikon CF Plan 5X (Ebay)	[5] AmScope Fluor Plan 4X
Magnification (10X)	s	s	s	-	-
Epi-Illumination	s	s	s	s	s
LWD (34mm)	s	s	-	-	-
"∞-Corrected"	s	s	s	-	s
Cost (~$885)	s	+	-	+	+
Thread Standard	s	s	s	s	-
SCORE:	**0**	**1**	**-2**	**-2**	**-2**


[1]


[2]

[3]

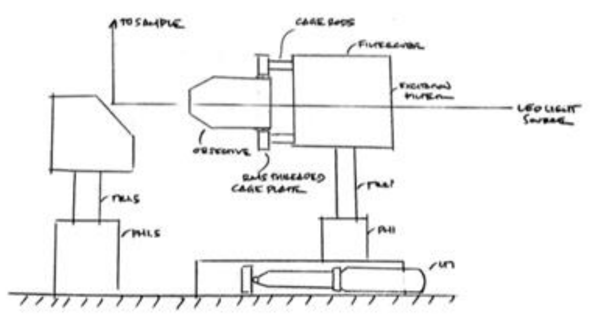
[4]

[5]

APPENDIX F
PUGH MATRICES (CONTINUED)

(2) Objective Lens Mounting

	ALTERNATIVES						
	Horizontal Configuration				Vertical Configuration		
CRITERIA:	[1] Caged Optics	[2] 2 Thread Adapters	[3] Machined Aluminum Block	[4] 3-D Printed Bracket	[5] Caged Optics	[6] 2 Thread Adapters	[7] Machined Aluminum Block
Modularity (Ability to Switch Filters	s	-	-	s	s	-	-
Distance of Focus Travel	s	s	s	s	-	-	-
Fluorescence Transmission	s	s	s	s	+	+	+
Cost	s	s	+	+	s	s	+
Time to Manufacture	s	s	-	-	s	s	-
Alignment Rigidity	s	-	+	+	s	-	+
SCORE:	**0**	**-2**	**0**	1	**0**	**-2**	**0**



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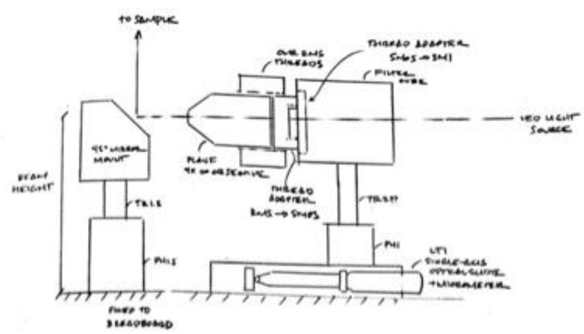

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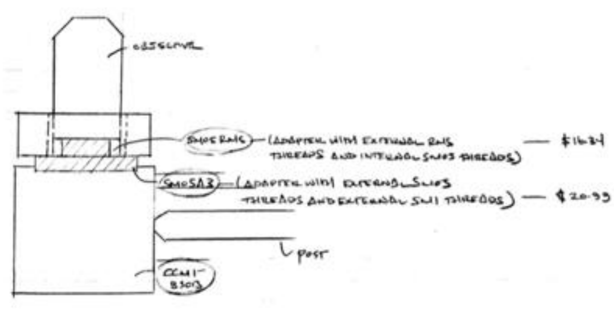
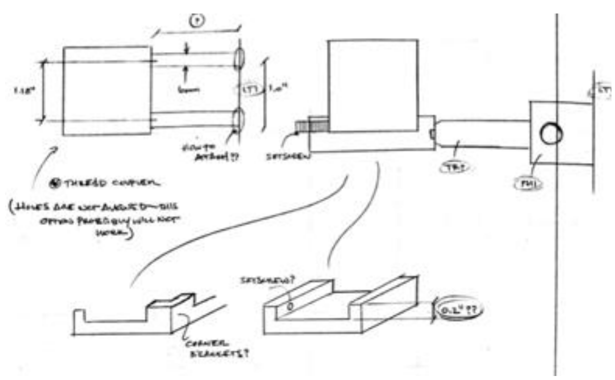


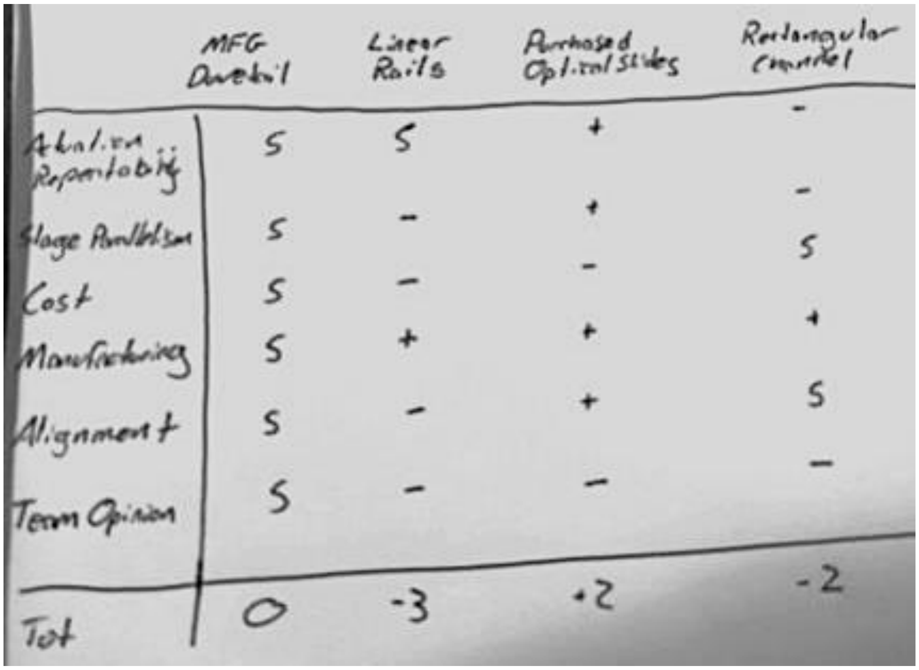
[6]



[7]

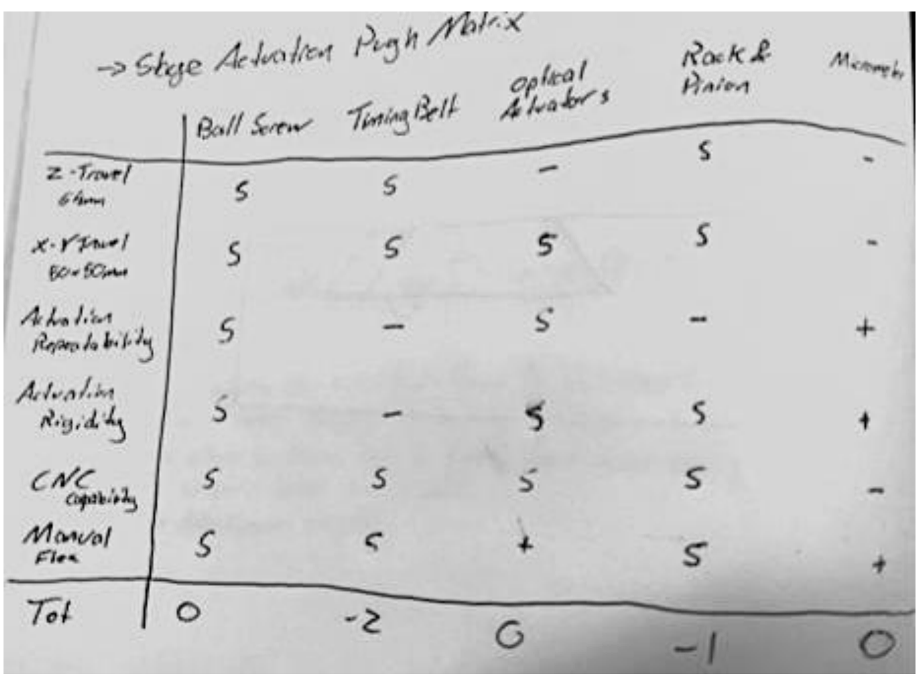
APPENDIX F
PUGH MATRICES (CONTINUED)

(3) Stage Form



	MFG Dovetail	Linear Rails	Purchased Optical Slides	Rectangular Channel
Actuation Repeatability	S	S	+	-
Stage Parallelism	S	-	+	-
Cost	S	-	-	S
Manufacturing	S	+	+	+
Alignment	S	-	+	S
Team Opinion	S	-	-	-
Tot	0	-3	+2	-2

(4) Stage Actuation



-> Stage Actuation Pugh Matrix

	Ball Screw	Timing Belt	Optical Actuators	Rack & Pinion	[illegible]
Z-Travel 64mm	S	S	-	S	-
X-Y Travel 50x50mm	S	S	S	S	-
Actuation Repeatability	S	-	S	-	+
Actuation Rigidity	S	-	S	S	+
CNC capability	S	S	S	S	-
Manual Flex	S	S	+	S	+
Tot	0	-2	0	-1	0

APPENDIX G
TABLE OF INDICATOR DYES

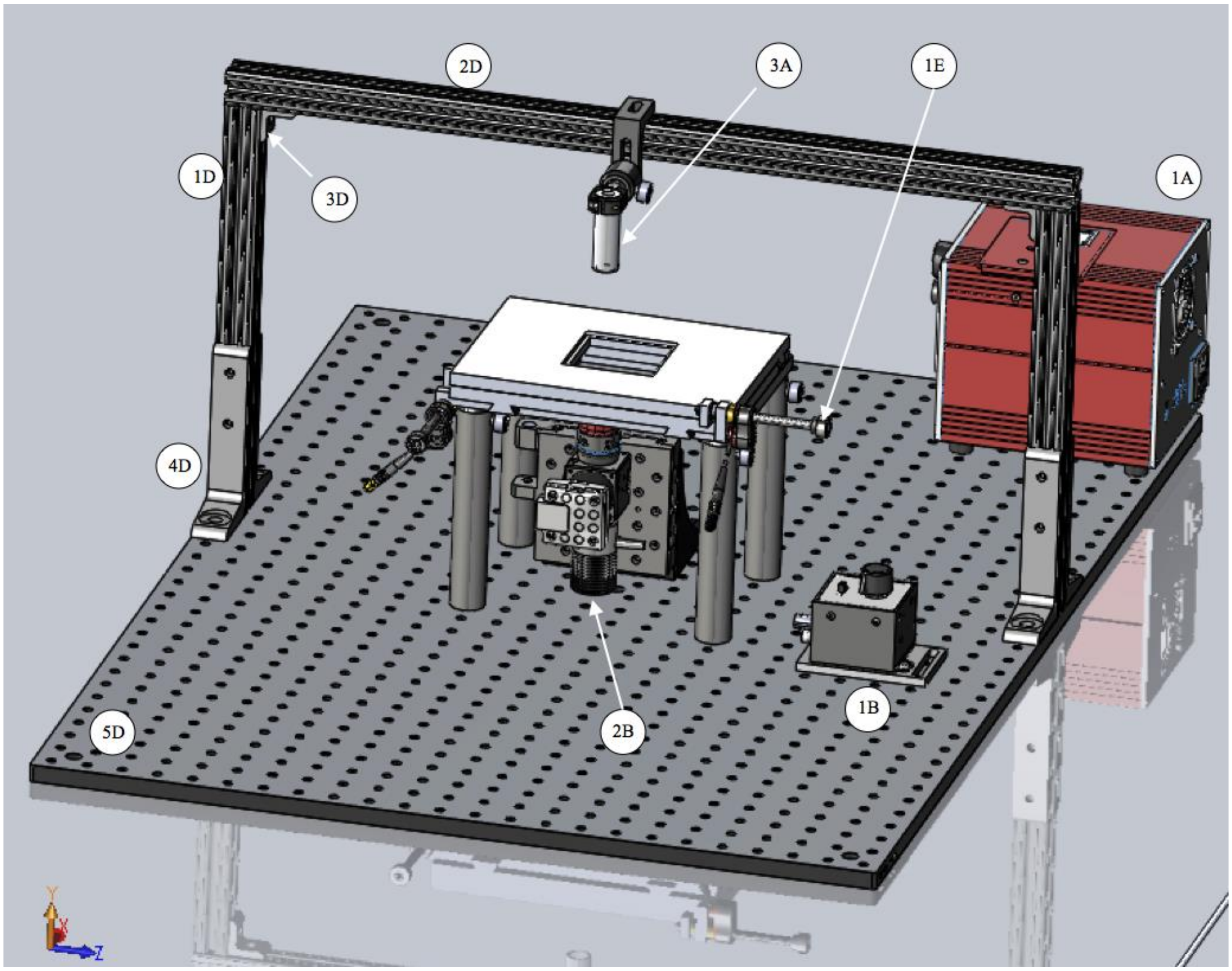
TABLE 11.1 Properties of Commonly Used Fluorescent Dyes[a]

Fluorochrome	Color Band[b]	Excitation (nm)	Emission (nm)	Filter Set[c]
Acridine orange	Cyan	502	525	FITC
Allophycocyanin	Red	621/650	661	Cy5
AMCA	UV	350	445	DAPI
Alexa Fluor 405	UV	401	421	DAPI
Alexa Fluor 488	Cyan	495	519	FITC
Alexa Fluor 568	Yellow	578	603	TxRed
Alexa Fluor 647	Red	650	665	Cy5
ATTO 488	Cyan	501	523	FITC
ATTO 550	Green	554	576	TRITC
ATTO 594	Yellow	601	627	TxRed
ATTO 740	Far-Red	740	764	Cy7
BODIPY FL	Cyan	503	512	FITC
BODIPY TMR	Green	542	574	TRITC
Cascade blue	UV	400	425	DAPI
Carboxy-SNARF-1 (low pH)	Green	548	587	TRITC
Cy2	Cyan	489	506	FITC
Cy3	Green	548	562	TRITC
Cy5	Red	650	670	Cy5
Cy7	Far-Red	710	805	Cy7
DAPI (bound to DNA)	UV	350	470	DAPI
$DiIC_{18}$ (bound to lipid)	Green	549	565	TRITC
$DiOC_6$	Cyan	484	501	FITC
Fluorescein (FITC)	Cyan	494	518	FITC
Fluo-3 (with calcium)	Cyan	485	503	FITC
FM 1-43 (bound to Lipid)	Cyan	473	578	Special
Fura-2 (with calcium)	UV	335	505	Special
Hoechst 33258, 33342	UV	352	461	DAPI
Indo-1 (with calcium)	UV	350	405/482	Special
Lissamine-rhodamine B	Yellow	575	595	TxRed
Lucifer yellow	Blue	425	528	Special
LysoTracker green	Cyan	504	511	FITC
LysoTracker red	Yellow	577	590	TxRed
MitoTracker red	Yellow	581	644	TxRed
Oregon green 488	Cyan	496	524	FITC
Oregon green 514	Green	511	530	FITC
Phycoerythrin-R	Green	565	578	TRITC
Propidium iodide	Green	520	610	TRITC
SYTOX green	Cyan	504	523	FITC
SYTOX orange	Green	547	570	TRITC
Tetramethylrhodamine	Green	540	578	TRITC

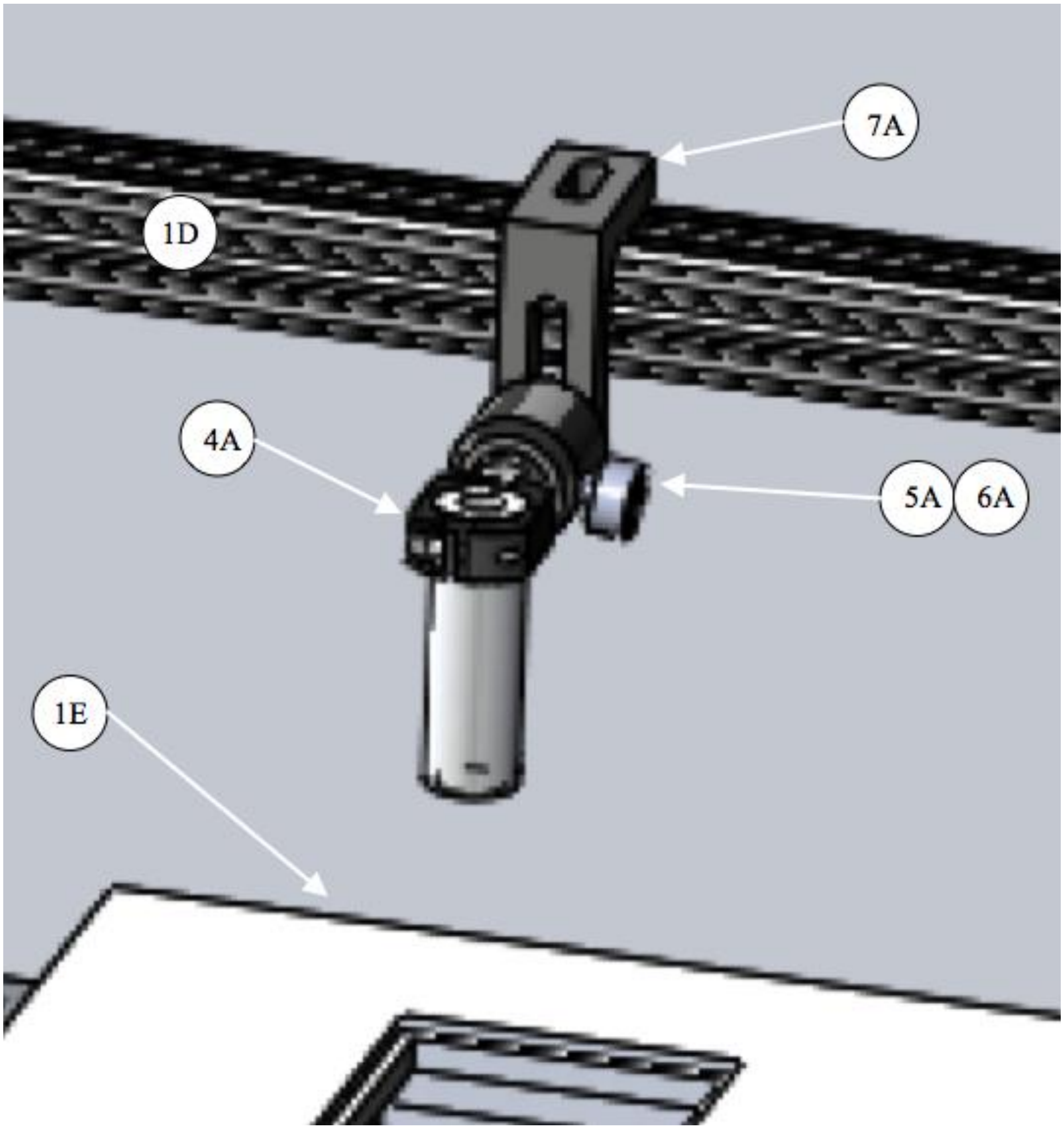
APPENDIX H

CONCEPT CAD – ISOMETRIC VIEW

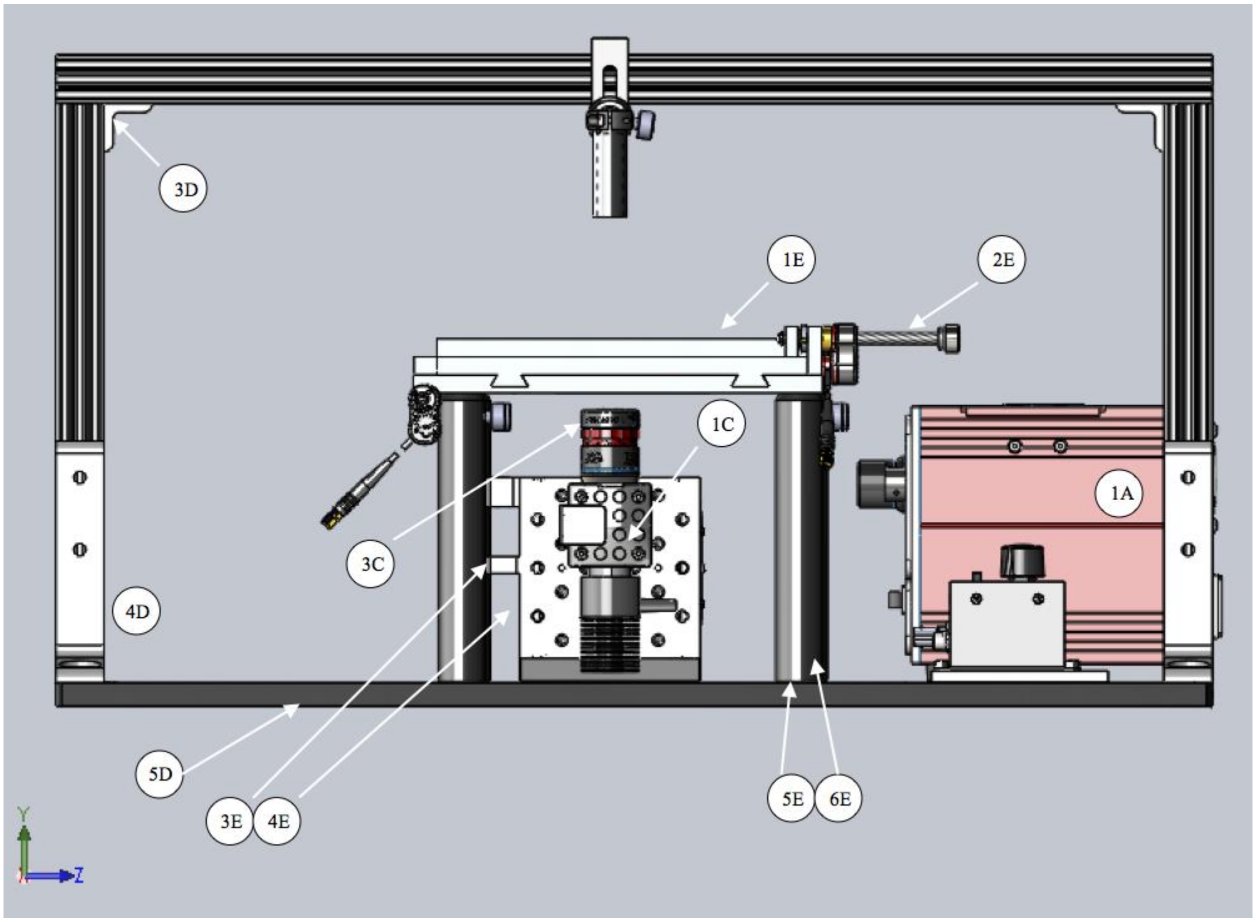
Note: All corresponding part labels are listed in the Conceptual Bill of Materials in Appendix [I].



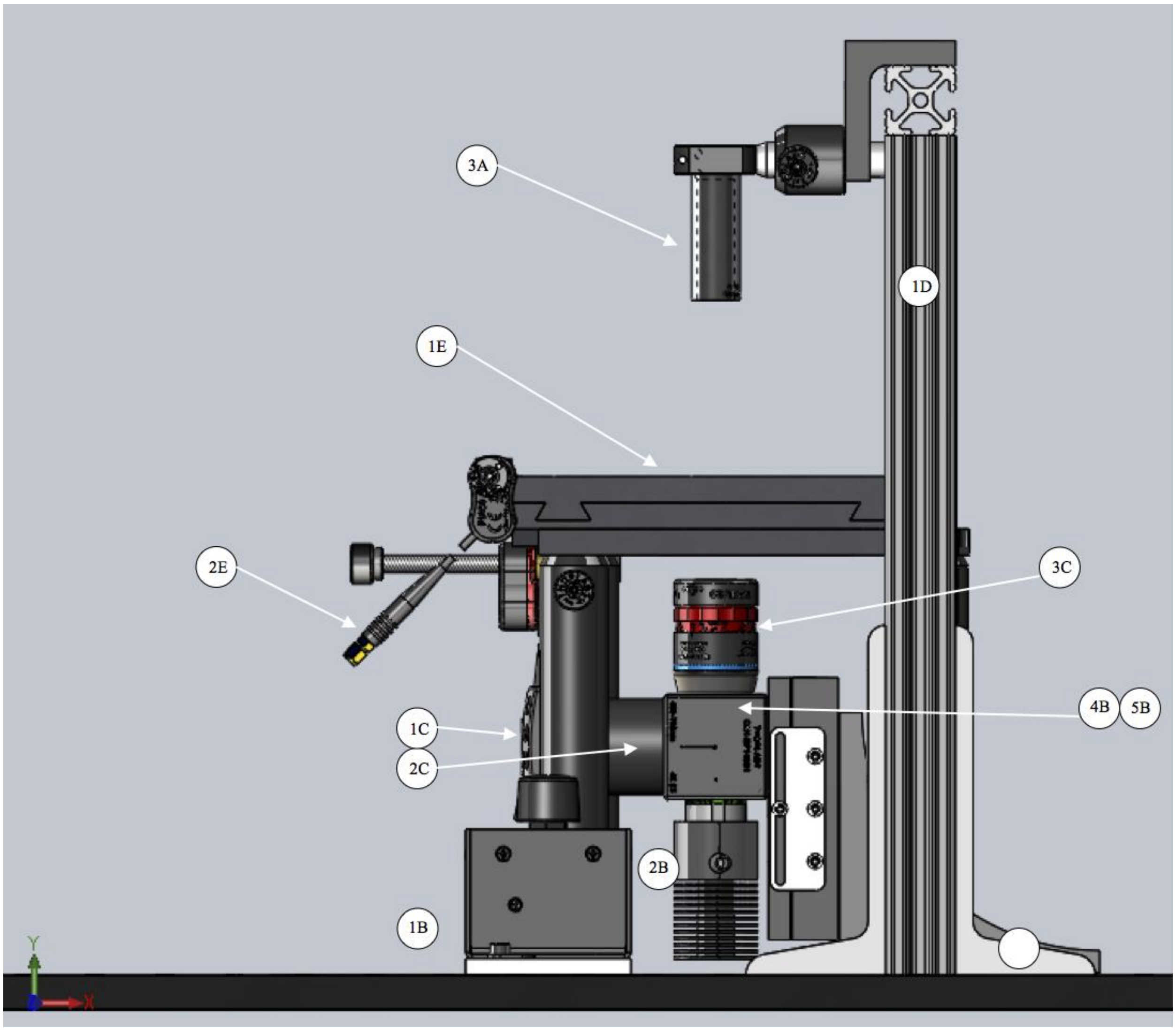
Appendix H
Concept CAD – Brightfield Illumination



APPENDIX H
CONCEPT CAD – FRONT VIEW



APPENDIX H
CONCEPT CAD – SIDE VIEW



APPENDIX I
CONCEPTUAL BILL OF MATERIALS

BRIGHTFIELD ILLUMINATION

PART	PART NO.	NAME	VENDOR	PRICE	#
1A	OSL2	Fiber Light Source	ThorLabs	$990.14	1
2A	OSL2FB	Fiber Bundle	ThorLabs	(included w/ source)	1
3A	OSL2COL	Collimation Package for Fiber Optic Bundle	ThorLabs	$98.47	1
4A	SM05RC	Slip Ring	ThorLabs	$21.31	1
5A	TR2	Ø1/2" Optical Post, L = 2"	ThorLabs	$5.35	1
6A	PH1	Standard Ø1/2" Post Holder, L = 1"	ThorLabs	$7.24	1
7A	AB90H	Slim Right-Angle Bracket	ThorLabs	$27.85	1

FLUORESCENCE ILLUMINATION

1B	LEDD1B	T-Cube LED Driver	ThorLabs	$323.55	1
2B	M505L4	Single-Color Cold Visible Mounted LED (Cyan)	ThorLabs	$296.50	1
3B	CAB-LEDD1	LED Connection Cable	ThorLabs	(included w/ driver)	1
4B	CCM1-BS013	Cube-Mounted, Non-Polarizing, 50:50 Beamsplitter	ThorLabs	$296.50	1
5B	MF530-43	FITC Emission Filter	ThorLabs	$257.54	1
6B	TR1.5	Ø1/2" Optical Post	ThorLabs	$5.12	1
7B	UPH1.5	Universal Ø1/2" Post Holder	ThorLabs	$32.74	1

IMAGING

1C	DCC1645C	USB 2.0 CMOS Camera, Color Sensor	ThorLabs	$387.92	1
2C	SM2L20	SM2 Lens Tube	ThorLabs	$32.31	1
3C	MY10X-823	10X Plan Mitutoyo Apochromat	ThorLabs	$1,895.20	1

FRAME

1D	47065T101	T-Slotted Framing (1ft)	McMaster-Carr	$5.84	2
2D	47065T101	T-Slotted Framing (2ft)	McMaster-Carr	$7.79	1
3D	47065T236	Corner Bracket	McMaster-Carr	$5.21	2
4D	47065T841	Mounting Foot	McMaster-Carr	$12.00	4
5D	MB2436	Aluminum Breadboard 24" x 36" x 1/2"	ThorLabs	$736.92	1

STAGE AND TRAVEL

1E	N/A	THK XY Stage	Ebay	$220.00	1
2E	PIA50	Piezo Inertia Actuator	ThorLabs	$550.80	2
3E	AP90	Right-Angle Mounting Plate	ThorLabs	$86.30	1
4E	XR50P	Linear Translation Stage, Side-Mounted Micrometer	ThorLabs	$769.15	1
5E	PH6	Standard Ø1/2" Post Holder, L = 6"	ThorLabs	$13.02	4
6E	TR6	Ø1/2" Optical Post, L = 6"	ThorLabs	$7.33	4

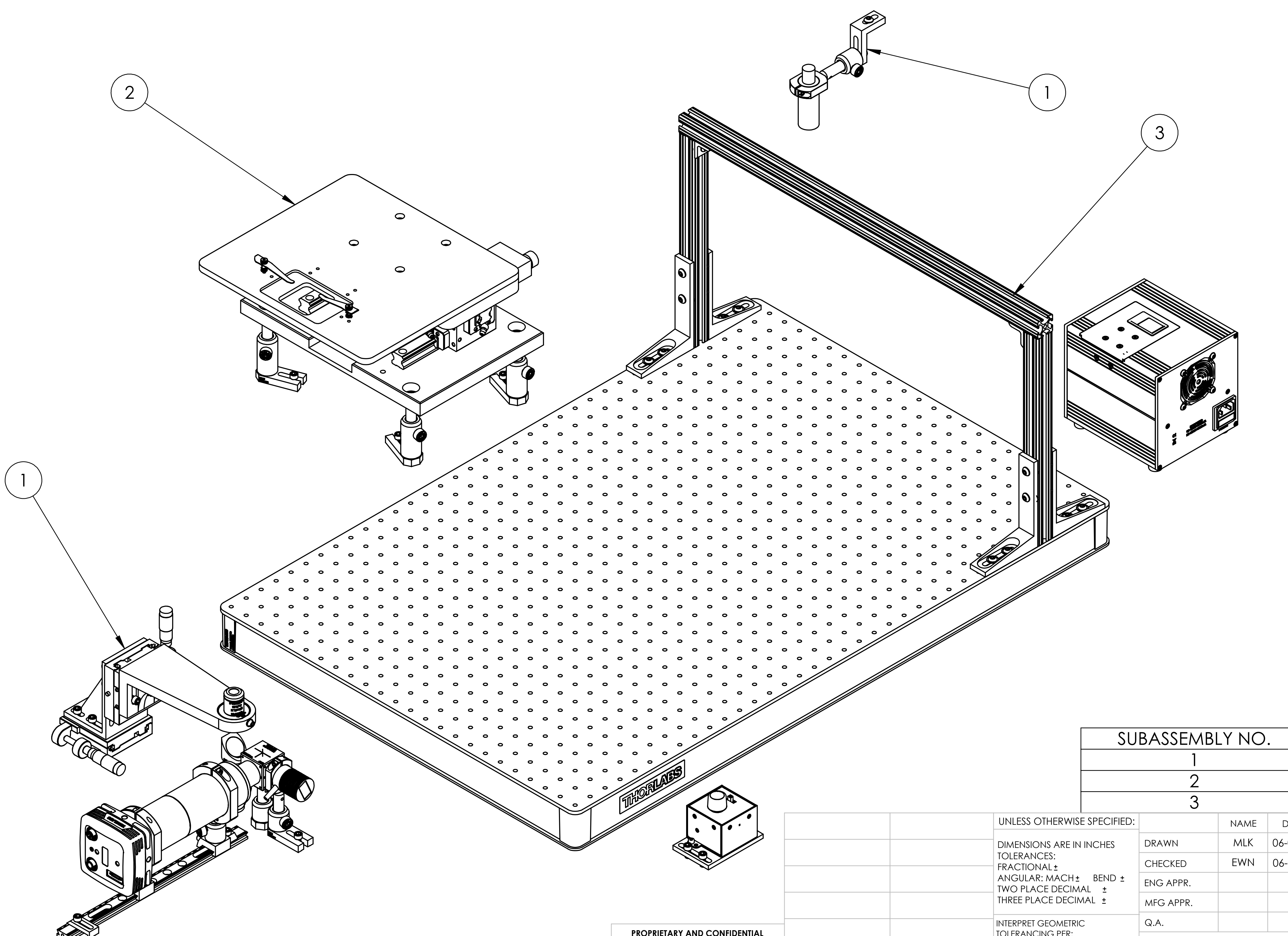
Note: Items in gray are already available for use in the Microfabrication Lab. Marked in green are items that we currently have access to, but will likely upgrade at a later time

