Microscope Low-Cost Motorized Stage

Biomedical Engineering 200/300: 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 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 was created, consisting of a worm system driven by two bipolar stepper motors. The worm system has one spur gear on the x-direction manual knob and one spur gear on the y-direction manual knob, each connected to a separate worm gear and stepper motor. 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 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. It must be integrated with Nikon Elements to be programmed to do a 30-minute automated imaging and stitching process. Finally, the device must be low-cost, within the budget of $100. See Appendix A for the full Product Design Specification.

Preliminary Designs

Design 1: Replaceable Stage

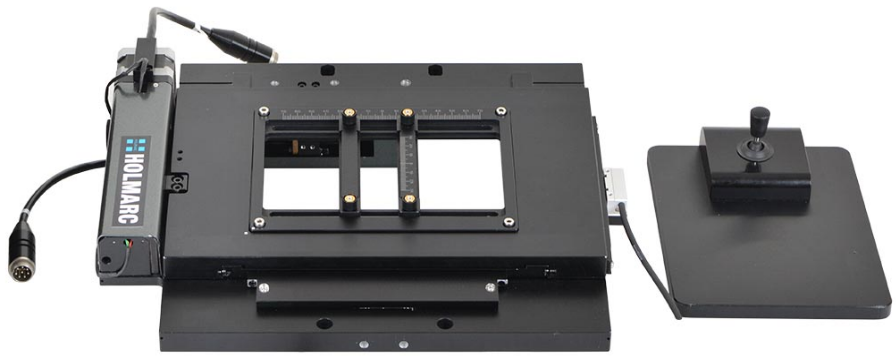


Figure 1. The Replaceable Stage. This stage would replace the current stage. The joystick on the right controls the stage movement [7].

The first alternate design is the Replaceable Stage design. This design would entail the complete removal and replacement of the current stage. Currently, the stage has a manual knob that controls the Y-axis and X-axis movement of the stage. Instead of trying to build a motorized design that connects to these knobs, with the Replaceable Stage the stage would be completely replaced. The benefits to this design idea are numerous. For one thing, if the group decided to fulfill this design, there would be complete freedom of how the stage was designed, how it moved, and how we incorporated the motor. This is in contrast to the other designs, which are limited by the current stages' knob mechanism. Unfortunately, this design also has some major drawbacks. Since there are two different types of microscopes, the Nikon Eclipse Ti-U and the Olympus IX71, the group would need to design two completely different stages with the specs and size of each individual microscope. This would be both expensive and time consuming. The expenses are partially due to the size of the stage being relatively large and the variety of materials that would likely be required to replace all functional aspects of the current design. Time constraints also prevent the design of the stage from being efficiently created and functional to the high standard of the current design.

Design 2: Worm Drive

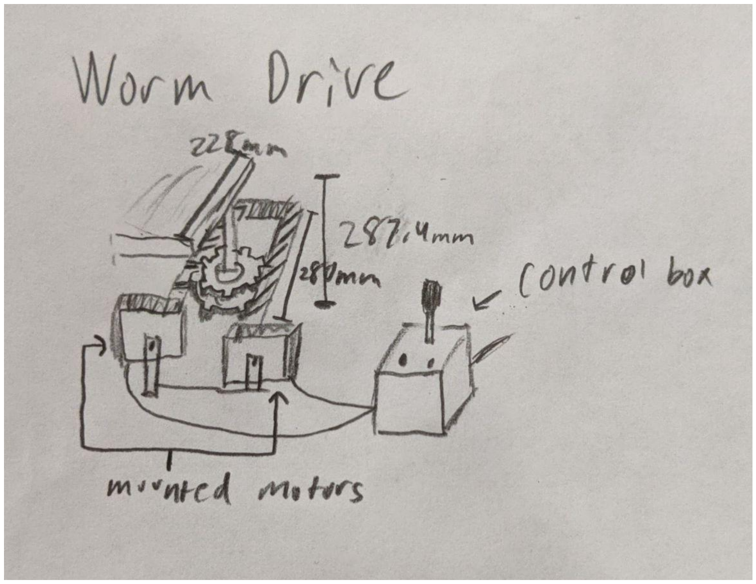


Figure 2. The Worm Drive consists of two worm gears attached to the manual joystick and controls the stage via rotation in the worm gears.

