Microscope Low-Cost Motorized Stage

Biomedical Engineering 301: Biomedical Engineering Design

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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.

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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 take advantage of the time-efficient methods. 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].

Using microscopes with manual translational knobs to take images can be a tedious, and non-uniform process, making it an inefficient system which can lead to 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

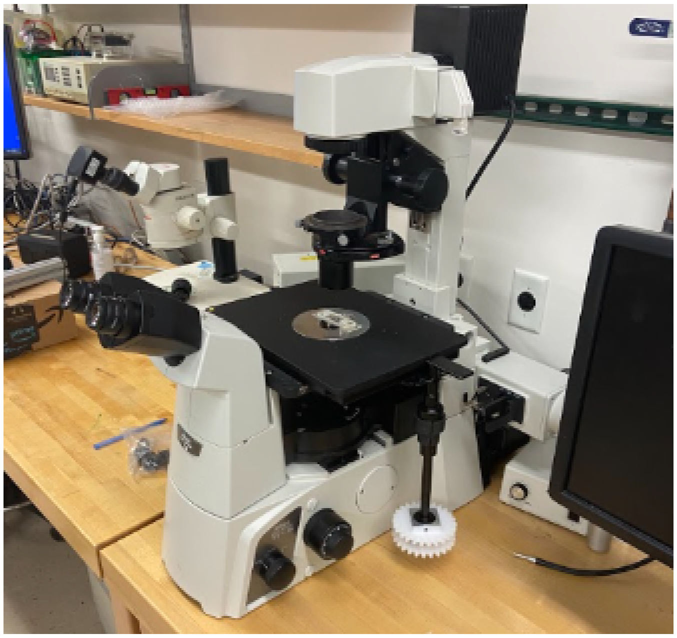


Figure 1. Picture of the microscope being used at the BME teaching labs at UW Madison.

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 motorized stage attachment that is detachable. The movement must have a resolution of 1 μm in order for the image stitching software to overlay the images properly. 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 to 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.

Last semester, the design consisted of the two gears held by metal gear holders on each the x- and y- direction knobs. They were turned by worm drives connected to motors. The motors sat on a plastic platform, and the platform was held by a rail system. The motors were manipulated by an Arduino program that controlled when and in which direction the motors would turn. However, issues arose with the former design as the motors were taped down to a platform along with a counterbalance to ensure the motors would not tip over. This semester, it was necessary to brainstorm, design, and fabricate a feasible way to secure the motors. Secure motors were necessary for accurate linear movements of the stage.

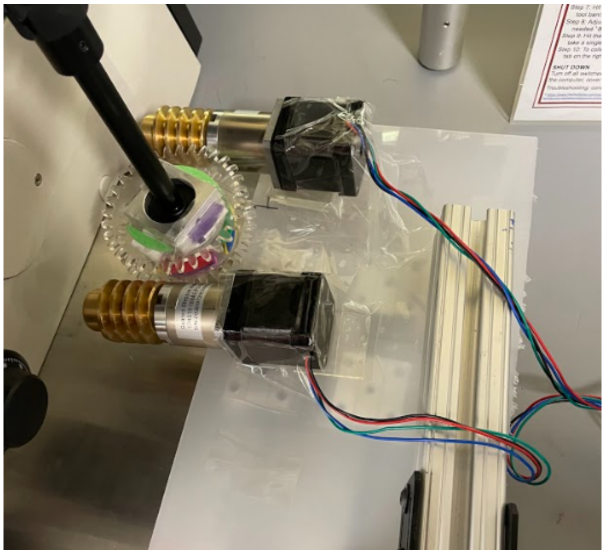


Figure 2. Top view of last year's final design. Stepper motors are connected to a rail system which can slide with the stage in the y-direction. Stepper motors are controlled with an Arduino Uno microcontroller and an Arduino program.The motors are being held down with tape and colored foam is used to give an appropriate amount of separation for the gears.