Appendix J

Bill of Materials

Level	Number	Description	Qty.	Cost	Ttl. Cost	Source	More Info.
		Lvl 0 *Lvl 1* *Lvl 2* *Lvl 3*					
0	010000	Final Assembly					
1	011000	Optical Train					
2	011100	Brightfield Illumination					
3	011101	Fiber-Lite 3100 Illuminator	1	$410.67	$410.67	Dolan-Jenner	3100 00283 1000000
3	011102	Model 3100-1 Gooseneck Single-Arm	1	included w/ source)		Dolan-Jenner	
3	011103	Focusing Lens	1	included w/ source)		Dolan-Jenner	LH-759
3	011104	Slip Ring for Ø1" Components	1	$27.59	$27.59	Thorlabs Inc.	C1RC
3	011105	Slim Right-Angle Bracket with Slots	1			3-D Print	DWG NUMBER
3	011106	Ø1/2" Post Holder, L = 1"	1	$7.24	$7.24	Thorlabs Inc.	PH1
3	011107	Ø1/2" Post, L = 2"	1	$5.35	$5.35	Thorlabs Inc.	TR2
2	011200	Fluorescence Illumination					
3	011201	T-Cube LED Driver	1	$323.55	$323.55	Thorlabs Inc.	LEDD1B
3	011202	Single-Color Mounted LED; 490mn	1	$206.68	$206.68	Thorlabs Inc.	M490L4
3	011203	LED Connection Cable	1	(included w/ driver)		Thorlabs Inc.	CAB-LEDD1
3	011204	Slip Ring for SM1 Lens Tubes	1	$25.10	$25.10	Thorlabs Inc.	SM1RC
3	011205	Ø1/2" Post Holder, L = 1"	1	$7.24	$7.24	Thorlabs Inc.	PH1
3	011206	Ø1/2" Post, L = 1.5"	1	$4.88	$4.88	Thorlabs Inc.	TR1
2	011300	Optics and Mounts					
3	011301	4X Plan F Infinity-Corrected Objective Lens	1	$71.99	$71.99	AmScope	PF4X-INF
3	011302	Objective Mounting Bracket	1			3-D Print	DWG NUMBER
3	011303	RMS Microscope Objective Mount, 8-32 Tap	1	$29.76	$29.76	Thorlabs Inc.	OMR-RMS
3	011304	1" Round, Protected Aluminum Mirror, 3.2mm Thick	1	$14.82	$14.82	Thorlabs Inc.	ME1-G01
3	011305	45° Mount Assembly for Ø1" Optics	1	$48.01	$48.01	Thorlabs Inc.	H45B2
3	011306	Cube-Mounted, Non-Polarizing, 50:50 Beamsplitter	1	$296.50	$296.50	Thorlabs Inc.	CCM1-BS013
3	011307	FITC Emission Filter; CWL = 530nm	1	$257.54	$257.54	Thorlabs Inc.	MF530-43
3	011308	SM1 Retaining Ring for Ø1" Lens Tubes and Mounts	1	$4.64	$4.64	Thorlabs Inc.	SM1RR
3	011309	SM1 Lens Tube, 1" Thread Depth	1	$14.68	$14.68	Thorlabs Inc.	SM1L10
3	011310	Ø1/2" Post Holder, L = 1"	1	$7.24	$7.24	Thorlabs Inc.	PH1
3	011311	Ø1/2" Post, L = 1"	1	$4.88	$4.88	Thorlabs Inc.	TR1
3	011312	Ø1/2" Post, L = 1.5"	1	$5.12	$5.12	Thorlabs Inc.	TR40/M
3	011313	8-32 Flanged Socket Head Screw, L = 3/8"	1	$3.27	$3.27	McMaster Carr	92235A507
2	011400	Imaging					
3	011401	Evolution LC Megapixel Firewire Camera Kit	1			Media Cybernetics	PL-A662
3	011402	Adapter with External C-Mount Threads and Internal SM2 Th	1	$27.59	$27.59	Thorlabs Inc.	SM2A31
3	011403	SM2 Lens Tube, 0.3" Thread Depth	1	$24.06	$24.06	Thorlabs Inc.	SM2L03
3	011404	SM2 Lens Tube, 0.5" Thread Depth	1	$26.53	$26.53	Thorlabs Inc.	SM2L05
3	011405	SM2 Lens Tube, 2" Thread Depth	1	$32.31	$32.31	Thorlabs Inc.	SM2L20
3	011406	SM2 Lens Tube, 3" Thread Depth	1	$38.09	$38.09	Thorlabs Inc.	SM2L30
3	011407	Tube Lens, f = 200mm, External SM2 Threads	1	$496.70	$496.70	Thorlabs Inc.	TTL200-A
3	011408	Adapter with External SM1 Threads and Internal SM2 Thread	1	$26.51	$26.51	Thorlabs Inc.	SM1A2
3	011409	Slip Ring for SM2 Lens Tubes	2	$31.92	$63.84	Thorlabs Inc.	SM2RC
3	011410	Dovetail Optical Rail, 12" Imperial	1	$80.90	$80.90	Thorlabs Inc.	RLA1200
3	011411	Dovetail Rail Carrier, 1" x 1"	2	$26.94	$53.88	Thorlabs Inc.	RC1
3	011412	Ø1/2" Post, L = 0.75"	2			Thorlabs Inc.	TR075
3	011413	Ø1/2" Post Holder, L = 1"	2			Thorlabs Inc.	PH1
3	011414	Table Clamp, RLA Series Optical Rails	2	$6.39	$12.78	Thorlabs Inc.	CL6
2	011500	Focus					
3	011501	Angle Bracket; 90°; 1/4-20 Slots; 1" Spacing	1			Newport Optics	360-90
3	011502	Linear Stage with SM-25 Micrometer	2			Thorlabs Inc.	423

1	012000	Stage					
2	012100	X-Y Stage	1	$220.00	$220.00	Ebay	N/A
3	012101	THK X-Y Bearing Block	2	Included	N/A		N/A
3	012102	THK Linear Rail XXX in	2	Included	N/A		N/A
3	012103	THK Linear Rail XXX in	1	Included	N/A		N/A
3	012104	Grease Nipple	4	Included	N/A		N/A
3	012105	Stage Top Plate	1	Included	N/A		Modified for Mounting
3	012106	Stage Bottom Plate	1	Included	N/A		N/A
2	012200	X-Axis Actuation System					012200
3	012201	X-Axis Actuator Mount	1	N/A	N/A	3-D Print	012201
3	012202	X-Axis Block	1	N/A	N/A	3-D Print	012202
2	012300	Y-Axis Actuation System					012300
3	012301	Y-Axis Actuator Mount	1	N/A	N/A	3-D Print	012301
3	012302	Y-Axis Block	1	N/A	N/A	3-D Print	012302
2	012400	Stage Spring Return					
3	012401	4" 0.438" OD Steel Extension Spring	2	(Pack of 6)	$10.33	McMaster Carr	N/A
3	012402	Spring Pin 1	4	N/A	N/A	Manufacture	012402
3	012403	Spring Pin 2	4	N/A	N/A	Manufacture	012302
3	012404	Spring Pin 3	4	N/A	N/A	Manufacture	012302
3	012405	Spring Pin 4	4	N/A	N/A	Manufacture	012302
3	012406	X-Axis Spring Block 1	1	N/A	N/A	3-D Print	012306
3	012407	X-Axis Spring Block 2	1	N/A	N/A	3-D Print	012307
3	012408	Y-Axis Spring Block 1		N/A	N/A		012308
3	012409	Y-Axis Spring Block 2		N/A	N/A		012309
2	012500	Sample Retaining					N/A
3	012501	IFM Stage Plate	1	$300.00	N/A	N/A	012601
3	012502	Microscope Slide Spring Clip, Qty:2	1	$45.72	$45.72	Thorlabs Inc.	SLH1
2	012600	Stage Mounting					N/A
3	012601	Ø1/2" Post Holder, L = 2"	4	$7.93	$31.72	Thorlabs Inc.	PH2
3	012602	Ø1/2" Post, L = 2"	4	$5.35	$21.40	Thorlabs Inc.	TR2
3	012603	Ø1/2" Post, L = 6" (Pack of 5)	1	$32.97	$32.97	Thorlabs Inc.	TR6-P5
3	012604	Mounting Base, 1" x 2.3" x 3/8"	8	$5.36	$42.88	Thorlabs Inc.	BA1S
3	012605	M4 x 0.7mm Socket Head Screw, 6mm Long (Pack of 100)	1	$8.80	$8.80	McMaster Carr	91290A139
3	012606	1/2" M6 Socket Head Cap Screw	2				N/A
3	012607	3/8" #10-32 Low Profile Socket Head Cap Screw	9	(Pack of 50)	$9.28	McMaster Carr	N/A
3	012608	3/8" #10-32 Flat Head Socket Cap Screw	3	(Pack of 50)	$8.62	McMaster Carr	N/A
3	012609	1.5" M6 Socket Head Cap Screw	1				N/A
2	012700	Stage Electronics					
3	012701	Arduino Uno	1				N/A
3	012702	5-pin Joystick	1				N/A
3	012703	Newport 850G Linear Actuator	2				N/A
3	012704	L293D H-Bridge	1				N/A
3	012705	DB25-M2 Female Input	2				N/A
3	12706	Arduino Mega	1	$38.50	$38.50	Arduino	N/A
1	013000	Frame					
2	013100	T-Slot					
3	013101	T-Slotted Framing (1ft)	2	$5.84	$11.68	McMaster Carr	47065T101
3	013102	T-Slotted Framing (2ft)	1	$7.79	$7.79	McMaster Carr	47065T101
3	013103	Corner Bracket	2	$5.21	$10.42	McMaster Carr	47065T236
3	013104	Mounting Foot	4			3-D Print	DWG NUMBER
3	013105	Honeycomb Optical Breadboard 24" x 36" x 2.3", 1/4"-20	1	$1,038.00	$1,038.00	Newport Optics	IG-23-2
3	013106	End Feed Single Nut with Button Head 1/4"-20 Thread Size (I	1	$1.85	$1.85	McMaster Carr	47065T139
3	013107	1/4"-20 Alloy Steel Socket Head Screw, 1/2" Long (Pack of 1	1	$11.38	$11.38	McMaster Carr	91251A537
3	013108	1/4"-20 Alloy Steel Flanged Button Head Screw (Pack of 10)	1	$7.58	$7.58	McMaster Carr	91355A178



SUBASSEMBLY NO.	DESCRIPTION
1	OPTICS
2	STAGE
3	FRAME

	NAME	DATE
DRAWN	MLK	06-03-20
CHECKED	EWN	06-03-20
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL

FINISH

DO NOT SCALE DRAWING

NEXT ASSY | USED ON

APPLICATION

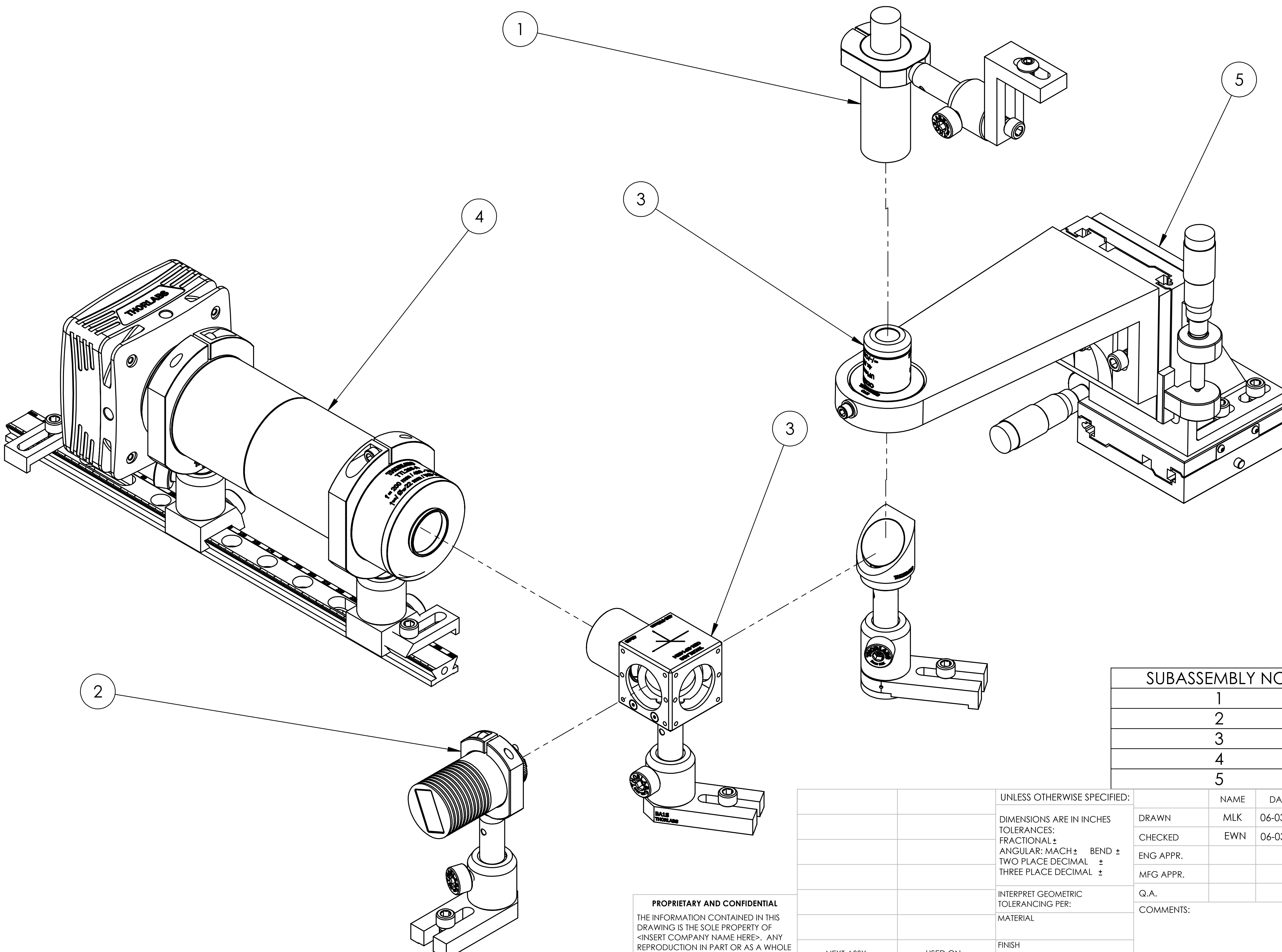
PROPRIETARY AND CONFIDENTIAL

TITLE:

FINAL ASSEMBLY

SIZE **B** DWG. NO. 01-0-0-00 REV 3

SCALE: 1:5 WEIGHT: SHEET 1 OF 1



SUBASSEMBLY NO.	DESCRIPTION
1	BRIGHTFIELD ILLUMINATION
2	FLUORESCENCE ILLUMINATION
3	OPTICS AND MOUNTING
4	IMAGING
5	FOCUS

PROPRIETARY AND CONFIDENTIAL

THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <INSERT COMPANY NAME HERE>. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF <INSERT COMPANY NAME HERE> IS PROHIBITED.

NEXT ASSY	USED ON
APPLICATION	

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL

FINISH

DO NOT SCALE DRAWING

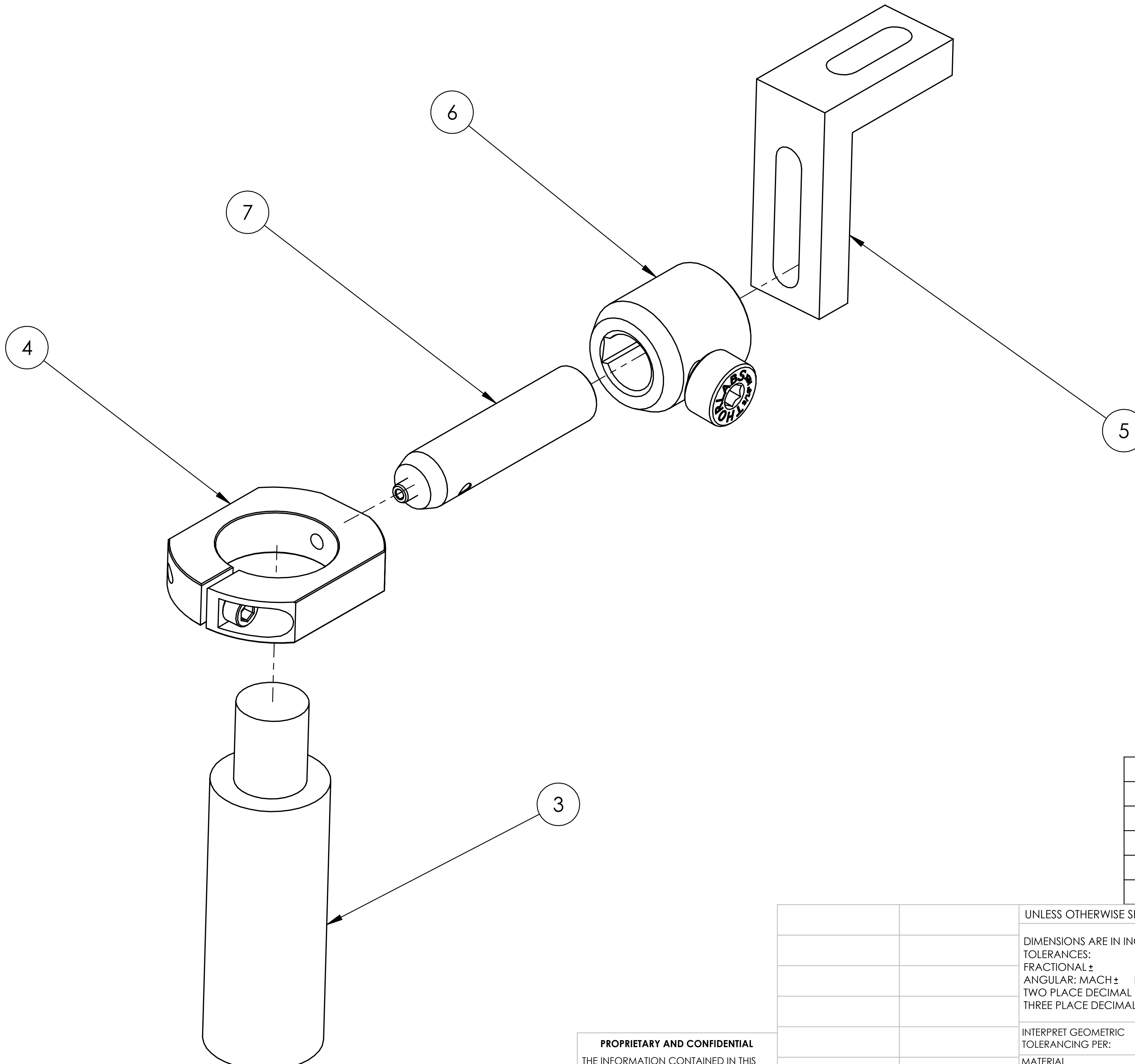
	NAME	DATE
DRAWN	MLK	06-03-20
CHECKED	EWN	06-03-20
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:

LEVEL 1 ASSEMBLY - OPTICAL TRAIN

SIZE	DWG. NO.	REV
B	01-1-0-00	3

SCALE: 1:2 WEIGHT: SHEET 1 OF 1



SUBASSEMBLY NO.	DESCRIPTION
3	FOCUSING LENS
4	SLIP RING FOR Ø1" COMPONENTS
5	SLIM RIGHT ANGLE BRACKET
6	Ø1/2" POST HOLDER, L = 1"
7	Ø1/2" POST, L = 2"

	NAME	DATE
DRAWN	MLK	02-01-20
CHECKED	EWN	02-03-20
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL

FINISH

DO NOT SCALE DRAWING

NEXT ASSY | USED ON

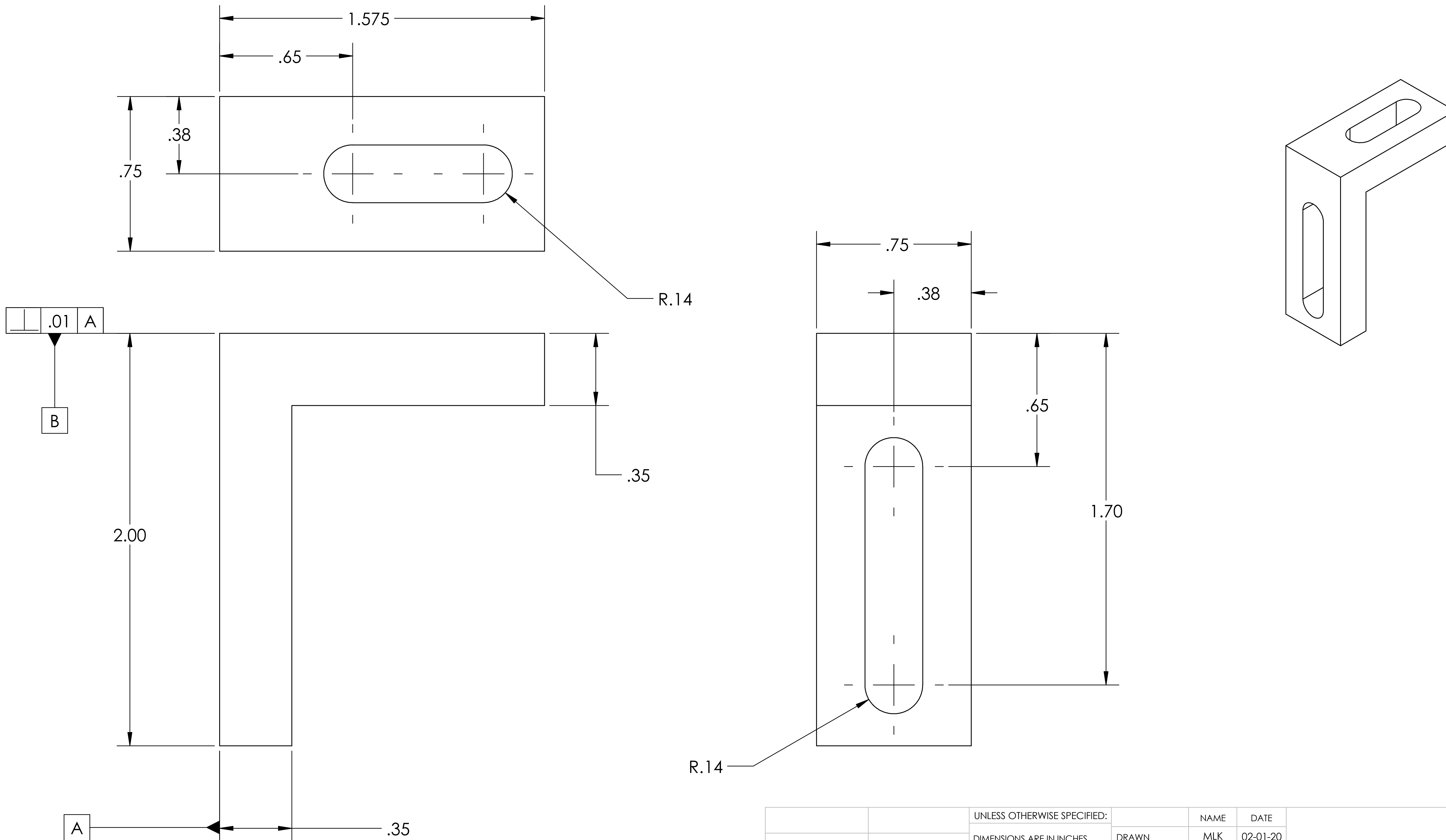
APPLICATION

TITLE:

LEVEL 3 SUBASSEMBLY - BRIGHTFIELD ILLUMINATION

SIZE **B** | DWG. NO. 01-1-1-00 | REV 2

SCALE: 1:1



PROPRIETARY AND CONFIDENTIAL

THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <INSERT COMPANY NAME HERE>. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF <INSERT COMPANY NAME HERE> IS PROHIBITED.

NEXT ASSY	USED ON
APPLICATION	

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL ± .1
TWO PLACE DECIMAL ±.05
THREE PLACE DECIMAL ±.005

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: PLA

FINISH: PRINTER QUALITY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	MLK	02-01-20
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

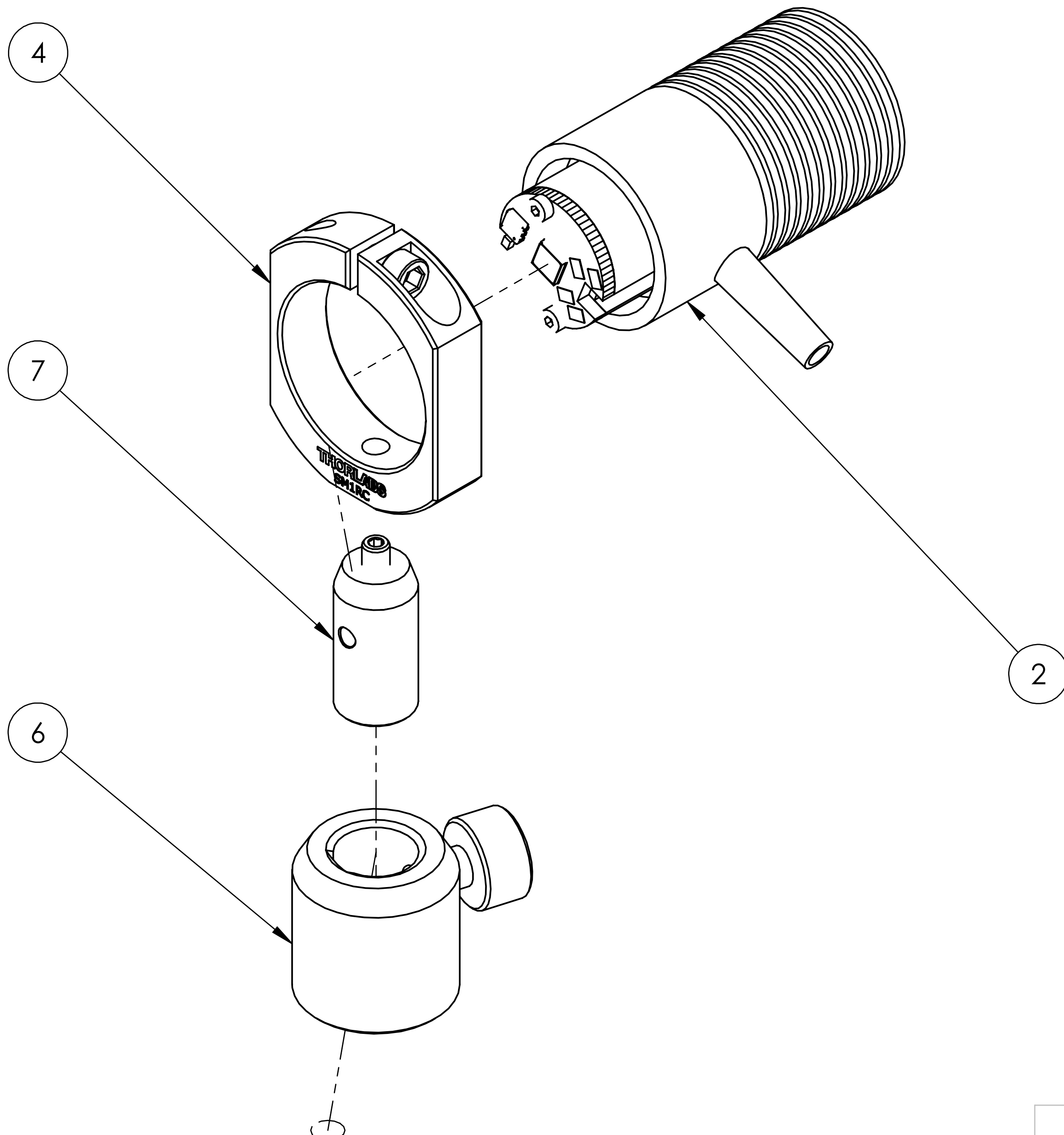
COMMENTS: THIS PART IS TO BE 3D PRINTED ON DATUM A.

TITLE:

SLIM RIGHT ANGLE BRACKET

SIZE	DWG. NO.	REV
B	01-1-1-05	2

SCALE: 2:1



SUBASSEMBLY NO.	DESCRIPTION
2	SINGLE-COLOR LED, 490nm
4	SLIP RING FOR SM1 LENS TUBES
6	Ø1/2" POST HOLDER, L = 1"
7	Ø1/2" POST, L = 1"

	NAME	DATE
DRAWN	MLK	02-01-20
CHECKED	EWN	02-03-20
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

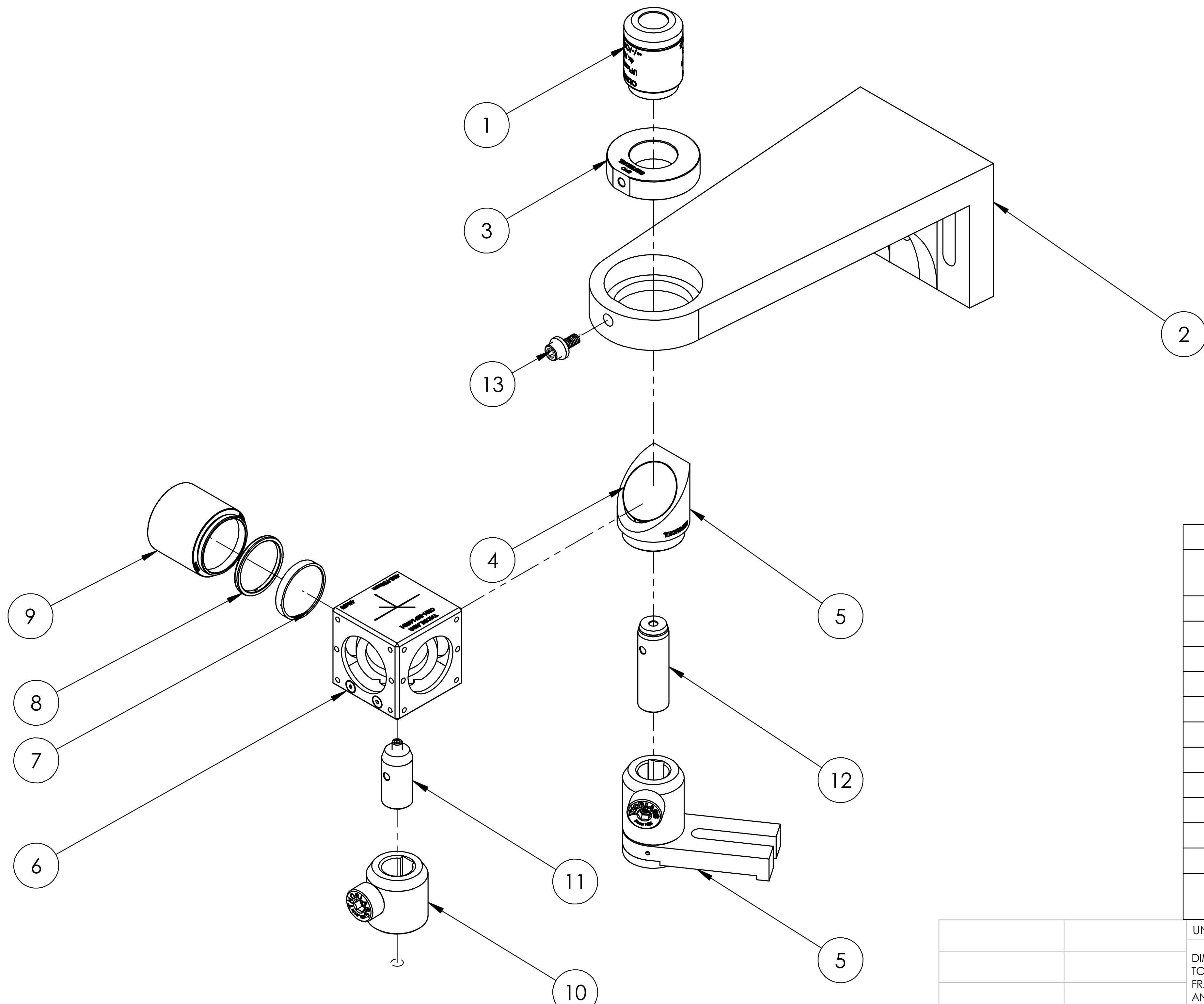
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL±
ANGULAR: MACH± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
FINISH
DO NOT SCALE DRAWING

NEXT ASSY | USED ON
APPLICATION

TITLE:
LEVEL 3 SUBASSEMBLY - FLUORESCENCE ILLUMINATION

SIZE **B** | DWG. NO. 01-1-2-00 | REV 2

SCALE: 1:1



SUBASSEMBLY NO.	DESCRIPTION
1	4X PLAN INFINITY CORRECTED OBJECTIVE LENS
2	OBJECTIVE MOUNTING BRACKET
3	RMS MICROSCOPE OBJECTIVE MOUNT
4	Ø1" ROUND, PROTECTE ALUMINUM MIRROR
5	45° MOUNT ASSEMBLY FOR Ø1" OPTICS
6	CUBE-MOUNTED 50:50 BEAMSPLITTER
7	FITC EMISSION FILTER, CWL = 530nm
8	SM1 RETAINING RING FOR Ø1" LENS TUBES
9	SM1 LENS TUBE, 1" THREAD DEPTH
10	Ø1/2" POST HOLDER, L = 1"
11	Ø1/2" POST, L = 1"
12	Ø1/2" POST, L = 1.5"
13	8-32 FLANGED SOCKET HEAD SCREW, L = 3/8"

UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL±
ANGULAR: MACH± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
FINISH
DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	MLK	02-01-20
CHECKED	EWN	02-03-20
ENG APPR.		
MFG APPR.		
Q.A.		

