INVERTED FLUORESCENCE MICROSCOPE (F13)
FINAL DESIGN REVIEW

Authors:

Trevor Blythe (tblythe@calpoly.edu)

Spencer Hann (shann@calpoly.edu)

Thomas Eggenberger (teggenbe@calpoly.edu)

Matthew Pfeiffer (mbpfeiff@calpoly.edu)

Sponsor:

Professor Hans Mayer

California Polytechnic State University

Mechanical Engineering

June 5, 2020

Statement of Disclaimer

Abstract

Team F13 is composed of Trevor Blythe, Spencer Hann, Matthew Pfeiffer, and Thomas Eggenberger. We are all majoring in mechanical engineering and in our final year of study here at Cal Poly San Luis Obispo. This project is a continuation of a 2019-2020 senior project. The previous team designed and built a functioning inverted fluorescence microscope (IFM) from scratch. This device was created as a lab tool for undergraduate students to be able to perform experiments on microfluidic devices constructed in Cal Poly's Microfabrication Laboratory. Although substantially functional, several design constraints had not yet been met. Our team has improved microscope robustness and functionality for practical undergraduate lab use. To do this, we set overarching goals including decreasing microscope footprint, increasing the accuracy of microscope positional repeatability, and improving user-friendliness. Within this Final Design Review report, the full design, manufacturing, and testing processes of this project are explicitly detailed, as well as project logistics, future suggestions, and project management.

Table of Contents

Table of Figures

Table of Tables

1 Introduction

This Project is a continuation of a 2019 mechanical engineering senior project, which designed and built a functional, low-cost, Inverted Fluorescence Microscope (IFM) from scratch. This device was intended for use as a research tool in the Cal Poly Microfabrication Laboratory. Although partially functional, several design challenges still needed to be overcome. The central objectives of this year's senior project team were to consolidate existing hardware into a lab-friendly footprint, simplify the microscope's user interface to an acceptable level for undergraduate lab work, and to diagnose and repair several problems with the microscope's repeatability and functionality.

The preexisting IFM was functional, but not fully prepared for student use. This microscope, with robotically actuated stage positioning is designed to replace the present manually adjustable microscope in the lab. This IFM is designed to outperform the present microscope in several ways. Namely, a computer-controlled stage, and both brightfield and fluorescence microscopy modes. The computer-controlled stage and digital microscope camera add the potential of programmable stage paths, automatic image or video capture sequences, fully automated studies, and more. As of the start of this project, the stage positioning does not meet repeatability specification, the command line user interface is difficult to use, and the electronics are loosely connected in a temporary manner. To address these issues, we have reevaluated, revised, and expanded on the preexisting designs.

This report begins by discussing the initial project state and preliminary project research performed in a background chapter. From there, detailed objectives and engineering specifications are outlined from a house of quality diagram and preliminary research are laid out in an objectives chapter. Following this, the concept design and development chapter describes the initial solution ideation process and early design considerations. After cautious narrowing, preliminary testing, and preliminary analysis, the final design chapter presents the final chosen designs and corresponding specifications. The next three chapters are the team's manufacturing plan, design verification plan, and project management, respectively. These three chapters dig into the details of how the final design has been effectively manufactured and tested. The final chapter of this report is a conclusion, in which general takeaways, future considerations, and general details of the process described, as well as a request for permission to move forward with manufacturing.

Due to the ongoing COVID-19 pandemic, access to on-campus resources and the ability for the team to meet in person is limited. A safety procedure has been developed and implemented for limited in-person interaction. To enable initial testing, the IFM was transported from the microfabrication laboratory on Cal Poly's campus to a spare room in a local team member's household. Figure 1 shows the IFM in its off-campus environment during the first quarter of design progress.

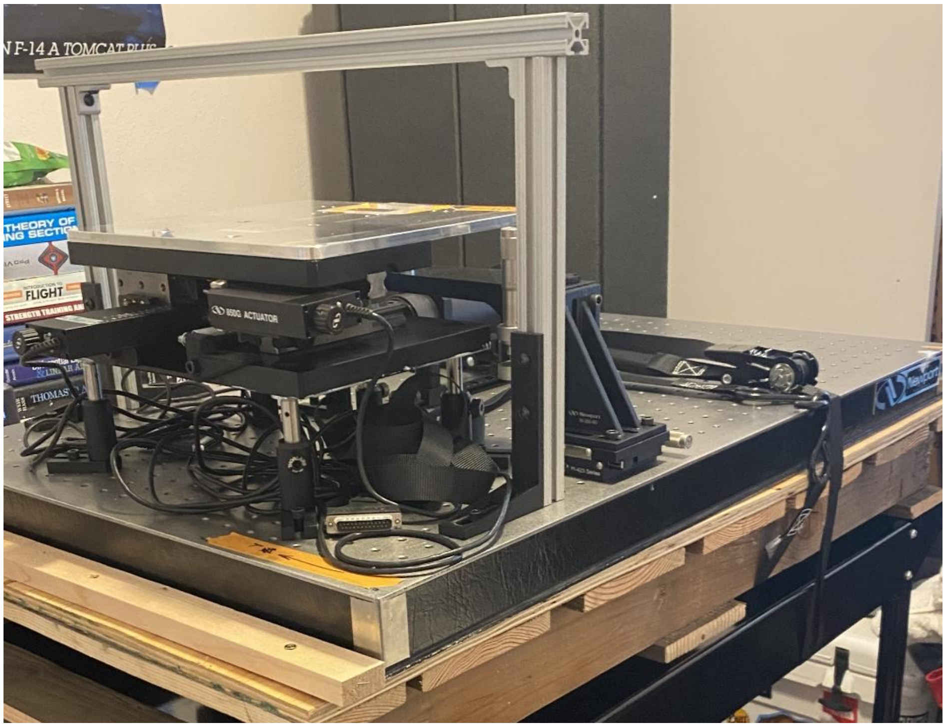


Figure 1: Initial IFM State after transportation to off-campus location.

Our team of four senior mechanical engineering students have a wide variety of skills and experience. With Matthew Pfeiffer and Trevor Blythe both pursuing a Mechatronics concentration, Spencer Hann pursuing an energy resources concentration, and Thomas Eggenberger pursuing a general concentration, our diverse skillset has enabled solution to as many design challenges as time and COVID-19 restrictions have permitted.

In this document, we will summarize the research performed to outline design constraints and specifications, quality assurance methods, initial design ideation methods, intended design direction, chosen manufacturing processes, testing procedures, relevant project logistics and timing, as well as outline general suggestions for future design iterations.

2 Background

In the background chapter, the initial performed project research to assist in specification decision-making and project objectives is broken into customer research, product research, and technical research. The customer research discusses central users use of the IFM, and necessary engineering specifications. Product research investigates existing IFMs, how they approach satisfying customer needs, and if the needs they satisfy have overlap with our customer needs. Lastly, the technical research describes initial research into specific technical functionality and components of the IFM for more thorough understanding of the microscope's inner workings and potential.

2.1 Customer Research

For initial design considerations, it was critical to select relevant engineering specifications, so an initial interview with our project sponsor, Professor Mayer, was conducted. From this, we learned the predominant use cases of the device. Most critical, undergraduate use for brightfield microscopy of microfluidic devices. Such a student should find the microscope simple to setup and use without much guidance other than a brief demonstration or access to a user's manual. To limit microscope downtime, loose electronics components should be safely housed and more securely assembled than the present breadboard arrangement allows. Because the microscope will be in a clean room, extreme environmental and spill resistance is unnecessary.

Beyond initial simplification of the user interface and repair of general functionality, Professor Mayer stressed the advantages of a digitally controlled stage. For more advanced research techniques, the digital stage allows for fully automated studies by computer numerical control (CNC) stage positioning paired with automatic image capture for user defined paths. Because these functions would be used by more advanced users, they are not of the highest priority.

Interviews were also conducted with the previous year's senior project team members to determine the current functionality of the microscope, and where improvements could be made. Team members Enoch Nicholson and Makenzie Kamei both said that the user experience of the microscope, in its current state, was unsuitable for lab use. The challenges of remote assembly due to the COVID-19 pandemic meant that there was little time available for product and user testing. The lack of final testing means that stage motion repeatability had an outstanding problem. There was a comprehensive manual to aid in the operation of the microscope, but its length and complexity could dissuade the average lab student from using the new microscope [1]. An isometric view of the previous team's full-system CAD model of the microscope is shown in figure 2 below.

The single most important design takeaway from our performed customer research is in relation to ease of operation. Undergraduate science students should be able to operate the microscope within 5 minutes of introduction. Speaking with undergraduate engineering students about their lab experiences, we found that students were intimidated by complex interfaces. Therefore, an ideal interface would be limited in size and located in one place on the machine. This would prevent an excess of controls and provide a more ergonomic user experience. Students also mentioned that a single page of high-level instructions was more helpful than a full user manual.

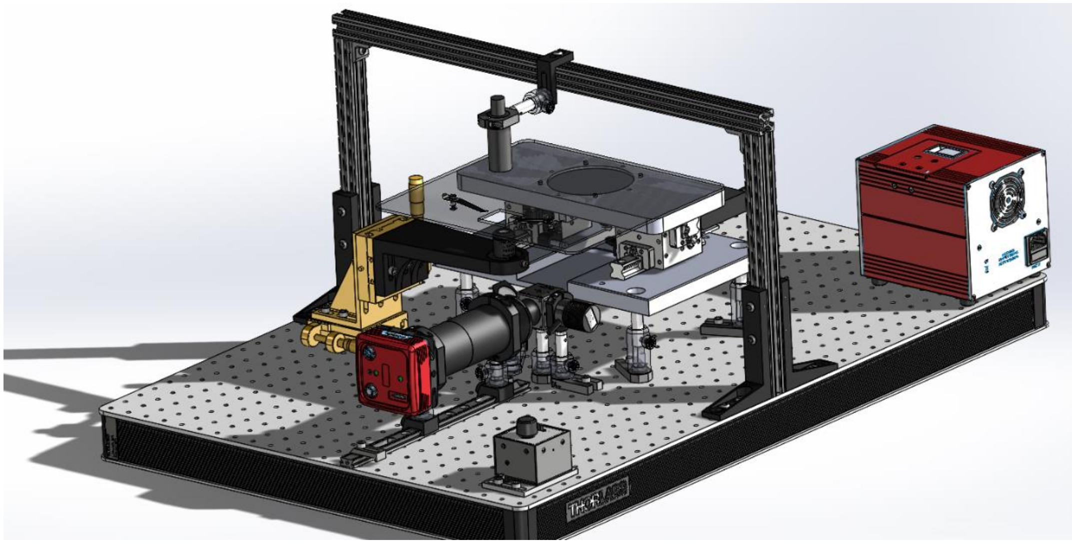


Figure 2: Microscope in its current configuration [1]

2.2 Product Research

Prior to diving into technical operation of microscopes of this variety, it was relevant to investigate the initial state of the IFM. At the time of initial inspection, brightfield illumination microscopy was possible, but a known stage repeatability error existed. When actuating the stage in a square pattern back to the same coordinate, a different stage coordinate position resulted. When using the microscope, all electronic components were loosely laid out next to the microscope, taking up optical breadboard space and acting as a hazard to microscope functionality. Although usable, the fiber-optic brightfield light source was loosely mounted and oscillated during normal operation, impeding visibility and focus on the microscope target. The command line prompt did not enable simple coordinate control and was intimidating for someone without familiarity to programming. Lastly, while the joystick control of the stage worked, the exposed circuitry made the device seem more intimidating and prone to damage than necessary.

While this may paint a bleak review of the initial microscope design, it is clear most components have been cautiously chosen for cost effectiveness, ease of manufacturability, ease of replacement, and effective functionality. The optical pathway is the microscope subsystem mechanical engineering students generally have the least experience with. However, the pathway developed by the previous team performs just as we hoped, with stiff component mounting brackets, easy accessibility, and a simple structural layout.

There were three specific commercial microscopes considered as comparable products. The Nikon Ti-2 [2], Olympus IXplore [3], and OPTIKA IM-3 [4]. These microscopes were all considerably more expensive than the IFM built by last year’s senior project team. These microscopes, in addition to being more expensive, also offered more features, especially the Nikon Ti-2. Many of these features—like automatic zoom or built in illumination—are designed to make the microscopes more convenient to use [3]. The OPTIKA was the only microscope researched that did not have a motorized stage. The automatic operation of the Z stage and built in illumination and fluorescence capabilities of all commercially available microscopes were not present in the IFM. All commercial microscopes researched also had a smaller footprint than the current IFM. Analyzed commercial microscope characteristics are in Table 1 below, compiled by the previous senior project team.

Table 1. Commercial Microscope Characteristics [1]

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 • X-LED illumination system with a 50k hour lifetime
OPTIKA IM-3	$4,706	• Camera port with multiple adapters • HBO or LED fluorescence • 250x160 mm fixed stage • Motorized 114x75 mm stage • Motorized long working distance universal condenser
Olympus IXPlore	Unknown	• Filter wheels and shutters • 8 position motorized encoded fluorescence mirror turrets • 200 x 260mm Stage

A robust light source is one of the most advertised features of the commercially available microscopes. Our initial light source was a standalone fiber-optic light mounted above the stage with a 3-D printed bracket. The illumination power of 30 watts is sufficient for capturing brightfield images, but a fluorescent light source is not present on our machine. Recommendations by the previous senior project team suggest the next step in adding fluorescence capabilities is purchasing a high power LED such as a ThorLabs Solis® series light and associated driver [1]. Unfortunately, fluorescence will be difficult to match with our budget because the combined cost of the light source and driver is over $1700 [5].

Additional product research entailed finding relevant patents for more examples of related product concepts. Although not as applicable to this project because we already have a mostly functional IFM, patents showing specific microscope control methods and sub-functions were analyzed. Appendix A contains a table several patents as well as details about each. Some considerable takeaways include previous team design choice reinforcement from patents showing similar microscope stage control methods [6] [7]. Another patent proposed use of linear encoders with motorized stage control for high locational precision CNC manufacturing application [7].

To position the microscope stage, the IFM uses Newport 850G actuators controlled by custom software on an Arduino® mega microcontroller [1]. Table 2 below shows Newport listed specifications [12]. For a full list of actuator specifications, see Appendix B. These linear actuators can only transmit force in one direction, so the return movement is externally applied to the actuator. In their initial mode of operation, the actuators are controlled by encoder counts, which were not yet calibrated to a specific unit scale [1]. This made it impossible to control the stage in fixed increments, limiting functionality. Additionally, the stage did not return to the same point after moving in both directions, indicating stage drift was an issue that needed to be addressed. The important actuator specifications are tabulated below.

Table 2. Newport 850-G Actuator Specifications

Specifications	
Bi-directional repeatability	Better than 1 micron
Encoder resolution	0.60514 micron
Encoder type	Magnetic rotational encoder 2KHz open collector

The initial Arduino® to actuator interface was a 25-pin breakout board connected to the parallel port of the actuator [12]. This breakout board was wired to the Arduino® through use of a breadboard [1]. The prototype-style wiring made the Microscope susceptible to damage by impact, vibrations, and spills. Therefore, an environmentally resistant electronics housing mounted to the optical breadboard was of interest.

2.3 Technical Research

Our technical research was focused on specific subsystem arrangements, component effectiveness, subsystem performance, and isolated electro-mechanical component output. The isolated component output is the process of isolating single components, just as a breakpoint enable when debugging code. This process was used to aid identification to the source of the actuated stage error and gave insight to solution ideation.

The IFM actuators can only actuate the stage in one direction. The return is currently actuated by springs mounted on 3-D printed brackets. It was initially believed these two different methods of actuation may have been a mechanical cause of the stage repeatability issues. The actuators also contact the stage with metal pins on plastic 3-D printed brackets, slight flex of the 3D printed surface was another considered contributor to the stage drift.

We talked to Professor Ridgely about standard approaches to CNC controls, and he believes that the use of the motor's rotary encoder present in the actuators is not ideal. The rotary encoder inside of each actuator is a direct measurement of where the stage has moved, there is gearing backlash, mechanical deflection, and losses that unseen by measurement of the motor in the actuator. Because of this, the feedback loop to control linear position of the stage is not closed.

A potential solution would be to use linear encoders on the stage itself, and then to update the Arduino® code to use this full-state feedback. Linear encoders are designed for one of two feedback types, absolute or incremental. Based on an initial read-through of the code, we could get away with an incremental encoder and limit switches to mark minimum and maximum positions. The downside of this versus absolute encoders is, homing sequence like with the initial microscope one would still be necessary. Incremental encoders can only provide relative position, not absolute. This means they do not provide information about where they are with respect to minimum and maximum travel to the Arduino® code. To account for this, the homing sequence would need to touch off a mechanical, infrared, or magnetic limit switch for each axis. The 850G actuators have built in limit switches internally on the motor shaft to prevent over-travel, providing the same homing mechanism for the rotary encoders in the actuators. Using these internal limit switches, and rotary encoders, the previous team developed the initial homing sequence and stage positioning code.

Other devices like 3D printers and CNC machines often use stepper motors instead DC motors. Like our Newport Actuators, stepper motors function like a DC motor paired with an encoder, but in one unit. The

DC motor with encoder knows how many revolutions it has turned by reading the encoder signals. A stepper motor senses position from many different windings inside the motor. A stepper motor can also be micro stepped, where the stepper moves a smaller distance than one full step. These discrete movements are possible with a stepper motor because of the large number of field windings that divide the rotation into small steps. However, the resolution of a DC motor paired with an encoder is generally much higher than that of a stepper motor.

3 Objectives

In the objectives chapter, project objectives are initially interpolated from a precise problem statement and boundary diagram presented to assist in problem identification and task prioritization. The quality function deployment (QFD) sub-section discusses the development and takeaways from the developed house of quality diagram. From here, the engineering specifications and risk assessment sub-section describes each chosen engineering specification from the QFD, project significance, and initial risk assessment.

3.1 Project Objectives

Cal Poly Microfabrication Laboratory Director Dr. Hans Mayer needs a computer controlled inverted fluorescence microscope with an intuitive user interface, and high-precision stage. The IFM should be suitable for use by both undergraduate and graduate students in the study of small-scale fluid mechanics and materials engineering devices. To document the project scope, we included a boundary diagram below in Figure 3, which outlines the subsystems of the microscope within group control that are not meeting requisite customer needs. For example, the programable control system could formerly only be accessed by entering code directly into the serial monitor of the Arduino® IDE application. For another example, the joystick override for manual stage control existed, but to access it the user had to again interact with the Arduino® command line prompt and use hardware with exposed circuitry, which is unsuitable for a fluid experiment environment.

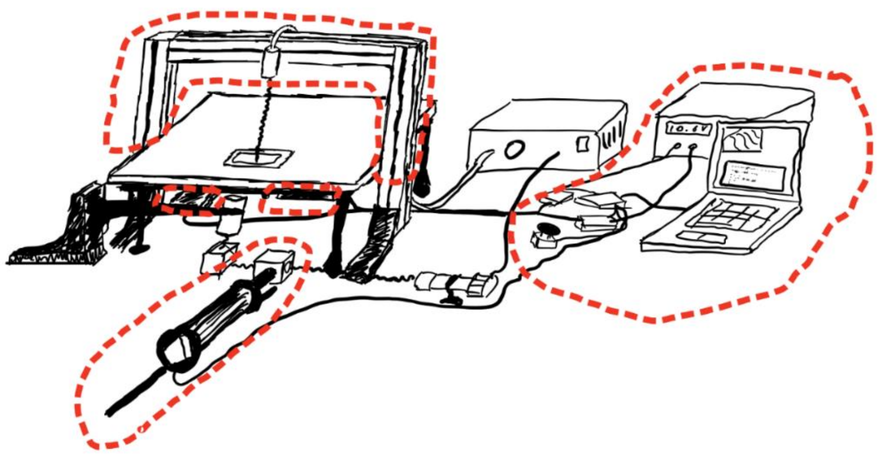


Figure 3: IFM Boundary Sketch

Our objectives were outlined by our sponsor in our first official meeting. Priority of milestones was centered around features and improvements that best prepare the microscope for use by students in on-campus lab experiments. Our first target was improving the overall footprint of the device to take up less lab workspace. Additionally, this reduces the perceived complexity for students, making it less initially intimidating, and more convenient to locate in a potentially crowded lab environment. We planned to achieve this in two ways. One, by creating a housing system for the currently exposed actuators and Arduino® boards used to control the device; second, by reconfiguring the optical lens tube and microscope position on the optical breadboard.

The second priority, and a precursor to all potential testing, was to specify a suitable alternative for the existing clean room power supply. The replacement must be smaller and fit underneath the microscope's shop cart, to achieve the first goal of reducing the footprint. The replacement still required to have some level of voltage variability for stage repeatability testing and data collection. These first two goals were achieved entirely by our project's Critical Design Review.

Our third-tier goals were to improve the microscope's user interface, simplifying the existing command line system into a graphical user interface (GUI), and a housed package for the Arduino® joystick. This objective also entailed development of a quick-start document, to briefly outline the processes from powering on the microscope, adjusting the objective lens, controlling software, and using the USB microscope camera.

Our fourth primary goal was to fix the present actuator repeatability issues by modeling the drift that occurred when moving the stage in a 'square' pattern. We considered adding an alternative feedback source to the Arduino® controller to correct for it, but decided it was not necessary. This would have required a lot of testing of and additions to the mechanical, electrical, and software sub-systems of the IFM. With this scale of testing necessary, this goal is set to be completed before our final design review. Table 3 displays a summarized list of our wants and needs, as provided by our sponsor, broken into three sections indicating the quarter in which we hope to meet them.

Table 3. Wants and Needs Summary

Needs	Wants
Create Controller Housing	Improve 3D Printed Hardware
Replace Power Supply	Purchase Shop Cart
Improve User Interface	Create Joystick Housing
Create User Manual	Add Programmable Stage Paths
Diagnose and Correct Stage Drift	Add Fluorescence Capabilities

It should be noted that as of the time of writing out the Final Design Review, all deadlines were achieved to satisfy relevant needs and several of the wants laid out by our project sponsor. We felt compelled to prioritize our goals to be in-line with our sponsors desires for the final product. If we were unable to complete all our tasks, we would have concentrated on the 'needs' category prior to satisfying 'wants.' This organization would prevent us from spreading ourselves too thin with the broad spectrum of design challenges laid out. We intend to deliver a final product that capable of providing undergraduate students with a usable microscope for lab experiments. The 'wants' category is intended for goals outside of the initial project scope.

3.2 Quality Function Deployment

To ensure that the design choices made accomplished the goals laid out by our sponsor, Quality Function Deployment (QFD) methods were employed. The House of Quality found in Appendix D serves to identify and quantify final design specifications. This occurs through comparative analysis of engineering specifications to consumer requirements and alternative products on the market. The attic of the house of quality requires identification of correlation between brainstormed engineering specifications. Strong correlations between specifications expose repetition, helping focus the integrity of design specifications. Additional house of quality analysis enables quantification of engineering specifications based on consumer requirements. The QFD process has proven to be a powerful organizational tool in the initial stages of the design process.

The conclusions drawn from our QFD were that all our specifications had a similar weight of around 10%, meaning all consumer needs have similar importance. Also, there were no specifications that negatively correlated with each other, suggesting low risk of one specification overriding another. This makes sense when considering how the subsystems interact on the IFM. The user interface being purely software, unaffected by the exterior of the microscope and controller containment unit. Additionally, many of the specifications did not correlate outside of their subsystem group, meaning our subsystems can be developed reasonably independently from one another.

Table 4. QFD engineering specification explanation.

QFD Spec #	Specification	Explanation
1	Stage Repeatability	The numerical precision of stage actuation through coordinate control mode
2	Instruction Time for General Use	The amount of time that it takes to teach someone to use the microscope.
3	Environmental Resistance	The durability of the microscope to laboratory abuse cases.
4	Footprint	The size of the footprint the full IFM system takes up.
5	Ease of Setup	Simplicity for a user to set up the microscope with a guide.
6	Technician Calibration Time	Amount of time it takes a technician to calibrate the microscope.
7	Part Replacement Expense	Replacement parts desired to be relatively cheap
8	Rigidity of Mounting Brackets	Optical and stage mount brackets stiff in place and not prone to oscillation/deflection.
9	Safety Considerations	Risks and hazards present with the final IFM design.
10	CNC Stage Control	Additional added microscope functionality
11	Simple Use Control	Simplicity of undergraduate microscope use for microfluidic studies.
12	Rigidity of Optics	Visible deflection and oscillation in view of the microscope camera.
13	Cleaning Accessibility	How simple the components are to access and clean when necessary.

Table 4 briefly outlines and explains each of the engineering specifications extracted from the House of Quality. These were all uncorrelated from each other, and either qualitatively or quantitatively measurable such that achievement of each can be specified. Quantitative necessity for each specification is outlined in the Engineering Specifications and Risk Assessment below.

3.3 Engineering Specifications and Risk Assessment

The QFD methods enabled us to come up with the engineering specifications listed in Table 5, as well as quantified targets for each. The Target, Tolerance, and Risk headers provide detail to our specific engineering specifications. Target outlines the quantified goal, or range of acceptability. Tolerance defines the target as either maximum acceptable, minimum acceptable, or an exact value. Risk defines the anticipated difficulty of each and perceived impact on specification on total project significance. These specifications are results of QFD analysis and provide technical goals as targets for the final design. Many of these specifications have a maximum tolerance. This is because without them, the microscope is realistically no more useful than the current lab microscope.

To measure stage control specifications, a calibration and testing procedure was requisite. Potentially, a mask with known feature sizes could be placed on the stage, where the stage repeatability and CNC control could be further quantified. The user experience targets have been measured by allowing undergraduate engineering students, to use the microscope. User experiences have been measured through use of surveys, and further qualitative testing has contributed to fine tuning of an intuitive microscope user experience.

Table 5. IFM design specifications.

QFD Spec #	Specification	Target	Tolerance	Risk
1	Stage Repeatability	10 [μm]	Max	H
2	Instruction Time for General Use	5 [min]	Max	M
3	Environmental Resistance	Impact and Spill Resistant	Target	M
4	Footprint	2’ x 2’ (Without Fluorescence)	Max	H
5	Ease of Setup	10 [min] Without Instruction	Max	M
6	Technician Calibration Time	5 [min] With Guide	Max	M
7	Part Replacement Expense	No Custom Shop Work Necessary (3D Printing Okay)	Target	L
8	Rigidity of Mounting Brackets	Meets Stage Repeatability Specifications	Min	L
9	Safety Considerations	No Pinch Points	Min	L
10	CNC Stage Control	Ability to Position to Multiple Targets	Target	H
11	Simple Use Control	Intuitive Joystick Operation	Target	M
12	Rigidity of Optics	No Visible Oscillation from Microscope Camera	Max	M
13	Cleaning Accessibility	No Power Tools Requisite	Target	M

In the table above, the high-risk parameters include the stage CNC control. This is where the previous senior project team had trouble and is at the interaction point between several subsystems. must perform correctly together. The other high-risk specification is the IFM footprint. There are many components that cannot be reduced in size including the stage, light source, and optical lenses. Because of this, packaging all sub-systems and subsequent components together in a small space represents a significant design challenge.

4 Concept Design Development

In the Concept Design Development chapter, the solution ideation process is thoroughly described as well as each chosen design direction. The first sub-section describes the functional decomposition process and functionality identification. Next, the Ideation sub-section describes the formulation process of a large quantity of potential solutions. Pugh matrices and weighted-decision matrices were used to begin sorting and narrowing the potential solutions as described under the Pugh Matrices and Weighted Decision Matrices sub-headers, respectively. As indented sections to the Weighted Decision Matrices sub-section, the chosen initial design direction for customer need satisfaction is described. Followed by a Results and Conclusions sub-chapter of the initial design process.

4.1 Functional Decomposition

We began our ideation process with a functional decomposition of the microscope to identify the different subsystems. We identified many subsystems in the microscope however only some of them are encompassed by our boundary diagram. For instance, the stage movement is a function that needs to be improved, but the physical stage with linear bearings is not something that is controllable within the constraints of this project. Therefore, we will need to address this function with different subsystems like the actuator mounting or Arduino® code.

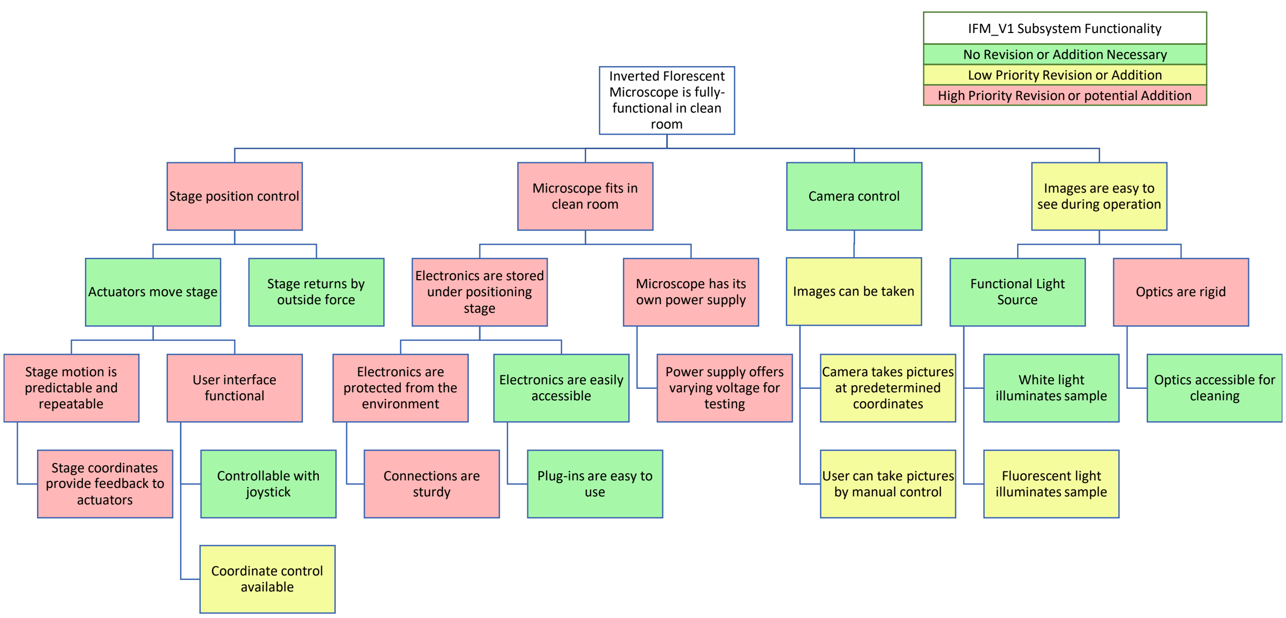


Figure 4: Function Tree with functionality prioritization

Several key identified functions for further consideration were stage illumination, component layout on the optical breadboard, electronic environmental resistance, and user interface. We identified these functions through meeting with Dr. Mayer where he thoroughly outlined the improvements that we will make to the IFM to prepare it for lab use by students. Once these functions were identified, we identified the subsystems that addressed these functions. Figure 4 shows our color-coded function decomposition tree, which we used to identify subsystems and functionality in need of design improvement. This enabled us to categorize the device and components into subsystems. From there, color coding revealed where our attention was needed the most. The color coding provides information about the priority of the microscope to function. Separate sub-systems can be analyzed independently.

Splits of the tree identify independence of sub-systems. For example, the way the user interacts with the computer does not change the control architecture or method of stage actuation. As these two subsystems are independent, ideation could be carried out without consideration to how they would interact with each other. The other two functions, component layout and electronic environmental resistance, are more closely related. Because the size of an electronics enclosure affects the footprint of the microscope, the solution ideation for each must be caried out in conjunction. These two ideation processes were carried out independently, but consideration was given to how the electronics enclosures might fit on the breadboard and how the breadboard layout would allocate space for the electronics.

4.2 Ideation

Ideation was initially carried out by brainwriting in OneNote. Under each function, generated lists of potential solutions and selection processes have been compiled in Appendices E-G. When the ideas stopped flowing, we moved to the next subsystem and continued to brain-write. After all the brainwriting was finished, we each began to build ideation models so that we could turn the ideas into more concrete solutions and better visualize how the ideas would work in real life and how some of the related ideas might fight together. In addition to prototyping our brainstormed ideas we also created mock-ups of the various electronics parts of the microscope, as seen in Figure 5, which we used to demonstrate how the electronic components would fit into different casing designs. These included the Arduino® Mega board, the 25 pin breakout boards for the actuators, and the developed protoboard with driver chip that would connect them all together. Pictures of all the ideation models can be found in Appendix F. We found that the mock-up electronic components were very helpful in ideating the different electronics enclosures that we came up with during our brainstorming.

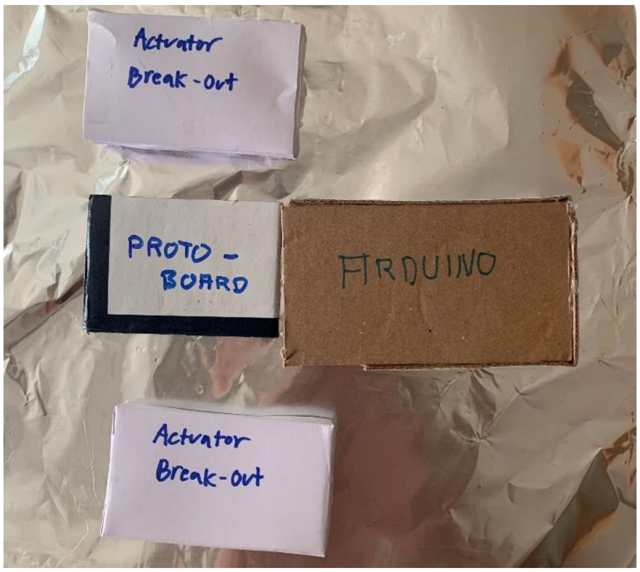


Figure 5: Arduino®, actuator breakout, and proto-board mockups.

In addition to the mock-up components, we also created ideation models of the new subsystems that would be present on the finished microscope these were the brightfield illuminator as well as the electronics enclosure. The brightfield illuminator is likely going to be an off the shelf component that will be mounted to illuminate the slide. The mounting apparatus is what we ideated. For our graphical user interface (GUI), the ideation models were images and ideas of the different ways that we could interact with the Arduino® system. Some of the ideas included a 7-segment display and button in addition to a computer-based GUI.

4.3 Pugh Matrices

After our ideation models were complete, we needed to quickly and effectively narrow down the number of potential solutions generated so we could choose the best designs. A Pugh matrix is an excellent way to do that. A Pugh matrix is a method of comparing a set of designs against a 'datum' design evaluated across the criteria deemed to be most important for each solution. If the design would perform better than the datum, it receives a '+', worse and it receives a '-', the same and it receives an 's'. After all criteria are evaluated, each design is scored by summing the ratings it received, with '+' being 1, '-' being -1, and 's' being 0, giving the datum design a score of 0. The highest scoring designs from this process should be the ones that best meet all the criteria. However, this process is very generalizing, it is important to listen to intuition and not throw-out designs just because they did not score the highest. If a favorite design did not make the cut, factors not represented by the Pugh matrix in play. The Pugh matrix for the electronics enclosure options is presented as Table 6, and the one for the brightfield illuminator mounting included as Table 7. All matrices are listed and presented in Appendix E. The conclusions from the Pugh matrices are discussed in Weighted Decision Matrices section below.

Table 6. Pugh Matrix for Electronics Enclosure

Concept	Protection from environment	Small Footprint	Ease of Access	Ease of Manufacturing	Access to ports	Mounting to Stage	Sum
1	+	+	+	-	+	+	4
2	+	+	S	-	+	+	3
3	+	+	S	-	+	+	3
4	+	S	+	-	+	+	2
5	+	+	+	-	+	-	2
6	+	+	+	-	+	+	4
Datum	S	S	S	S	S	0	0

Figure 6 below presents the each of the concept sketches for the above Pugh Matrix. The two options selected to move forward were the simple box and lid design and the design with a clamshell hinging lid. The server-rack style, PC-tower style, and box on rails were all decidedly too complicated. None of those designs would contribute enough new and useful functionality to outweigh the work necessary. Two narrow a decision between these two options further, a more focused selection method became necessary.

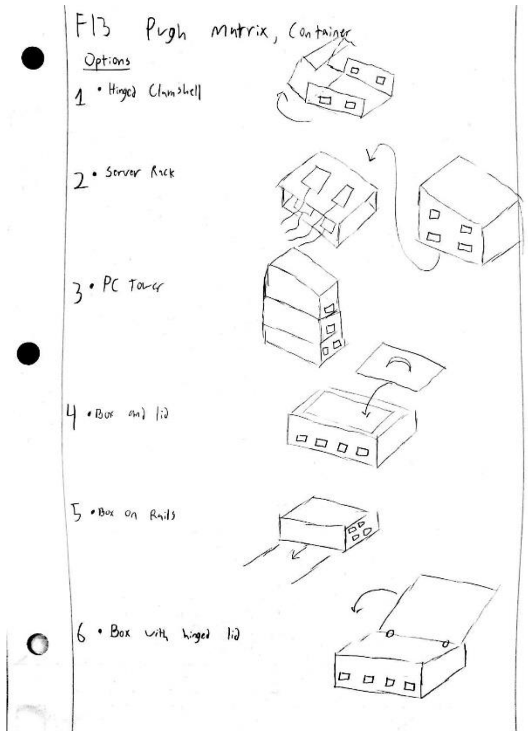


Figure 6: Sketches of Designs used in the Pugh Matrix.

For the electronic enclosure, the design concepts 1 and 6 were chosen to move on. The Pugh Matrix is excellent at pointing out overcomplications in designs. More complicated electronics enclosure ideas were identified in the matrix as just as functional as the simplest of electronics enclosures. This aided in preventing potentially wasteful manufacturing processes.