Preliminary Designs

Design 1: One-Rail System

Figure 3. 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

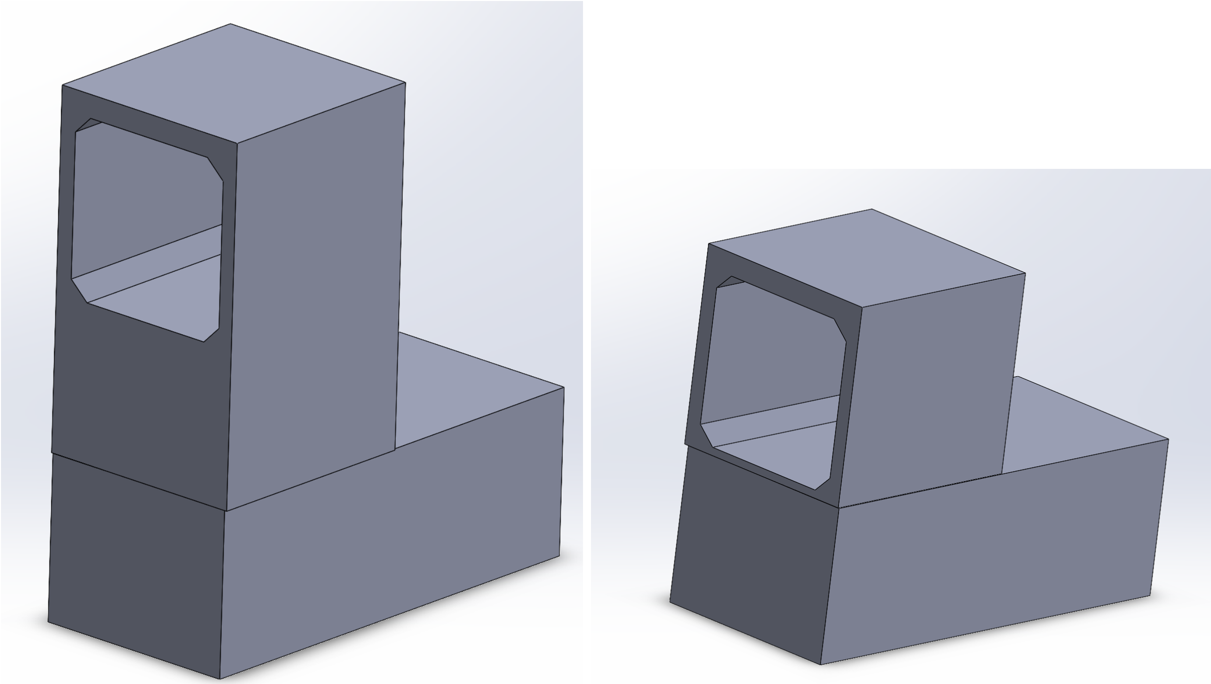


Figure 4. The two-rail system. The motor slips into its casing, which sits on two blocks that enclose the bearings, which slide on the rails.

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

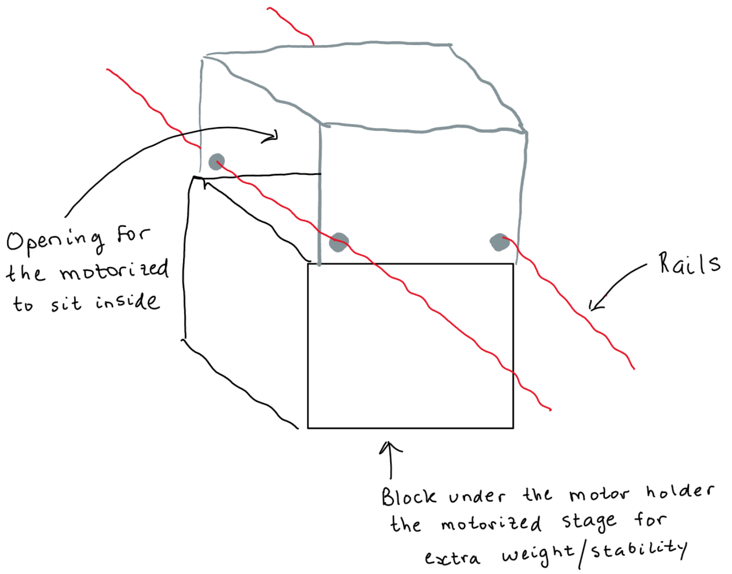


Figure 5. The Tarp Design. The motor slips through the opening on the left side of the covering and the support box below is meant to add some weighted stability of the motor upon operation of the microscope.

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

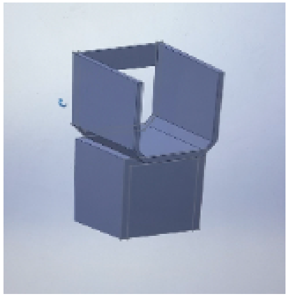
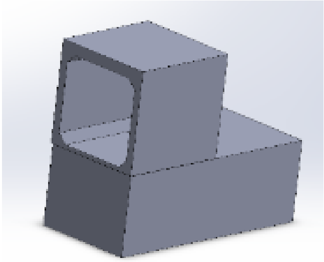
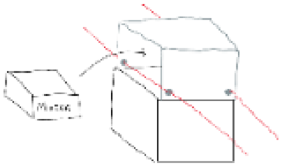
Design Criteria	Design 1: One Rail System 		Design 2: Two Rail System 		Design 3: The Tarp 	
	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Stability (25)	3/5	15	5/5	25	4/5	20
Balance (20)	2/5	8	4/5	16	3/5	12
Detachability (20)	5/5	20	5/5	20	1/5	4
Compactness (15)	5/5	15	2/5	6	4/5	12
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 6. 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 3D printed stabilizing devices so that the stage will not drift during movement. There were two motor holders printed, a device to lock the manual control knob in place at the joint, and two devices to raise the gear system to be flush with the worm drive gears on the motors. All of the 3D printed pieces are made with PLA material. An acrylic bar holds the two motor systems together.

