Microscope Low-Cost Motorized Stage

Biomedical Engineering 301: Biomedical Engineering Design

Date: March 2nd, 2022

Client: Dr. John Puccinelli, PhD, Department of Biomedical Engineering, UW-Madison

Advisor: Dr. Colleen Witzenburg, PhD, Department of Biomedical Engineering, UW-Madison

Team Members: Mark Nemcek - Team Leader, Nate Burkard - Communicator, Corey Steinhauser - BWIG and BSAC, Siddharth Kulkarni - BPAG

Abstract

The biomedical engineering teaching labs at the University of Wisconsin-Madison have two inverted fluorescent microscopes. These microscopes are the Nikon Eclipse Ti-U and the Olympus IX71. Both of these inverted fluorescence microscopes are currently controlled using manual translational control knobs. These manual control knobs do not allow for automated imaging and automated stitching of images. Integrating a motorized stage allows for a range of functions including time-lapse imaging, automated tracking, and image mosaic creation. The current commercially available options for motorized hardware for the stages of microscopes are too expensive. The overarching goal of this project is to design, program, and fabricate a lower cost motorized stage to be used for inverted fluorescent microscopes to allow for automated imaging and automated stitching that can be integrated with the Nikon Elements imaging software in the teaching labs. The mechanism must cost less than $100 and the resolution of the stage's movement should be around 1 um. A fabricated prototype will be created to help stabilize the motors responsible for operating the microscope. The stepper motors are connected to a rail system which can slide with the stage in the y-direction. Stepper motors are controlled with an Arduino Uno microcontroller and an Arduino program.

Table of Contents

Body

Introduction

In the current biological research community, innovative technology and more efficient research methods has been essential for progress. Improving research methods with technology can make results more accurate and time-efficient. As important as improving research methods and technology is, it is equally important that the technology is easily accessible and at low-cost, for more people to be able to use. The more people with access to the efficient and affordable technology, the quicker the development of research will be.

Microscopes are a type of technology used to help researchers see microscopic organisms and other types of cell biology. They are a key visualization tool used throughout several areas of research and development of drugs. Combining microscopy and imaging allows for permanent images to be taken of a microscopic sample, to evaluate at later times. Although imaging for future use is crucial for gathering and presenting research, it is not always intuitive. The ability to understand the microscope and all it is capable of can be critical for maximizing the potential use of imaging microscopy. Creating a more intuitive type of imaging microscopy can speed up the research process and lead to faster results.

Furthermore, automating the imaging process will allow imaging with a click of a button. Computer software and motors that control the stage can take images and stitch them together creating a large image of the entire sample, while still being able to zoom in on particular parts of the sample to see them in full focus. While there are some versions of microscopes with automatic imaging capabilities, these designs are expensive and therefore not always accessible for research. A tool or attachment for a microscope is needed to add automatic imaging to current microscopes.

Current designs for an automatic imaging tool include more affordable replaceable stages made by research groups [1]. These designs are useful, but can be difficult to standardize over several different types of microscopes, since stage size can vary, and having a replaceable stage may not be the easiest to attach and remove. More expensive products made by companies called Echo and Prior Scientific also come up with solutions. The Echo in specific, has many useful imaging features including imaging and stitching, but comes as a whole new microscope, and costs around $70,000 making it not feasible [2].

The biomedical engineering teaching tabs have two inverted fluorescence microscopes that use stages controlled by translational knobs. Manipulating these manual translational knobs to take images can be a tedious, and non-uniform process, making it an inefficient system to create sub-par images. Creating a cost-efficient method to automate imaging and stitching can benefit the BME teaching lab with a more efficient way to make accurate images.