Table 7. Pugh Matrix for Lighting Solution

Solution	Rigidity	Complexity	Complexity of new parts	Robustness	New Functionality	Sum
Existing mount for fiber optic light source (80-20 "gantry style" with 3-d printed mount fiber optic source located next to stage)	S	S	n/a	S	S	0
Existing fiber optic source with gantry style 80-20 mounting,	+	S	S	S	+	2

Solution	Rigidity	Complexity	Complexity of new parts	Robustness	New Functionality	Sum
Existing fiber optic source with gantry style 80-20 mounting, improved 3-D printed parts like brackets improved mounting for fiber optic for z adjustability	+	S	S	n/a	S	0
Existing fiber optic source with crane style 80-20 mounting,	S	+	S	S	S	1
Existing fiber optic source with tripod style 80-20 mounting	+	-	-	+	S	0
New (compact) fiber optic source with on stage mounting	+	+	+	-	S	2
Under stage lighting with new optical path	+	-	-	S	S	-1
Mounting linkage for z adjustability	S	-	-	S	+	-1
Fiber optic source with carne style	S	-	S	S	+	0

The designs that were chosen for the brightfield illuminator mount were all revisions of the existing design. Re-designing brackets for the gantry mount would address rigidity concerns with minimal redesign necessary. This decision ultimately reduced cost, manufacturing time, and made use of IFM components created by the prior team.

4.4 Weighted Decision Matrices

Pugh Matrices are great for narrowing down lots of ideas quickly, but they fail to address how well designs meet criteria and achieve final specification. A Weighted Decision Matrix (WDM) gives every criterion a weight out of 10 to rate how relative functional significance. Each idea is then given a grade for how well each criterion is satisfied. A designs total score is determined by multiplying each criterion by the relative weight of that criterion and summing the values. This process provides much more detailed evaluation of how well designs will perform and gives more insight to potentially successful designs than Pugh matrices. We used WDMs to evaluate the best designs for each the four functions we chose to focus on for the ideation process. The full WDMs can be found in Appendix section F and the results of the matrices are explored below.

4.4.1 Electronics Enclosure

Safely containing the Arduino® and actuator breakouts is one of the most important goals Dr. Mayer outlined for us due to the importance of these components to the function of the device and the potential splash hazards of being used in a laboratory with fluid experiments. We produced some very good models during the ideation prototype session and using the weighted decision matrix we were able to come up with a best solution of box with a hinged lid. This design is shown in Figure 7. We will now need to decide between using a 3-D printer that our sponsor has access to, or an online service that specializes in manufacturing electronic containment boxes to produce the final version of this design. We have discussed these options with both our coach and sponsor and will be choosing a direction before PDR.

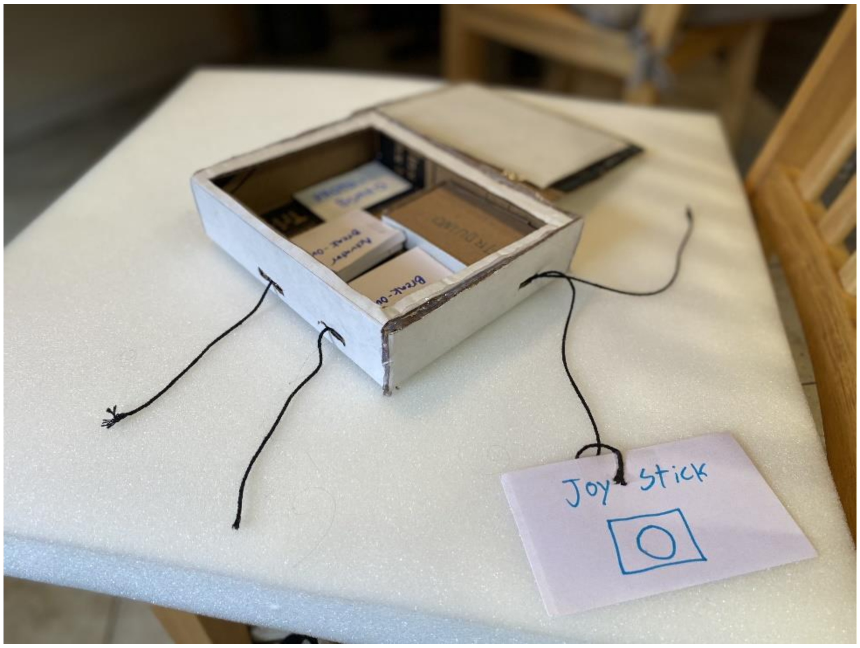


Figure 7: Completed Ideation model of hinged lid electronics enclosure

4.4.2 Lighting

For this decision matrix, there were two options. Design 1 is the same implementation as the previous team provided, but with re-designed stiffening 3D brackets. This design scores well, but not the maximum for the design criteria, except for the new functionality criterion. As it is the existing design, it does not add any new functionality, so it scores a zero. Design 2 is a crane style mount with the added functionality of being able to swing out of the way. Because of this motion it is not as rigid and therefore is worse than the current option and the added functionality does not make this a worthwhile design. The third option of on-stage mounting scored the highest but there may be implementation issues as the actuators might not be able to move the stage with the added weight.

After consulting with Dr. Schuster, it was brought to our attention that there may be an issue with vibrations due to the small jerky movements of the stage. The flexible fiber-optic conduit might shake and cause uneven illumination of the slide. Because of this further testing is required and the two solutions of revamping the existing 80-20 structure and the on-stage mounting will be pursued in parallel. Sketches of the two solutions are shown in Figure 8 below.

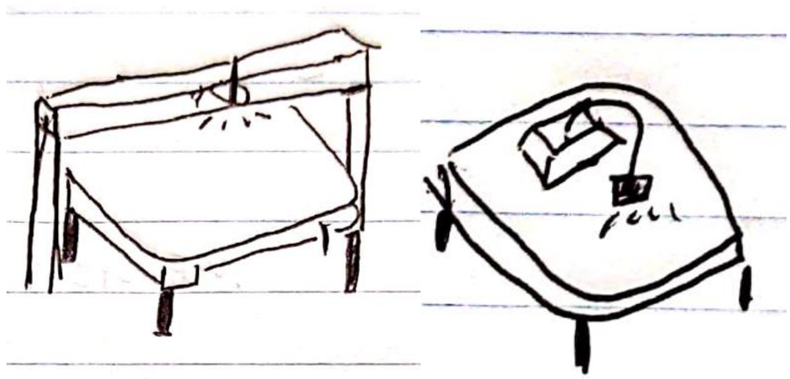


Figure 8: Sketches of existing lighting solution(left) and the on-stage mounting (right)

4.4.3 Optical Component Layout

One of the central microscope goals laid out by our sponsor is to take up less space in the microfabrication lab. One way in which this can be achieved is by modifying the current position of optical components and the orientation of the microscope on the optical breadboard.

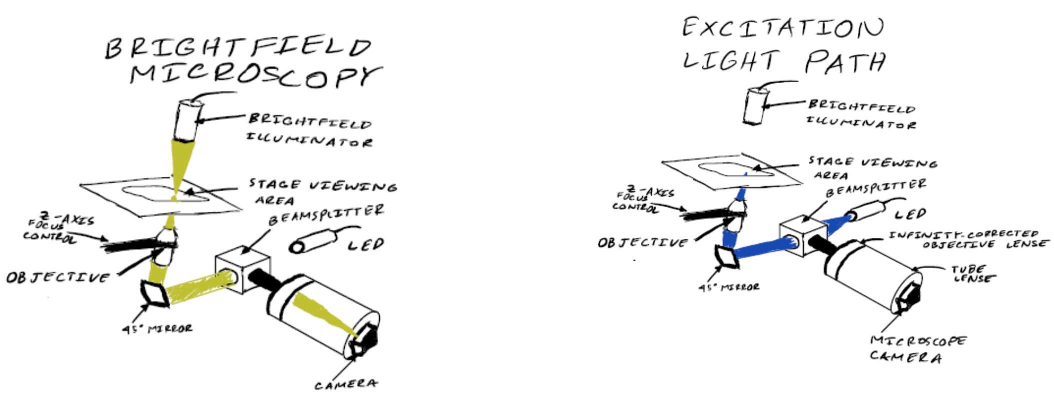


Figure 9: Brightfield microscopy and excitation optical pathways and components

In the current microscope layout on the optical breadboard, the tube lens and camera attachment sit jutting out from the microscope stage on a long slotted rail mount, requiring significantly more space on the breadboard than the rest of the microscope assembly. Figure 9 shows a layout of the optical components in their current orientation, the optical pathways of the brightfield luminescence and excitation light are respectively highlighted in yellow and blue. These sketches were developed based on models in the previous IFM team's FDR and observation of the physical system [1]. The dovetail mounting rail the tube lens and camera assembly sit on is not included in these models.

Fortunately, the 45-degree mirror that translates the optical pathway perpendicular to the objective can be rotated any direction, enabling simple repositioning of the components that control the optical pathway parallel with the breadboard. Because the components between the tube lens and objective have infinity corrected lenses, the beam splitter, tube lens, and camera assembly could theoretically be placed an infinite distance away from the 45-degree mirror. However, increasing the distance between the 45-degree mirror and beam splitter or brightfield LED increases the sensitivity to improper alignment. Because of this, it is desirable to keep these distances as small as possible.

After initial ideation, six potential solutions were chosen. Three of these solutions keep the microscope orientation in its present portrait configuration on the optical breadboard, the other three solutions consider rotating the microscope stage for simpler locating in the laboratory. Each configuration has the tube lens located as it is currently, rotated left along the stage, and rotated right along the stage. Initially it was considered the stage could be raised, and the tube lens could be positioned underneath the microscope stage. Our team decided the space under the stage would better serve as an electronics housing location.

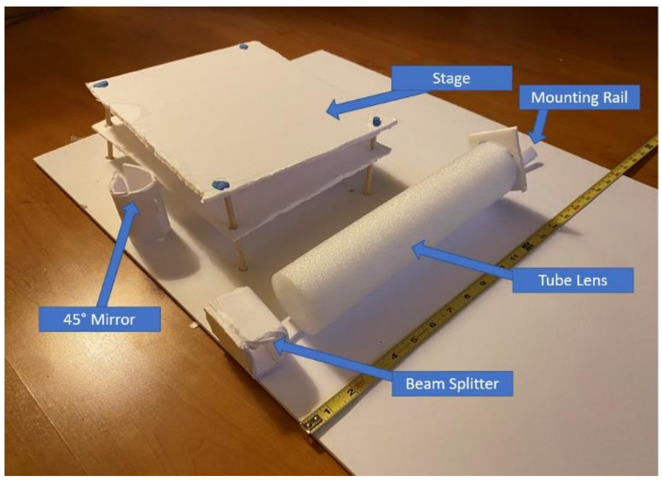


Figure 10. Proposed optical component layout prototype.

Two weighted decision matrices were developed to compare specification achievability for various orientations of the microscope on the breadboard, and optical components relative to the microscope. With the same specification weights for each matrix, the lowest scoring layout was the present microscope layout. The highest scoring layout is the first landscape layout, this is where the optical tube lens is runs parallel to the right side of the stage as shown in figure 10. This seems the most efficient layout, because less of the optical breadboard space is consumed and the stage faces out the long end of the breadboard as opposed to towards the center. Additionally, there is a large amount of space accessible for future fluorescence microscopy add-ons.

4.4.4 User Interface

For the user interface, we are referring to the way in which the user interacts with the functions controlled by the Arduino® microcontroller. As it stands now, these functions are accessed by a command line on a connected computer, and by a small joystick connected directly to the Arduino®. The first issue to be addressed is the command line, because it is overly complex to operate, and so a simple graphical user interface (GUI) was mocked up. This will allow for simplicity of use for a new user, but with a depth of functions for more experienced users. Once a GUI has been successfully implemented, there may be room for additional controls to be added, in the form of physical buttons, and a small LCD screen. This will allow for quick access to the most used functions, and easy access to important information.

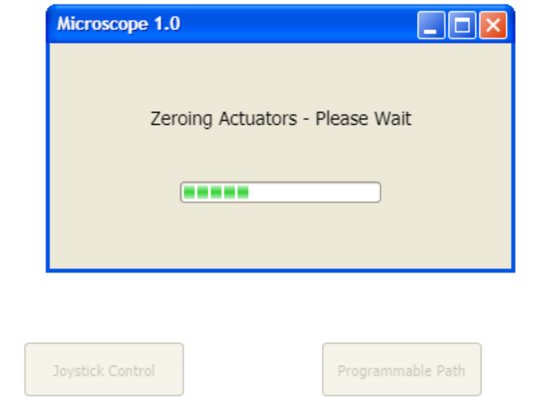


Figure 11: Concept prototype of the Graphical User Interface

4.4.5 Electronics Assembly

The previous team left the Arduino® and actuator breakouts connected via a breadboard, which worked for prototype-style testing, however breadboards are not very robust, and connections can be disrupted through improper handling. Our end goal for the wiring is to replace this breadboard with a custom printed circuit board (PCB), with an intermediary step of building a hardwired protoboard to test our wiring map and be able to perform testing in the meantime. Figure 12 shows the protoboard we created and the soldering map we followed. We still need to fully test this protoboard before we can connect it to the rest of the hardware to make sure we did not create any potentially damaging shorts.

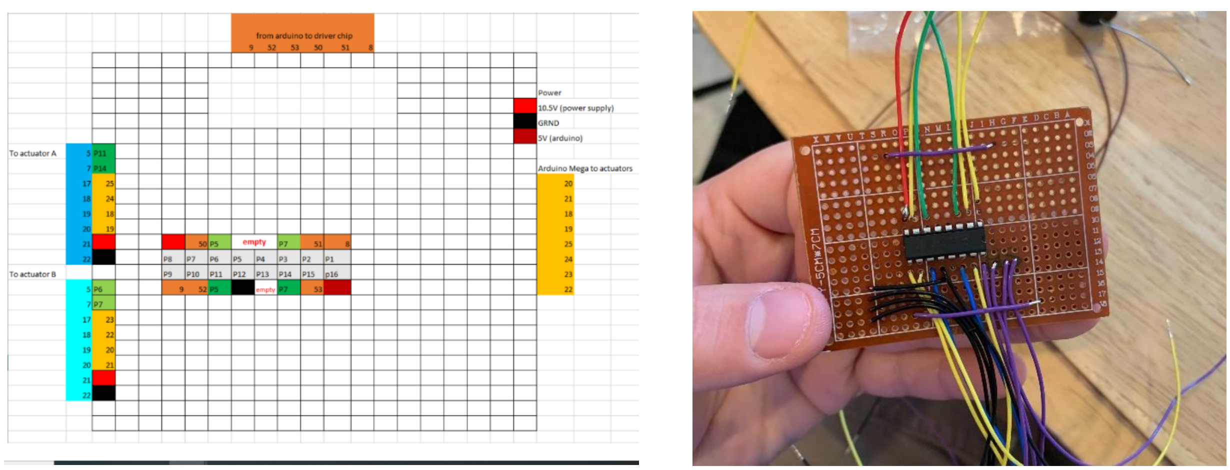


Figure 12: Protoboard (right) and soldering map (left)

4.5 Testing Plan

To solve the issues with the stage repeatability the error that is present in the stage needs to be quantified. Currently the stage in command line mode cannot form a square by entering commands of +X +Y -X -Y. This could be a result of the actuators not moving the stage the same amount in each direction or one actuator moving a different amount for each encoder count. These issues can be isolated through testing of the actuators.

To determine the absolute position of the stage a microgrid will be used. A grid with dot spacings of 0.060" will be printed on engineering paper and placed on the microscope stage. The camera will be turned on a screenshot will be captured after each actuator movement. These images will be compared and the known spacing of the dots will be used to quantify the actual movement of the stage. These actual movements will be compared to those sent by the Arduino® control system to determine the error.

The stage will be tested over various actuation lengths to determine if the encoder has an error that accumulates over a long distance. The stage will be actuated in all four quadrants to determine if the actuators experience errors at extremes of their movement. After these tests the actuators will be swapped, and the tests repeated to determine if an error is local to a particular actuator or if it is local to the stage. Once the error is characterized, we will be able to determine the appropriate action to take. After meeting with Eduardo who wrote the code for the IFM last year we have a suspicion that actuator B is moving slower that actuator A for a given input voltage meaning that at the extremes of stage movement the actuator is not moving its prescribed distance. These testing procedures can be seen in Appendix I

4.6 Results and conclusions

After analyzing the results of the WDMs we decided on our best solutions for each subsystem. As discussed in section 4.4 the subsystems were all mostly independent so the choice of one optimal subsystem did not exclude others. For the lighting there are two solutions that will be developed in parallel. Reinforcement of the existing 80-20 structure or an on-stage mount for the fiber optic light source. For the electronics enclosure, the chosen design was a box with a hinged lid. This design offers a lot of flexibility both in where the connectors are placed on the box as well as where and in what orientation the enclosure is placed on the stage. This flexibility is great because the stage layout selected with the tube lens tucked underneath the stage means that for a low footprint the enclosure might have to be mounted vertically next to the stage or horizontally under it. For the GUI the decision was made to proceed with the joystick and GUI on the computer, forgoing the extra displays or buttons for a single screen for the user to look at with all of the information that they would need.

Designing for safety of end users and team members in all facets of this project is the first and foremost priority of our team. Presently, no designs considered add any notable risks to the microscope that do not already exist. The only existing notable hazards are fall hazards of the optical breadboard, and the potential energy stored in the spring returns when extended. Another minor risk is pinch point creation when the spring returns are extended. All these risks can and have been easily mitigated, but plans are also in place to ensure there is little to no risk in use of the final microscope revision.

5 Final Design

To solidify design choices, the Final Design chapter presents detailed descriptions of the chosen design direction, justification of design choices, and relevant detailed analysis. While the practical implementation of explained designs is saved for the Manufacturing Plan, further system safety, maintenance, repair, and cost considerations are summarized here as well.

5.1 System Redesign

For satisfaction of the key objectives outlined in the Objectives chapter, we have chosen to perform full and partial redesigns for all sub-systems of the microscope. The electrical subsystem will be modified with a new printed circuit board, a 3D printed enclosure, and a new mounting point below the microscope stage. To modify the optical and mounting systems: the optical components will be reconfigured on the optical breadboard; the full microscope assembly will be translated; and the brightfield will be repositioned on a redesigned gantry-style frame above the stage. To fix the coordinate repeatability and improve its position, a new control method will be implemented to the appropriate functions of the Arduino® code. Each individual adjustment mentioned is described in much greater detail in the individual subsystem headers below. Designs chosen for full detailed documentation, are those which were outlined as most critical "needs" by our project sponsor. Additional system designs and additions from the "wants" category permitted by time will be documented within the design changes subsection.

5.2 Electrical Subsystem

For satisfactory footprint reduction, environmental resistance, and general design robustness, the electrical system has been redesigned. In its initial state, the IFM electrical sub-system consisted of an Arduino® Mega micro-controller, a breakout board for each of the two linear actuators, and an electrical breadboard with an L293Da TI-L293D motor-driver IC. All components were loosely connected and spread across the optical breadboard. While functional, the next step in producing a lab-ready microscope is to establish secure and permanent connections and protect electrical components from misuse. This will be accomplished by replacing the electrical breadboard and breakout boards with more permanent electrical components and housing them beneath the microscope stage. Details and justification of these decisions are presented in the sections below.

5.2.1 Electronics Assembly

A PCB was chosen to facilitate more environmentally secure electrical connections, simpler electrical troubleshooting, a cleaner wiring setup, and enable physical mounting to an electronics housing. Previously, the L293 motor-driver is soldered to a protoboard with additional solder connections between the Arduino® microcontroller, two 25-pin actuator connection breakout boards. The protoboard was implemented as a structural prototype to ensure wiring diagram accuracy. This intermediate step gives proof-of-concept for printed circuit board (PCB) functionality.

There are three categories of electrical connections present in the PCB CAD model, figure 13. Traces connecting pins from the microcontroller ("AM" solder pads) directly to the actuator breakout connections ("A" and "B" pins respectively for actuators A and B). There are traces connecting the microcontroller and actuator breakout connections to the L293D chip in the middle, and there are traces providing power supplies from the microcontroller and external power supply to the chip.

The Arduino® connects to the "AM +5[V]" power supply and "GND" to provide the initial pulse-width modulation signal. The external power supply connects to the "+VCC" and "GND" for the desired scaled signal size. The motor driver handles the upscaling of the signal using the signal of the 5[V] power source and the scale of the external power source to drive the linear actuator motors. At this point, the external power source is a variable voltage power supply to enable simple testing. This power supply could be replaced in the future with a fixed voltage source without requisite PCB design. The school-year the PCB was designed: "2020-2021," and "REV. A" are included at the base of the PCB for clarity if other boards are designed in the future.

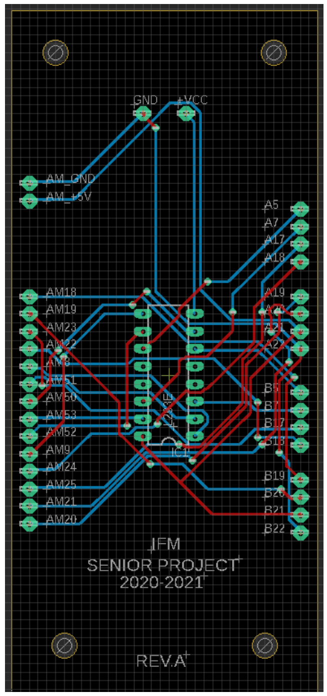


Figure 13 Eagle CAD PCB model.

5.2.2 Electronics Enclosure

Typical microscope operation will be in a clean room; because of this, significant weather, dust, and corrosion protective measures for the IFM electrical sub-system are unnecessary. However, even with a PCB, general use with exposed circuitry could result in component damage, and subsequent loss of microscope functionality. To combat this, an electronics housing will be used store the electrical components safely underneath the microscope stage.

As presented in initial design ideation, a simple hinged-lid custom housing was chosen to enclose the electronics. The custom housing has been design based around several key constraints. These consist of space available beneath stage, component connection orientation, and general accessibility. To determine a maximum available size, we used the existing microscope SolidWorks® model produced by the previous senior project team to create a maximum size. The maximum housing envelope is 8x4 inches of footprint and 3 inches of vertical space. These dimensions give adequate clearance to the four optical posts that support the stage, allowing the electronics housing to be easily removed for access if needed. Figure 14 below shows maximum sized electronics enclosure in green beneath the microscope stage.

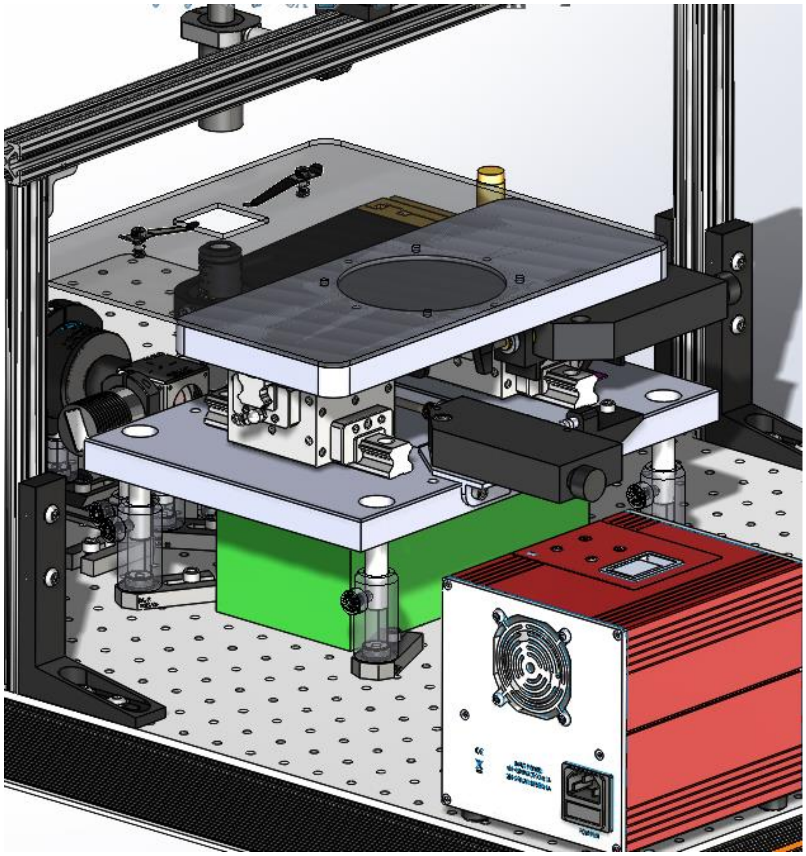


Figure 14. Maximum envelope of space for electronics packaging.

Once the maximum envelope of the enclosure was determined detailed design work began by development of a SolidWorks® model created in coordination with the PCB. electronic components have 3.5-3.6 [mm] mounting holes, allowing for mounting with standard M3 machine screws. These holes were used to locate standoffs that allow for rigid mounting of the 4 essential electronics components, the Arduino® Mega, PCB, and two 25 pin breakout boards. A screenshot of the enclosure's CAD model shown below in Figure 15.

The positioning of the PCB in the middle of the enclosure was consciously chosen such that connections from one side of the microcontroller to the actuators can be embedded within the PCB. This minimizes the wire lengths needed and will prevent complicated wiring in the housing.

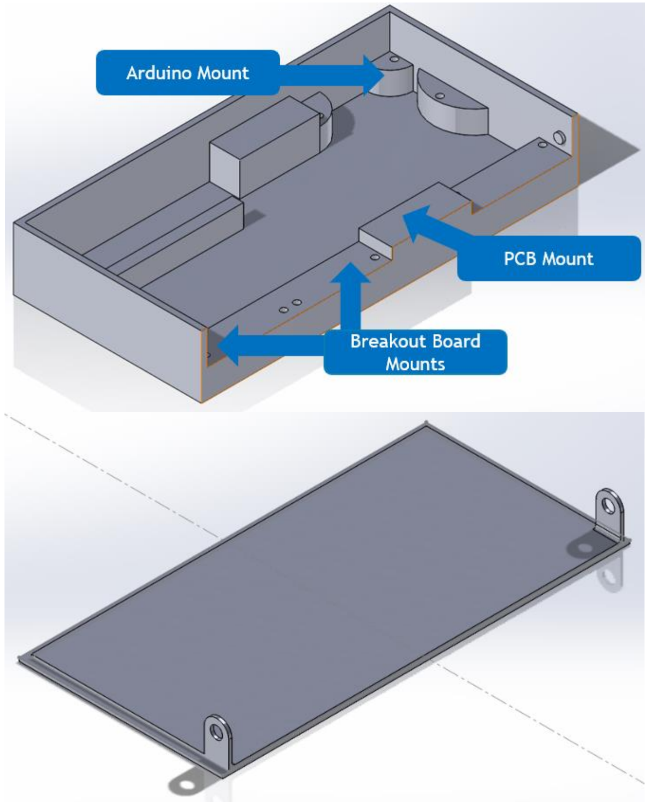


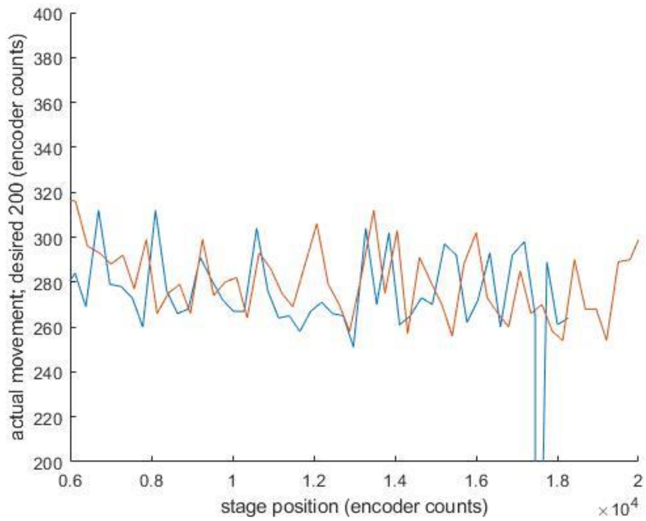
Figure 15. SolidWorks® electronics housing CAD model.

5.3 Actuator Controls

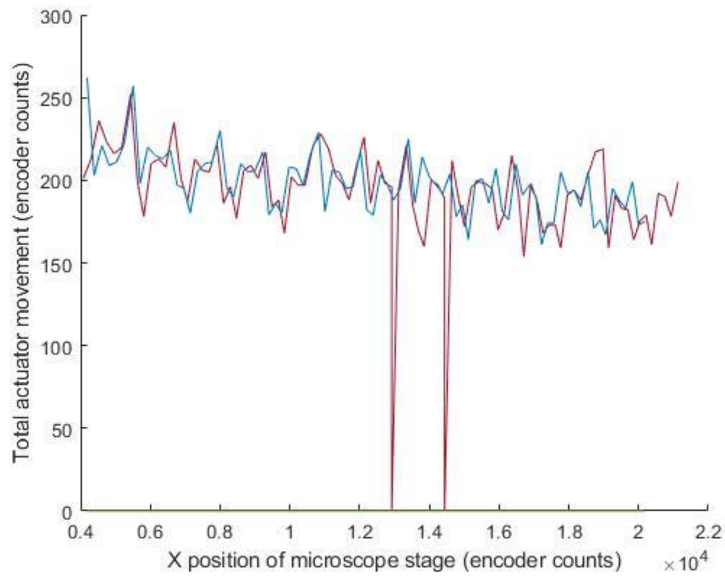
Currently, the actuators are controlled by an Arduino® C++ script written by Eduardo Miranda from the previous year's senior project team. The actuators function in two modes; joystick mode, and coordinate control mode. In joystick mode a manual joystick module is used by the operator to coarsely actuate the stage in its four principal directions. The joystick is excellent for simple stage positioning to look at a particular feature, but difficult for finely adjust the image or moving the stage a known distance. To move the stage a set distance, the programmable coordinate control (PCC) uses the serial interface of the microcontroller to move a specified axis (x or y), a fixed number of rotary encoder-counts.

The previous team's PPC code is functional but has room for improvement. For starters, encoder-counts are not a useful measurement to an operator, only to the actuator encoder. Positional feedback in either millimeters or micrometers would match the scale of microfluidic devices. To convert between the two-unit systems, a conversion from encoder counts to microns in the actuator specifications will be used and checked via physical testing as discussed in the Design Verification chapter.

The second and central area for improvement lies in the repeatability of the microscope actuation in PPC mode. When prompted to move a specified number of encoder-counts, the actual position of each microscope axis is always greater than the requested movement, causing the stage to overshoot the target. These error trends are highlighted in figures 16 and 17.



<u>Figure 16. Data from Y position error repeatability test.</u>



<u>Figure 17. Data from X position error repeatability test.</u>

Figure 17 shows the results of two repeatability tests across the full actuation range of the X axis travel. If the repeatability were within specification, the results of the test would be a horizontal line at 100 encoder counts. Every time the encoder moves 100 counts, the position change would be consistent. The outliers presented as significant spikes in the data can be accredited to typos in PCC during repeatability testing. In both two separate trials, when prompted to move 100 encoder-counts, the actuator consistently overshot by 75 microns. This falls outside the desired repeatability specification of 10 microns for each axis. However, this error is remarkably consistent across the trials. The maximum deviation during the test was 15 microns, much closer to the repeatability specification.

Initially, it was assumed the repeatability error was not consistent enough to code out, and linear encoders would need to be added to the system to meet specification. After analyzing the cost of linear encoders, it was decided to better quantify the nature of the error. Because the error is predominantly consistent, we have made the decision not to add linear encoders to the system. Linear encoders with a resolution of less than 10 microns would use almost all our remaining budget, and likely not provide much better repeatability than eliminating the error using the code to correct the overshooting.

To correct the repeatability error, the consistent error from the target actuation distance will be subtracted from the prompted code. Further, implement of a slow-down as the actuator approaches its target will reduce the tendency to overshoot. Details of code implementation are further described in the Manufacturing Plan chapter.

5.4 Optical Component Placement

In coordination with one of the central project goals: microscope footprint reduction, the microscope will be reconfigured on its current optical breadboard. The staging area will face the long side of the 2’x3’ optical breadboard. This reconfiguration will result in less space taken on the current optical breadboard and enable more convenient microscope positioning in the microfabrication lab. Depending on if one is available in the lab, the full microscope will be transitioned to a smaller optical breadboard.

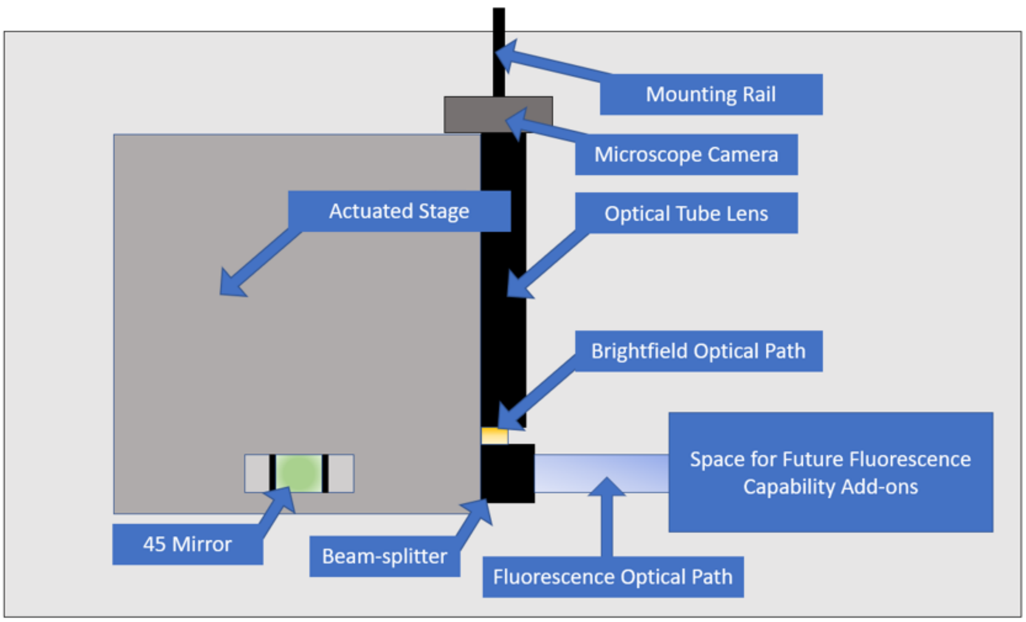


Figure 18. New optical component layout.

Figure 18 above shows all the optical components that would require repositioning on the optical breadboard to achieve full microscope rotation. Fortunately, the previous senior project team chose to purchase infinity corrected lenses for each of the optical components in the diagram above. Infinity corrected lenses are designed such that the light rays are near perfectly parallel, and not have a specific focal length. This means that with proper alignment, components could theoretically be infinite distances from one another and maintain functionality. Because of this, no optical analysis is necessary to determine the distances between components. It is possible that with the reconfiguration, the dovetail mounting rail will hang off the back of the optical breadboard as shown above. Should this be the case, the rail can be cut, replaced with a shorter rail, or positioned accordingly in the lab.

In addition to reducing the microscope footprint, the optical component reconfiguration will provide space for future addition of a fluorescence LED source, and a dedicated user interface device.

5.5 Brightfield Illuminator Repositioning

To further reduce the footprint of the microscope, the fiber-optic brightfield illuminator will be mounted above the stage using 80-20 modular hardware, and a new fiber-optic cable of more appropriate length will be purchased.

Because sample view will be on an external monitor, there is no need to maintain the ergonomics of a traditional microscope with an eye-piece. Also, by locating the Illuminator above the stage, it will be less likely for a user to bump the light source and change the illumination of the sample. A CAD model for visualization of the gantry-style 80-20 structure is shown in figure 19 below.

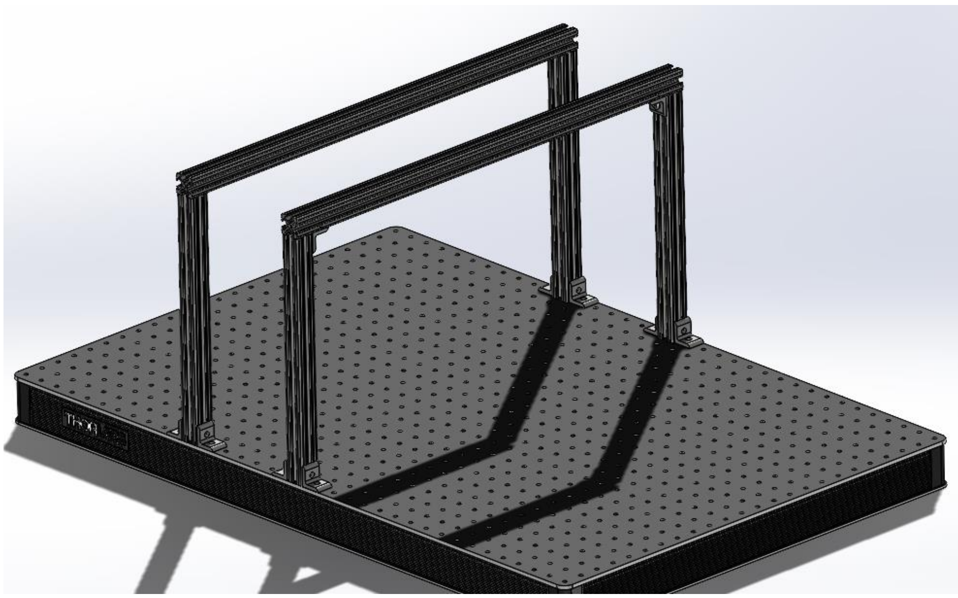


Figure 19. mock-up of the new brightfield illuminator mount.

5.6 Final Design Considerations

While microscope functionality and performance in the lab a focal point of our designs, safety is always top priority. The following sections discuss alternative critical aspects of the chosen design direction such as safety, maintenance and repair, and a cost analysis.

