FINAL REPORT: MICROSCOPE LOW COST MOTORIZED STAGE

December 13th, 2023

BME 300/200

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Abstract

Inverted fluorescence microscopes are a highly utilized piece of laboratory equipment used in life sciences research. Manually controlled versions of these microscopes are much cheaper than the market-available motorized alternatives, but are not as ideal for teaching and high-throughput research scenarios. The client, Dr. Puccinelli, tasked the team to create a device capable of adding motorization, automation, and image sequencing capabilities to these microscopes. Through organized brainstorming, structured evaluation ideas, and well-executed fabrication, the team created a working final prototype. Proof-of-concept related findings showed great results; the prototype is capable of moving the stage by user inputs through a circuit-integrated joystick as well as by inputting values into the Arduino IDE. While much better than any previous prototype in every regard except cost, testing still revealed drawbacks in the areas of precision, accuracy and automation, which future groups will look to fine-tune in an attempt to make this product lab-ready. The accomplishments of this semester shed optimistic light on a project that has been torn down and built back up under the reign of each new group attempting to meet client criteria. With a solid base and a functional prototype, future teams will start on more sturdy legs than ever before, and hopefully achieve levels of prototype competency not yet seen in this project's short history.

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I. **Introduction**

Motivation

In biological research, microscopes are essential for their pivotal role of helping researchers by magnifying microscopic objects, allowing close examination of details that can't be seen with human eyes alone. Moreover, the integration of motorized stages in microscopes has significantly improved research practices that involve microscopes. These advanced microscopes bring several benefits to researchers, making their work more efficient and precise, especially when studying dynamic biological processes. For example, the automated functionality of motorized microscopes improves the efficiency of data collection. Researchers can program the microscope to automatically capture images at specific locations, freeing up time for more in-depth analysis and interpretation of results. This accelerates the research process and allows scientists to explore larger datasets. However, microscopes equipped with motorized stages typically come at a significantly higher cost compared to their manual counterparts. This poses a significant challenge to accessibility, particularly for budget-constrained and educational laboratories. The motivation behind developing an affordable motorized microscope stage is to broaden access to a more extensive audience by embracing a cost-effective design that eliminates financial barriers. Moreover, the device should be easy to refabricate. This approach aims to enhance the overall experience for both students and staff using the Biomedical Engineering teaching lab. Additionally, the incorporation of image sequencing capabilities into the motorized microscope stage is expected to significantly boost its versatility, making it more robust for applications in both teaching and research.

Existing Devices and Current Methods

While the market currently offers commercial motorized stage replacements for conventional microscopes, these options, usually provided by well-established microscopy manufacturers, often boast advanced capabilities. Unfortunately, their performance comes at a steep price, rendering them financially unfeasible for many laboratories. An illustrative example is the Nikon TI-U, a non-motorized fluorescent microscope in use at the Biomedical Engineering teaching lab, which can be upgraded to a motorized version for a staggering $70,000-$80,000 [1]. The exorbitant cost of these commercial alternatives has prompted the exploration of more affordable solutions.

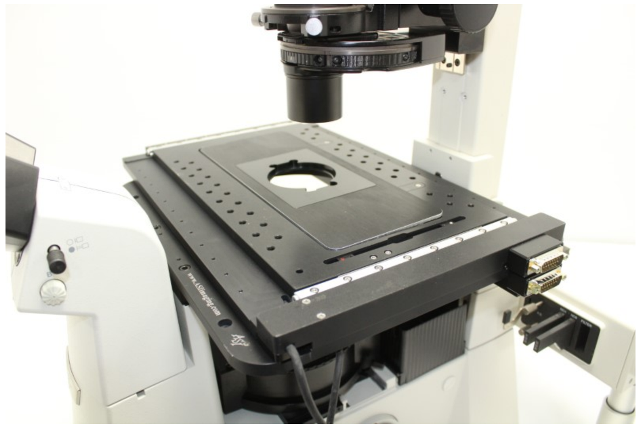


Figure 1: Nikon Ti-U Inverted Fluorescence Motorized Microscope [1].