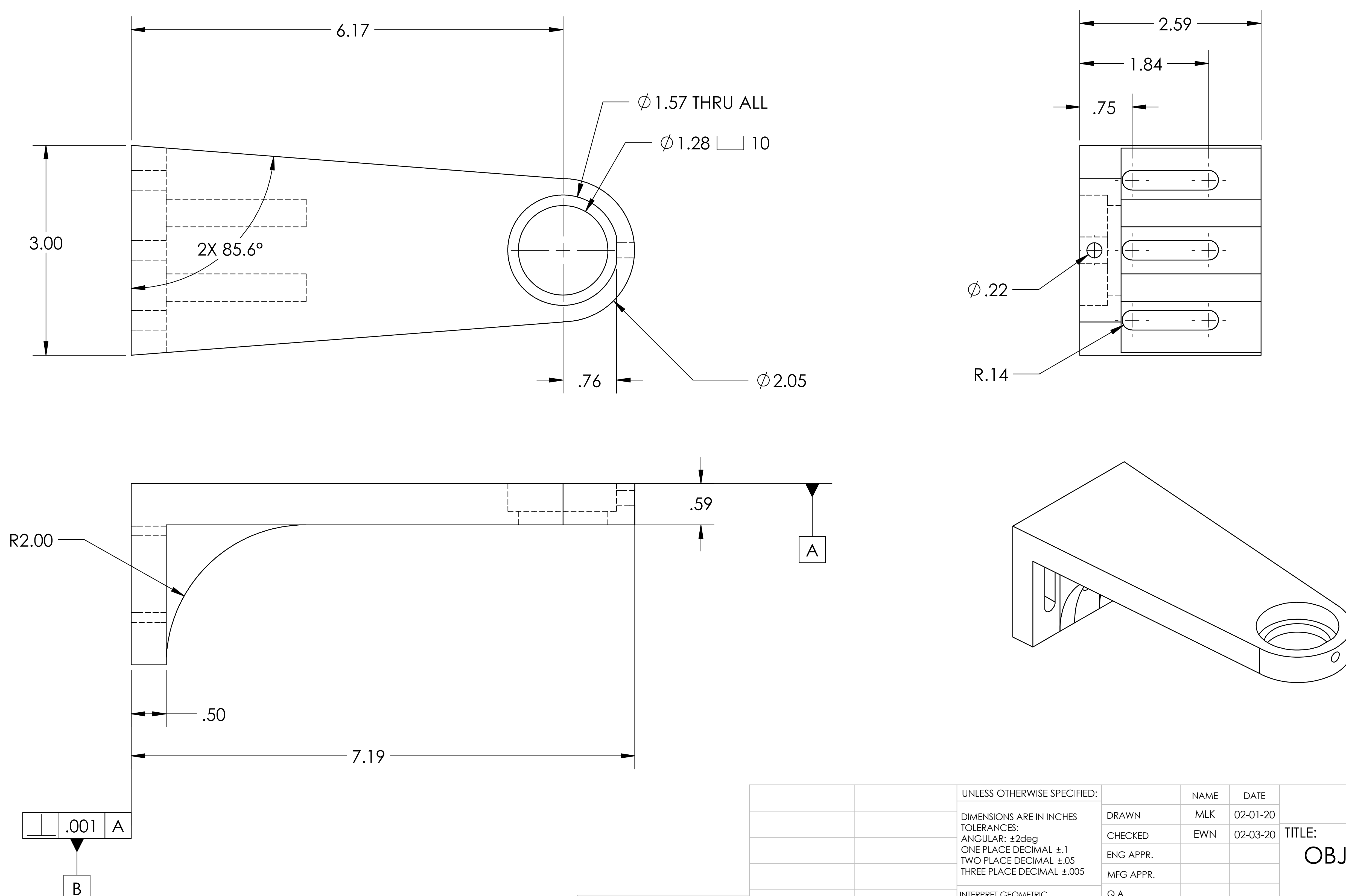
COMMENTS:

TITLE:
LEVEL 3 SUBASSEMBLY - OPTICS AND MOUNTING

SIZE B DWG. NO. 01-1-3-00 REV 2

SCALE: 1:2

NEXT ASSY | USED ON
APPLICATION



UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL ±.1
TWO PLACE DECIMAL ±.05
THREE PLACE DECIMAL ±.005

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: PLA

FINISH: PRINTER QUALITY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	MLK	02-01-20
CHECKED	EWN	02-03-20
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS: THIS PART IS TO BE 3D PRINTED ON DATUM A.

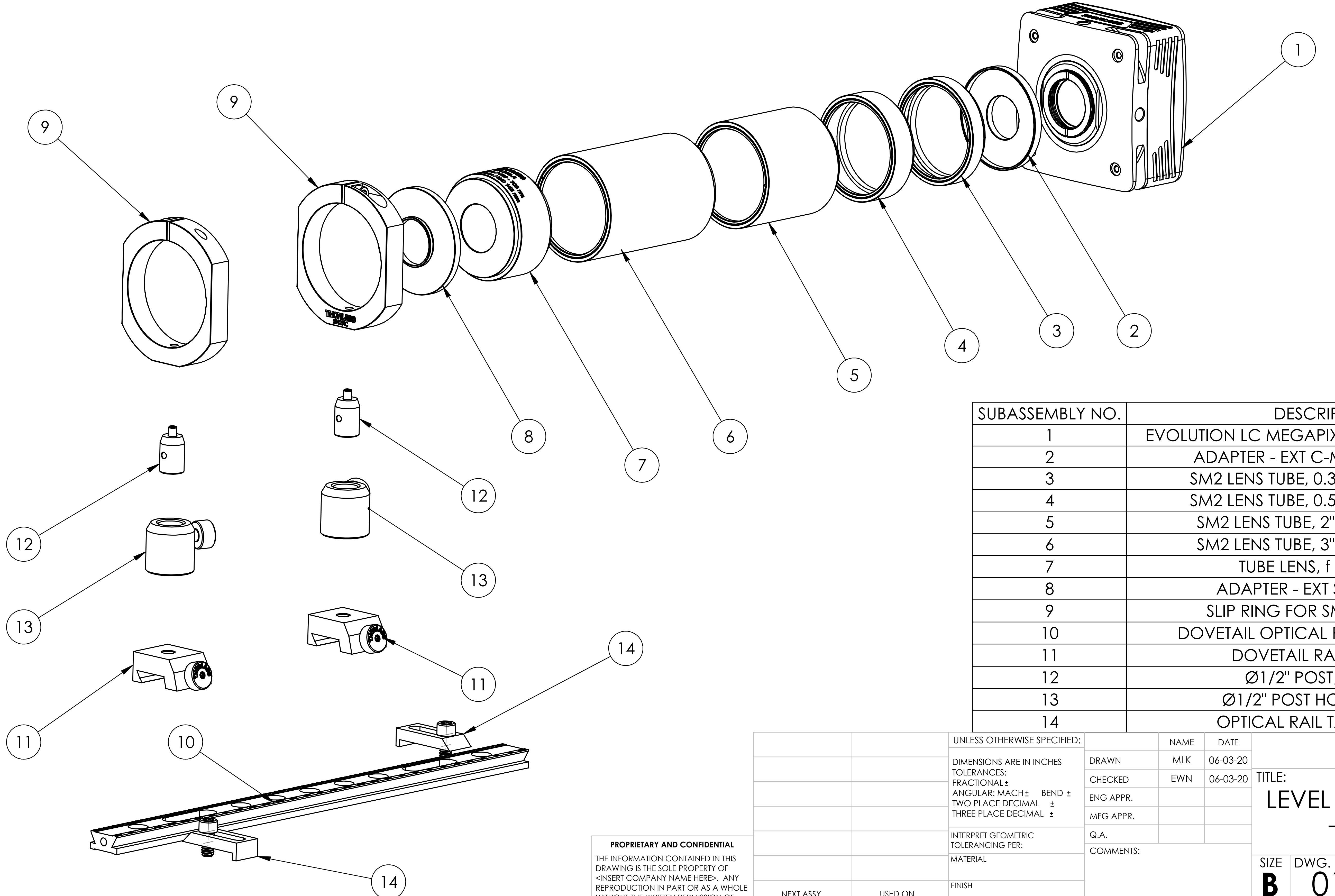
TITLE:

OBJECTIVE MOUNTING BRACKET

SIZE: B | DWG. NO. 01-1-3-02 | REV 2

SCALE: 2:3

NEXT ASSY	USED ON
APPLICATION	



SUBASSEMBLY NO.	DESCRIPTION
1	EVOLUTION LC MEGAPIXEL FIREWIRE CAMERA
2	ADAPTER - EXT C-MOUNT; INT SM2
3	SM2 LENS TUBE, 0.3" THREAD DEPTH
4	SM2 LENS TUBE, 0.5" THREAD DEPTH
5	SM2 LENS TUBE, 2" THREAD DEPTH
6	SM2 LENS TUBE, 3" THREAD DEPTH
7	TUBE LENS, f = 200 mm
8	ADAPTER - EXT SM1; INT SM2
9	SLIP RING FOR SM2 LENS TUBES
10	DOVETAIL OPTICAL RAIL, 12" IMPERIAL
11	DOVETAIL RAIL CARRIER
12	Ø1/2" POST, L = 0.75"
13	Ø1/2" POST HOLDER, L = 1"
14	OPTICAL RAIL TABLE CLAMP

NEXT ASSY	USED ON
APPLICATION	

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL

FINISH

DO NOT SCALE DRAWING

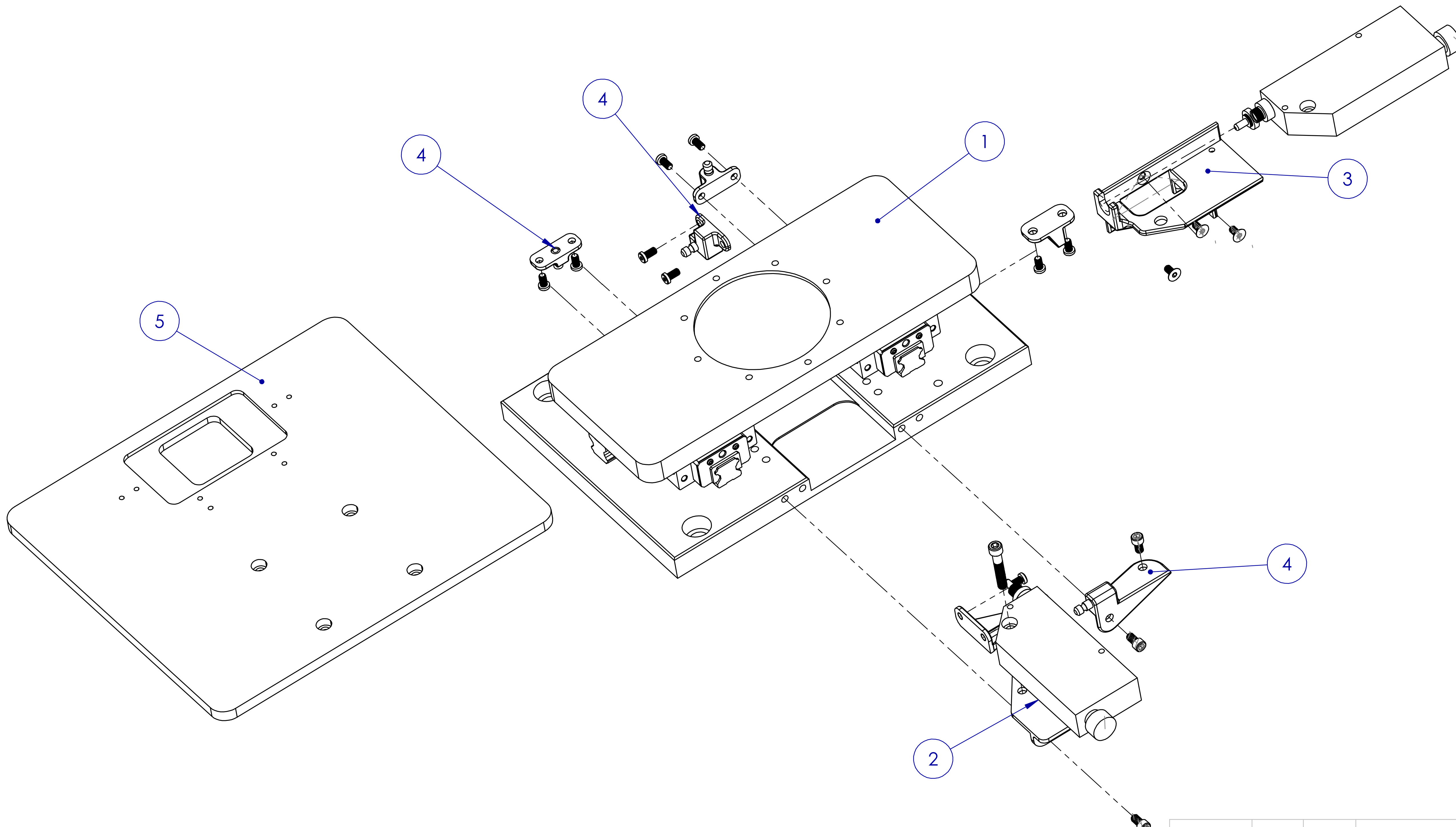
	NAME	DATE
DRAWN	MLK	06-03-20
CHECKED	EWN	06-03-20
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:

LEVEL 3 SUBASSEMBLY - IMAGING

SIZE	DWG. NO.	REV
B	01-1-4-00	3

SCALE: 1:2 WEIGHT: SHEET 1 OF 1



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	012100	THK X-Y STAGE	1
2	012200	X-AXIS ACTUATION SYSTEM	1
3	012300	Y-AXIS ACTUATION SYSTEM	1
4	012400	SPRING RETURN	1
5	012501	SAMPLE PLATE	1

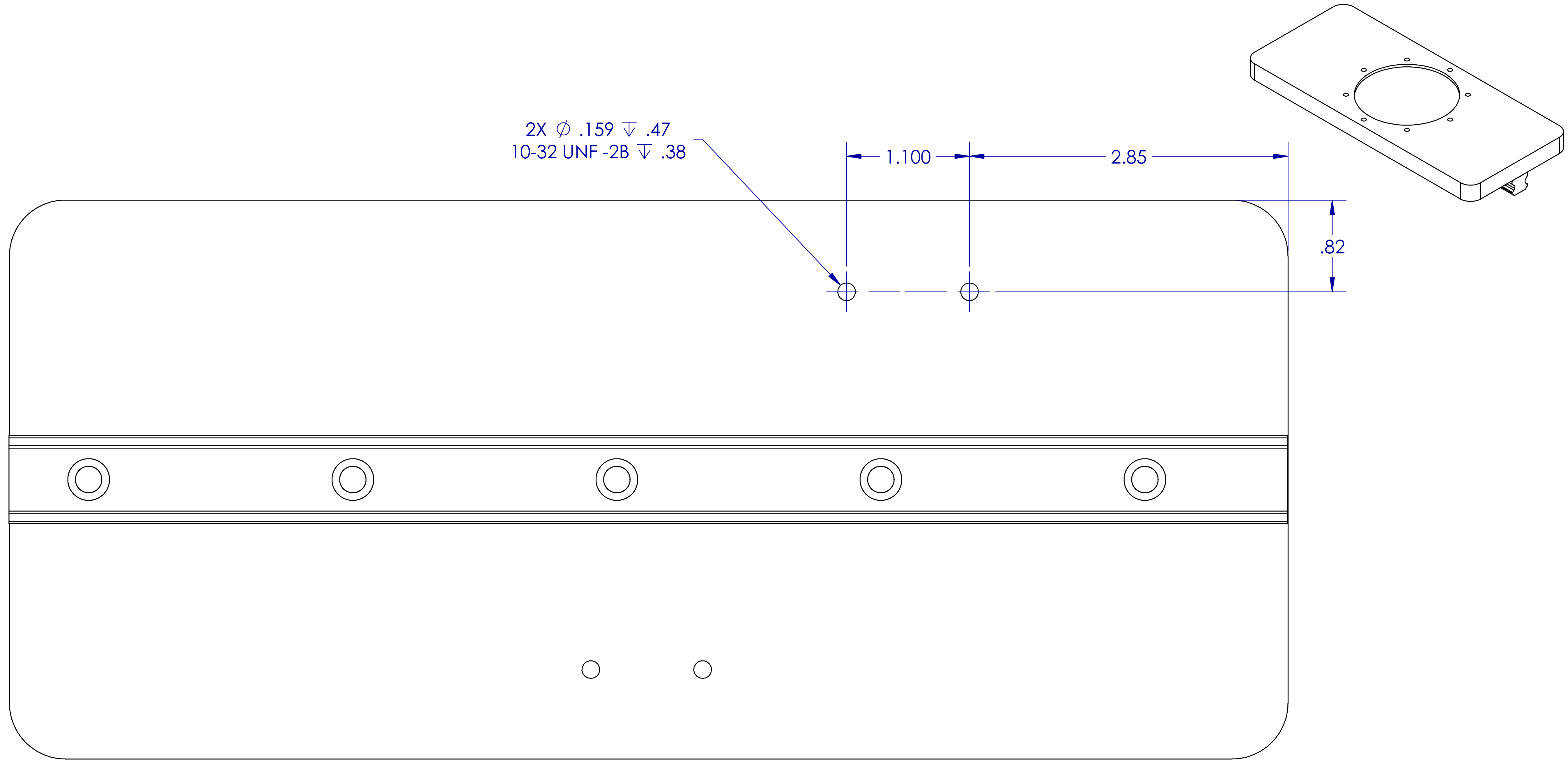
	NAME	DATE
DRAWN	EWN	6-3-2020
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:

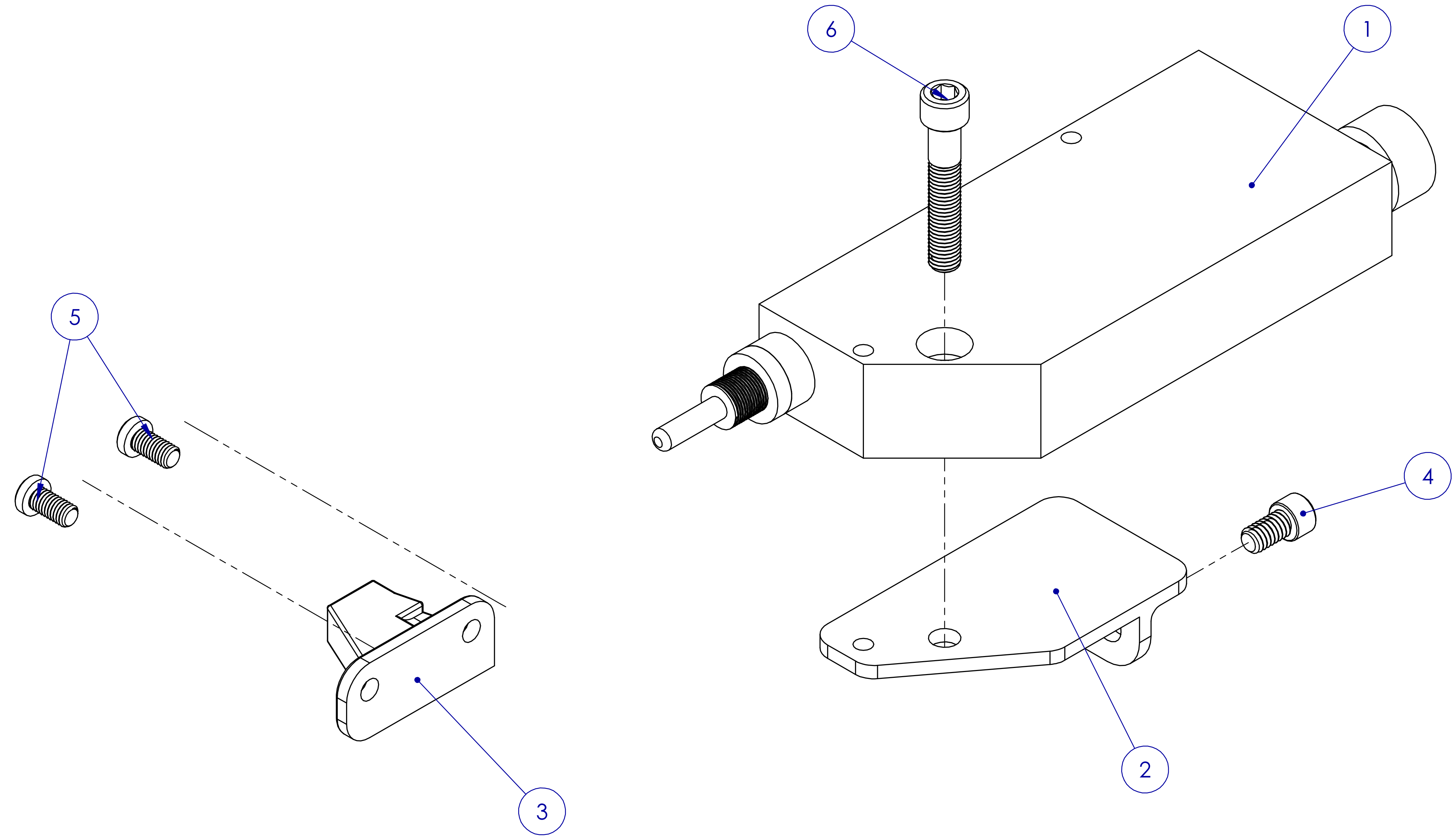
STAGE ASSEMBLY

SIZE	DWG. NO.	REV
B	01-2-0-00	2

SCALE: 1:3 | SHEET 1 OF 15



UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	2-4-20	TITLE:
	CHECKED			STAGE PLATE MODIFICATIONS
	ENG APPR.			
	MFG APPR.			
INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.			
MATERIAL	COMMENTS: - THIS DRAWING LOCATES THE ADDITIONAL MOUNTING HOLES WE ADDED TO THE STAGE PLATE - HOLES ON STAGE BOTTOM			SIZE **B** DWG. NO. 01-2-1-05 REV 2
FINISH				
DO NOT SCALE DRAWING	- CRITICAL HOLE RELATIONS CHAINED			1:1 WEIGHT: SHEET 2 OF 15



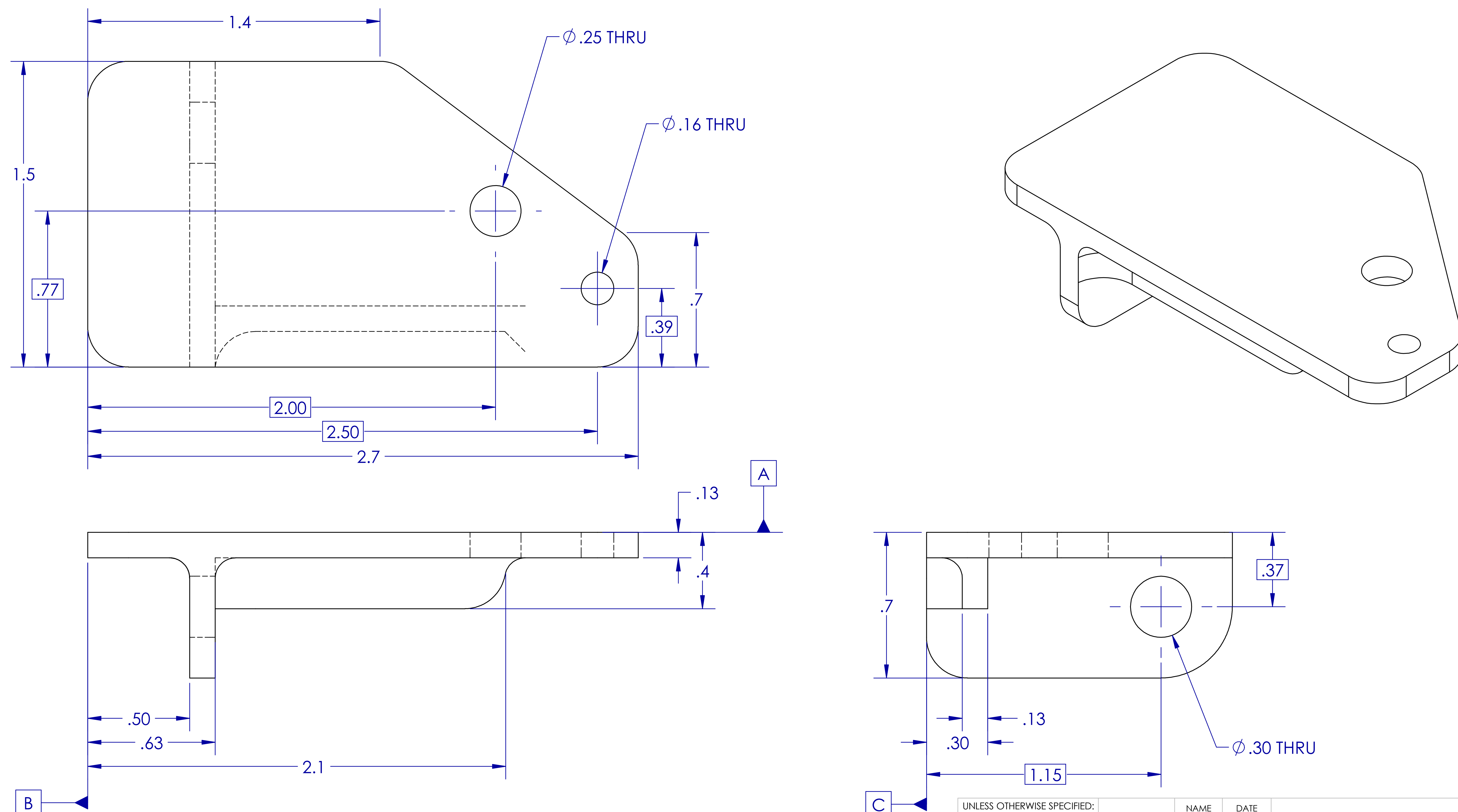
ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	012703	NEWPORT 850G LINEAR ACTUATOR	1
2	012201	X-AXIS ACTUATOR MOUNT	1
3	012202	X-AXIS BLOCK	1
4	012606	1/2" M6 SOCKET HEAD CAP SCREW	1
5	012607	3/8" #10-32 LOW PROFILE SOCKET CAP SCREW	2
6	012609	1 1/2" M6 SOCKET HEAD CAP SCREW	1

	NAME	DATE
DRAWN	EWN	6-3-2020
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:
X-AXIS AC. SYSTEM

SIZE B | DWG. NO. 01-2-2-00 | REV 2

SCALE: 1:1 | SHEET 3 OF 15



UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL: ±.1
TWO PLACE DECIMAL: ±.05
THREE PLACE DECIMAL: ±.005

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: PLA

FINISH: PRINTER QUALITY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	EWN	6-2-2020
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

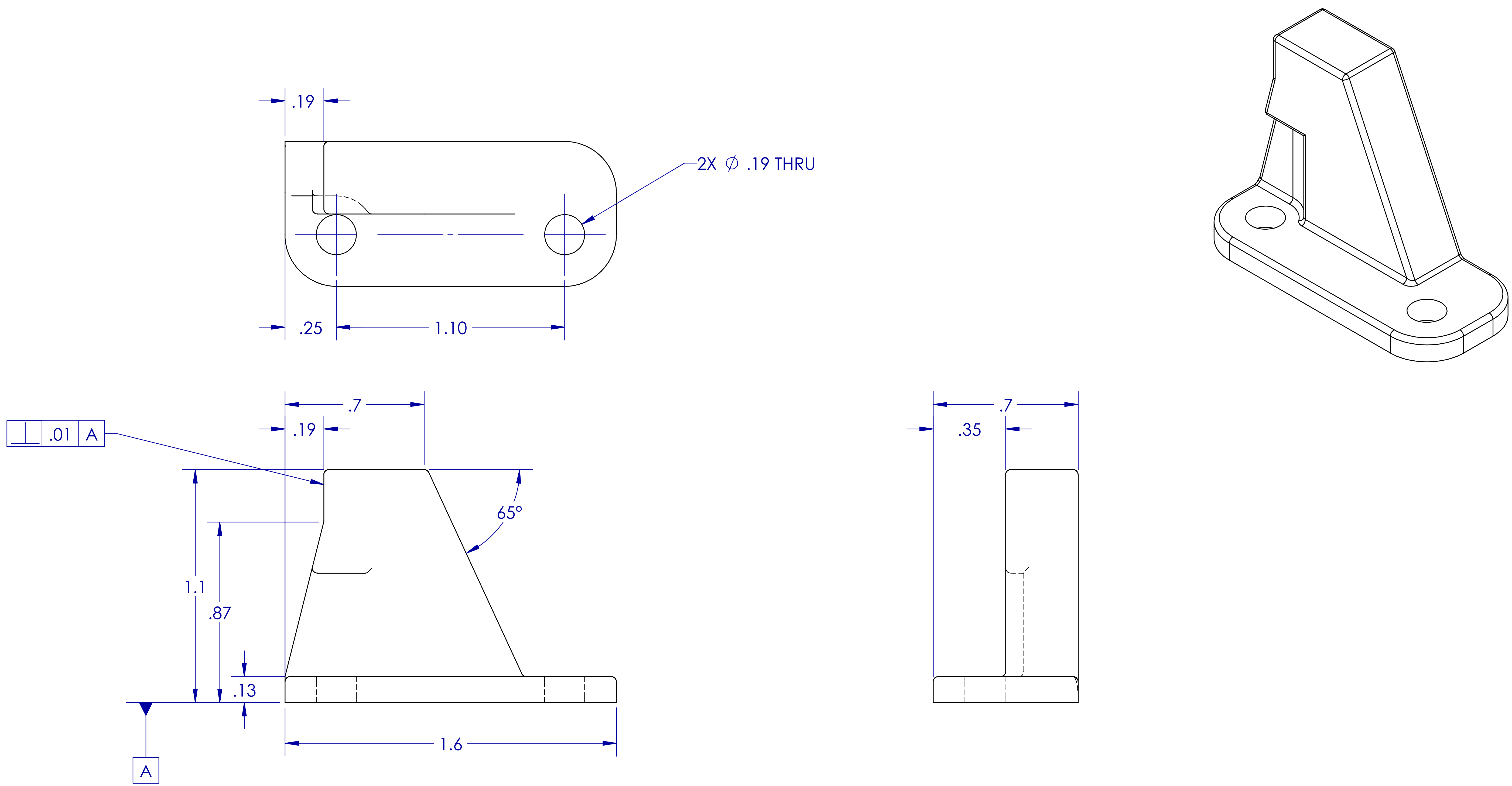
COMMENTS:
This part is 3D printed.
Print on Datum A.
The dimesions shown are for refference only. Contact Dr. Mayer hmayer@calpoly.edu for the up to date STL file.

TITLE:

X-AXIS AC. MOUNT

SIZE	DWG. NO.	REV
B	01-2-2-01	3

SCALE: 2:1 WEIGHT: SHEET 4 OF 15



UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL: ±.1
TWO PLACE DECIMAL: ±.05
THREE PLACE DECIMAL: ±.005

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: PLA

FINISH: PRINTER QUALITY

DO NOT SCALE DRAWING

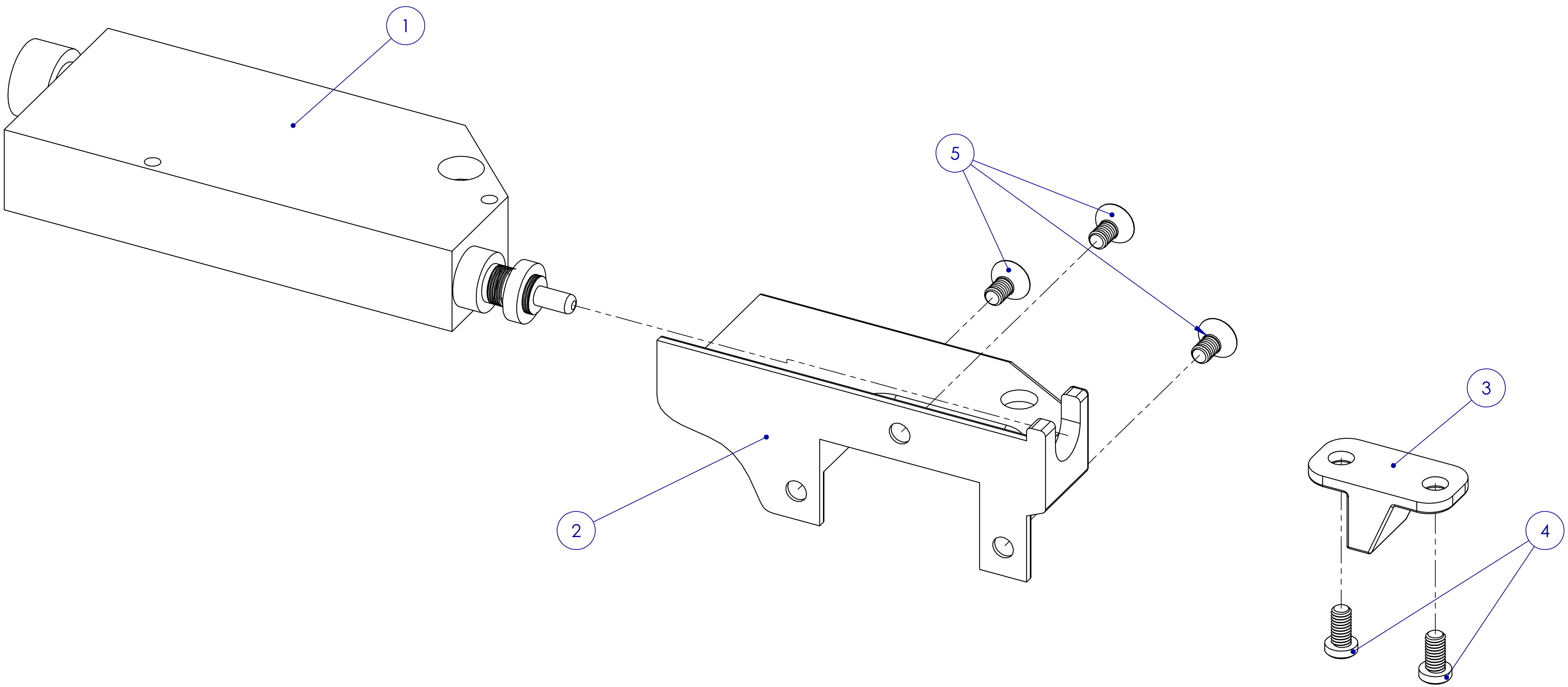
	NAME	DATE
DRAWN	EWN	6-2-2020
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:
THIS PART IS 3D PRINTED.
PRINT ON DATUM A.
THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.