The second alternative design that the team is considered is the Worm Drive. This design attaches to the current knobs that manually control the movement of the stage in the X and Y direction. This design uses two motors that are fixed in place. Each motor would rotate a separate worm gear. One of the motors would be assigned to the X-axis knob and the other would be assigned to the Y-axis knob. As the motor spins the worm would spin the so-called worm wheel gears that are attached to the manual knobs. This design has many advantages. Firstly, since the manual knobs are very similar on both the Nikon Eclipse Ti-U and the Olympus IX71 microscope, it would be very easy to make one design that works with both microscopes. Secondly, this design would require a lot less expenses than that of the Replaceable Stage design. Since the group has a budget of $100, it is important that the design not be overly expensive. Finally, this design would solve an issue that the previous group was having with their design. When the manual knob in the Y-direction is turned, the entire arm containing both knobs moves. Since the motors are not attached to the arm in this design and the worm gears allow for translation along the y axis without decoupling the gears from the worm wheels, this issue is mitigated. The worm gears also provide a very high degree of accuracy due to the high gear ratio relative to the size of the gears. While the Worm Drive does have many positives, there are also a few negatives associated with this design as well. One negative of the Worm Drive design is that the team would have to undo some of the work done by the past group in order to go through with this design. The group would have to create all new gears and would not be able to to use the current gears or motor attachment that are in place right now. It also has the issue of increased friction between the worm gears and the gears themselves due to increased surface contact [8].

Design 3: Attachable Gearbox

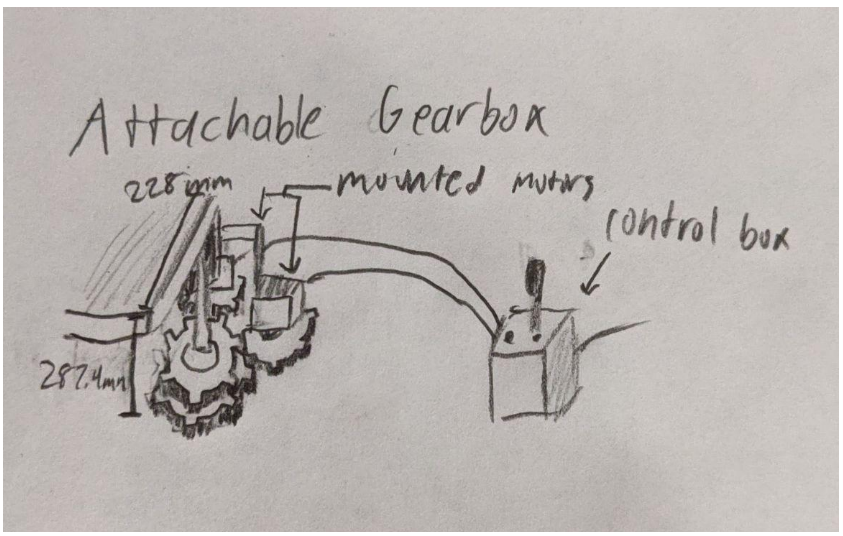


Figure 3. The Attachable Gearbox Design. The motor is attached to the stage on the left. The gears are attached to the stage knob and the motor shaft. The control box on the right has a joystick to control the stage.

The third and final design is called the Attachable Gearbox. This design is similar to the design which was previously being worked on by the past group. Similar to the Worm Drive design, the Attachable Gearbox uses two motors, one for movement in the X direction and one for movement in the Y direction. Unlike the Worm Drive design however, the motors are attached to the arm of the microscope which contains the manual knobs. These motors turn a set of gears, which are attached to the gear on the manual control knobs. Therefore, when the motors are activated there is control over both the X and Y direction of movement. This design has many benefits. One of the main benefits of this design is that the team would not have to design new gears or motor attachments. This would save a lot of time and energy. One main disadvantage to this design is that as the stage moves in the Y-direction, the entire arm moves with it. This causes issues when it comes to wiring as well as the gears staying together. Unless the team can come up with an effective solution to this problem, it would be a major inhibitor for continuing with this Attachable Gearbox design. Another main disadvantage is the additional strain placed on the stage itself. Since the stage is not designed with additional attachments in mind, the addition of two fairly heavy stepper motors to the stage may interfere with prolonged functioning of the stage.

