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Microscope Low-Cost Motorized Stage
Final Report
BME 200/300

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Abstract

The current inverted fluorescent microscopes inside the biomedical engineering teaching lab have fixed stages. This means they can only be moved using two control knobs, one for the x-axis and one for the y-axis. The traditional, manual movement of the stage is inconsistent and prone to error, and these inconsistencies prevent the use of advanced techniques such as panoramic imaging. A new designed motorized stage is of necessity capable of automatic movement, self-corrected positioning, and a joystick for precise manual control. The current competing designs of motorized microscope stages are too expensive to be supported by our experimental teaching lab thus a low cost motorized stage is crucial for educators and students utilizing the fluorescent microscope. Our goals in this project were centered around those outlined by our client, including: keeping the cost of the project under our $100 budget, and achieving a resolution of movement of 1 micrometer. The final design encompassed a meshed spur gear system, where as a motor turns it rotates a spur gear, which turns a spur gear on the control knob, thereby translating the stage. The gears on the control knob meshed with gears on stepper motors, which were used to drive the translation of the stage. Results of the testing showed an average standard error of 0.4 µm, with an accuracy of 1.6442±0.9464 µm. As a whole, testing of the final meshed spur gear design showed high accuracy and low standard error, ensuring reliable and consistent translation of the microscope stage.

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Introduction

A. Motivation

Figure 1: Nikon TI-U Fluorescent Microscope [1]

Motorized microscope stages are commonly used in bioimaging and other cell culture laboratories. They allow advanced imaging techniques, such as time lapse microscopy and image stitching, to be completed quickly and accurately. Automating microscopy would mean that newer users can be trained more quickly on the techniques, thus allowing them to focus their expertise on research as opposed to the menial tasks of imaging and stitching them. This would help advance the research. Automation also ensures more consistent results and will increase the replicability of the experiment, as it will simplify more complicated multi-step experiences. However microscopes with motorized stages are often significantly more expensive than their non-motorized counterparts. This creates a large barrier to accessibility, particularly for low-budget and teaching laboratories.

With a low-cost automated microscope, fluorescent microscopy would no longer be exclusive to highly-funded laboratories, which will help close the research gap initially between the low-cost labs and eventually between countries, since fluorescent microscopy will now be accessible. Therefore, teaching labs and institutes with lower fundings can provide better advanced curriculums and help their students understand not only microscopy techniques but engineering and problem solving techniques as well. Teaching labs and institutes with higher fundings can use this solution to further improve their experiences with microscopy and potentially better microscopy for industries. It is also imperative to note that fluorescent microscopy will also become more accessible to people with disabilities which prevent them from incorporating this technique into their lives. [2]

B. Current Solutions

Currently, there exist two major solutions to motorized stage microscopy. The solution is to purchase the motorized version of a fluorescent microscope. This is often too large an expense for new teaching labs and low funded schools. For example, the non-motorized fluorescent microscope that is currently used in the BME teaching lab is a Nikon TI-U, which retails at around $16,000 used [3]. The motorized version of this microscope (Figure 1) is $19,000 used, or around $70,000 new [4]. To address the need for a more cost effective motorized microscope stage, other designs have been developed.

One of these such designs is the open-source project Openstage (Figure 2) [5]. Openstage is a design for a low cost motorized microscope stage created for a multiphoton microscope. The project was able to achieve 1µm accuracy in the x and y axes. However the budget of the Openstage is significantly higher than this project, at $1000.

C. Problem Statement

The BME teaching lab in the Engineering Center Building currently has two inverted fluorescence microscopes. These microscopes are non-motorized and must be operated manually, and as a result are limited in their capabilities. Purchasing a motorized microscope is cost-prohibitive, as they can cost tens of thousands of dollars. Therefore, our design has a budget of under $100. By motorizing the current microscopes, advanced techniques such as photo stitching and time lapse microscopy could be performed. To perform these imaging methods, a high degree of control is needed over the position of the stage. The design aims to translate the stage to 1µm precision. To control the stage easily, it should interface with the existing software that is used to control the microscope.

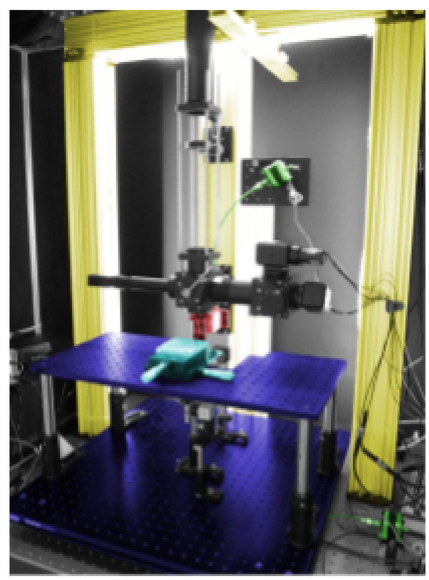


Figure 2: Openstage Motorized Stage [4]

Background

A. Physiology and Biology

Fluorescent microscopes are an optical microscope that uses fluorescence to study biological samples.They use high intensity light sources to excite fluorescent species. The specimen reflects back a shorter wavelength that is then measured [6]. The fluorescent microscope uses filters to match the specific wavelength range of the specimen that is being measured [7]. The functions of the Fluorescent microscope include measuring cell metabolism. This is done by exciting the fluorescent ROS (reactive oxygen species) NADH and FAD which fluorescent molecules. Measuring the wavelength they readout allows for researchers to measure the metabolic rates of specific samples of cells [8]. At the teaching lab here there are 2 inverted fluorescent microscopes. The Nikon Ti- U and the Olympus IX71. The microscopes are currently controlled by manual translation control knobs. The knobs move in both the x and y directions so therefore 2 motors are required to move each individually. Affordable solution, easily attached or removed and integrated with Nikon Elements. The impact of fluorescent microscopes is crucial to the development of technology and advancement of science. Making the process of imaging and analyzing cells more accurate and time efficient would help advance the research field and make it more accessible for low funded labs to conduct important experiments.

B. Client Information

The client for the project is Dr. John Puccinelli, who works as an Associate Chair of the Undergrad Program and Associate Teaching Professor for University of Wisconsin-Madison Department of Biomedical Engineering.

Dr. Puccinelli instructs Biomedical Engineering students who need to learn many techniques such as usage of fluorescent microscopy. The client has requested a low-cost motorized stage for use in teaching labs.

C. Design Specifications

The motorized stage must be compatible with the rest of the microscope. The software that the microscope uses include: Nikon elements, micromanager and the software to automate the locations. The wand of the motorized stage must not inhibit the rest of the microscope functions including the focus or the objectives. The client would like the motorized stage to also be electronic, small and enclosed. The motorized stage will likely be used in a research setting for researchers to image their samples. The motors must be calibrated to accurately and precisely translate to an inputted location of the sample. Specifically, the stage system should have a 1 μm resolution when inputting locations, with a ±0.1 μm error. The stage must also maintain the distance between the sample and the lens to ensure that the focus of the microscope remains the same and all effects of gravity are negated. To achieve this accuracy and precision, in addition to effectively calibrating the motors, that design that holds the motors must also be stable and its resistance to movement will prevent the buildup of error throughout its use. The joystick must also be reliable where the stage translations should correspond to its use, and this error should also fall within the ±0.1 μm range. The budget for this project should be within $100. The full Product Design Specifications are available in Appendix A.

Figure 3: Teaching Lab Microscope

Considering that the stage is primarily designed to be placed in a Biological Safety I classified lab, it has to agree with all the standards of equipment to be placed in the laboratory. Since the intended goal for this project is for it to be replicable and compatible with all microscopes, the stage has to follow the ISO 21073:2019 standards, which provides the metrics used to assess image performance in microscopy. Therefore, the design will not be made so as to not interfere with the resolution of the microscope, the uniformity of the imaging, the scanning frequency and field number of the confocal scan optics [9].

A.

In addition to the constraints outlined by the client, physical constraints due to lack of space were experienced during the design process. The microscope wand lies to the right of the stage, where there is not much room to integrate a motorized mechanism to drive the translation of the stage. This is best illustrated in Figure 3, which shows the tight constraints to work within outlined in orange. The figure also shows the direction of translation of the entire stage when the y-axis knob is turned, represented by the blue arrows.