TITLE:
X-AXIS BLOCK

SIZE	DWG. NO.	REV
B	01-2-2-02	2

SCALE: 2:1 WEIGHT: SHEET 5 OF 15



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	012703	NEWPORT 850G LINEAR ACTUATOR	1
2	012301	Y-AXIS ACTUATOR MOUNT	1
3	012302	Y-AXIS BLOCK	1
4	012607	3/8" #10-32 LOW PROFILE SOCKET HEAD CAP SCREW	2
5	012608	3/8" #10-32 FLAT HEAD SOCKET CAP SCREW	3

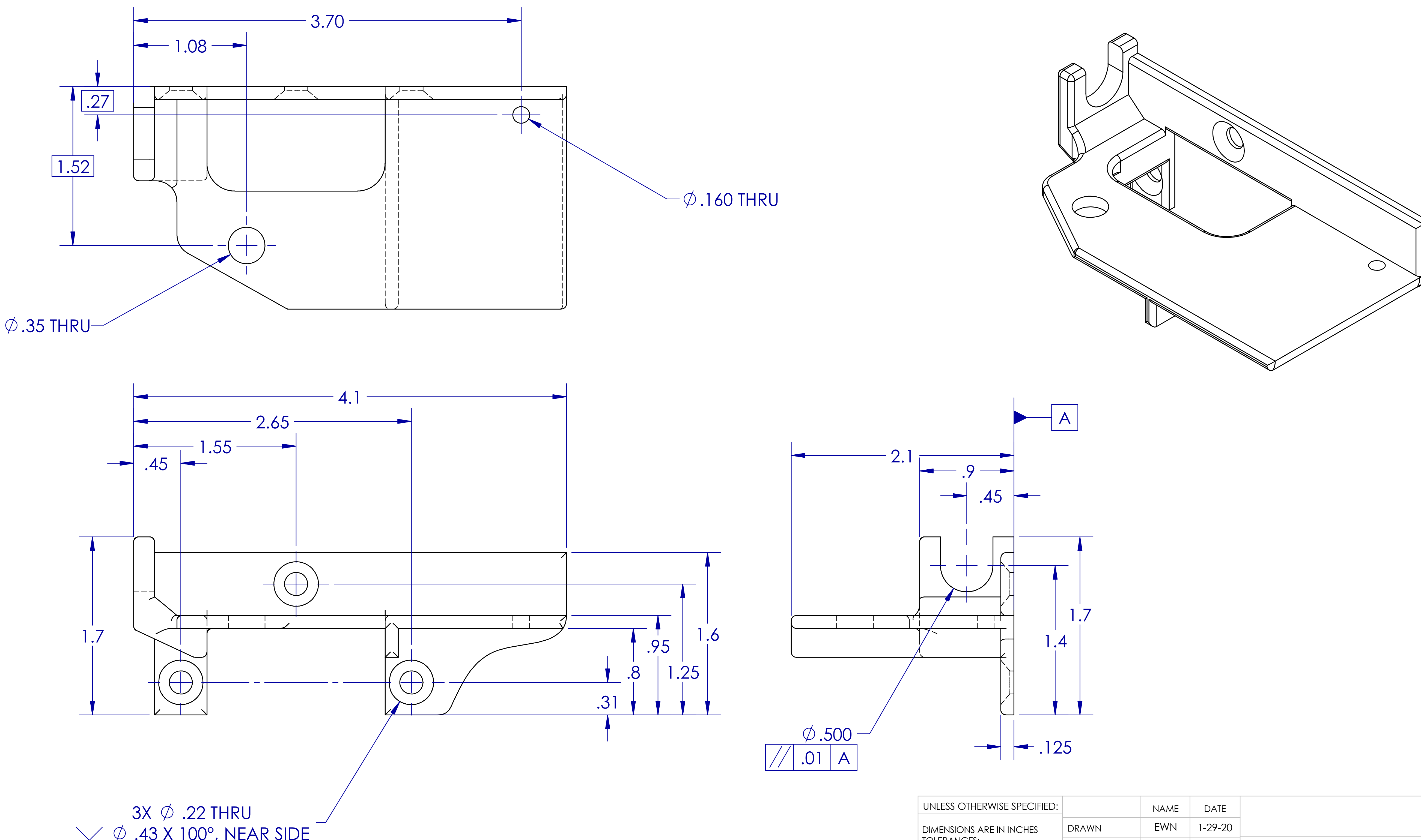
	NAME	DATE
DRAWN	EWN	2-1-20
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:

Y-AXIS AC. SYSTEM

SIZE **B** DWG. NO. 01-2-3-00 REV 1

SCALE: 1:1 SHEET 6 OF 15



UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL: ±.1
TWO PLACE DECIMAL: ±.05
THREE PLACE DECIMAL: ±.005

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: PLA

FINISH: PRINTER QUALITY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	EWN	1-29-20
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

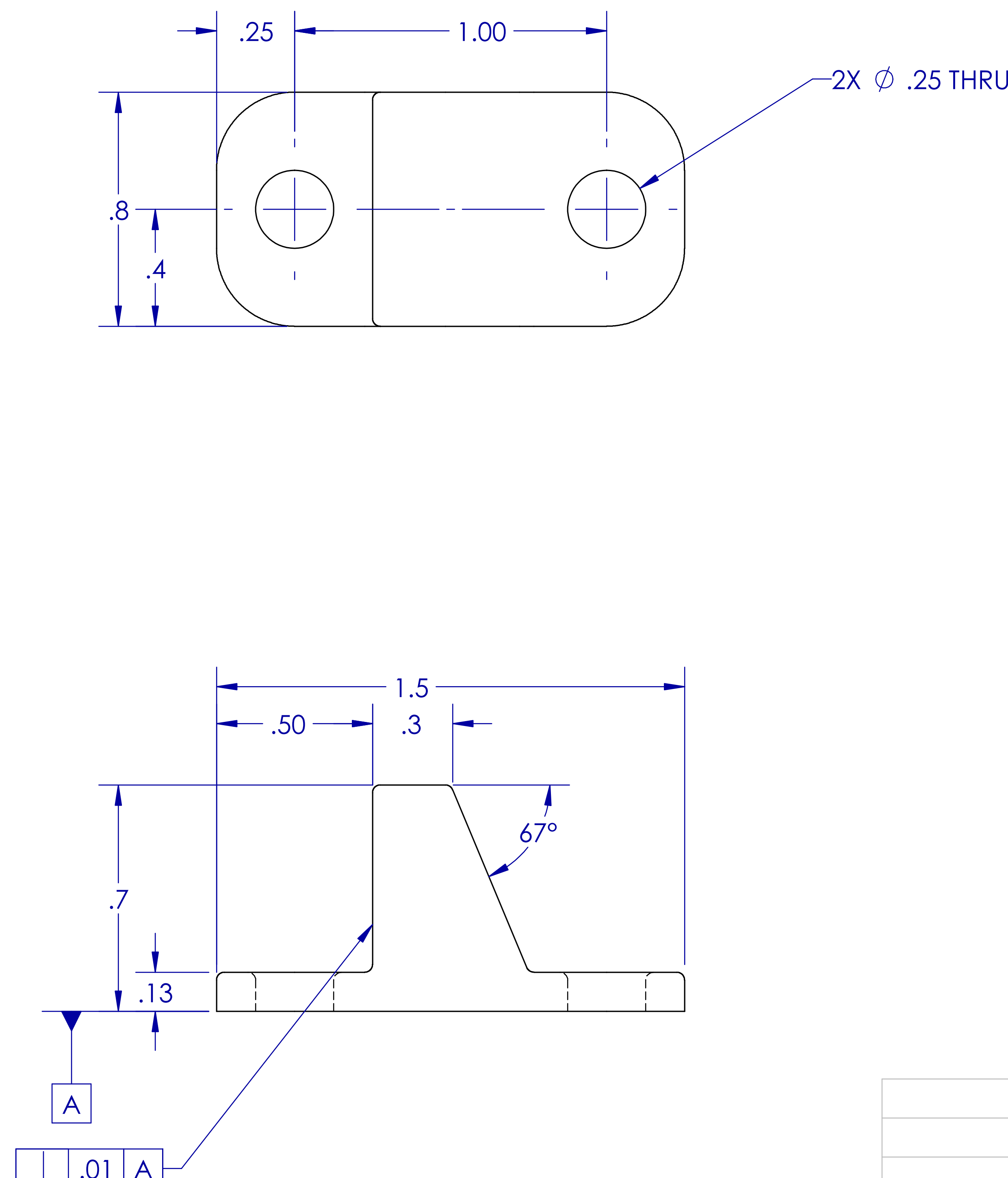
COMMENTS:
THIS PART IS 3D PRINTED.
PRINT ON DATUM A.
THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.

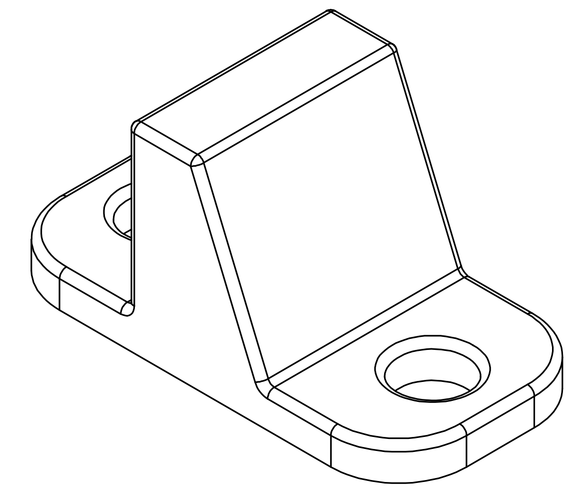
TITLE:

Y-AXIS BLOCK

SIZE	DWG. NO.	REV
B	01-2-3-01	2

SCALE: 1:1 WEIGHT: SHEET 7 OF 15



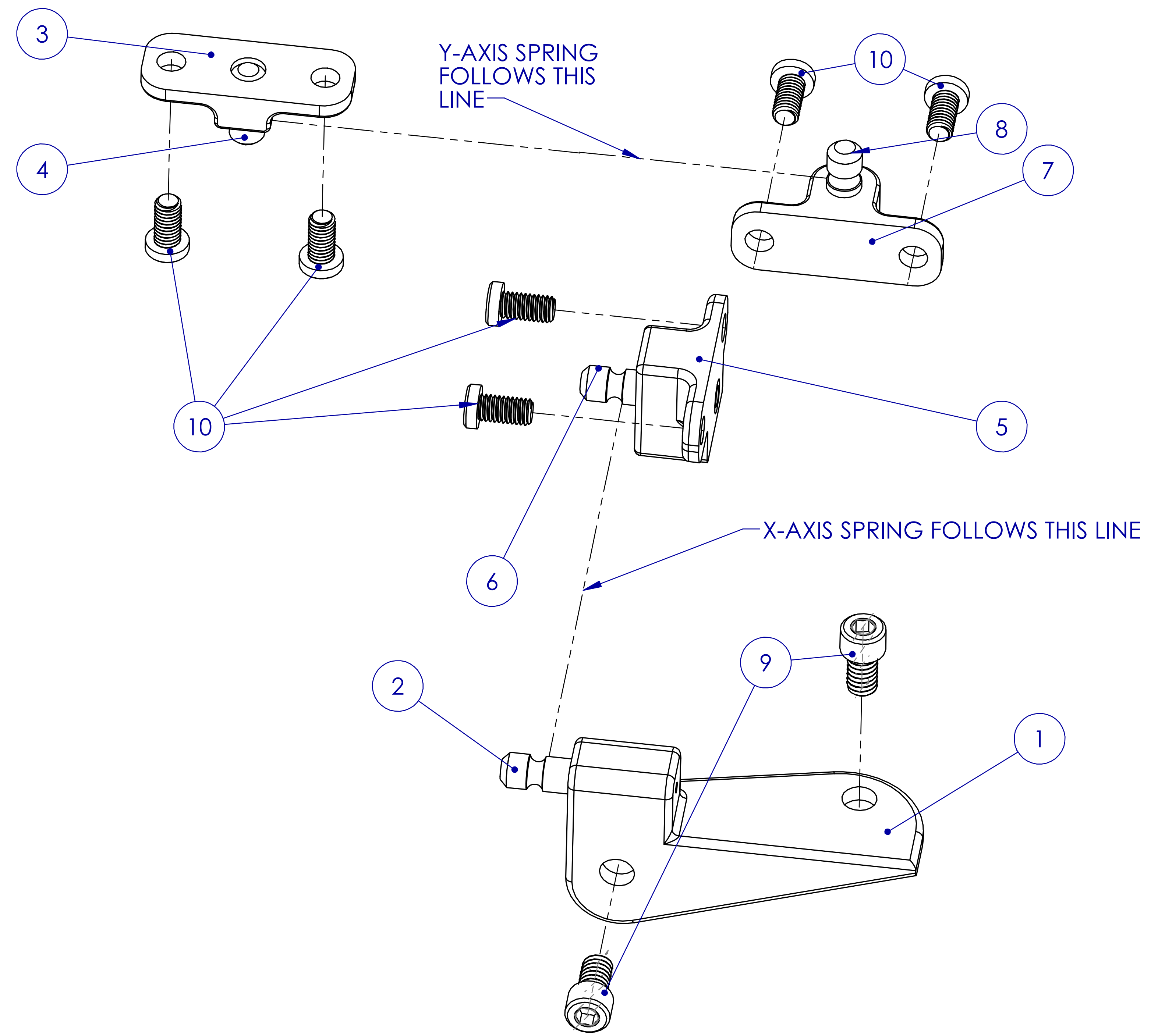


		UNLESS OTHERWISE SPECIFIED:		NAME	DATE
		DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	6-2-2020
			CHECKED		
			ENG APPR.		
			MFG APPR.		
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.		
		MATERIAL PLA	COMMENTS: THIS PART IS 3D PRINTED. PRINT ON DATUM A. THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.		
NEXT ASSY	USED ON	FINISH PRINTER QUALITY			
APPLICATION		DO NOT SCALE DRAWING			

TITLE:

Y-AXIS BLOCK

SIZE	DWG. NO.	REV
B	01-2-3-02	2
SCALE: 2:1	WEIGHT:	SHEET 8 OF 15



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	012406	X-AXIS SPRING MOUNT 1	1
2	012402	SPRING PIN 1	1
3	012408	Y-AXIS SPRING MOUNT 1	1
4	012403	SPRING PIN 2	1
5	012407	X-AXIS SPRING MOUNT 2	1
6	012404	SPRING PIN 3	1
7	012409	Y-AXIS SPRING MOUNT 2	1
8	012405	SPRING PIN 4	1
9	012606	1/2" M6 SOCKET HEAD CAP SCREW	2
10	012607	3/8" #10-32 LOW PROFILE SOCKET HEAD CAP SCREW	5

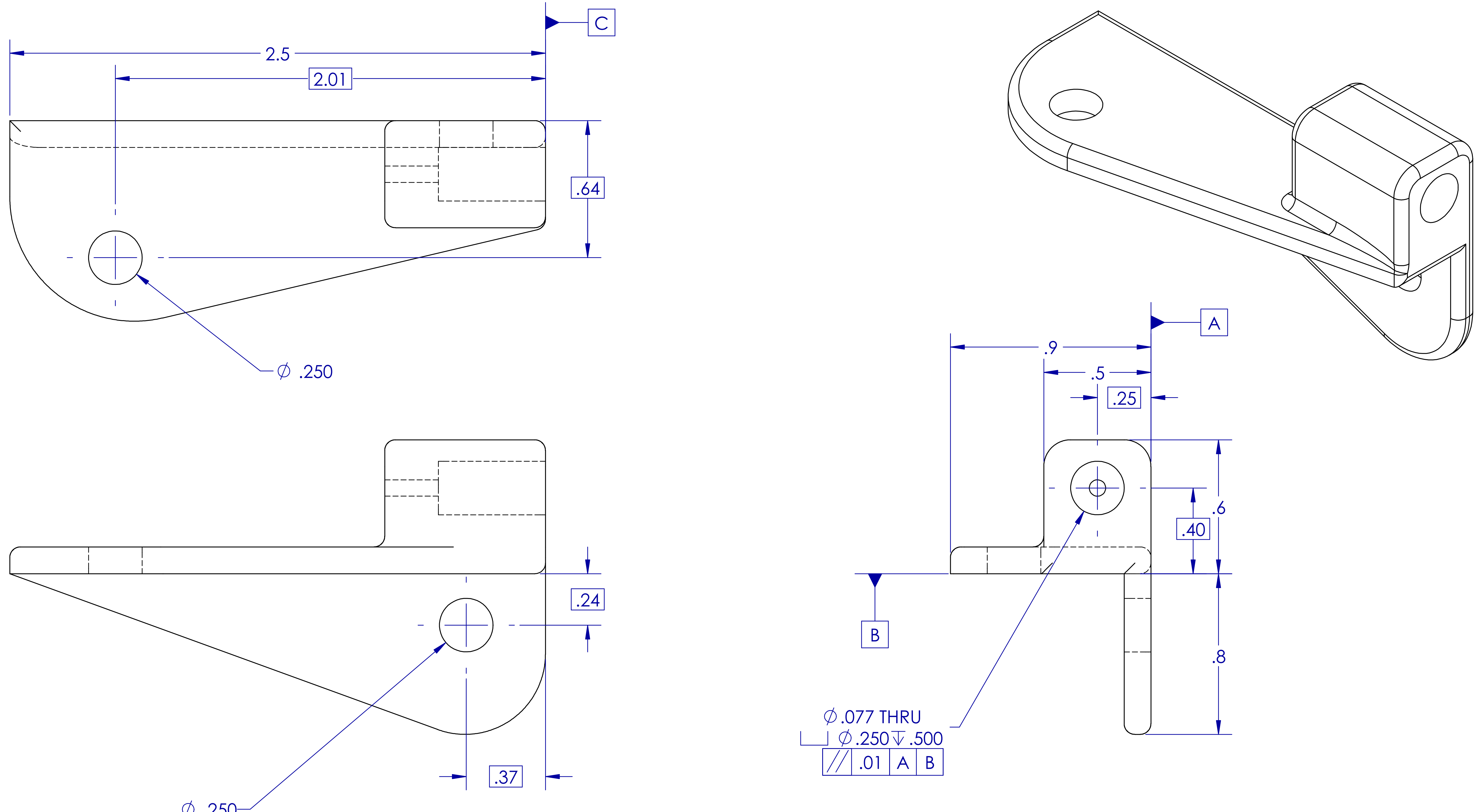
	NAME	DATE
DRAWN	EWN	6-3-2020
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:
- SPRING PINS ARE TO BE PRESS FIT INTO MOUNTING BRACKETS IN INDICATED HOLES
- PRESS FLUSH WITH REAR SIDE OF HOLE
- SPRING GROOVE SHOULD BE VISIBLE

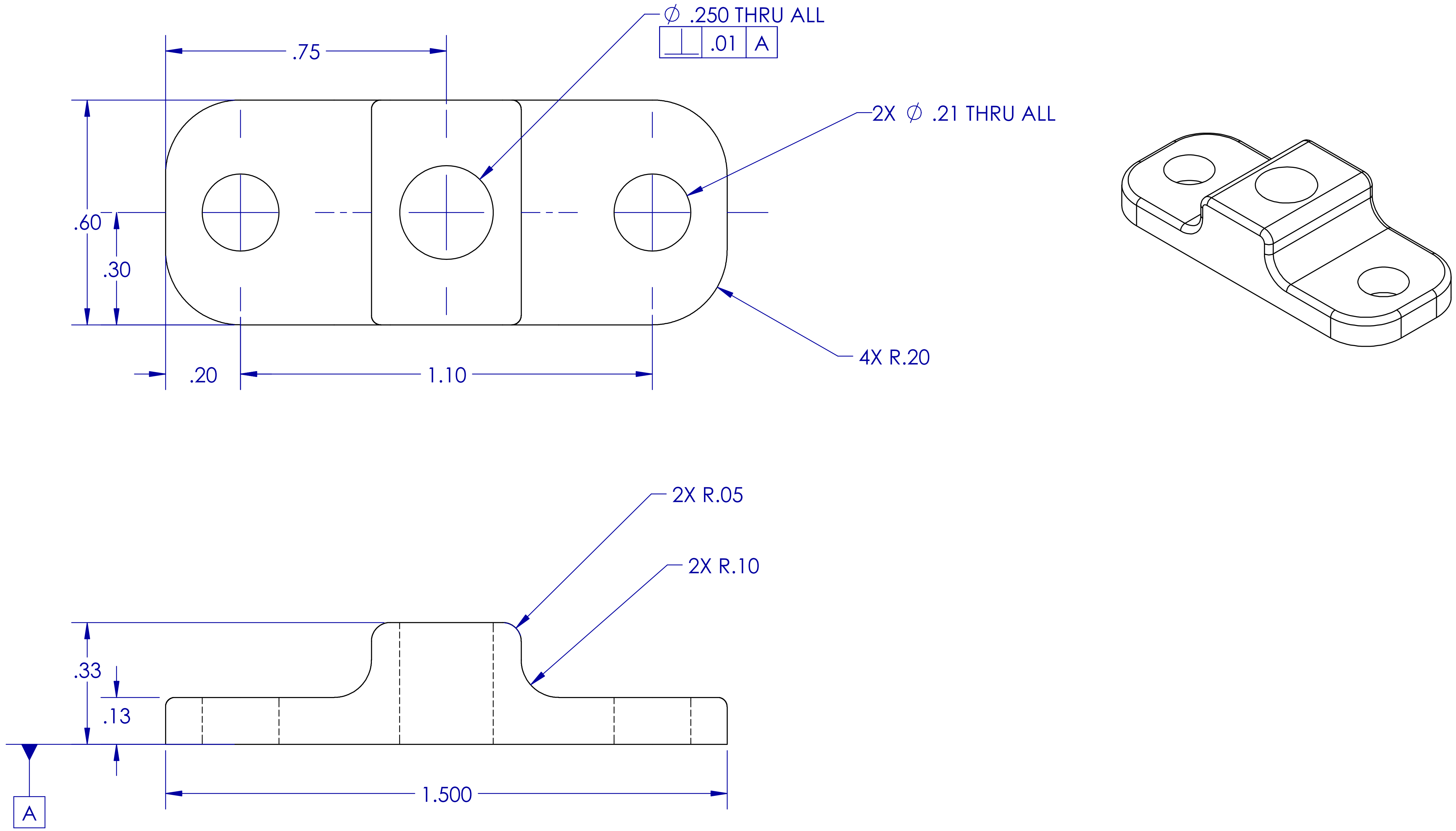
TITLE:
SPRING RETURN

SIZE	DWG. NO.	REV
B	01-2-4-00	2

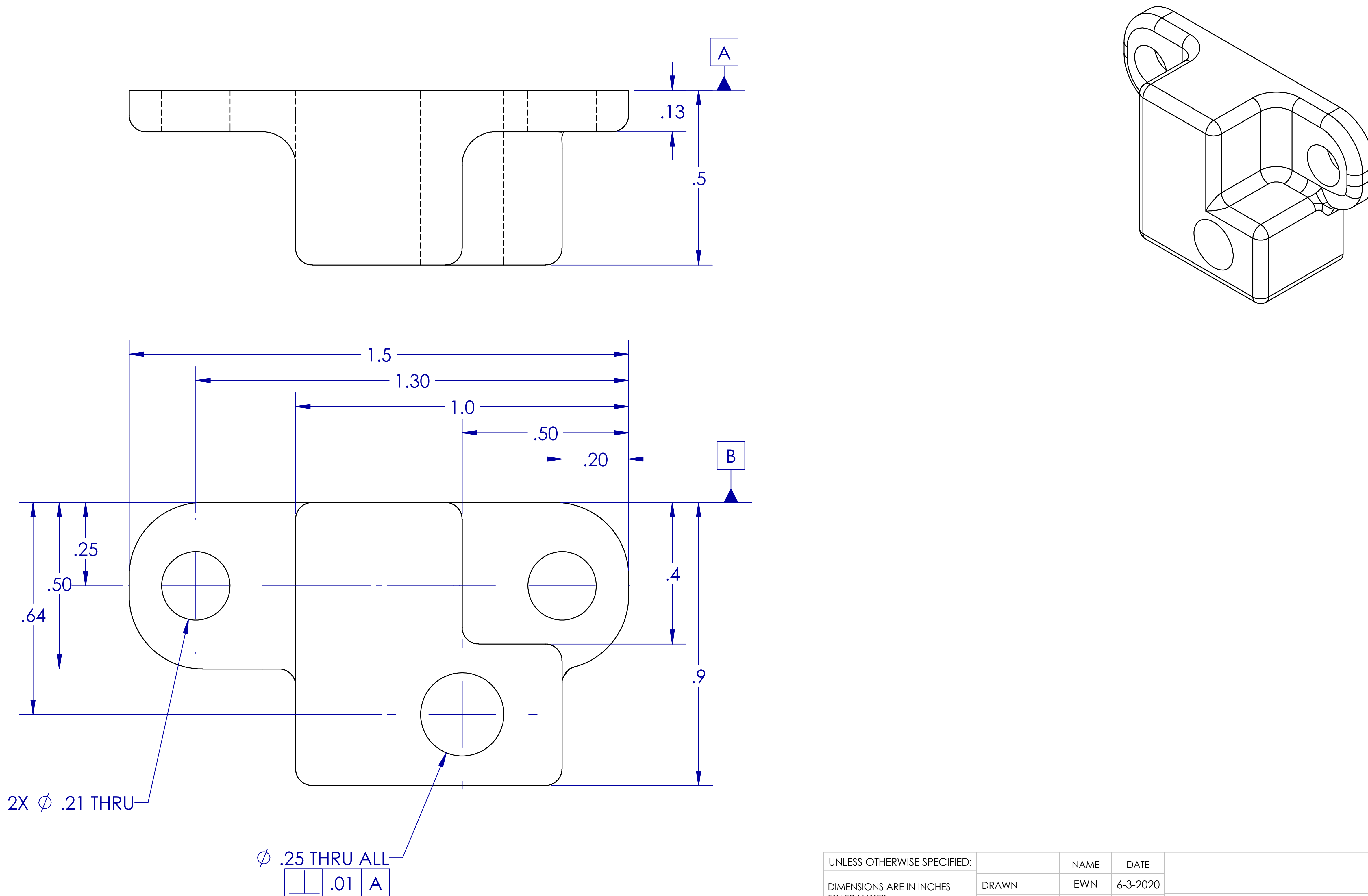
SCALE: 1:1 SHEET 9 OF 15



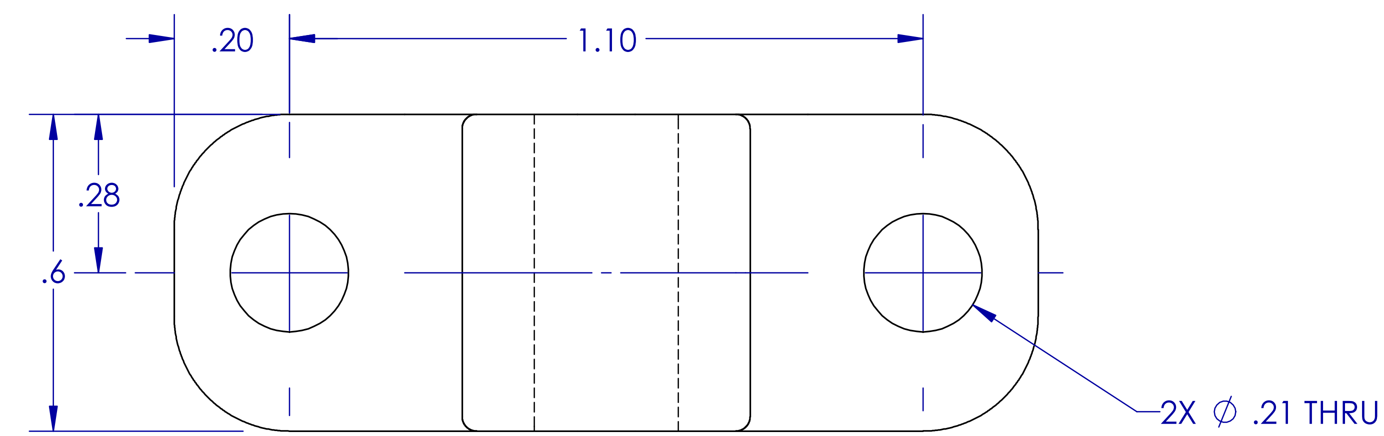
UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	6-2-2020	TITLE: X-AXIS SPRING MOUNT 1
	CHECKED			
	ENG APPR.			
	MFG APPR.			
INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.			
MATERIAL PLA	COMMENTS: THIS PART IS 3D PRINTED. PRINT ON DATUM A. THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.			SIZE B · DWG. NO. 01-2-4-01 · REV 2
FINISH PRINTER QUALITY				
DO NOT SCALE DRAWING				SCALE: 2:1 · WEIGHT: · SHEET 10 OF 15

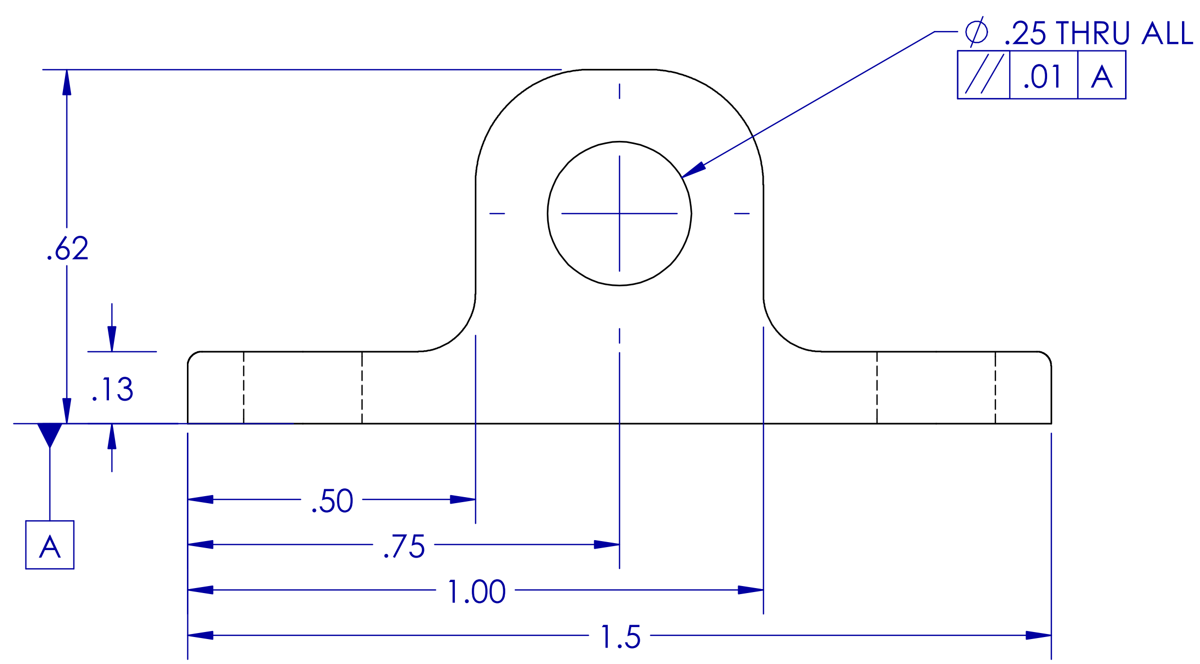


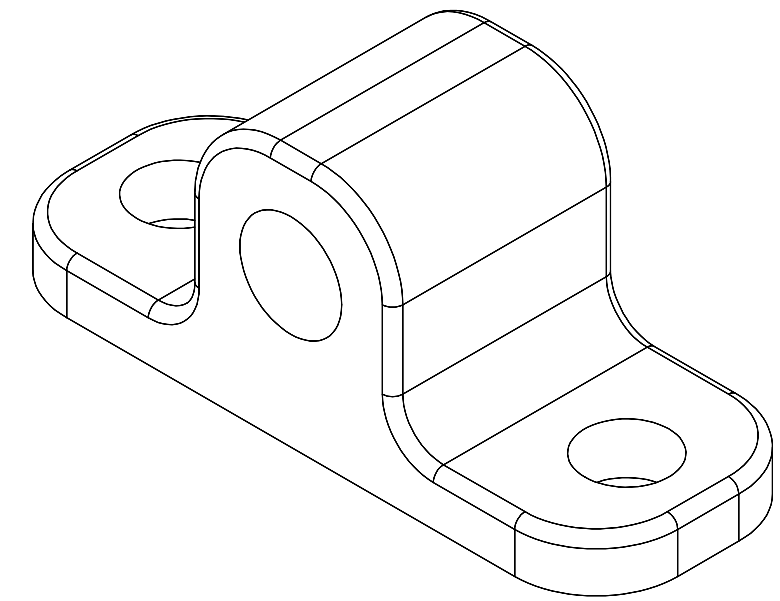
UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	1-29-20	TITLE:
	CHECKED			Y-AXIS SPRING MOUNT 1
	ENG APPR.			
	MFG APPR.			
INTERPRET GEOMETRIC TOLERANCING PER: ASME Y14.5	Q.A.			
MATERIAL PLA	COMMENTS: THIS PART IS 3D PRINTED. PRINT ON DATUM A. THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.			SIZE B / DWG. NO. 01-2-4-02 / REV 1
FINISH PRINTER QUALITY				
DO NOT SCALE DRAWING				SCALE: 3:1 / SHEET 11 OF 15