Preliminary Design Evaluation

Design Matrix

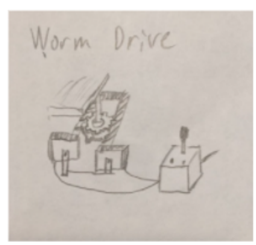
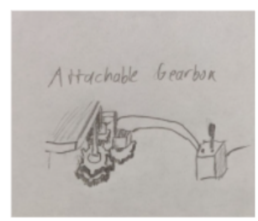
	Replaceable Stage		Worm Drive		Attachable Gearbox	
Design Criteria						
	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Accuracy (25)	5/5	**25**	4/5	20	3/5	15
Cost (20)	1/5	4	3/5	**12**	3/5	**12**
Detachability (20)	2/5	8	4/5	**16**	3/5	12
Ease of Use (15)	3/5	9	5/5	**15**	5/5	**15**
Longevity (10)	5/5	**10**	4/5	8	5/5	**10**
Safety (5)	4/5	**4**	3/5	3	3/5	3
Ease of Fabrication (5)	1/5	1	3/5	3	5/5	**5**
Total (100)		**61/100**		**77/100**		**72/100**

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 Worm Drive Design scored the highest overall.

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. Accuracy was given the most consideration in the design matrix due to the general importance of accuracy as it relates to mechanisms in the lab. The thought process here was that if a motorized state compromised the accuracy of the overall system, it would be of virtually no use in the lab.

The team also gave increased consideration to the projected cost of each project given the emphasis placed on the element of cost by the client. 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.

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 less 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.

Another measure that the team thought was important to consider with each design was how easy it would be to use. Given that this device is intended to be used in labs on campus, it is important that the product be intuitive and not complicated to use so that it can be utilized by all those who work in the lab rather than a select few with a specific skill set or training.

Longevity was given consideration in the design matrix due to the fact that the client had mentioned the desire for the product to "last forever," but it was not given exceptionally high consideration because the team did not foresee any significant differences in longevity between designs. In terms of materials, each design requires similar parts, but the differences in longevity were attributed mainly to the possibility of shifting in the mechanism to cause malfunctions sooner in the Worm Drive design compared to the others.

Safety is something that should always be kept in mind for any design, but given that the nature of the project does not suggest a high-risk mechanism regardless of the design, it was not found suitable to give safety extraordinary weight in the design matrix. The thought here was that all design options in the matrix entail similar risks which are relatively low to begin with.

Ease of fabrication was a consideration that held importance to the team given the timeline for when the product will ideally be fabricated. The team thought it was worth contemplating how realistic it would be to fabricate each design as getting a physical prototype is a goal of the semester. However, ease of fabrication was not weighted as highly as the other categories of evaluation due to the simple fact that just because a design is easy to fabricate does not mean that it is better in functionality or other, more important, aspects.

Proposed Final Design

Based off of the design matrix, the group decided that the Worm Drive would be the fabricated design. The Worm Drive Design exceeded the score of the Attachable Gearbox in the design evaluation by a relatively small margin. This resulted primarily from the notion that Worm Drive design excelled in many of the criteria the group weighted most heavily in the matrix. In all three of the categories of accuracy, detachability, and longevity, the design scored a four on a five point scale. These categories were important because they reflected specific preferences voiced by the client. Namely, the need for the microscope to have a resolution between 1-10 µm, be detachable, and last forever. The Worm Drive design also scored joint first in cost effectiveness, and scored full points in ease of use. This is beneficial to the design because students will be using the microscope frequently, so it should be intuitive to maximize accessibility on campus. Through careful completion of the design evaluation criteria as it related to each preliminary design, it became apparent that the Worm Drive design was the preferred design for the team to proceed with.

Fabrication Development Process

Materials

There are various materials necessary to create a functioning prototype of the Worm Drive Design. The group from last year left behind two stepper motors which were used to spin the worm gears, and two laser cut gears that attach to the x and y knobs with gear holders. The first thing that the group this year needed to purchase were the worm drive gears themselves. The group ordered two worm drive gears to spin the laser cut gears and turn the x and y knobs. The gears from last year's group did not fit with the teeth of the worm drive, so new laser cut gears had to be manufactured. The new laser cut gears were free and fit well with the worm drive gears. The worm drive gears, however, could not connect to the stepper motors, so an adapter had to be 3D printed so they could be attached. The group also had to purchase a frictionless rail system for the motors to rest on top of in order for the system to move with the stage and the knobs. The rails were attached to a sheet of acrylic which had the motors mounted on top of it. Additionally, the group used an Arduino Uno as well as wiring for operating the motors with a computer. These items were already owned by members of the group. Finally, the group needed a joystick device that was used to control the motors and therefore control the movement of the stage. This joystick was purchased online.