Preliminary Designs

A. Design 1 - Worm Drive Gear System (Last Year's Design):

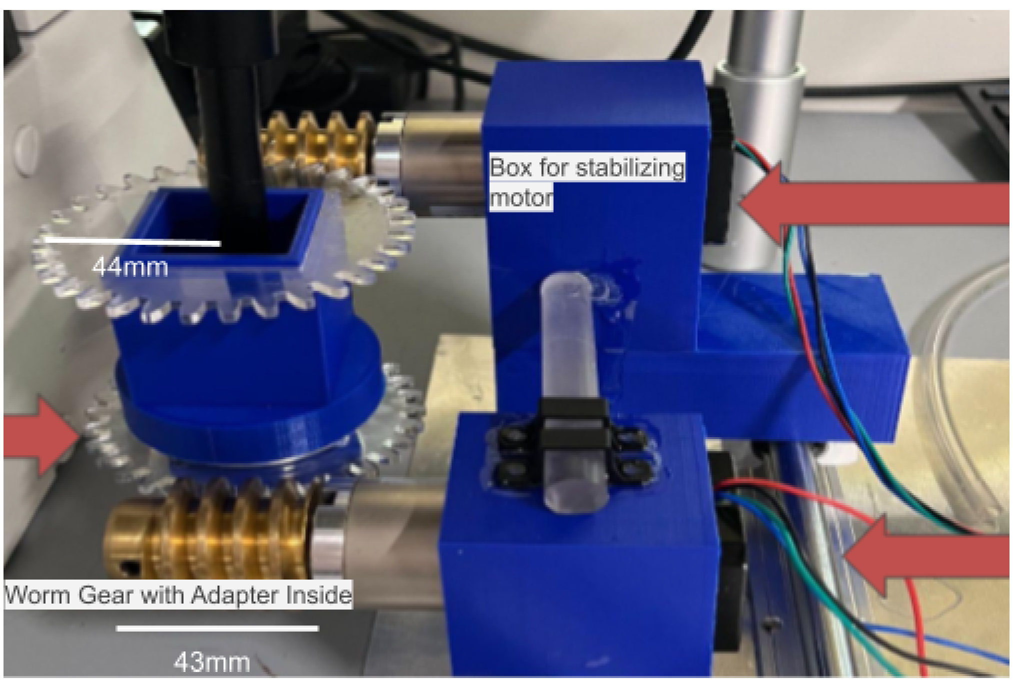


Figure 4: Preliminary Design 1 - Worm Drive Gear System

The Worm Drive Gear System (Figure 4) encompasses two worm drive gears each respectively connected to two spur gears that are attached directly onto the knobs that translate the stage. Two steppers motors are used to individually turn each worm drive gear, which rotates the spur gear, thereby turning the knob and moving the stage. The system sits on a linear sliding rack, since as the y-axis knob turns, the wand with the knobs moves as well, and thus the prototype must move with it. This design was fabricated by last year's BME design team, and our focus would be to improve upon three major aspects of this prototype including decreasing its size, improving its resolution and accuracy, and interfacing the motors with a joystick and the Nikon computer software for the microscope.

B. Design 2 - Chain and Sprocket:

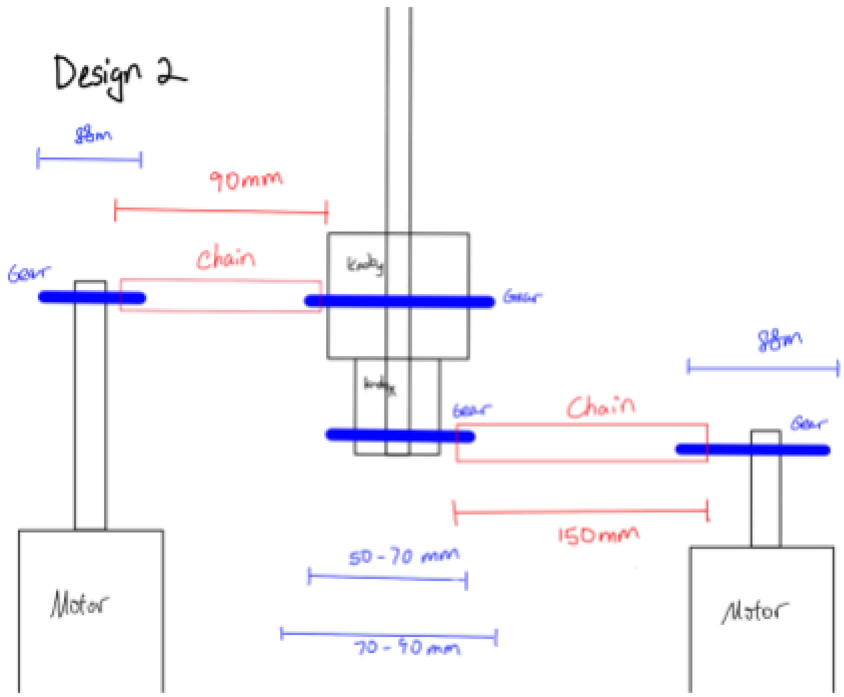


Figure 5: Preliminary Design 2- Chain and Sprocket

The Chain and Sprocket Design (Figure 5) is similar to the Worm Drive Gear System except it replaces the worm drive gear with a spur gear that is connected to the knob's gear through a chain. Notably, in this design the spur gears would not be directly in contact with one another. As the motor is activated, a spur gear will turn thereby rotating the chain which is in tension and allowing the knob gear to also rotate and translate the stage. The primary advantage of this design is in the stability of the system, where the teeth of the gear fall into place in the holes of the chain, making misalignment more difficult between the gears as the system slides on the linear rack. Removing the worm drive gears and positioning the motors vertically also decrease the bulkiness of the design. However, these advantages also present themselves as a potential weakness, since the chain itself would be tedious to fabricate and faulty fabrication of the chain would only increase the likelihood that the system jams and fails.

C. Design 3 - Meshed Spur Gears Design:

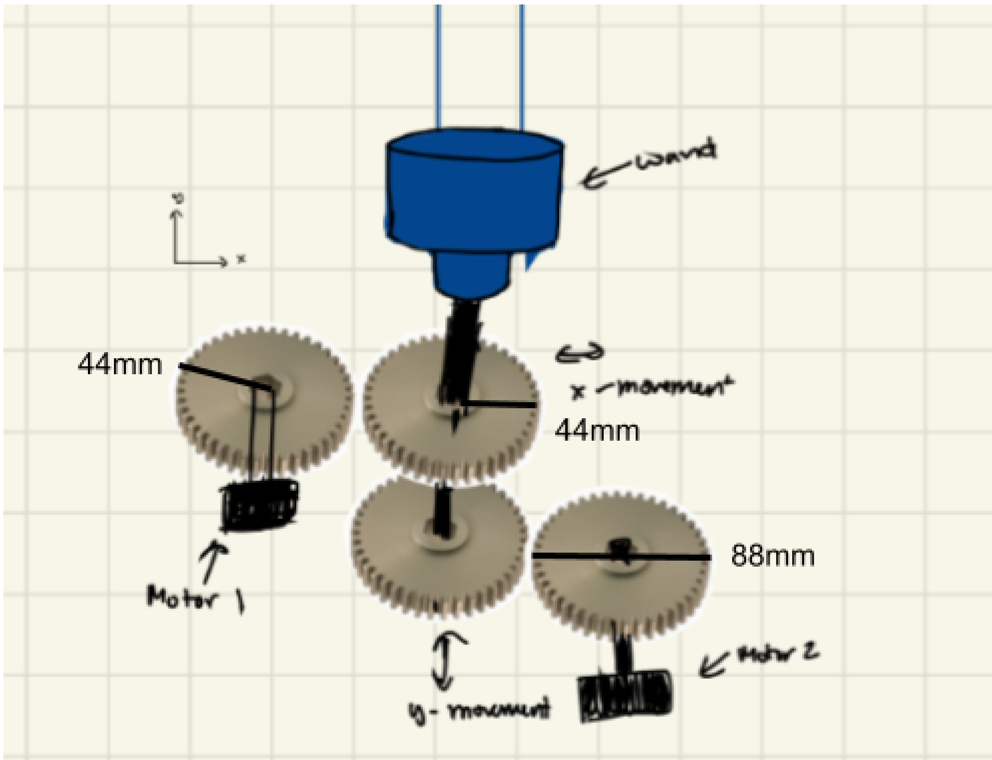


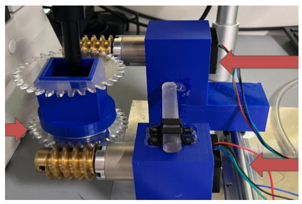
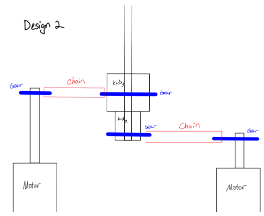
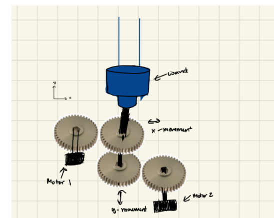
Figure 6: Preliminary Design 3 - Meshed Spur Gear Design

The Meshed Spur Gear Design (Figure 6) has the same functionality as the Chain and Sprocket Design yet it eliminates the need for a chain by placing the gears in direct contact with one another. Each motor is directly connected to a spur gear. As the gear rotates, its horizontal alignment and connection with a knob spur gear would allow the knob to turn thereby moving the stage. The principal advantage of this design is that it eliminates the bulkiness associated with the use of worm drive gears and a chain, and by fine tuning the gear ratios between the spur gears it could be possible to improve the resolution and accuracy of the system. The main drawback of this design is in the potential for misalignment between gears. As the x knob rotates, the stage is translated horizontally, and the system itself will move on a rack. However, now the fluidity of this movement must be considered to prevent sudden breaks that cause the gears to fall out of place which was not a major issue with the chain and sprocket system. The advantages and disadvantages for each design are quantitatively evaluated through a defined set of criteria in the next section.