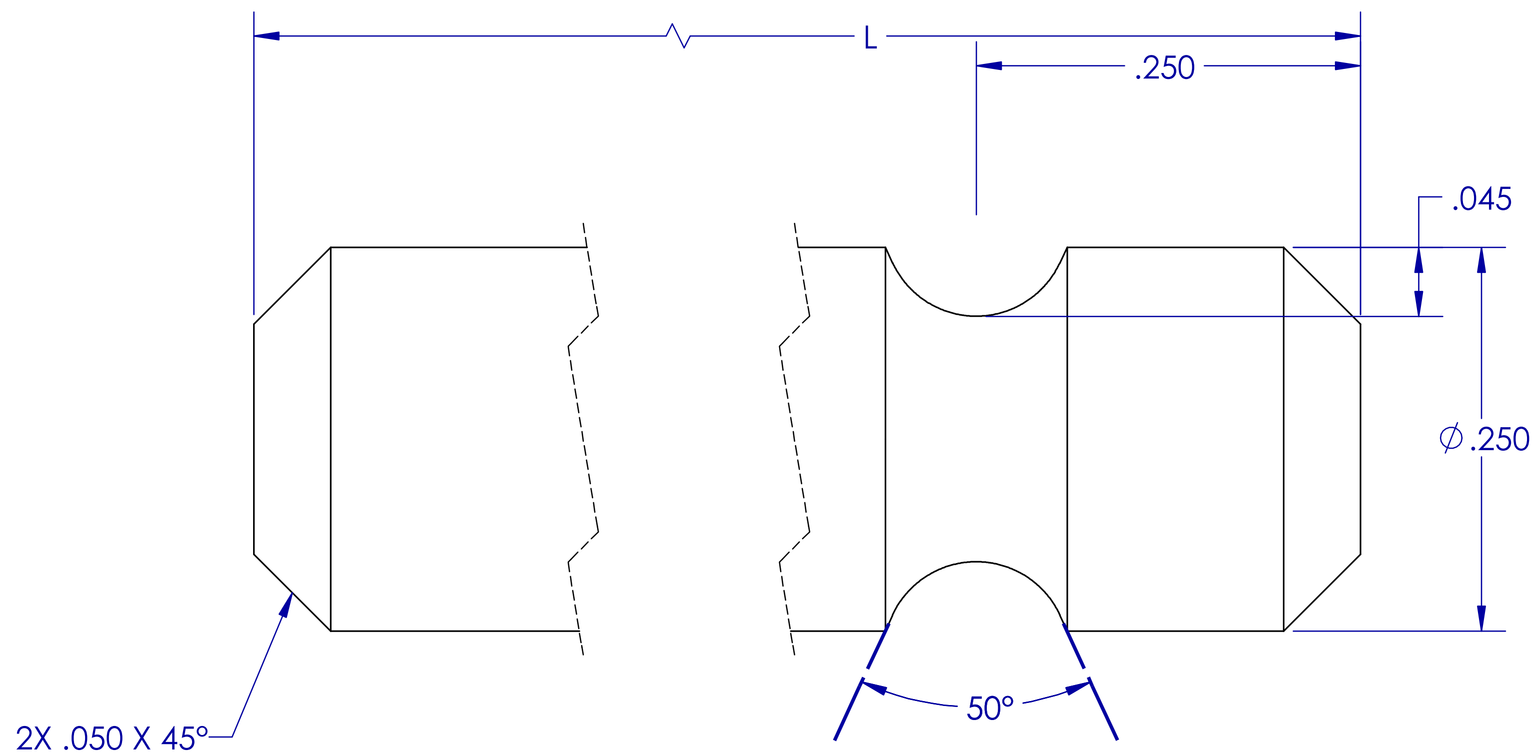
UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	6-3-2020	TITLE: X-AXIS SPRING MOUNT 2
	CHECKED			
	ENG APPR.			
	MFG APPR.			
INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.			
MATERIAL PLA	COMMENTS: THIS PART IS 3D PRINTED. PRINT ON DATUM A. THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.			SIZE B · DWG. NO. 01-2-4-03 · REV 2
FINISH PRINTER QUALITY				SCALE: 3:1 · WEIGHT: · SHEET 12 OF 15
DO NOT SCALE DRAWING				







		UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
		DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	6-3-2020	
			CHECKED			TITLE: Y-AXIS SPRING MOUNT 2
			ENG APPR.			
			MFG APPR.			
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.			
		MATERIAL PLA	COMMENTS: THIS PART IS 3D PRINTED. PRINT ON DATUM A. THE DIMENSIONS SHOWN ARE FOR REFFERENCE ONLY. CONTACT DR. MAYER (hmayer@calpoly.edu) FOR THE UPDATED STL FILES.			SIZE B	DWG. NO. 01-2-3-04	REV 1
NEXT ASSY	USED ON	FINISH PRINTER QUALITY				
APPLICATION		DO NOT SCALE DRAWING				SCALE: 3:1 WEIGHT: SHEET 13 OF 15



PIN LENGTHS	
PIN	L
1	1.00
2	.65
3	.97
4	.92

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
ANGULAR: ±2deg
ONE PLACE DECIMAL: ±.1
TWO PLACE DECIMAL: ±.05
THREE PLACE DECIMAL: ±.005

MATERIAL: A36 HR ROD

FINISH

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	EWN	4-29-20
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

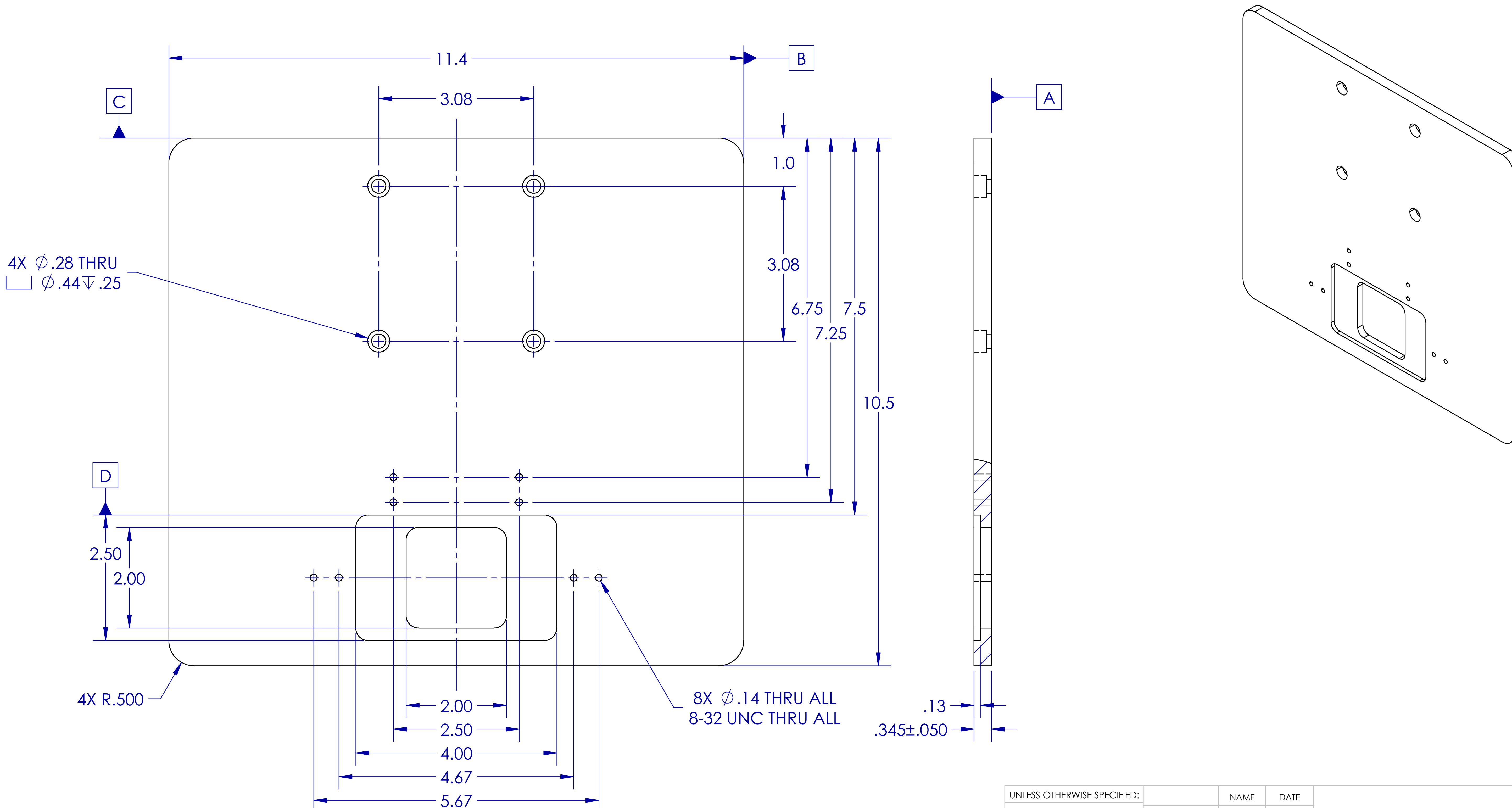
COMMENTS:
- THIS PART IS TO BE TURNED FROM 1/4" STEEL ROD STOCK

- NOTCH SIZE / SHAPE IS NON-CRITICAL. IT SHOULD SECURELY RETAIN A .054" OD WIRE LOOP.

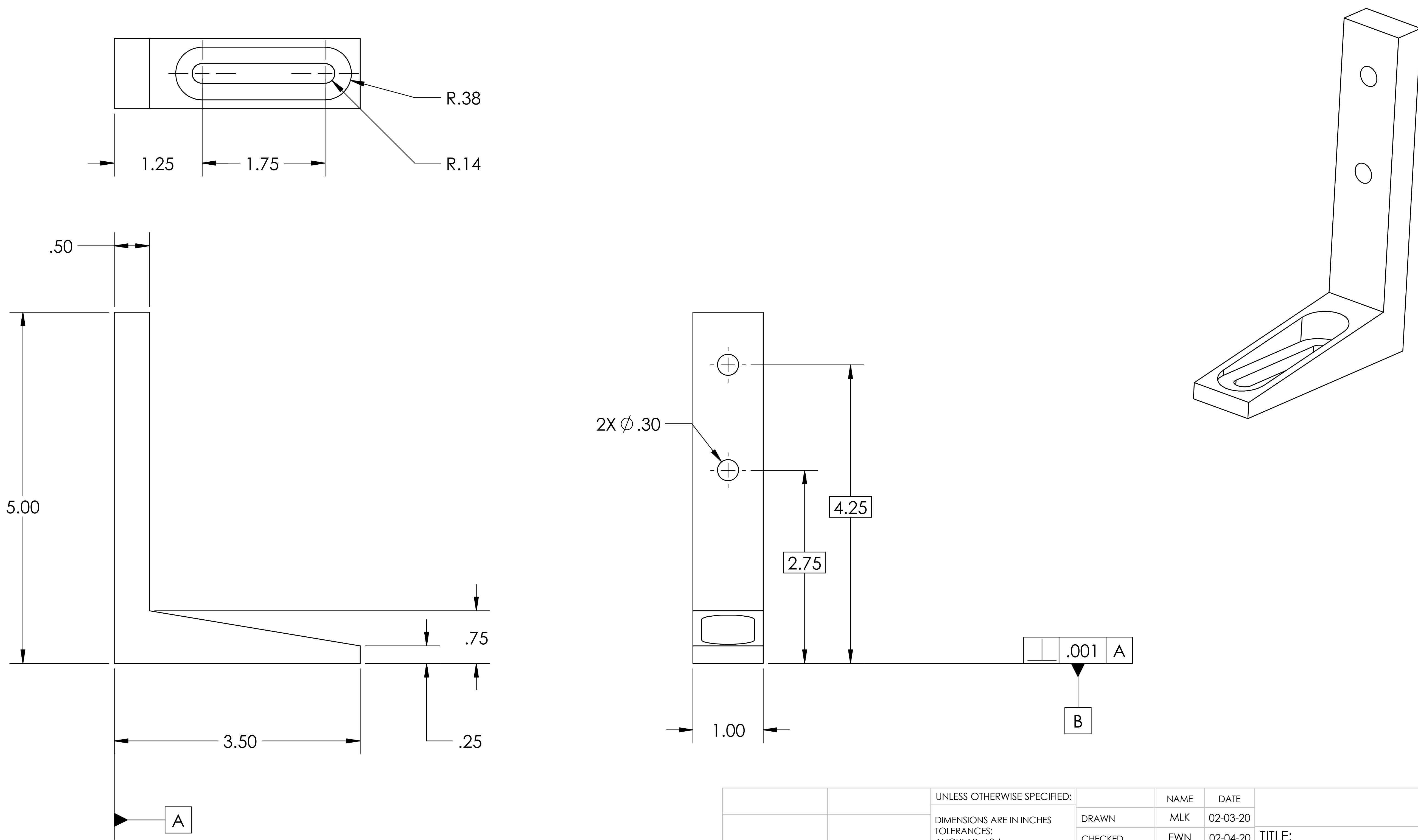
TITLE: SPRING PINS

SIZE B | DWG. NO. 01-2-3-05 | REV 2

SCALE: 8:1 | SHEET 14 OF 15



UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL: ±.1 TWO PLACE DECIMAL: ±.05 THREE PLACE DECIMAL: ±.005	DRAWN	EWN	6-3-2020	TITLE:
	CHECKED			IFM STAGE PLATE
	ENG APPR.			
	MFG APPR.			
	Q.A.			
MATERIAL 6061-T6	COMMENTS: RAW STOCK IS .375' FINISHED PLATE SHOULD BE AS THICK AS POSSIBLE WHILE ACHIEVING PARALLELISM TOLERANCE			SIZE B / DWG. NO. 01-2-5-01 / REV 2
FINISH BLACK ANODIZE T-II				SCALE: 1:2 / SHEET 15 OF 15
DO NOT SCALE DRAWING				



		UNLESS OTHERWISE SPECIFIED:		NAME	DATE	
		DIMENSIONS ARE IN INCHES TOLERANCES: ANGULAR: ±2deg ONE PLACE DECIMAL ±.1 TWO PLACE DECIMAL ±.02 THREE PLACE DECIMAL ±.005	DRAWN	MLK	02-03-20	
			CHECKED	EWN	02-04-20	TITLE: FRAME MOUNTING FOOT
			ENG APPR.			
			MFG APPR.			
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.			
		MATERIAL PLA	COMMENTS: THIS PART IS TO BE 3D PRINTED ON DATUM A.			SIZE B	DWG. NO. 01-3-1-04	REV 2
NEXT ASSY	USED ON	FINISH PRINTER QUALITY				
APPLICATION		DO NOT SCALE DRAWING				SCALE: 2:3

APPENDIX L
PSUEDO CODE

Set variable VRxpin to the analog pin A0 as a constant integer
Set variable VRypin to the analog pin A1 as a constant integer

//For the first 850G actuator
Set the Arduino digital pin 11 to function for the 1st enable pin (pin 9) on the L293D
Set the Arduino digital pin 12 to function for the positive motor voltage pin (pin 10) on the L293D
Set the Arduino digital pin 13 to function for the negative motor voltage pin (pin 15) on the L293D

//For the second 850G actuator
Set the Arduino digital pin 11 to function for the 2nd enable pin (9) on the L293D
Set the Arduino digital pin 12 to function for the motor voltage pin (pin 3) on the L293D
Set the Arduino digital pin 13 to function for the motor voltage pin (pin 4) on the L293D

Make a set up class to initialize the properties of each of the pins designated
    Set the VRxpin to Input
    Set the VRypin to Input
    Set the 1st enable pin to Output
    Set the 1st positive motor voltage pin to Output
    Set the 1st negative motor voltage pin to Output
    Set the 2st enable pin to Output
    Set the 2nd positive motor voltage pin to Output
    Set the 2nd negative motor voltage pin to Output
    Set the serial prompt to read the board at 115200 bits per second

Create a Saturation Block function take an input (x) and change its value accordingly
    Create float variable y
    Create float variable z
    If x is greater than or equal to 383 and less than or equal to 640
        Make x equal to 0 and return its value
    If instead less than 383
Make y equal to x subtracted by 383 and then divided by 383 and then multiplied by 200
Return y
    Or instead greater than 640
Make z equal to x subtracted by 640 and then divided by 640 and then multiplied by 200
Return z

Create a function to move the first actuator out with the input as the speed
    Have the enable pin sent the speed as a value between 0 and 255
    Have the positive motor pin set to a voltage low
    Have the negative motor pin set to a voltage high

Create a function to move the first actuator in the input as the speed
    Have the enable pin sent the speed as a value between 0 and 255
    Have the positive motor pin set to a voltage high
    Have the negative motor pin set to a voltage low

Create a function to stop the first actuator movement
Have the enable pin send a zero to the actuator
Set the positive motor pin to a voltage low
Set the negative motor pin to a voltage low

Create a function to move the second actuator out with the input as the speed
Have the enable pin sent the speed as a value between 0 and 255
Have the positive motor pin set to a voltage low
Have the negative motor pin set to a voltage high

Create a function to move the second actuator in with the input as the speed
Have the enable pin sent the speed as a value between 0 and 255
Have the positive motor pin set to a voltage high
Have the negative motor pin set to a voltage low

Create a function to stop the second actuator movement
Have the enable pin send a zero to the actuator
Set the positive motor pin to a voltage low
Set the negative motor pin to a voltage low

Create a loop that continuously cycles through the functions within it
Create a variable to grab value from first actuator
Create a placeholder variable to grab value from the second actuator

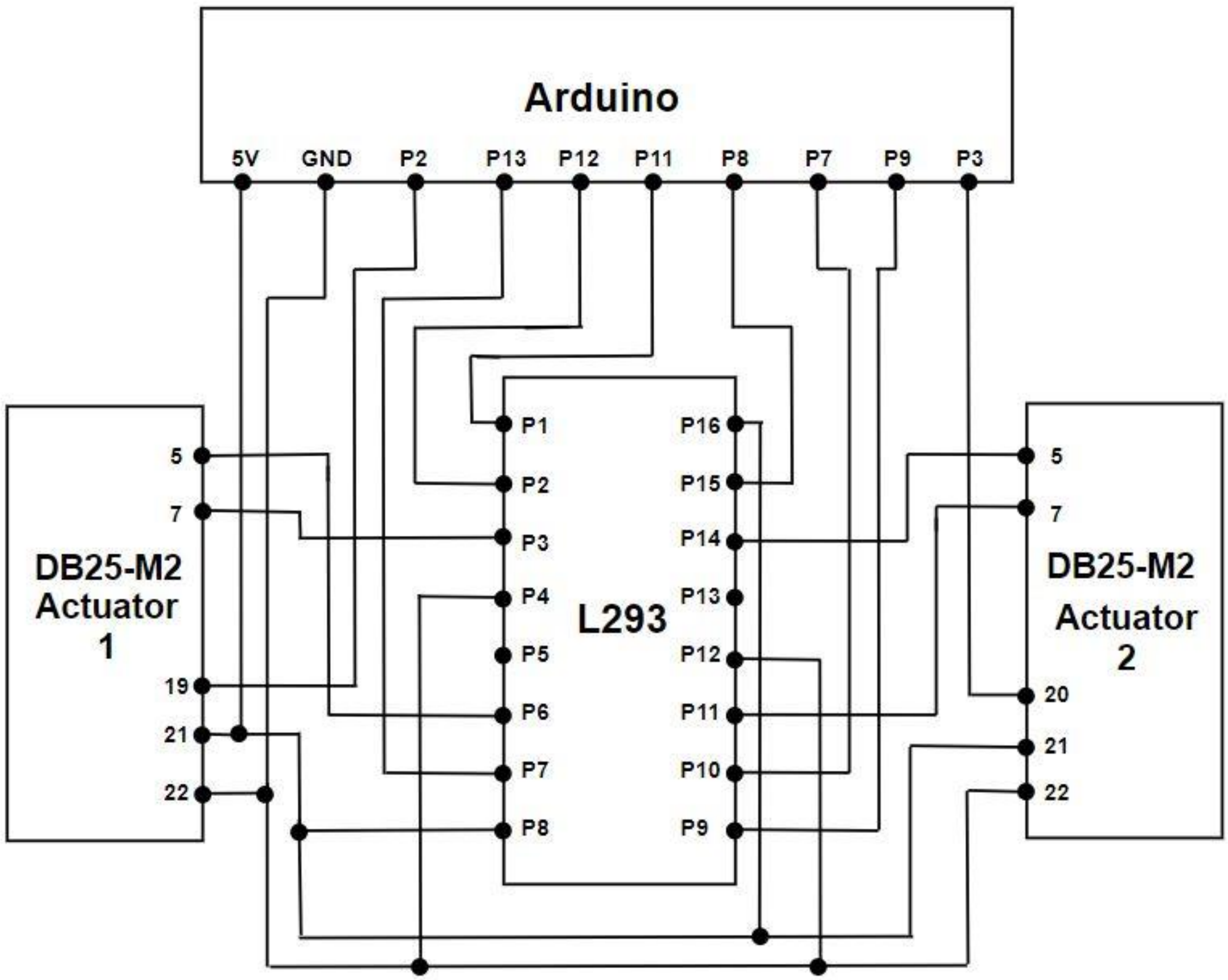
Read from the VRxpin the value from joystick, transform it from the Saturation function and set it equal to first variable
If the first variable is greater than zero, call the move out function and input the variable
If the first variable is less than zero, call the move in function and input the absolute value of the variable
If neither, call the stop movement function

Create a delay of 25 milliseconds to provide time for another reading

Read from the VRypin the value from joystick, transform it from the Saturation function and set it equal to second variable
If the second variable is greater than zero, call the move out function and input the variable
If the second variable is less than zero, call the move in function and input the absolute value of the variable
If neither, call the stop movement function