Methods

To begin 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. While progress was made in this area, the joystick was not fully integrated for use in the y-direction. The contents of this aspect of the design are discussed in more detail in the following section.

Final Prototype

There were five main components that were included in the final prototype: the gear-knob attachment, worm drive gear, stepper-motor to worm-drive adapter, two linear rails, and the Arduino with the electrical circuitry. The gear-knob attachment contained gears that were spun by motors using worm drives. Linear rails with a platform held the motors, and the motors were controlled by an Arduino Uno and electrical circuitry.

1. Gear-knob Attachment

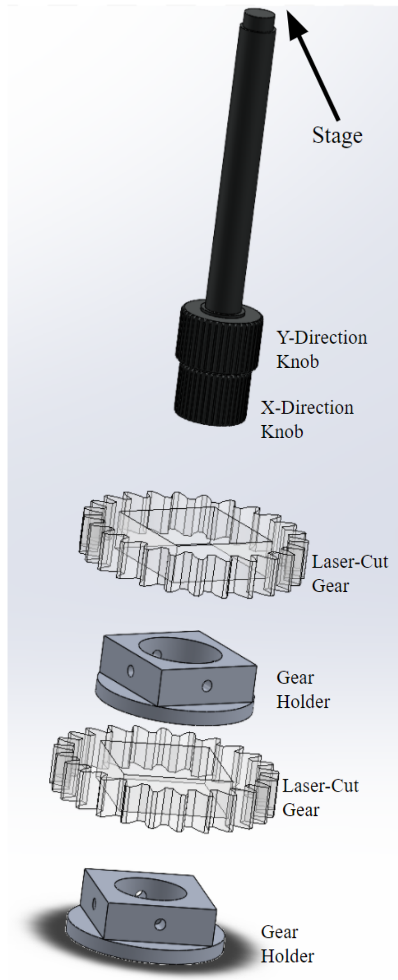


Figure 5. An exploded view of the gear-knob attachment, including the two gear holders and two laser cut gears that sat on the x and y direction knobs.

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

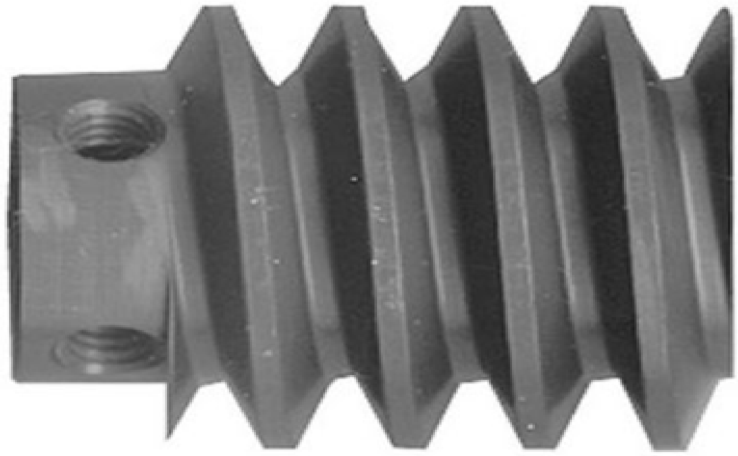


Figure 6. The worm drive gears that were used in the final design.

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

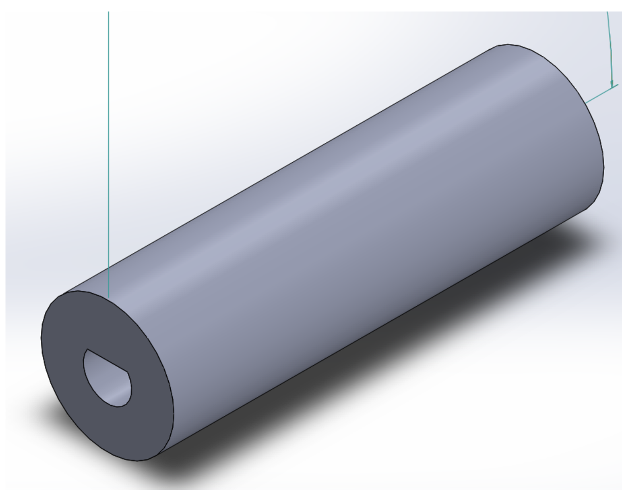


Figure 7. The adapter that connected the worm drive to the motors.