Preliminary Design Evaluation

A. Design Matrix

Table 1: Preliminary Design Matrix

Criteria	**Design 1: Worm Drive Gear System** 	**Design 2: Chain and Sprocket** 	**Design 3: Meshed Spur Gears** 
Functionality (25)	4/5 * 25 = 20	3/5 * 25 = 15	4/5 * 25 = 20
Size (25)	2/5 * 25 = 10	4/5 * 25 = 20	5/5 * 25 = 25
Ease of Fabrication (20)	5/5 * 20 = 20	3/5 * 20 = 12	4/5 * 20 = 16
Cost (20)	5/5 * 20 = 20	3/5 * 20 = 12	4/5 * 20 = 16
Aesthetics (5)	4/5 * 5 = 4	5/5 * 5 = 5	3/5 * 5 = 3
Safety (5)	5/5 * 5 = 5	4/5 * 5 = 4	5/5 * 5 = 5
Total = 100	**79 / 100**	**69 / 100**	**85 / 100**

The design matrix (Table 1) above was created by our team to organize and effectively evaluate our ideas. The three designs were each graded by specific determined criteria and then weighted by the significance and relativity of the criteria to the project. The sections with the most priority are functionality and size as they are highly essential to the client.

The Worm Drive Gear System scored highly in many categories, with perfect scores in ease of Fabrication, Cost, and Safety. With the product already made, its fabrication is close to none. In addition, with all the parts already included, the cost would also be minimal. The previous semester's design scored 4/5 in Functionality as well since it was the closest to being ready to use except needing connected software. This design scored a 4/5 in Aesthetics as it is bulky and its wiring was left in a bundle to the side. Overall, this design scored the second highest.

The Chain and Sprocket design scored a 5/5 in Aesthetics, as its moving gears and chains may make the design look clean and polished. However, this design scored lower in all other criteria. The added chain caused a score of 3/5 in Functionality and 4/5 in Size as the two chains will make the design larger and could cause problems for the user if the chains slip off the gears. Next, the chains create added costs compared to the other designs and would make ease of fabrication slightly more difficult in order to match the chains' size to the correct gears. Therefore, the Chain Drive scored a 3/5 in both respective categories. This design scored lower in Safety than the other two designs as it is possible for the user to get an object tangled in the open chains.

Lastly, the Meshed Spur Gear design scored the highest in the combined categories. The third design scored well in Functionality, Size, and Safety. Without chains or a worm drive, having only gears on gears would make the design smaller and minimalistic. This led to 5/5 scores in Size and Safety, as the design would be more straightforward for the user, especially for a classroom setting. This design scored lower in Ease of Fabrication and Cost than Design 1, as the Design 3 has to be made from scratch unlike the previous Design. However, purchasing only gears instead of chains in addition is less cost-worthy.

B. Proposed Final Design

Based on the design matrix, the proposed final design was the Meshed Spur Gear Design. This design scored higher in comparison to the others and was decided to move forward with as it best meets the clients needs. With winning scores in Functionality and Size, the criterias weighed the heaviest, the Meshed Spur Gears simplistic design was found to be the best match for the team to construct and appease the client. After meeting with our advisor, it was decided that our first plan of action was to calculate the gear ratios to see how accurate we could get in movement before we began fabrication.

Fabrication/Development Process

A. Knob Testing and Gear Ratio Calculations

To translate the stage with a 1 micron translation step size, the angle needed to turn the x and y axis knobs to translate the stage 1 micron needs to be experimentally determined since these parameters are not publicly available. Experimentally, for each control knob, the strategy for testing was to turn the knob 360 degrees, using a tape marker and visual observations to ensure it has arrived back at its original starting points. A metric ruler was used to measure the distance the stage translated in this 360 degrees rotation. Then given the ratio of knob angle to translation distance, we can then do the appropriate conversions to determine the knob angle necessary to turn it exactly 1 micron. Three trials were done for the y axis knob, and exactly the same results were achieved each time, and four trials were done for the x axis knob simply because there was some variation and an extra trial was of interest to mitigate potential user error. The data and calculations are in Appendix H. For the y control knob, to translate the stage exactly 1 micron, the knob would have to turn 0.01125 degrees. For the x control knob, to translate the stage exactly 1 micron, the knob would have to turn 0.0192 degrees.

Next, the appropriate gear ratios were calculated to ideally achieve a 1 micron translation precision. The formula below enables us to input the desired total pitch diameter of both gears when meshed to then retrieve the required gear ratio such that the step angle of the motor corresponds to the angle the knob must turn for a 1 micron precision.

$$Large\ Gear\ Diameter\ = \frac{Total\ Gear\ Diameter}{1 + (1\ micron\ knob\ angle/motor\ step\ angle)}$$

The derivation of the formula is shown in Appendix C. The derivation overall rests on the constraints considered including the step angle of the motor, the angle the control knobs must turn to translate the stage 1 micron, and the maximum diameter a gear can be to prevent physical conflict with the microscope.

The formula was then used with the 1 micron knob angle experimental values to determine the diameters of each of the meshed spur gear systems for the x and y axis control knobs. For the x control knob, the pitch diameters of the knob and motor spur gears are 80.75 mm and 27.563 mm respectively. For the y control knob the pitch diameters of the knob and motor spur gears are 67.5 mm and 13.5 mm respectively

Final CAD Design of Meshed Spur Gear System

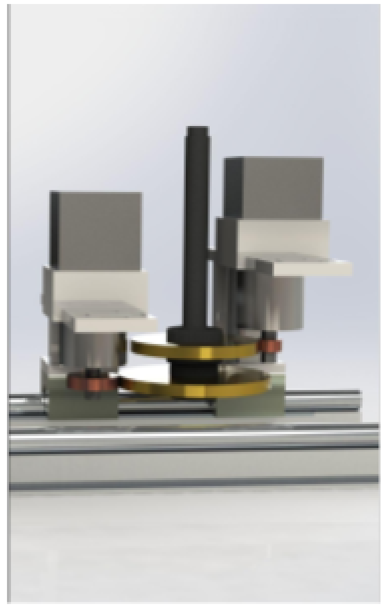


Figure 7: Final Prototype CAD Render

The final CAD design is shown above (Figure 7). The design encompasses four total gears of the diameters previously calculated to achieve a 1 micron translation precision, along with a motor base to hold two motors vertically upside down. A spur gear is attached to each of the motors that meshes with a spur gear on the knob, and when each of the motors rotates it turns the attached spur gear, thereby rotating the spur gear on the control knob which translates the stage. The system itself will rest parallel to the inverted fluorescent microscope below the control knob wand which can be further seen in the final prototype. Like noted before, when the y axis control knob turns, interestingly the wand moves with the stage as well, and thus a linear rack is necessary such that when the wand moves the entire system can slide along and follow the wand. The wand does not move when the stage translates in the x direction simply by the design of the microscope.

Materials

Multiple components from the previous iterations of the project were reused in our current design. Of these, the most important parts were the two stepper motors, the arduino microcontroller with all the electrical components for its connections, the linear rail system on which the motors moved. The spur gears were laser cut using acrylic plastic. All the supports for the motors and the motor bases were 3D printed in PLA plastic. A new joystick was bought and attached to a small breadboard. The code for the project was written in the Arduino IDE. The total costs of the materials are listed in Appendix B.

B. Methods

Mechanical Fabrication

The gears were fabricated with the laser cutter using 0.25 inch acrylic plastic. The high precision and low kerf of the laser cutter, the 2 dimensional design of the gears, and the ease of use for prototyping made the laser cutter the optimal method for fabrication. In practice, the gears were fabricated through five different iterations until achieving a set of gears that was ready for testing. A figure of the major improvements made through each iteration is shown below. Notably, on the first iteration, the inverse of the module of the gears (# of teeth/unit diameter) was too high meaning there were too many teeth on the gears and the gears could not mesh with one another. The inverse module parameter was decreased for the second iteration such that there would be less teeth for each gear and the gears were in turn able to mesh well, yet they did not fit tightly onto the motors and the control knobs. Measurements of the control knob diameters and the motor shaft were recorded again and this problem was solved on the third iteration, where the gears meshed well and also had a better fit on the control knobs. However the smaller gears could not fit on the motor, and through the last two iterations, the module was not changed, but the center holes were adjusted to achieve a better fit. Figure 6 shows the iterative design process of the gears.