5.6.1 Safety

The two largest safety concerns when using the microscope are the potential pinch points present on the stage, and the potential for heavy components to fall. To ensure the microscope is safe, the Design Hazard Checklist presented in Appendix J has been updated since CDR to reflect hazard consideration of changed designs. Additionally, Appendix K presents a Design Failure Mode and Effects Analysis, which ensures cautious consideration to potential failure mode and repercussions have been considered.

The potential pinch points discussed in the Design Hazard Checklist near the bearing blocks that support the actuators and, on the actuator-to-stage interface, where springs are extended and retracted. To reduce the likelihood of fingers getting caught, the stage will be positioned such that the stage positioning location is well away from potential pinch-points. Also, to move the actuators, input is required either through the joystick or the command line interface. This means a single user cannot easily have his or her hands on the microscope and actuate the stage at the same time. This advantage of the current system will need further consideration when automated stage control is implemented. When two or more people are using the microscope, appropriate lab procedures will ensure that when the microscope is being used someone will not have their hands near the microscope's pinch points.

The next significant design concern is the potential for the 8 [lb] brightfield illuminator housing to fall when mounted above the microscope. To combat this, the illuminator housing will be rigidly attached to the 80-20 aluminum frame. This will prevent instability caused by bumping the component or microscope bench. To prevent some of the heavier components from falling, the microscope will be located on a bench in the Cal Poly Microfabrication Lab, and all components will be secured from axial motion on top of the bench.

Additionally, end caps will be purchased to cover exposed ends of the 80-20, which sometimes have sharp corners. This would eliminate a potential cut and scratch hazard, making it safer to lean over the stage.

5.6.2 Maintenance, and Repair

The maintenance intensive items of the scope include lubrication of the slides with grease, maintaining the cleanliness of the optical components, and periodic repeatability checks. There are no consumable items present in the IFM. Because operation is in a clean room environment, the frequency of grease addition and optical component cleaning is significantly decreased. The optical components should be dusted once a quarter, and the slides should be greased every year. These maintenance items will be included in the operating manual of the IFM. With the addition of new stage actuation controller, a calibration procedure has been developed and placed in the user manual, found in appendix O. After running reliability tests to quantify error, we determined that the actuator movement error was related to the return spring force and the speed of actuator operation, which is set in the code. To remedy this, we added a speed differential function to the code, causing the actuators to slow down as they approached their target coordinates. We also determined that while the stage cannot be moved to an exact set of coordinates, it does accurately report the coordinates of its current position using the linear encoders embedded in the actuators.

If the microscope becomes damaged and needs repair, the documentation provided by the senior project provides a parts list in case a component is damaged and needs to be replaced. Because most component additions are to be 3D printed, and full CAD models are given, part replacement will be simple to do in-house at Cal Poly. Since the electrical sub-system is most prone to damage, two extra PCBs have been ordered to subsidize the initial purchase and provide backups in case of damage.

The highest risk components in terms of costly repair are the optical components and the actuators. Because the actuators are on the older side, they may be difficult to replace with the original part. However, the existing electrical controls system and new 3D printed mounts could be retrofitted to new linear actuators with internal rotary encoders if necessary.

5.5.3 Cost Analysis

Because most high-cost optical and electrical components have already been provided by the Cal Poly Microfabrication Lab and the previous IFM senior project team, our team has not had many major expenses. Some notable expenses include the anodized 80-20 frame and mounting hardware, which were purchased from McMaster-Carr, and the PCBs, which were ordered from Oshpark. Table 8 presents tracked budget spending and total costs throughout the course of the project. Several components not reflected in the Indented Bill of Materials exist in this table, because several components were ordered that were not expected within the outline of the initial design. This table has been updated since CDR to reflect full budget spent, what it was spent on, and how much is remaining.

Table 8. Budget tracking and order sheet.

(Quantity) Product	Cost (Full Quantity)	Additional Notes	Seller	Order No.	Budget Remaining After Purchase
Shop Cart	$60.00	24" x 36"	Harbor Freight	1	$1,400.00
(10) Protoboard	$5.99	5 x 7 cm	Amazon	1	$1,394.01
(10) DB37 Connectors	$9.75	solder cup connections	Amazon	2	$1,384.26
T-Slotted Framing	$15.58	Single Four Slot Rail, Silver, 1" High x 1" Wide, Solid (2 ft length)	McMaster-Carr	3	$1,368.68
(2) T-Slotted Framing	$22.10	Single Four Slot Rail, Black, 1" high x 1" wide, Solid (2 ft length)	McMaster-Carr	4	$1,346.58
(4) T-Slotted Framing	$26.92	Single Four Slot Rail, Black, 1" high x 1" wide, Solid (1 ft length)	McMaster-Carr	4	$1,319.66
(4) T-Slotted Framing	$4.80	End Cap for 1" High Single Rail	McMaster-Carr	4	$1,314.86
(12) Black Corner Bracket	$67.20	1" Long, for 1" High Rail T-Slotted Framing	McMaster-Carr	4	$1,247.66
(12) Silver Corner Bracket	$62.52	1" Long for 1" High Rail T-Slotted Framing	McMaster-Carr	4	$1,185.14
(4) T-Slotted Framing	$24.76	Antislip Leveling Mount for 1" High Single Rail	McMaster-Carr	4	$1,160.38
(2) Dovetail Rail Carrier	$50.00	N/A	Thorlabs	4	$1106.50

(Quantity) Product	Cost (Full Quantity)	Additional Notes	Seller	Order No.	Budget Remaining After Purchase
(1) Custom PCB Prototype	$40.00	N/A	Osh Park	5	$1,066.50
(1) Dupont Connector Kit	$15.99	N/A	Amazon	6	$1,090.51
(4) Ti-l293D Motor Driver Ics	$16.44	N/A	Mouser Electronics	6	$1,074.07
(1) Braided cord Sleeve	$7.50	N/A	Amazon	6	$1,066.57
(4) T-Slotted Framing End Caps	$4.80	N/A	McMaster-Carr	6	$1,061.77
Heat Shrink Tubing	$3.99	N/A	Amazon	6	$1,057.78
Female Barrel Connectors	$4.84	N/A	Amazon	6	**$1,052.94**

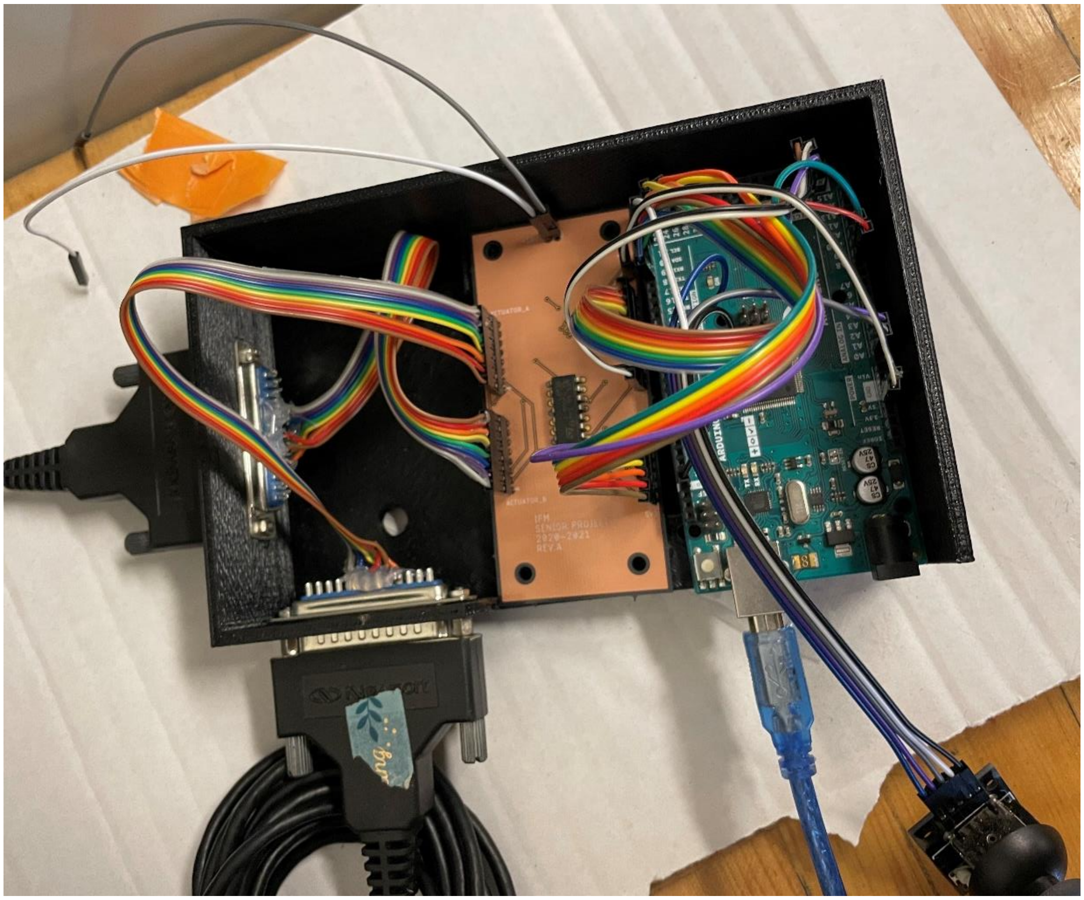
A full Indented Bill of Materials is listed in Appendix L with previously expected costs, there has been no overrun of budget. This excess budget enabled addition of lower priority improvements, like anodizing the stage and purchasing, and will enable purchasing of a dedicated computer for the microscope's operation after the completion of our project.

5.7 Design Changes after CDR

While the design direction remained the same throughout the project, there were some changes made to specific elements of the final design, especially the electronics housing. Since CDR: the PCB layout was revised as discussed in the manufacturing section; the variable voltage power supply was removed and replaced with a laptop power supply; the 3D printed enclosure was modified for better fit; the microscope was transitioned to a smaller optical breadboard; and a GUI was fully developed for simple joystick mode. Greater detail of design implementation for those changes mentioned above are captured in the Manufacturing Plan chapter.

After 3-D printing the electronics enclosure as planned, it became apparent that a second revision was necessary. The hinges were not sturdy enough and broke immediately after printing, so they were eliminated from the final design. The rabbet along the edges of the lid was deemed sufficient to secure the lid. Also, the Arduino® and PCB were located too close to the edges of the enclosure to insert machine screws, so the wall thickness was reduced to accommodate them. This also reduced some of the warping that was in experienced printing the first enclosure. Additional holes were added prior to printing for rigid mounting to the optical breadboard. Finally, the size of the enclosure was reduced as we determined that it would make more sense to store the long cords of the actuators outside of the enclosure. A picture of the revised housing with electronics installed is included in Figure 20.

Additionally, the optical breadboard configuration and microscope layout presented in the Critical Design Review was insufficient for a smaller breadboard. More information is provided to full breadboard transitioning in the Manufacturing Plan chapter.



<u>Figure 20. The second revision of the electronics enclosure.</u>

6 Manufacturing Plan

The Manufacturing Plan chapter describes our team's material choices, manufacturing processes, and iterative design testing procedures selected to achieve the desired microscope specifications. This chapter has been updated to describe practical design changes made to the initial plan to complete our verification prototype.

6.1 Repeatability Error Correction

To account for the stage repeatability error, we chose to modify the existing closed-loop control model for stage actuation built into the backend code. The current model reads the serial input of the desired movement and adds that value to the existing encoder position to create a target encoder value to reach. The motor then linearly actuates the stage while the Arduino® continuously checks the rotary encoder for the target value in a while loop. This while loop originally ran with a delay of 0.1 seconds between each iteration. Once the target value is reached, power is cut to the motor and the Arduino® reports the actual position of the encoder. This initial design resulted in consistent target overshoots.

The new control scheme uses a similar closed-loop control method, but with some key changes to the actuator speed control. Because the microscope uses a spring return for the stage, the force required to move the stage a given distance increases as the spring extends. To account for this, the microscope controller uses a speed differential function to adjust the actuator voltage for the linear spring force. During repeatability testing, we found the positional overshoot was more significant when the spring was less stretched, and less significant when extended. This indicated that the speed differential function was not calibrated correctly.

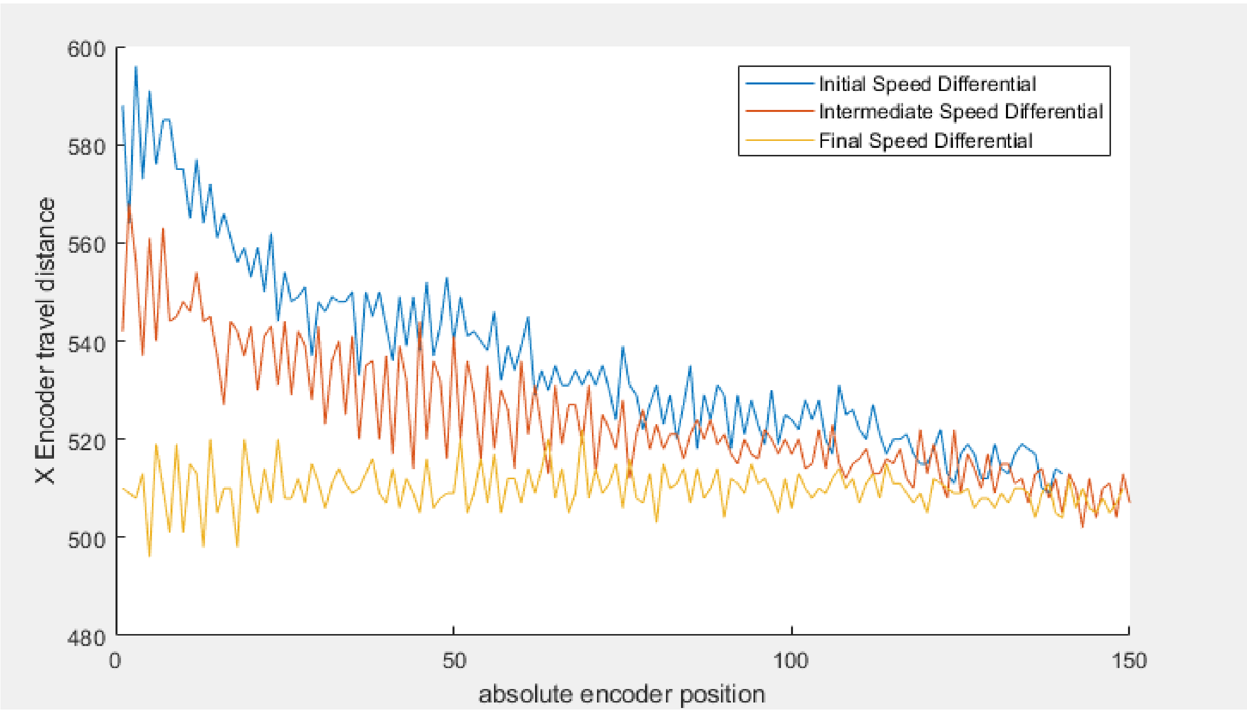


Figure 21 Error Reduction through Speed Diferential Function

Figure 21Figure 20 above shows the original speed differential function repeatability results in yellow, an intermediate in orange and final function repeatability results in blue. This graph is of total positional error versus the location on the microscope axis. The error of interest is represented by the steepness of the line in the early region, a perfect speed-differential would result in a perfectly horizontal line with consistent error over the entire distance of actuation. What this indicated was that early in the travel, the actuator was accelerating faster than the rate change in force applied by the spring. Manipulating this speed differential through testing nearly eliminated this contribution to total repeatability error.

Additionally, the sample rate of the encoder was increased by decreasing the delay of the while loop. This allowed the encoders to report position in smaller increments, decreasing the potential for overshoot of the target value. Figure 22 below shows a moderate decrease in overshoot error as a function of the decreased delay in the while loop. The yellow line on top is the original error as a function as a function of total encoder distance. As in the previous graph, a perfectly tuned control scheme would result in a horizontal line.

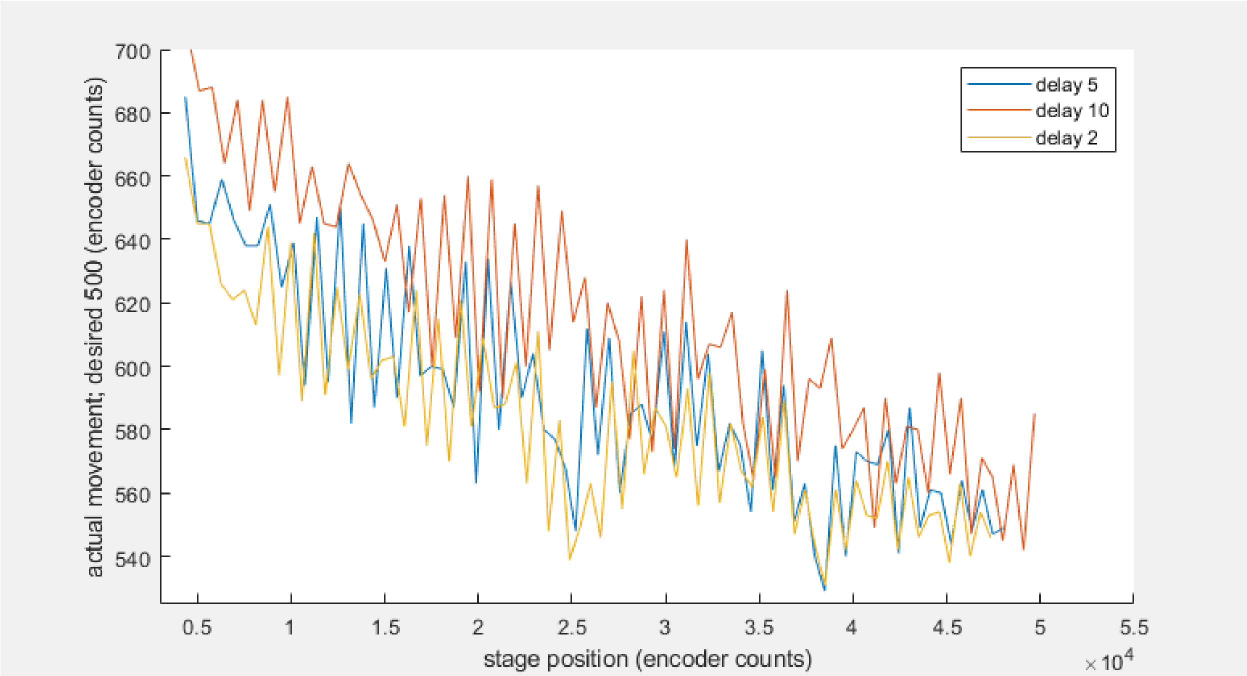


Figure 22 repeatability error reduction with delay

You can see as the delay is decreased from 10 with the yellow line to 5 with the blue line and finally red with the 2 microsecond delay, the error is indeed decreasing but from 5 to 2 microseconds the change is negligible, so the delay was not reduced further.

The control scheme was not modified in the positive direction as satisfactory repeatability numbers were realized by modifying the speed differential function and reducing the delay. However, in the negative direction the actuators continued to overshoot by an unacceptable 90 microns. To combat this the negative control scheme was modified so that after actuation a correction is made in the positive direction. This resolved the excessive error in the negative direction and increased the overall useability of the microscope. Figure 23 illustrates the corrective measures added to the code for actuation in the negative direction.

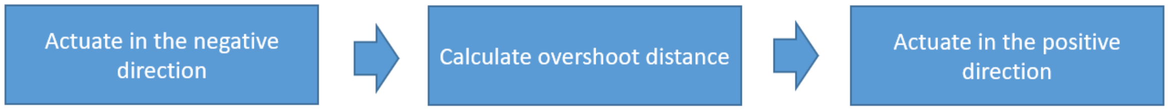


Figure 23 new negative direction actuator control flowchart.

6.2 Electronics Assembly Manufacturing

The electronics hardware for the IFM stage control consists of the two actuator breakouts, the Arduino® Mega controller, a simple dual-potentiometer joystick, and an IC controller board. The components all interact with each other through a central wiring harness which was updated from a bread board, to a protoboard, to the final design iteration which uses a custom PCB. We created this PCB to replace the protoboard for increased system durability and ease of component replacement in the case of failure. Figure 12 from structural prototype development displays both the intermediate soldering map (left), and the finished proto-board product (right). After several successful test sessions with the IFM using our wiring map, we determined that a printed circuit board (PCB) can be functionally integrated. A sample wiring map of the whole electronics assembly can be seen in **Error! Reference source not found.**. It s

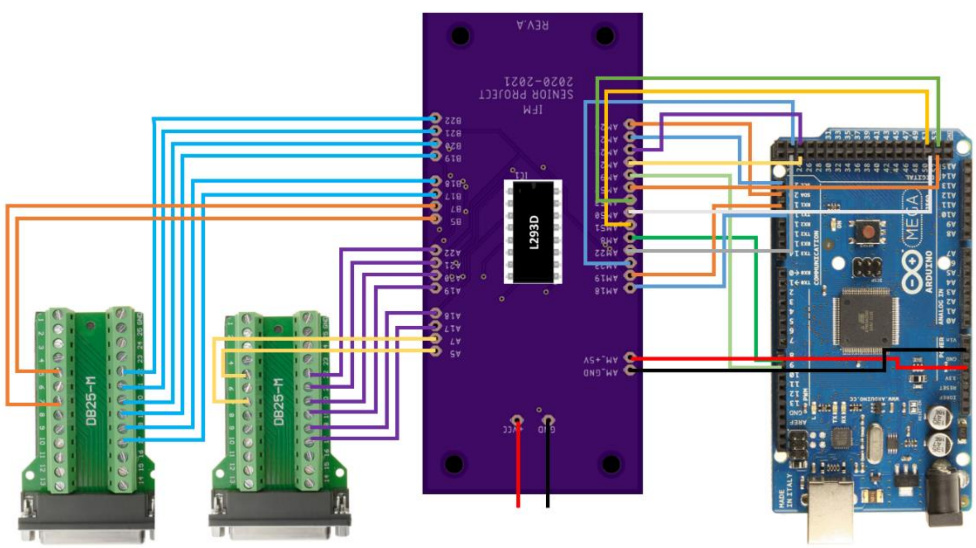


Figure 24. Wiring map of electrical subsystem.

hould be noted that **Error! Reference source not found.** does not represent the completed wiring diagram; we were able to bypass the actuator breakouts by soldering wires directly to the exposed contacts of the 25 pin connectors, reducing the number of components and decreasing the size of the electronics enclosure.

We ordered the PCB from a domestic manufacture, OSH Park, to reduce the lead time. Due to the nature of ordering these PCBs, we were forced to purchase a minimum of three copies. This is beneficial to us because should a board be damaged during soldering or mounting, several spares exist. In addition to ordering these PCBs, we also ordered new ICs to replace the one we soldered into the proto board. This was not an issue as these ICs are available at a low cost with little lead-time necessary.

The PCBs arrived on time and as expected. We soldered the L293D driver chips to each of the three boards and also soldered pins to facilitate connections to the Arduino® and the actuator breakout connectors. our manufacturing process we were able to remove the actuator breakout boards in favor of directly soldering ribbon cables to the 25 pin connectors. We did this to reduce enclosure size and system complexity. We also constructed ribbon cables to plug into the pin breakouts on the PCB to making the wiring set up neater and to allow for easy disassembly. The custom ribbon cables and 25 pin breakouts are shown in Figure 25 below.

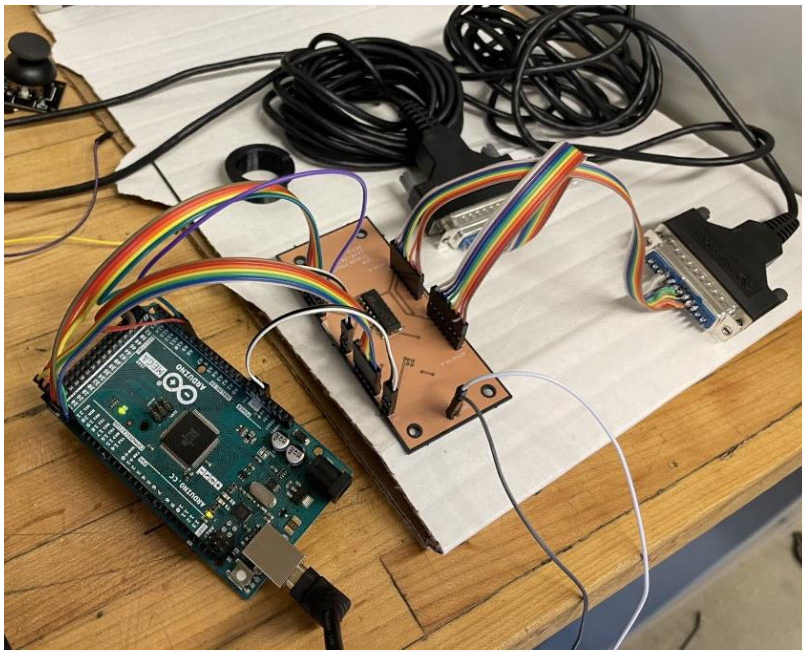


Figure 25 Completed PCB wiring with custom ribbon cables.

We verified that our wiring map was consistent with the existing functional microscope and wired the PCB into the electronics system. The microscope functionality was reduced after inputting the PCB so we began fault analysis. Knowing that our pre-existing wiring was functional we used a multimeter to verify that the connections from the Arduino® to PCB and then from the PCB to breakout boards were correct. Then we checked the PCB connections and determined that the L293D driver chip was not properly grounded to the rest of the electronics. We solved this issue by soldering a jumper wire from the ground pins of the driver chip to the 5V ground of the Arduino®. This ensured all components had a common ground. A figure of the revised PCB is shown below. After this correction, the microscope was once again at full functionality so we soldered all three boards with the jumper wire the position of which can be seen in Figure 26 below.

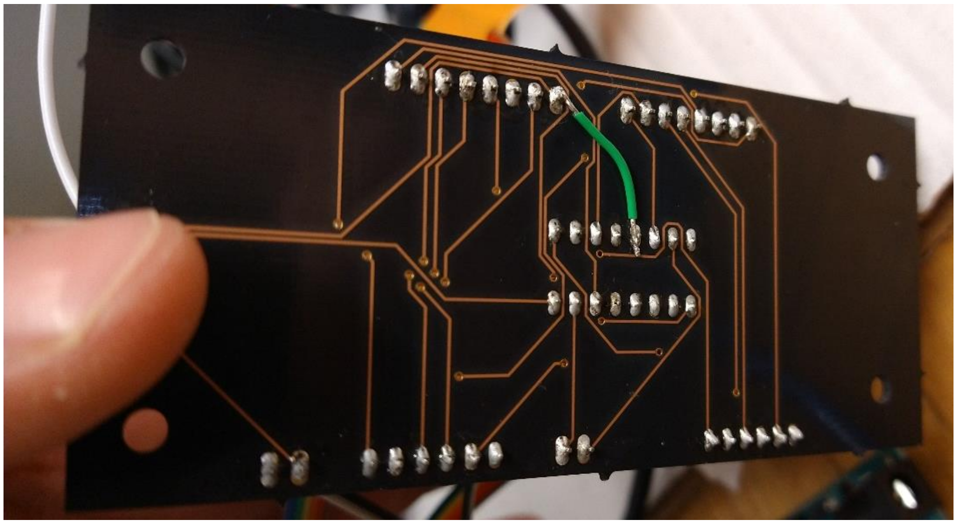


Figure 26 Jumper wire to resolve grounding issue.

6.3 Brightfield Illuminator Mount

When manufacturing the gantry-style illuminator frame, we highly prioritized light-source rigidity and frame stiffness. There were several issues present with the existing gantry, primarily due to flex in the 3D printed brackets used to connect the 80-20 frame to the optical breadboard. To improve the gantry, we replaced the 3D printed brackets with aluminum 80-20 brackets for the interface between the beams and the optical breadboard, providing a significant improvement in rigidity. We also added a third bracket on each leg of the gantry to resist rotation about the two independent axes. The cross bar of the gantry remains the same design, an 80-20 beam connected by a single bracket to both vertical supports.

To create a mounting platform for the brightfield illuminator, another cross-platform gantry like the original was added. Providing another point of contact for rigid connection will limit oscillation of the lighting system and prevent the potential for the light source to fall. Because the illuminator housing weighs only about 8 pounds, the solid aluminum 80-20 frame is more than capable of handling present loads. Figure 27 shows the updated gantry on the previous CAD model for the microscope. Because this model was developed prior to the transition to a smaller optical breadboard, the optical components and stage position are not accurate to the current microscope layout.

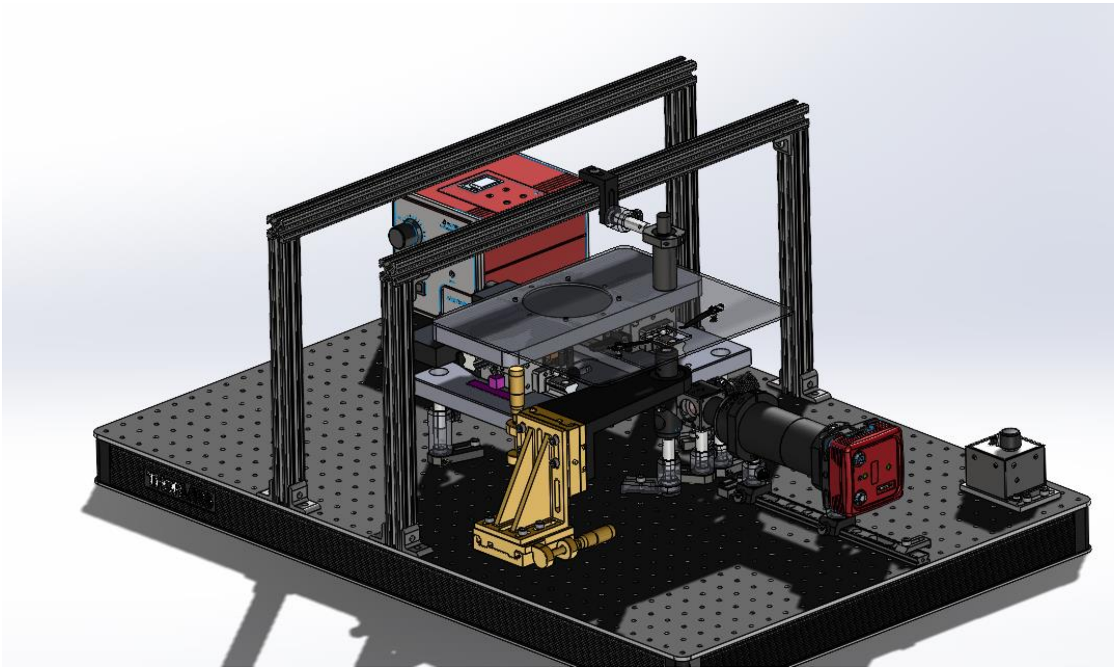


Figure 27 Integrated CAD of the new illuminator mounting.

Table 9. Additional Components for new gantry illuminator mount

Component	Quantity	Price per Unit (McMaster Carr)
80-20 Beam (6ft Length)	2 (Black Anodized)	$28.30 7.52
90 deg Bracket	14	$ 5.62
End Caps	4	$1.20

After communicating our plans to modify the gantry and purchase more 80-20 components to our sponsor, it was suggested that we purchase black anodized components as opposed to the current brushed aluminum. This would minimize any potential glare during fluorescence testing. Professor Mayer also provided us with a new subtask that can be pursued independently of other design decisions; finding a shop somewhere close to San Luis Obispo with the capability to anodize the whole stage black. We located a company in Santa Barbara called Neal Feay who offered to anodize our stage for free. They did a spectacular job, and the finished result can be seen in Figure 28. There is some dust on the stage in this photo, the anodizing is perfectly matte when clean. Anodizing all reflective components makes the microscope more conducive to fluorescence capabilities further along in microscope development.

The black 80-20 gantry was assembled as planned and the original 3-D printed brackets that attached the gantry to the optical breadboard were repurposed to secure the brightfield illuminator to the top of the new gantry. The final assembly is shown as Figure 28 below. Note the 3-D printed brackets that secure the brightfield illuminator to the gantry.

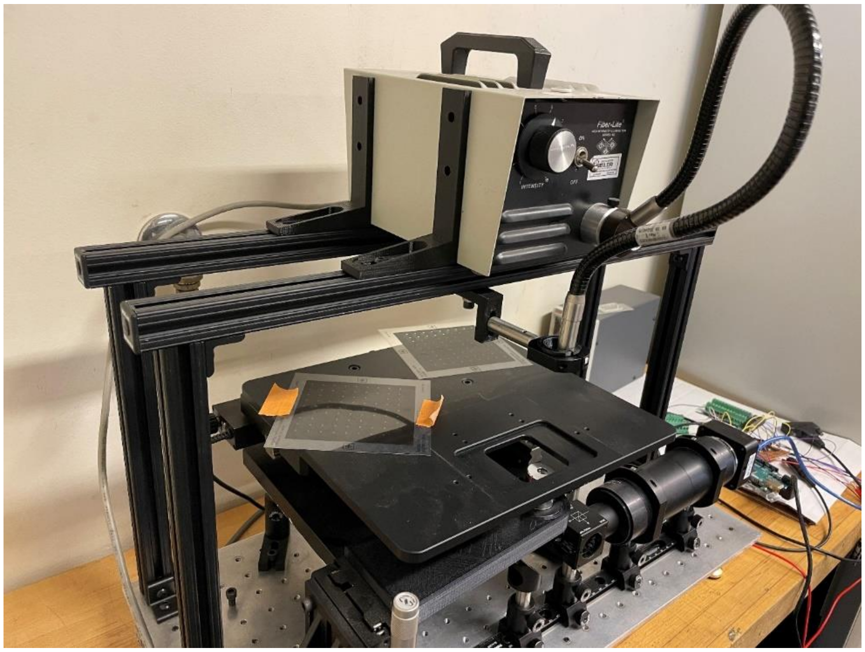


Figure 28 Completed Gantry Assembly

Once the brightfield illuminator was secured the fiber optic cable needed to be secured as well. An existing gooseneck was modified to position the fiber optic cable over the lens. A new 3-D printed bracket was designed to attach the fiber optic cable and position it directly over the sample for consistent and adjustable illumination. Another adapter was also 3-D printed and reduces the diameter of the gooseneck so the end of the cable will fit snugly. A detail photo is included as Figure 29.

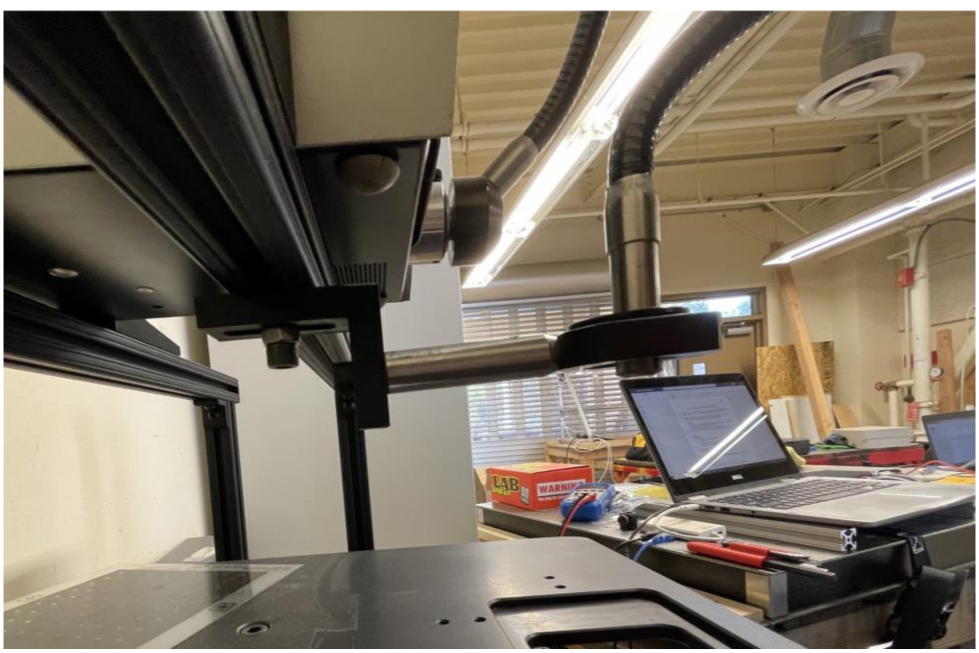


Figure 29 Detail of gooseneck

6.4 Footprint Reduction

One of our primary objectives was to reduce the footprint of the IFM so it would take up less of the valuable bench space in the clean room. We were able to achieve this by transitioning all the components from the original optical breadboard to a much smaller one for a reduction in footprint of 6 sqft down to 2 sqft; a 66% reduction. Transitioning to the smaller breadboard required some active problem solving outside of rearranging the components as described in the final design chapter. The new optical breadboard is a 1/4" aluminum plate with tapped holes spaced 1 inch apart in both axes. When the leveling feet were installed we noticed some slight bowing in the middle of the plate. This is illustrated in Figure 30, where we positioned a straight edge over the plate and measured the deflection to be 2mm. In order to keep our optical components aligned and maintain the standards of precision required for this project, we needed to resolve this issue.