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 8. 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

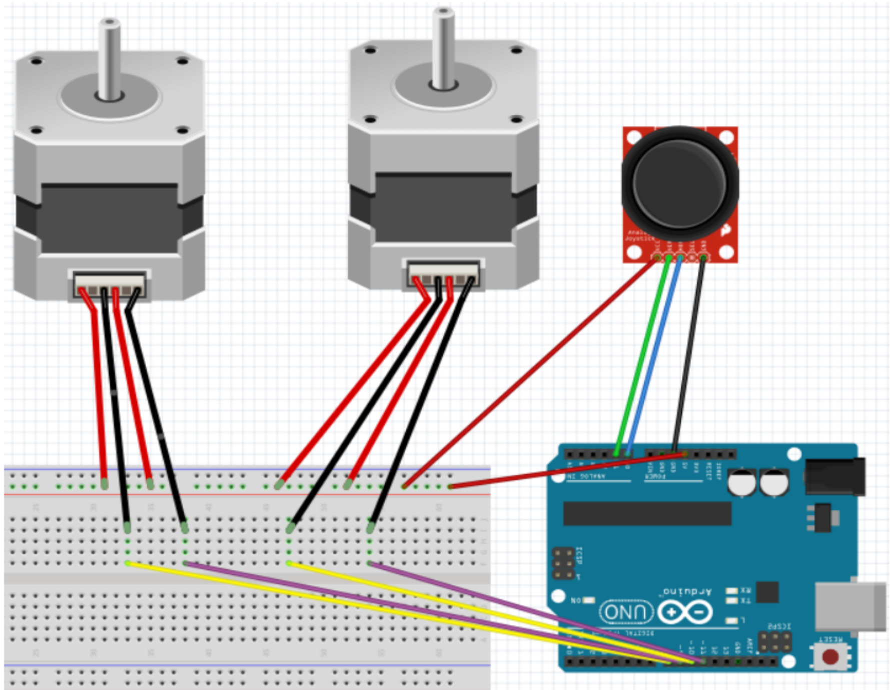


Figure 9. The electric circuit. An Arduino Uno microcontroller (pictured in the bottom right) reads the voltage outputs from the joystick (top right) and sends impulses to the stepper motor drivers (not shown above). The drivers would then turn the stepper motors (top).

The components in the circuit include an Arduino Uno microcontroller, a 2-axis analog thumb joystick, two stepper motors, and two drivers for the stepper motors. The stepper motors are both powered by a 12V power supply, and the Arduino is powered by a laptop. The Arduino sends a constant 5V to PUL+(+5V) and DIR+(+5V) pins to each driver and the joystick. The joystick contains two output pins, one for x-direction and one for y-direction. When tilting the joystick in the positive direction for either axis, the respective output pin increases its voltage output. The respective output pin decreases in voltage when the joystick is pushed in the negative direction. The output voltages are read by analog input pins on the Arduino and when the voltage goes above a certain threshold, impulses are sent from a digital pin on the microcontroller to the PUL-(PUL) pin on the driver, turning the stepper motor. When the voltage goes below a certain threshold, a second digital pin sends constant voltage to the DIR-(DIR) to turn the motor in the opposite direction.

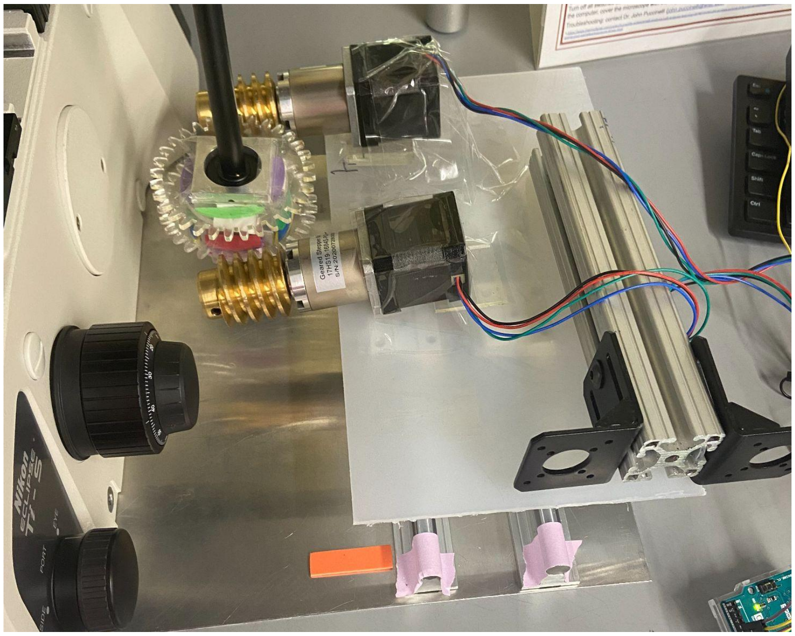


Figure 10. The mechanical part of the design. The stage knob with the gear attachment is shown on the left, with the stepper motors and worm drive gears.