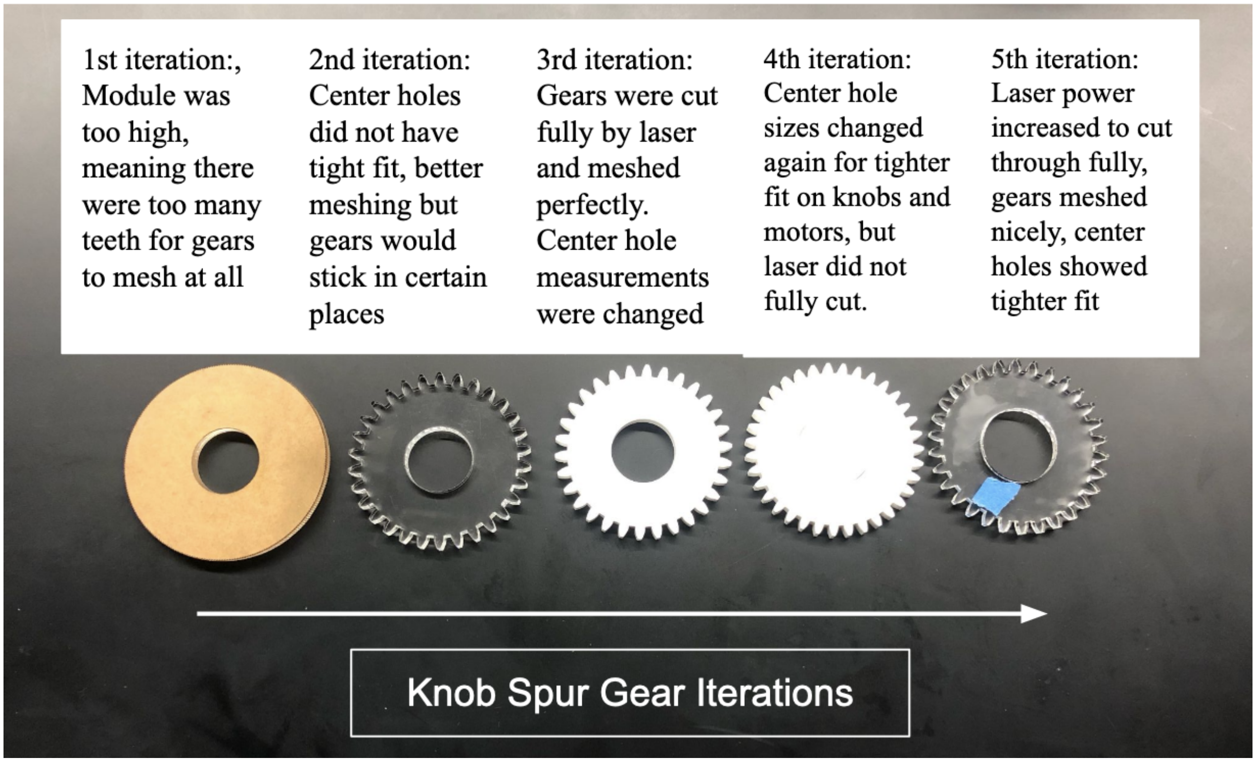


Figure 8: Knob Spur Gear Progression through 5 iterations of fabrication

The motor base being the design that holds the motors and rests on the linear rack was fabricated using the 3D printer. PLA was an appropriate material for the design and with the ability to control parameters including the infill and easily reprint for prototyping, 3D printing was the most practical choice for the motor base fabrication. The motor base went through three iterations shown in the figure below, where in the first iteration the part that held the motors had square holes that were too small and they did not fit onto the pillars of the motor base, and the motor base itself was not wide enough for it to not physically conflict with the gears. On the second iteration, both these issues were solved for where the motor base was widened, and the pillars were redesigned to be slightly smaller at the top and larger at the bottom for a more stable fit. For iteration 3, more support was added such that the motor base fits tightly on the pillars Figure 9 shows the design process of the motor mounts.

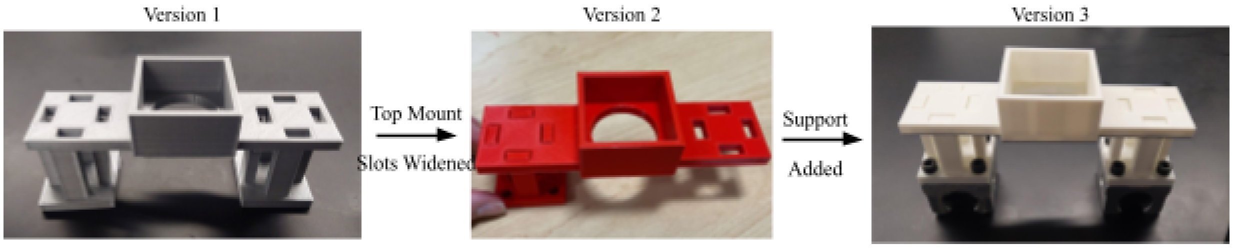


Figure 9: Motor Mount Prototypes

Lastly, with each of the individual parts fabricated, in assembling the prototype, first the linear racks were positioned correctly relative to the control knob and parallel to the microscope. Two of the linear slides were placed on each of the linear racks, then the motor bases were fastened on top of each of the linear slides as shown in the CAD. The part that held the motors upside down sat tightly onto the pillars that were fastened to the linear slides, and because of the pressure along with gravity, extra fasteners were deemed unnecessary here. Each of the two smallest gears were then fit tightly onto the motor ends. The motors themselves were then placed upside down into each of the square boxes that held them, and here as well, additional fasteners were not required for stability. The last two gears were then tightly positioned on the control knobs, where the top knob gear was placed first then the bottom knob gear. The linear slides holding each of the motor bases were then brought closer to the control knob to allow the gears to mesh. Minor adjustments were then made to enable the gears to mesh properly.

Electronics Fabrication

We used a specific type of motor called a stepper motor which is extremely efficient for speed control, precise positioning and repeatability of movement. Stepper motors are better in this way due to their high pole count. They are also able to have high torque at low speeds and although they are more expensive than a regular DC motor they are still relatively cheap and available. This is why we used the previous team Stepper motors for our electronics design. We ran 12 Volts of power through the stepper motors. Along with the stepper motors we required the drivers for the stepper motor. The drivers for the stepper motor also required wiring. The stepper motor drivers convert the pulse signals from the controller into motor motion in order to achieve precise positioning. The motor driver is also important as it is the controller by itself cannot provide the current required by the motor. Therefore we set the motor driver to a current limit of 1 amp, using the DIP switches on the side of the motor controller. There are a couple signals in the stepper motor and drive connections. There is pulse positive and negative and Direction both positive and negative and there is also EN+ and EN-. We needed to set the configuration switch at the top of the stepper driver. The switches correlated with different current settings.

We also considered a relay design initially as an alternative to fixing the last semester electronics design. Relays are a way to control the motors which work with the computer type circuits that switch relatively high currents or voltages to “ON” to “OFF” which is how the relay switch circuit is required to control it. This was going to be one of our backup designs if our initial design failed. We used an arduino microcontroller to control the drivers and the stepper motors for the circuit. We had to code the arduino to the specific pins that the motors were attached to (Appendix F). Initially we just wanted to code the motors to move forward 3 and backward 3 step sizes. Each step size was coded to 1 μm. We had code in order to make the motors move by output of the joystick but were still in the process of testing its compatibility with the rest of the circuit. The final circuit design is shown in Figure 10.