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 was 3D printed to help stabilize the motor and prevent error and drift during movement. Two motor holders were 3D printed, which held the motors in place and did not allow them to shift during use. Another device was 3D printed to lock the manual control knob in place at the joint. Since the manual control knob is a ball in socket joint, it shifted greatly during motorized movement. Therefore, locking in the manual control knob is a necessity. Additionally, we 3D printed a device to raise the gear system to be flush with the worm drive gears on the motors. The two motors are held together with a piece of acrylic. In future semesters, the team would like to further test the joystick. While we did get the joystick to run, we did not have time to test its accuracy and reliability. Finally, in future semester the team would like to use µManager software will be used to integrate both the Nikon elements software and Arduino Uno software to create a fully automated device.

Final Prototype

1. Gear-knob Attachment

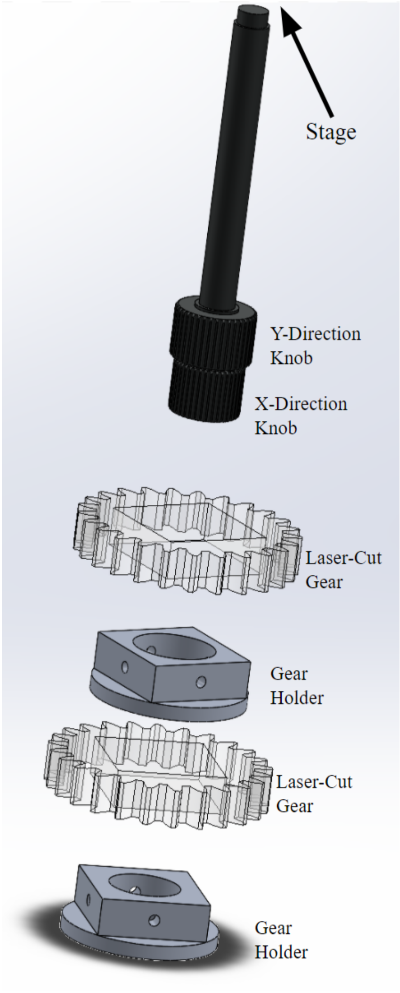


Figure 7. An exploded view of the gear-knob attachment, including the two gear holders and two laser cut gears that sat on the x and y direction knobs.

The gear knob attachment included the set gear holders and the laser cut gears. This attachment was how the manual control knob could be spun with motors. Gear holders used set screws to stabilize one gear holder onto the y-direction knob, and the other on the x-direction knob. Laser-cut gears sat upon each of the gear holders, with a diameter to teeth ratio of 88mm:32 teeth.

2. Worm-Drive Gear

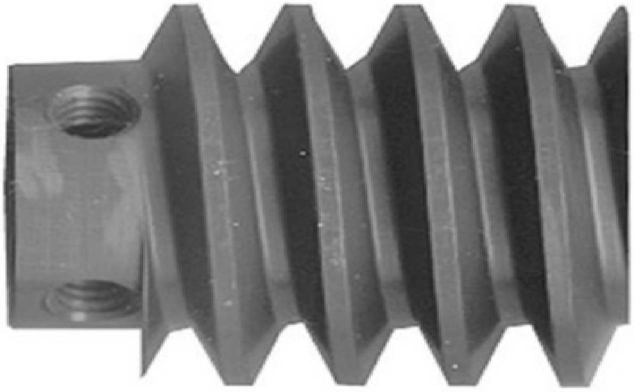


Figure 8. The worm drive gears that were used in the final design.

The worm drive was attached to the motors, and spun the laser-gut gears, moving the stage. The two brass worm drives were each 1.5” x 1.7” x 1.5”.

3. Stepper-Motor to Worm-Drive Adapter

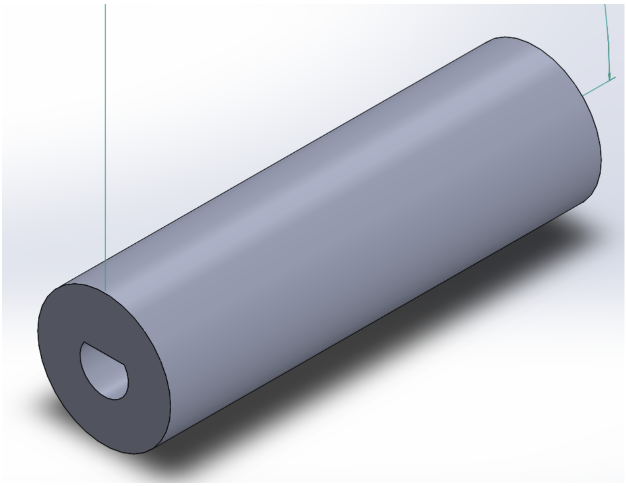


Figure 9. The adapter that connected the worm drive to the motors.

The stepper-motor to worm-drive adaptor is utilized since the stepper-motor shaft is too small for the worm drives. An adapter is needed to allow the motor shaft to fit snugly in the worm drive. The adapter made out of CPE+ plastic had an outside diameter of 15.5 mm and length of 40 mm. The motor shaft hole inside the adapter had a full diameter of 8 mm, and length of 7 mm from the flat edge to the far edge of the hole.

4. **Linear Rails**

Figure 10. Two linear rails that were used in the final design.

Two non-friction linear rails hold a platform carrying the motors with the worm drives that directly connect to the laser-cut gears on the manual control knob. The rails move in the y-direction to allow the motors to move with the manual control knob as the y-direction knob is turned.