Background

At the BME teaching labs there are two inverted fluorescence microscopes, the Nikon Ti-U and Olympus IX71. Fluorescence microscopy is ideal for imaging samples in biology labs because it allows the imaging of targeted, single cells using a naturally fluorescent protein or antibody as a fluorescence tag [3]. The Nikon Ti-U comes equipped with TI-SR Rectangular Mechanical Stages [4] and the Olympus IX71 comes with IX-MVR Mechanical stages [5]. Both stages can be controlled manually using the stage knobs in the x and y directions. Ideally, a motorized stage would be used because of its accuracy in movement and its capability for automated imaging. However, obtaining a motorized microscope stage can be very expensive. To image, software called Nikon Elements Basic Research is capable of processing, measuring, and analyzing images [6]. The integration of a motorized microscope stage with the Nikon Elements Basic Research software makes collecting imaging data easier and more time efficient, by allowing for automated imaging and stitching. An imaging device that can be easily detachable from the microscope will create a more affordable solution for a motorized stage with automating imaging and stitching. The client for this design project, Dr. John Puccinelli, the Associate Chair of the Undergraduate Program at University of Wisconsin-Madison wants a device that can be easily attached or removed to the microscope and the movement must have a resolution of 1 µm. To obtain this accuracy, a structural support system must be put in place for the motor to allow it to frictionlessly move along the rail system while the microscope is being operated. All the different components of the device must be integrated with Nikon Elements to be programmed to do a 30-minute automated imaging and stitching process. The device must be low-cost, within the budget of $100. See Appendix A for the full Product Design Specifications. Our preliminary designs and design matrix were made to brainstorm ways we can stabilize the two motors that attach to each gear on the manual control knobs. As the stage moves in the y-direction, the manual control knob moves in the same direction, so a linear rail system is required to hold the motors to the gears as the manual control knob moves. Our design matrix compares the variables stability, balance, detachability, compactness, ease of fabrication, cost, and weight between the three proposed designs for the linear rail system, to determine which design provides the most stability to the motors, while maintaining the client's ease of use.

Preliminary Designs

Design 1: One-Rail System

Figure 1. The One-Rail System. The bottom clamps on a bearing on the linear rail and the top holds the motor.

The first design is the One-Rail Design. The One-Rail Design works by clamping the bottom half onto the bearing on the linear rail. The top portion of the design would hold the motor tightly, which would ensure the motors would not be moving side to side. Any movement of the motor can cause drift, making the entire device much less accurate than intended. The biggest advantage the One-Rail Design has is that it's small and compact. This makes it easier to fabricate and less bulky, helping with the design's detachability. It also means it is easier to fabricate and uses less material, costing less. However, its small size and the fact it only attaches at one bearing makes it less stable than the other designs. The stepper motors and their attached worm drive gears are heavy and tip forward easily. Overall, the One-Rail Design is favorable because of its small size, but it is unfavorable in the most important aspect, its stability.

Design 2: Two-Rail System

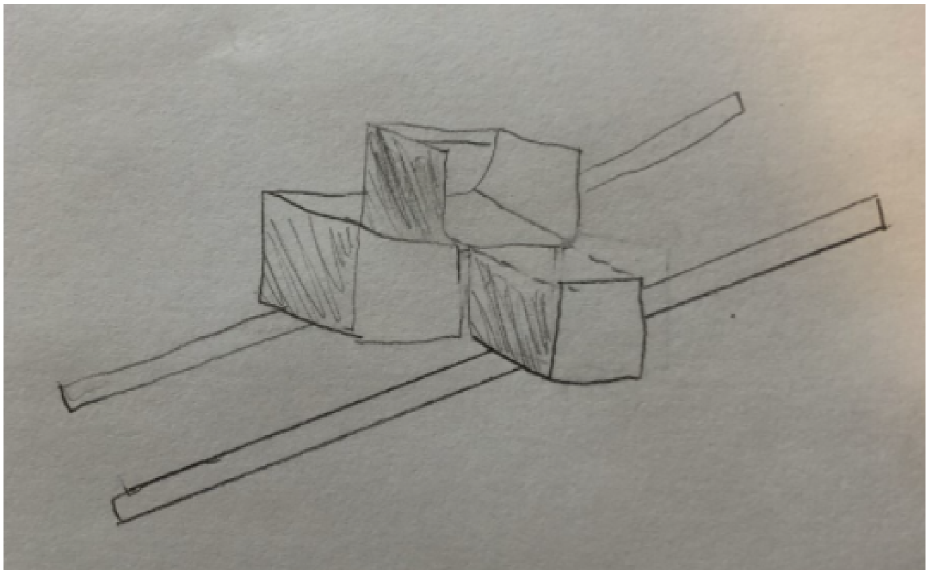


Figure 2. The two-rail system. The motor slips into its casing, which sits on two blocks that enclose the bearings, which slide on the rails.