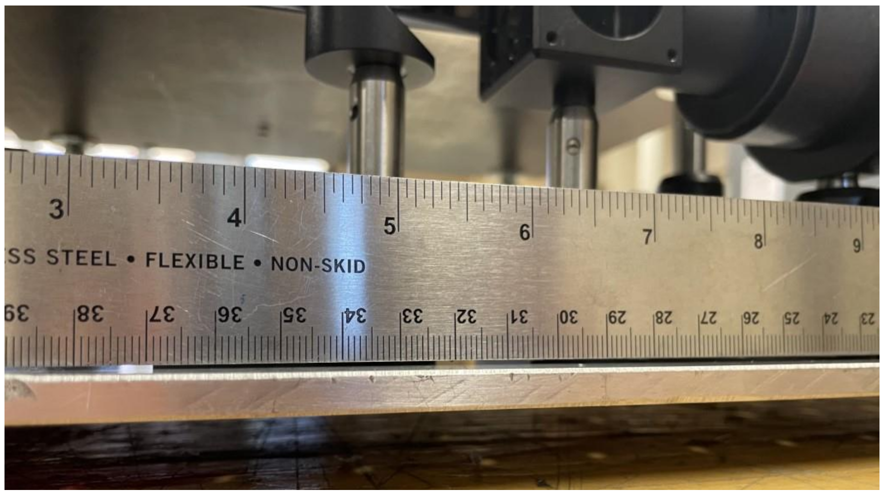


Figure 30 Deflection of un-reinforced breadboard

To address this unacceptable deflection, we added two 80/20 beams to the bottom to increase the plate rigidity by increasing the second moment of area along the axis of bending. The beams are connected at six points along the span of the plate, with two 90-degree brackets on either side of the beam at both ends of the plate and in the middle. A detail of the arrangement is shown as Figure 31. To ensure that we were not introducing any deflection, we supported the plate from the center by a wooden block during beam installation.

Figure 31 80-20 support beams in installed location

6.5 Joystick Housing and Cable

The simplest control method of the IFM, and the one most likely to be used in undergraduate labs, is the joystick control mode. The 2 axis potentiometer joystick with a center click button is connected to the Arduino® headers with female to male pin cables, which connect to the male pin headers on the joystick. The joystick hardware was mounted on an exposed PCB, which was fine for us to work with in the senior project lab environment but was not robust enough to meet our durability and professionalism standards for the finished project in the clean room. The joystick we are using is a high-quality clone of the very common and standardized PS2 joystick, so it was very easy to find existing joystick housings on the internet. To reduce cost and lead time we opted for a 3d printed hand-held version found on thingverse for free, which we printed in the mustang 60 shop, also for free. A ribbon cable was constructed in the same way as the ones used on the PCB and a sheath was added for abrasion and strain resistance. A detail of the finished joystick and its position on the stage is shown in Figure 32. The sheath and ribbon cable are secured to the inside of the joystick housing with epoxy so that no strain loads are transferred to the connection points, and the other end of the cable is secured to the bread board with a 3D printed plate, for the same reasons. Brief qualitative testing of this cable system determined that it was able to survive significant accidental tension loads without any damage.

Figure 32 Detail of the joystick and connecting cable.

6.6 Graphical User Interface (GUI)

Because other major goals were accomplished for this project in a timely manner, we were able to develop and incorporate a functional graphical user interface as shown in Figure 33 below.

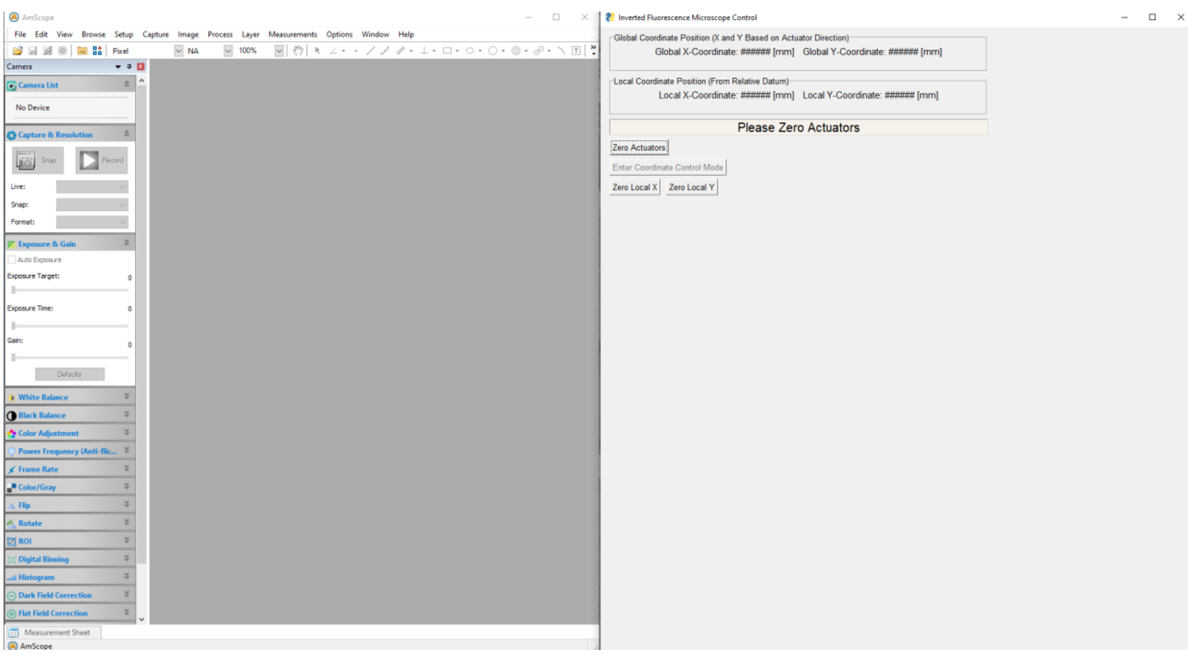


Figure 33. GUI and AmScope® on startup.

The graphical user interface developed for the microscope control was made in Python using the PySimpleGUI library that uses python native and tKinter libraries to enable simple graphical user interface development. The IFM controlling GUI operates using Serial port of the computer controller to communicate with the code Arduino® code. This required restructuring of the Arduino® code to incorporate a finite state machine. Another finite state machine was developed within the GUI code. Both the modified Arduino code and GUI make use of a central communication state where critical information for operation is sent and read. This information is then sorted appropriately and informs the code what functional state to drop into.

At the time of project conclusion, the GUI for the microscope can zero the actuators, launch joystick mode, and function with full coordinate feedback in mm. Both a live global and local coordinate system give detail about the position of the microscope. To begin, the local coordinate system is matched to the global coordinate system. To measure features, or move based on known relative geometry, buttons “Zero X Coordinate” and “Zero Y Coordinate” enable the ability to set a datum at a particular point in either the X or Y axis of the microscope.

6.7 Outsourced Components

Most of the manufacturing work was performed in house by the IFM team, with use of custom 3D printed parts and existing components. We did however completely outsource the manufacturing of our PCB. It is not within the scope of this project or the Cal Poly machine shops to custom manufacture PCBs, so we have chosen Oshpark as an external vender. Oshpark charges five dollars per square inch of PCB for three PCBs, guaranteeing shipping after 12 days. Because the microscope was fully functional with the proto-board, this lead time was not an issue towards other project progress. We also outsourced the anodization of the existing microscope stage to reduce reflection. Like the PCB manufacturing, anodizing is not a manufacturing operation supported by the Cal Poly machine shops so will be outsourced to the vendors citing in Table 10.

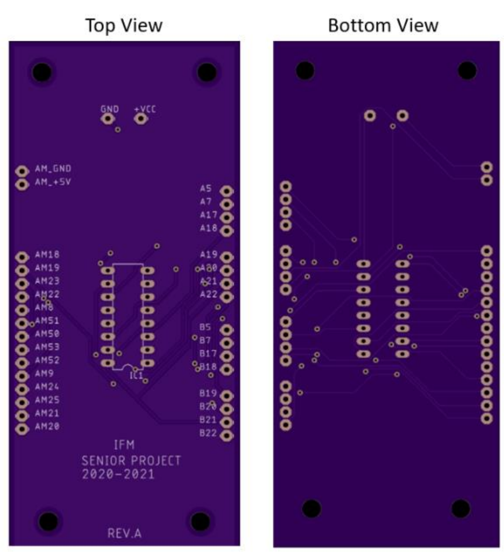


Figure 34 Outsourced Oshpark PCB model

Table 10. Anodizing Venders

Company	Location
Elite Metal Finishing LLC	Oxnard
Neal Feay	Goleta
Advanced Powder Coating and Sandblasting Inc	Paso Robles
Western Metal Refinishing	Oceano

Table 10 displays a cursory list of shops we inquired with to get quotes for a custom one-off anodized part. We ended up going with Neal Feay, who did an exceptional job and waived the cost for their services as a donation to support our senior project. Alternatively, if we had been unable to find a shop that could anodize our part within our budget, we were going to explore powder coating as an alternative. This was less preferable as powder coating changes the dimensions of the part by a non-negligible amount however we could have worked around this if needed.

7 Design Verification Plan

In this chapter we will discuss our design specifications, design verification, and required testing. Individual tests, appropriate measurements, facilities needed, equipment needed, and general timing is presented in the Design Verification Plan in Appendix N. Environmental resistance must be verified by observing the microscopes' reaction to transportation and verifying that all electronics and connections remain secure during movement. The highest priority specification for testing was stage repeatability. A target goal of 10 [µm] repeatable positional accuracy was set by our team and sponsor for each axis. To test this, the stage was run through a pre-determined series of movements and its ability to return is measured using the actuators internal encoders. These repeatability tests are explicitly outlined in appendix I. The optics needed to be sufficiently rigid, without any observable deflection or oscillation during normal operation. To verify this, we ran the microscope at maximum speed, and carefully observed the view of the microscope through the microscope camera. Any noticeable rigidity issues were recorded on a test sheet for further improvement. The microscope has two use cases that needed testing: CNC control and simple joystick control. To test these, first we attempted to “break” the programming by switching between modes. As we switched between modes, we tested each, running the microscope through an array of programmed paths, and then through several paths using the joystick. To test instruction time and simplicity of general use, we were assisted by students who have not used the microscope yet. We trained them to use the microscope in simple joystick mode and verified that they can fully use the microscope with 5 minutes of instruction time. We also requested that they fill out a feedback survey detailing the different tasks associated with using the microscope. They ranked these tasks from most to least difficult on a scale of 1-10. This feedback allowed us to ensure that all students can use the microscope and that our operations manual is appropriately concise. To test the technician calibration time, we needed Professor Mayer’s assistance. We verified that the calibration documentation is sufficient for him to easily calibrate the microscope without our assistance, and that he can do so within 10 minutes.

DVP&R - Design Verification Plan (& Report)

Project: Inverted Fluorescence Microscope (F13) — Sponsor: Professor Hans Mayer — Edit Date: 4/22/2021

TEST PLAN										TEST RESULTS	
Test #	Specification	Test Description	Measurements	Acceptance Criteria	Required Facilities/Equipment	Parts Needed	Responsibility	TIMING		Numerical Results	Notes on Testing
								Start date	Finish date		
1 Repeatability Testing	Stage Repeatability	Use a reticle style mask with markings every 10 microns to quantify repeatability. See explicit repeatability testing procedure.	Repeatable Position Range	No visible drift or repeatability issues for either axis.	In-person microscope access.	Grid pattern mask.	Thomas	2/11/2021	4/20/2021	Average error of 1 micron total variance of 20 microns each direction	Repeatability testing procedding as expected Encoder Resolution testing requires furthur investigation
2 Undergraduate Usage Test	Instruction Time for General Use Ease of Setup Safety Considerations Simple Use Control	Have an undergraduate member of Dr. Hawkins' research lab use the microscope with limitted instruction.	Time to setup; Rating of ease of use ; Pass/fail ability to use microscope effectively	Less than five minutes of instruction to learn how to use the microscope in joystick mode	In-person meeting with undergraduate and microscope access.	Microscope and Lab computer	Spencer	5/21/2021	4/22/2021 (setup time) 5/24/221	less than the goal time of 2 min	easy to set up, power on and follow onscreen prompts General use of microscope revealed inconsitencies in error messages; negative flags; actuator zeroing vocabulary and user manual instructions reccomendations documented
3 Code Reliability Test	Code functionality CNC path Control	This test is to verify that the code works reliably, regardless of user input. It also has edge cases including actuating the actuators 0 units in PPC mode	Qualitative analysis of breakpoints in code	Microscope delivers appropriate error messages and does not become inoperable	In-person microscope access In final configuration	Microscope and Lab computer	Team	5/21/2021	5/24/2021	1 error in PPC mode over all user testing	repeatability numbers as expected
4 Microscope Robustness Test	Environmental Resistance Rigidity of mounting brackets Safety Considerations Rigidity of Optics	When transporting microscope, observe reaction to vibration from travel. Ensure all connections remain secure and electronics are safely housed.	Included checklist in test procedure	Microscope remains fully functional after transportation. IFM remains undamaged by typical use	In-person microscope access.	Microscope and Lab computer	Team	2/10/2021 Pending Microscope setup in Final configuration Re-Test needed in Lab environment	5/10/2021	Average robustness of components of 8.5	Everything as expected all safety items received a NO (non issue) in checklist
5 Technician Microscope Use Cases	Technician Calibration Time Cleaning Accesibility	Have Professor Mayer or Dr. Hawkins' perform any relevant calibration and cleaning procedure and record time it takes.	Time User feedback of cleaning procedures	Calibration consistently takes <10 [min] start to finish. Cleaning has no unresolved issues	In-person microscope access in lab position. In person meeting with Professor Mayer or Dr. Hawkins.	Microscope and Lab computer Setup in Microfluids Lab	Spencer	5/21/2021	5/24/2021	Technician setup time of less than 5 min	Spencer performed test as stand in for Dr. Hans and Dr. Hawkins. he was able to run the caliration and setup procedures correctly without prior functional knowledge

Figure 35. Summary table of all tests performed on IFM and their results

7.1 Resistance to Environmental Factors

Figure 36. Matthew transporting IFM to his house using a rolling tool cart.

We picked it up and shook it around gently. This device will not be transported often but all of the hardware components are securely attached to the breadboard. The only concern is the tethered joystick, which is free to swing around during movement. It would be beneficial to 3D print a mounting clip to the 80-20 frame to hold the joystick during transport or when not in use. A transportation procedure has been added to the user manual to provide step by step instructions for moving the IFM safely.

A test procedure was also developed to quantify the robustness of the microscope in various aspects of its use the full procedure can be seen in the appendix. The average robustness of the components was 8.5 and all the safety concerns were addressed. These results met our passing criteria.

7.1.1 Optical Rigidity:

Because the microscope magnifies the image, any vibrations present in the stage will be magnified through the optical train and drastically reduce the quality of the image. As such we determined the rigidity and robustness of the mounts for the optical system to be a high priority. The brightfield illuminator is mounted above the stage on a gantry constructed from 80-20 the brackets were changed from flexible 3-D printed ones to solid aluminum sourced from McMaster-Carr and the illuminator was rigidly mounted to the gantry with 3-D printed bracketry. The optical components were also all repositioned onto a common rail to reduce the relative motion of components. Figure 37 below shows the new common rail optical system.

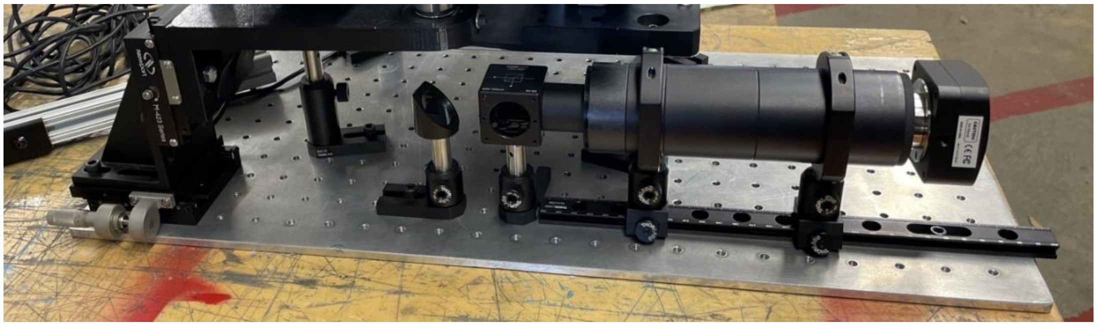


Figure 37. Common rail optical system.

It was also important that the optical breadboard itself be rigid we found that the aluminum plate was flexing and so we installed 80-20 bracketry to prevent the flexing which would amplify any vibration and decrease image quality.

Testing included rigorous actuation of the microscope to try and excite any natural frequencies present in the scope and stage. Testing revealed that there are none present. We also bumped into the stage simulating a situation that although undesirable would nonetheless be encountered in typical IFM use. Again the vibrations present did not affect the image in the camera. An image is included below as Figure 38 of the image as seen on the computer monitor. You can see the resolution and magnification is such that we can resolve the security threads present on a dollar bill.

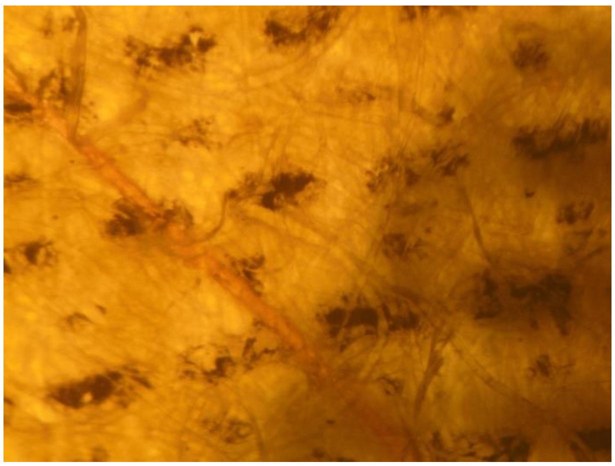


Figure 38. Security threads on a dollar bill

7.2 Stage Position Reliability:

For our microscope to be considered functional when in the programmable path mode of operation, we determined that 10 microns of accuracy was required in each axis for any positional change. Although the microscope was functional the repeatability values were unacceptable with the microscope consistently overshooting by almost 100 microns in the positive direction and 200 microns in the negative direction. After the code improvements described in the manufacturing section testing was carried out to determine the final reliability.

The reliability testing was carried out by using the programable path functionality of the microscope a desired actuation distance would be entered. Distances of 100 and 200 encoder counts were chosen as initial test values on both axis in both the X and Y directions. This isolation of variables would allow us to determine if the repeatability issues were isolated to a particular actuator, particular actuation direction and if the issues was consistent across different actuation distances. It was also important to track the error across the full range of microscope travel to be able to verify the speed differential function was correct. Manual tests were run the first couple times by repeatedly entering command line prompts to move the actuators in increments of 100 or 200 encoder counts through their full range of travel and recording the actual actuated distance. This became tedious and an automatic testing procedure was implemented. The workflow is shown below in Figure 39 and consists of a testing function implemented into the Arduino® code and an excel sheet and MATLAB script to process the data.

Figure 39. Automated testing workflow

The automated testing generated statistics including average error and total variation as well as maximum error across the entire actuation distance of the microscope. The error was also plotted to better determine error trends and a sample plot is shown below as Figure 40.

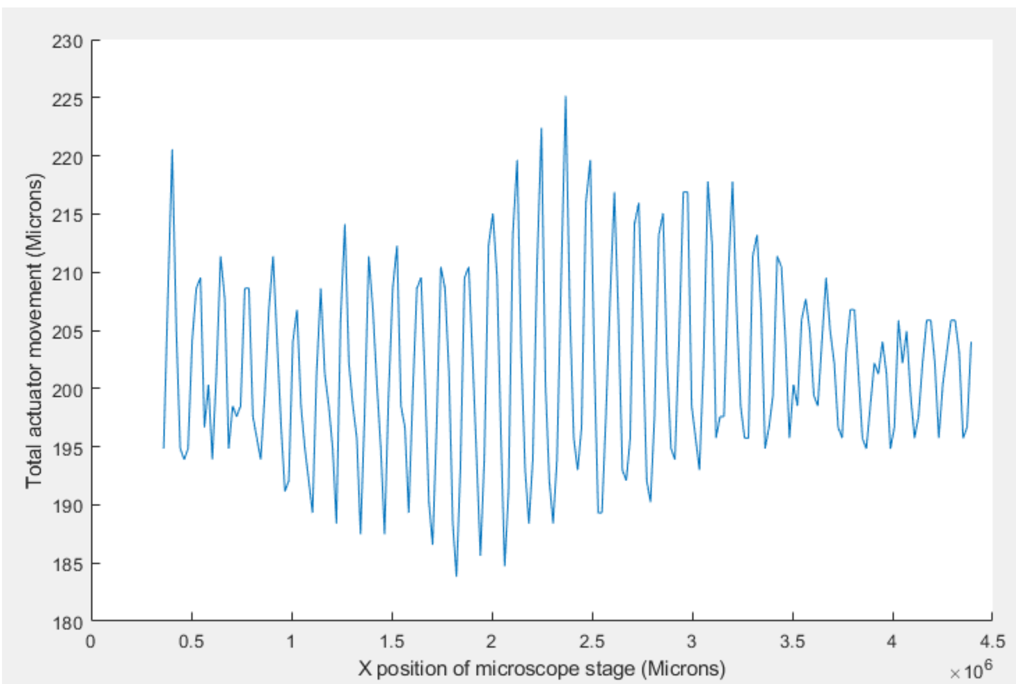


Figure 40. Final X axis 200 microns repeatability data

As you can see the error follows a cyclical pattern, the total variance is 20 microns in either direction and the average error is only 1 Micron. The cyclical pattern of the error is approximately 1200 encoder counts which corresponds to 1 revolution of the actuator motor. We determined that this variance is likely due to a bent shaft either on the motor or somewhere in the gear train. Repairing the actuators was deemed outside the scope of this progress and our specification of less than 10 microns of repeatability error was met.

In addition to reliability, the microscope internally uses units of encoder counts to control the actuators however these units are not useful for maneuvering the IFM around a microfluidic device whose features are micrometers apart to accomplish this we devised a test to equate encoder counts to microns. Using a special diode mask provided by our sponsor Prof. Mayer we were able to recognize features of known size and distance apart we then actuated the stage a known number of encoder counts and used the resulting distance measured in microns to come up with a conversion factor. An image illustrating this process is shown below as Figure 41.

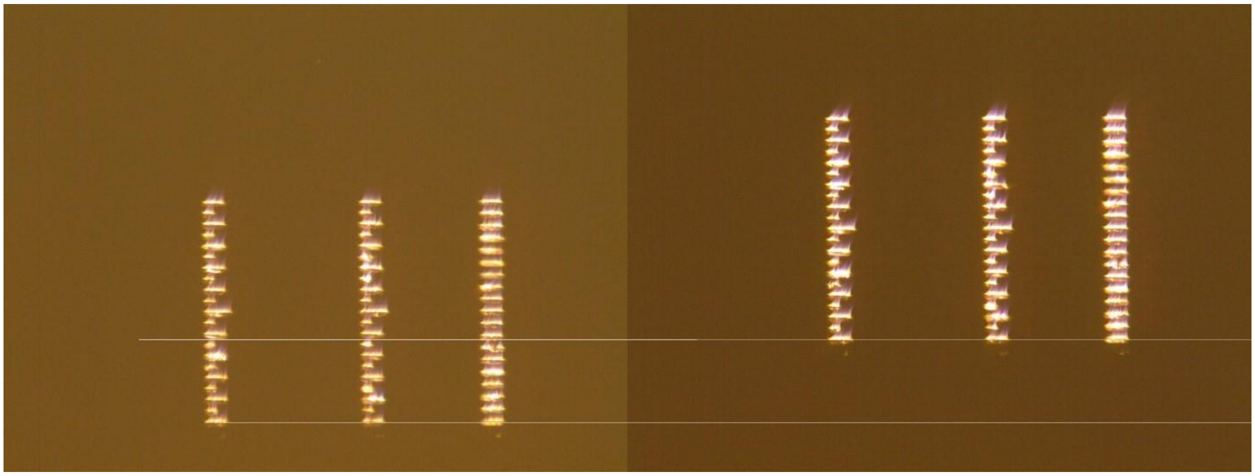


Figure 41. Conversion process of encoder counts to microns

The original position of the IFM is shown on the left and the final position is shown on the right. There is some uncertainty due to the optical uncertainty of using a scale with gradations every 13 microns and due to the exposure settings of the camera the edges of the scale are also not perfectly crisp and some approximation was required to determine the measured position of the stage. Uncertainties related to the manufacture of the mask and differences between the designed dimensions and actual dimensions were neglected. The uncertainty in the measured position of the stage was determined to be ±6.8μm with calculations included in appendix N design verification and testing. The uncertainty in the encoder values was also considered. The encoders are static during this test and during the repeatability testing we determined that as the encoders are zeroed every time the IFM is used the uncertainty in the encoders was taken to be zero. This total uncertainty is larger than expected but with repeatability values required to be less than 10 microns we deemed this uncertainty acceptable.

7.3 Software Testing:

The purpose of a test procedure for the team-developed software is to expose the limitations of the code any bugs that may impact user experience or microscope functionality. Among this procedure, steps were taken to viscously select as many buttons on the GUI as possible and attempt to “break” the running code or find a software path that may contain blocking code. Blocking code is code that prevents the functional finite-state machine loop from operating cooperatively or performing multiple operations at the same time.

From this software testing, several design considerations were exposed. First, when the GUI window was closed and reopened, no signal was sent to the Arduino® code to reset the microscope and zero the actuators. Because of this, there was miscommunication occurring between the finite-state-machines of the GUI code and Arduino® code. To prevent further “desynchronization” of these two finite state machines different states were chosen so that the GUI state is always different than the internal state of the IFM this redundancy prevents a situation where the Arduino® controller is expecting signals from the GUI that will never be sent because the GUI thinks that the microscope is in a different state.

In the command line structure, the Arduino® serial communication was also tested both by sending incorrect data for which appropriate error messages are given and by sending too much data all at once. For instance, when asking for a simple “x” or “Y” value to select an actuator entering the value ”xy” results in an appropriate error message not the simultaneous selection of both actuators. In addition, when asking for a letter input both upper and lowercase letters are acceptable values to reduce user confusion.

7.4 User Control Feedback/Instruction Time:

The purpose of user control and instruction time feedback is to streamline our microscope designs by point but as of the day we are turning in this draft we believe to be at a point where we can begin that phase. Our user manual, Joystick control with feedback of global and local coordinates, and command line control are all functionally complete. The users reported that the user manual did not contain explicit enough language and figures for them to open the software’s required for IFM operation without significant trial and error. To fix this we added separate sections for the AmScope® camera software, Arduino® serial communication, and GUI. Additionally, the users struggled with finding the correct balance of lighting for the samples, particularly with regards to adjusting the camera exposure settings in AmScope® and tuning the physical focus of the objective using the micrometer. We solved this by adding more detail to the instructions and creating sections for the issues in the FAQ section of the user manual.

8 Project Management

The following chapter lays out the timing of the project to achieve all relevant objectives. The resolution of the Gantt Chart has been increased by breaking down larger goals into smaller tasks with specific responsible team members. The major milestones and progress of objectives during the project are described, and new subtasks and subsequent steps are laid out.

This project is defined primarily by the series of subtasks we identified during function decomposition in the Concept Design Chapter. The highest priority tasks being those which required to prepare this microscope for lab use. Table 11 provides a breakdown of the main tasks and their associated project leads. These are the individuals responsible for designating various tasks and assigning deadlines within the project to the rest of the team, giving each person a more direct responsibility and improving team performance. Our project overview can be seen in Figure 42, which is a simplified version of our Gantt chart in Appendix C. Our full Gantt chart has all the subtasks for each group task as well as the team member(s) responsible for each sub task. At the time of publishing this preliminary design review we have completed the tasks of transporting the IFM, obtaining dedicated power supply, ideation, and weighted decision matrix creation, and are currently working on the tasks of soldering the proto-board and creating an improved user interface.

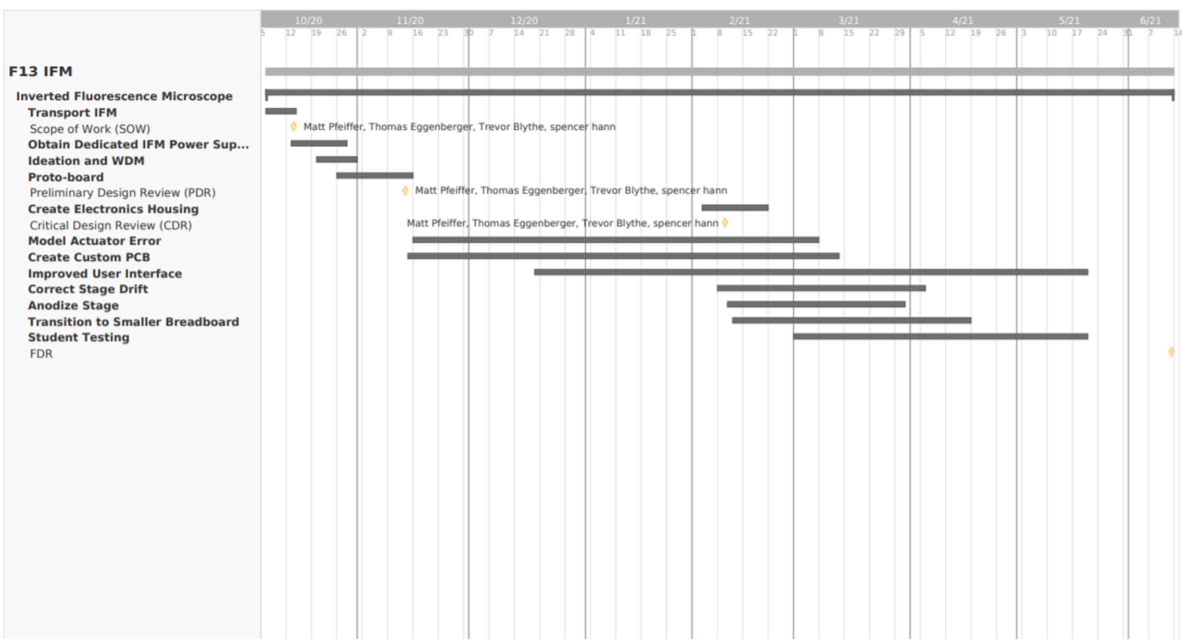


Figure 42. Simplified Gantt chart outlining our major project milestones through Final Design Review.

Something unique to this project is the independence that subtasks have from each other regarding relative impacts of design decisions which allows us to work on different subtasks simultaneously. This is represented in the task overlaps and is beneficial to us as it reduces the amount of slowdown caused by bottlenecks such as shipping time. If we are waiting on something for one task, there is almost certainly another task we can work on in the meantime. The task leads assigned in Table 11 are in responsible for making sure that the other team members always have tasks to do that will get the team closer to reaching a milestone.

Table 11. Major project milestones and their associated project lead

Milestone	Coordinator
Controller Housing	Trevor
User Interface	Matthew
User Manual	Spencer
Drift Correction	Thomas

Table 12 displays the wants and needs we outlined with our sponsor at the beginning of this project as well as a color key indicating progress on each of the tasks.

Table 12. Needs and Wants Timeline

Needs	Wants
Before PDR	
Create Controller Housing	Improve 3D Printed Hardware
Replace Power Supply	Purchase Shop Cart
Before CDR	
Improve User Interface	Create Joystick Housing
Create User Manual	Add Programmable Stage Paths
Before FDR	
Diagnose and Correct Stage Drift	Add Fluorescence Capabilities
Color Code	
Complete	
In Progress	
Not Started	

These milestones consist of many different subtasks that will need to be accomplished. We have successfully transported the IFM off campus and it is being stored in a spare room at Matthew Pfeiffer's house. We have been able to access it a few times since bringing it off campus by following our safe operating procedures which can be seen in appendix H. Table 13 displays the detailed list of subtasks required for each project milestone, our timeline for which is outlined in detail in the Gantt chart in appendix C.

Figure 43. Transporting the IFM to Matthew Pfeiffer's house for testing.

Table 13. Breakdown of Subtasks for Major Project Goals

Subtask	Subtask steps
Transport IFM to safe working location	• Submit safe operating procedure to both Dr. Mayer and Dr. Schuster. • Spencer needs to be authorized to go on campus to help Dr. Mayer load it into his truck. • Trevor needs to finish building the wood carrying platform to rest the IFM on. • Matthew needs to clear a spot in is house where the IFM can sit safely, low foot traffic area. • Thomas needs to coordinate day and time for pickup with Spencer and Dr. Mayer, should be about an hour time slot.
Design and manufacture controller housing	• Research existing Arduino® casings options, 3D printed is preferred. • Determine 3 configurations of interior dimensions based on size of Arduino®, bread/busboard, 2 actuator controller boards. • CAD up a case design with appropriately sized holes in the side for actuator connectors, power cables. • Create mechanical mounting systems for all boards such that they are secured, but can be removed with relative ease. • Send CAD files to Dr. Mayer for 3D printing with his personal printer. • Test first prototype housing, document and improvements and iterate, hopefully just once.
Replace the current power supply with a more compact version	• Determine the voltage and current required to power Arduino® and actuators. • Search for cheap solutions on ebay, etc. • Send a viable, cost effective solution to Dr. Mayer for purchasing along with any connecter breakouts we need to plug into the controller housing. • Should have some level of voltage variability in case the voltage being supplied to actuators is affecting positioning repeatability. • Submit selected power supply to Dr. Mayer for ordering.
Create a GUI for the Arduino® controller	• Research how to make GUI for Arduino®. • Determine most frequently used features and make them most accessible.

Subtask	Subtask steps
Create User Guide	• Compile list of most important features and controls needed for undergraduate lab experiments. • Create list of control commands. • Compile into user-friendly guide.
Reduce positioning repeatability error to less than 10 microns in each axis	• Get transparent mask to put over the objective, basically just a 2 axis, very small ruler that we can use to quantify drift. This requires purchasing through Dr. Mayer. • Design testing procedure and data sheets. • Preform tests to determine drift amount based on distance traveled on either axis, and when there's movement on both axis. • Research the ideal operating voltage for the actuators and make sure it is met, play with voltage and record drift, see if there are changes. • Use drift data to create a model to predict drift, code it into Arduino® controller to reduce it. • During testing, observe the mechanical systems involved in moving the stage, are the return springs mounted poorly? Is there flex in the actuator plunger plates? Take notes during testing and if there seem to be any mechanical inconsistencies, fix them with stiffer 3D printed parts.

9 Conclusion

The goal of this project was to improve the hardware, software, and user interface of this inverted fluorescent microscope so that it has an intuitive user interface and high-precision stage. These improvements will allow the microscope to be used in the Microfabrication Lab for undergraduate lab experiments.

After researching the problem as presented, we determined a path forward, that enabled us to create a computer controlled inverted florescent microscope with an intuitive user interface and high-precision stage with consistent positioning repeatability. Our completed product allows the Microfabrication Lab users to perform research at a higher level than the previously available hardware allows.

After defining the objectives for our project, we completed the design work required to accomplish our goals. This work included ideating different solutions for the functions that were identified by our research including the controller housing, user interface, user manual, drift correction as well as the brightfield illumination. For the controller housing we decided on a 3D printed hinged lid enclosure.

The user interface has been updated to include a GUI to interact with the Arduino® in real time and provide an intuitive user experience. The GUI is functional but still needs improvement, so Professor Mayer will enlist the help of a computer science student to complete that portion of the project.

The drift correction was tested and found to be repeatable. This meant we were able to account for it by modifying the Arduino® code to include a better control scheme, which accounts for the error and reduces the drift to within our specifications. After correction action is taken the error will be re-tested using the transparency masks with known distances to determine the actual repeatability of the microscope. The user manual includes a quick start guide as well as instructions for safe handling, transportation, and other more complex functions of our product. The brightfield illumination will be accomplished with a modification of the existing 80-20 structure.

In the future, are many additional functional improvements that should be considered. Primarily, they are related to improving the functionality with the software and GUI. It would be ideal for the video feed from the microscope camera to be displayed within the GUI, so that the entire workflow takes place in a single piece of software. There are also additional functions that can be implemented, including a more thoroughly implemented programmable path. Using such programmable path code, a button could enable return to local coordinates, and another could enable image capture and save to a local device. Another possibility is the implementation of edge detection and more advanced Computer Vision methods. These could improve image quality, enable the microscope automatically seeks out certain features, or to include a "legend" of the microscope bed, providing live visual updates of where the image is located. This would involve a zoomed-out view of the bed, and then a rectangle, which represents the current view. This would give the user a tool to help them understand the orientation they are currently in. Additional improvements to microscope functionality would be adding general image processing tools like exposure adjustments, contrast adjustments, zoom, sketch tools, and image overlays. This microscope in its current state would benefit immensely from contribution of someone with experience in computer programming, GUI development, and computer vision.

Some additional hardware adjustment should be considered. Locating a fluorescence illuminator under the stage and rotating the beam-splitter would enable a fluorescence illumination mode. Anodizing the optical breadboard or building a full enclosure around the microscope would further reduce luminous pollution and improve image quality. Designing a more robust focusing mechanism would simplify initial setup. Additional consideration should be put to sample securing on the microscope stage. If placed at an angle in its current setup, the microscope stage positioning will not track along a straight horizontal or vertical line. Lastly, consideration to an objective turret or other simple objective switching device would simplify the process of swapping magnifying objectives.

With that, we would like to formally thank both our project sponsor, Dr. Hans Mayer, and faculty advisor, Dr. Peter Schuster, for allowing us the opportunity to work on this project.

References

[1] Brown, T., Kamei, M., Miranda, E., & Nicholson, E. (2020). *INVERTED FLUORESCENCE MICROSCOPEFINAL DESIGN REPORT* (Rep.). San Luis Obispo, CA: Cal Poly Mechanical Engineering Department.

[2] "Eclipse Ti2 Series: Eclipse Ti2-E." Nikon Instruments Inc. URL: https://www.microscope.healthcare.nikon.com/products/inverted-microscopes/eclipse-ti2-series. Accessed 9/28/2020

[3] "IM-3." OPTIKA Microscopes. URL: https://www.optikamicroscopes.com/optikamicroscopes/product/im-3/. Accessed 9/28/2020

[4] "IXplore Standard." IXplore Standard -High-Quality Imaging | Olympus Life Science, Olympus. URL: https://www.olympus-lifescience.com/en/microscopes/inverted/ixplore-standard/. Accessed 9/28/2020

[5] Solis® High Powered LEDs for Microscopy. (n.d.). Retrieved October 27, 2020, from https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=8986

[6] Zeineh, J. A. (1999). System and method for remote navigation of a specimen. Washington, DC: U.S. Patent and Trademark Office.