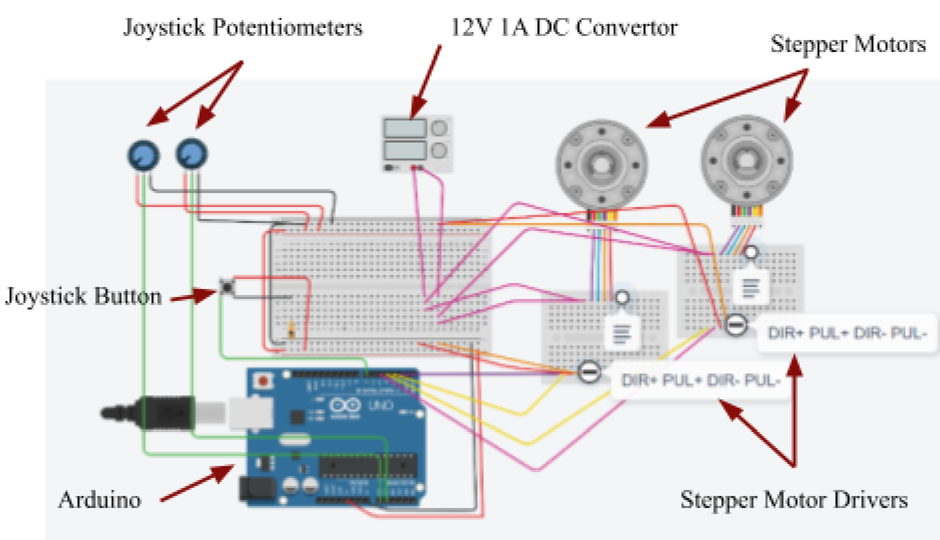


Figure 10: Tinkercad Circuit Design

C. Final Prototype

Mechanical Final Prototype

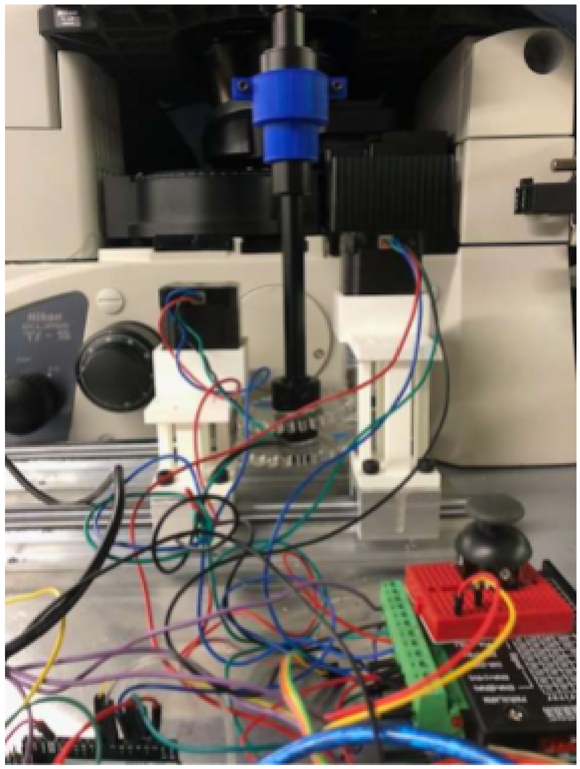


Figure 11: Final Prototype

The final prototype attached to the microscope is shown above (Figure 11). The final prototype follows the CAD design , where for each control knob, there is a stepper motor with a spur gear attached, which is meshed with a spur gear on the control knob. When the motor turns, the attached spur gear rotates, thereby rotating the spur gear on the control knob which can translate the stage. The gears were fabricated to theoretically achieve a 1 micron translation step size when the motor turns at its smallest step angle. The linear rack allows the entire system to move with the wand when the y axis control knob turns. Overall, the final prototype was successfully assembled in accordance with the CAD design, and the mechanical section can now proceed into testing.

Electronics Final Prototype

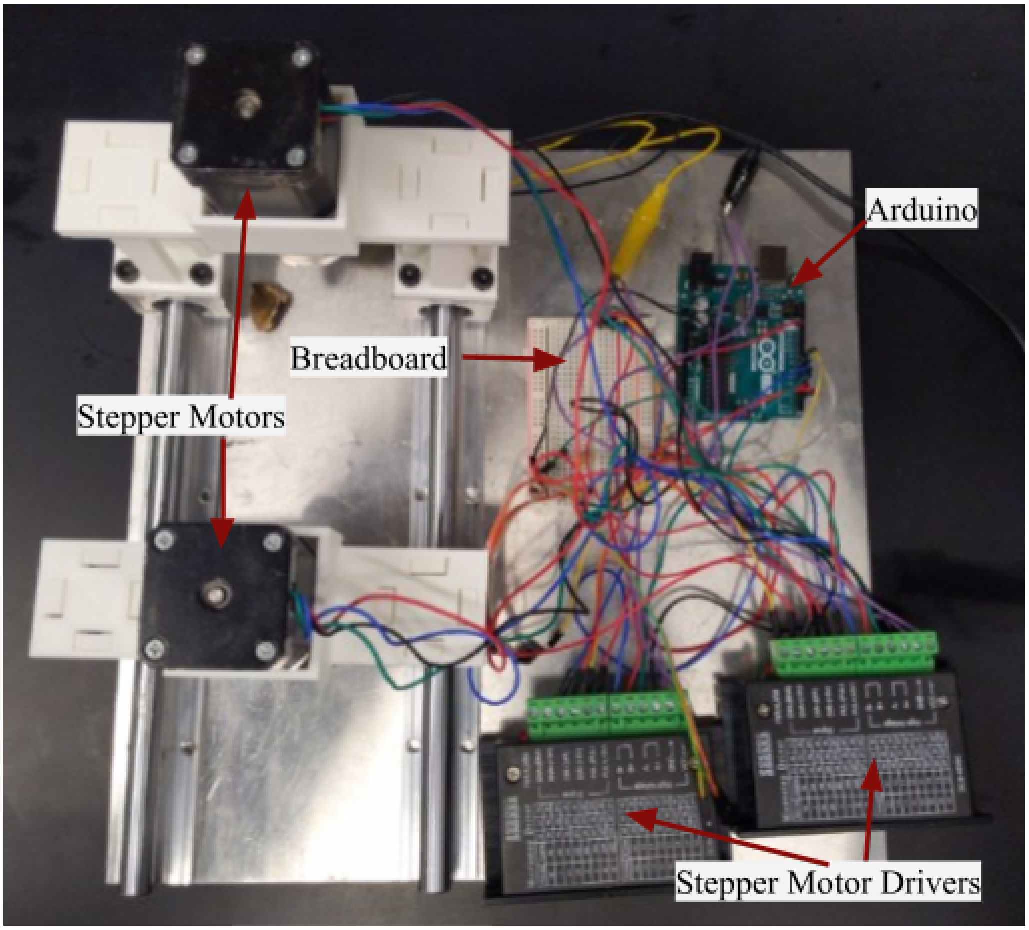


Figure 12: Prototype Circuit Design

In our final prototype (Figure 12) the arduino microcontroller had the arduino code uploaded. The arduino microcontroller had the pins attached to the drivers and the driver motors were connected to the stepper motors. There was 5 volts of power running through the microcontroller. On the board there was wiring for the ground and for the voltage. The pins for the drivers were connected to 2,3,4,5 on the digital pins. 2 and 4 were ones without the pwm. 3 and 5 were pins that were used as a pwm so they had the power also running through them. In total the arduino board had the power supply that ran the code and supplied enough power to send the signal through the pins. There was also a 5V and ground pin. There were also analog pins that we used for the joystick because of the fact that it was a type of potentiometer. There was a reset button and the serial in and out buttons. The digital pins were the most important as that was what we used to communicate a signal to the driver and the stepper motors.

In our final design we used drivers and stepper motors which enables us to get accurate positioning and accurate rotation angle and speed control using pulse signals. Stepper motors generate high torque with a compact body. The way that stepper motors work is that they take pulse signals in and convert them to motor motion. A pulse signal is the electrical signal which is based off the voltage level change that happens when it is turned on and off. Each time we turn the signal on and off that counts as a pulse. Each time the signal is on the voltage is high and each time the signal is off the signal is low. This way the amount of rotation is proportional to the number of pulses of the stepper motor and its rotations. The speed is also controlled in this way because the speed is proportional to the pulse speed. So if there is a higher pulse speed which means a higher frequency then the stepper driver is able to do more rotations per minute. This is the function of the driver so that it controls the pulse signals that it gets from the arduino. And then translate the pulses to the stepper motor so it can control the speed with greater precision. This way there are 2 ways to control the precision of the stepper motor. One way is through the code and the other is through the driver. The final piece of the electronics is the stepper motors which finally mesh the mechanical components as well. There are gears attached to the stepper motors which are then attached to the rest of the design allowing for the stage to be moved.

D. Testing

In deciding what aspects of the prototype should be tested, the main goals of the PDS were considered. The primary measure of success, as defined by the client, was the precision of the motorized stage to a 1 μm precision. Secondarily, the team chose to test the accuracy of translation of the stage. When constructing a protocol to test the precision of the device, the team ran into difficulties with obtaining a measuring device that could quantify such a small distance. As a result, the accuracy of the device was tested extensively, using the following method. A more detailed description, including screenshots, reference Appendix D.