The second design is the Two-Rail design. The Two-Rail design consists of Two-Rails, a bearing on each of the rails, and a 3D printed plastic that connects the bearings on each rail, and the motor. The 3D printed plastic has two parts, one being each of the nesting blocks, and the other being the motor casing. The two nesting blocks clamp on the blocks, and the motor casing sits on top and holds the motor in place. In contrast to the One-Rail design, Two-Rails being connected to the motor casing would provide further stabilization of the motors, and more balance to the heavy motors. Increased stabilization and balance of the motors is imperative for the accuracy of the automated microscope stage, as a small shift in the motors can cause drift, leading to inaccurate motor movements and images that will not be able to be stitched. However, with increased stability and balance of the motors comes increased weight and cost, and less detachability and compactness. Weight, cost, detachability and compactness are all variables that need to be considered, as they can affect the client's ease of use.

Design 3: The Tarp

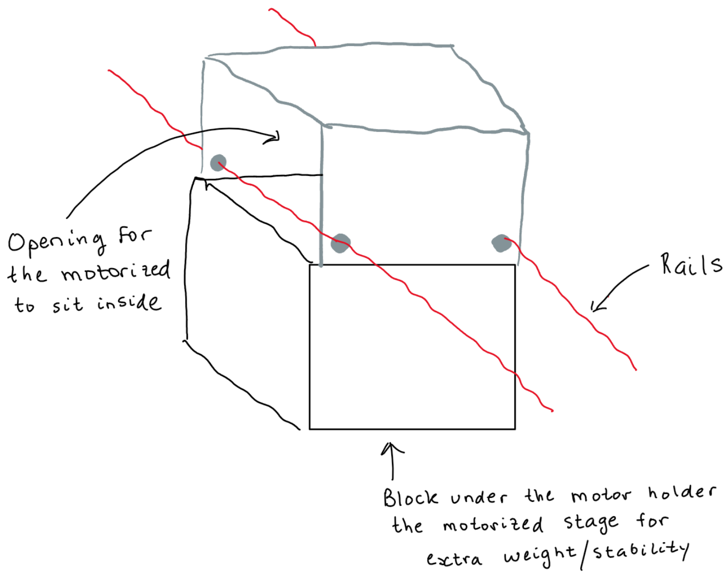


Figure 3. The Tarp Design. The motor slips through the opening on the left side of the covering and the support box below is meant to add some weighted stability of the motor upon operation of the microscope.

The third and final design is called the Tarp Design. This design is meant to implement some aspects of the One-Rail and Two-Rail designs shown above. Similar to the One-Rail design, the Tarp design has a little nested opening that the motor can slide into and effortlessly move along the frictionless rails upon operation of the microscope. Unlike the One-Rail and Two-Rail designs, however, the Two-Rails in which the device will be operating on are meant to go through the "tarp" part of the device in order to add balance and prevent the possibility of tipping and falling of the motor upon movement on the rails. The support box located underneath the Two-Rails is also meant to add to this stability in order to, first, prevent tipping of the device, and, second, prevent any bending of the material holding the motor up. As mentioned before, a main advantage of this design is to ensure the balance of the motor upon operation as well as prevent any tipping of the device upon operation. A couple disadvantages of this includes the cost of material required to build the device. Lot of material would be needed to build the support block underneath the rails as well for the "tarp" covering. Additionally, the opening for the rails to slip into the device would need to be measured precisely in order to prevent drift. Even with these openings being cut precisely, there is a huge possibility that this part of the device is at risk of damage if treated without care. If treated in such a way, drifting of the motors upon operation would likely result in poor resolution.