[7] Wilson, C. (2006). Motorized system and method of control. Washington, DC: U.S. Patent and Trademark Office.

[8] Meyer, M., Keydell, W., Mollring, F. K., & Weber, K. (1979). *U.S. Patent No. US4210384A*. Washington, DC: U.S. Patent and Trademark Office.

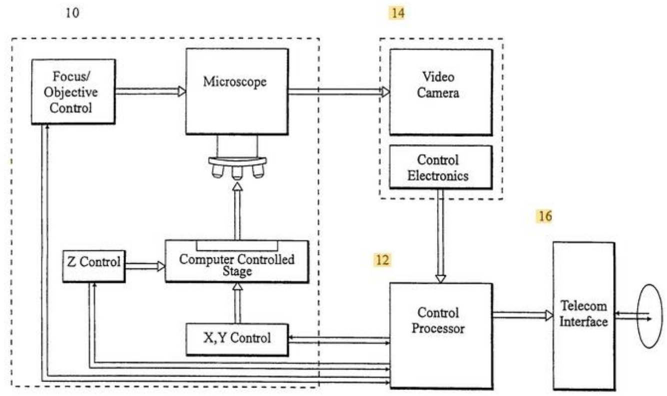
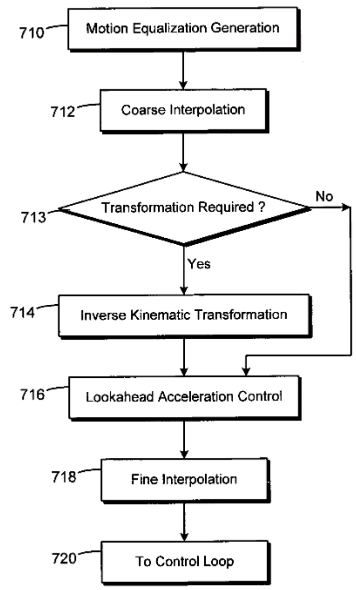
[9] No, K. F., Tan, H. A., Low, M. L., & Chin, Y. L. (2004). *U.S. Patent No. US7180430B2*. Washington, DC: U.S. Patent and Trademark Office.

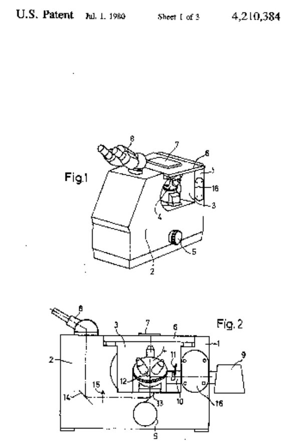
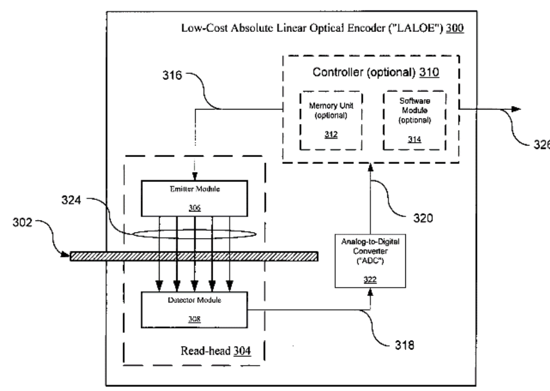
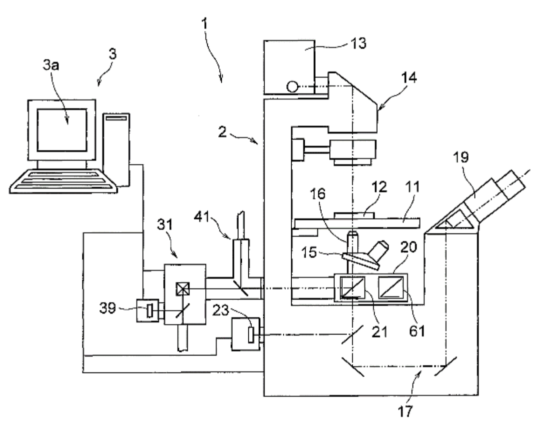
[10] Nihoshi, T. (2009). Microscope apparatus with fluorescence cube for total-internal-reflection fluorescence microscopy. Washington, DC: U.S. Patent and Trademark Office.

[11] D. Hanafi, M. Tordon and J. Katupitiya, "An active axis control system for a conventional CNC machine," *Proceedings 2003 IEEE/ASME International Conference on Advanced Intelligent Mechatronics (AIM 2003)*, Kobe, Japan, 2003, pp. 1188-1193 vol.2, doi: 10.1109/AIM.2003.1225511.

[12] Newport Corporation. (1999). 850G: Linear Series Actuator. Retrieved from ftp://download.newport.com/MotionControl/Archive/Positioner%20Manuals/850G.PDF Accessed 10/5/2020

Appendix A – Preliminary Patent Research

Patent Number	Patent Title	Description	Picture
7916916	System and method for remote navigation of a specimen	A system and method of microscopy control to move a microscope and camera associated with the microscope to view a specimen.	
7348748	Motorized system and method of control	A generic patent for motorized method of control cartesian coordinate position control of a generic stage. In this patent the application seems to be manufacturing processes.	

Patent Number	Patent Title	Description	Picture
4210384	Inverted-design optical microscope	This is an initial patent for an inverted microscope design.	
7180430	Low-cost absolute linear optical encoder	This patent describes an absolute low cost linear encoder. The function of this encoder is to determine absolute position along a linear path and for correction.	
8014065	Microscope apparatus with fluorescence cube for total-internal-reflection fluorescence microscopy	This patent discusses a cube-shaped apparatus that sits in the optical pathway and acts to collimate laser light and direct it to a sample for total internal reflection. Basically, enabling use as a fluorescence microscope.	FIG. 1 

850G Series Linear Actuator

User Manual

Newport

Product Warranty

Because we are confident that they will meet your high standards, our products carry the following warranty, effective for a period of one year from the original invoice date unless otherwise stated in the product literature.

- Products will be free of defects in material and workmanship.
- Products will meet the specifications stated in this document.

If you find any defects in material or workmanship or a failure to meet specifications within the warranty period, return the product to us clearly marked with a Return Authorization Number (RA#) and we will either repair or replace it at our discretion.

Our warranty excludes products that have been improperly installed or maintained, modified or misused. Notification of claim must occur within the warranty period. Newport's liabilities are limited as set forth in our standard terms and conditions, copies of, which is available upon request.

Non-Warranty Repairs

If a product needs repairing after the one-year warranty period expires; we will first provide an estimate of repair charges and then repair the product upon receiving authorization from you. Repairs are warranted for 90 days.

Part No. 27481-01, RevA
IN-12981 (12-98)

Table of Contents

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.

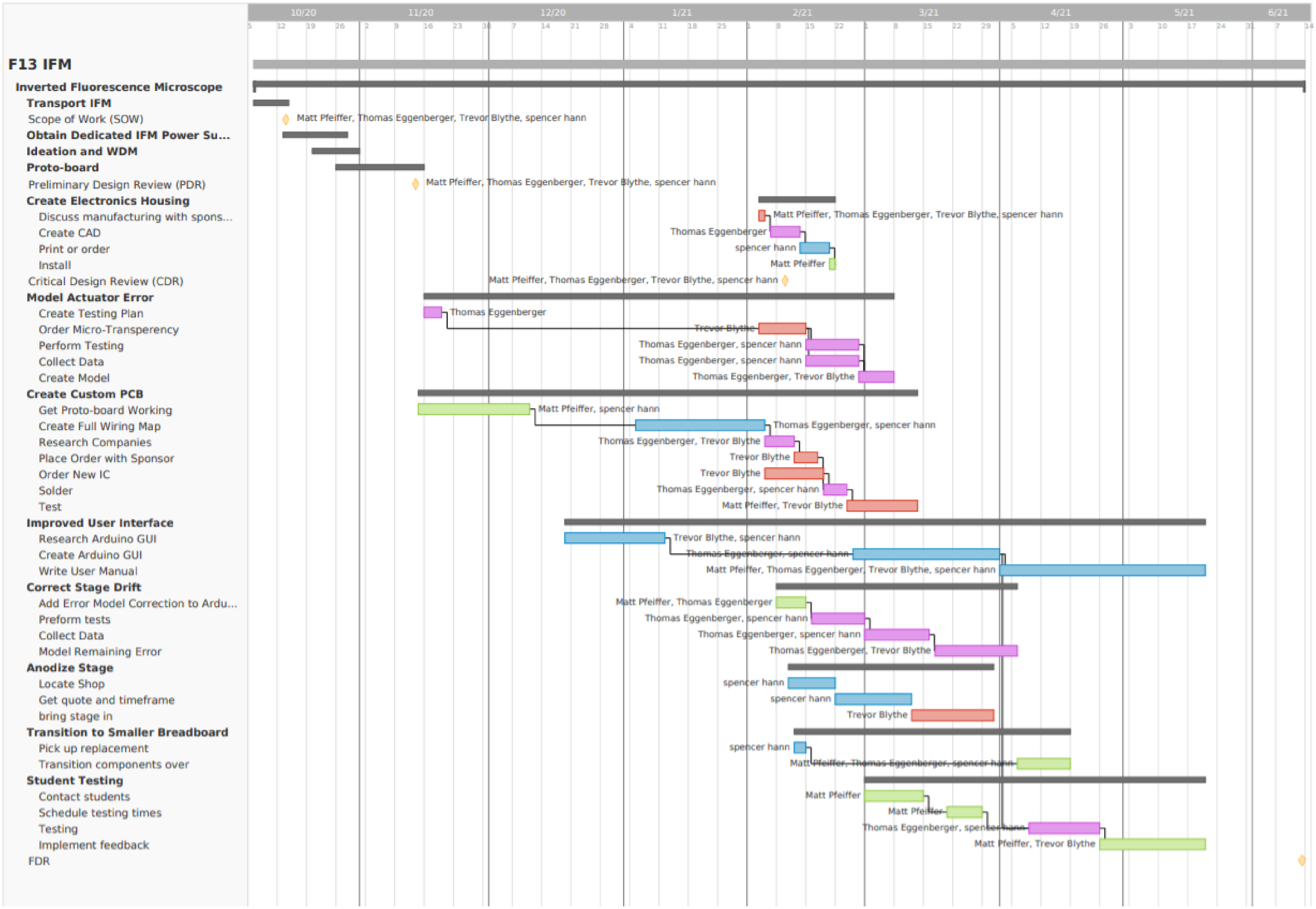
Backlash	< 20 micron typical with external load of 2 lbs. (1 kg) minimum
Accuracy	< 0.1% of travel, cumulative
Bi-directional	Repeatability: Better than 1 micron when backlash is compensated by controller (standard actuators) *1
Encoder	Magnetic, 2KHz; open collector, quadrature output, +5V to +12V supply
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 C – Gantt Chart



Appendix D – House of Quality

Correlations	
Positive	+
Negative	–
No Correlation	

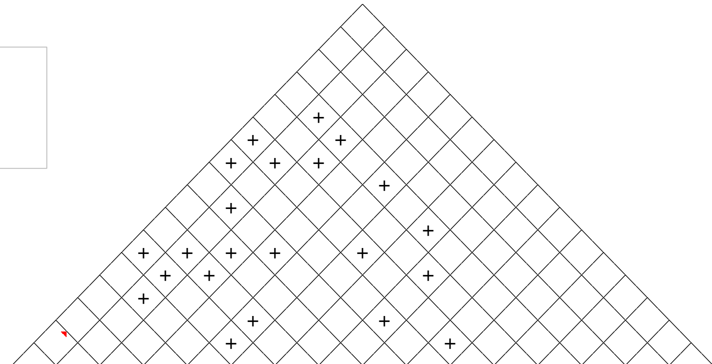
Relationships	
Strong	●
Moderate	○
Weak	▽

Direction of Improvement	
Maximize	▲
Target	◇
Minimize	▼

QFD House of Quality

Project: Fluorescence Microscope (F13)

Revision Date: 10/06/2020

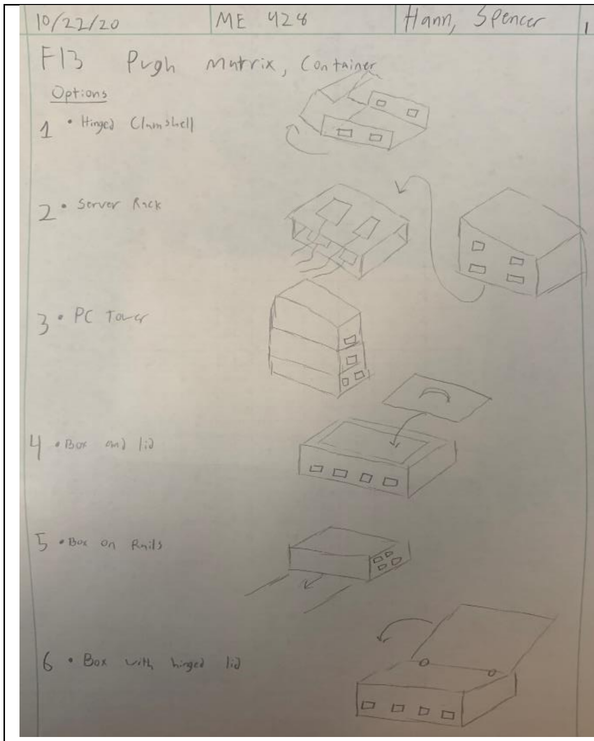


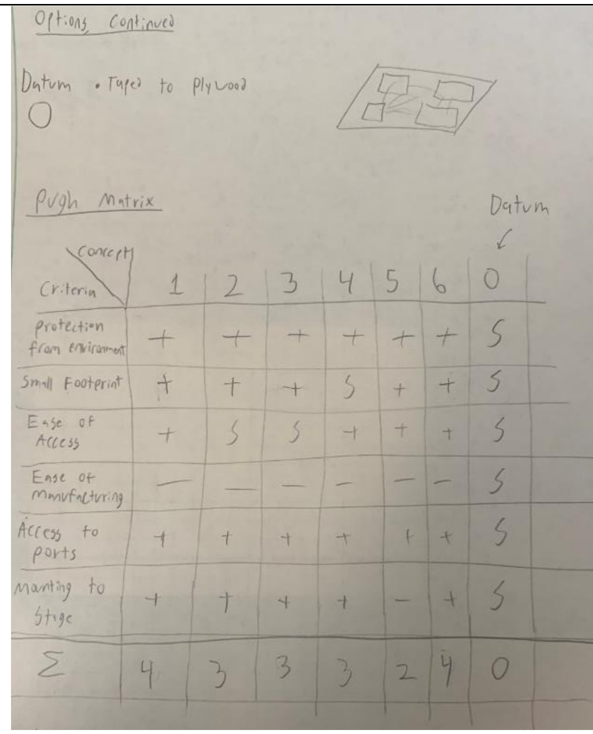
Row #	Weight Chart	Relative Weight	Undergraduate Research	Graduate Research	Instructor/Technician		Maximum Relationship	WHAT: Customer Requirements (Needs/Wants) / HOW: Engineering Specifications (Tests)	Stage Repeatability	Use with Limitted instruction	Environmental Resistance	Footprint	Ease of Setup	Ease of Calibration	Part Replacement	Rigid Mounting Brackets	Safety	CNC Stage Control	Simple Control	Rigid Optics	Cleaning				Present Microscope Prototype	Current Manual Microscope	Nikon TI2-E Microscope	Optika IM-3 Microscope		Row #
								Column #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						
								Direction of Improvement	▼	▼	◇	▼	▼	▼	◇	◇	◇	◇	◇	◇	◇									
1		9%	4	9	9		9	Precise stage position control	●	●	▽	▽	○	○	○	●	●	●	●	○	▽				2	3	3	3		1
2		9%	9	6	6		9	Compact Electronics Enclosure	▽	○	●	●	●	▽	●	●	●	▽	▽	▽	●				1	3	3	3		2
3		5%	3	3	8		8	Easy Connection Points	▽	●	●	●	●	▽	●	●	●	▽	▽	●	●				1	3	3	3		3
4		6%	4	4	8		8	Sturdy Optical Mounts	○	▽	●	▽	▽	○	○	●	●	○	●	●	●				3	5	5	5		4
5		8%	6	6	7		7	Small Footprint	▽	▽	●	●	○	▽	●	○	●	▽	▽	▽	○				3	4	4	5		5
6		5%	4	4	4		4	Aesthetically Pleasing	●	●	○	●	●	○	●	○	○	○	●	●	○				2	4	5	5		6
7		9%	8	6	7		7	Graphic User Interface	○	●	▽	▽	▽	●	▽	●	▽	●	●	▽	▽				1	0	5	0		7
8		6%	2	6	8		8	Easy Calibration	●	○	▽	▽	○	●	▽	▽	▽	○	●	○	▽				1	2	3	4		8
9		4%	3	3	5		5	Easily Portable	▽	○	○	●	●	▽	○	▽	●	▽	▽	▽	○				1	2	3	3		9
10		5%	3	5	5		5	Stand-alone System	▽	○	○	○	●	○	○	▽	○	▽	▽	●	○				3	5	5	5		10
11		7%	3	8	6		8	Programmable Control	●	○	▽	▽	▽	○	▽	▽	▽	●	○	▽	▽				2	0	5	0		11
12		11%	9	9	9		8	Joytstic Control	●	○	○	○	○	○	○	▽	▽	○	●	▽	▽				4	0	5	0		12
13		5%	2	7	3		8	Auto-Image Capturing	●	○	▽	▽	▽	▽	▽	○	▽	●	○	●	○				0	0	5	0		13
14		7%	6	7	3		7	Fluorescence Capability	●	▽	▽	▽	▽	▽	○	○	▽	●	●	●	●				0	0	5	5		14
15		4%	1	1	10		10	Low Cost	○	○	○	○	○	○	○	○	▽	▽	▽	○	○				5	1	0	0		15
16		0%																												16

WHO: Customers — NOW: Curr. Products

HOW MUCH: Target Values	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Target Values	10 Microns	5 Min Instruction	Impact Resistant	2'x 2'	3 min Without Instruction	5 min Operation	No custom shop work needed	Meets Stage repeatability	No Pinch points, Easy to stop	Able to position to multiple targets	Joystick operation and easy to use	Minimize visible deflection/oscillation	Easy to access Optical Components			
Max Relationship	5	5	5	5	5	5	3	5	5	5	5	5	4			
Technical Importance Rating	531.1	424.6	386.6	392.1	406.2	313	409.2	460.8	451.1	442.1	543.7	405.2	381.8	0	0	0
Relative Weight	10%	8%	7%	7%	7%	6%	7%	8%	8%	8%	10%	7%	7%	0%	0%	0%
Curr. Products: Present Microscope Prototype	0	1	0	0	0	0	4	0	5	0	3	3	3			
Curr. Products: Current Manual Microscope	5	5	5	5	5	5	3	5	5	0	0	5	4			
Curr. Products: Nikon TI2-E Microscope	5	5	5	4	5	5	3	5	5	5	5	5	3			
Curr. Products: Optika IM-3 Microscope	5	5	5	5	5	5	3	5	5	0	0	5	4			
Column #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Appendix E - Pugh Matrices





Criteria \ Concept	1	2	3	4	5	6	0
Protection from environment	+	+	+	+	+	+	S
Small Footprint	+	+	+	S	+	+	S
Ease of Access	+	S	S	+	+	+	S
Ease of Manufacturing	–	–	–	–	–	–	S
Access to ports	+	+	+	+	+	+	S
Mounting to Stage	+	+	+	+	–	+	S
Σ	4	3	3	3	2	4	0

Design sketches and Pugh Matrix for electronics enclosure. This Pugh matrix evaluates the designs created during the ideation process for the electronics enclosure solution. The datum is set as the current electronics arrangement method which is simply taping the boards to a small plywood square. This is an untraditional approach as the datum is usually set as one of the designs predicted to be on the higher-scoring side. The result is all our designs scored positively, with designs 1 and 6 scoring the highest. Design 1 is a hinged clamshell style box with the ports located on opposite sides, and design 6 is a simple box with a flat hinged lid. Design 6 leaves us the freedom to locate the ports on all four vertical sides of the box.

Solution	Rigidity	Complexity	Complexity of new parts	Robustness	New Functionality
Existing mount for fiber optic light source (80-20 "gantry style" with 3-d printed mount fiber optic source located next to stage)	S	S	n/a	S	S
Existing fiber optic source with gantry style 80-20 mounting, improved 3-D printed parts like brackets improved mounting for fiber optic for z adjustability	+	S	S	s	+
Existing fiber optic source with crane style 80-20 mounting,	S	+	S	S	S
Existing fiber optic source with tripod style 80-20 mounting	+	-	-	+	S
New (compact) fiber optic source with on stage mounting	+	+	+	-	S
Under stage lighting with new optical path	+	-	-	S	S
Mounting linkage for z adjustability	S	-	-	S	+
Existing fiber optic source with crane style 80-20 mounting swing arm	S	-	S	S	+

Pugh matrix evaluating design options for the light mounting fixture. The design options are briefly described in the left column with options 2 and five scoring the highest with a total score of 2.

Optical Breadboard Layout Pugh Matrix

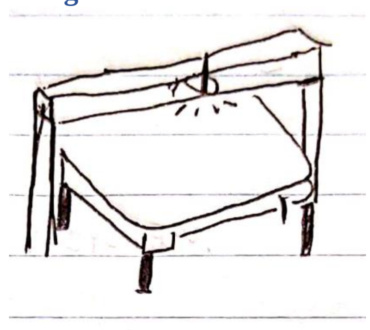
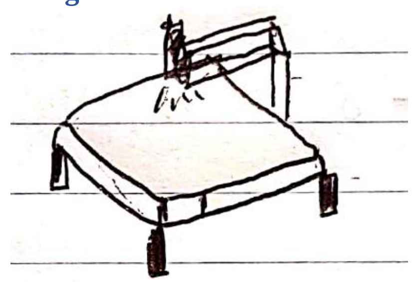
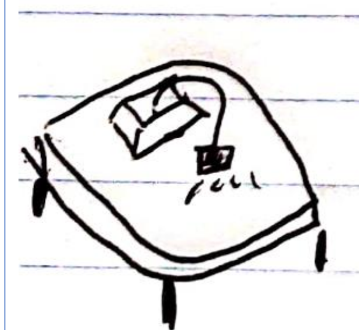
PUGH MATRIX

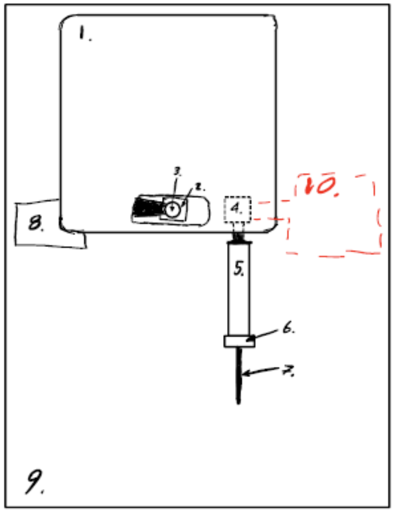
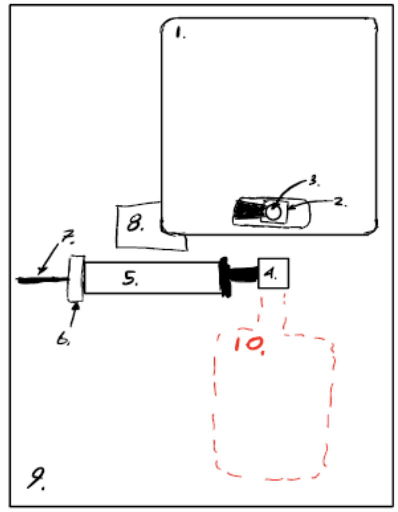
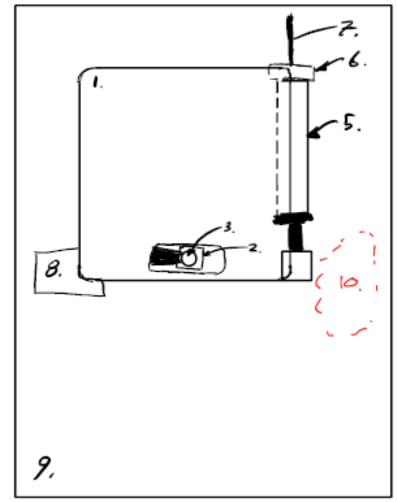
SOLUTION	LEVEL OF COMPACTNESS	EASE OF TRANSITION	SPACE FOR FUORESCENCE COMPONENTS	ACCESSIBILITY
CURRENT LAYOUT	DATUM	DATUM	DATUM	DATUM
PORTRAIT LAYOUT 2	+	S	+	S
PORTRAIT LAYOUT 3	+	S	−	
LANDSCAPE LAYOUT 1	+	−	+	S
LANDSCAPE LAYOUT 2	+	−	S	S
LANDSCAPE LAYOUT 3	S	−	+	S

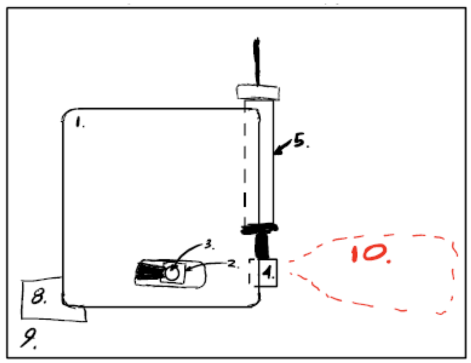
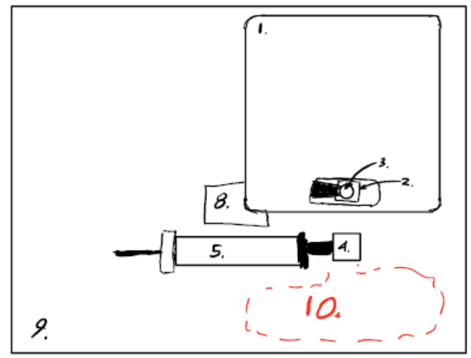
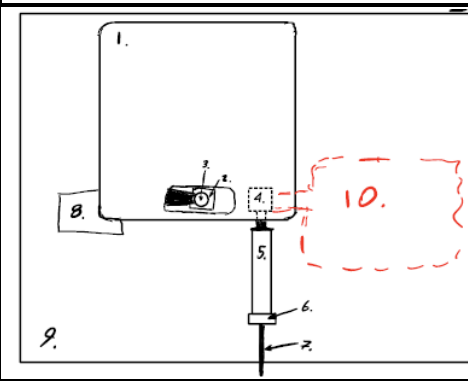
THE HIGHEST RANKED LAYOUT IS THE FIRST LANDSCAPE LAYOUT. IN THIS DESIGN THE ENTIRE MICROSCOPE IS SHIFTED 90° ON THE BREADBOARD AND THE BULKY TUBE LENSE OPTICAL PATH IS RELOCATED TO PARTIALLY UNDER THE ACTUATED RIGHT SIDE OF THE STAGE. THIS MAKES THE MICROSCOPE MUCH MORE COMPACT, PLACES THE FRONT OF THE STAGE IN A CONVENIENT LOCATION FOR LOCATING THE OPTICAL BREADBOARD IN THE LAB, AND CREATES A LOT OF SPACE FOR OTHER COMPONENTS TO FIT ON THE BREADBOARD. THE MAIN DRAWBACKS OF THIS ARE THE REPOSITIONING OF THE MICROSCOPE AND NECESSARY REDESIGN OF THE BRIGHTFIELD ILLUMINATOR MOUNT.

Appendix F – Weighted Decision Matrices

Weighted Decision Matrix for lighting

	Design 1			**Design 2**			**Design 3**		
									
	Gantry with improved mounting			Crane with swing arm			On stage mounting		
Criteria	Weight	Score	Total	Weight	Score	Total	Weight	Score	Total
Rigidity	0.25	7	1.75	0.25	5	1.25	0.25	8	2.5
Complexity to Design	0.1875	8	1.5	0.1875	7	1.3125	0.1875	8	1.5
Complexity to Manufacture	0.1875	9	1.6875	0.1875	8	1.5	0.1875	8	1.5
Robustness (impact & environmental resistance)	0.25	8	2	0.25	6	1.5	0.25	6	1.5
New functionality	0.125	0	0	0.125	7	0.875	0.125	0	0
	Total score		6.9375	Total score		6.4375	Total score		6.5

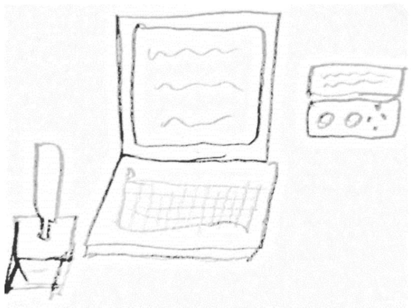
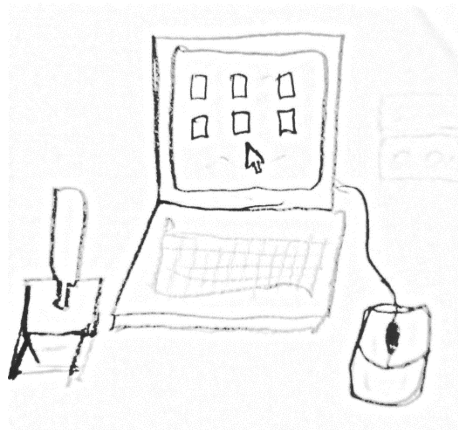
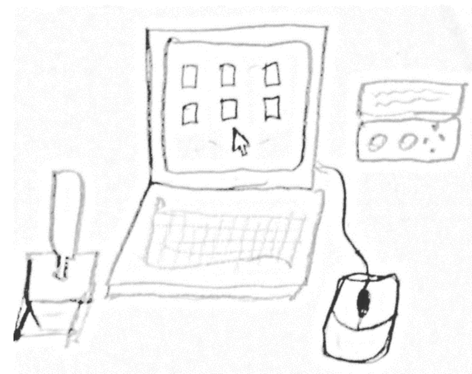
Portrait Optical Breadboard Layout Weighted Decision Matrix				
Specification	Weight	Portrait Layout 1 (Current) 	Layout 2 	Layout 3 
Environmental Resistance	1	2	2	3
Footprint	4	2	4	5
Part Replacement	3	4	4	3
Cleaning Accessibility	3	4	4	3
Position In Lab	2	1	2	3
Fluorescence Add-on Space	4	3	5	3
TOTAL	85	48	66	59

Landscape Optical Breadboard Layout Weighted Decision Matrix				
Specification	Weight	Landscape Layout 1	Landscape Layout 2	Landscape Layout 3
				
Footprint	4	5	4	2
Environmental Resistance	1	3	2	2
Part Replacement	3	3	4	4
Cleaning Accessibility	3	3	4	4
Position In Lab	2	4	3	2
Fluorescence Add-on Space	4	5	3	5
TOTAL	85	69	60	58

KEY

1. ACTUATED STAGE WITH SAMPLE VIEWING CUTOUT
2. 45° MIRROR
3. OBJECTIVE MAGNIFIER
4. BEAMSPLITTER
5. TUBE LENSE
6. CAMERA
7. DOVETAIL RAIL
8. Z-AXIS OBJECTIVE CONTROL
9. OPTICAL BREADBOARD
10. POTENTIAL BROADBAND LED & DRIVER SPACE

User Interface Weighted Decision Matrix

									
	Joystick + Command Line + External control panel and LCD			Joystick + GUI			Joystick + GUI + External control panel and LCD		
Criteria	Weight	Score	Total	Weight	Score	Total	Weight	Score	Total
Ease of use	3	1	3	3	2	6	3	3	9
Ease of implementation	2	3	6	2	2	4	2	1	2
Flexibility of use	3	1	3	3	2	6	3	2	6
Cost	1	2	2	1	3	3	1	2	2
Manufacturability	2	1	2	2	2	4	2	1	2
Total			16			23			21

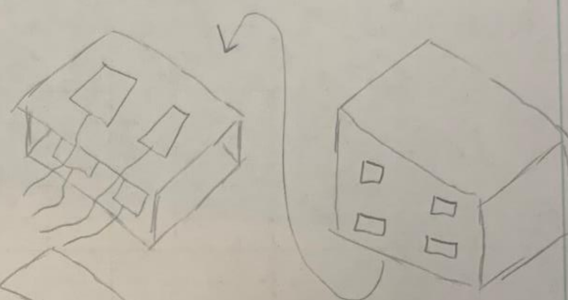
10/22/20 | ME 428 | Hann, Spencer | 1

F13 Pugh matrix, Container

Options

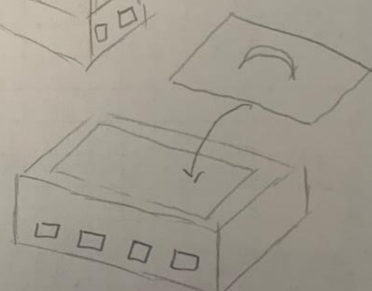
1 • Hinged Clamshell

2 • Server Rack

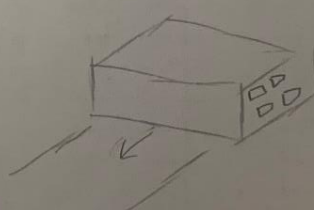


3 • PC Tower

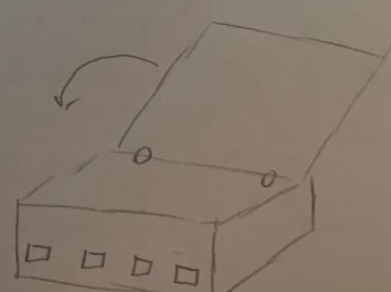
4 • Box and lid



5 • Box on Rails



6 • Box with hinged lid



10/27/20 | ME 424 | Hann, Spencer

Weighted Decision Matrix For Electronics containment Unit

I have chosen to evaluate options 1, 3, and 6 from the pugh matrix, since these sub systems don't affect any of the other sub systems I can select the best one with out considering the whole system.

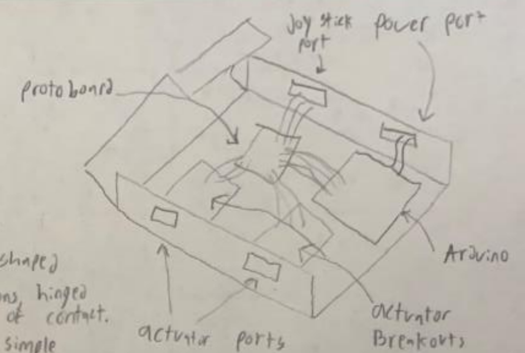
Note: weighting uses whole numbers for easier math

		Options					
		1 Hinged Clam shell		3 PC Tower		6 Box with hinged Lid	
Criteria	Weighting	Score	Total	Score	Total	Score	Total
protection from environment (splashes)	9	4	36	8	72	10	90
Small Footprint	7	7	49	10	70	7	49
Ease of acces to components	5	9	45	3	15	8	40
Ease of manufacturing	8	8	64	4	32	9	72
Access to ports	4	9	36	7	28	9	36
How well does it latch (close)	6	5	30	8	48	9	54
Total			260		265		341

Disscusion: The WDM shows that design option 6, Box with hinged lid. This matches the team and I's initial intuition. This design offers lots of flexibility with where we can locate the ports and its low height means we can position it under the stage or vertically on its side to reduce the foot print on the optical bread-board. We will proceed with generating solid models of this design, with several different versions to test different port locations. The goal is to have this design 3-D printed so we will keep that in mind while modeling.

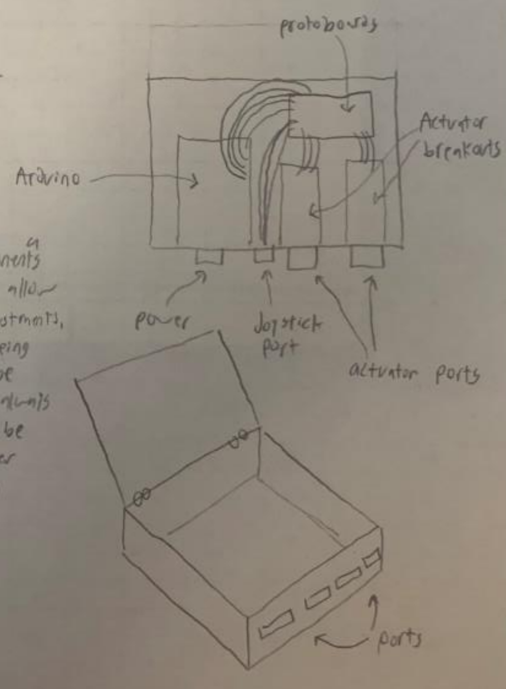
Top Ideas

1. Hinged Clamshell



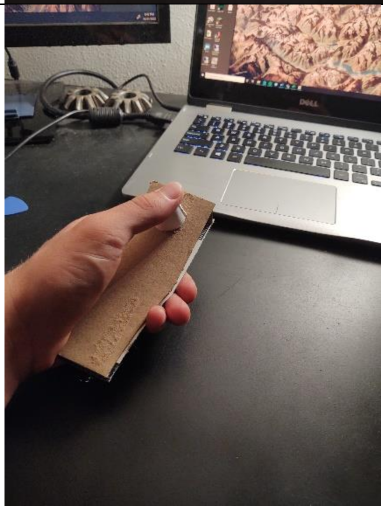
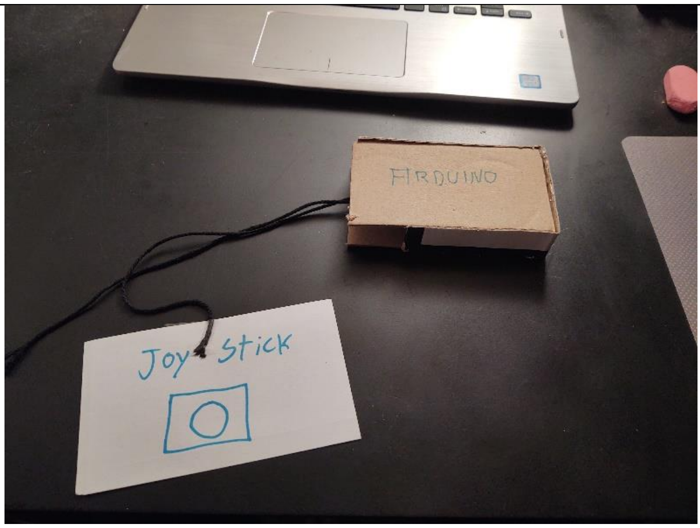
This design is a simple 'U' shaped box with a 'U' shaped lid of the same dimensions, hinged along one of the lines of contact. I will provide a very simple layout of the internal components along with really easy access. The ports are on opposite sides of the box which could allow for a more efficient wiring layout. This design could be difficult to make waterproof if that is something our sponsor asks for.