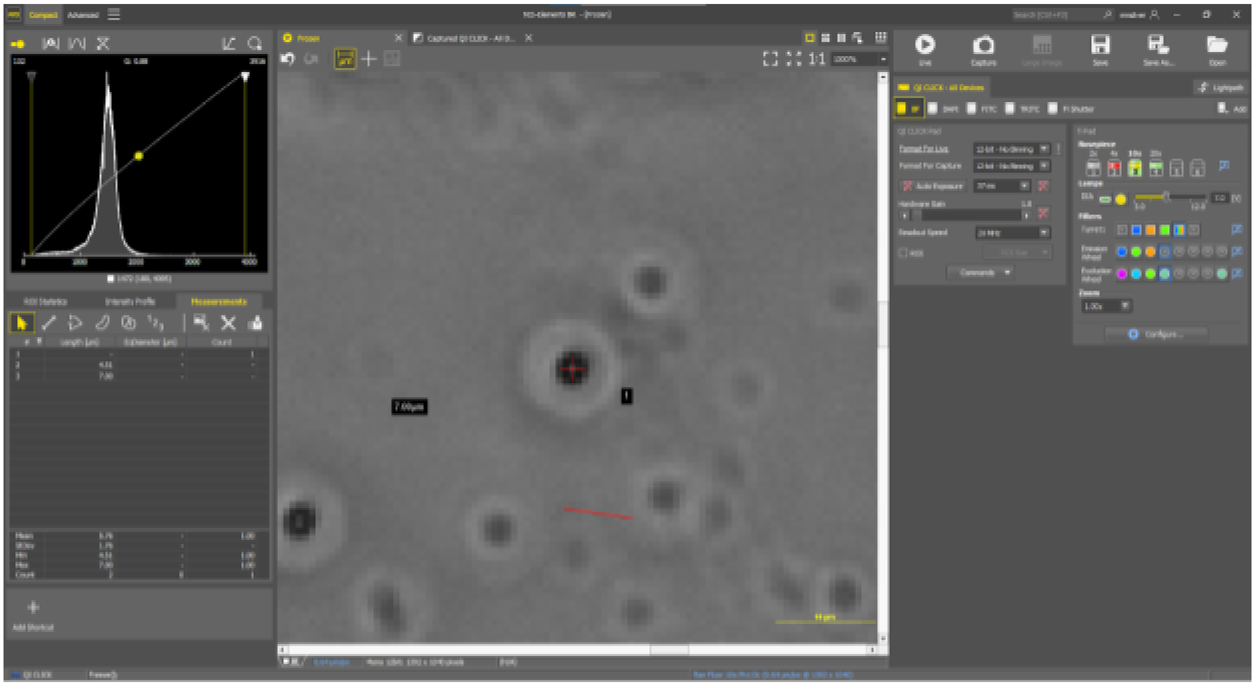


Figure 13: Nikon Elements Software Live View

To confirm the accuracy of the device, 6 µm fluorescent beads, similar to these purchasable from ThermoFisher [10], were used as a focusing point. The inverted fluorescence microscope was powered on and focused on one of the beads at 20x. The Nikon Elements software was used on the imaging computer to track and measure the beads (Figure 13). Running the Arduino testing code (available in Appendix E), the X motor was run for 2500 steps (2500 µm) in one direction, and then the same amount in the opposite direction. The distance from the starting point (the middle of the selected bead) to where the stage ended its translation, was measured in microns. If the stage did not translate enough to reach the target (i.e. undershooting) the measurement was recorded as positive, and vice versa. In total, 12 trials were run. The raw testing data is available in Appendix G. This test measured the accuracy, and therefore the error, of the stage's translation. To quantify the error found, the confidence interval and derived statistics were calculated.

Results

To determine the error in accuracy, the 95% confidence interval and other statistical indicators were calculated using VassarStats [11]. The 95% confidence interval of the sample was 1.6442±0.9464 µm, meaning that the stage would be able to translate within that range 95%

of the time. The mean of the sample was 1.64 µm, this is the mean distance that the microscope undershot the target by. Finally, the standard error of the sample was 0.4302 µm. The t-test value at 95%, or $t_{crit}(.05)$, was 2.2. The small error of the sample, as well as its' mean are shown in Figure 14.

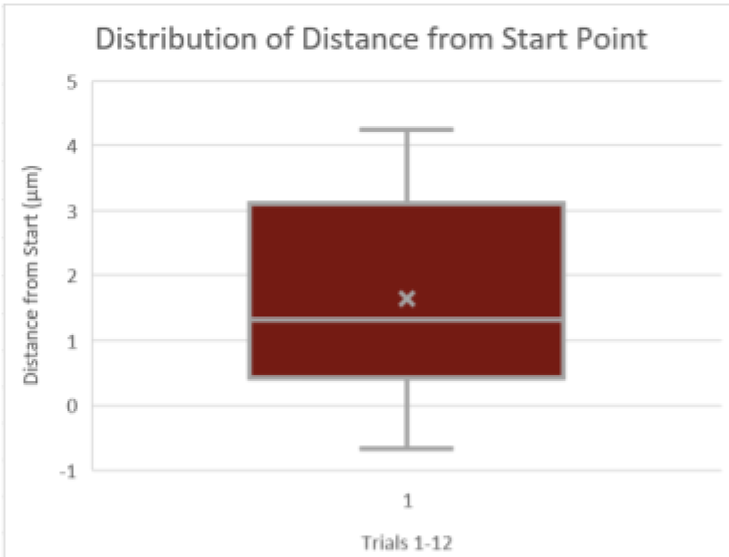


Figure 14: Mean, Minimum, and Maximum of Dataset

When observing the trends in data over time (trial number), an interesting trend appears. The error seems to increase over the testing time. This is best observed in Figure 15, which shows a roughly linear trend in the error. This change in error is likely due to a faulty connection between the motor and the motor driver. During the testing, the motor was observed to turn on and off sporadically. The wiring issue was attempted to be resolved by securing the connections, but the error still was observed.

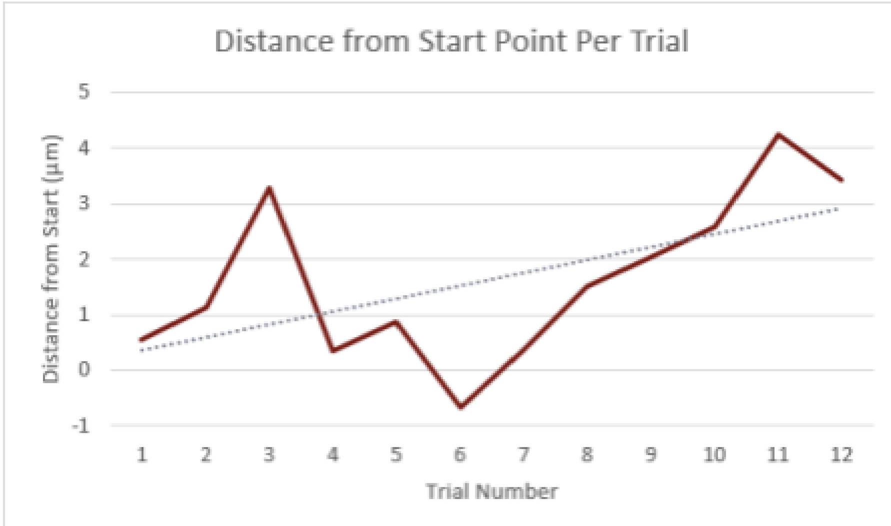


Figure 15: Trend in Error Throughout Testing

Discussion

Automated imaging would reduce the amount of manual time needed for imaging and stitching the images. This would mean that the researcher would be able to devote more time towards the actual experiment. When used in a teaching lab, it will allow the students to learn microscopy techniques better. Therefore, it is important to develop a low-cost automation system for the fluorescent microscope. Utmost care was taken to ensure that the final design of the project agreed with our initial PDS criteria, however, the major areas of our focus was to ensure that the bulkiness of the system was reduced and that we achieved the desired accuracy during the imaging.

The size of the automation system was reduced by about half by orienting it along the vertical axis with the wand of the microscope placed between the gears so it can be moved effectively without it being a completely external system. From our results for testing the accuracy of the system, we see that the mean error was around 1.664 µm with a 95% CI of 1.6442±0.9464. The standard error was about 0.4302 µm. It was seen that we did not completely achieve our target goal of 1 µm accuracy, however, while testing, the motor turned on and off which could have affected the accuracy, considering that we initially achieved an accuracy of around 0.5 µm. Moreover, a number of other factors could have affected the accuracy of the model, for example, it was observed that touching the table while the test was being run bumped the slide on the microscope stage and changed its positioning.

The mechanical aspect of the model was fairly stable therefore, the likelihood of it affecting the consistency of the model is very low. The model was fabricated to ensure that it withstands all usual wear and tear in a typical teaching laboratory setting. It was also ensured that the model will be low cost and replicable. The spending for the entire project was just over $50 which was well under our specified budget.

There are actually no ethical concerns that arise with the usage of the prototype with the microscope, however it is imperative to ensure that all components are handled with care so as to ensure that the electrical components are quite fragile. A possible solution would be the addition of an electrical circuit holding box. Two future goals that we hope to achieve with this project are the effective integration of a joystick to allow an option for manual control of the stage if needed and the ability of the user to change the speed of the imaging process as required by the size of the sample and the integration of the system with the Nikon Elements imaging software.

Conclusion

The current inverted fluorescent microscope in the teaching lab has fixed stages with manual movements through knobs located on the side of the stage connected through a wand sticking out. The current method lacks precision and ease of control. The team's client, Dr. John Puccinelli, wants to integrate the stage system with an automatic motorized system capable of automatic positioning and movements through translational control knobs while keeping the cost low. The design could potentially influence future advancements in microscopy and is primarily aimed for use in teaching laboratories and other under-funded labs.

The team decided on the gear-to-gear design, consisting of two systems of gears each having two gears attached to both the motor, the manual knob on the microscope, and to each other. This method of gear automatic movements will be controlled through the central system program by arduino to interface with software and be used through the joystick. This gear-to-gear system will prevent displacement of gears compared to other designs and achieve higher accuracy and maintain the low cost.

The fabricated system initially had a high accuracy of around 0.5 μm with the error increasing as the testing proceeded. This could have been attributed to faulty wiring, error in the arduino or due to the faults in the motors. In the future, the consistency of the model has to be worked on. Integration of the joystick and making it more adaptable with the design still requires more work.. This would allow the user to control the speed of the movement of the stage for imaging. Integration of the final design with the Nikon Imaging software was not feasible given the limited timeframe that the team was provided with. This remains a largely unexplored avenue, which is something that will be considered in the future.