5. **Arduino and Electrical Circuitry**

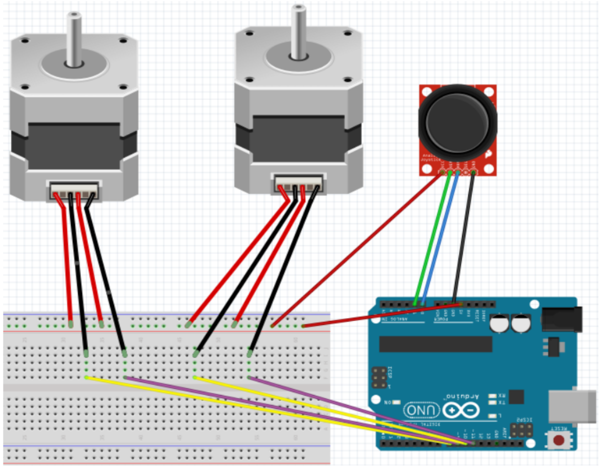


Figure 11. The electric circuit.

An Arduino Uno microcontroller (pictured in the bottom right) reads the voltage outputs from the joystick (top right) and sends impulses to the stepper motor drivers (not shown above). The drivers would then turn the stepper motors (top).

6. Motor Holder Stabilizers

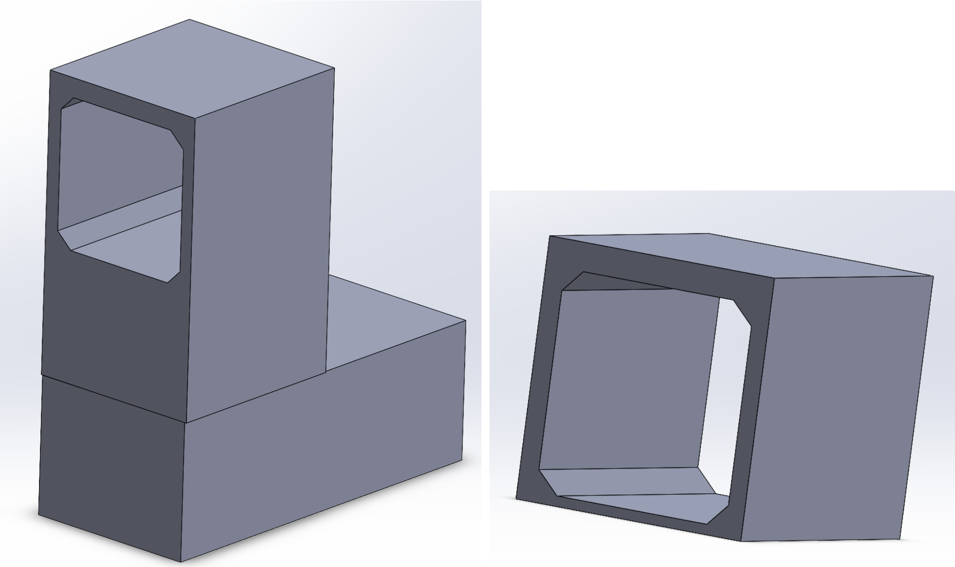


Figure 12. Two 3D printed devices that hold the motors.

These devices hold the motors ensuring stability and no shifting during the use of the prototype. These devices attached to the rail system so the motors could slide as the manual control knob moved.

7. Manual Control Knob Stabilizer

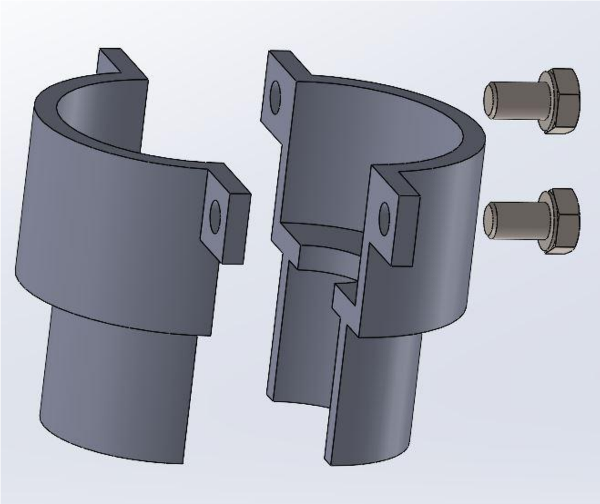


Figure 13. Manual control knob stabilizer device.

These devices clasp together around the manual control knob joint. The manual control know joint is a ball and socket so it shifts easily during the use of the microscope, which makes the gear system come apart. The stabilizer locks the control knob in a straight position.