Create a delay of 25 milliseconds to provide time for another reading

APPENDIX M
OLD WIRING DIAGRAM



Appendix N
Actuator Control Code

```
const byte numChars = 32;          //Number of characters for received
char receivedChars[numChars];   // an array to store the received data
static int index = 0;   //Used to determine the index of characters in the array for turning them
into an
                                        integer type
long int val = 0;   //Current set up in showNewNumber prevents outside of -90000 and 90000

boolean Move_flag = false;   //Determines whether a actuator is able to move
boolean neg_flag = false;      //Determines if the serial input is negative
boolean newData = false;      //Determines whether newData had been entered into the serial
monitor
boolean EndMark_flag = false; //Determines if the end of the serial input is detected

boolean X_flag = false;  //Actuator selection flag for X axis
boolean Y_flag = false;  //Actuator selection flag for Y axis
boolean NEGnum_flag = false; //Used to determine which direction the actuators move

long int dataNumber = 0; //Used for taking serial monitor input and moving the actuator to a
new
                                                encoder location
int character = 0;  //Used for Actuator Axis selection, X and Y only.

int state = 0;  //State of the system (Zeroing, Joystick, PPC)

//Joystick Analog reading pins
const int VRxpin = A0;
const int VRypin = A1;

//Joystick Switch Pin
const int SWpin = 2;

//850 G on 1-8 side of L293D
int en1 = 8;   //Enables Pulse Width Modulation (PWM) to control motor speed, Pin 1 on
L293D
int in1 = 51;  //First Driver Input for HIGH/LOW inputs from Arduino, Pin 2 on L293D
int in2 = 50;  //Second Driver Input for HIGH/LOW inputs from Arduino, Pin 7 on L293D
int revlimA = 22;   //Reverse Limit Switch for Actuator A, terminal 18
int forlimA = 23;   //Forward Limit Switch for Actuator A, terminal 17
static int pinAA = 20;   //Encoder Channel A for Actuator B, terminal 19. Used for hardware
interrupts
static int pinAB = 21;   //Encoder Channel B for Actuator B, terminal 20. Used for hardware
interrupts
```

```
volatile long int EncAencoderPos = 0;   //Variable value for current encoder reading. Provides
signed
                                                      reading of a 32-bit number

//850 G on 9-16 side of L293D
int en2 = 9;   //Enables Pulse Width Modulation (PWM) to control motor speed, Pin 9 on
L293D
int in3 = 52;   //First Driver Input for HIGH/LOW inputs from Arduino, Pin 10 on L293D
int in4 = 53;   //Second Driver Input for HIGH/LOW inputs from Arduino, Pin 15 on L293D
int revlimB = 24;   //Reverse Limit Switch for Actuator B, terminal 18
int forlimB = 25;   //Forward Limit Switch for Actuator B, terminal 17
static int pinBA = 18;   //Encoder Channel A for Actuator B, terminal 19. Used for hardware
interrupts
static int pinBB = 19;   //Encoder Channel B for Actuator B, terminal 20. Used for hardware
interrupts
volatile long int EncBencoderPos = 0;   //Variable value for current encoder reading. Provides
signed
                                                      reading of a 32-bit number

//---------------------Actuator A Channel A Encoder ------------------------

void AencChA(){
/* Description: Counts each time Actuator A’s encoder triggers a change in its Channel A’s
voltage
 */
  cli(); //stop interrupts happening before we read pin values
  if (digitalRead(pinAA) == HIGH){
    if(digitalRead(pinAB) == LOW){
      EncAencoderPos --; //Moving In, index - 1
      sei();             //restart interrupts
    }
    else{
      EncAencoderPos ++; //Moving Out, index + 1
      sei();             //restart interrupts
    }
  }
  else{
    if(digitalRead(pinAB) == HIGH){
      EncAencoderPos --; //Moving In, index - 1
      sei();             //restart interrupts
    }
    else {
      EncAencoderPos ++; //Moving Out, index + 1
      sei();             //restart interrupts
    }
  }
```

```
}

//---------------------Actuator A Channel B Encoder ------------------------

void AencChB(){
/* Description: Counts each time Actuator A's encoder triggers a change in its Channel B's
voltage
 */
  cli(); //stop interrupts happening before we read pin values
  if (digitalRead(pinAB) == HIGH){
    if(digitalRead(pinAA) == HIGH){
      EncAencoderPos --;  //Moving In, index - 1
      sei();              //restart interrupts
    }
    else{
      EncAencoderPos ++;  //Moving Out, index + 1
      sei();              //restart interrupts
    }
  }
  else{
    if(digitalRead(pinAA) == LOW){
      EncAencoderPos --; //Moving In, index - 1
      sei();             //restart interrupts
    }
    else {
      EncAencoderPos ++;  //Moving Out, index + 1
      sei();              //restart interrupts
    }
  }
}

//---------------------Actuator B Channel A Encoder ------------------------

void BencChA(){
/* Description: Counts each time Actuator B's encoder triggers a change in its Channel A's
voltage
 */
  cli();   //stop interrupts happening before we read pin values
  if (digitalRead(pinBA) == HIGH){
    if(digitalRead(pinBB) == LOW){
      EncBencoderPos --;   //Moving In, index - 1
      sei();             //restart interrupts
    }
    else{
      EncBencoderPos ++;    //Moving Out, index + 1
      sei();              //restart interrupts
```

```
    }
  }
  else{
    if(digitalRead(pinBB) == HIGH){
      EncBencoderPos --;   //Moving In, index - 1
      sei();             //restart interrupts
    }
    else {
      EncBencoderPos ++;   //Moving Out, index + 1
      sei();             //restart interrupts
    }
  }
}

//---------------------Actuator B Channel B Encoder ------------------------

void BencChB(){
/* Description: Counts each time Actuator B's encoder triggers a change in its Channel B's
voltage
 */
  cli();   //stop interrupts happening before we read pin values
  if (digitalRead(pinBB) == HIGH){
    if(digitalRead(pinBA) == HIGH){
      EncBencoderPos --;  //Moving In, index - 1
      sei();              //restart interrupts
    }
    else{
      EncBencoderPos ++;  //Moving Out, index + 1
      sei();              //restart interrupts
    }
  }
  else{
    if(digitalRead(pinBA) == LOW){
      EncBencoderPos --; //Moving In, index - 1
      sei();             //restart interrupts
    }
    else {
      EncBencoderPos ++; //Moving Out, index + 1
      sei();             //restart interrupts
    }
  }
}

//---------------------Actuator A Basic Movement Functions ------------------------

void MoveAOut(int speed){
```

```
/* Description: Moves Actuator A out at a set speed determined by the Speed Differential
function
 */
  analogWrite(en1, speed); //Writes to Enable 1 pin 8 bit value for speed
  digitalWrite(in1, LOW);  //Writes Input 1 a low value
  digitalWrite(in2, HIGH); //Writes Input 2 a high value
}

void MoveAIn(int speed){
/*Description: Moves Actuator A in at a set speed determined by the Speed Differential
function
 */
  analogWrite(en1, speed); //Writes to Enable 1 pin 8 bit value for speed
  digitalWrite(in1, HIGH); //Writes Input 1 a high value
  digitalWrite(in2, LOW);  //Writes Input 2 a low value
}

void StopA(){
/* Description: Stops actuator motion by with a preset zero for the speed
 */
  //Stops movement for Actuator A
  analogWrite(en1, 0);     //Enable 1 pin 8 bit value for speed set to zero
  digitalWrite(in1, LOW);  //Writes Input 1 a low value
  digitalWrite(in2, LOW);  //Writes Input 2 a low value
}

//---------------------Actuator B Basic Movement Functions ------------------------

void MoveBOut(int speed){
/* Description: Moves Actuator B out at a set speed determined by the Speed Differential
function
 */
  analogWrite(en2, speed); //Writes to Enable 2 pin 8 bit value for speed
  digitalWrite(in3, LOW);  //Writes Input 3 a low value
  digitalWrite(in4, HIGH); //Writes Input 4 a high value
}

void MoveBIn(int speed){
/* Description: Moves Actuator B in at a set speed determined by the Speed Differential
function
 */
  analogWrite(en2, speed); //Writes to Enable 2 pin 8 bit value for speed
  digitalWrite(in3, HIGH); //Writes Input 3 a high value
  digitalWrite(in4, LOW);  //Writes Input 4 a low value
}
```

```
void StopB(){
/* Description: Stops actuator motion by with a preset zero for the speed
 */
  analogWrite(en2, 0);     //Enable 2 pin 8 bit value for speed set to zero
  digitalWrite(in3, LOW);  //Writes Input 3 a low value
  digitalWrite(in4, LOW);  //Writes Input 4 a low value
}

void setup() {
  // set up Joystick pins for input
  pinMode(VRxpin, INPUT);
  pinMode(VRypin, INPUT);
  pinMode(SWpin, INPUT);
  digitalWrite(SWpin, HIGH);

  //Set up pins for Actuator A for output
  pinMode(en1, OUTPUT);
  pinMode(in1, OUTPUT);
  pinMode(in2, OUTPUT);

  //Set up pins for Actuator B for output
  pinMode(en2, OUTPUT);
  pinMode(in3, OUTPUT);
  pinMode(in4, OUTPUT);

  //Set up limit switches for both Actuators
  pinMode(forlimA, INPUT_PULLUP);
  pinMode(revlimA, INPUT_PULLUP);
  pinMode(forlimB, INPUT_PULLUP);
  pinMode(revlimB, INPUT_PULLUP);

  //Actuator A, Encoder Output
  pinMode(pinAA,INPUT_PULLUP);
  pinMode(pinAB,INPUT_PULLUP);
  attachInterrupt(digitalPinToInterrupt(pinAA),AencChA,CHANGE);
  attachInterrupt(digitalPinToInterrupt(pinAB),AencChB,CHANGE);

  //Actuator B, Encoder Output
  pinMode(pinBA,INPUT_PULLUP);
  pinMode(pinBB,INPUT_PULLUP);
  attachInterrupt(digitalPinToInterrupt(pinBA),BencChA,CHANGE);
  attachInterrupt(digitalPinToInterrupt(pinBB),BencChB,CHANGE);

  Serial.begin(9600);
  Serial.println("<Arduino is ready>");
}
```

```
void loop() {
   int SW_read;   //Variable to read switch pin
   int X_dir = 0; //Variable for Joystick in X direction
   int Y_dir = 0; //Variable for Joystick in Y direction

   SW_read = digitalRead(SWpin); //Read switch pins digital input
   stateChange(SW_read,state);  //Check switch to alter state
   if(SW_read == LOW){           //If Switch button is pressed
      while(SW_read == LOW){    //While the button is pressed
        SW_read = digitalRead(SWpin);  //Read the switch pin
        if(SW_read == HIGH){    //If the button is released
          break;                //Break the loop and continue with code
        }
      }
   }
   switch (state){
     case 0:
        Serial.println("Initial State: Click Enter to start Zeroing the actuators");
        while (Serial.available() == 0){} //Wait till Serial Monitor detects an input
        Serial.read();              //Remove newline created when clicking enter
        Serial.print("Zeroing the X Axis Actuator\n");
        XZeroing(revlimB,forlimB);  //Zeroing the X axis while monitoring both limit switches
        Serial.print("Zeroing the Y Axis Actuator\n");
        YZeroing(revlimA,forlimA);  //Zeroing the Y axis while monitoring both limit switches
        state++;                    //Increase state variable to move to case 1 (Joystick)
        Serial.println("Done, Moving to Joystick state");
        delay(50);
     break;

     case 1:
        //Change Satblock to act as a flag instead
        X_dir = SatBlock(analogRead(VRypin)); //Reads the analog input from VRy and put it
through a
                                              Saturation Block
        Y_dir = SatBlock(analogRead(VRxpin)); //Reads the analog input from VRx and put it
through a
                                              Saturation Block
        LimitSwitchA(revlimA,forlimA,Y_dir);  //Moves Actuator A (Y_axis) while checking
for limit
                                              switch activation
        LimitSwitchB(revlimB,forlimB,X_dir);  //Moves Actuator B (X_axis) while checking
for limit
                                              switch activation
        delay(50);
     break;
```

```
      case 2:
        StopA();
        StopB();
        InitPPCselect();
        if (X_flag == true){  //If X selected
          Serial.println("Enter a target value for X axis");
          while (Serial.available() == 0){}  //Wait till Serial Monitor detects an input
          MoveXEncoder();
        }
        else if(Y_flag == true){  //If Y selected
          Serial.println("Enter a target value for Y axis");
          while (Serial.available() == 0){}  //Wait till Serial Monitor detects an input
          MoveYEncoder();
        }
        delay(50);
      break;
    }
}

void stateChange(int sw, int s){
/* Description: Checks if the joystick switch has been activated and which state the Arduino is
 *                  currently in to switch to the next mode. The inputs are the Switch read value
(int sw)
 *                  and the current 3 states (int s).
 */
  if (sw == LOW){
      if(s == 0){
        state++;  //Add 1 to state to move to Joystick Case
        Serial.println("Joystick Case");
        delay(300);
      }
      else if(s == 1){
        state++;  //Add 1 to state to move to Joystick Case
        Serial.println("Path Code Case");
        //Display current encoder counts on switch
        delay(300);
      }
      else if(s == 2){
        state--;  //Subtract 1 from state to move to Joystick Case
        Serial.println("Joystick Case");
        delay(300);
      }
  }
}
```

```
void InitPPCselect(){
/* Description: Waits for an Actuator to be selected in the Programmable Path mode. This
function also
 *                    allows for the joystick switch to be activated in order for the user to switch to
the
 *                     Joystick mode.
 */
  int SW_read;        //Variable to store switch read (HIGH/LOW)
  int switch_flag = 0; //Flag activated during switch activation

  Serial.println("Select Actuator to move (X/Y)");
  while (Serial.available() == 0){  //While waiting for an input to be detected
      SW_read = digitalRead(SWpin); //Read switch pins digital input
      if(SW_read == LOW){           //If Switch button is pressed
        Serial.print("Switching to Joystick State\n");
        state--;         //Change to Joystick state
        switch_flag = 1; //Activate switch flag
        while(SW_read == LOW){  //While the button is pressed
          SW_read = digitalRead(SWpin); //Read the button pin
          if(SW_read == HIGH){  //If the switch reads a high
            break;   //Break the loop
          }
        }
        break; //Break the loop
      }
  }
  if (switch_flag == 0){ //If the switch is not activated and serial monitor detected an input
    while (newData != true){  //If newData has not been detected
      recvWithEndMarker();  //Cycle through to read next character from serial port
    }
    selectActuator();  //Determine Actuator selected
    if (character == 0x58){  //If character is equal to ASCCI "X"
      X_flag = true;   //Set X_flag to move to target step
    }
    else if(character == 0x59){  //If character is equal to ASCCI "X"
      Y_flag = true;   //Set X_flag to move to target step
    }
  }
  else{
    switch_flag = 0;
  }
}

//---------------------General Actuator Movement Functions------------------

void YZeroing(int rlimsw, int flimsw){
```

```
/* Description: Zeros the Y axis actuator (Actuator A) while monitoring both limit switches. It
uses the
 *                    functions MoveAOutToTarget and MoveAIn.
 */
  int x = 0;      //Variable for reading LimitSwitch
  long int y = 0; //Variable for encoder target input
  x = digitalRead(rlimsw);   //Read limit switch output
  while (x == LOW){          //While the reverse limit is not activated
    x = digitalRead(rlimsw); //Read limit switch output
    if(x == LOW){            //If the reverse limit switch is not activated
      MoveAIn(175);          //Move A at a set speed -----Change if Actuator B is moving faster
    }
    else if (x == HIGH){     //If the reverse limit switch is activated
      Serial.print("Triggered Setpoint\n");
      break;                 //Break while loop
    }
  }
  StopA();             //Stop movement of Actuator A
  delay(500);          //Delay between print
  EncAencoderPos = 0;  //Set Encoder position to zero
  Serial.println("Encoder has been zeroed");
  delay(500);          //Delay between prints
  y = 3500;            //Number of encoder counts
  Serial.println("Moving Actuator to Zero Mark");
  MoveAOutToTarget(flimsw,y); //Move Actuator A to target while checking forward limit
switch
}

void XZeroing(int rlimsw, int flimsw){
/* Description: Zeros the X axis actuator (Actuator B) while monitoring both
 *             limit switches. It uses the functions MoveBOutToTarget and
 *             MoveBIn.
 */
  int x = 0;      //Variable for reading LimitSwitch
  long int y = 0; //Variable for encoder target input
  x = digitalRead(rlimsw);   //Read limit switch output
  while (x == LOW){          //While the reverse limit is not activated
    x = digitalRead(rlimsw); //Read limit switch output
    if(x == LOW){            //If the reverse limit switch is not activated
      MoveBIn(175);          //Move B at a set speed---Actuator has had trouble moving at 150
previously
    }
    else if (x == HIGH){     //If the reverse limit switch is activated
      Serial.print("Triggered Setpoint\n");
      break;                 //Break while loop
    }
```

```
  }
  StopB();           //Stop movement of Actuator B
  delay(500);        //Delay between prints
  EncBencoderPos = 0; //Set Encoder position to zero
  Serial.print("Encoder has been zeroed\n");
  delay(500);        //Delay between prints
  y = 3500;          //Number of encoder counts
  Serial.println("Moving Actuator to Zero Mark");
  MoveBOutToTarget(flimsw,y); //Move Actuator B to target while checking forward limit
switch
}

void MoveYEncoder(){
/* Description: Moves the Y axis actuator when the Arduino is in the Programmable Path
mode.
 *            It uses recWithEndMarker, showNewNumber, MoveAOutToTarget and
MoveAInToTarget
 *            functions.
 */
    long int y = 0;
    while (newData != true){ //If newData has not been detected
      recvWithEndMarker();   //Cycle through to read next character from serial port
    }
    showNewNumber();      //Turn characters entered into an integer
    y = dataNumber;       //Load y with new integer
    Y_flag = false;       //Actuator selection flag changed to prevent re-entry
    if(Move_flag == true){      // If Move_Flag is true
      if (NEGnum_flag == false){ //And the number is not negative
        MoveAOutToTarget(forlimA,y); //Move Actuator to target and check if the forward limit
switch
                                                    is entered
        Move_flag = false;      //Once completed, disable Move_flag
      }
      else {                    //If the number is negative

        MoveAInToTarget(revlimA,y); //Move Actuator to target and check if the forward limit
switch is
                                                  entered
        Move_flag = false;    //Once completed, disable Move_flag
        NEGnum_flag = false;  //Disable number flag
      }
    }
}

void MoveXEncoder(){
```

```
/* Description: Moves the X axis actuator when the Arduino is in the Programmable Path
mode.
 *             It uses recWithEndMarker, showNewNumber, MoveBOutToTarget and
MoveBInToTarget
 *             functions.
 */
  long int x = 0;
  while (newData != true){  //If newData has not been detected
    recvWithEndMarker();    //Cycle through to read next character from serial port
  }

  showNewNumber();          //Turn characters entered into an integer
  x = dataNumber;           //Load x with new integer
  X_flag = false;           //Actuator selection flag changed to prevent re-entry

  if(Move_flag == true){    //If actuator is allowed to move
    if(NEGnum_flag == false){  //If the dataNumber is negative
      MoveBOutToTarget(forlimB,x);  //Move Actuator B out to target while checking forward
limit
                                                  switch
      Move_flag = false;            //Move flag is turned off
    }
    else {
      MoveBInToTarget(revlimB,x);  //Move Actuator B into target while checking reverse limit
switch
      Move_flag = false;           //Once completed, disable Move_flag
      NEGnum_flag = false;         //Disable number flag
    }
  }
}

//------------------------Encoder Movement for Actuator A------------------------

void MoveAOutToTarget(int flimsw,long int target)  {
/* Descrription: : Moves Actuator A using the MoveAOut function and detects whether the
target
 *                    encoder count is reached (long int target), or the forward  limit switch (int
flimsw) has
 *                    been activated to stop actuator motion using the StopA function.
 */
  Serial.print("Moving A forward  ");
  Serial.print(target);
  Serial.print(" encoder counts\n");

  long int x = 0;  //New encoder count to reach
  int y = 0;       //Limit switch variable
```

```
  int f = 0;       //Speed Differential variable

  x =  EncAencoderPos + target; //Make new total encoder count to reach

  while (EncAencoderPos < x){  //While the current encoder count is less than the new count
    y = digitalRead(flimsw);   //Read forward limit switch
    if (y == LOW){             //If forward limit switch is not active
      f = SpeedDifferential(EncAencoderPos);  //Calculated required extrusion speed
      MoveAOut(f);             //Set moving out speed to speed found above
      delay(10);
    }
    else if(y == HIGH){ //If forward limit switch is not active
      Serial.println("Forward Limit Switch Activated, Stopping");
      break;           //Exit out of loop
    }
  }
  StopA();     //Stop movement of actuator
  delay(250);  //Delay for print

  Serial.print("target found at ");
  Serial.print(EncAencoderPos);
  Serial.print("\n");
}

void MoveAInToTarget(int rlimsw,long int target){
/* Description: Moves Actuator A using the MoveAIn function and detects whether the target
encoder
 *                    count is reached (long int target), or the reverse limit switch (int rlimsw) has
been
 *                    activated to stop actuator motion using the StopA function.
 */
  Serial.print("Moving A backward ");
  Serial.print(target);
  Serial.print("\n");
  long int x = 0;  //New encoder count to reach
  int z = 0;       //Limit switch variable
  float f = 0;     //Speed Differential variable

  x =  EncAencoderPos + target; //Make new total encoder count to reach

  while (EncAencoderPos > x){ //While the current encoder count is less than the new count
    z = digitalRead(rlimsw);  //Read reverse limit switch
    if (z == LOW){            //If reverse limit switch is not active
      f = SpeedDifferential(EncAencoderPos);  //Calculated required extrusion speed
      MoveAIn(f);             //Set moving out speed to speed found above
      delay(10);
```

```
    }
    else if(z == HIGH){  //If forward limit switch is not active
      Serial.println("Reverse Limit Switch Activated, Stopping");
      break;             //Exit out of loop
    }
  }
  StopA();     //Stop movement of actuator
  delay(250);  //Delay for print

  Serial.print("target found at ");
  Serial.print(EncAencoderPos);
  Serial.print("\n");
}

//------------------------Encoder Movement for Actuator B------------------------

void MoveBOutToTarget(int flimsw,long int target)  { // Moving shaft out
/* Description: Moves Actuator B using the MoveBOut function and detects whether the target
 *                  encoder count is reached (long int target), or the forward  limit switch (int
flimsw) has
 *                  been activated to stop actuator motion using the StopB function.
 */
  Serial.print("Moving B forward  ");
  Serial.print(target);
  Serial.print(" encoder counts\n");
  long int x = 0;  //New encoder count to reach
  int y = 0;       //Limit switch variable
  int f = 0;       //Speed Differential variable

  x =  EncBencoderPos + target; //Make new total encoder count to reach

  while (EncBencoderPos < x){  //While the current encoder count is less than the new count
    y = digitalRead(flimsw);   //Read forward limit switch
    if (y == LOW){             //If forward limit switch is not active
      f = SpeedDifferential(EncBencoderPos);  //Calculated required extrusion speed
      MoveBOut(f);             //Set moving out speed to speed found above
      delay(10);
    }
    else if(y == HIGH){ //If forward limit switch is not active
      Serial.println("Forward Limit Switch Activated, Stopping");
      break;            //Exit out of loop
    }
  }
  StopB();     //Stop movement of actuator
  delay(250);  //Delay for print
```

```
  Serial.print("target found at ");
  Serial.print(EncBencoderPos);
  Serial.print("\n");
}

void MoveBInToTarget(int rlimsw,long int target){
/* Description: Moves Actuator B using the MoveBIn function and detects whether the target
encoder
 *                count is reached (int target), or the reverse limit switch (int rlimsw) has been
activated
 *                to stop actuator motion using the StopB function.
 */
  Serial.print("Moving B backward ");
  Serial.print(target);
  Serial.print("\n");
  long int x = 0;  //New encoder count to reach
  int z = 0;       //Limit switch variable
  float f = 0;     //Speed Differential variable

  x =  EncBencoderPos + target; //Make new total encoder count to reach

  while (EncBencoderPos > x){ //While the current encoder count is less than the new count
    z = digitalRead(rlimsw);  //Read reverse limit switch
    if (z == LOW){           //If reverse limit switch is not active
      f = SpeedDifferential(EncBencoderPos);  //Calculated required extrusion speed
      MoveBIn(f);           //Set moving out speed to speed found above
      delay(10);
    }
    else if(z == HIGH){ //If forward limit switch is not active
      Serial.println("Reverse Limit Switch Activated, Stopping");
      break;           //Exit out of loop
    }
  }
  StopB();      //Stop movement of actuator
  delay(250);   //Delay for print

  Serial.print("target found at ");
  Serial.print(EncBencoderPos);
  Serial.print("\n");
}

//---------------------------Joystick Motion-----------------------------

void LimitSwitchA(int rlimsw, int flimsw,int speed){
/* Description: Moves the Y axis actuator in the Joystick state while checking whether the
limit
```

```
 *                    switches (int rlimsw & int flimsw) have been activated. The speed (int speed)
is
 *                    a flag for which direction the joystick is pointing.
 */
  int x = 0;  //Forward Limit Switch reader variable
  int y = 0;  //Reverse Limit Switch reader variable
  int f = 0;  //Speed Differential calculated value variable
  x = digitalRead(flimsw);  //Read forward limit switch
  y = digitalRead(rlimsw);  //Read reverse limit switch
  if (speed > 0){
    if (x == LOW){      //Move out Actuator A
      f = SpeedDifferential(EncAencoderPos);  //Calculate speed from Encoder A Position
      MoveAOut(f);
    }
    else if(x == HIGH){ //Front Limit Switch Activated for Actuator A
      Serial.print("Y Axis forward limit reached, Reverse Now! \n");
      StopA();          //Stop Actuator B motion
    }
  }
  else if (speed < 0){
    if (y == LOW){      //Move in Actuator A
      f = SpeedDifferential(EncAencoderPos);  //Calculate speed from Encoder A Position
      MoveAIn(f);       //Move Actuator A out by calculated speed
    }
    else if (y == HIGH){ //Reverse Limit Switch Activated for Actuator A
      Serial.print("Y Axis reverse limit reached, Forward Now! \n");
      StopA();           //Stop Actuator A motion
    }
  }
  else {
    StopA();  //Stop Actuator A motion
  }
}

void LimitSwitchB(int rlimsw, int flimsw,int speed){
/* Description: Moves the X axis actuator in the Joystick state while checking whether the
limit
 *                    switches (int rlimsw & int flimsw) have been activated. The speed (int speed)
is
 *                    a flag for which direction the joystick is pointing.
 */
  int x = 0;  //Forward Limit Switch reader variable
  int y = 0;  //Reverse Limit Switch reader variable
  int f = 0;  //Speed Differential calculated value variable
  x = digitalRead(flimsw);  //Read forward limit switch
  y = digitalRead(rlimsw);  //Read reverse limit switch
```

```
  if (speed > 0){
    if (x == LOW){  //Move out Actuator B
      f = SpeedDifferential(EncBencoderPos); //Calculate speed from Encoder B Position
      MoveBOut(f);  //Move Actuator B out by calculated speed
    }
    else if(x == HIGH){ //Reverse Limit Switch Activated
      Serial.print("X Axis forward limit reached, Reverse Now!\n");
      StopB();  //Stop Actuator B motion
    }
  }
  else if (speed < 0){
    if (y == LOW){        //Move in Actuator B
      f = SpeedDifferential(EncBencoderPos); //Calculate speed from Encoder B Position
      MoveBIn(f);         //Move Actuator B in by calculated speed
    }
    else if (y == HIGH){  //Forward Limit Switch Activated
      Serial.print("X Axis forward limit reached, Forward Now! \n");
      StopB();            //Stop Actuator B motion
    }
  }
  else {
    StopB(); //Stop Actuator B motion
  }
}

//---------------------------Saturation Block----------------------------

int SatBlock(float x) {
/* Description: Receives input from the Joystick and acts as flag to determine which axis
moves. float x
 *                    is a floating number with a range between 0-1023.
 */
  float y;  //Initializes a floating number for return
  float z;  //Initializes a floating number for return
    if (x >= 383 && x <= 640){  //x equals zero if input is between 383 and 640
      x = 0;
      return x;
    }
    else if (x < 383){          //If input is less than 383
      y =  1;//*((x - 383)/383); //Run math to change input into percentage then multiply by -255
      return y;
    }
    else if (x > 640){          //If input is greater than 640
      z = -1;//*((x - 640)/383); //Run math to change input into percentage then multiply by -255
      return z;
    }
```

```
}

//---------------------Serial Monitor Read/Decode------------------------

void recvWithEndMarker() {
/* Description: Reads from the Serial Monitor then input entered and
 *             organizes the input into an array
 */
   static byte ndx = 0;   //Storage Index
   char endMarker = '\n'; //End marker from Serial Inputs
   char rc;               // Temporary char storage

   if (Serial.available() > 0) { //Wait for Serial port to read entered input
      rc = Serial.read();      //Reads 1 byte from Serial port
      if (rc != endMarker) {      //If end marker not detected, index data
         receivedChars[ndx] = rc;
         ndx++;                       //Increase storage index
         if (ndx >= numChars) {//If Storage Index larger than array size
            ndx = numChars - 1; //Set index to last array space for overwrite
         }
      }
      else {
         receivedChars[ndx] = '\0'; // terminate the string
         index = ndx;     //Make char sorter index equal to storage index
         ndx = 0;        //Set storage index to zero for next data
         newData = true;  //Set newData flag to true
      }
   }
}

void showNewNumber() {
/* Description: Receives the array constructed by the recWithEndMarker function and
attempts to
 *                   construct an integer from the ASCII values present in the array
 */
   if (newData == true) { //If newData is ready
      dataNumber = 0;
      int i = 0;  //Index for while loop to be compared to array index
      int x = 0;  //Variable to hold next array input
      while (i < index+1){     //While i is less than the character storage index + 1
        if (receivedChars[i] >= 0x30 && receivedChars[i] <= 0x39){
         //If the character in the array location is between 0 and 9 in Hex
         val = val*10;                //Multiply value by 10
         x =  receivedChars[i] - 0x30; //Subtract character by 30 hex
         //Serial.println(x);         //Print digit
```

```
    val = val + x;               //Add digit to value
    i++;                         //increase index by 1
    x = 0;                       //Set x equal to zero for next array location
  }
  else if(receivedChars[i] == 0x2D){
    Serial.println("Negative Number");
    neg_flag = true;     //If a negative sign is detected, set neg_flag to true
    NEGnum_flag = true;  //and set NEGnum_flag to true
    i++;                 //Increase index by 1
  }
  else if(receivedChars [i] == '\0'){ //If the array location contains a NULL
    i++;   //Increase index by 1
    x = 0; //Set x equal to zero for next iteration
  }
  else{
    Serial.print("Yo, your number is dogshit, write a new one\n"); //Reasonable Response
    val = 0;  //Bad number detected, set value to 0 and exit loop
    break;    //Exit out of the loop
  }
}
if (neg_flag == true){ //If negative number detected, multiply value by -1
  val = val*-1;
  if(val < -90000){   //If less than -90000,
    Serial.print("Number too small, Enter a larger one\n");
    dataNumber = 0;  //Sets number to zero for next iteration
    val = 0;         //Sets value to zero for next iteration
    Move_flag = false;
  }
  else{ //If with threshold
    dataNumber = val; //Set value equal to dataNumber
    val = 0;          //Set value to zero for next iteration
    Move_flag = true; //Allows selected actuator to move
  }
}
else{ //If negative number not detected
  if(val > 90000){ //If number is larger than 90000
    Serial.print("Number too big, Enter a smaller one\n");
    dataNumber = 0;    //Sets number to zero for next iteration
    val = 0;           //Sets number to zero for next iteration
    Move_flag = false; //Prevents selected actuator from moving
  }
  else{
    dataNumber = val; //Set value equal to dataNumber
    val = 0;          //Set value to zero for next iteration
    Move_flag = true; //Allows selected actuator to move
  }
```

```
        }

        newData = false;  //Set newData to false to allow for next data input
        neg_flag = false; //Set neg_flag to allow for next calculation of a negative value
    }
}

void selectActuator() {
/* Description: Acts similar to the showNewNumber but instead checks whether the array
holds an X
 *                 or a Y.
 */
    if (newData == true) { //If newData is ready
        character = 0;     //Variable set to zero for next iteration
        if (receivedChars[0] == 0x58){  //Compares receivedChar to X in hex
          Serial.println("X Axis Selected");
          character = receivedChars[0]; // X placed into character
        }
        else if(receivedChars[0] == 0x59){ //Compares receivedChar to Y in hex
          Serial.println("Y Axis Selected");
          character = receivedChars[0]; // Y placed into character
        }
        else{
          Serial.print("You dumb as shit, try again.\n"); //Reasonable print for failed character
        }
      }
      newData = false;  //Set newData to false to allow for next data input
}

int SpeedDifferential(float x){
/*Description: Receives the current encoder count and calculates the speed for encoders.
 *          NOTE: The numbers below can be manipulated as long as the final result (float y)
results in
 *          255 as the maximum possible output.  The 88100 in the denominator of float m
represents the
 *          maximum amount encoder countsthe actuator can travel. This has been verified
through
 *          multiple tests at varying speeds.
 *
 *          As a general rule, if 255 is wanted to be the maximum speed at full extension, the
slope must
 *          coincide with the selected y intercept to maintain a linear relationship.
 */
  float b = 175;          //Y-Intercept, Set speed is 150 for 5V or 175
  float m = 80.0/88100.0; //Slope of speed differential, 80.0
  float y;                //Final speed output variable
```

```
  y = m * x + b;
  return y;

 //At 5V, b = 175, m =  80.0/88100,  At 10.5V, try to use b = 150, m =  105.0/88100
 //---- 10.5V can be manipulated to be slower at start (best lower limit estimate is b = 72 and m
= 183/88100
}
```

APPENDIX O
VENDOR INFORMATION

VENDOR INFORMATION						
PART NO.	**PART**	**PRICE PER**	**QTY.**	**PRICE TOTAL**	**VENDOR**	**CONTACT**
PF4X-INF	4X Plan F Infinity-Corrected Objective Lens	$71.99	1	$71.99	AmScope	1-888-950-2888
N/A	X-Y Stage	$220.00	1	$220.00	Ebay - Silicon Valley Techparts Surplus	1-408-564-6264 info@svtechparts.com
47065T101	T-Slotted Framing (1" x 1") (L = 1ft.)	$5.84	2	$11.68	McMaster-Carr	1-562-692-5911 la.sales@mcmaster.com
47065T101	T-Slotted Framing (1" x 1") (L = 2ft.)	$7.79	1	7.79		
47065T139	End Feed Single Nut with Button Head 1/4"-20 Thread (Pack of 4)	$1.85	4	$7.40		
47065T236	T-Slot Corner Bracket	$5.21	2	$10.42		
90377A119	Oversized Washer for #8 Screw (Pack of 25)	$6.67	1	$6.67		
91253A001	3.8" #10-32 Flat Head Socket Cap Screw	$8.62	1	$8.62		
91290A139	M4 x 0.7mm Socket Head Cap Screw, 6mm Long (Pack of 100)	$8.80	1	$8.80		
91355A178	1/4"-20 Flanged Button Head Screw, 5/8" Long (Pack of 10)	$7.58	1	$7.58		
92220A172	3/8" #10-32 Low Profile Socket Head Cap Screw (Pack of 50)	$9.28	1	$9.28		
92235A507	8-32 Flanged Socket-Head Screw, L = 3/8"	$3.27	1	$3.27		
9654K163	4" 0.438" OD Steel Extension Spring (Pack of 6)	$10.36	1	$10.36		
BA1S-P5	Mounting Base (1" x 2.3" x 3/8") (Pack of 5)	$24.10	2	$48.20	Thorlabs Inc.	1-973-300-3000 sales@thorlabs.com
C1RC	Slip Ring for Ø1" Components	$27.59	1	$27.59		
H45B2	45° Mount Assembly for Ø1" Optics	$48.01	1	$48.01		
ME1-G01	Ø1" Round, Protected Aluminum Mirror, 3.2 mm Thick	$14.82	1	$14.82		
MF530	FITC Emission Filter, CWL = 530nm	$257.54	1	$257.54		
OMR	RMS Objective Mount, 8-32 Tap	$29.76	1	$29.76		
PH1-P5	Ø1/2" Post Holder, L = 1" (Pack of 5)	$36.21	1	$36.21		
PH2-P5	Ø1/2" Post Holder, L = 2" (Pack of 5)	$39.65	1	$39.65		
SLH1	Microscope Slide Spring Clip (Pack of 2)	$45.71	1	$45.71		
SM1A2	Adapter with External SM1 Threads and Internal SM2 Threads	$26.51	1	$26.51		
SM1L10	SM1 Lens Tube, L = 1"	$14.68	1	$14.68		
SM1RC	Slip Ring for SM1 Lens Tube	$25.10	1	$25.10		
SM1RR	Retaining Ring for SM1 Lens Tubes	$4.64	1	$4.64		
SM2A31	Adapter with External C-Mount Threads and Internal SM2 Threads	$27.59	1	$27.59		
SM2L03	SM2 Lens Tube, L = 0.3"	$24.06	1	24.06		
SM2L05	SM2 Lens Tube, L = 0.5"	$26.53	1	$26.53		
SM2L20	SM2 Lens Tube, L = 2"	$32.31	1	$32.31		
SM2L30	SM2 Lens Tube, L = 3"	$38.09	1	$38.09		
SM2RC	Slip Ring fro SM2 Lens Tubes	$31.92	1	$31.92		
TR1-P5	Ø1/2" Post, L = 1" (Pack of 5)	$21.97	1	$21.97		
TR1.5	Ø1/2" Post, L = 1.5"	$5.12	1	$5.12		
TR2-P5	Ø1/2" Post, L = 2" (Pack of 5)	$24.06	1	$24.06		
TTl200-A	Tube Lens, f = 200mm	$496.70	1	$496.70		
UPH1	Ø1/2" Universal Post Holder, L = 1"	$32.20	1	$32.20		
		SUBTOTAL:		**$1,762.83**		

Note: This list includes the fluorescence filter, which was not purchased. Actual expenditures are lower and shown in Appendix [Q].

APPENDIX P
VENDOR-SUPPLIED SPECIFICATION AND DATA SHEETS

PART NO. 011101

Fiber-Lite® **Dolan-Jenner**

Model 3100
30 Watt Small-Footprint Illuminator

The 3100 is a light weight and compact 30 Watt quartz halogen illuminator with a remote plug-in-the-wall transformer. The light source uses an EKZ lamp, which provides upwards 10,000 footcandles of intense, cold illumination. The 3100 has a standard Dolan-Jenner nosepiece, which allows the interface of all Fiber-Lite standard and custom fiber optic light guides. Precise light level settings are obtained via the 4-position, solid state, intensity control switch.

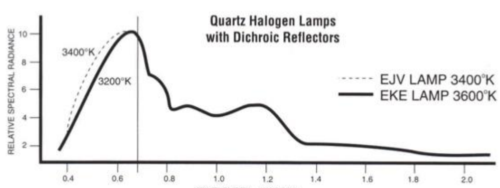
The Model 3100 illuminator is typically used for co-axial illumination in microscopy applications. Available in either single gooseneck or ring light configurations, the Model 3100 is Dolan-Jenner's smallest footprint illuminator.

Ring light Systems

The Model 3100 Ring light systems provide high intensity uniform illumination at high magnifications and long working distances without light adjustments when refocusing or when zoom features are used. Eliminating the clutter and head radiation of conventional light sources and offering better uniformity than LED rings, the Model 3100 ring light system is the ideal solution where 360° of shadow free illumination is required.

Single Arm Systems

The Model 3100-1 "Gooseneck" system is a single arm assembly, featuring a self-supporting, flexible light guide assembly that allow users to position the lighting at an optimal angle of incident lighting. This system also includes the LH-759 focusing lenses to optimize the intensity to the spot where light is needed most. These qualities make the Model 3100-1 a very versatile system for supplying high intensity and cold illumination in both laboratory and harsh environments.



Model 3100

- **Small, Compact Footprint**
- **Compatible with all Light Guides**
- **Lamp Life up to 10,000 Hours**

Model 3100 Features:

- Compact, Hard-Mountable to Specialty Equipment
- 10,000 Footcandles
- Intense, Cold Illumination
- 4-Position, Solid State Intensity Control
- Light Weight & Rugged, High-Impact Plastic Housing
- 2 Year Warranty

Configurations:

- 25mm ID accepts "SX" and "MX" series adapters
- Available in Single Gooseneck and Ring Light

Model 3100

30 Watt Small-Footprint Illuminator

Dolan-Jenner®

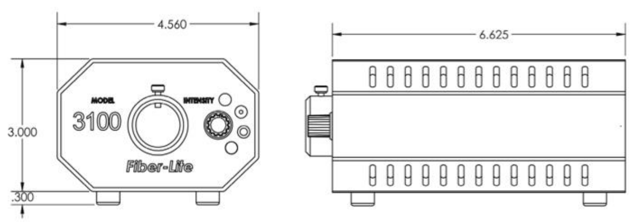
ORDERING INFORMATION

Model & P/N	Description
3100 002831000000	30 Watt Lamp, 115 V AC Illuminator
3100-1 660000051001	30 Watt Lamp, 115 V AC Illuminator w/ BGG 1823 Single Gooseneck, (1) LH759 Focusing Lense, SX-10 ADAPTER
3100-A37P 660000051004	30 Watt Lamp, 115 V AC Illuminator w/ A3739P Ring Light, SX-10 Adapter

GENERAL SPECIFICATIONS

Performance Data	
Lamp Output	10,000 footcandles at fiber optic intersection plane
Electrical Data	
Input Voltage	115 V AC, 60Hz
Environmental Data	
Operating Temp.	5 - 40°C
Cooling	Fan Cooled
Physical Description	
Dimension	3" x 4.5" x 6.5"
Weight	4.5 lbs.
Intensity Control	4-Position Solid State Switch
Adapters	SX-type adapter series
Lighting Properties	
Lamp	30 Watt, 10.8 V, Type EKZ qtz halogen
Color Temp.	3100° Kelvin
Lamp Life	200 hrs. full intensity 10,000 at min. intensity
Fiber Optic Interface	25mm (A Style)
Certifications	
None	

DIMENSIONS



SYSTEMS

	3100-A37P	3100-1	3100
Fiber Optic Assembly Included	60mm ID Ring Light with 36" Cable	Single Gooseneck with Focusing Lens	None
Fiber Optic Assembly	0.55NA High Quality Glass Fibers (A3739P)	0.55NA High Quality Glass Fibers (BGG1823)	None
Optional IR Filter Available	No	No	No
61010 3rd Ed. Safety Rated	No	No	No
Warranty	2 Years	2 Years	2 Years

5 Appendix

5.1 Technical Data

Specification	
LED Current Range	0 ... 1200 mA
LED Current Limit Range	200 ... 1200 mA
LED Forward Voltage	min. 11 V; typ. 12 V
Current Ripple	8 mA
Current Ripple Frequency	570 kHz
Modulation Input Impedance	10 kΩ
Modulation Mode [2) 3)]	
Modulation Frequency Range	typ. 0 ... 5 kHz (Sine Wave)
Modulation Form	Arbitrary
Input Voltage Range	0 ... 5 V
Zero Set Point Offset	10 ... 40 mV; typ. 24 mV
Slew Rate	13.6 mA/µs
Decay Rate	13.1 mA/µs
Trigger Mode [2)]	
Modulation Frequency Range	0 ... 1 kHz
Duty Cycle Range	20 ... 80 % @ 1 kHz 2 ... 98 % @ 100 Hz 0.2 ... 99.8 % @ 10 Hz
Modulation Form	Square Wave / PWM
Logic Input levels	TTL (Min H-Level: 2 V; Max L-Level: 0.55 V)
Slew Rate	18 mA/µs
Rise Time (10% -> 90%)	51 µs
Turn-on Dead Time	57 µs
Decay Rate	12 mA/µs
Fall Time (90% -> 10%)	79 µs
Turn-off Dead Time	14 µs
Power Supply	
Line Voltage (Ext. Power Supply)	100 ... 240 VAC (-10 %, +10 %)
Line Frequency (Ext. Power Supply)	50 ... 60 Hz
Supply mains over Voltage	Category II (Cat II)
Input Voltage (LEDD1B chassis)	15 V DC
Power Consumption (max)	15 VA

General	
Operating Temperature Range 1)	0 - 40 °C
Storage Temperature Range	-40 to 70 °C
Relative Humidity	Max. 80% up to 31 °C decreasing to 50% at 40 °C
Pollution Degree (indoor use only)	2
Operation Altitude	< 3000 m
Dimensions (W x H x D) - without operating elements - with operating elements and baseplate	 60 x 47 x 60 mm³ 60 x 73 x 104 mm³
Weight	240 g

[1]) non-condensing

[2]) Specifications for the modulation and trigger modes depend on the forward voltage and capacitance of the connected LED.