Preliminary Design Evaluation

Design Matrix

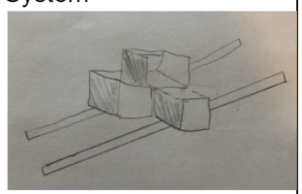
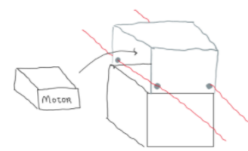
Design Criteria	Design 1: One Rail System 		Design 2: Two Rail System 		Design 3: The Tarp 	
	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Stability (25)	3/5	15	5/5	25	4/5	20
Balance (20)	2/5	8	4/5	16	3/5	12
Detachability (20)	5/5	20	5/5	20	1/5	4
Compactness (15)	5/5	15	2/5	6	4/5	12
Ease of Fabrication (10)	4/5	8	3/5	6	2/5	4
Cost (5)	5/5	5	3/5	3	2/5	2
Weight (5)	5/5	5	2/5	2	3/5	3
Total (100)		**76/100**		**78/100**		**57/100**

Figure 4. The Design Matrix. The matrix evaluates the three preliminary designs (at the top) based on seven criteria (on the left). The boxes highlighted in yellow show which design scored best in each category. The Two-Rail System scored the highest overall.

The main purpose of the project is to find a lower cost alternative to the options currently available, something that was important to reflect in the evaluation of the preliminary designs. After narrowing down the team's preliminary ideas for the design, a list of criteria was developed to evaluate the top three designs and compare them to one another.

Stability, balance and detachability were given the most consideration in the design matrix due to their general importance in the accuracy of the device. Stability and balance were considered to be very important for the team. Since the device was actually running last semester, the main goal of this semester was to make the device more accurate. To do this the motors need to be much more stable and balanced than they were last semester, which relied mostly on tape and a counterbalance for these two criteria. Detachability has to do with the ease with which the device would be able to be removed from the microscopes in the lab. This struck the team as significant because creating a prototype which requires complex changes in the structure of the microscope itself might result in increased complication and a worse ease of use. The idea here was that creating a device that could be easily interchanged between microscopes would likely yield the best results in terms of functionality in the lab.

The team also gave increased consideration to the compactness and ease of fabrication as these two criteria will have a large overall effect on the feasibility of completion in the semester. Too big of a device may be difficult to print and is going against the overarching goal of creating a small support device for the motors. Given the small timeframe and the relatively low budget for this project, creating a design that is cost efficient will allow for proper testing and inform future groups about any design updates that need to be completed in future semesters. Additionally, weight is an important factor to consider because too heavy of a device may cause impedance of the device to move upon the rail system. This can lead to poor resolution of the stitched images upon testing.

Proposed Final Design

Based off of the design matrix, the group decided that the Two-Rail System would be the fabricated design. The Two-Rail System exceeded the score of the Tarp by a large margin and the One-Rail System by a small margin. Overall, the main benefit of having Two-Rails for the system is the increased stability and balance of the motors, as it can sit on the middle of two bearings instead of one. The Two-Rail System ranked a five for stability and a four for balance on a five-point scale, in comparison to a three for stability and a two for balance for the One-Rail

System. Detachability was rated similarly between the One and the Two-Rail Systems, but the One-Rail System edged the Two-Rail System in compactness, ease of fabrication, cost, and weight. These four factors all had to do with the ease of use, and were important in giving our client an easier time setting up our attachment. However, the importance of the stability and balance of the motors to limit the drift of the stage and to obtain accurate stage movement outweighed the importance of the client's ease of use, and ultimately led us to decide to move forward with the Two-Rail System.