One such alternative is the Openstage, an open-source project presenting a three-axis drive motorized stage system with a stand-alone controller unit [2]. Despite its considerably lower cost compared to commercial options, the Openstage offers performance that rivals or even surpasses more expensive counterparts. Impressively, its accuracy in the X and Y directions is reported to be 1µm or better. The stage's motion can be controlled either through a handheld controller or a programmed interface, with the added flexibility of easily modifiable controller software. However, the project's total cost hovers around $1000, which, while more economical, might still be a financial challenge for some. Additionally, the design is criticized for being somewhat bulky, as detailed in the PDS (Appendix A).

Another contender in the realm of cost-effective designs is the Openflexture Delta Stage [3]. This innovative stage utilizes 3D printing for its construction, and its electronic components are housed in the base, powered by a Raspberry Pi. Operating in the X, Y, and Z axes with three stepper motors, this compact design is suitable for placement on a laboratory bench or within a microbiological safety cabinet. Notably, the Openflexture Delta Stage comes at a more palatable price point of around $336, making it a more accessible option when compared to both commercial and the Openstage alternatives.

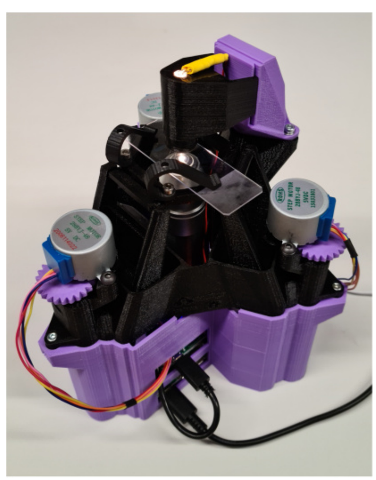


Figure 2: Openflexture Delta Stage [3].

Problem Statement

The UW-Madison BME teaching lab currently houses two inverted fluorescent microscopes. While these contemporary pieces of equipment are functional in their current state, they leave a great deal to be desired when it comes to laboratory efficiency. Motorized alternatives do exist, however, cost continues to be a factor limiting the exposure students have to this technology. With a working budget of approximately $100, our team seeks to optimize the precision and automation of these microscopes through the creation of a novel microscope stage moving device. This design will allow for sequential, automated imaging of coordinate-based locations on slides. The creation of this device will increase the consistency of measurements, reduce the amount of time spent during data collection, and lower the learning curve for operating this equipment. To remain competitive, design prototypes should be automated in both the X and Y directions and retain high precision and accuracy in translations (1 µm resolution).

II. <u>**Background**</u>

Design Research

The BME Teaching Lab at UW-Madison houses, amongst many other pieces of equipment, two inverted fluorescence microscopes-one of which can be seen in Figure 3. The "inverted" in the name is due to the flipped arrangement of certain parts of the microscope relative to what is normally seen. These microscopes house an objective lens below the stage, with a condenser and light source located above the stage [4]. In addition to an altered arrangement of componentry, inverted fluorescence microscopes also have a unique imaging mechanism that utilizes fluorescent dyes. Dyes are placed within the sample by researchers and excited through the use of a halogen lamp as well as LEDs and lasers. Differences between input and output emission are used to generate high resolution imagery of things like living cells and tissue culture [5].

Figure 3: Nikon TI-U inverted fluorescence microscope used in the BME Teaching Lab [6]

This variety of microscope has become very popular in the scientific community, specifically amongst researchers as well as in general laboratory use across a variety of categories of science. This points to the significance of creating a device that can modify microscopes without previously integrated mechanized systems at a low cost; many in the scientific community would benefit from the reduced costs of equipment that such a device would provide.