[3]) Specifications are valid for a current limit of 1.2 A.

All technical data are valid at 23 ± 5°C and 45 ± 15% rel. humidity (non condensing)

PART NO. 011205

Mounted LED, 490 nm

M490L4

Description

Thorlabs' M490L4 Mounted LED has a nominal wavelength of 490 nm, outputs more than 205 mW of power, and is mounted to the end of a Ø30.5 mm heat sink. This LED needs to be supplied with a constant current that must not exceed 350 mA. The current source must be able to deliver this current at a forward voltage of 3.8 V.

Specifications

Specification	Value
Color	Blue
Nominal Wavelength	490 nm
Bandwidth (FWHM)	26 nm
Viewing Angle (Full Angle)	128°
Emitter Size	1 mm x 1 mm
Test Current for Typical LED Power	350 mA
Maximum Current (CW)	350 mA
Electrical Power	1330 mW
Typical Lifetime	>10 000 h
Operating Temperature (Non-Condensing)	0 to 40 °C
Storage Temperature	-40 to 70 °C
Risk Group[a]	RG2 – Moderate Risk Group

a. According to the standard IEC 62471:2006, Photobiological Safety of Lamps and Lamp Systems

M490L4				
	Symbol	Min	Typical	Max
Peak Wavelength[a]	λ_p	480 nm	490 nm	500 nm
LED Output Power[a,b]	P_{out}	205 mW	240 mW	-
Forward Voltage[a]	V_F	-	3.2 V	3.8 V
Maximum Irradiance[b]	E_e	-	2.5 µW/mm^2	-

a. When Driven with the Test Current

b. Measured at a Distance of 200 mm

Operating Instructions

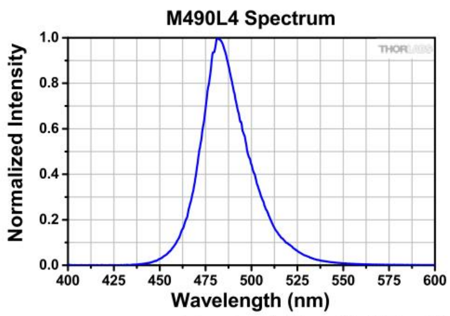
Be sure to provide air ventilation in order to avoid overheating, drops in optical power, and reduced lifetime. Each LED has a characteristic switch-on behavior, which depends on the LED properties and environment conditions. An important criterion is the heat dissipation. The M490L4 has a unique thermal and heat sink design that reduces the power decay to a minimum.

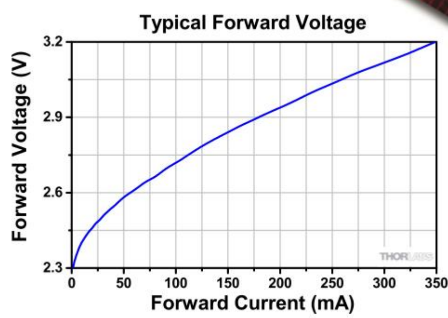
M490L4's male connector is a standard M8 x 1 sensor circular connector. Pins 1 and 2 connect to the LED. Pins 3 and 4 are used for the internal EEPROM. Only use these connections when using a Thorlabs LED driver.

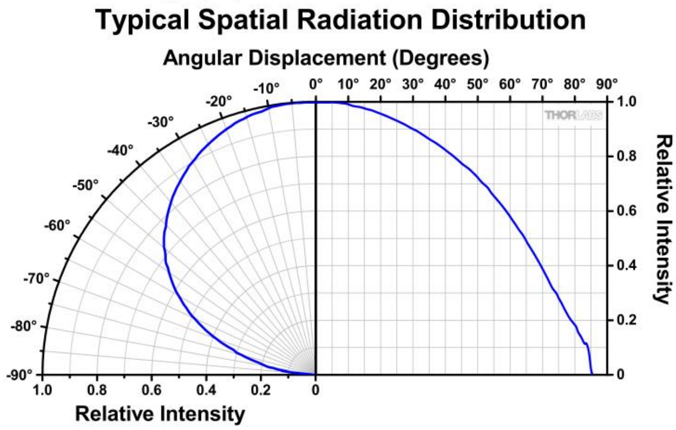
After an LED is switched on, it will warm up which can cause a decay in optical power. The heat sink of the M490L4 provides good thermal management, reducing the loss of power as the LED reaches its equilibrium temperature.

February 14, 2018
MTN006737-S01, Rev C

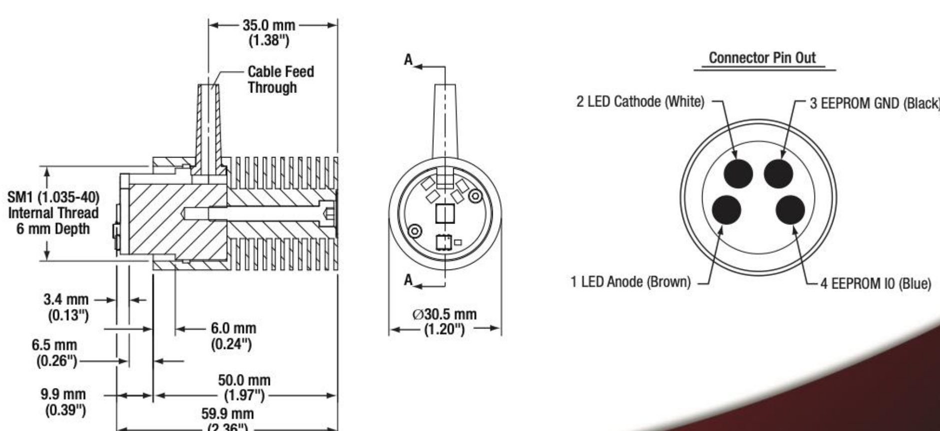
Performance Plots







Drawings



PART NO. 011301

Technical Specs

Axes of Travel	X
Maximum Stage Travel	25 mm
Thread Type	1/4-20
Load Capacity	156 N
Vertical Load Capacity	67 N
Angular Deviation	<200 µrad
Material	Aluminum
Bearings	Single-Row Ball Bearings
Drive Location	Side drive
Platform Size	3.0 x 3.0 in.
Height	1.0 in.
Recommended Adjustment Screw	AJS100-1
Recommended Vernier Micrometer	SM-25
Recommended DM Differential Micrometer	DM-25L
Recommended Digital Micrometer	DMH-1
Recommended TRA Motorized Actuator	TRA25CC or TRA25PPD
Recommended TRB Motorized Actuator	TRB25CC or TRB25PP
Recommended LTA Motorized Actuator	LTA-HS
Travel Requirement	12.7 to 25.4 mm

PART NO. 011305

This is a 4X plan-fluor objective lens for high-resolution biological and fluorescence applications. Semi-apochromatic optical corrections improve resolution and color accuracy by realigning blue, green, and red wavelengths. Plan field corrections further improve image quality by providing edge-to-edge sharpness. Along with improvements in resolution, the optics provide better transmittance in the ultraviolet and near-infrared bands for use in fluorescence microscopy.

The lens is designed for use with AmScope upright, infinity-corrected microscopes, as well as other microscopes using RMS mounts and 45mm parfocal distance.

Compatibility

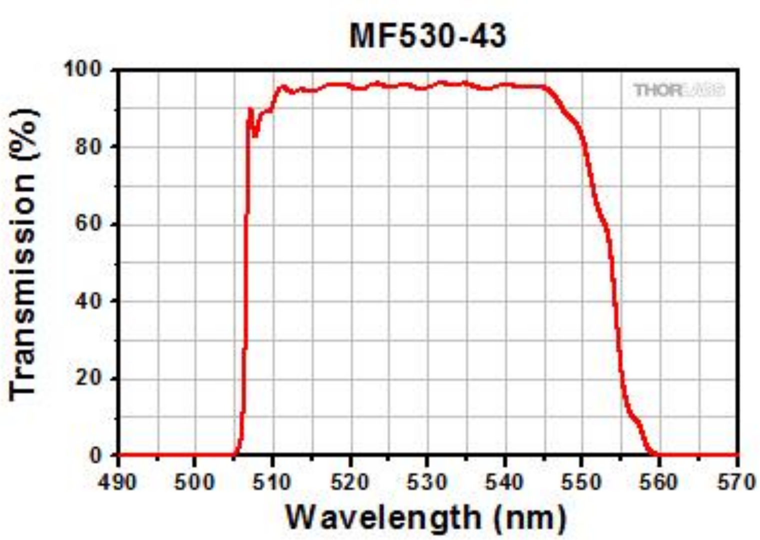
Optical System	Infinite
Reference Focal Length	180mm
Mounting Thread	RMS 20.32mm
Parfocal Distance	45mm

Lens Specifications

Magnification	4X
Numerical Aperture	0.13
Corrections	Plan, semi-apochromatic
Cover-glass Thickness	—
Nosecone	Fixed
Immersion Medium	—
Working Distance	16.3mm

PART NO. 011306

MF475-35	MDF-FITC	FITC	Excitation	475 nm	35 nm	-	-	-	
MF530-43			Emission	530 nm	43 nm	-	-	-	
MD499			Dichroic	-	-	R_{avg} > 90% 470 - 490 nm	T_{abs} > 90% 508 - 675 nm	R_{abs} < 2% from 400 to 800 nm	



PART NO. 011401

FROM IMAGES TO ANSWERS®

Evolution™ LC Megapixel Firewire Camera Kit

The Digital Alternative to Analog Video Microscopy

Overview

Evolution LC Digital Kits provide a cost-effective solution for image capture, enhancement, and reporting. These cameras offer high-resolution megapixel capability with the convenience of FireWire (IEEE 1394) to capture low latency video streams and digital still images at four times the resolution of video cameras.

Evolution LC Description

The Evolution LC monochrome and color cameras use the FireWire (IEEE 1394) digital bus protocol to streamline the capture, digitization and processing of megapixel color video streams. These cameras are designed to simplify real-time uncompressed video streaming and digital still image acquisition while maintaining uncompromising video and digital still-image quality. One FireWire cable can manage power distribution, PC-based control and real-time video streaming capabilities of the attached camera.

Evolution LC Features

- All-in-one megapixel FireWire C-mount cameras
- Stream uncompressed video or capture still images
- FireWire digital interface simplifies installation and eliminates the need for framegrabbers
- Flexible and full-featured CMOS image sensor with high resolution 1.3 megapixel array (1280 × 1024 pixels)
- Up to 14 fps at 1280 × 1024 resolution, 30 fps at 640 × 480

Evolution LC Features (cont.)

- FireWire cable carries video data, camera commands and power— no additional cables required.
- Image-Pro® driver controls for:
  - Gamma correction, signal gain, exposure time
  - Imager subwindow size and position
  - Image flip and mirror
  - Choice of 8-bit or 10-bit data and data range

FROM IMAGES TO ANSWERS®

Evolution LC Camera Specifications	
Resolution	1280 x 1024
CCD Type	Kodak KAC-1310 CMOS
Pixel Size	6.0 µm × 6.0 µm
Full Well Capacity	40,000 e-
Dark Current	6250 e-/pixel/sec
Optical Interface	c-mount
Image Color Depth	8 or 10-bit
Frame Rate	Up to 14 fps at 1280 × 1024 resolution, 30 fps at 640 × 480
Interface	IEEE 1394 FireWire
Operating System	Windows® 2000 & XP

Works Seamlessly with Image-Pro Software

The Evolution MP kit comes with an Image-Pro family driver that requires one of the following Image-Pro family applications:

Image-Pro® Express
Perfect for basic imaging, Image-Pro Express offers numerous capture and enhancement options. Includes an easy upgrade path to Image-Pro Discovery or Image-Pro Plus.

Image-Pro® Discovery
More advanced than Image-Pro Express, Image-Pro Discovery includes added measurement and analysis capabilities. Includes an easy upgrade path to Image-Pro Plus.

Image-Pro® Plus
The ultimate imaging software package. Image-Pro Plus includes all of the functionality of Image-Pro Discovery along with added analysis tools and the ability to write customized macros.

Experience a Fully Integrated Solution

Don't risk valuable time and money by attempting to use poorly integrated software and hardware components. Evolution LC Digital Kits provide guaranteed compatibility from one source, at a price that is easy on your budget.

Our Worldwide Dealer Network is There to Assist You. For a Dealer In Your Area, Contact us Today!

Evolution™ LC DIGITAL KIT An Integrated Solution

MediaCybernetics From Images to Answers®

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8484 Georgia Avenue, Suite 200
Silver Spring, MD 20910 USA
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Fax: +31.715.730.640
UK: +44.(0).118.979.4065
Fax: +44.(0).118.979.7999
Asia Pacific: +65.6245.4965
Fax: +65.6245.4967

PART NO. 011505

Technical Specs

Broadband Damping	Integrated Damping including constrained layer core, damped working surface and composite edge finish
Width	24 in.
Length	36 in.
Thickness	2.3 in.
Working Surface	0.134 in. thick 400 Series ferromagnetic stainless steel
Surface Flatness	±0.006 in. over 2 ft. square
Microlocks	No
Mounting Holes	1/4-20
Mounting Hole Type	Cut (not rolled) threads with countersink
Mounting Hole Pattern	1.0 in. grid
Mounting Hole Borders	0.5 in. borders
Hole Sealing Type	Easy clean non-corrosive high impact polymer conical cup, 0.75 in. deep
Core Design	Trussed honeycomb, vertically bonded closed cell construction, 0.010 in. Steel sheet materials, 0.030 in. triple core interface
Maximum Dynamic Deflection Coefficient	17×10^{-4}
Maximum Relative Motion Value	13×10^{-7} in.
Deflection Under Load	15×10^{-5} in.
Weight	91 lb

Specifications

Encoder Resolution		Part Number
Standard Actuators:	0.05101µm	850G, 850GV6
High Speed Actuators:	0.60514µm	850G-HS
Low Speed Actuators:	0.007985µm	850G-LS

Nominal Gearbox Ratio and Maximum Speed	
Standard Actuators:	262:1 ratio (1624 motor); 500µm/sec.
High Speed Actuators:	22:1 ratio (1624 motor); 6000µm/sec.
Low Speed Actuators:	1670:1 ratio (1516 motor); 78µm/sec.

Backlash	< 20 micron typical with external load of 2 lbs.(1 kg) minimum
Accuracy	< 0.1% of travel, cumulative
Bi-directional	Repeatability: Better than 1 micron when backlash is compensated by controller (standard actuators) *1
Encoder	Magnetic, 2KHz; open collector, quadrature output, +5V to +12V supply
Absolute cyclic pitch	Error < 1 micron
Time to reach full speed	< 50 msec at max. speed and acceleration settings
Max. Side Load	5 lb. (2.3 kg) at full shaft extension
Max. Axial Load	18 lb. (8 kg) standard and low speed actuators
Cable	12 foot (3.6 m) cable integral to actuator terminated with 25-pin male Dsub connector

Specifications (Continued)

Temperature Range	
Storage Temperature	0°F to +120°F
Operating Temperature	40°F to + 100°F
Actuator Case	Black anodized aluminum
Vacuum Compatibility	Special-order vacuum compatible versions for operation to 10-6 Torr, temperature range restricted as stated above

* NOTE: Backlash can be compensated by MotionMaster and PMC200 Series Controllers. Cumulative, monotonic error due to leadscrew pitch error or mounting errors can be compensated via the CO command in MotionMaster Controllers, and via the coupling ratio parameter in PMC200 Series Controllers.

APPENDIX Q
FINAL PROJECT BUDGET

iBOM PART NO.	VENDOR PART NO.	PART	PRICE PER	QTY	PRICE TOTAL	VENDOR
011104	C1RC	Slip Ring for Ø1" Components	$27.59	1	$27.59	ThorLabs Inc.
011204	SM1RC	Slip Ring for SM1 Lens Tube	$25.10	1	$25.10	
011303	OMR	RMS Objective Mount, 8-32 Tap	$29.76	1	$29.76	
011304	ME1-G01	Ø1" Round, Protected Aluminum Mirror, 3.2 mm Thick	$14.82	1	$14.82	
011305	H45B2	45° Mount Assembly for Ø1" Optics	$48.01	1	$48.01	
011308	SM1RR	Retaining Ring for SM1 Lens Tubes	$4.64	1	$4.64	
011309	SM1L10	SM1 Lens Tube, L = 1"	$14.68	1	$14.68	
011310	PH1-P5	Ø1/2" Post Holder, L = 1" (Pack of 5)	$36.21	1	$36.21	
011311	TR1-P5	Ø1/2" Post, L = 1" (Pack of 5)	$21.97	1	$21.97	
011312	TR1.5-P5	Ø1/2" Post, L = 1.5" (Pack of 5)	$23.04	1	$23.04	
011402	SM2A31	Adapter with External C-Mount Threads and Internal SM2 Threads	$27.59	1	$27.59	
011403	SM2L03	SM2 Lens Tube, L = 0.3"	$24.06	1	$24.06	
011404	SM2L05	SM2 Lens Tube, L = 0.5"	$26.53	1	$26.53	
011405	SM2L20	SM2 Lens Tube, L = 2"	$32.31	1	$32.31	
011406	SM2L30	SM2 Lens Tube, L = 3"	$38.09	1	$38.09	
011407	TTL200-A	Tube Lens, f = 200mm	$496.70	1	$496.70	
011408	SM1A2	Adapter with External SM1 Threads and Internal SM2 Threads	$26.51	1	$26.51	
011409	SM2RC	Slip Ring for SM2 Lens Tubes	$31.92	2	$63.84	
011410	RLA1200	Dovetail Optical Rail, 12" Imperial	$80.90	1	$80.90	
011411	RC1	Dovetail Rail Carrier, 1" x 1"	$26.94	2	$53.88	
011413	TR075	Ø1/2" Post, L = 0.75"	$4.88	2	$9.76	
011414	CL6	Table Clamp, RLA Series Optical Rails	$6.39	2	$12.78	
012401	PH2-P5	Ø1/2" Post Holder, L = 2" (Pack of 5)	$39.65	1	$39.65	
012403	BA1S-P5	Mounting Base (1" x 2.3" x 3/8") (Pack of 5)	$24.10	2	$48.20	
012602	SLH1	Microscope Slide Spring Clip (Pack of 2)	$45.71	1	$45.71	
012603	TR6-P5	Ø1/2" Post, L = 6" (Pack of 5)	$32.97	1	$32.97	
013101	47065T101	T-Slotted Framing (1" x 1") (L = 1ft.)	$5.84	2	$11.68	McMaster-Carr
013102	47065T101	T-Slotted Framing (1" x 1") (L = 2ft.)	$7.79	1	$7.79	
013103	47065T236	T-Slot Corner Bracket	$5.21	2	$10.42	
013106	47065T139	End Feed Single Nut with Button Head 1/4"-20 Thread (Pack of 4)	$1.85	4	$7.40	
N/A	N/A	Stage Plate Machining Job - Outsourced	$200	1	$200.00	John Gerrity (Santa Maria)

TOTAL:	$1,542.59

APPENDIX R

FAILURE MODES AND EFFECTS ANALYSIS

													Action Results				
System	**Function**	**Potential Failure Mode**	**Potential Effects of the Failure Mode**	**Severity**	**Potential Causes of the Failure Mode**	**Current Preventative Activities**	**Occurence**	**Current Detection Activities**	**Detection**	**Priority**	**Recommended Action(s)**	**Responsibility & Target Completion Date**	**Actions Taken**	**Severity**	**Occurence**	**Detection**	**RPN**
Microscopy	Bright Field	Cannot resolve clear image (dim)	No Image, Poor Image	8	a) fiberoptic illuminator not bright enough b) components not aligned c) camera not sensitive enough	a) plan optical pathway b) carefully select optical components for purchase	3		1	24							
	Fluorescence	Cannot resolve clear image (dim)	No Image, Poor Image	6	a) LED not bright enough b) components not aligned c) camera not sensitive enough	a) plan optical pathway b) carefully select optical components for purchase	5		1	30	a) test objective lens with tube lens	Makenzie (03/01/20)		4	3	1	12
		Weak or indetectible fluorescence	No fluorescent signal	7	a) LED not bright enough b) not enough indicator c) mismatched fluorescence components	a) plan optical pathway b) inventory available LEDs c) inventory available filters	6		1	42							
	Focus	Cannot resolve clear image (unfocused)	Poor image	8	a) damaged components b) components not aligned		4		1	32							
		Inconsistent focus		6	a) stage not parallel b) stage damaged		3		1	18							
		Optical pathway deflection	Component damage, component misalignment	8	a) single-axis stage cannot handle load imposed by attached optical components		4	a) perform FEA on objective bracket	1	32							
Stage	Move Sample	No Motion	No Imaging Window	5	a) stage is too heavy for actuators to push b) software issues c) misaligned rails d) damaged bearings	a) verify actuator can push stage b) test stage travel	2		1	10			a) verify actuator can push stage (12/5/19)				
		Stage falls off	component damage	9	extreme travel		1		1	9	a) install travel stops	Enoch (03/20/20)		2	2	1	4
		Resticted Motion	Reduced Imaging Window	4	a) Misaligned rails b) Damaged Bearings c) optical componenet collision	a) plan optical componenet placement b) test stage travel	3		1	12							
Appearance	Look Good	looks unprofessional	poor user experience	2	a) inconsistent components b) inelegant attachments		2		2	8							

APPENDIX S
DESIGN HAZARD CHECKLIST

Y	N	
x		1. Will any part of the design create hazardous revolving, reciprocating, running, shearing, punching, pressing, squeezing, drawing, cutting, rolling, mixing or similar action, including pinch points and sheer points?
	x	2. Can any part of the design undergo high accelerations/decelerations?
	x	3. Will the system have any large moving masses or large forces?
	x	4. Will the system produce a projectile?
x		5. Would it be possible for the system to fall under gravity creating injury?
	x	6. Will a user be exposed to overhanging weights as part of the design?
	x	7. Will the system have any sharp edges?
	x	8. Will any part of the electrical systems not be grounded?
	x	9. Will there be any large batteries or electrical voltage in the system above 40 V?
	x	10. Will there be any stored energy in the system such as batteries, flywheels, hanging weights or pressurized fluids?
	x	11. Will there be any explosive or flammable liquids, gases, or dust fuel as part of the system?
	x	12. Will the user of the design be required to exert any abnormal effort or physical posture during the use of the design?
	x	13. Will there be any materials known to be hazardous to humans involved in either the design or the manufacturing of the design?
	x	14. Can the system generate high levels of noise?
	x	15. Will the device/system be exposed to extreme environmental conditions such as fog, humidity, cold, high temperatures, etc.?
	x	16. Is it possible for the system to be used in an unsafe manner?
	x	17. Will there be any other potential hazards not listed above? If yes, please explain on reverse.

Description of Hazard	Planned Corrective Action	Planned Date	Actual Date
Moving slides have the potential to cause finger entrapment.	Purchase a commercially available microscope stage that has accommodated for these potential pinch points.	11/2019	11/2019
Optical breadboard is heavy (~250lbs.) and could cause injury if dropped.	Store it on a cart with edges and ensure the cart is bottom-heavy enough to avoid tip over.	10/2019	10/2019

APPENDIX T
OPERATORS' MANUAL

The Inverted Fluorescence Microscope is a complex system with various critical components when operating in conjunction can introduce complicacy of operation. The following includes instructions for basic microscope use, a list of common problems and troubleshooting measures to achieve desired image quality, guidelines for system maintenance, and detailed specifications of critical components.

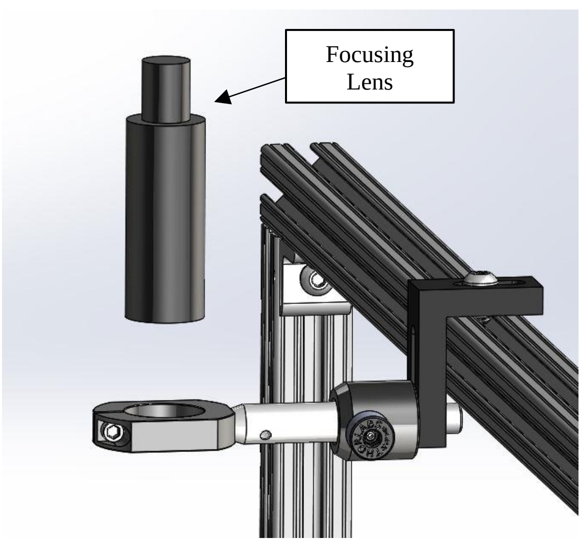
OPERATION

I. Selecting Illumination / Switching Modes

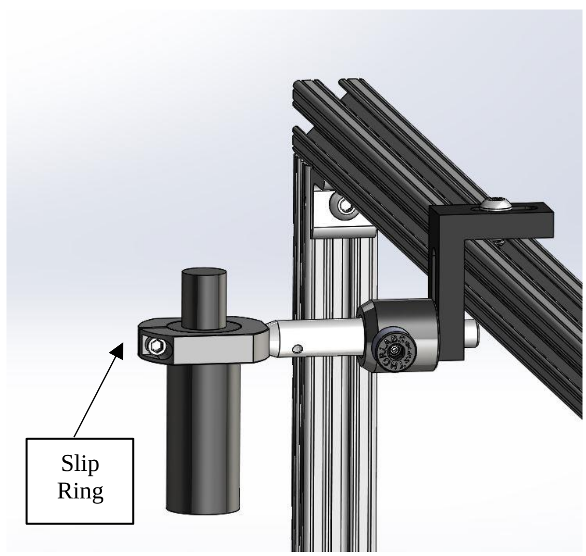
This microscope is capable of two operation modes: (1) brightfield and (2) fluorescence. Their light paths are overlapping but are not designed to operate simultaneously. Below are instructions for the basic setup of each mode.

Brightfield Mode

(1) Turn the Dolan-Jenner Fiber Lite Illuminator ON to its high intensity setting. Ensure the LED is OFF. It will not be used in this mode.
(2) Orient the focusing lens at the end of the fiber optic cable within the slip ring so that it illuminates the sample at a distance of XX" from the sample (*Note: this is an approximate specification; this distance will likely vary slightly when focusing the stage*).
(3) Once focusing lens is in desired position, tighten the slip ring with an Alan key.



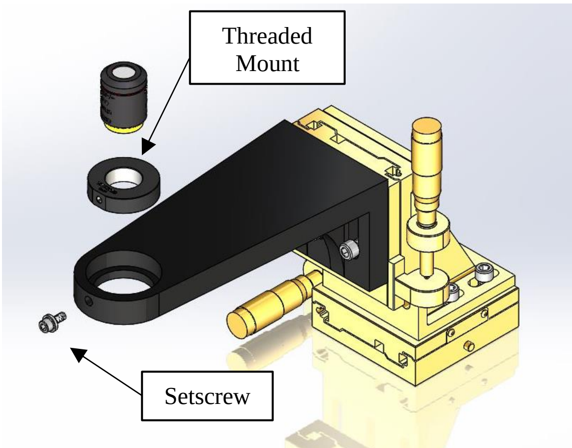
Locate slip ring for brightfield focusing lens attachment.



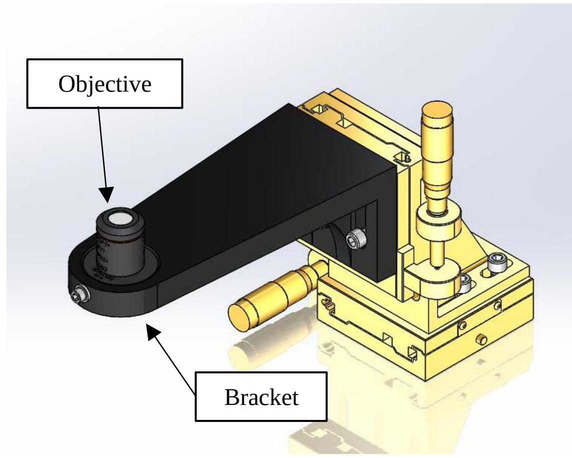
Attach brightfield focusing lens and tighten slip ring.

(4) In brightfield mode, the filter cube assembly should not include an emission filter. Locate the 1" diameter lens tube attached to the filter cube. If the fluorescence emission filter is installed, unscrew the lens tube and remove the filter and its retaining ring. Return the empty lens tube to its position in the optical path.

(5) Turn on the camera.
(6) Couple RMS-threaded objective lens to its compatible lens mount.
(7) Position objective lens (in its mount) in bracket, ensuring setscrew is tightened and the objective is fixed. Coarsely measure and approximate the objective location with the concave front lens one working distance from the sample. Follow focusing guidelines (Section X) to focus the objective and obtain desired image quality.



Place objective lens, coupled to the ThorLabs mount, in PLA bracket. Flats should be parallel.

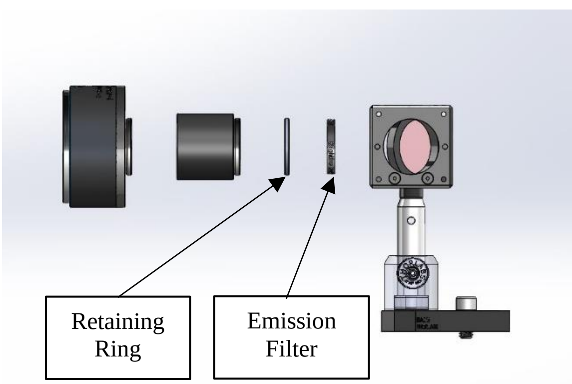


Tighten the setscrew to fix the objective in the desired position.

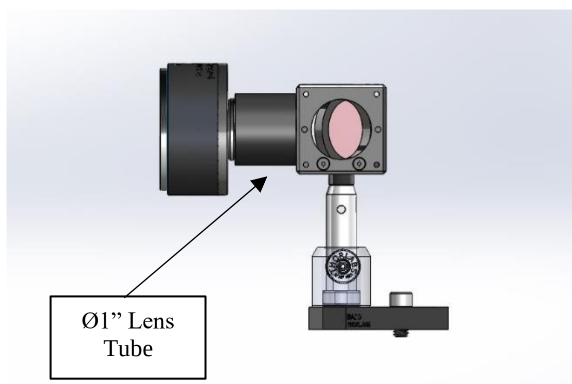
(8) Adjust camera settings to achieve desired image resolution, exposure, etc.

Fluorescence Mode

(1) Turn the LED driver ON, operating in Trigger Mode as denoted by "TRIG." Ensure the Dolan-Jenner Fiber Lite Illuminator is OFF. It will not be used in this mode.
(2) In fluorescence mode, the filter cube assembly includes an emission filter. Locate the 1" diameter lens tube attached to the filter cube. If the fluorescence filter is not installed, unscrew this tube and screw the filter into its retaining ring. Fix this into the lens tube and return the tube and filter assembly to its position in the optical path.



Locate required emission filter components for fluorescence mode.



Assemble components.

(3) Turn on the camera.
(4) Follow steps (6) and (7) of Brightfield Mode to place the objective lens.
(5) Adjust camera settings to achieve desired image resolution, exposure, etc.

II. Focusing the Objective

Focusing the objective should be performed in brightfield mode. While looking at the computer screen image, move the microscope slide until it is in the center of the objective's field of view (FOV). Use the micrometer on the horizontal slide beneath the objective to make any finer adjustments. Once the sample is in the desired lateral position, the image must now be focused.