Given the opportunity to improve our project, we would most likely alter the design since the manufacturing process of the custom gears proved to be more challenging than expected. In the context of the same design, the gears could be further refined to increase the accuracy and precision of translation. The mechanical components could also become more compact, which will require further creativity and deliberation given the large size of the step motors required to achieve a 1 micron translation precision. In regards to electronics, we would also focus more on increasing the replicability of the software components of the project to make it more intuitive. This would encompass incorporating a joystick to work with both the stepper motors and the Nikon software. Thus, further refinement in manufacturing and electronics is needed to increase accuracy and replicability. As a whole, with a near 1 micron translation step size and a cost of under $100, the team's prototype of a gear-to-gear system for automatic positioning and movement of the stage in a microscope has the potential to improve precision and ease of control in teaching laboratories and other under-funded labs.

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Appendix

A. Product Design Specifications

Microscope Low-Cost Motorized Stage - BME 200/300 Section 304

Product Design Specifications
September 22nd, 2022

Client: Dr. John Puccinelli

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Team:			
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Function:

Contemporary inverted fluorescent microscopes are equipped with manual control knobs that enable a researcher to translate the microscope stage freely in the x and y directions. This manual control system presents a series of challenges to a user in recording and stitching sample images and in relocating or refocusing on specific sample areas. A manual knob is also generally more prone to error and tedious to operate when translating within the sample. A new motorized stage control system is needed that can automate the translation of a sample. The motorized stage with the options of a joystick or computer interface would enable a user to seamlessly and precisely move between areas of the sample. Programmed translations would also allow for the recording and stitching of numerous images. Fundamental specifications for the design include that it be low in cost (<$100) and have a high precision and accuracy in translations (1 μm resolution). As a whole, current inverted fluorescence microscopes necessitate a low cost motorized system for stage translations that would allow for the automated recording and stitching of images and seamless precise translations.

Client Requirements:

- Cost of the product to be within $100.
- Use 3D printing and laser cutting.
- Capable with Nikon Elements imaging software.
- Resolution of the movement is around 1 μm.

Design Requirements:

1. Physical and Operational Characteristics

a. *Performance requirements:*

The motorized stage must be compatible with the rest of the microscope. The software that the microscope uses include: Nikon elements, micromanager and the software to automate the locations. The wand of the motorized stage must not inhibit the rest of the microscope functions including the focus or the objectives. The client would like the motorized stage to also be electronic, small and enclosed. The motorized stage will likely be used in a research setting for researchers to image their samples.

b. *Safety:*

The motorized stage must be safe to the microscope and the operator of the device. The stage can be properly clean after use by the operator without any difficulty. The material of the motorized stage must be safe and should not cause any harm to the operator.

c. *Accuracy and Reliability:* The motors must be calibrated to accurately and precisely translate to an inputted location of the sample. Specifically, the stage system should have a 1 μm resolution when inputting locations, with a ±0.1 μm error. The stage must also maintain the distance between the sample and the lens to ensure that the focus of the microscope remains the same and all effects of gravity are negated. To achieve this accuracy and precision, in addition to effectively calibrating the motors, that design that holds the motors must also be stable and its resistance to movement will prevent the buildup of error throughout its use. The joystick must also be reliable where the stage translations should correspond to its use, and this error should also fall within the ±0.1 μm range.

d. *Life in Service:* The stage must be functional for at least 10,000 cycles of imaging and should only undergo damage due to regular wear and tear. Updates to the software for how the stage translation system interacts with the computer interface may need to be done if a new or updated version of the computer interface is installed.

e. *Shelf Life:* Power off when not in use and store properly. If using batteries, store in a dry environment to prevent from heat source to secure a higher lifespan. f. *Operating Environment:* The microscope will be used in a lab class environment for experiments. Presumably under room temperature and normal conditions. No direct exposure to sunlight and limited shakes and tilts followed by proper microscope use guidelines. Cover the device with a plastic cover to ensure no dust or corrosion gets in contact.

g. *Ergonomics:* The entire equipment used must be small and not bulky to ensure the comfortable usage of the microscope. The mechanism of the stage must not interfere with the actual functioning of the microscope and must integrate with the software used. The equipment should be easy to carry and fit for storage. h. *Size:* The product should be as small and compact as possible. It should not get in the way of microscope operation. The components must be enclosed, but still able to access for maintenance.

i. *Weight:* The product must be lightweight and able to easily lift off and reattach back on. Under 10lbs is ideal, in order for any user to move the product throughout the shop floor or between labs.

j. *Materials:* A 3D printer will primarily be used from UW's makerspace to print plastic prototypes. The printing method chosen will most likely be FDM/FFF methods [1]. In addition, a laser cutter from Makerspace will be needed. The model included is the Universal ILS9.150D [2]. An analog 2-axis thumb joystick for operating the device will be purchased. A low cost option is a joystick with select button and breakout board, used by the 2022 Spring BME students. Any materials that cost over a total of $100 should not be used.

k. *Aesthetics, Appearance, and Finish:* The product must look simple and understandable enough for students to use or learn from. While the product should be low cost, it should also appear compatible and designed to complement the microscope. The product's finish should be smooth to the touch for operators.

2. Production Characteristics

a. *Quantity:* The client would like two universal units for the motorized stage that is functioning satisfactorily for the two microscopes in the teaching lab.

b. *Target Product Cost:* The ideal cost of the unit should be under $100, including the fabrication and installation. The budget, however, is flexible.

3. Miscellaneous

a. *Standards and Specifications:* As the device will not be mass produced, there are no manufacturer-required standards that it must abide by. However, there are still useful tools which can be used to test the accuracy and precision of our device. One of these is the Pelcotec™ LMS-20G Magnification Calibration Standard [3]. This is a piece of soda glass with a grid of 10 µm divisions. Using this calibration tool, the ability of the stage to move to a specific position and back can be tested.

b. *Customer:* The client, Dr. Puccinelli, would like to utilize the fluorescence microscopes in the teaching labs for both the basic and advanced courses that he teaches. As a result, the device should be removable, as to allow Dr. Puccinelli to instruct students on how to use the microscope normally. When teaching the advanced courses, Dr. Puccinelli can reattach the device, and utilize the motorized slide for more complex experiments.

The client would also prefer that the device is small and does not interfere with the normal usage of the microscope. Finally, Dr. Puccinelli would prefer if the motorized slide interfaced with the native software of the microscope, allowing him to utilize the predefined functions to stitch photos together and create timelapses.

c. *Patient-related concerns:* As the device will be used in a teaching lab, there are no patient-related concerns to be addressed.

d. *Competition:* Many other motorized slide solutions are available for purchase for research usage. These designs are often very costly and complex, which limits their usage to large, well funded research groups. Our design serves to fill the gap, and increase access to motorized microscope stages. By creating a low cost alternative, labs with tighter budgets, such as a teaching lab, will be able to perform more advanced experiments.

One of the inverted fluorescence microscopes that the BME design lab operates is the Nikon TI-U. This microscope is also available with a motorized slide, for an additional cost [4]. The motorized version of the microscope, purchased from a third party website, costs $3000 more than the base model [5]. This price difference is monumental, particularly when considering the large startup costs associated with purchasing lab equipment. Our design aims to cost under $100, 30 times cheaper than if purchased through the microscope manufacturer.

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B. Material Costs:

Materials	Place Purchased	Quantity	Cost
PLA Plastic for 3D Prints	Makerspace	334 g	$26.72
Four-way Joystick with Button	Makerspace	1	$4.75
18in x 24in x 0.25in Clear Acrylic Sheet	Makerspace	1	$18.00
M5 12mm Bolts	Makerspace	16	$1.60
Previous Years: Gearbox Nema 17 Stepper Motors 2X SBR12 Linear Rail Guide Arduino Uno Breadboard Stepper Motor Driver Nema TB6600 boards	 StepperOnline.com Amazon Makerspace Makerspace Amazon	 2 2 1 1 2	 $62.76 $30.74 $10.00 $3.00 $19.96
Total (Out of a $100 Budget)			**$177.53**