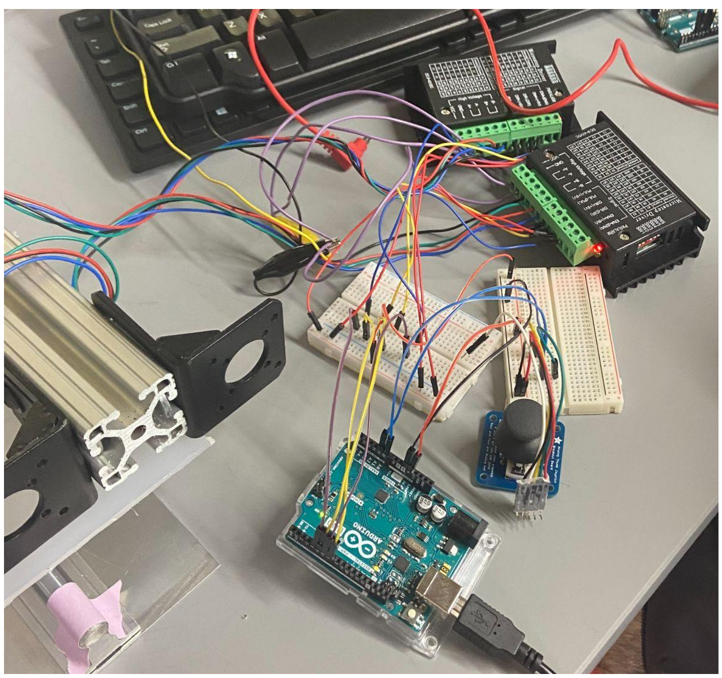


Figure 11. The circuit part of the design. The Arduino connects to the drivers that the stepper motors are attached to.

Testing

Using the final design prototype, the team was able to utilize the Nikon Ti-U to assess the efficacy of the design. The testing procedure consisted of taking a reference picture through the microscope of a slide that contained dots of known sizes (6 um). Next, the stepper motor was set to a constant speed and allowed to run for 15 seconds in one direction. Once the motor had run for 15 seconds, a second picture was taken. The two photos were inserted into ImageJ and then overlaid. After overlaying the images, the distance traveled was calculated by scaling the images using the known 6 um dots. This process was repeated for a total of 5 trials to test the reliability of the prototype. A mean speed was calculated by dividing the mean movement by the 15 seconds time taken for the movement.

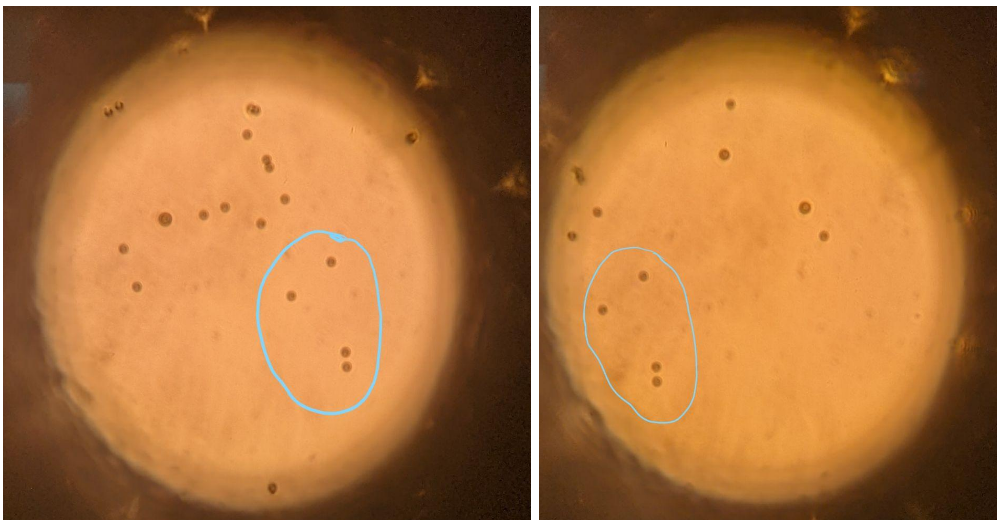


Figure 12. A reference image (left) and a second image after 15 seconds of movement (right) are shown.