Fabrication Development Process

Materials

There are various materials necessary to create a functioning prototype of the automated stage. The group from last year left behind two stepper motors and two laser cut gears that attach to the x and y knobs with gear holders. Last semester the team bought a frictionless rail system as well as worm gears. Laser cut gears were made and the team 3D printed an adapter so that the worm gears could be attached to the stepper motors. Additionally, last semester the team provided an Arduino Uno microcontroller along with the wiring for operating the motors automatically. Finally, last semester the team bought a joystick device that will be used to control the motors and therefore control the movement of the stage. This semester the team wants to 3D print the stabilizing device so that the stage will not drift during movement. Additionally, a device is needed to raise the gear system to be flush with the worm drive gears on the motors. Acrylic will likely need to be purchased for this purpose.

Methods

In last semester's fabrication, two worm drive gears, a low friction rail system, and a joystick were ordered online. Two acrylic gears were laser cut to mesh with the worm drive gears and fit on the existing gear attachments from the previous semester's design. In order to attach the worm drive gears to the D-shaft of the two stepper motors, an adapter was 3D printed for each motor using CPE+. Pictures of each of these components are included in the following section. At this point in the fabrication process, the team started developing both the physical and electronic components of the design on parallel timelines. To assemble the mechanism, the gear holders with the laser cut gears were fastened to the knobs of the microscope. The worm drives, secured to the stepper motors using the 3D printed adapters, were placed on the rail system using a sheet of acrylic at a height which allowed for movement of each gear respectively. Adjustments in the height of the motors and gears were made incrementally as the team progressed with fabrication. In terms of electronics, the code for the stepper motors was developed using an Arduino Uno Microcontroller, allowing for isolated movement of the stage in both the x and y directions. Once the code was developed, the team started implementing the use of the previously mentioned joystick as the source of electronic input.

For this semester, a stabilizing device will be 3D printed to help stabilize the motor and prevent error and drift during movement. Additionally, the team will attempt to solder the joystick to create better connection to the electronics board. After completing the solder, further coding would be developed to better integrate the joystick with the motors. Acrylic will be laser cut to raise the gear system to be flush with the worm gears. Finally, the µManager software will be used to integrate both the Nikon elements software and Arduino Uno software to create a fully automated device.

Testing

The team wants to test the accuracy of our improved design using a hemocytometer. Using the Nikon elements imaging software along with µManager software, the group would set the stage to move a certain distance and capture a photo. Then, using the grid system of the hemocytometer, the distance that the stage actually moved could be checked. Using this method the group could find the error and drift that the stage produced while moving.

Conclusion

The teaching lab in the Engineering Centers Building of the University of Wisconsin Madison has two inverted fluorescent microscopes. The group was tasked with creating a low cost motorized microscope stage for these microscopes. The current microscopes are controlled by manual translational knobs that must be spun to move the stage. These manual knobs do not allow for automated imaging or image stitching. The team decided on a Two-Rail motor stabilizing system for the final design. This design consists of two motors, which each control one worm drive gear, that will be balanced on the Two-Rails of the frictionless rail system purchased last semester and placed adjacent to the microscope. One worm drive gear would control the movement in the x-direction and the other would control movement in the y-direction. Ultimately, the goal would be to have these motors be controlled by a joystick or other control mechanism and the resolution of the stages' movement should be 1-10 um. The final components of the prototype would need to successfully move the stage in a controlled and repeatable manner.The final design as well as the Nikon Ti-U will be used for testing. The motorized stage should also be implemented with the Nikon Elements software and capable of recording images and stitching them together automatically.

References

[1] Bhakti, T., Susanto, A., Santosa, P. and Widayati, D., 2021. *Design of Motorized Moving Stage with Submicron Precision*. [online] Citeseerx.ist.psu.edu. Available at: <https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.589.55&rep=rep1&type=pdf> [Accessed 15 October 2021].

[2] Discover-echo.com. 2021. *Revolve Fluorescence Microscope by Echo*. [online] Available at: <https://discover-echo.com/revolve> [Accessed 15 October 2021].

[3] K. Thorn, “A quick guide to light microscopy in cell biology,” *Molecular biology of the cell*, 15-Jan-2016. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4713126/. [Accessed: 20-Oct-2021].