C. Derivation of Gear Diameter Formula

$$\frac{d_{large}}{d_{small}} = \frac{small\ \ gear\ rotations}{large\ gear\ rotations} = how\ many\ times\ small\ gear\ turns\ when\ large\ gear\ turns\ 360°$$

$$\frac{d_{large}}{d_{small}} = \frac{x^{\circ}}{y^{\circ}}$$

$$\frac{d_{large}}{d_{total} - d_{large}} = \frac{x^{\circ}}{y^{\circ}} = \frac{motor\ step\ angle}{1\ \mu m\ knob\ angle}$$

$$\frac{d_{large}}{d_{total} - d_{large}} = \frac{x^{\circ}}{y^{\circ}}$$

$$d_{large} = \frac{x^{\circ}}{y^{\circ}}(d_{total} - d_{large})$$

$$d_{large} = \frac{x^{\circ}}{y^{\circ}}(d_{total}) - \frac{x^{\circ}}{y^{\circ}}(d_{large})$$

$$d_{large} + \frac{x^{\circ}}{y^{\circ}}(d_{large}) = \frac{x^{\circ}}{y^{\circ}}(d_{total})$$

$$d_{large}(1 + \frac{x^{\circ}}{y^{\circ}}) = \frac{x^{\circ}}{y^{\circ}}(d_{total})$$

$$d_{large} = \frac{d_{total}(\frac{x^{\circ}}{y^{\circ}})(\frac{y^{\circ}}{x^{\circ}})}{(1 + \frac{x^{\circ}}{y^{\circ}})(\frac{y^{\circ}}{x^{\circ}})}$$

$$d_{large} = \frac{d_{total}}{1 + (\frac{y^{\circ}}{x^{\circ}})}$$

$$d_{total} = d_{large} + d_{small}$$

D. Testing Protocol

Testing Protocol for Accuracy of Motorized Stage

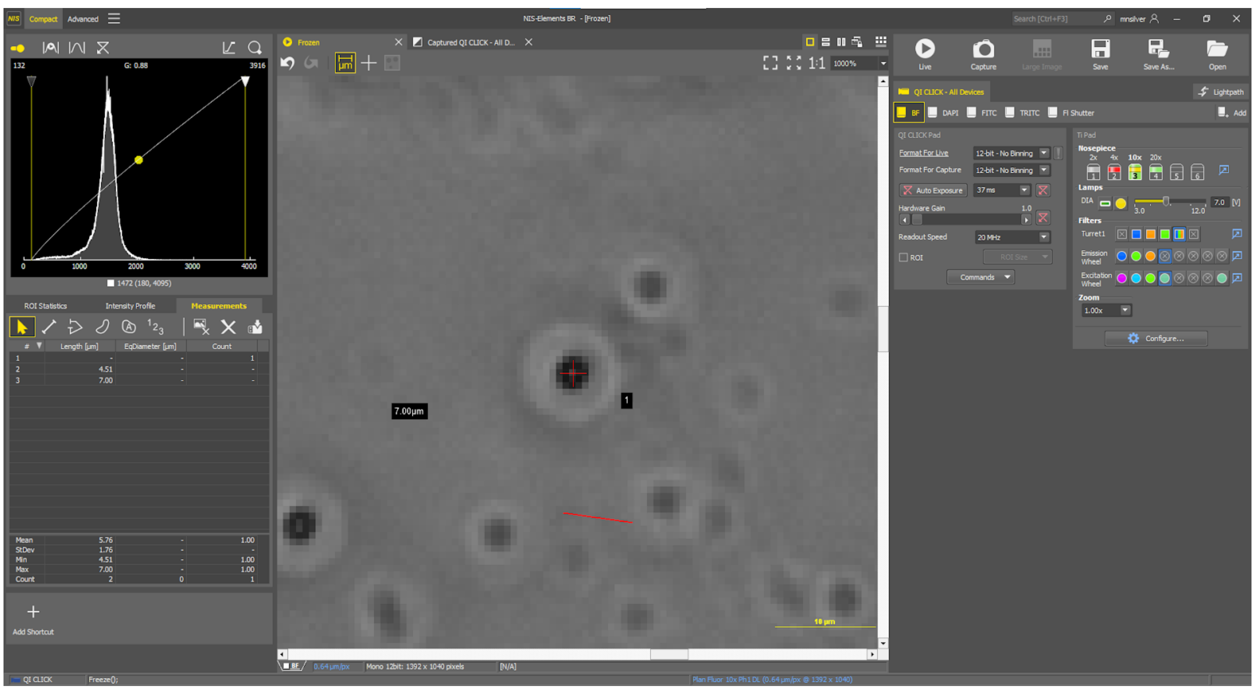
1. Turn on inverted fluorescence microscope and computer with Nikon Elements software.

2. Set microscope to bright-field imaging, and set optical path selector to "Camera" setting

3. Plate 6 µm beads, such as these (https://www.polysciences.com/default/fluoresbrite-yg-microspheres-600m), on a glass slide

4. Place the slide on a microscope stage and focus on the 20x magnification setting.

5. Open Nikon Elements, and click on "Live View" button to enable the microscope camera feed. The computer screen should look like this.



6. Select a bead in frame as the starting point, and mark with the crosshair tool.

7. Run Arduino testing code (under "Project Files"), which will move the stepper motor 2500 steps in one direction and then the same number in the reverse direction.

8. Measure the difference between the starting location (the crosshairs) and where the stage stopped moving.

9. Record this distance (in microns) in a spreadsheet.

E. **Arduino Testing Code**

```
#define DRIVERX_PUL 5
#define DRIVERX_DIR 4

const int buffer = 0.1; // joystick buffer region/tolerance
const int speed = 60; // sleep time between pulses
const int distance = 2500; // distance to move left/right
void setup() {
  // put your setup code here, to run once:
  pinMode(DRIVERX_PUL, OUTPUT);
  pinMode(DRIVERX_DIR, OUTPUT);
}


void loop() {
  for(int i = 0; i < distance; i++){
        digitalWrite(DRIVERX_DIR, HIGH); // X DIRECTION CLOCKWISE
        digitalWrite(DRIVERX_PUL, LOW);
        delayMicroseconds(speed);
        digitalWrite(DRIVERX_PUL, HIGH);
  }
  for(int i = 0; i < distance; i++){
        digitalWrite(DRIVERX_DIR, LOW); // X DIRECTION COUNTER-CLOCKWISE
        digitalWrite(DRIVERX_PUL, LOW);
        delayMicroseconds(speed);
        digitalWrite(DRIVERX_PUL, HIGH);
  }
}
```

F. Arduino Operational Code

```
#define JOY_X A0
#define JOY_Y A1
#define DRIVERX_PUL 5
#define DRIVERX_DIR 4
#define DRIVERY_PUL 3
#define DRIVERY_DIR 2

const int buffer = 0.1; // joystick buffer region/tolerance
const int speed = 60; // sleep time between pulses
int xValue;
int yValue;

void setup() {
  // put your setup code here, to run once:
  pinMode(DRIVERX_PUL, OUTPUT);
  pinMode(DRIVERX_DIR, OUTPUT);
  pinMode(DRIVERY_PUL, OUTPUT);
  pinMode(DRIVERY_DIR, OUTPUT);

  pinMode(JOY_X, INPUT_PULLUP);
  pinMode(JOY_Y, INPUT_PULLUP);
}

void loop() {
  // put your main code here, to run repeatedly:
  xValue = analogRead(JOY_X);
  yValue = analogRead(JOY_Y);

  // X value low
  if (xValue < ( (1024 / 2) - (buffer * 1024) )) {
          digitalWrite(DRIVERX_DIR, HIGH); // X DIRECTION CLOCKWISE
          digitalWrite(DRIVERX_PUL, LOW);
          delayMicroseconds(speed);
          digitalWrite(DRIVERX_PUL, HIGH);
  }
  // X value high
  else if (xValue > ( (1024 / 2) + (buffer * 1024) )) {
          digitalWrite(DRIVERX_DIR, LOW); // X DIRECTION COUNTER CLOCKWISE
          digitalWrite(DRIVERX_PUL, LOW);
          delayMicroseconds(speed);
          digitalWrite(DRIVERX_PUL, HIGH);
```

```
  }
  // Y value low
  else if (yValue < ( (1024 / 2) - (buffer * 1024) )) {
        digitalWrite(DRIVERY_DIR, HIGH); // Y DIRECTION CLOCKWISE
        digitalWrite(DRIVERY_PUL, LOW);
        delayMicroseconds(speed);
        digitalWrite(DRIVERY_PUL, HIGH);
  }
  // Y value high
  else if (yValue > ((1024 / 2) + (buffer * 1024))) {
        digitalWrite(DRIVERY_DIR, LOW); // Y DIRECTION COUNTER CLOCKWISE
        digitalWrite(DRIVERY_PUL, LOW);
        delayMicroseconds(speed);
        digitalWrite(DRIVERY_PUL, HIGH);
  }
}
```

G. Raw Testing Data

Trial	Distance from target at end of translation (microns)
1	0.54
2	1.13
3	3.28
4	0.36
5	0.86
6	-0.66
7	0.38
8	1.51
9	2.04
10	2.6
11	4.25
12	3.44

H. 1 Micron Knob Angle Testing Data

Y knob(top knob):
Trial 1:

0.7 cm starting
3.9 cm ending

Trial 2:
0.8 cm starting
4 cm ending

Trial 3:
0.7 cm starting
3.9 cm ending

Average difference: 3.2 cm
Ratio: 3.2 cm / 360 degrees rotation
To turn 1 micron:
360/(3.2*(10^4))
0.01125 degrees/micron

X knob(bottom knob):

Trial 1:
31.9 cm starting
30.1 cm ending

Trial 2:
32 cm starting
30.1 cm ending

Trial 3:
32 cm starting
30.15 cm ending

Trial 4(simply because some variation was noticed, and another trial was of interest):
30.15 cm starting
28.2 cm ending

Average difference: 1.875 cm
Ratio: 360 degrees / 1.875 cm
To turn 1 micron: 0.0192 degrees/micron