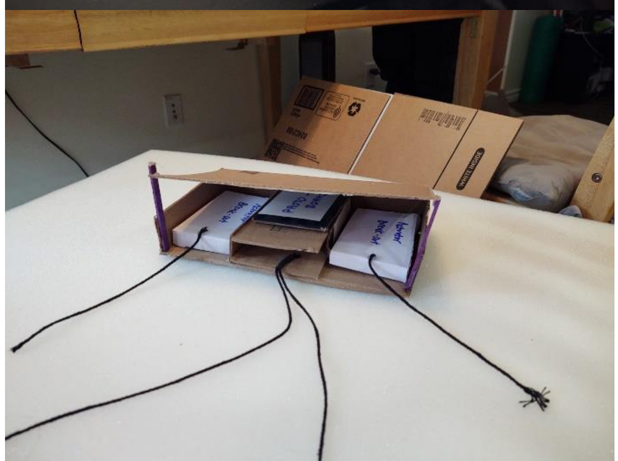
2. Box with Hinged lid



This design is a simple box with a flat hinged lid with all components layed out flat inside. This will allow easy access for repairs and adjustments. The ports are sketched as being all on one side which could be a little crowded but we can always change this. This design will be easier to manufacture and easier to put a waterproof seal on.

Appendix G – Ideation Models

Description	Image
Mock up of protoboard using the dimensions Spencer supplied. Used in mocking up electronics placement	
Model of handheld joystick suitable for use with both hands and could be held some distance away from the microscope.	
Mock-up of Arduino® Mega using dimensions from Spencer.	

Electronics box with a hinged lid with and without electronics inside.	 
Large flight stick style controller.	 

Appendix H- Off Campus Work Plan

Purpose

The primary goals of the project are to test the microscope in a way that minimizes potential risk of COVID transmission from team member to team member. The guidelines that follow are designed to accomplish this goal.

Background

To facilitate our understanding of the microscope's construction and operation and to perform benchmark testing our team must interact with the microscope. In spring quarter 2020 the previous senior project team interacted with the microscope virtually by having Prof. Mayer live stream working on the microscope in the clean room. After consulting with the previous senior project students and Prof. Mayer we decided that for the project to proceed on time and without undue effort on behalf of Prof. Mayer. We would move the microscope to a secure, off-campus location for testing and review. The location is an empty room in Matthew Pfeiffer's house. There we will be able to work on the scope in person.

Guidelines

- Understanding that the easiest way to minimize risk is to not work in person we will consider each task and evaluate if it can be done virtually or by a single team member
- Team members will not participate in in-person activities if they have tested positive for COVID, been in contact with someone who has, or are experiencing symptoms
- To maintain appropriate social distancing only one person will be working on the microscope at a time
- Masks will be worn at all times
- If more than one person is working on the scope the work will take place outdoors (Spencer's backyard)
- Before and after each activity with the microscope team members will sanitize their hands
- When it will not cause damage to the microscope or components the scope will be wiped down with sanitizing spray after use
- Team members will complete the Qualtrics COVID questionnaire or a paper substitute before coming to work on the microscope
- Testing activities will be logged in Microsoft Teams so if contact tracing becomes necessary records are available

Signatures

______________________________ ______________________________

______________________________ ______________________________

Appendix I Testing Manual for Stage Repeatability And Protoboard

TEST PROCEEDURE

Test Description:	Actuator repeatability test using the camera and microgrid paper to determine the stage position
Date Created:	11-5-20
Date Revised:	11-9-20

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. The travel will be measured by a micro

2.0 Facilities & Equipment:

The testing will be conducted on the IFM located on-campus in Bonderson

Table 1: Components used for testing

Equipment	Use
Arduino® Control System	This system will control the actuators using the command line interface
Newport 850G Actuators	These are the actuators whose precision will be tested
DC Power Supply	The power supply will increase the force delivered and allow for full actuation of the stage
IFM	The stage is equipped with mounting brackets for the actuators and a microgrid paper to determine position.
Soldered Protoboard	To connected driver chip to Arduino®

3.0 Safety Considerations:

No safety considerations are necessary for this test.

4.0 Data Collection:

Draw a square of various sizes at various different locations to determine in what directions and over what distances the actuators experience error the error can be quantified by the dots on the microgrid that are 0.060” apart in a triangle spacing. Screenshots are also taken after each actuator movement to compare the expected movement to the actual movement

To swap the A and B actuators will determine if an issue is localized to each actuator. The various sizes of square will determine if an issue is present at a particular stage location or over a particular region of the stage.

5.0 Testing Procedure:

Quantifying Stage repeatability

Using Grid on engineering paper characterize the repeatability of stage for various encoder count sizes

Draw a square using command lines of +X +Y -X -Y where X and Y are the same value

Square size (Encoder Counts)
100
200
300
400
500
600
700

Quantifying Stage repeatability

Using Grid on engineering paper characterize the repeatability of stage for various encoder count sizes

Draw a square using command lines of +X +Y -X -Y where X and Y are the same value of 500 encoder counts perform test at various starting positions, near center of the board and near each corner

Stage Position
Center
-x,-y
+x,-y
-x, +y
+x, +y

Swap actuator A for actuator B by plugging them into different ports and repeat tests to see if

Quantifying Stage repeatability with SWAPPED ACTUATORS

Using Grid on engineering paper characterize the repeatability of stage for various encoder count sizes

Draw a square using command lines of +X +Y -X -Y where X and Y are the same value

Square size (Encoder Counts)
100
200
300
400
500
Square size (Encoder Counts)
600
700

Quantifying Stage repeatability with SWAPPED ACTUATORS

Using Grid on engineering paper characterize the repeatability of stage for various encoder count sizes

Draw a square using command lines of +X +Y -X -Y where X and Y are the same value of 500 encoder counts perform test at various starting positions, near center of the board and near each corner

Stage Position
Center
-x,-y
+x,-y
-x, +y
+x, +y

TESTING DATA SHEET

Personnel: ______________ ______________

____________@calpoly.edu ____________@calpoly.edu

______________ ______________

____________@calpoly.edu ____________@calpoly.edu

Date: ______________________

Location: ______________________

Test Description: ____

Fill out the data sheet for each trial

Square size (Encoder Counts)	X Error Direction	X Error Size	Y Error Direction	Y Error size
100				
200				
300				
400				
500				
600				
700				

Stage Position	X Error Direction	X Error Size	Y Error Direction	Y Error size
Center				
-x,-y				
+x,-y				
-x, +y				
+x, +y				

SWAPPED ACTUATORS

Square size (Encoder Counts)	X Error Direction	X Error Size	Y Error Direction	Y Error size
100				
200				
300				
400				
500				
600				
700				

Stage Position	X Error Direction	X Error Size	Y Error Direction	Y Error size
Center				
-x,-y				
+x,-y				
-x, +y				
+x, +y				

TEST PROCEEDURE

Test Description:	Testing of the Soldered Protoboard
Date Created:	11-2-20
Date Revised:	11-3-20

1.0 Introduction:

The purpose of this test is to verify the soldered Protoboard is functioning as intended. There are many different things to verify including the solder joints, the wiring as well as the connections to the Arduino® Mega and the 25 breakout boards that go to the actuators.

2.0 Facilities & Equipment:

The testing will be conducted on the IFM located at Matthew Pfeiffer's house

Table 1: Components used for testing

Equipment	Use
Soldered Protoboard	Component being tested
Multimeter	The multimeter will be used to
DC Power Supply	The power supply will increase the force delivered and allow for full actuation of the stage
IFM	The stage is equipped with mounting brackets for the actuators and a microgrid paper to determine position.
Soldered Protoboard	To connected driver chip to Arduino®

3.0 Safety Considerations:

No safety considerations are necessary for this test.

4.0 Data Collection:

The multimeter will be used to determine whether the soldered connections are indeed connected and whether or not the voltages present are correct. These will be qualitative measurements and will be judged on a go, no-go criteria outlined in the testing procedure.

5.0 Testing Procedure:

Verification of L293D soldering

Check all pins against wiring diagram and solder map

Ensure that pins are not shorted together with visual inspection and multimeter

Verification of operation before connecting actuators

Check all pins against wiring diagram and solder map

Ensure that pins are not shorted together with visual inspection and multimeter

Connect to and power on Arduino®

Ensure Arduino® is communicating with laptop

Turn on power supply to 10.5 volts

Hit "enter" to begin zeroing process

Using multimeter see that voltage across 5&7 of the actuator connector pins is 10.5 volts -10.5 volts supplied to driver chip but 10.5 volts is not seen across the pins of the actuators, Voltage measures 3.4-3.5 volts can hear actuator wanting to move

Power off & shut down

Verification of actuator movement

Plug in actuators to protoboard making sure that actuator B with the special tape goes to its connector

Plug in joystick to Arduino®

Visual inspection & compare to wiring diagram

Turn on power supply to 10.5 volts

Hit "enter" to begin zeroing process

Ensure that the actuators are moving, hit the limit switches and don’t crash the stage

Use joystick to move stage

Use command line to move stage

Appendix J Risk Analysis

Y	N	
	a	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?
	a	2. Can any part of the design undergo high accelerations/decelerations?
	a	3. Will the system have any large moving masses or large forces?
	a	4. Will the system produce a projectile?
a		5. Would it be possible for the system to fall under gravity creating injury?
	a	6. Will a user be exposed to overhanging weights as part of the design?
	a	7. Will the system have any sharp edges?
	a	8. Will any part of the electrical systems not be grounded?
	a	9. Will there be any large batteries or electrical voltage in the system above 40 V?
a		10. Will there be any stored energy in the system such as batteries, flywheels, hanging weights or pressurized fluids?
	a	11. Will there be any explosive or flammable liquids, gases, or dust fuel as part of the system?
	a	12. Will the user of the design be required to exert any abnormal effort or physical posture during the use of the design?
	a	13. Will there be any materials known to be hazardous to humans involved in either the design or the manufacturing of the design?
	a	14. Can the system generate high levels of noise?
	a	15. Will the device/system be exposed to extreme environmental conditions such as fog, humidity, cold, high temperatures, etc?
	a	16. Is it possible for the system to be used in an unsafe manner?
	a	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
If not mounted or positioned appropriately, it may be possible for the microscope and optical breadboard to fall and cause injury.	When moved back into the Microfabrication lab, the microscope will have its own designated cart. The microscope will either be mounted directly to the cart, or the cart will be sized such that the system is fully constrained by walls. The system is never to be exposed to an open edge.	4/24/2020	
Small amounts of energy are stored in each of the springs for the stage return. Spring returns may also act as pinch point if body parts inappropriately placed under the actuated stage.	A warning sticker will be placed on the stage as well as a caution note in the user guide to inform user of the caution. An alternative approach would be to place a guard in this location, but it seems unnecessary as there is minimal risk of even minor injury. The groves that the springs rest in will be checked to ensure minimal risk of slipping and releasing stored energy.	4/24/2020	

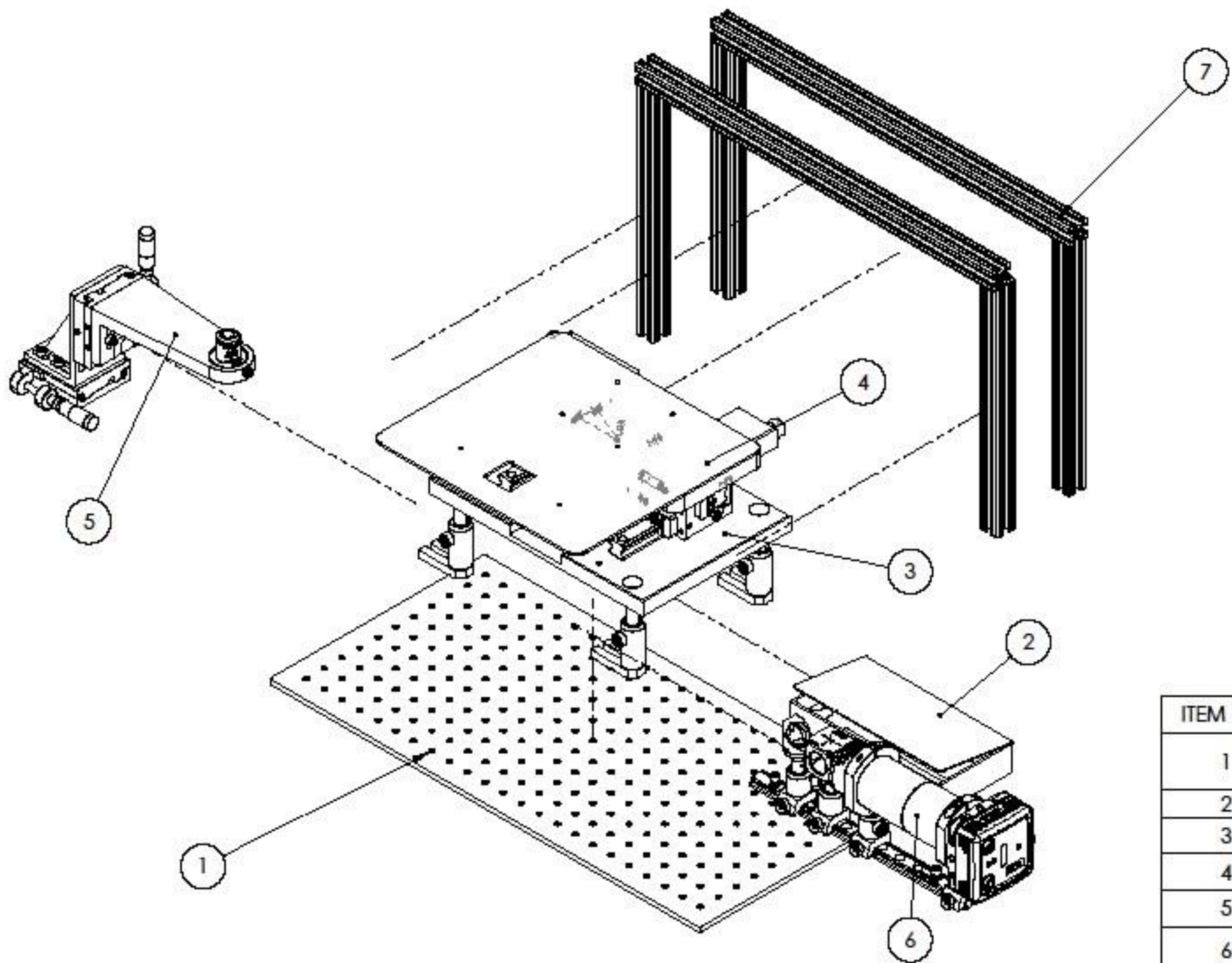
Drawing Package

Inverted Fluorescent Microscope (F13)

Indented Bill of Material (iBOM)

Color Coding	
No Modification	
Modifying	
Non-Existing	

Assembly Level	Part Number	Description					Qty	Cost	Ttl Cost	Existing?	Source	More Info
		Lvl0	*Lvl1*	*Lvl2*	*Lvl3*	*Lvl4*						
0	01000	Final Assy									------	
1	01100		Stage Assembly								------	
2	01110			Machined stage			1	$300.00	$300.00	Y	Sponsor	Machined Aluminum
2	01120			Linear Slide Blocks			2	$400.00	$800.00	Y	Sponsor	
2	01130			Microscope Baseplate								
3	01131				Optical Breadboard		1	-	-	Y	Sponsor	Aluminum Plate
3	01132				Silver 80-20 Corner Bracket (With Fasteners)		12	$5.21	$62.52	N	McMaster-Carr	Part No. 47065T236
3	01133				Silver anodized 80-20 2ft section		2	$7.79	$15.58	N	McMaster-Carr	Part No. 47065T101
2	01140			Optical Mounting posts							------	
3	01141				3-D printed base		4	-	-	Y	Sponsor	
3	01142				Dovetail Optical Mounts		2	$26.94	$53.88	N	Thorlabs	Part No. RC4
3	01143				Optical Post		4	$15.00	$60.00	Y	Thorlabs	
3	01200		Illumination Assy								------	
3	01210			Brightfield Illumination							------	
4	01211				Fiber optic Illuminator & cable		1			Y	Sponsor	Shorten Cable Length
	01212				Black 80-20 Corner Bracket (With Fasteners)		12	$5.60	$67.20	N	McMaster-Carr	Part No. 47065T831
	01213				Black anodized 80-20 1ft section		4	$6.73	$26.92	N	McMaster-Carr	Part No. 47065T503
2	01214				Black anodized 80-20 2ft section		2	$11.05	$22.10	N	McMaster-Carr	Part No. 47065T503
3	01220			Fluorescent Illumination			1					
1	01221				Fluorescent Illuminator		1	TBD	TBD	N	Sponsor	Not in project scope
1	01300		Optics and Mounts									
2	01301			4X Plan F Infinity-Corrected Objective Lens			1	$71.99	$71.99	Y	AmScope	PF4X-INF
2	01302			Objective Mounting Bracket			1	-	-	Y	3-D Print	DWG NUMBER
2	01303			RMS Microscope Objective Mount, 8-32 Tap			1	$29.76	$29.76	Y	Thorlabs Inc.	OMR-RMS
2	01304			1" Round, Protected Aluminum Mirror, 3.2mm Thick			1	$14.82	$14.82	Y	Thorlabs Inc.	ME1-G01
2	01305			45° Mount Assembly for Ø1" Optics			1	$48.01	$48.01	Y	Thorlabs Inc.	H45B2
2	01306			Cube-Mounted, Non-Polarizing, 50:50 Beamsplitter			1	$296.50	$296.50	Y	Thorlabs Inc.	CCM1-BS013
2	01307			FITC Emission Filter; CWL = 530nm			1	$257.54	$257.54	Y	Thorlabs Inc.	MF530-43
2	01308			SM1 Retaining Ring for Ø1" Lens Tubes and Mounts			1	$4.64	$4.64	Y	Thorlabs Inc.	SM1RR
2	01309			SM1 Lens Tube, 1" Thread Depth			1	$14.68	$14.68	Y	Thorlabs Inc.	SM1L10
2	01310			Ø1/2" Post Holder, L = 1"			1	$7.24	$7.24	Y	Thorlabs Inc.	PH1
2	01311			Ø1/2" Post, L = 1"			1	$4.88	$4.88	Y	Thorlabs Inc.	TR1
2	01312			Ø1/2" Post, L = 1.5"			1	$5.12	$5.12	Y	Thorlabs Inc.	TR40/M
2	1313			8-32 Flanged Socket Head Screw, L = 3/8"			1	$3.27	$3.27	Y	McMaster-Carr	92235A507
1	01400		User Interface									
2	01410			Joystick Housing			1	-	-	N	3-D Print	Format TBD
2	01420			Computer Monitor			1	-	-	Y	Sponsor	
	01430			Dedicated Microscope Computer			1	-	-	Y	Sponsor	
	01511			Graphical User Interface			1	-	-	N	Tkinter	Custom Software
1	01500		Electronics Subsystem									
2	01510			Wiring Subsystem								
3	01511				PCB		5	$5.00	$25.00	N	Oshpark	Custom Order
3	01512				Jumper Cables (Pack of Varying Color)		1	-	-	Y	Sponsor	
3	01513				Actuator Breakouts		2	-	-	Y	Sponsor	
3	01514				Pin Housing Connector Kit (Dupont Connectors)		1	$17.00	$17.00	N	Amazon	Clarks 1181 Pcs.
3	01515				Microcontroller (Arduino Mega)		1	-	-	Y	Sponsor	
2	01520			Electronics housing			1	TBD	TBD	N	3D Print	
2	01521			Electronics housing lid			1	TBD	TBD	N	3D Print	
2	01530			Power Supply			1	$40.00	$40.00	N	Sponsor	



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	01130	STUDENT FABRICATED OPTICAL BREADBOARD	1
2	1500	ELECTRONIC SUBSYSTEM	1
3	1100	ACTUATED STAGE	1
4	1110	MACHINED AND ANODIZED STAGE	1
5	1300	OPTICS AND MOUNTS	
6	1301	INFINITY CORRECTED OBJECTIVE LENS	1
7	1212	80-20 MOUNTING STRUTS	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:
FRACTIONAL± .25
ANGULAR: MACH± 1°
TWO PLACE DECIMAL ±.01
THREE PLACE DECIMAL ±.001
INTERPRET GEOMETRIC TOLERANCING PER: ANSI Y14.5
MATERIAL
FINISH:
DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	TE	3/10/21
CHECKED	TB	3/12/21
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

TITLE:
IFM ASSEMBLY

SIZE B | DWG. NO. 1000E | REV C
SCALE: 1:10 | WEIGHT: | SHEET 1 OF 1

Notes

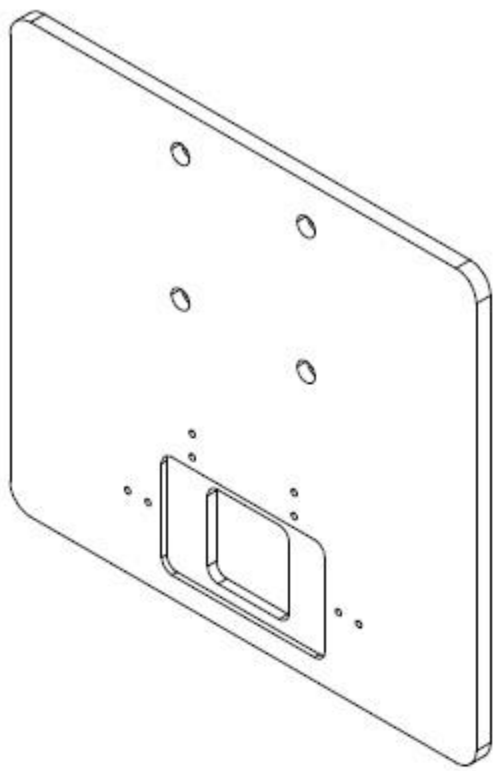
Many of the parts present on our microscope are from a previous senior project team. We have considered their microscope as an existing product documented in their senior project report which has been included in the reference section. The pre-existing parts listed in green on the Indented Bill of Materials are listed under different part numbers as the previous senior project team included below is a reference table between the two systems

Table 1 Part Number Reference Table

Previous Team	Current Team
012100	01120
013105	01130
011311	01141
011310	01143
013102	01212
011401	01300
011402	01301
011403	01302
011404	01303
011405	01304
011406	01305
011407	01306
011408	01307
011409	01308
011410	01309
011411	01310
011412	01311
011413	01312
011414	01313

Machined Aluminum Stage

Part no 1110 From previous senior project team part no. 12501 Modified by anodizing to black color existing plate shown below



Brightfield Illumination

Part no.01213 (1’ length) &01214 (2’ length) 80-20 struts
MacMaster Part no. 47065T503

Framing Type	T-Slot
T-Slot Framing Component	Rail
Rail Profile	Single
Single Rail Profile Style	Four Slot
Rail Height	1"
Rail Width	1"
Rail Construction	Solid
T-Slot Width	0.256"
Material	6105 Aluminum
Temper	T5
Finish	Anodized
Color	Black
System of Measurement	Inch
RoHS	RoHS 3 (2015/863/EU) Compliant
REACH	REACH (EC 1907/2006) (06/25/2020, 209 SVHC) Compliant
DFARS	Specialty Metals COTS-Exempt
Country of Origin	United States
USMCA Qualifying	No
ECCN	EAR99

Brightfield Illumination

Part no.01133 80-20 struts MacMaster Part no. 47065T101

Single Four Slot Rail, Silver, 1" High x 1" Wide, Solid

Framing Type	T-Slot
T-Slot Framing Component	Rail
Rail Profile	Single
Single Rail Profile Style	Four Slot
Rail Height	1"
Rail Width	1"
Rail Construction	Solid
T-Slot Width	0.256"
Material	6105 Aluminum
Temper	T5
Finish	Anodized
Color	Silver
System of Measurement	Inch
Length	2 ft.
RoHS	RoHS 3 (2015/863/EU) Compliant
REACH	REACH (EC 1907/2006) (01/19/2021, 211 SVHC) Compliant
DFARS	Specialty Metals COTS-Exempt
Country of Origin	United States
USMCA Qualifying	No
Schedule B	761090.0050
ECCN	EAR99
Related Product	Touch-Up Paint for Silver Rails (12 oz. Can)

Part no. 01212 80-20 mounting hardware MacMaster Part no. 47065T831

Framing Type	T-Slot
T-Slot Framing Component	Structural Bracket
Bracket Type	Corner
Corner Bracket Style	Corner
For Rail Profile	Single
For Rail Height	1"
Length	1"
Material	Anodized Aluminum
Mounting Fasteners Included	Yes
Mounting Fastener Installation Type	End Feed
Mounting Fastener Thread Size	1/4"-20
Mounting Fastener Thread Length	1/2"
System of Measurement	Inch
Color	Black
RoHS	RoHS 3 (2015/863/EU) Compliant
REACH	REACH (EC 1907/2006) (06/25/2020, 209 SVHC) Compliant
DFARS	Specialty Metals COTS-Exempt
Country of Origin	United States
USMCA Qualifying	No
Schedule B	761090.0050
ECCN	EAR99

Part no. 01132 80-20 mounting hardware McMaster part no 3136N2

Framing Type	T-Slot
T-Slot Framing Component	Cap and Seal
Cap and Seal Type	End Cap
For Rail Profile	Single
For Rail Height	1"
Includes	Push-In Rivet
Material	Plastic
System of Measurement	Inch
Color	Black
RoHS	RoHS 3 (2015/863/EU) Compliant
REACH	REACH (EC 1907/2006) (06/25/2020, 209 SVHC) Compliant
DFARS	Specialty Metals COTS-Exempt
Country of Origin	Peoples Republic of China
Schedule B	392690.9988
ECCN	EAR99

Brightfield Fiber optic Illuminator

Part no. 01221 Provided by sponsor but found at website linked below Fiber-Lite 180 Illuminator

Fiber-Lite 180 Illuminator, 150 watt Halogen fiber optic light source — Dolan-Jenner (dolan-jenner.com) Key features

- adjustable intensity, standalone unit , fiber optic compatibility

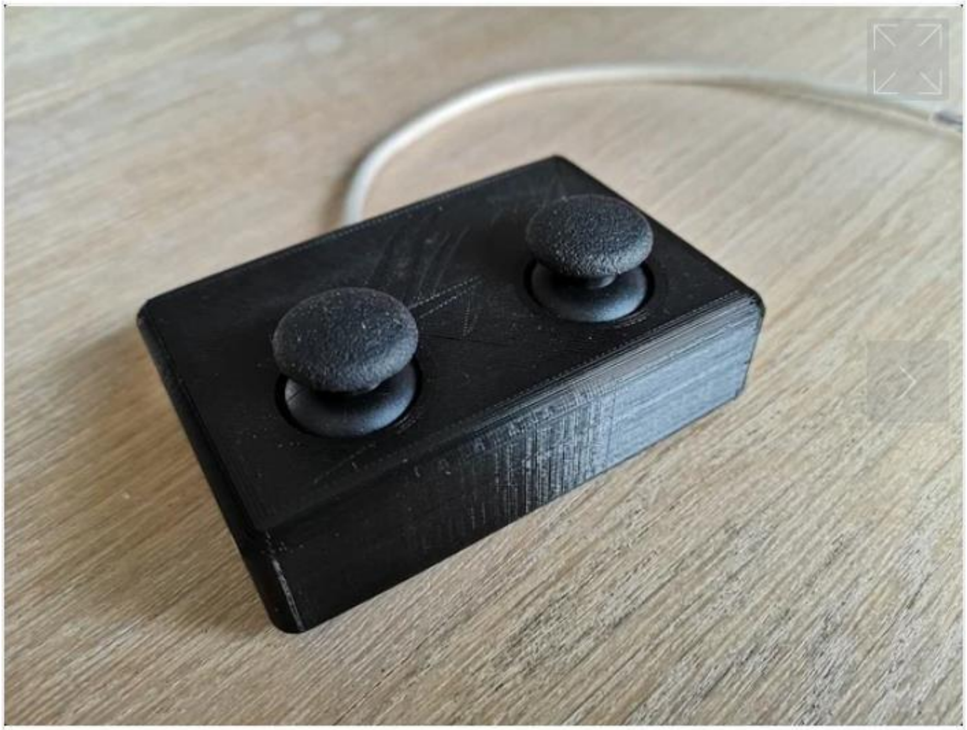
Joystick housing

Part no. -01410 3-D printed from thingverse .com link provided below

[Joystick Case by Zorglup - Thingiverse]

Key features-

Compatible with Arduino joystick, 3-D printed



Optical Mounting Posts

Part no. 01142 Dovetail mounts for optical rail used to position 45 degree mirror and beam splitter Thorlabs part no. RC4

Dovetail Rail Carrier, #8 (M4) Counterbore

Zoom

- 0.60" x 1.00" (15.2 mm x 25.4 mm) Carrier Platform
- #8 (M4) Counterbored Hole for Mounting

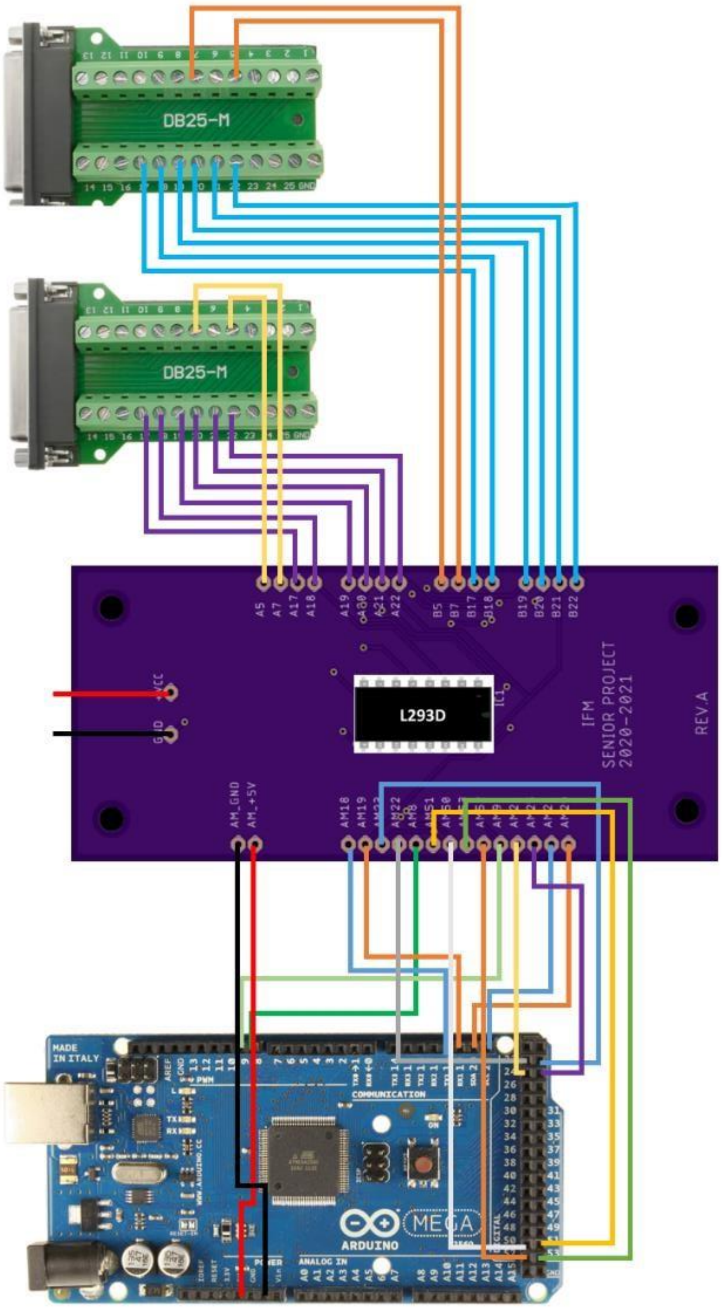
The RC4 Rail Carrier is designed to mount optomechanical components on an RLA dovetail rail using a #8 (M4) counterbore. The carrier can be placed onto the rail anywhere along its length without requiring access to the rail ends. The spring-loaded plunger built into the locking thumbscrew provides wobble-free translation as the carrier slides along the rail.