[4] “Nikon Inverted Microscope ECLIPSE Ti-U Ti-U/B Ti-S Ti-S/L100 Instructions,” *eliceirilab.org*. [Online]. Available: https://eliceirilab.org/sites/default/files/2016-09/Nikon%20Eclipse%20Ti-U%20Manual.pdf. [Accessed: 19-Oct-2021].

[5] “Instructions IX71/IX51 - University College Cork.” [Online]. Available: https://www.ucc.ie/en/media/academic/anatomy/imagingcentre/icdocuments/OLYMPUSIX71_manual.pdf. [Accessed: 20-Oct-2021].

[6] “NIS-elements: NIS-elements basic research,” *Nikon Instruments Inc.* [Online]. Available: https://www.microscope.healthcare.nikon.com/products/software/nis-elements/nis-elements-basic-research. [Accessed: 11-Oct-2021].

[7] Holmarc.com. 2021. *Microscope Translation Stage*. [online] Available at: <https://www.holmarc.com/microscope_translation_stage.php> [Accessed 15 October 2021].

[8] Roymech.org. 2021. *Worm Gears - Roy Mech*. [online] Available at: <https://roymech.org/Useful_Tables/Drive/Worm_Gears.html> [Accessed 19 October 2021].

Appendix

Appendix A: Product Design Specification

Microscope Low-Cost Motorized Stage Product Design Specifications

February 11th 2022

Client: Dr. John Puccinelli
Advisor: Dr. Colleen Witzenburg

Team Members:
Mark Nemcek (Team Leader) mtnemcek@wisc.edu
Nate Burkard (Communicator) njburkard@wisc.edu
Corey Steinhauser (BWIG and BSAC) steinhauser2@wisc.edu
Siddharth Kulkarni (BPAG) sskulkarni4@wisc.edu

Function:

Inverted fluorescence microscopes are currently controlled using manual translational control knobs. These manual control knobs do not allow for automated imaging and automated stitching of images. Our goal is to design, program, and fabricate an attachment to motorize a stage to be used for inverted fluorescent microscopes to allow for automated imaging and automated stitching that can be integrated with the Nikon Elements imaging software. This attachment must cost less than $100 and the resolution of the stages' movement should be around 1 um.

Client Requirements:

- The movements of the stage should be able to be controlled by joystick or computer software.
- The program should be able to perform automated imaging and stitch images together.

- Team must create a motorized mechanism that moves and controls the stage.
- The movements of the stage should be within a resolution of 1-10 microns in x and y direction.
- There needs to be a fast and slow mode for the joystick.
- Should be powered by a wall outlet, and there needs to be a switch to turn the device on and off.

Design Requirements:

1. **Physical and Operational Characteristics:**

   a. *Performance Requirements:* The product must be able to automatically take pictures, and stitch them together. This device will be used often, and should be easy to put on and remove as an attachment. Should be powered by a wall outlet, but needs to have a switch to turn the device off. The device should be able to take images and stitch it in a 30 minute cycle. Need to increase structural stability . Additionally, a stabilizing device would be needed to hold the motors in place without tape.

   b. *Safety:* Keep parts away from the edge of the table as it is heavy and could pose a hazard to the user. Additionally, it is vital that any high-voltage elements be insulated and well organized, as to not cause any danger to the user.

   c. *Accuracy and Reliability:* The stage should have an ideal movement resolution of around 1 um. The client specifically requested that the stage have a resolution between 1 and 10 um. Cannot drift during imaging cycles to prevent faulty imaging.

   d. *Life in Service:* The microscope stage should be able to be used for as long as the microscope is in use. Since the microscopes have never had to be replaced in the past, the goal for our shelf life would be forever. A quantifiable goal would be at least 20 years of quality use.

   e. *Shelf Life:* When not in use, the device should be stored at room temperature and in a dry environment. The device will not require batteries as it will use standard wall power to run.