As research was conducted regarding the efficiency and accuracy of the device, it was discovered that gear meshing and the reduction of deflection should be a very high priority goal during the production of the device. Gear meshing can be described as the interaction between the teeth of two gears, as one tooth from one gear is inserted in between two teeth of another gear, they become meshed. Solid and consistent meshing is what makes an accurate and reliable gear system, and this consistency is why gears have been chosen to create a relationship between the motors and control knobs despite alternative solutions such as belts being available. However, consistent gear meshing can be difficult to accomplish, especially amongst gear systems with more than 2 gears. As research was conducted to determine the best gear setup for the design, there were two major factors that influenced the final decision, size and gear functionality. This can be seen in more detail in the PDS (Appendix A). It was already established from our Client's design criteria that the device as a whole should not take up excessive space on the countertop where the microscope is located. Research was also conducted to determine common pitfalls that occur when working with gears, which were determined to be gear meshing, gear lockup, and gear deflection. Originally, this year's team met with a Mechanical Engineering professor, Doctor Christopher Westphal, to discuss gearing as well as the designs that were being developed at that time in regards to the gear setup. Initially, a three year design was going to be used as it was thought it would reduce the size of the overall design by having smaller gears. However, Doctor Westphal highlighted that a three gear design, especially with 3D printed gears, was prone to gear lockup. Gear lockup would greatly reduce the accuracy and efficiency of the device, so a two gear system was used instead. As previously mentioned, the ability of the gears to mesh is also crucial for an efficient and effective gear system that prevents backlash and loss of motion. Figure *4* below is a diagram that shows an example of effective gear meshing with minimal backlash. Backlash can be described as the effect that unfilled space between two meshing gears has on the loss of motion in a gear system[7]. This contributes to a loss of motion in the overall device. The effects of the loss of motion are highlighted in the testing results in *Section VI.*

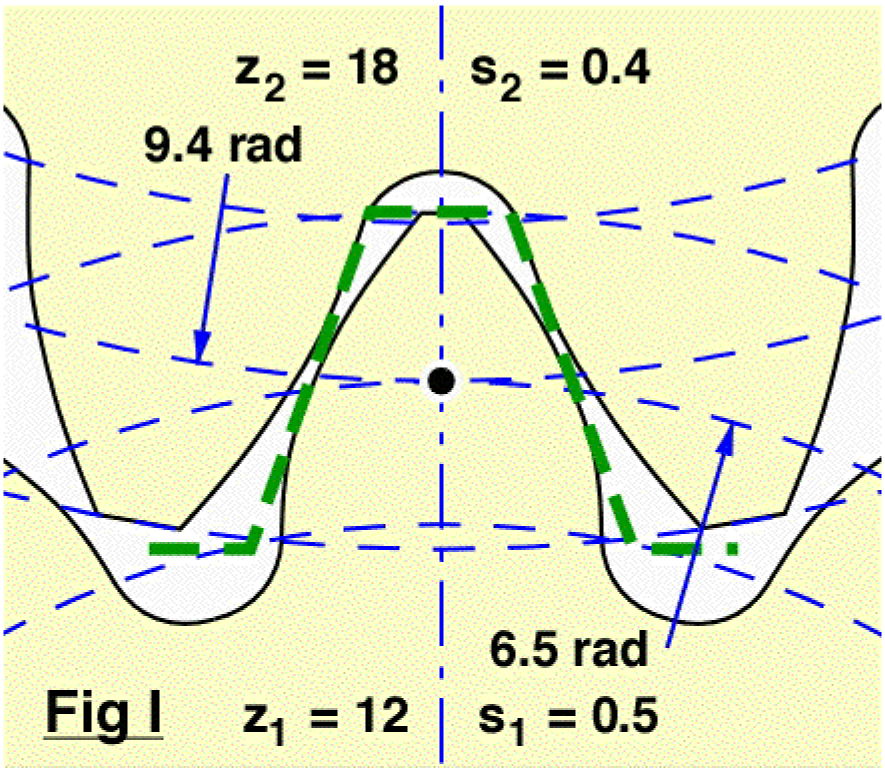


Figure 4: showcase of gear meshing and backlash [8]

Design History

The Low Cost Motorized Microscope stage design has had several iterations prior to our assignment to this project. Each of these designs has made steps towards creating a consistently functional final project, but none have done so to completion. Previous groups have taken several different approaches to solving the issue of integration motorization into the design of the microscope. Each of the previous designs, as well as this year's final Prototype have used the already existing manual control knobs as the medium for manipulation of the stage. Each team has also been faced with producing a solution to one of the more difficult aspects of this project to overcome, that being the Y-axis travel of the stage. As the stage is moved by the manual control knobs in the X-axis direction, the manual control knob shaft that hangs from the stage is not moved, however, when the stage is moved in the Y-axis direction, the manual control knob moves with the stage. Since the desired outcome of this project is to create a device that manipulates the stage in both the X and Y directions, the movement of the manual control knobs increases the complexity of reaching our goal. Due to the movement of the control surfaces (the manual control knobs), a solution must be created in order to keep the device's movement relative to the control knobs.