(1) Note the working distance of the objective lens. The objective this microscope was designed for has a working distance of 16.3mm. This is the distance from the objective lens to the sample. Carefully use a straightedge or calipers to make a rough estimate of this distance.
(2) Use the micrometer on the vertical linear "focus" slide to coarsely move the objective to this location.
(3) While watching the image on the screen, slowly turn the micrometer on the vertical slide until the image comes into focus.

III. Replacing the Objective

This microscope was designed using the specifications for a 4X magnification, 16mm-WD objective lens (detailed Specification Table in Section X) is but has the modularity to be compatible with alternative lenses of working distances up to 40mm.

When it is desired to operate the microscope with a different objective, carefully replace it using the following procedure:

(1) Use the coarse adjustment knob on the z-axis slide to slowly guide the objective assembly downwards and away from the sample. Continue turning until the slide "bottoms out," to allow maximum space underneath the stage.
(2) Loosen the setscrew holding the objective to the bracket.
(3) Carefully lift the objective, still in its mount, from the bracket, taking care to avoid collisions with any of the stage components above.
(4) Unthread the objective from the RMS mount and stow in proper storage container.
(5) Add thread adapters to the objective mount, if necessary.
(6) Attach new objective lens to mount, place in bracket with flats parallel, and fix with the setscrew. Tighten the setscrew.
(7) Follow focusing procedure to focus the new objective lens.

IV. Electronic Manipulation

At the start of operation, the electronic components of the system will need the DC power supply to be connected and raised to 10.5V. Afterwards, the IFMActuatorControl.io file must be opened and sent to the Arduino Mega to reboot the system with new unaltered variables. The system will commence an initialization process and then give control to the user as show in the finite state diagram below. Details of each mode/state of the system is detailed along with how to switch between each mode/state.

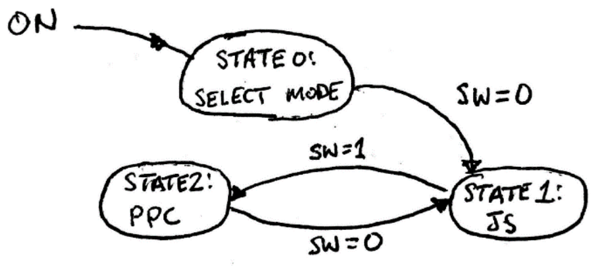


Figure 1. Arduino® Mega finite state machine diagram

Initial Mode

(1) Open the program file IFMActuatorControl.io
(2) Click on the right arrow to upload the code from the computer to the Arduino® Mega
(3) Click CTRL+SHIFT+M to open up the Serial Monitor. On startup, the Serial Monitor should print "<Arduino is ready>"
(4) The control system will execute a prewritten command to zero the actuators by retracting them until the reverse limit switches are activated.
(5) After the limit switches have been activated, the actuators will be extruded 3500 encoder counts, which is the estimated distance to reach the zero markings on the actuators

Joystick Mode

(1) Once the actuators have been zeroed, the control system will switch into Joystick mode.
(2) The joystick will move in the Cartesian coordinate system shown in the diagram below.

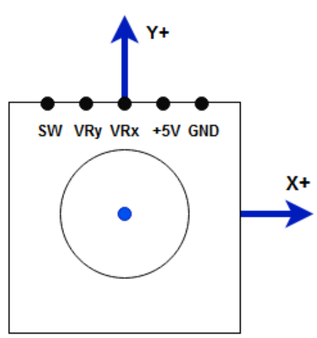


Figure 2. Joystick Cartesian Map

(3) The actuators will move forward or backwards until the forward and reverse limit switches are activated, respectively. In the event a limit switch is activated, a prompt will be printed on the Serial monitor.
(4) The switch can be pressed to switch to the programmable path mode, where a prompt will be printed to state which mode the control system is in.

Programmable Path Mode (PPC)

(1) Once entering the programmable path mode, a prompt will print stating "Select Actuator to move (X/Y)"
(2) Type a capital X or Y and press ENTER to select the axis to operate.
(3) After pressing ENTER, a prompt will ask for the target value for the respective axis.
(4) Type in a number between -90000 and 90000 and click ENTER to move the actuator. A negative number will retract the actuator and a positive number will extrude the actuator.
(5) The actuator will move to the entered amount of encoder counts. Once the target encoder count is reached, a prompt will be printed stating the current encoder count for the actuator manipulated.
(6) Both actuators will stop when the limit switches are activated. The Arduino® Mega will print a prompt stating which limit switch was activated and print the current encoder count.
(7) In the Select Actuator prompt, the joystick switch can be pressed, causing the control system to return to Joystick Mode.

The actuator speeds are controlled by the SpeedDifferential function. The function operates by taking the current encoder count and outputs a speed that increases linearly in proportion to said encoder count. The values in the function can be manipulated to have a lower speed at the maximum encoder count or a different starting point. Regardless of what is changed, the maximum value must not exceed 255 as it will cause and overflow and have the actuators change direction.

TROUBLESHOOTING

Table 1. Troubleshooting Solutions

Problem	Possible Cause(s)	Solution
Image not clear	Specimen is in incorrect position	Re-position specimen
Poor image sharpness or contrast	Specimen slide is dirty	Clean specimen slide
	Dirt or debris on the objective lens / microscope optics are not clean	Clean objective / tube lens
Poor brightfield illumination	Brightfield focusing location is misaligned	Re-position brightfield focus
	Microscope optics are not clean	Clean objective / tube lens / focusing lens
	Specimen is not placed level	Re-position specimen
Cannot focus	Objective not placed at proper working distance	Adjust z-axis micrometer with the fine adjuster until image becomes focused
Dim or undetectable fluorescence	Excessive transmission losses	Shroud light path between optical components with lens tubes
	Too many reflective surfaces	
	Surrounding light is too bright	Turn off lights in room
	Improper camera settings	Decrease frame rate / increase exposure time of camera
	Mismatched fluorescence components	Change LED source, filter cube, or indicator dye to make a compatible fluorescence set
Actuator will not fully extend	More voltage is required to motors	Increase voltage (DO NOT EXCEED 12V)
	Speed Differential is not reaching maximum value or overflowing	Adjust the SpeedDifferential (See simple y = mx + b math in function). Do not exceed 255 as the maximum value ouput
		Rotate actuator knobs manually; the encoder counts will still be recognized

Actuator system is not responding	User held onto the switch for too long User entered an invalid input	Restart the system by clicking on the right arrow at the top of the file for the Arduino® code
Actuator is not moving after zeroing	SpeedDifferential initial value is not large enough.	Increase float b and adjust float m accordingly in the code

MAINTENANCE

I. Cleaning the Optics

This microscope in intended for use with a dry objective lens of working distances 15-40mm; specifications for the primary objective lens are detailed in Section X. In general, an objective with a smaller concave front lens and a smaller working distance is more susceptible to the collection of dust or debris that will result in poor image quality.
Similar soiling of any refractive or reflective surface in the light path (includes the objective. 45º mirror, beamsplitter, emission filter, tube lens, and camera lens) may result in reduced image quality results; hence, it may occasionally be necessary to clean the optical components. Note that the less disassembly required for cleaning, the better. So, it is important to first identify the component that is causing image inadequacy.

Materials Needed
- Air Blower or Aspirator
- Optical Lens Tissue
- Solvent or Lens Cleaner
    - Distilled Water
    - 90%+ Pure Isopropyl Alcohol

Process
(1) The primary likely cause of an obscured or unclear image is the accumulation of dust. As a first remedy, remove dust using an aspirator bulb. If dust remains, use a higher-pressure air blower such as a compressed air can.
(2) For dirt or fingerprint residue, lens tissue can be used as a single-use brush. Tear a piece of lens tissue, using the frayed, feathery ends to dust the lens.
(3) For stubborn residue, use of a cleaning solvent may be necessary. Start by using distilled water; if residue persists, try isopropyl alcohol. Note that a minimum purity of 90% isopropyl alcohol should be used. Rubbing alcohol (at only 70% purity) is not sufficient and may cause damage to lenses [32]. Apply one to two drops of solvent to a lens tissue, gently place the tissue on the lens surface, and wait a few seconds to allow the solvent to dissolve the residue. Drag the tissue, parallel to the surface, away from the optic.

    Note: It is best to orient the optic in such a way that the lens tissue can be dragged upward for removal to avoid solution or dissolved dirt from collecting at an edge or corner. To carefully and thoroughly clean the objective lens, it may be necessary to remove it from its mount.

II. Soldering Broken Jumper Wires

If one of the jumper wires has a broken header pin, the following provides two courses of action, in preferential order:

(1) Replace the jumper wire with one of a similar color.
(2) If there are no similar colored wires, soldering the wire can prove to be a quick fix. For soldering, be sure to strip the wire to expose at least 0.5 cm of wire. The temperature for the soldering iron should be set to around 350ºC (662ºF) to prevent the insulation from receding while soldering and the wire from burning.

The wires are color coded to assist the user in determining which pin pairs conduct which function.

Table 2. Wire Colors

Wire Color	**Description**
Blue	Driver Inputs from Arduino Digital Ports
Orange	Driver Outputs from L293D to ports 5 and 7 on DB25 adapter
Brown	Driver Enables controlled by Arduino PWM
White	Encoder channel reading from Arduino PWM/Digital
Yellow	V_{cc} for L293D pins 8 and 16
Green	Heat Sink/GND from negative power rail
Grey	Reverse Limit Switch read from Arduino Digital
Purple	Forward Limit Switch read from Arduino Digital
Red	Positive Terminal
Black	Negative terminal

Because some of the wires share the same color but connect for different functions, the pin to pin relation for each component is documented at the end of this manual in Tables 8-11.

III. Power Supply to the L293D

The DC power supply should have its positive terminal in the same breadboard connection row as Pin 8 of the L293D. This pin acts as the power supply for the motors. The negative terminal should be placed on the same ground rail as all the components. The voltage supplied to the L293D motor driver must not exceed 12V as it will damage the actuators. The L293D pin layout is displayed towards the end of this manual in Figure 1.

IV. Storage

To avoid the necessity of cleaning the optics, potentially risking damage or further contamination and eliminating the complications of disassembly, the following preventative measures should be taken.

- Store microscope in a dry place
- Cover with dust cover when not in use
- Avoid touching optics with bare fingers

With regards to electronics, do not remove any components from the enclosure unless necessary for wire replacements. In the event of wires need to be replaced, the wiring diagram of the electronic system is shown below.

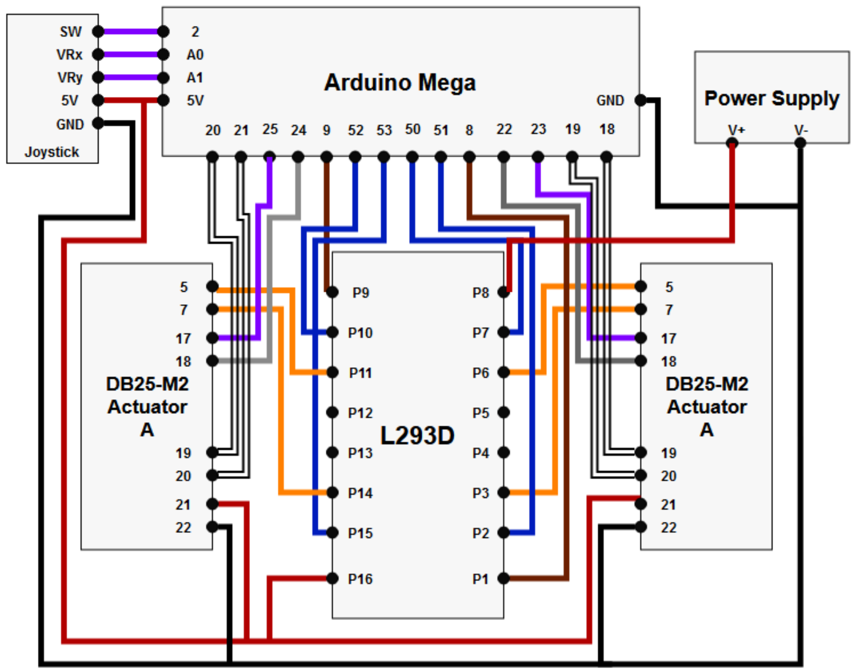


Figure 3. Full Arduino Wiring diagram

Since the microscope is intended to be permanently housed in Cal Poly's Microfabrication Laboratory, a clean room, these actions are primarily precautionary.

V. Pin Correspondence

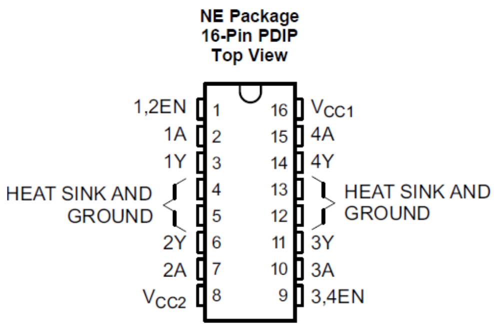
The following tables detail the pin relationships between components in the Arduino® control system. Figure 4 displays the datasheet for the L293D for better visual of the component as well as specific designations for the pins.

Table 3. L293D to Actuator Pin Correspondence

L293D	Actuator A	Actuator B	Description
3	7		Negative Motor Terminal Input
6	5		Positive Motor Terminal Input
11		7	Negative Motor Terminal Input
14		5	Positive Motor Terminal Input

Table 4. Arduino Mega to L293D Pin Correspondence

Arduino Mega	L293D	Description
8	1	PWM line to control speed of Actuator A
9	9	PWM line to control speed of Actuator B
51	2	HIGH/LOW Logic for 1st driver input of Actuator A
50	7	HIGH/LOW Logic for 2nd driver input of Actuator A
52	10	HIGH/LOW Logic for 1st driver input of Actuator B
53	15	HIGH/LOW Logic for 2nd driver input of Actuator B
GND	12	Grounds the L293D
5V	16	Supplies the logic of the motor driver. Do not exceed 5V
GND	5	Grounds the L293D
-	8 (PS+)	Power Supply voltage to the motors. Do not exceed 12V



Pin Functions

PIN		TYPE	DESCRIPTION
NAME	NO.		
1,2EN	1	I	Enable driver channels 1 and 2 (active high input)
<1:4>A	2, 7, 10, 15	I	Driver inputs, noninverting
<1:4>Y	3, 6, 11, 14	O	Driver outputs
3,4EN	9	I	Enable driver channels 3 and 4 (active high input)
GROUND	4, 5, 12, 13	—	Device ground and heat sink pin. Connect to printed-circuit-board ground plane with multiple solid vias
V_{CC1}	16	—	5-V supply for internal logic translation
V_{CC2}	8	—	Power VCC for drivers 4.5 V to 36 V

Figure 4. Pinout diagram taken from Texas Instruments Datasheet

Table 5. Arduino Mega to Actuators Pin Correspondence

Arduino Mega	Actuator A	Actuator B	Description
20		19	Encoder Channel A
21		20	Encoder Channel B
18	19		Encoder Channel A
19	20		Encoder Channel B
25		17	Forward Limit Switch
24		18	Reverse Limit Switch
23	17		Forward Limit Switch
22	18		Reverse Limit Switch

Table 6. Arduino Mega to Joystick Pin Correspondence

Arduino Mega	Joystick	Description
2	SW	Reads Switch input for changing states
A0	VRx	Analog reads inverse Y direction
A1	VRy	Analog reads inverse X direction
5V	+5V	Supplies joystick with 5V
GND	GND	Grounds joystick

COMPONENT SPECIFICATIONS

I. Purchased Optical Components

The following components have been purchased and assembled within the system.

- Objective Lens: AmScope 4X Infinity-Corrected Plan Fluor

Table 7. Objective Lens Specifications

Classification	Optical System	Magnification	Numerical Aperture	Working Distance
Achromatic Objective	Dry	4X	0.13	16.3mm

- Fluorescence Light Source: M490L4 – 490 nm Mounted LED

Table 8. Fluorescence Light Source Specifications

Color	Nominal Wavelength	Bandwidth	Output Power
Blue	490nm	26nm	240mW

II. Recommended Optical Components

The following components are part of the final microscope design but have not yet been purchased. In further development of the system, these components are suggested for optimum operation.

- Camera: AmScope 5MP USB 2.0 Color CMOS C-Mount Microscope Camera with Reduction Lens

Table 9. Camera Specifications

Pixel	Connectivity	Resolution	Application	Reduction Lens
5.0MP	USB 2.0 or 3.0	2592x1944	PC and Mac Display	0.5X

- Indicator Dye(s): Fluorescein and AlexaFluor 488

Table 10. Indicator Dye Specifications

	Fluorescein	AlexaFluor 488
Excitation Max.	490nm	490nm
Emission Max.	525nm	525nm

Note: Both Fluorescein and AlexaFluor 488 will work with the specified LED and emission filter. AlexaFluor 488 has slightly higher initial brightness and photostability.

- Fluorescence Emission Filter: MF530-43 FITC Emission Filter, CWL = 530nm

Table 11. Fluorescence Emission Filter Specifications

Nominal Wavelength	Bandwidth
530nm	43nm

APPENDIX U
TESTING PROCEDURES

This appendix contains a copy of the four formal test procedures we created for our project. Also included are blank data sheets in case these tests need to be carried out in the future. We were only able to complete the stage bracket test. Because of this, that stage bracket test is the only one with a scan of our hand recorded testing data. The tests appear in the following order, they are separated to facilitate printing:

1. IFM Optics Testing
2. IFM Stage Bracket Testing
3. IFM Actuator Testing
4. IFM Stage Parallelism Testing

TEST PROCEDURE 1

Test Description:	Optical train alignment and component compatibility verification
Date Created:	03-02-20
Date Revised:	03-02-20

1.0 INTRODUCTION:

This test is intended to confirm adequate operation and compatibility of each individual optics component within the optical train subassembly. Components will be added on one-by-one to ensure the camera can resolve an image.

2.0 FACILITIES & EQUIPMENT:

This test will be performed in the Cal Poly Microfabrication Lab Metrology Room, utilizing the available optical breadboard, purchased ThorLabs optical components, provided camera and light sources, and optical mounts and fixtures designed and produced using additive manufacturing techniques.

Table 2.1: Components to be Tested in Sequence

Order:	**Component Name:**
1	Camera (Baseline Shutter Speed)
2	Tube Lens
3	Fluorescence Emission Filter (TBD)
4	Beamsplitter
5	45º Mirror
6	Sample
7	Objective Lens

For testing purposes, the “sample” used with be a piece of acrylic with different-sized dots to ranging from ___ microns to ___ microns. The acrylic will be mounted to a linear slide

3.0 SAFETY CONSIDERATIONS:

This test procedure does not entail critical concern for personal safety; there will be no moving parts, flying debris, or other glaring hazards.

However, test personnel should operate test conditions with consideration for preventing any damage to the delicate optics.

Table 3.1: Testing Concerns and Actions (Preserving the Optics)

Concern:	**Action:**
Dropping Components	Fasten all necessary components to the breadboard prior to testing to ensure sturdy alignment.
Fingerprints on Optics	Wear gloves while handling lenses and mirrors.

4.0 DATA COLLECTION:

The data collected from this test is primarily qualitative; however, we can take note of the camera shutter speed that results in what the team regards an “acceptable” image following the addition of each component.

The inability of the camera to resolve an acceptable image within the camera’s shutter speed capabilities may warrant:

- Purchase of a higher-quality camera
- Measures taken to shroud the optics (purchasing additional lens tubes) to eliminate transmission losses

5.0 TESTING PROCEDURE:

As a baseline, the camera will be tested with the tube lens, sample, and objective lens to first confirm that the assembly of the most basic primary components will resolve an image. The parts will be placed flush with one another (with the exception of the 16.3mm working distance of the objective so that the baseline test reports results at maximum transmission.

From there, components will be added one-by-one within the infinity distance between the tube lens and the objective lens.

The following test procedure outlines the steps that will be taken prior to testing of the optical train following the addition of each sequential component.

Step:	**Description:**
1.00	Assemble parts for testing.
1.01	Tighten each component using Alan keys.
1.02	Fix to optical breadboard with ¼”-20 fasteners.
2.00	Hook up camera to assembly.
2.01	Screw camera to C-mount adapter.
2.02	Attach camera USB to computer. *(This step will differ if the camera uses FireWire – we are currently waiting on our sponsor to provide us with more information about the camera.)*
3.00	Orient light source to shine light through the partial optical train assembly. The light source should be collimated and close to the sample. *Note: this step is the same for brightfield and fluorescence, only with a different light source.*

4.00 Use camera to capture first image of sample.

5.00 Reduce exposure time (increase shutter speed) to the lowest setting at which test personnel deem the image quality to be satisfactory. Record this value.

6.00 Repeat test scenario for samples (dots) of different sizes and for each component added.

TESTING DATA SHEET

Personnel: Trevor Brown tbrown33@calpoly.edu | Eduardo Miranda ermirand@calpoly.edu
Makenzie Kamei mkamei@calpoly.edu | Enoch Nicholson ewnichol@calpoly.edu

Date: ______________________

Location: Cal Poly Microfabrication Laboratory – Metrology Room

Test Description: Optical train alignment and component compatibility verification

Brightfield Mode: Broadband Fiber-Optic Illuminator

Optics Component	Image Resolved? (Y/N)	Camera Shutter Speed	Notes
Baseline			
Beamsplitter			
45º Mirror			

Fluorescence Mode: Single-Wavelength LED

Optics Component	Image Resolved? (Y/N)	Camera Shutter Speed	Notes
Baseline			
Fluorescence Emission Filter			
Beamsplitter			
45º Mirror			

Process Notes and Comments:

TEST PROCEDURE 2

Test Description: Load capacity verification for PLA stage components

Date Created: 03-02-2020

Date Revised: 03-05-2020

1.0 INTRODUCTION:

The intent of this test is to confirm that the PLA components of the stage subsystem can withstand up to two times the load that they could possibly experience in use. The stage actuators can generate a maximum of 18 lbs of force, so each bracket will be tested to 40 lbs.

2.0 FACILITIES & EQUIPMENT:

This test will be performed in either the Mustang 60 Machine Shop or the Hangar Machine Shop. Both facilities provide access to the necessary fixturing equipment.

Table 2.1: Equipment needs

Equipment:	**Use:**
Table Mounted Vise	Holding mounting bracket & securing ratchet strap
Ratchet Strap	Applying a test force to each bracket
Spring Scale	Quantifying the test load
Mounting Bracket	Securely hold each bracket for testing
Mounting Hardware	Screws & nuts for connecting PLA parts to metal mounting bracket
Weight Clevis	Apply ratchet strap force to the bracket in a way that mimics the stage actuators
Paracord Safety Line	Hold scale and ratchet strap securely in the event of a bracket failure

3.0 SAFETY CONSIDERATIONS:

This test involves the loading of plastic brackets to twice their service load. There is the potential for a failure that could result in flying components. Adhering to the following safety guidelines will minimize risk of injury for test personnel.

Table 2.2: Safety Concerns & Actions

Safety Concern:	**Action:**
Flying debris	All test personnel must wear **safety glasses.**
Projectiles launched towards operator	The operator handling the ratchet strap must wear a **face shield** during testing.
Slipping test bracket	1) Test personnel must ensure that both vises are **securely tightened** before testing. 2) A **safety line** must be tied between the spring scale and first vise. This line will catch the system if the bracket breaks or the vise slips

3.0 DATA COLLECTION:

The test data collected here is qualitative. This test is intended to confirm part safety. Because the loads generated by the Microscope are less than 20 lbs and the hazards associated with bracket failure are minimal, quantitative failure testing is unnecessary. For each bracket, test personnel must confirm that the bracket can withstand a load of 40 lbs without gross permanent deformation.

5.0 TESTING PROCEDURE:

Step:	**Description:**
1.00	Secure part for testing
1.01	Screw PLA bracket securely to metal mounting bracket using hardware.
1.02	Clamp metal mounting bracket tightly in the vise, and align so that the bracket can be loaded along the design load axis.
2.00	Connect loading device
2.01	Attach ratchet strap beneath the vise so that the bracket is loaded vertically.
2.02	Hook spring scale to free end of ratchet strap
2.03	Attach weight clevis to free end of spring scale
3.00	Couple loading device to bracket

3.01 Position weight clevis on PLA bracket and slightly tension ratchet strap so that the system holds (1-3 lbs).

Step:	**Description:**
3.02	Tie paracord safety line between vise holding mounting bracket and the closes spring scale hook. Ensure that the safety line is only tight 1 – 1.5” beyond the 0 lb load point.
4.00	Conduct load test
4.01	Apply force incrementally by advancing the ratchet strap. Stop when 40 lbs is reached on the spring scale.
4.02	Use the release on the ratchet strap to remove the tension from the test device.
4.03	Remove PLA part and inspect for damage. Note findings.
5.00	Repeat steps 1-4 for all PLA stage parts (do not test X or Y Axis Actuator Mounts).
6.00	Uncouple test device and clean test area

TESTING DATA SHEET

Personnel: ______________ ______________

____________@calpoly.edu ____________@calpoly.edu

______________ ______________

____________@calpoly.edu ____________@calpoly.edu

Date: ______________________

Location: ______________________

Test Description: Load capacity verification for PLA stage components

Bracket:	**Successful Loading:**	**Notes:**
X-Axis Push Block		
Y-Axis Push Block		
X-Axis Spring Holder 1		
X-Axis Spring Holder 2		
Y-Axis Spring Holder 1		
Y-Axis Spring Holder 2		

PROCESS NOTES & COMMENTS:

TESTING DATA SHEET

Mechanical Engineering CAL POLY

Personnel: TREVOR BROWN
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Enoch Nicholson
enichol @calpoly.edu

MACKENZIE KAMEI
mkamei @calpoly.edu

________ @calpoly.edu

Date: 3-5-20

Location: Hangar

Test Description: Load capacity verification for PLA stage components

Bracket:	Successful Loading:	Notes:
X-Axis Push Block	✓	Slight indentation, tested to 42 lbs
Y-Axis Push Block	✓	Minimal indentation, tested to ~50 lbs
X-Axis Spring Holder 1	✓	BRACKET SURVIVED WITH NO VISIBLE DAMAGE BRACKET DEFLECTED OFF OF TEST FIXTURE DURING LOADING BECAUSE FASTENER WAS TOO THIN.
X-Axis Spring Holder 2	✓	NOTICEABLE DEFLECTION OF SPRING PIN, non permanent NO NOTICEABLE DEFLECTION OF PLA BRACKET
Y-Axis Spring Holder 1	—	—
Y-Axis Spring Holder 2	—	—

Process Notes & Comments:
Will test Y-Axis Brackets at a later date

TEST PROCEDURE3

Test Description:	Actuator repeatability test using the built-in encoders
Date Created:	03-12-20
Date Revised:	03-12-20

1.0 Introduction:

The purpose of this test is to verify the repeatability of the actuators' movement. The actuators use encoders to determine the distance traveled by the actuator plungers and they will be compared to measured travel.

2.0 Facilities & Equipment:

The testing will be conducted in the Cal Poly Microfabrication Lab's Metrology Room and will use the electrical control system designed by the team.

Table 2.1: Components used for testing

Equipment	**Use**
Arduino Control System	This system will control the both actuators using a joystick
Newport 850G Actuators	These are the actuators whose4 precision will be tested
DC Power Supply	The power supply will increase the force delivered and allow for full actuation of the stage
Stage and Brackets	The stage is equipped with mounting brackets for the actuators

3.0 Safety Considerations:

No safety considerations are necessary for this test.

4.0 Data Collection:

The actuators will be moved back and forth between different distances and each of the encoder readings will be recorded at each position. This will be done for both actuators at the following distances: 0.5, 1, 1.5 and 2-inches actuation.

5.0 Testing Procedure:

Mount the actuators to the stage and load the spring returns. Turn on the DC power supply and turn up the voltage to 10.5 volts. Zero the actuators to both of their reverse limit switches, Move the actuator to each of the desired locations using the indicator on the actuators. Record the encoder counts and reverse to reverse limit switches and record the final encoder count. Repeat for each of the distances.

TESTING DATA SHEET

Personnel: ______________ ______________

____________@calpoly.edu ____________@calpoly.edu

______________ ______________

____________@calpoly.edu ____________@calpoly.edu

Date: ______________________

Location: ______________________

Test Description: ____

Fill out the data sheet for each trial

Distance	X Actuator		Y Actuator	
in	Forward Count	Reverse Count	Forward Count	Reverse Count
0.5				
1				
1.5				
2				

TEST PROCEDURE 4

Test Description:	Stage Parallelism with Base
Date Created:	03/02/20
Date Revised:	--

1.0 INTRODUCTION:

The intent of this test is to ensure that the stage remains parallel with the base, so that the sample will remain the same distance from the objective throughout the motion of the stage.

2.0 FACILITIES & EQUIPMENT:

This test requires the use of a dial indicator and the stage in its final position on the stage. The test will be performed in the metrology room attached to the microfabrication lab.

3.0 SAFETY CONSIDERATIONS:

This test does not require any safety procedures or protective equipment.

4.0 DATA COLLECTION:

The stage will be moved throughout the full range of motion that it will see during use. The dial indicator will remain motionless as the stage moves under it. A reading will be taken every 10mm in a grid pattern, i.e. x will move 10mm, and repeat until the full range of x is covered, shift 10mm in the y, and repeat the 10mm motion in the x until all points are covered.

5.0 TESTING PROCEDURE:

Zero the dial indicator and place on the stage. Start at the starting position of the stage. Advance in the x direction by 10mm. Repeat until the stage has moved fully in the x direction. Return to starting position. Move the stage 10mm in the y direction. From here, move in 10mm increments in the x direction. Once the stage has moved completely in the x direction again, move back to the starting x position for this run and move 10mm more in the y direction. Repeat until stage has moved fully within the y direction. At each location, record the value on the dial indicator in the provided data table.

TESTING DATA SHEET

Personnel: ______________ ______________

_____________@calpoly.edu _____________@calpoly.edu

______________ ______________

_____________@calpoly.edu _____________@calpoly.edu

Date: ______________________

Location: ______________________

Test Description: ____

Fill out the data sheet for the listed positions

		Y (mm)					
		0	10	20	30	40	50
X (mm)	0						
	10						
	20						
	30						
	40						
	50						

APPENDIX V
DESIGN VERIFICATION PLAN

Senior Project DVP&R													
Date: 02/03/2020		Team: TEEM	Sponsor: Dr. Mayer and Dr. Hawkins			Description of System: Inverted Flourescence Microscope					DVP&R Engineer: Trevor Brown		
TEST PLAN										TEST REPORT			
Item No	Specification #	Test Description	Acceptance Criteria	Test Responsibility	Test Stage	SAMPLES		TIMING		TEST RESULTS			NOTES
						Quantity	Type	Start date	Finish date	Test Result	Quantity Pass	Quantity Fail	
1	1	Resolving an image	Min 10μm	Makenzie	FP	1	Sys	4/3/2020	4/7/2020				
2	2	Able to travel in Z direction	Min 64mm	Makenzie	FP	1	Sub	4/3/2020	4/7/2020				
3	3	Be able to travel in X-Y directions	Min 50x50mm	Eduardo	FP	1	Sub	4/3/2020	4/7/2020				
4	4	Repeatability of Actuation	Max 50μm	Eduardo	FP	1	Sub	4/3/2020	4/7/2020				
5	5	Parallelism	0μm±25μm	Enoch	FP	1	Sys	4/3/2020	4/7/2020				
6	6	Clearance	Min 50mm	Trevor	FP	1	Sys	4/3/2020	4/7/2020				
7	7	Budget	Max $3,000	Makenzie	FP	1	Sys	4/3/2020	4/7/2020				
8	8	Footprint	Man 2x3 ft	Trevor	FP	1	Sys	4/3/2020	4/7/2020				
9	10	Detectable Flourescence	Any, No Fail	Makenzie	FP	1	Sys	4/3/2020	4/7/2020				

Senior Project DVP&R Post COVID-19													
Date: 05/28/2020		Team: TEEM	Sponsor: Dr. Mayer and Dr. Hawkins			Description of System: Inverted Flourescence Microscope						DVP&R Engineer: Trevor Brown	
TEST PLAN													
Item No	Specification #	Test Description	Acceptance Criteria	Test Responsibility	Test Stage	SAMPLES TESTED		TIMING		TEST RESULTS			NOTES
						Quantity	Type	Start date	Finish date	Test Result	Quantity Pass	Quantity Fail	
1	1	Resolving an image	Focusing an Image	Makenzie	FP	1	Sys	5/28/2020	5/28/2020	Pass	1	0	Pass
2	2	Able to travel in Z direction	Any, No Fail	Makenzie	FP	1	Sub	5/28/2020	5/28/2020	Pass	1	0	Pass
3	3	Be able to travel in X-Y directions	Allow most of Sample window to be viewed	Eduardo	FP	1	Sub	5/28/2020	5/28/2020	Pass	1	0	Pass
4	4	Repeatability of Actuation	Any, No Fail	Eduardo	FP	1	Sub	5/28/2020	5/28/2020	Pass	1	0	Pass
5	7	Budget	Max $3,000	Makenzie	FP	1	Sys	5/28/2020	5/28/2020	Pass	1	0	Pass
6	8	Footprint	Max 2x3 ft	Trevor	FP	1	Sys	5/28/2020	5/28/2020	Pass	1	0	Pass