8. Gear Extenders

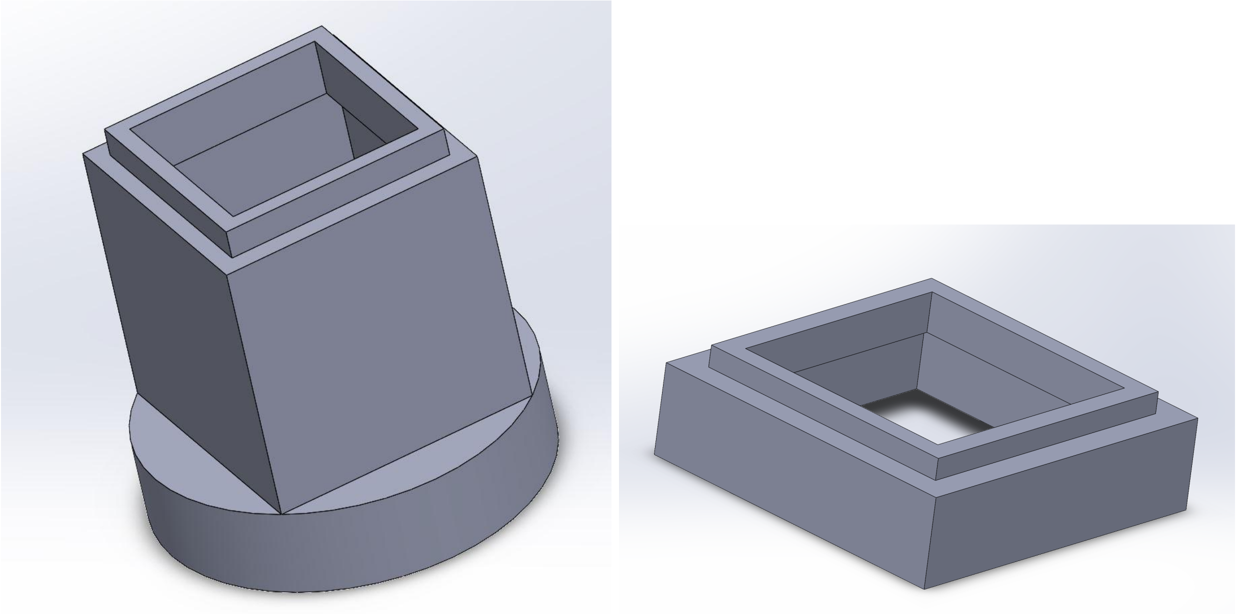


Figure 14. Two gear extender devices.

These gear extender devices were used separate the acrylic gears on the manual control knob. This was important to solve two issues of the prototype. The first issue was that the worm gears were too low, so they were not flush with the acrylic gears. The second issue was that two acrylic gears were too close together, so the worm gears were hitting and turning both acrylic gears.

9. Final Combined components image:

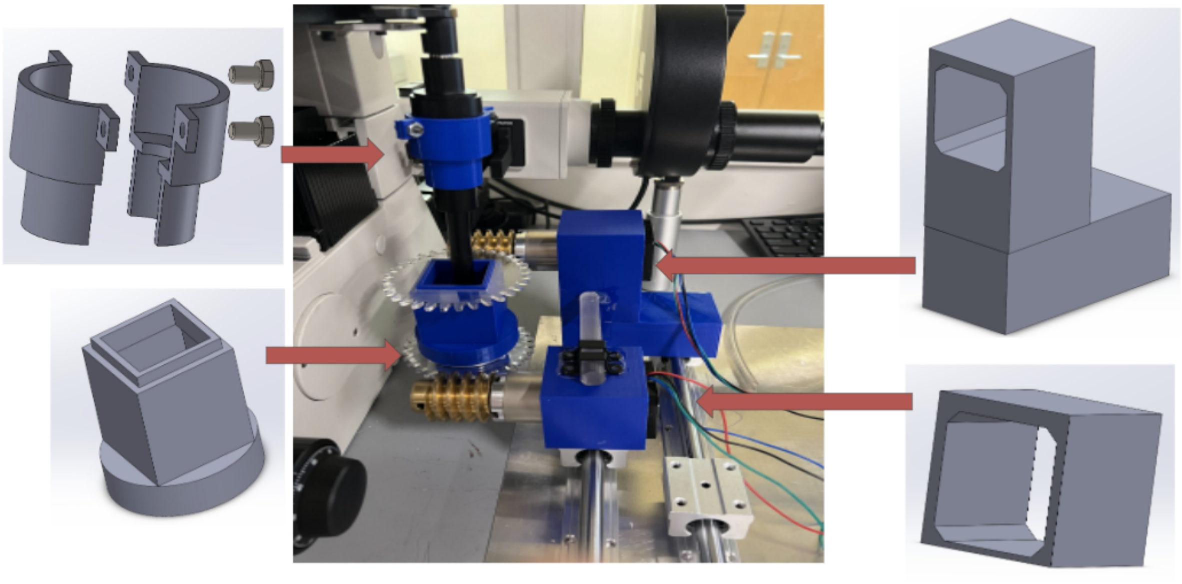


Figure 15. Image of all the final components and attachments put together with the teaching-lab microscope.

The figure above of the final prototype shows a front view of all the gears and the attachments stabilizing the motors and connected to the microscope. The other images on the outside show four images showing the SOLIDWORKS designs of all the stabilizers. The top-left image shows the stabilizer for the manual control knob. The bottom-left Solidworks image shows the attachment of the gear separator to allow each motor to attach to separate gears. Lastly, the two images on the right show the motor stabilizers. The center image shows the entire set up with the microscope, attachments, arduino, and projected microscope image all together.