Workarounds that have been developed by previous groups include a linear rail system to allow the device to slide along the side of the microscope as the stage moves in the Y direction, as well as a housing unit that hangs from the underside of the stage that acts to support each component of the device. Previous groups have encountered issues with both of these methods. Both are shown below in Figure 5 and Figure 6.

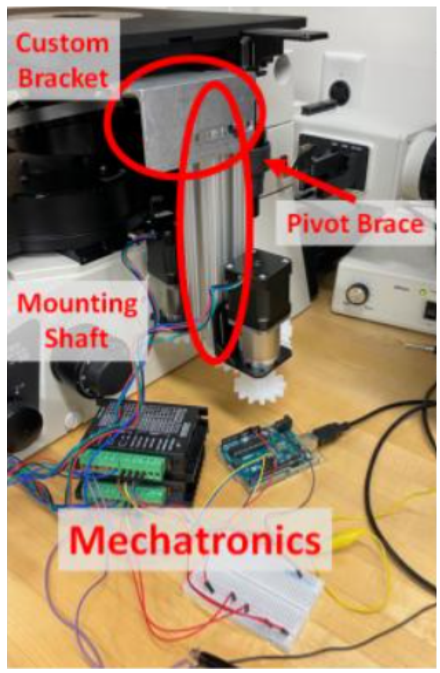


Figure 5: Fall 2020- Spring 2021 stage-mounted final design

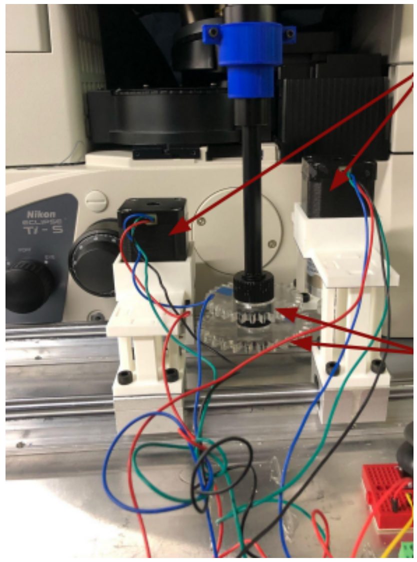


Figure 6: Fall 2022 linear rails final design

The Linear Rail System has been shown to produce inaccuracies due to the movement of the gearing as well as motor components of the device. Previous groups have utilized a faulty rail system that allows rotation around the centroidal axis of the rail, this allowed for deflection of gears as well as overall instability of the device. The hanging housing unit has proved to be very heavy, which can play a role in creating inaccuracies of the microscope's ability to focus as a heavy object on one side of the stage can cause it to tilt, which can cause the microscope to render blurry images.

Client Information

The client, Dr. John Puccinelli, is the Associate Chair of the Undergraduate Program and Associate Teaching Professor for University of Wisconsin-Madison Department of Biomedical Engineering. He oversees the BME Teaching Lab in ECB 1002 and is responsible for the purchasing and management of all equipment inside. Dr. Puccinelli has tasked our team with the creation of a device to motorize and automate the inverted fluorescence microscopes in the teaching lab to offer a more robust teaching and research experience to biomedical engineering students and staff at UW-Madison.

Design Specifications

Through collaboration between the client and our team, a Product Design Specifications (Appendix A) document was created. This document can be found in its entirety in *Section A* of the Appendix, but will also be described here. Our client requested that this device motorize the stage of the microscopes with a maximum fabrication budget of $100. This device must be easily attachable and detachable so it can be used as an accessory, rather than being permanently integrated into the microscope's design. Final prototypes of this semester should be capable of moving the stage of the microscope as directed and be accurate to within a micron (1 μm) of the intended movement input by the user. Inputs from the user should either be directed by a joystick integrated into the circuit or by inputting coordinate-based locations into a user interface (UI) the team would create. The client would be satisfied with inputting these values into the arduino IDE for proof of concept and testing, but would like a more streamlined UI in a final design. This design, at Dr. Puccinelli's request, will interact with the preexisting manual control knob of the microscope. This knob is located very close to many other mechanical structures of the microscope such as other adjustment knobs on the side of the microscope. With this in mind, designs should be compact and modeled in a way such that they won't interfere with functions of the microscope when attached.