Results

Using the testing method seen above data for five trials was collected. A reference picture and a second picture after 15 seconds were taken for each trial and distance traveled was calculated through ImageJ. Through these five trials there was a mean of 171.6 um of movement in 15 seconds with a standard error of 9.53 um. This translated to a mean speed of 11.44 um/second.

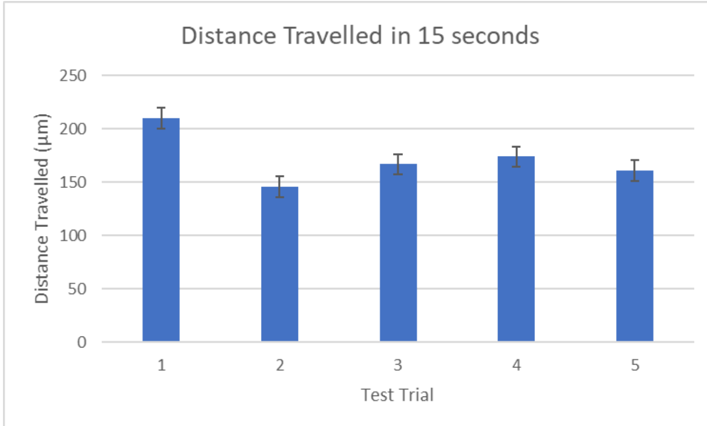


Figure 13. A graph representing the distance traveled by the stage in 15 seconds in each of the five trials. The 5 trials are on the x-axis and the distance traveled in μm is on the y-axis.

Discussion

Automated image stitching would reduce the long and inconvenient hours required to manually record and stitch images. The motorized stage would be able to run overnight, and capture snapshots of a sample throughout the night, not requiring any assistants to come into the lab in the middle of the night. The motorized stage also allows for lab assistants, who may be physically unable to manipulate the manual stage, to operate the device easily using a preprogrammed pathway or the manual joystick instead of the manual knobs. This device would also increase efficiency in a lab setting allowing for multiple tests to be performed without increasing the workload of individual people. Morale in the lab can also be improved due to the very tedious task of manually moving the microscope stage micrometers at a time being fully automated. The motorized stage still requires some improvements in order to reduce error and increase longevity and ease of use. Some error was observed due to the motors drifting from the laser-cut gears allowing for the worm drive gear to spin without causing the stage to translate for a brief moment before coming back in contact. Other sources of error stem from the method of testing. A picture taken by hand allows for some drifting, where the viewing angle is slightly different after the elapsed time, changing the measurements done by ImageJ slightly.

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 the Worm Drive for the final design. This design consists of two motors which each control one worm drive gear. 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 prototype successfully moved the stage in a controlled and repeatable manner, though some degree of error was present. Using premade worm drive gears and rails allowed for other time and resources to be devoted to the coding and functionality of the final prototype. The final design as well as the Nikon Ti-U were used for testing. Through the testing the team found that the prototype could translate the microscope stage in both the x and y direction with a good margin of consistency. In the future the motors can be stabilized and better attached to the rails, which would reduce the error present in the testing due to separation between the worm drive gears and laser cut gears. The joystick can also be fully implemented to work in both directions with variable speed as well. The motorized stage should also be implemented with the Nikon Elements software and capable of recording images and stitching them together automatically. Finally, in the future the team wants to increase the stepper motor RPM through improvements in the circuit or code or possibly buying new motors.

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].

Appendices

Appendix A: Product Design Specification

Product Design Specification

September 24th

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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. This project's goal is to design, program, and fabricate a 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. The stage 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.

   b. *Safety:* It is important for the team to keep moving elements of the stage enclosed, such as gears. 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.

2. **Production Characteristics:**

a. *Quantity:* The client has 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:* The customer would like to have a 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=Cj0KCQj

w1ouKBhC5ARIsAHXNMI_3xPRL8j0lS98sKdirvi69QyfNvPLfS-GGZ8iVymvGb_l_qPeDi7caAgf1EALw_wcB#headerAnchor. [Accessed: 24-Sep-2021].