Testing

Speed Determination Testing

To test for the accuracy of distance traveled, the speed that the stage moved in each direction had to be determined. Speed determination testing started with a photograph of the sample with 6μm dots was taken at one position. Then, the motor was set to a constant speed for 2.5 seconds and another photograph was taken of the sample at position 2. The two images were imported and overlaid in ImageJ to calculate the distance traveled by a dot on the slide. Based on the distance traveled, the speed in each direction was determined.

Accuracy of Distance Traveled Testing

After the speed was determined, code was developed to set the motors to move 100µm. The slide with the 6µm dots was photographed before and after the motor movement. The two images were imported into ImageJ and the actual distance traveled of one dot on the slide was determined. Three tests were run in each direction and the difference between the projected and actual distance traveled was quantified with a percent error.

Results

Speed Test

	Seconds	Distance (um)	Test Trial	Speed (um/s)	Average (um/s)
Shift Up (+y)	2.5	84.11	1	33.644	38.224
	2.5	96.34	2	38.536	
	2.5	106.23	3	42.492	
Shift Down (-y)	2.5	104.93	1	41.972	39.044
	2.5	118.22	2	47.288	
	2.5	69.68	3	27.872	
Shift Left (+x)	2.5	77.08	1	30.832	29.714
	2.5	75.223	2	30.0892	
	2.5	70.552	3	28.2208	
Shift Right (-x)	2.5	83.556	1	33.4224	27.66426667
	2.5	58.002	2	23.2008	
	2.5	65.924	3	26.3696	

Table 1. Table showing data on the distance and time in order to find the speed of the motor during experimentation.

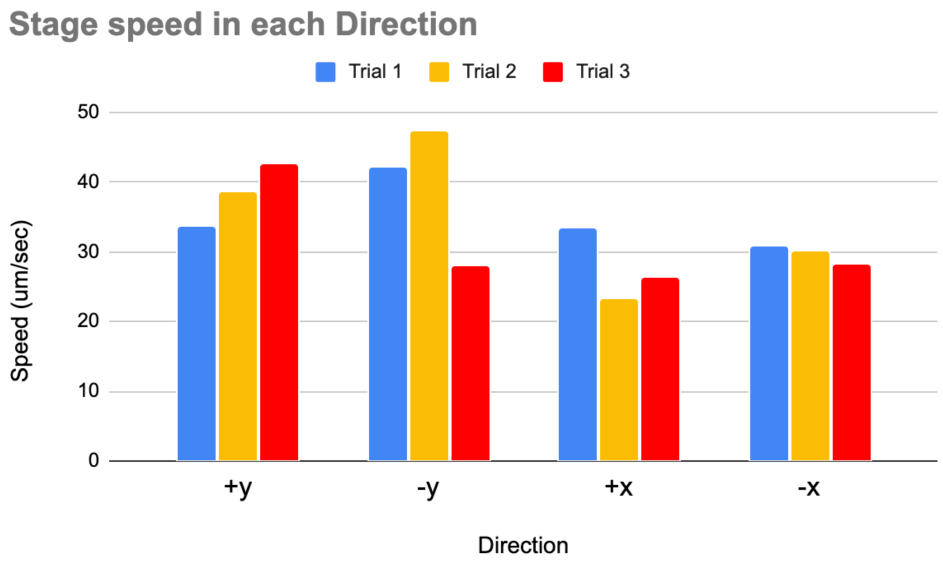


Figure 16. Graphical representation of the speed test results of each trial in each direction tested.

Accuracy Test

	Projected Distance (um)	Distance (um)	Test Trial (um)	Average	SD	% Error
Shift Up	100	132.465	1	103.5636667	25.8628009	3.563666667
	100	95.626	2			
	100	82.6	3			
Shift Down	100	108.963	1	113.8486667	8.441446815	13.84866667
	100	123.596	2			
	100	108.987	3			
Shift Left	100	94.318	1	95.64833333	9.0233525	4.351666667
	100	105.263	2			
	100	87.364	3			
Shift Right	100	78.318	1	92.69066667	16.08684684	7.309333333
	100	110.068	2			
	100	89.686	3			

Table 2. Table showing the actual distance traveled by the microscope stage compared to the projected distance it was meant to travel.