III. Preliminary Designs

This Year's Team brainstormed several designs to try and overcome the issue of the stage's Y direction travel. Previous years' designs as well as the clients design criteria were considered in order to create the following designs. These decisions were supplemented by additional research in order to gather more information to produce better refined preliminary designs.

Spur Gear Design

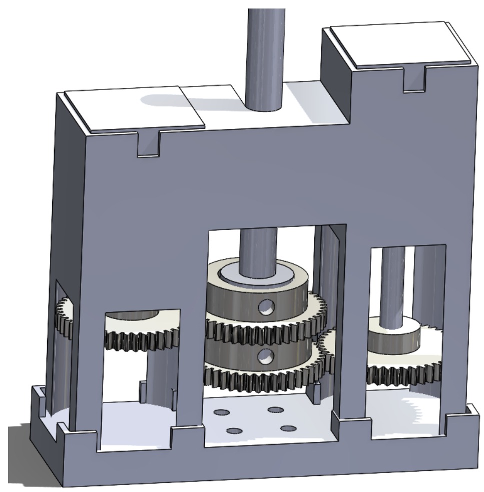


Figure 7: Spur Gear Design

The spur gear design is attached directly under the stage and interacts with the existing control knob. In this design each motor is directly connected to a spur gear. When it rotates, its horizontal alignment and connection with a spur gear would move the control knob to turn, thereby moving the stage in the X and Y direction. The main advantage of this design is that it reduces the size of the device and the space it occupies on the countertop.

Worm Drive Design

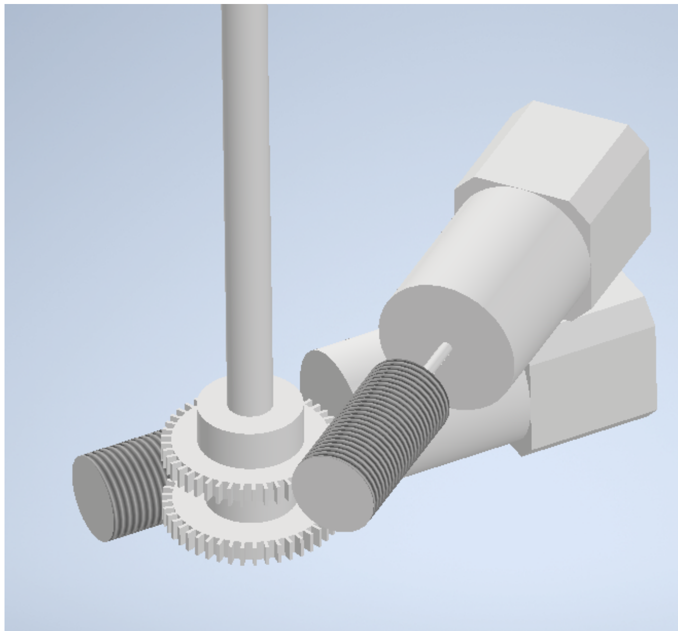


Figure 8: Worm Drive Design

The worm drive design interfaces with the existing manual control knob in order to move the stage. In this design, the manual knob will have spur gears added so that the worm gears can turn the knob. The worm gears will be placed on top of one another to reduce the amount of space used and will be angled so they can easily mesh with their respective spur gear.