Based on your currency / country selection, your order will ship from Newton, New Jersey

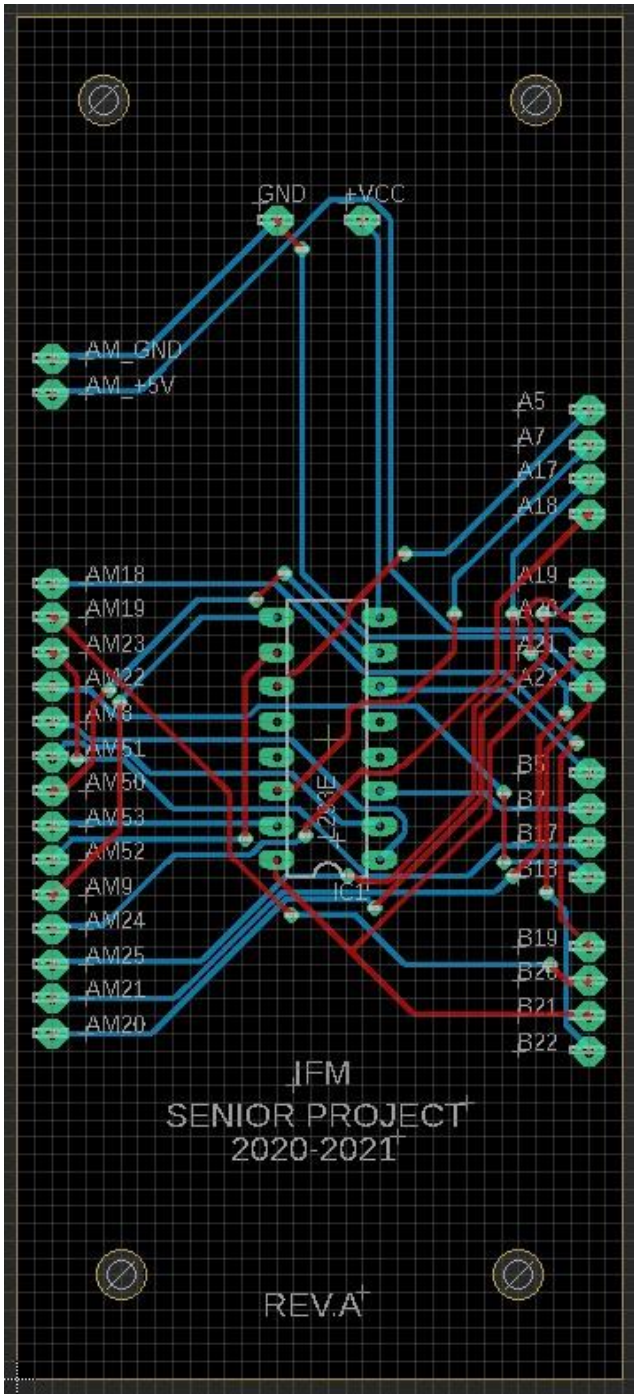
+1	Qty	Docs	Part Number - Universal		Price	Available
+1			RC4	Dovetail Rail Carrier, 0.60" x 1.00" (15.2 mm x 25.4 mm), #8 (M4) Counterbore	$26.94	Today

Add To Cart

Wiring Diagram of Completed Electronics Assembly



PCB cad model made using Eagle software



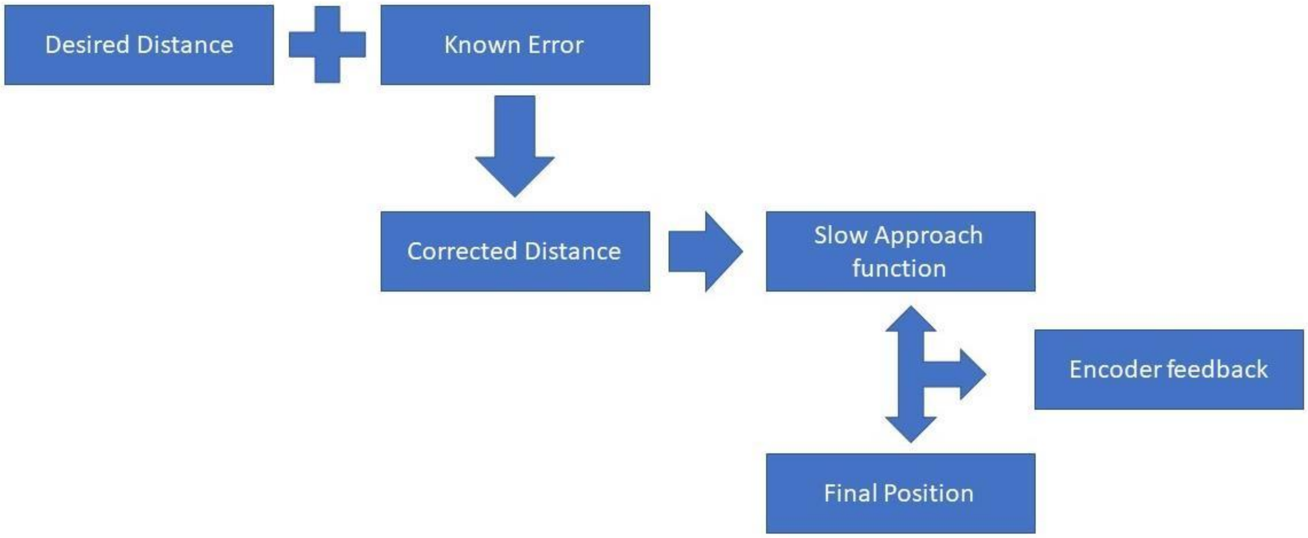
Pin connectors for Arduino

Part no 01514

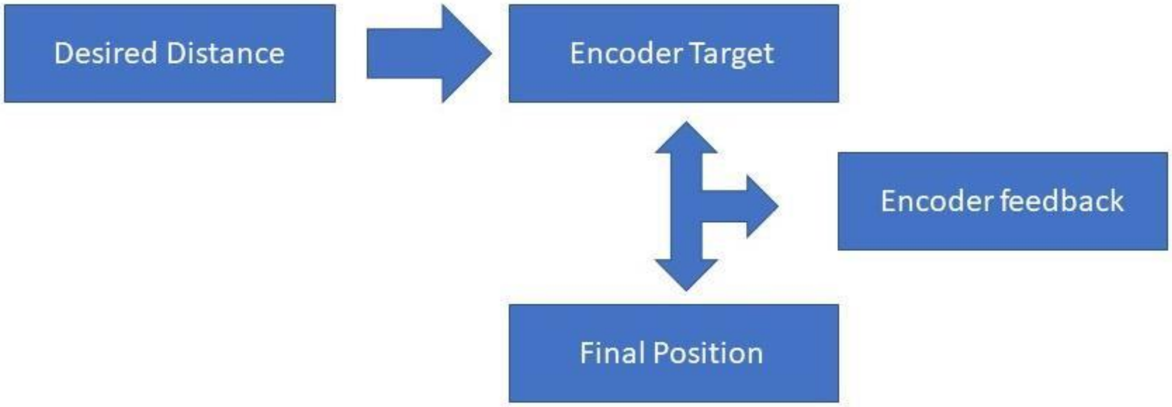
Amazon link [Amazon.com: Dupont Connector Kit - 1004pcs Crimp Connector Kit with 2.54 mm Crimp]

[Pin Connector Housings, Single Row Male Headers, Male/Female Crimp Pins and Ribbon Cable from Plusivo: Industrial & Scientific]

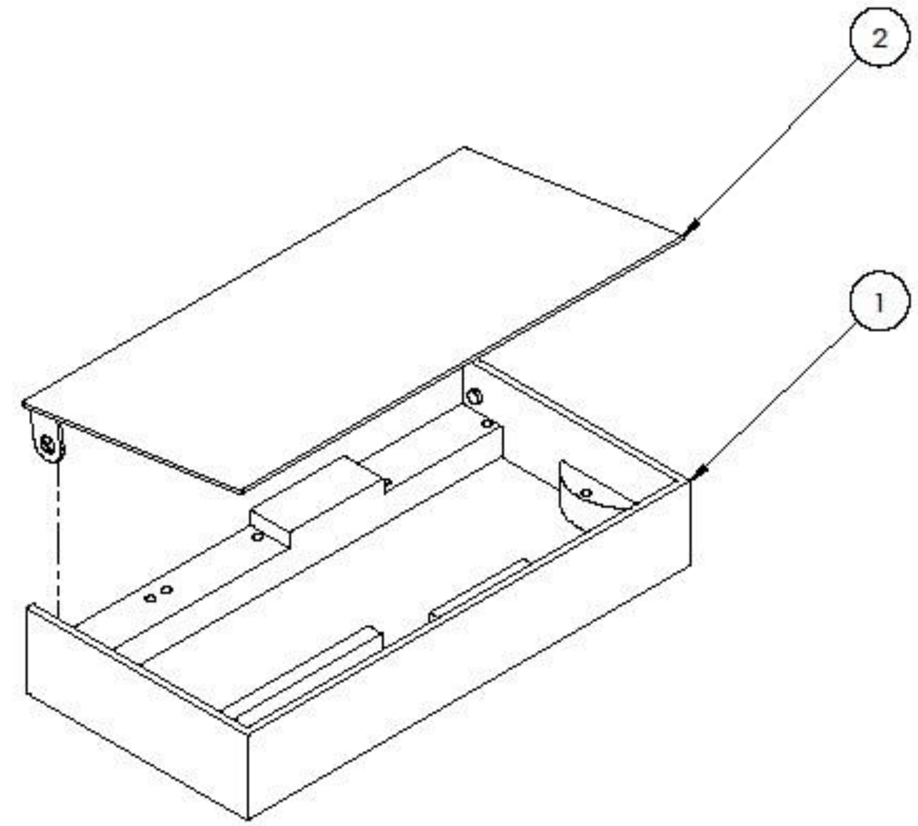
Pseudo code flowcharts describing the software changes to the Arduino control system



New Control Flowchart



Old Control Flowchart



ITEM NO.	PART	PART NUMBER	QTY.
1	EGG_3DPRNT_ELECTRONICS_ENCLOSURE	01520	1
2	EGG_3DPRNT_ELECTRONICS_LID	01521	1

PROPRIETARY AND CONFIDENTIAL

THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF F13 MICROSCOPE. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF F13 MICROSCOPE 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: ANSI Y14.5

MATERIAL

FINISH

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	TE	2/11
CHECKED	MP	2/12
ENG APPR.		
MFG APPR.		
Q.A.		

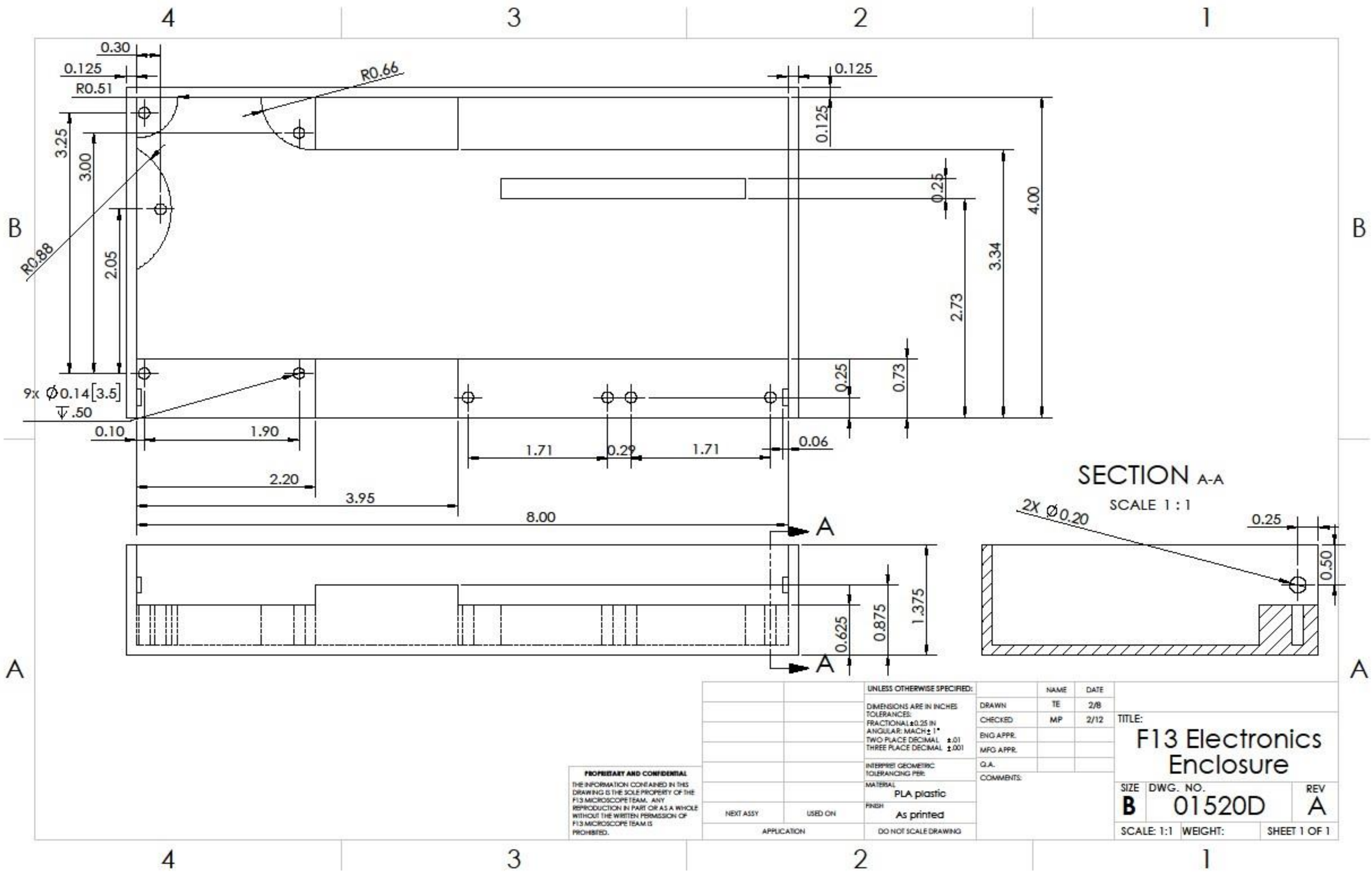
COMMENTS:

TITLE:

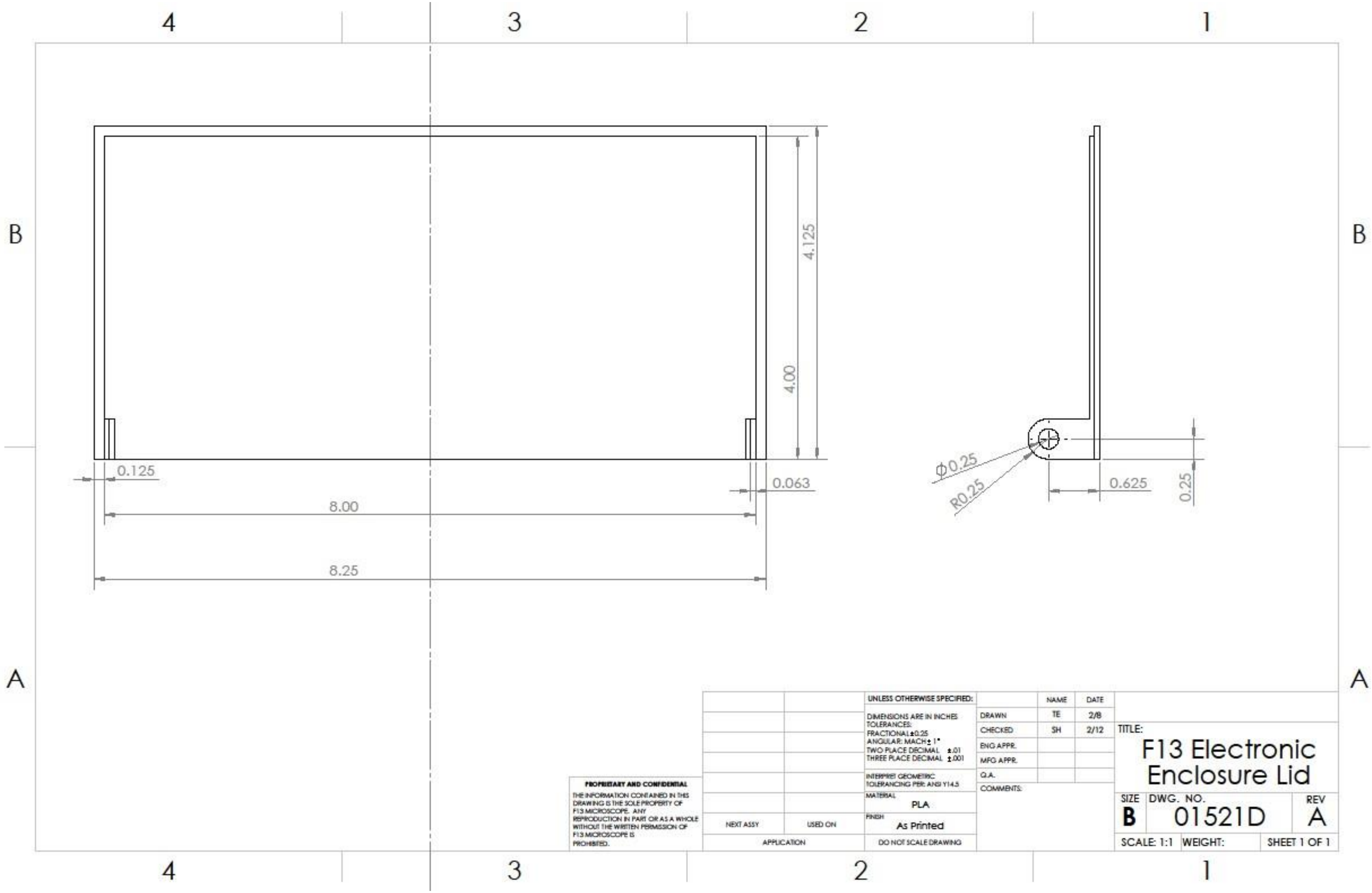
Housing Assembly

SIZE **B** DWG. NO. 01520A REV A

SCALE: 1:2 WEIGHT: SHEET 1 OF 1



0.30
0.125
R0.51
R0.66
0.125
0.125
3.25
3.00
R0.88
2.05
0.25
2.73
3.34
4.00
0.25
0.73
9x Ø0.14 [3.5]
↧.50
0.10
1.90
1.71
0.29
1.71
0.06
2.20
3.95
8.00
A
A
0.625
0.875
1.375
SECTION A-A
SCALE 1 : 1
2X Ø0.20
0.25
0.50
PROPRIETARY AND CONFIDENTIAL
THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF THE F13 MICROSCOPE TEAM. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF F13 MICROSCOPE TEAM IS PROHIBITED.
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL±0.25 IN
ANGULAR: MACH± 1°
TWO PLACE DECIMAL ±.01
THREE PLACE DECIMAL ±.001
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
PLA plastic
FINISH
As printed
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
TE
2/8
CHECKED
MP
2/12
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
F13 Electronics Enclosure
SIZE
B
DWG. NO.
01520D
REV
A
SCALE: 1:1
WEIGHT:
SHEET 1 OF 1



4
3
2
1
B
A
4.125
4.00
0.125
0.063
8.00
8.25
Ø0.25
R0.25
0.625
0.25
PROPRIETARY AND CONFIDENTIAL
THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF F13 MICROSCOPE. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF F13 MICROSCOPE IS PROHIBITED.
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL±0.25
ANGULAR: MACH± 1°
TWO PLACE DECIMAL ±.01
THREE PLACE DECIMAL ±.001
INTERPRET GEOMETRIC TOLERANCING PER: ANSI Y14.5
MATERIAL
PLA
FINISH
As Printed
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
TE
2/8
CHECKED
SH
2/12
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
F13 Electronic Enclosure Lid
SIZE
B
DWG. NO.
01521D
REV
A
SCALE: 1:1
WEIGHT:
SHEET 1 OF 1

Power Supply

Part no. 01530 Variable voltage power supply purchased from amazon link below

https://www.amazon.com/Adjustable-Eventek-KPS3010D-Switching-Regulated/dp/B073TW8H2S/

- DC Power Supply Adjustable: 0-30 volts and 0-10 amp outputs; the Back-lit LED display accuracy: 0.1 V and 0.01A , Could be used for electro etching
- Constant voltage and current operation mode (C.C and C.V. automatic conversion);small volume,light weight and stable output
- Intelligent temperature control with built-in thermo-sensor, effectively reduce noise and prolong the life of product
- Multiple protections: limit current protection, thermal protection, voltage overload protection, short-circuit protection

Appendix L

Design Failure Mode and Effects Analysis

Product: ______________________________ Prepared by: ______________________________

Team: ______________________________ Date: ______________ (orig)

Inverted Fluorescence Microscope (F13) FMEA												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	Criticality
stage/ increase functionality	Stage binds/crashes	Microscope becomes unuseable, damage to actuators,	8	Poor maintenance of linear bearings, unnaceptable misalignment when assembling, Electronics Malfunction, User Error	Physical limit switches present and wired on actuators, separate limit switches coded in software	1	test stage movement endpoints and limit switches	2	16	inspect stage at regular intervals as part of maintenance procedure	Spencer Continuous Procedure				
	Stage Mounts Fail	Microscope becomes unuseable, damage to optical components	9	improper installation, user damage	Installation and adjustment care	1	Stage seems non-rigid.	2	16	proper scope set-up	Matt January 6th	Scope is properly setup to prevent stage mount failure.			
Stage Controller/increase functionality	Stage Does not Actuate	Stage does not actuate, Reduced functionality	7	1) Actuator Receives wrong voltage. 2) Actuator mounts fail or binding occurs. 3) Actuator Motor Faiilure	1) Thorough actuator documentation research. 2) Discussion with previous team. 3) Keep actuator pathways clear and easily accessible for cleaning.	3	Stage doesn't actuate	2	48	testing plan in progress	Thomas February 2nd	Testing in progress			
	Position control norepeatable	Impossible for coordinate control microscopy, stage position feedback, and autocorrect capabilities.	3	1) Gear backlash, mount deflection, and other mechanical elements inhibit precise positional control. 2) Software and calibration procedure do not hone positional repeatibility. 3) Actuator rotary encoder or stage linear encoder failure.	1) Use of linear encodor to account for mechanical repeatability issues. 2) Thorough linear/rotary encoder controlling research and theory. 3) Thorough linear/rotary encoder research for appropriate design choices.	5	Repeatablity testing procedure with lithography masks	6	90	testing plan in progress	Thomas February 2nd	Testing in progress			
Decreased Lab Footprint	Unable to fit on smaller optical breadboard	Microscope takes up more space than necessary in microfabrication lab	2	1) Optical component layout non-optimal 2) Electronics housing too large (doesn't fit under stage) 3) Non-compact user interface	1) Find optical component of appropriate scale for sponsor needs. 2) Find layout for all components to mount to the optical breadboard.	5	Component layout does not fit on 1x2 breadboard we are trying to move to	2	20	perform layout analysis as part of detailed design	Matt January 21st				
Images can be taken/seen on monitor	Objective unable to be porperly focused	Cant observe sample	8	Optics improperly aligned	None	2	Image Clarity	3	48	ensure optics are properly aligned after reducing footpring, install guards	Trevor January 21st				
	Camera stops working	Cant observe sample	8	1) Camera got wet 2) Cable damaged 3) Camera software issue	None	1	Camera functionality.	3	24	none	Matt (No Action)				

Appendix L

Design Failure Mode and Effects Analysis

Product: ____________________________ Prepared by: ____________________________

Team: ____________________________ Date: _______________ (orig)

Inverted Fluorescence Microscope (F13) FMEA													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	Criticality
Electronics housed from the environment	Box is not environmentally protective	1) Improper electrical microscope operation 2) actuators get shorted	8	1) box not waterproof 2) box not secured to breadboard	None	3	Test splash proof before installing components. Mount to stage	2	48	Develop enclosure as part of detailed design	Spencer January 21st				
Brightfield Light source properly illuminates	Mounts are not solid	-Small vibration present - poor image quality	6	1)improper assembly 2) improper design of mounts	Mounting screws are tight	4	Vibration testing	2	48	redesign mounts	Thomas February 2nd				
	Illumintor does not function	-Slide impossible to see - poor image quality	6	1) illuminator not plugged in 2) Illuminator malfunction	Illuminator maintained properly	1	Illuminator does not illuminate	2	12	none	Thomas Continuous Procedure				
Fluoresence light source	Illumintor does not function	-Slide impossible to see - poor image quality	6	1)LED inorrectly wired 2)optical path blocked	None	2	Illuminator does not illuminate	2	24	none	Matt Continuous Procedure				
Stage moves accurately in joystick mode	Joystick breaks/loses functionality	- stage cannot move - stage hits limit switch	7	1)Wires become disconnected 2) encoder or thermal drift 3) actuator undervoltage	Electronics housing and assembly development.	5	Joystick no longer controls stage	2	70	testing plan in progress	Trevor February 2nd	testing in progress			

Appendix M Computer Code

Full code can be downloaded from
[Arduino and GUI code for the Cal Poly IFM senior project (github.com)]

```
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;  //Used to determine if the serial input is negative
boolean newData = false;  //Used to determine whether newData had been entered into the serial monitor
boolean EndMark_flag = false; //Used to determine 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 test_flag = false; //Selection flag for test case

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.
long int convert = 0.919; //number of encoder counts per micron used for PPC mode
long int correction = 0; //correction value to allow for negative overshoot

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 the speed of the motors, 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 A, terminal 19. Used for hardware interrupts
static int pinAB = 21; //Encoder Channel B for Actuator A, 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 the speed of the motors, 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("Initializing the X Axis Actuator\n");
          XZeroing(revlimB,forlimB);  //Zeroing the X axis while monitoring both limit switches
          Serial.print("Initializing 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, IFM in Joystick state");
          Serial.println("To change to programable path mode click the joystick");
          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
          //Serial.println(X_dir);
          //Serial.println(Y_dir);
          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 in Microns");
              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 in Microns");
          while (Serial.available() == 0){}  //Wait till Serial Monitor detects an input
          MoveYEncoder();
        }
        else if(test_flag == true){ //if in test mode is selected
          Serial.println("Select an Actuator to test");
          InitPPCselect();
            if(X_flag == true){ //if x actuator has been selected
              testXactuator();
            }
            else if(Y_flag == true){ //if Y actuator has been selected
              testYactuator();
            }
        }
        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
 *             state (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 PPC 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)or click the joystick to enter joystick control mode");
  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 = 2;        //Change to Joystick state
      switch_flag = 1; //Activate switch flag
      break;
      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 || character == 0x78){  //If character is equal to ASCCI "X"
      X_flag = true;   //Set X_flag to move to target step
    }
    else if(character == 0x59 ||character == 0x79){  //If character is equal to ASCCI "Y"
      Y_flag = true;   //Set Y_flag to move to target step
    }
    else if(character == 0x54 ||character == 0x74){  //If character is equal to ASCCI "T"
      test_flag = true;  // Set test_flag to move to test mode
    }
  }
  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 initialized");
  delay(500);         //Delay between prints
  y = 3500;           //Number of encoder counts
  dataNumber = 3500*0.919; //convert to microns for serial print
  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 initialized\n");
  delay(500);        //Delay between prints
  y = 3500;          //Number of encoder counts
  dataNumber = 3500*0.919; //convert to microns for serial print
  Serial.println("Moving Actuator to Zero Mark");
  MoveBOutToTarget(flimsw,y); //Move Actuator B to target while checking forward limit witch
}

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/0.919;        //Load y with new integer and change units to encoder counts
  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;  //Do not reset flag to enable corrective re-entry into MoveAOutToTarget
    }
  }
}

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/0.919;     //Load x with new integer and change units to encoder counts
 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;       //Do not reset flag to enable corrective re-entry into MoveAOutToTarget
   }
 }
}

//------------------------Encoder Movement for Actuator A------------------------
//------------------------(Y axis)----------------------------------------------

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.
 */
long int x = 0;  //New encoder count to reach
int y = 0;       //Limit switch variable
int f = 0;       //Speed Differential variable

  if(NEGnum_flag != true){//if  not entering as a negative correction & print as usual
      Serial.print("Moving Y actuator forward  ");
      Serial.print(dataNumber);
      Serial.print(" Microns \n");

      x =  EncAencoderPos + target; //Make new total encoder count to reach
    }
  else{ // if entering as a negative correction do not print and set X to the correction value
      x = EncBencoderPos + correction;
      Serial.print("Correction of   ");
      Serial.print(correction);
      Serial.print(" Microns required \n");
    }

  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(2);
      //Serial.println(EncAencoderPos);
    }
    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
  NEGnum_flag = false; //reset flags
  //Serial.println(f);
  Serial.print("target found at ");
  Serial.print(EncAencoderPos*0.919);
  Serial.print(" Microns \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 Y actuator backward ");
  Serial.print(dataNumber);
  Serial.print(" Microns \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(0.5*f);      //Set moving in speed to 50% the speed found above because the actuators move faster in the
reverse direction
      delay(2);
      //Serial.println(EncAencoderPos);
    }
    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

  correction = (x - EncBencoderPos) * 0.919; //calculates overshoot value in microns

//  Serial.print("target found at ");
//  Serial.print(EncAencoderPos*0.919);
//  Serial.print(" Microns \n");
  MoveAOutToTarget(forlimA,correction);
}

void testYactuator(){
  /*Description: Tests Y actuator repeatability across a chosen
   * encoder count range
   */
 Serial.println("Enter a target value for Y axis repeatability in encoder counts");
  long int y = 0;
  long int rep=0;
  //int k=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
        int k = 0;
        rep = (88100 - EncAencoderPos) / y;
        Serial.print("test will repeat ");
        Serial.print(rep-20);
        Serial.print( "times \n");
        for (k = 0; k <= (rep-20); k++){
          MoveAOutToTarget(forlimA,y);
```

```
        delay(50);
      }
    }
    else {                    //If the number is negative
      int k = 0;
      rep=(EncAencoderPos)/-y;
      Serial.print("test will repeat ");
      Serial.print(rep-20);
      Serial.print( "times \n");
      for (k = 0; k <= (rep-20); k++){
        MoveAInToTarget(revlimA,y);
        delay(50);
      }
    }
   NEGnum_flag = false;    //Disable number flag
   }
  Move_flag = false;        //Once completed, disable Move_flag
  test_flag = false;        //Once completed, disable test_flag
}

//------------------------Encoder Movement for Actuator B------------------------
//-----------------------------------(X axis)-----------------------------------

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.
 */
  long int x = 0;  //New encoder count to reach
  int y = 0;       //Limit switch variable
  int f = 0;       //Speed Differential variable

  if (NEGnum_flag != true){      //if  not entering as a negative correction print as usual
    Serial.print("Moving X actuator forward  ");
    Serial.print(dataNumber);
    Serial.print(" Microns \n");

    x =  EncBencoderPos + target; //Make new total encoder count to reach
  }
   else{     // if entering as a negative correction do not print and set X to the correction value
    x = EncBencoderPos + correction;
    Serial.print("Correction of   ");
    Serial.print(correction);
    Serial.print(" Microns required \n");
   }

  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(2);
```

```
      //Serial.println(EncBencoderPos);
    }
    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
  NEGnum_flag = false; //reset flags

  Serial.print("target found at ");
  Serial.print(EncBencoderPos*0.919);
  Serial.print(" Microns \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 X actuator backward ");
  Serial.print(dataNumber);
  Serial.print(" Microns \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(0.5*f);        //Set moving in speed to 20% the speed found above because the actuators move faster in the
reverse direction
      delay(2);
      }
    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

  correction = (x - EncBencoderPos) * 0.919; //calculates overshoot value in microns

//  Serial.print("target found at ");
//  Serial.print(EncBencoderPos*0.919);
//  Serial.print(" Microns \n");
  MoveBOutToTarget(forlimB,correction);
```

```
}

void testXactuator(){
/* Description: Tests the repeatability of the Y actuator by moving it a set number of encoder
counts and then repeating that action a desired number of times
 */
  Serial.println("Enter a target value for X axis repeatability in encoder counts");
  long int y = 0;
  long int rep = 0;
  //long int k = 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
    X_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
        int k = 0;
        rep= (88100 - EncBencoderPos)/y;
        Serial.print("test will repeat ");
        Serial.print(rep-20);
        Serial.print(" times \n");
        for (k = 0; k <= (rep - 20); k++) {
          MoveBOutToTarget(forlimB,y);
          delay(50);
          k++;
        }
      }
      else {                    //If the number is negative
        int k = 0;
        rep= (EncBencoderPos)/-y;
        Serial.print("test will repeat ");
        Serial.print(rep-20);
        Serial.print(" times \n");
        for (k = 0; k <= (rep - 20); k++) {
          MoveBInToTarget(revlimB,y);
          delay(50);
          k++;
        }
      }

    NEGnum_flag = false;  //Disable number flag
    }
   Move_flag = false;     //Once completed, disable Move_flag
   test_flag = false;     //Once completed, disable test_flag
}

//---------------------------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(2*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(2*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(1.5*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(1.5*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 inappropriate, 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;
        neg_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 || receivedChars[0] == 0x78){  //Compares receivedChar to X in hex
      Serial.println("X Axis Selected");
      character = receivedChars[0]; // X placed into character
    }
    else if(receivedChars[0] == 0x59 || receivedChars[0] == 0x79){ //Compares receivedChar to Y in hex
      Serial.println("Y Axis Selected");
      character = receivedChars[0]; // Y placed into character
    }
    else if(receivedChars[0] == 0x54 || receivedChars[0] == 0x74){ //Compares receivedChar to T in hex
      Serial.println("Test mode Selected");
      character = receivedChars[0]; // T placed into character
    }
    else{
      Serial.print("That letter is not one of the availible options, 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 counts
 *        the 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 = 61;        //Y-Intercept, Set speed is 150 for 10.5V or 175 for 5V
 float m = 169/88100.0; //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 manipilated to be slower at start (best lower limit estimate is b = 72 and m = 183/88100
 //At 12V to keep actuator voltage away from it's 12V max b=130 and m=100 keeping m+b below 255
}
```

Appendix N Design Verification Plan and Completed Test Procedures

DVP&R - Design Verification Plan (& Report)

Project: Inverted Fluorescence Microscope (F13) | Sponsor: Professor Hans Mayer | Edit Date: 4/22/2021

Test #	Specification	Test Description	Measurements	Acceptance Criteria	Required Facilities/Equipment	Parts Needed	Responsibility	TIMING Start date	TIMING Finish date	Numerical Results	Notes on Testing
	TEST PLAN									**TEST RESULTS**	
1 Repeatability Testing	Stage Repeatability	Use a reticle style mask with markings every 10 microns to quantify repeatability. See explicit repeatability testing procedure.	Repeatable Position Range	No visible drift or repeatability issues for either axis.	In-person microscope access.	Grid pattern mask.	Thomas	2/11/2021	4/20/2021	Average error of 1 micron total variance of 20 microns each direction	Repeatability testing procedding as expected Encoder Resolution testing requires furthur investigation
2 Undergraduate Usage Test	Instruction Time for General Use Ease of Setup Safety Considerations Simple Use Control	Have an undergraduate member of Dr. Hawkins' research lab use the microscope with limitted instruction.	Time to setup; Rating of ease of use ; Pass/fail ability to use microscope effectively	Less than five minutes of instruction to learn how to use the microscope in joystick mode	In-person meeting with undergraduate and microscope access.	Microscope and Lab computer	Spencer	5/21/2021	4/22/2021 (setup time) 5/24/221	less than the goal time of 2 min	easy to set up, power on and follow onscreen prompts General use of microscope revealed inconsitencies in error messages; negative flags; actuator zeroing vocabulary and user manual instructions reccomendations documented
3 Code Reliability Test	Code functionality CNC path Control	This test is to verify that the code works reliably, regardless of user input. It also has edge cases including actuating the actuators 0 units in PPC mode	Qualitative analysis of breakpoints in code	Microscope delivers appropriate error messages and does not become inoperable	In-person microscope access In final configuration	Microscope and Lab computer	Team	5/21/2021	5/24/2021	1 error in PPC mode over all user testing	repeatability numbers as expected
4 Microscope Robustness Test	Environmental Resistance Rigidity of mounting brackets Safety Considerations Rigidity of Optics	When transporting microscope, observe reaction to vibration from travel. Ensure all connections remain secure and electronics are safely housed.	Included checklist in test procedure	Microscope remains fully functional after transportation. IFM remains undamaged by typical use	In-person microscope access.	Microscope and Lab computer	Team	2/10/2021 Pending Microscope setup in Final configuration Re-Test needed in Lab environment	5/10/2021	Average robustness of components of 8.5	Everything as expected all safety items received a NO (non issue) in checklist
5 Technician Microscope Use Cases	Technician Calibration Time Cleaning Accesibility	Have Professor Mayer or Dr. Hawkins' perform any relevant calibration and cleaning procedure and record time it takes.	Time User feedback of cleaning procedures	Calibration consistently takes <10 [min] start to finish. Cleaning has no unresolved issues	In-person microscope access in lab position. In person meeting with Professor Mayer or Dr. Hawkins.	Microscope and Lab computer Setup in Microfluids Lab	Spencer	5/21/2021	5/24/2021	Technician setup time of less than 5 min	Spencer performed test as stand in for Dr. Hans and Dr. Hawkins. he was able to run the caliration and setup procedures correctly without prior functional knowledge

TEST PROCEDURE 1

Test Description:	Actuator repeatability test using actuator's internal rotary encoders to determine relative position of the actuator plunger and therefore the stage. Using this encoder to characterize the error between a desired movement and a=the actual movement.
Date Created:	11-5-2020
Date Revised:	3-4-2021

1.0 Introduction:

The purpose of this test is to verify the repeatability of the actuators' movement. The actuators use rotary encoders to determine the distance traveled by the actuator plungers and they will be compared to a desired travel value entered using the Programable Path Control mode of the Arduino code. Because of the codes design the

2.0 Facilities & Equipment:

The testing will be conducted on the IFM located in the senior project room at Bonderson on Cal Poly's campus

Table 1: Components used for testing

Equipment	**Use**
Arduino Control System	This system will control the actuators using the command line interface
Laptop Computer	Used to interface with the Arduino and view the command line interface to read the encoder data from the Arduino.
Laptop Software	*Calibration excel.xlsm* and *calibration.m* are both required for data processing and can be found in the team's onenote
Newport 850G Actuators	These are the actuators whose precision will be tested
DC Power Supply	The power supply will increase the force delivered and allow for full actuation of the stage
IFM	The stage is equipped with mounting brackets for the actuators and a microgrid paper to determine position.
Soldered Protoboard	To connected driver chip to Arduino

3.0 Safety Considerations:

Shop attire including long pants and closed toed shoes are a requirement for working in the senior project room.

Pinch hazards are present when the microscope stage is moving so do not touch the microscope while it is moving

4.0 Data Collection:

To determine the error between the desired actuator movement and the actual movement we will be using the Programable Path Control or PPC mode of the Arduino control system. In this mode a desired path described in length by number of encoder counts and direction by the actuator axis will be sent to the actuator. The actuator will then follow this path but due to the controller design will overshoot. Measuring this overshoot across various

To collect the difference in desired and actual movement the serial output of the Arduino will be processed by an excel sheet to scrub the text from the serial output and then a MATLAB script to graph the error and the desired actuator movement.

5.0 Testing Procedure:

Quantifying Stage repeatability

1. Turn on Arduino and interface with your laptop computer running the latest version of the control software pulled from teggenbe.bitbucket.io.
2. Power up the external power supply and set to 12v
3. Set up the microscope by following the on-screen prompts to zero the stage and set it to programmable path mode by clicking the joystick button.
4. When prompted to select an actuator to move enter “T” as the serial command to enter the testing mode
5. Follow the command line prompts and enter “X” encoder when prompted for a controller and 200 encoder counts when prompted for a target value
6. Copy and paste the serial output into the excel file and apply the *onlynums* function.
7. Run the MATLAB code ensuring that it is reading from the correct range of excel data.
8. Save the generated figure .

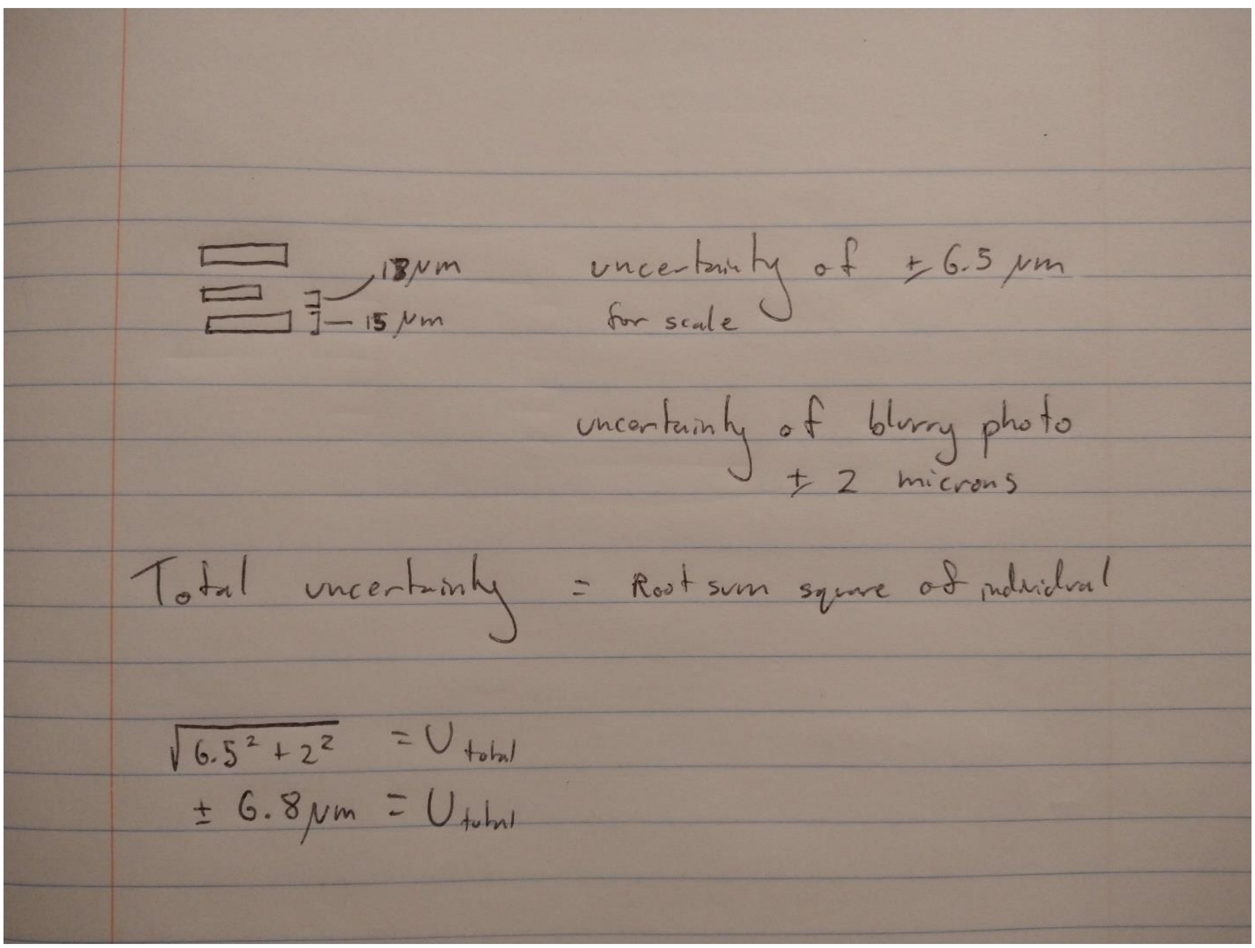
To increase the resolution of the data and characterize the error across a range of actuator travel ranges and directions modify the value of 200 encoder counts in step 4 to include all the values found below in the table. A positive error value indicates overshoot.