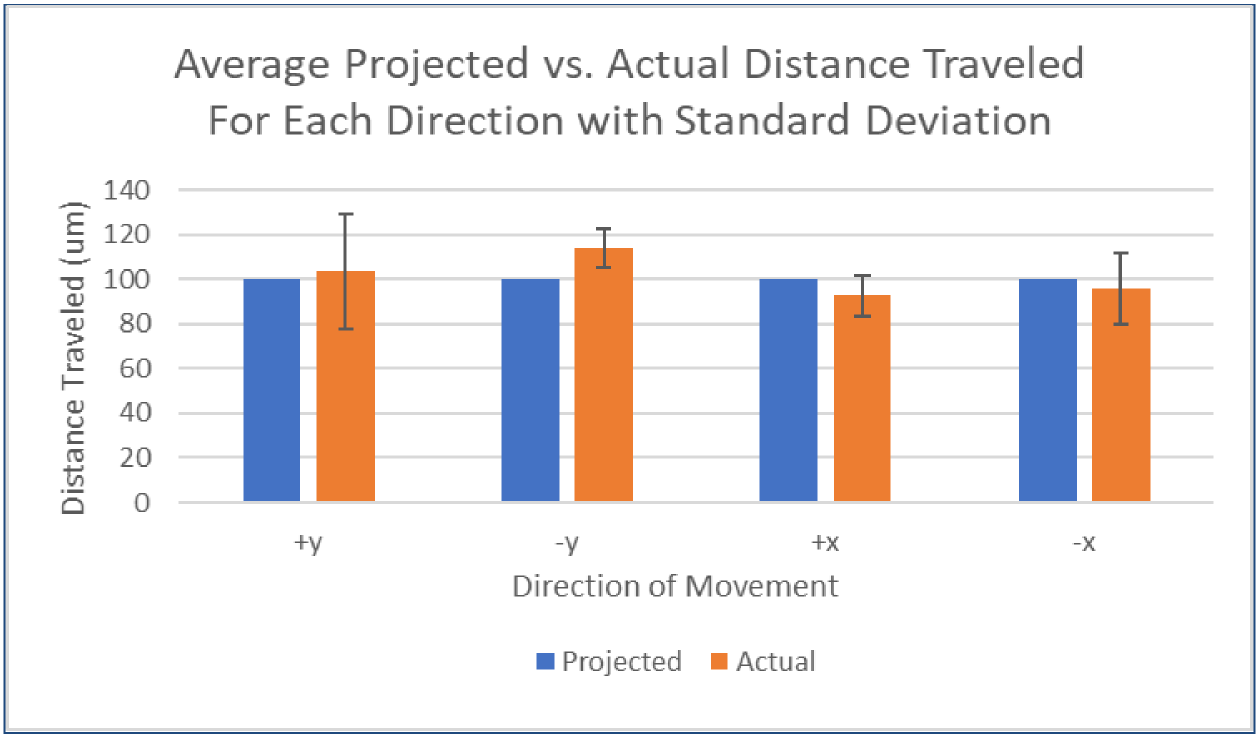


Figure 17. Graphical representation of the accuracy test showing comparisons in the distance traveled vs the projected distance the stage was supposed to travel.

Discussion

Upon analysis of the data gathered from the experimentation process, the 1-10 um resolution was not obtained for all directional movements of the microscope stage. While the distance moved in the +y direction, +x direction, and -x direction was under 10 micrometers, the accuracy of the distance moved in the -y direction was well above the specified range. The results of the speed test showed significant differences in the speed of the stage in the x and y directions. The x directions moved around a speed of 27 um/sec while the speed in the y direction moved around a speed of 38-39 um/second. Ideally, under the same conditions, the stage was supposed to move the same speed regardless of the direction it was specified to move. However, there was a significant improvement in the results of these tests compared to last semester's work. The resolution, while not completely hit, was closer to the specified range. Implications of our improved results create improvements in the stage movement and accuracy can allow scientists to perform tissue analysis, examine forensic evidence, study the role of a protein within the cell, and study atomic structures efficiently. Separate applications from the client's desires, including taking images of a cell migrating through a sample, could be performed with our device. If the speed of a sample in a cell was known, the stepper motors can be adjusted respectively to move that speed to take pictures of that cell as it moved.

The main criteria for this project was to make a low cost solution for the stabilization of the microscope motors. Overall, the cost of the designs this semester was $26.88. However, the total cost of the materials used from this semester as well as prior semester came to $220.27. Furthermore, the overall printed prototypes were effective in stabilizing the motors on the stage and connecting them to the microscope. No tipping of the motors occurred and no tape was used to further hold any additional components.

There are no serious ethical concerns that need to be addressed with the printed prototypes as they are not dangerous to handle or operate. However, the microscope and circuitry involved to operate it should be handled carefully as they are expensive equipment that are fragile if not properly balanced and handled properly. A big change that needs to be made to the design is the connecting bar that is holding the two stabilizing devices together. The bar was not precisely placed at a location that connects the two stabilizing devices properly and allows for proper fixation of the gear and the motors together.

There are a number of sources of error that could have accounted for the lack of accuracy in 1-10 um resolution. For example, as the motors were being operated, the motor shifted upwards and caused the fixed position of the motor and gear to change, which may have accounted for a slower rotation of the gear. Additionally, the bar that held the two stabilizing devices together was poorly constructed and may have caused one of the motors to not move with the system upon operation. Therefore, there would have been less fixation of the second motor and its respective gear which could cause a slower rotational movement of the gear. Lastly, new rails systems would need to be used because there was inbuilt friction that was causing the system to not move properly during testing.

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.