Appendix B: Team Expenses

Team Expenses

Item	Quantity	Price
2X SBR12 Linear Rail Guide	1	$30.74
AllPoints 26-4004 – GEAR, BRASS WORM - MAIN SHAFT BRASS WORM GEAR.	2	$16.08 (Price incorporates shipping cost)
Analog 2-axis Thumb Joystick with Select Button + Breakout Board	1	$16.72
3D Printing Makerspace	2	$2.08
	Total:	$81.71

Appendix C: Arduino Uno Code for Bipolar Stepper Motors With Joystick

```
int xPul = 8;
int xDir = 9;
int yPul = 10;
int yDir = 11;

void setup() {
```

```
  // set digital pins to output
  pinMode(xPul, OUTPUT);
  pinMode(xDir, OUTPUT);
  pinMode(yPul, OUTPUT);
  pinMode(yDir, OUTPUT);
}

void loop() {
  int analogValX = analogRead(A0); // reads ADC value from pin A0
  float voltageValX = analogValX * 5 / 1023.0; // converts ADC to voltage
  int analogValY = analogRead(A1); // reads ADC value from pin A0
  float voltageValY = analogValY * 5 / 1023.0; // converts ADC to voltage

  digitalWrite(xPul, LOW);
  digitalWrite(yPul, LOW);

  if(voltageValX > 4) { // checks if the joystick is tilted in positive x direction
    digitalWrite(xDir, LOW); // sets motor to turn the stage in positive x direction
    while(voltageValX > 4) {
      digitalWrite(xPul, HIGH);  // sends impulse to driver
      delay(1);
      digitalWrite(xPul, LOW);
      analogValX = analogRead(A0);  // reads voltage output from joystick x output
      voltageValX = analogValX * 5 / 1023.0;
    }
  }
  else if(voltageValX < 1.5) { // checks if joystick is tilted in negative x direction
    digitalWrite(xDir, HIGH); // sets motor to turn stage in negative x direction
    while(voltageValX < 1.5) {
      digitalWrite(xPul, HIGH); // sends impulse to driver
      delay(1);
      digitalWrite(xPul, LOW);
      analogValX = analogRead(A0); // reads voltage output from joystick x direction
      voltageValX = analogValX * 5 / 1023.0;
    }
  }
  else { // joystick is not tilted on x axis
    digitalWrite(xPul, LOW);
    digitalWrite(xDir, LOW);
  }

  if(voltageValY > 4) { // checks if joystick is titled in positive y direction
```

```
    digitalWrite(yDir, LOW); // sets motor to turn stage in positive y direction
    while(voltageValY > 4) {
      digitalWrite(yPul, HIGH); // sends impulse to driver
      delay(1);
      digitalWrite(yPul, LOW);
      analogValY = analogRead(A0); // reads voltage output from joystick y direction
      voltageValY = analogValX * 5 / 1023.0;
    }
  }
  else if(voltageValY < 1.8) { // checks if joystick is titled in negative y direction
    digitalWrite(yDir, HIGH); // sets motor to turn stage in positive y direction
    while(voltageValY < 1.8) {
      digitalWrite(yPul, HIGH); // sends impulse to driver
      delay(1);
      digitalWrite(yPul, LOW);
      analogValY = analogRead(A0); // reads voltage output from joystick y direction
      voltageValY = analogValY * 5 / 1023.0;
    }
  }
  else {  // joystick is not tilted on y axis
    digitalWrite(yPul, LOW);
    digitalWrite(yDir, LOW);
  }

  delay(1);
}
```

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

```
int xPul = 8;
int xDir = 9;
int yPul = 10;
int yDir = 11;

void setup() {
  // initialize all digital pins to output
  pinMode(xPul, OUTPUT);
  pinMode(xDir, OUTPUT);
  pinMode(yPul, OUTPUT);
  pinMode(yDir, OUTPUT);
}
```

```
void loop() {

   digitalWrite(xDir, LOW); // set motor to turn stage in positive x direction

   for (int i = 0; i < 10000; i++){ // loops 10000 times, each lasting 1.5 microseconds for a total
of 15 seconds
     digitalWrite(xPul, HIGH); // sends impulse to driver
     delayMicroseconds(750);
     digitalWrite(xPul, LOW);
     delayMicroseconds(750);
   }

   digitalWrite(xDir, HIGH); // set motor to turn stage in negative x direction

   for (int i = 0; i < 10000; i++){ // 15 seconds
    digitalWrite(xPul, HIGH); // sends impulse to driver
     delayMicroseconds(750);
     digitalWrite(xPul, LOW);
     delayMicroseconds(750);
  }

}
```