Test Procedure 1

Desired Distance	Actuator Tested	Complete?	Range	Average Error
200	X	Yes	48	+25
-200	X	Yes	172	+221
200	Y	Yes	27	+7
-200	Y	Yes	137	+114
100	X	Yes	38	+17
-100	X	Yes	161	+153
100	Y	Yes	27	+5
-100	Y	Yes	82	+95

Once these figures are generated the error should be characterized and analysis can begin to code that error out.

Error calculations for Micron to encoder conversion factor


13 μm
15 μm
uncertainty of ± 6.5 μm for scale

uncertainty of blurry photo ± 2 microns

Total uncertainty = Root sum square of individual

$\sqrt{6.5^2 + 2^2} = U_{total}$

$\pm 6.8 \mu m = U_{total}$

Test Procedure 1

Matlab Code for processing

```
%% Thomas Eggenberger Calibration data manipulation

% senior project microscope f13


%% y calibration


close all; clear all; clc


%fid = fopen('Major GPA.xlsm')


longcaldata=readmatrix('Calibration
excel.xlsm','Sheet','Y200','Range','F1:F337');


selector=longcaldata > 250;


caldata=longcaldata(selector);


error=zeros(length(caldata)-1);


for n = 1:length(caldata)-1

   error(n)=caldata(n+1)-caldata(n);

end


longcaldata1=readmatrix('Calibration
excel.xlsm','Sheet','Y200','Range','G1:G291');


selector1=longcaldata1 > 250;


caldata1=longcaldata1(selector1);


error1=zeros(length(caldata1)-1,1);
```

```
for n = 1:length(caldata1)-1
   error1(n)=caldata1(n+1)-caldata1(n);
end
figure(2)
hold on
axis([0.6E4,2E4,200,400])
plot(caldata1(2:length(caldata1)),error1)
plot(caldata(2:length(caldata)),error)
xlabel('stage position (encoder counts)')
ylabel('actual movement; desired 200 (encoder counts)')




%% x calibration
longcaldata=readmatrix('Calibration
excel.xlsm','Sheet','X100','Range','E1:E416');

selector=longcaldata > 250;

caldata=longcaldata(selector);

error=zeros(length(caldata)-1);

for n = 1:length(caldata)-1
   error(n)=caldata(n+1)-caldata(n);
end

longcaldata1=readmatrix('Calibration
excel.xlsm','Sheet','X100','Range','F1:F460');

selector1=longcaldata1 > 250;
```

Test Procedure 1

```
caldata1=longcaldata1(selector1);


error1=zeros(length(caldata1)-1,1);


for n = 1:length(caldata1)-1
   error1(n)=caldata1(n+1)-caldata1(n);
end
figure(1)
hold on
plot(caldata1(2:length(caldata1)),error1)
plot(caldata(2:length(caldata)),error)
xlabel('X position of microscope stage (encoder counts)')
ylabel('Total actuator movement (encoder counts)')
axis([0.4E4,2.2E4,150,300])
```

TEST PROCEDURE 2

Test Description:	Undergraduate Usage Test created by ME 429, Spencer Hann, F13
Date Created:	11-5-2020
Date Revised:	5-1-2021

Purpose:

The purpose of this test is to gauge the amount of time required for an undergraduate engineering student to prepare for and run a simple mock laboratory experiment. We will then be collecting qualitative feedback on improvements we can make to further streamline the lab operation process.

Scope:

This will be testing both the software and hardware components of the user interface system, specifically the user manual, graphical user interface, and the control joystick.

Equipment:

Three undergraduate volunteers (upper classman, microfab lab experience preferred)

Test slide, most likely the lithography masks we already have

The completed IFM setup including dedicated computer, joystick, and operation manual.

Two senior project members as observers, with timers and computers to take notes and record how long each task takes each student.

Hazards:

Covid

Pinch Points

PPE Requirements:

Since this test involves other students working together in a lab environment COVID-19 safety precautions must be taken.

All participating students are required to have been following the twice per week testing requirement set by Cal Poly as well as be cleared to access campus through the online questionnaire.

All participants must wear masks at all times and remain 6 feet apart.

All physical user interface components such as the keyboard, joystick, light source switch, etc. will be sanitized in between each user.

Users will be warned verbally and in the operation manual to keep away from moving stage to avoid pinch points.

Test Procedure 2

All students will be required to wear close toed shoes and full-length pants per Cal Poly general lab safety procedures.

This device is very safe to operate and there aren't very many specific safety procedures.

Facility:

Senior project room in Bonderson

Procedure:

1. Arrange IFM and computer on bench.
2. Power all the systems on and run calibration procedure.
3. Preform the mock test prepared for the test subjects to make sure all systems work.
4. Turn off all systems and return IFM to 'storage position.'
5. Bring in first test subject.
6. Provide them with verbal safety warnings, operation manual, and mock testing procedure.
7. Ask them to behave as if they were in class, focusing on working but not rushing to complete the test.
8. Time them on the startup, test, and shutdown procedure times.
9. Record all questions asked during the process.
10. Have them answer the survey questions.
11. Note any failures to return IFM to 'storage position' and correct them.
12. Bring in the next test volunteer and repeat.

Results:

Setup time: reading the manual, powering on the computer, microscope, lightsource, opening up the Arduino interface and running the calibration procedure, placing and securing the mask on the stage.

From 1 to 10, how easy was the operation manual to understand?

From 1 to 10, how easy was it to launch the software on the computer?

From 1 to 10, how confident do you feel in using the IFM again?

Do you have any suggestions for improvements to simplify the overall usage process?

Test Date(s):

Test Results:

Performed By:

TEST PROCEDURE 3

Test Description:	Code Reliability test Created by Trevor Blythe
Date Created:	2-8-2020
Date Revised:	5-1-2021

Purpose: The purpose of this test is to verify that the code works reliably, regardless of user input. The test is to make sure that the programming has been completed in such a way, that functionality is not broken by unexpected inputs. If properly completed, this test should only need to be performed once, however, the test procedure will likely need to be updated as the code is updated, and new potential break points are discovered.

Scope: This test will verify the function of the entirety of the code. This includes the reliability of the control loop, and the user input and interface.

Equipment:
-IFM microscope with actuators and stage present, along with the imaging lens, and camera to view the microscope stage.
-Laptop computer with Arduino IDE software, Amscope Labview software, and SolidWorks eDrawings Package software.
-Two USB A to USB 2.0 cables to connect Arduino and Amscope camera to computer.

Hazards:
-Pinch Hazards
    -keep hands away from IFM in operation.
-Electrical shock
    -keep hands away from exposed electronics on protoboard.
    -keep fluids away from the device.

PPE Requirements:
Shop attire, including long pants and closed toed shoes.

Facility:
Senior Project room 108 of Bonderson, where IFM is housed.

Testing Procedure

1. Power on microscope
2. Input test, performed as microscope is zeroing.

   a. Press enter, escape, and other keys, in a systematic manner, to verify that these keystrokes don't stop the zeroing process
   b. Use the joystick directionally, and press the joystick button, to verify that it doesn't stop the zeroing process.
3. Joystick mode test
   a. Put the microscope into joystick mode.
   b. While the microscope is in joystick mode, start pressing unnecessary keys, to verify that it does not unexpectedly exit from joystick mode.
   c. Press a key while simultaneously using the joystick directionally, to verify that it does not unexpectedly exit from joystick mode.
   d. Press the joystick button while using the joystick directionally, to verify that it doesn't unexpectedly exit from joystick mode.
4. Programmable path mode
   a. Put the microscope into programmable path mode.
   b. Program a path.
   c. As the microscope is undertaking this path, press errant keys, to verify that it doesn't unexpectedly exit from programmable path mode.
   d. As the microscope is undertaking this path, also use the joystick, to verify that it doesn't unexpectedly exit from programmable path mode.

Results: The test has only successfully been passed when all of these options are performed without breaking any of the features being tested.

#	Bug description	Possible cause?
1	When running in PPC after inputing random values the program has to read through each character that was entered	Serial input is stored for longer than anticipated
2		
3		
4		
5		
6		

Test Procedure 3

Test Date(s):4/13/21

Test Results: As noted above

Performed By: Thomas Eggenberger & Trevor Blythe

TEST PROCEDURE 4

Test Description:	*Microscope Robustness Test Procedure* Created by Matthew Pfieffer
Date Created:	1-15-2020
Date Revised:	5-1-2021

Purpose: This is a predominantly observational test to ensure consideration to specifications relating to general microscope robustness and general tolerance to environmental conditions. This test combines some of the specifications from the DVPR because these specifications can be tested in a short amount of time and should all be checked in the same microscope configuration. This procedure will test the microscopes ease of setup, footprint and rigidity of optics specifications.

Scope: This test is to be thought of more as a quality assurance method for more observational and subjective microscope specifications as presented in the House of Quality and DVPR.

Equipment:
-IFM microscope with actuators and stage present, along with the imaging lens, and camera to view the microscope stage. Microscope should be seated in
-Laptop computer with Arduino IDE software, Amscope Labview software.
-Two USB A to USB 2.0 cables to connect Arduino and Amscope camera to computer.

Hazards:
-Pinch Hazards
    -keep hands away from IFM in operation.
-Electrical shock
    -keep hands away from exposed electronics on protoboard.
    -keep fluids away from the device.

PPE Requirements:
Microfabrication lab general clean-room PPE.

Facility:
Microfabrication laboratory access. For testing in same environment as microscope is to be permanently located.

Testing Procedure
1. Setup microscope for operation in joystick mode.
2. Have copy of the Robustness Test Data Sheet available for completion.

3. Perform each test and ranking mentioned on the data sheet.
4. Supplement data sheet rankings with pictures and comments.

1. *Desired results with required uncertainty*

- All ranked features higher than 7/10.
- All safety considerations receive “NO”.

2. Diagram of apparatus and instrumentation

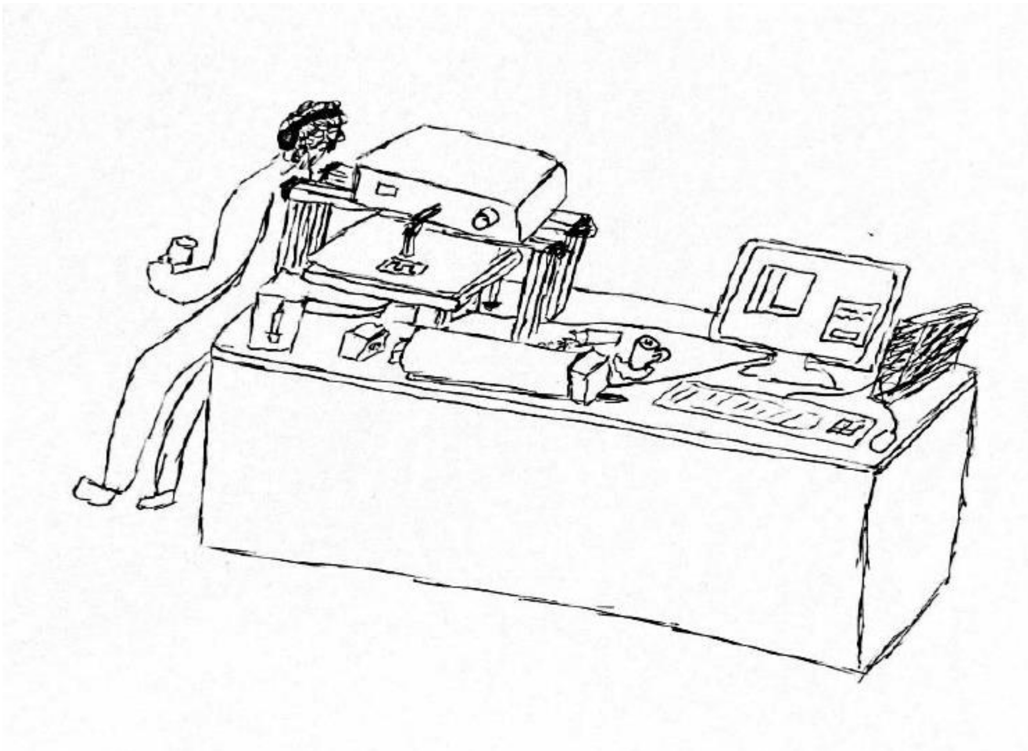


Figure 44. Sample Abuse Case

3. Priority list of measurements to be undertaken.

- Rankings of system and subsystem design-robustness
- Yes/No observational specification verification.

Test Date(s): Pending Microscope Completed Manufacturing
Test Results:
Performed By: Matthew Pfeiffer

Robustness Test Procedure Data Sheet:

Optical Mount Rigidity (Rank each out of 10):

- No visible deflection or oscillation. Rank 1-10 ***(____/10)***
- Sturdiness of optical mounts. Rank 1-10 ***(____/10)***
- Optical components easy to access and clean. Rank 1-10 ***(____/10)***

Electrical System Robustness (Rank each out of 10):

- Electrical component concealment: Rank 1-10 ***(____/10)***
- Cable management: Rank 1-10 ***(____/10)***
- Ease of cable connection and wire robustness (i.e. are there sharp angles, will cables wear over time if improperly located?): Rank 1-10 ***(____/10)***
- Electrical component spill protection: Rank 1-10 ***(____/10)***

Mechanical Component Inspection (Rank each out of 10):

- Light source capable of taking someone leaning or setting other objects on: Rank 1-10 ***(____/10)***
- Optical components protected from potential spill/bumping. Rank 1-10 ***(____/10)***
- Optical components protected from dropped components. Rank 1-10 ***(____/10)***
- Optical component alignment sturdy and not prone to change. Rank 1-10 ***(____/10)***

Design Modularity (Rank each out of 10):

- Parts easily sourced and replaceable. Rank 1-10 ***(____/10)***
- Components easy to access and clean? Rank 1-10 ***(____/10)***
- General microscope maintenance uncomplicated? Rank 1-10 (____***/10)***
- Design generally predictable/well-documented? Rank 1-10 ***(____/10)***
- Design incorporates future addition well. Rank 1-10 ***(____/10)***

Safety considerations (Yes or NO) [Note: All must receive NO by completion] :

- Is the microscope prone to tipping in lab environment? ***(Yes No)***
- Are there exposed high-voltage electrical components? ***(Yes No)***
- Do cords cause a potential tripping hazard? ***(Yes No)***
- Light source is prone to falling from bump or leaning? ***(Yes No)***
- Are all components of considerable weight rigidly attached? ***(Yes No)***
- Is the microscope prone to uncontrolled actuation? ***(Yes No)***

TEST PROCEDURE 5

Test Description:	*Technician Microscope use cases*
Date Created:	1-15-2020
Date Revised:	5-1-2021

Purpose: The purpose of this test is to test the microscope functionality with the lab technicians who will be maintaining the microscope and instructing students in it's use. The specifications tested will be cleaning accessibility and technician calibration time.

Location: The Microscope is currently located in Bonderson senior project room and should be tested in the small breadboard configuration with the latest version of the Arduino code.

Equipment

Table 1: Components used for testing

Equipment	**Use**
Arduino Control System	This system will control the actuators using the command line interface
Laptop Computer	Used to interface with the Arduino and view the command line interface to read the encoder data from the Arduino.
Laptop Software	*Calibration excel.xlsm* and *calibration.m* are both required for data processing and can be found in the team's onenote
Newport 850G Actuators	These are the actuators whose precision will be tested
DC Power Supply	The power supply will increase the force delivered and allow for full actuation of the stage
IFM	The stage is equipped with mounting brackets for the actuators and a microgrid paper to determine position.
Soldered Protoboard	To connected driver chip to Arduino

Safety Considerations:

Shop attire including long pants and closed toed shoes are a requirement for working in the senior project room.

Pinch hazards are present when the microscope stage is moving so do not touch the microscope while it is moving

Procedure:

A:To fulfil the calibration time test specification the test must take less than ten minutes to perform. Follow and time the procedure below.

Test Procedure 5

1. Start a timer
2. Restart the Arduino to re-zero the actuators
3. Open the Amscope software to place a cursor (reticle) on the microscope image
4. Using the joystick and or programable path mode place the reticle on a feature
5. Capture and image using the snap function of the Amscope software
6. Use the programmable path mode to draw a square in the X and Y directions
    a. Enter X 400 Microns, Y 400 μm, X-400 μm, Y-400 μm in programmable path mode to draw the square
7. Using the capture function capture another image
8. Compare images and determine if repeatability is acceptable if not consult user manual
9. Stop timer

Record Final Time:

B: Ensure cleaning of the microscope is easy clean the microscope and note any issues below including hard to reach areas or areas where disassembly is required

Notes:

Test performed on:

Test performed by:

Inverted Fluorescent Microscope (F13)

This is a digitally controlled microscope with a stage that moves on two horizontal axis, a digital optics system located underneath the sample, and a dual source illumination system (white light from above, fluorescent light from below). This device was designed to be used to observe samples produced in the micro-fabrication laboratory, specifically micro-fluidic devices.

Safety Considerations

- Prior to operating the microscope, it is important to ensure loose clothing, jewelry, and long hair are secured and free from the spring return on the underside of the stage.
- Ensure that all components of the microscope are securely attached to the stage or gantry above the stage such that is at risk of falling and causing component damage or user injury.
- Ensure that all cables are clear of the actuated stage motion and of the floor around the microscope.
- When finished operating the microscope. Be sure to unplug the microscope control box from the computer to cut the power to the Arduino microcontroller.

Component Breakdown

1. **Light Source**

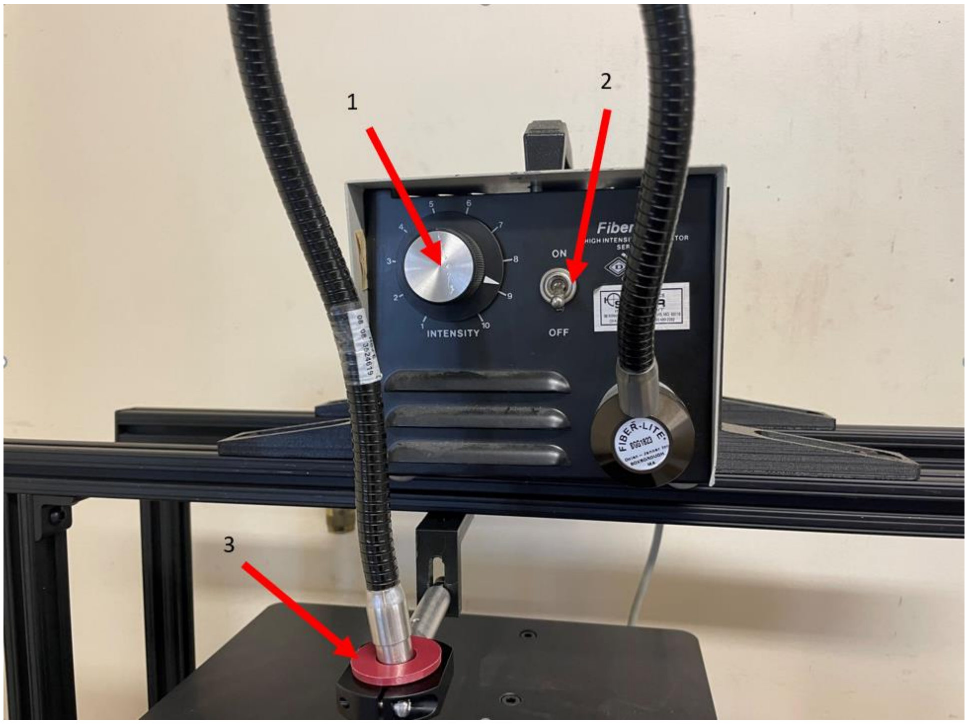


Figure 1: The visible-spectrum light source used to illuminate the samples from behind the objective. The intensity of this light source is controlled by the dial labeled as object 1 in the figure, which can be used in conjunction with the exposure setting in the camera software to achieve a clear, well-lit picture. The on-off toggle switch labeled as object 2 is used to power on the light source. Object 3 is the bracket used to position the fiber-optic cable above the sample and can be adjusted in 3-axis to achieve the desired lighting.

2. Fiber Optic Cable

The fiber optic cable directs the brightfield illumination onto and through the sample to provide enough illumination for the camera to provide a good image of the sample. Ensure that the fiber optic cable is positioned in the gooseneck to properly illuminate the sample

3. Joystick

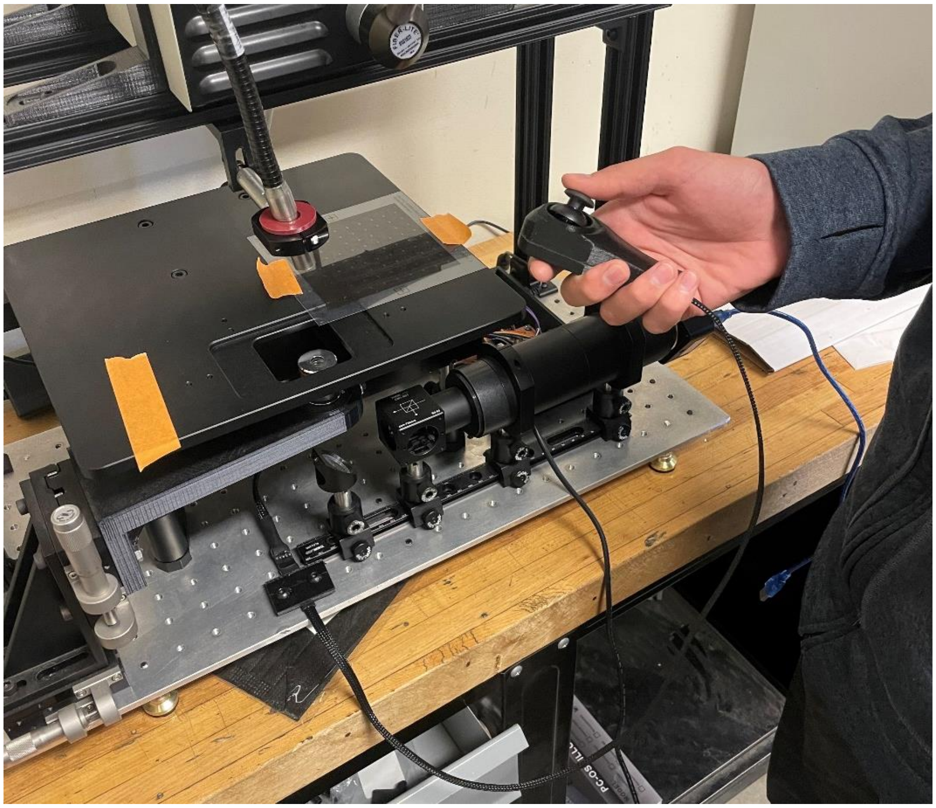


Figure 3: The joystick in use. The joystick is secured to the optical breadboard with a 3D printed plate to prevent any accidental tension from damaging the wiring. Care should still be taken to avoid yanking on the cable which may damage the scopes functionality. Pressing directly down on the joystick is how to switch between 'joystick' mode and 'programable path' mode and is referenced as the 'Joystick button' in the later parts of the manual.

4. Stage

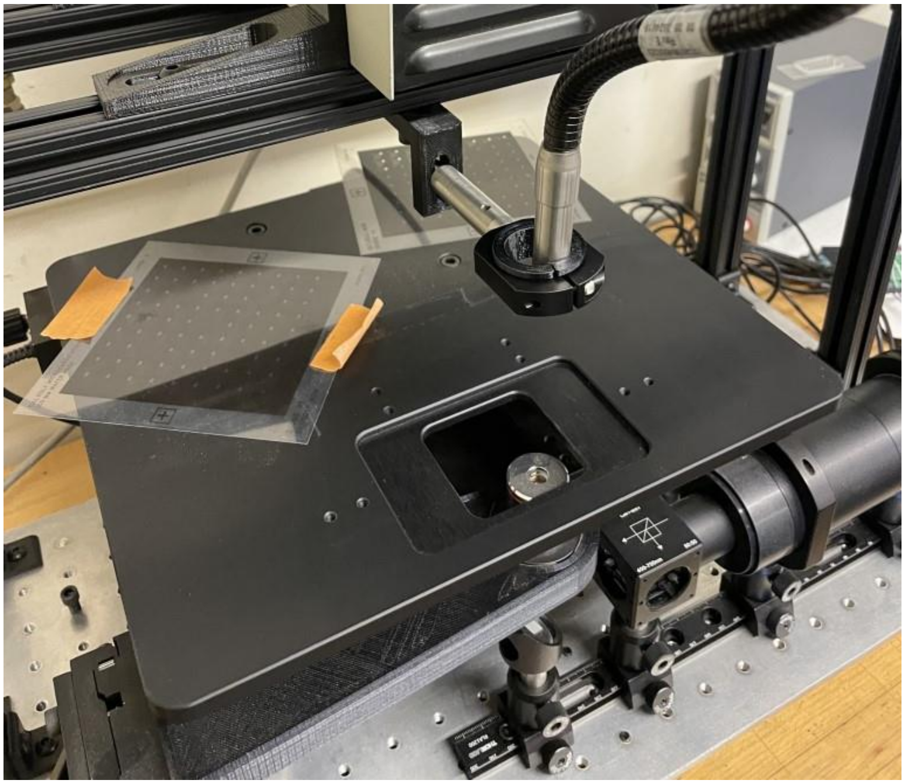


Figure 4: The stage the whole black plate seen in this photo. The indented rectangular portion around the cut-out is where samples are to be placed for viewing.

5. Actuators A & B

These actuators located at the rear of the microscope stage electronically actuate the stage by pushing against the bearing blocks of the stage. A spring return system is used as the actuators do not have positive movement in the negative direction. These actuators are connected with 25 pin connectors to the electronics assembly. During normal operation these actuators will not need to be directly accessed

6. Objective

The 35mm infinity corrected objective lens does the initial focusing of the sample image the image is then magnified in the tube lens and then captured by the camera. During normal operation ensure that the lens remains clean and free from scratches.

7. Camera

The Camera mounted at the end of the tube lens is controlled with the AmScope software loaded on the lab computer. This software allows for the digital adjustment of the camera exposure, which is utilized to obtain a clear, well-lit image. It also allows for the overlay of lines and digital measuring tools which are useful in the analysis of samples. The AmScope user manual, linked below, highlights how to use all the functions of the software starting at page 31 in the PDF.

https://www.amscope.com/software/AmScope/MU-Series-Complete-Manual-Complete.pdf

It is Also important to note that the camera has a correct orientation: the white FCC label on the side of the camera closest to the USB1.0 port should be facing out, towards the user, with the sticker side completely vertical.

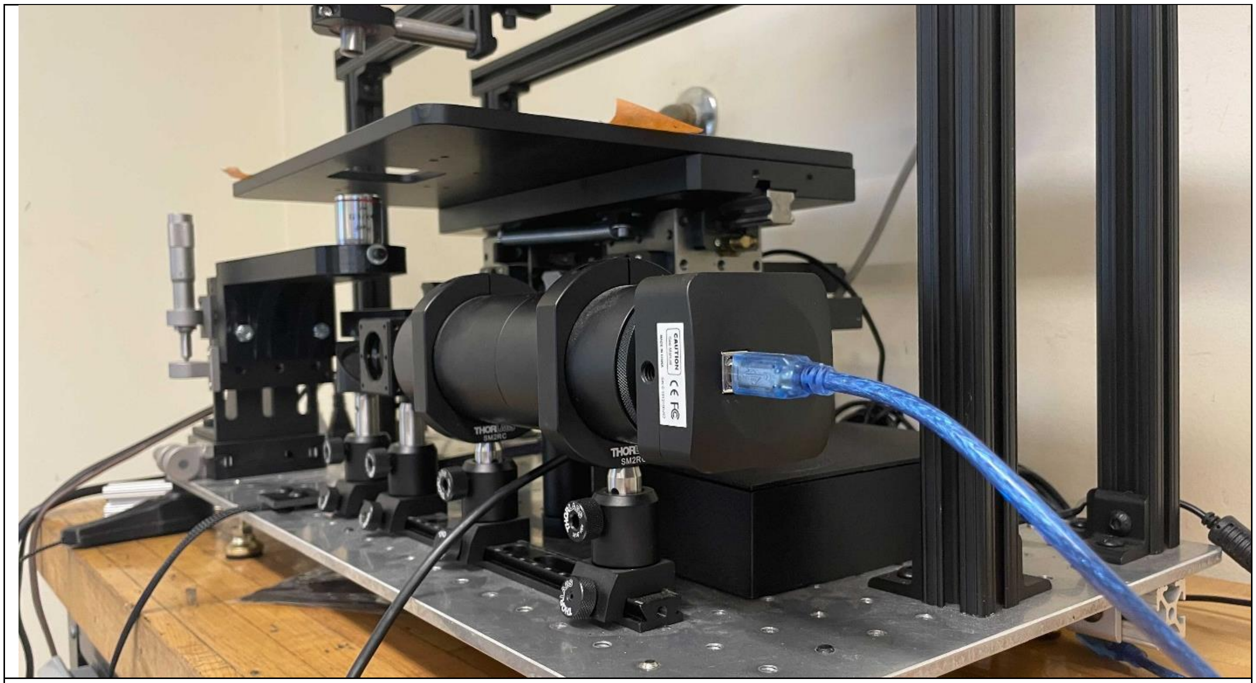


Figure XXX: This is how the AmScope camera should look when the microscope is in use

8. Objective Mount

The Objective is mounted on a cantilever 3-D printed mount. This mount is attached to the breadboard with a focusing assembly from Thorlabs. If the image on the camera appears out of focus twist the micrometer to adjust the focal length of the lens and bring the sample into focus. As different samples are placed on the stage the focus will need to be adjusted.

General Hardware and Software Setup Guide (Cold Start)

1. Make sure the system is appropriately powered on by plugging in the power supply to the back of the electronics control unit, turning on the surge protector.
2. Power on the control unit located under the stage by attaching the USB cable to the computer.
3. Plug the USB microscope camera cable attached to the optical tube lens into the computer.
4. Power on the light source using the switch and dial seen in figure 1.
5. Log into the computer using the guest account, which does not require a password.
6. Open the “IFM Control” software on the computer desktop. Note, executing this program will open both the IFM control software and the Amscope microscope camera.
7. To view the camera, select the MU-300 microscope camera from the Camera list on the left hand side of the Amscope software.

Microscope already on (Hot Start)

8. Click the button that reads “Zero Actuators,” Once the actuators are zeroed, the global and local coordinates of the microscope are displayed on the top of the IFM software, and Joystick Control Mode is active. By default, the local coordinates are set to the same value as the global coordinates. To set a datum, the local x and local y values can be zeroed at any time by clicking the “zero local x” or “zero local y” buttons.
9. When completed with microscope use, ensure that either the computer is shut off, or that the blue Arduino USB cable is disconnected from the computer to cut the power to the Arduino. This prevents potential accidental stage actuation.

Joystick Control Mode

Joystick Control Mode provides simple microscope translation for qualitative positional observation using a handheld joystick attached to the microscope stage. The Amscope software provides built-in image analysis and screen capture tools.

1. Once the actuators are zeroed, the Joystick control mode is automatically initiated. If coordinate control mode is active, the Control Toggle button reading “Enter Joystick Control Mode” can be pressed to transition back to Joystick Mode from Coordinate Control Mode.
2. When the status bar reads “Joystick Mode Active”, the handheld joystick can be used to move the stage in the X and Y directions.
3. At the top of the IFM control software, the global and local coordinates provide a reference for the scale of travel. To set a datum point, a local zero can be set for either the x or y axes.
4. The Amscope Software can be used to perform image analysis, and capture screenshots of specific microscope views.

Coordinate Control Mode

Coordinate Control Mode enables quantitative geometric-based image analysis. Desired coordinate changes can be submitted to the microscope for the stage to travel to.

1. From Joystick Control mode, coordinate control mode can be toggled by clicking the mode toggle button that reads “Enter Coordinate Control Mode.”
2. When Toggled, two new fields open, prompting the user to enter an integer number of micrometers to translate each actuator. These fields correspond to the x and y axes of the microscope, respectively.
3. After the value desired is entered, the “Submit” button must be clicked. Note: while the actuator is in travel, all buttons and submissions are temporarily disabled.
4. If the value is not an integer, or is outside the range of travel, the software will raise an error code in the status bar to indicate the problem and reset the submission field.

Command Line Operation

The command line operation is used when the GUI is nonfunctioning or you would like to troubleshoot the microscope. The command line can be accessed by opening the Serial Monitor functionality of the Arduino IDE. Once the command line is open simply follow the prompts to use the microscope.

Adjusting the Objective

The objective is the metal cylinder located beneath the stage and is what provides the focusing for the optics system. The position of the objective can be adjusted using the two micrometers located to the left of the objective. The focal point of the objective is fixed and so changing its distance from the sample in the stage will change its focus, so only use the vertical adjustment to change focus. Past the objective the optics train is infinity corrected so no more focusing can be done.

Focusing the Optics

To focus the optics, the barrel micrometer at the front of the microscope must be rotated until the comes into focus. Because the return of the objective z-axis sometimes gets stuck, the 3D printed objective arm may need to be physically pushed down into the barrel micrometer when backed away from the sample.

If it appears that the image cannot be focused it may be because the automatic gain in the camera is not adjusted properly. To adjust the gain unchceck the “automatic exposure” box in the amscope software and manually adjust the exposure until the image comes into focus.

<u>Future Design and Additions</u>

Fluorescence Illuminator Addition

For addition of a fluorescence illuminator to the microscope, the stage will need to be raised slightly. This can be accomplished by loosening the set screws in each of the legs, physically lifting the stage and actuator supports, and re-tightening them at the desired height. Additionally, the beam splitter will need to be dispatched, flipped 180◦ about the axis of the tube lens, and reattached. These component adjustments will allow for mounting of a fluorescence illuminator beneath the non-actuated stage. Care should be made in aligning the fluorescence illuminator such that the full optical pathway is created.

Software and CAD model Development

For full detailed view of the software, the IFM_Design folder on the designated microscope computer desktop provides all CAD models and both the code for the front-end graphical user interface and the back-end code flashed onto the Arduino microcontroller.

Troubleshooting

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
	Exposure setting in the camera software is set wrong	Adjust the camera exposure in both directions untill a well-lit, clear image is achieved.
	Dirt or debris on the objective lens / microscope optics are not clean	Clean objective / tube lens

Problem	Possible Cause(s)	Solution
Poor brightfield illumination	Brightfield focusing location is misaligned Microscope optics are not clean Specimen is not placed level	Re-position brightfield focus Clean objective / tube lens / focusing lens 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 Too many reflective surfaces Surrounding light is too bright Improper camera settings Mismatched fluorescence components	Shroud light path between optical components with lens tubes Turn off lights in room Decrease frame rate / increase exposure time of camera 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 Speed Differential is not reaching maximum value or overflowing	Increase voltage (DO NOT EXCEED 12V) 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

Transportation

This procedure is designed to instruct the reader on the safe transportation of all hardware related to the IFM. In addition to this manual, please use common sense and remember that this is a delicate instrument with many fragile components.

1. Before attempting to move anything, completely power down the microscope componenents by:
    a. Shutting down the computer and monitor
    b. Turning off and unplugging the brightfield illuminator
    c. Disconnecting the usb cables from the Arduino and camera from both ends
    d. Disconnecting the 12v power supply from the both the electronics enclosure and the wall
2. At this point there should be no cables leaving the optical breadboard, and the only two cables connected to the electronics enclosure should be the black 25-pin actuator cables.
3. Remove the brightfield illuminator from the gantry above the stage and transport it seperately.
4. Remove any samples or leftover materials from the stage.
5. Ensure that there are no loose items left sitting on the breadboard.
6. Tighten all thumbscrews on all the optical posts to finger tightness to ensure they don’t shift during transport.
7. The IFM is ready to be lifted. Due to the weight, two people are required to lift it using both hands and standing on opposite sides of the breadboard. Fingers should be hooked under the breadboard but not under the 80-20 support beams. Placing fingers under the beams can potentially create pinch points when setting down the IFM
8. Lift and move the IFM slowly, while keeping it as horizontal as possible. Placing it on a wheeled cart is recommended for long distance transportation. Note that if placed on a cart, straps should be used to secure the breadboard to the cart to prevent it from sliding.
9. Before placing the IFM on any surface, make sure the surface is clean, dry, level, and able to support the weight of the IFM and its auxiliary components.
10. When the IFM is in its new location, you may begin reassembling the components. Begin by connecting the computer and monitor to power and eachother, as well as connectin the mouse and keyboard to the computer.
11. Then connect the IFM to power using the 12v power supply.
12. Connect both the Arduino and camera to the computer using the two USBa to USB2.0 cables.
13. Finally, place the brightfield illuminator back on the gantry and connect it to power.