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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

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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

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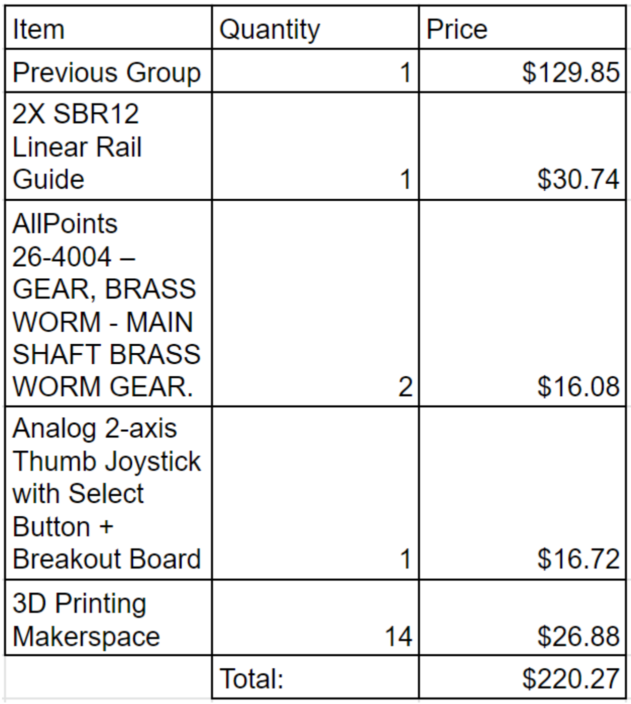
Appendix B: Team Expenses



Item	Quantity	Price
Previous Group	1	$129.85
2X SBR12 Linear Rail Guide	1	$30.74
AllPoints 26-4004 – GEAR, BRASS WORM - MAIN SHAFT BRASS WORM GEAR.	2	$16.08
Analog 2-axis Thumb Joystick with Select Button + Breakout Board	1	$16.72
3D Printing Makerspace	14	$26.88
	Total:	$220.27

Appendix C: Arduino Uno Code for Speed Testing

```
// Define pins

int driverY_PUL = 7;    // PUL- pin
int driverY_DIR = 6;    // DIR- pin
int driverX_PUL = 5;    // PUL- pin
int driverX_DIR = 4;    // DIR- pin

void setup() {

  // Set all digital pins to output
  pinMode(driverY_PUL, OUTPUT);
  pinMode(driverY_DIR, OUTPUT);
  pinMode(driverX_PUL, OUTPUT);
  pinMode(driverX_DIR, OUTPUT);
}

void loop() {

  digitalWrite(driverY_DIR, HIGH); // Controls the direction on the motor being used

  // 2273 iterations at .0011 sec/iteration = 2.5 sec
  for (int i = 0; i < 2273; i++){
```

```
    digitalWrite(driverY_PUL, HIGH);
    delayMicroseconds(550);
    digitalWrite(driverY_PUL, LOW);
    delayMicroseconds(550);
  }

  delay(2000); // delay to take picture and see how far it traveled
}
```

Appendix D: Arduino Uno Code for Bipolar Stepper Motors Without Joystick

```
// Speed in the up direction = 38.2 um/sec
// Speed in the down direction = 39.0 um/sec
// Speed in the right direction = 27.7 um/sec
// Speed in left direction = 29.7 um/sec


// Define pins
int driverX_PUL = 7;
int driverX_DIR = 6;
int driverY_PUL = 5;
int driverY_DIR = 4;

void setup() {

  // Set all digital pins to output
  pinMode(driverY_PUL, OUTPUT);
  pinMode(driverY_DIR, OUTPUT);
  pinMode(driverX_PUL, OUTPUT);
  pinMode(driverX_DIR, OUTPUT);

}

void loop() {

  // This loop is 100 um STAGE RIGHT
  digitalWrite(driverX_DIR, HIGH);
  for(int i = 0; i < 3282; i++) { // 100 (um) / 27.7 (um/sec) / 0.0011 (sec) = 3282 iterations
    digitalWrite(driverX_PUL, HIGH);
    delayMicroseconds(650);
    digitalWrite(driverX_PUL, LOW);
    delayMicroseconds(650);
  }
```

```
  // This loop is 100 um STAGE DOWN
  digitalWrite(driverY_DIR, LOW);
  for(int i = 0; i < 2331; i++) { // 100 (um) / 39.0 (um/sec) / 0.0011 (sec) = 2331 iterations
    digitalWrite(driverY_PUL, HIGH);
    delayMicroseconds(550);
    digitalWrite(driverY_PUL, LOW);
    delayMicroseconds(550);
  }


  // This loop is 100 um STAGE LEFT
  digitalWrite(driverX_DIR, LOW);
  for(int i = 0; i < 3061; i++) { // 100 (um) / 29.7 (um/sec) / 0.0011 (sec) = 3061 iterations
    digitalWrite(driverX_PUL, HIGH);
    delayMicroseconds(550);
    digitalWrite(driverX_PUL, LOW);
    delayMicroseconds(550);
  }

    // This loop is 100 um STAGE UP
  digitalWrite(driverY_DIR, HIGH); // 100 (um) / 38.2 (um/sec) / 0.0011 (sec) = 2380 iterations
  for(int i = 0; i < 2380; i++) {
    digitalWrite(driverY_PUL, HIGH);
    delayMicroseconds(550);
    digitalWrite(driverY_PUL, LOW);
    delayMicroseconds(550);
  }

  delay(1000);
}
```