   f. *Operating Environment:* This device should be able to withstand similar temperatures to the microscope at 0℃-40℃ and less than 60% Humidity [1]. The device will be used inside where it will spend most time at room temperature, so it does not need to withstand a fluctuating temperature or environment.

   g. *Ergonomics:* The mechanical elements should not be able to be manipulated manually and should only be controlled using the provided controller or designed software.

h. *Size:* Should be able to be easily attached and removed and should not inhibit the movement of the stage in any direction. If we decide to replace the current stage plates, the new plates must not be taller than the current plates, otherwise the inverted fluorescent microscope will be inaccurate.

i. *Weight:* The weight of the stage should be small enough that it does not affect the balance or the mechanical properties of the microscope.

j. *Material:* There are not any restrictions, however typically light weight aluminum is used. Given the emphasis on keeping costs low, finding a material that functions well while also minimizing overall costs will be beneficial.

k. *Aesthetics:* Stage should be black in color, so it does not reflect light from the inverted fluorescence microscope. Stage should not be too bulky, as it needs to be able to be used practically with a classroom. Possible improvements could include removing/hiding the multi-colored foam pieces and exposed wires.

2. **Production Characteristics:**

a. *Quantity:* The client wants us to aim for an end goal of two units since there are two similar microscopes in the teaching lab, but he would be happy if we made one as long as it is functioning as desired.

b. *Target Product Cost:* The product must be less than $100. Client stated if necessary the group could go slightly over the target product cost, but does not expect this to be necessary.

3. **Miscellaneous:**

a. *Standards and Specifications:* Microscope stages do not need FDA approval as they are device class 1, which makes them exempt [2]. Nikon Ti-U Inverted Fluorescence Phase Contrast Microscope Pred Ti2 is the microscope that we will be using. Standard microscope safety procedures should not be compromised by the product.

b. *Customer:* Our customer would like us to have our design able to be controlled by a joystick as well as a computer program that can operate independently.

c. *Patient-related concerns:* Needs to be intuitive so that students who use the teaching lab will be able to use it for years to come. With the ongoing pandemic, the device needs to be able to be easily cleaned.

d. *Competition:* A couple of companies are selling work that is similar to our own. One of these companies is Zaber [3]. Some other companies doing this type of work are Prior Scientific [4] and Echo [5].

Works Cited: IEEE format

[1] "ECLIPSE Ti2 SERIES: SPECIFICATIONS," *Nikon Instruments Inc.* [Online]. Available: https://www.microscope.healthcare.nikon.com/products/inverted-microscopes/eclipse-ti2-series/specifications. [Accessed: 22-Sep-2021].

[2] "Product classification," *accessdata.fda.gov*, 20-Sep-2021. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=5177. [Accessed: 24-Sep-2021].

[3] *Zaber Technologies*. [Online]. Available: https://www.zaber.com/products/scanning-microscope-stages?gclid=Cj0KCQjwqKuKBhCxARIsACf4XuFgEuegCULlyzycG19HIr-bf7WOgpQ68wCHPqkBGRQRDXsFb2vVrIkaAlLpEALw_wcB. [Accessed: 23-Sep-2021].

[4] "Microscope motorized stages, XY stages, precision stages," *Prior Scientific*. [Online]. Available: https://www.prior.com/product-category/motorized-stages?gclid=Cj0KCQjw1ouKBhC5ARIsAHXNMI9_AsBqKR83FdP0QIOnJo6ztnDI9AJOTtT2zUPyAKtAoAT4fysqFrsaAqnMEALw_wcB. [Accessed: 24-Sep-2021].

[5] "Redefining microscopy for life science research," *Discover Echo*. [Online]. Available: https://discover-echo.com/?utm_source=google&utm_medium=search&utm_campaign=11380688541&utm_term=motorized+microscope+stage&utm_content=505993872680&gclid=Cj0KCQjw1ouKBhC5ARIsAHXNMI_3xPRL8j0lS98sKdirvi69QyfNvPLfS-GGZ8iVymvGb_l_qPeDi7caAgf1EALw_wcB#headerAnchor. [Accessed: 24-Sep-2021].