Linear Rails Design

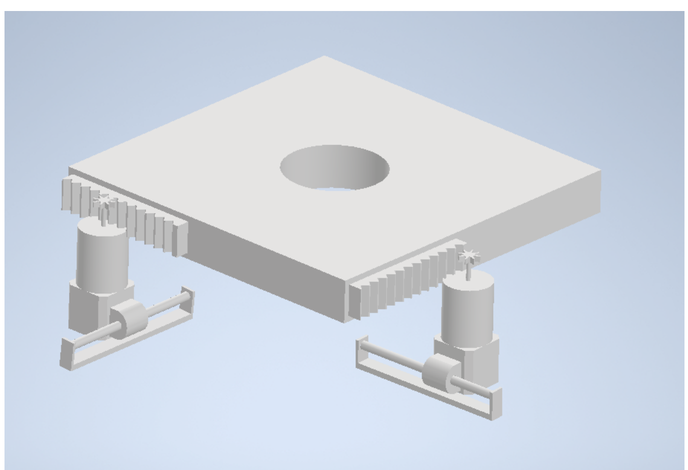


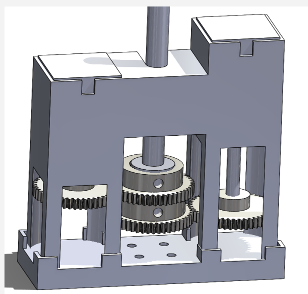
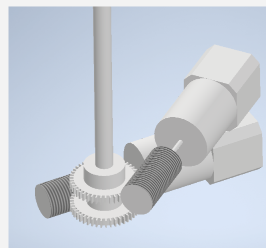
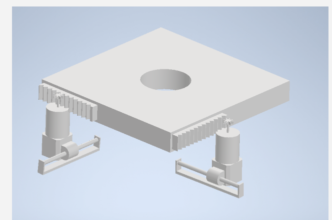
Figure 9: Linear Rail Design

The last design has many major differences to the previous designs. The linear rail design uses stepper motors to directly move the stage. It accomplishes this by having a spur gear on each stepper motor and linear gears attached to the stage. The spur gears can then move the stage by moving the linear gears. In order to keep the spur gear in contact with its linear gear when the stage is moving, there are linear rails attached to each stepper motor. These linear rails let the motor freely move with the stage.

IV. <u>Preliminary Design Evaluation</u>

Design Matrix

Table 1: A design matrix comparing the three designs.

Design Categories (Weight)	**Design 1: Spur Gears** 		**Design 2: Worm Drive** 		**Design 3: Linear Rails** 	
Projected Performance (25)	4/5	20	3/5	15	5/5	25
Compatibility (20)	5/5	20	3/5	12	3/5	12
Cost (20)	4/5	16	4/5	16	3/5	12
Ease of Fabrication (15)	4/5	12	4/5	12	3/5	9
Longevity (10)	4/5	8	4/5	8	5/5	10
Safety (5)	4/5	4	4/5	4	5/5	5
Total Points:	80		67		73	

Criteria Explanations

The following criteria were created by the team to evaluate each of the designs in a holistic manner based on requirements detailed in the Product Design Specifications (Appendix A).

The projected Performance of the motorized microscope stage is evaluated on the design's capability to adjust the stage in the x and y axis consistently, precisely, and efficiently. This category is scored the highest in the design matrix due to the high sensitivity and accuracy of the client requests. Specific considerations for this evaluation are outlined in the *Performance Requirements* section of the PDS.

Compatibility assesses how effectively the device interacts with the microscope's structural and mechanical elements, considering factors such as the microscope's limited surface area and where the device is mounted. As stated in the PDS's *Size, Weight, Ergonomics, and Spatial Configuration* sections, the device should be light, and compact and not interfere with existing mechanical structures of the microscope.

Cost is evaluated on the total price of the materials and associated fabrication costs, such as machinery use fees. Cost is ranked higher in the design matrix due to the client's emphasis on remaining under budget and allowing the project to be accessible for those of all economic backgrounds. The total expenses should aim to remain under $100, as outlined in the *Cost* section of the PDS.

The Ease of Fabrication category evaluates how easily the device will be able to be fabricated, along with how easy it is for other people to replicate the design in the future. The considered factors are outlined in the *quantity* section of the PDS. This category is less important because although this creates a design that can be easily reproduced by others, the main objective is to make a solution for the BME teaching lab specifically. Therefore, while still being considered, it is not ranked as highly as other metrics in the design matrix.

Longevity refers to the durability and lifespan of the components and overall device. It encompasses the ability of the device to withstand continuous usage over an extended period without significant deterioration in performance. A design scoring high longevity ensures that the materials, motors, and other essential elements are robust enough to endure repeated movements and usage, thereby extending the functional lifespan of the device. This consideration is not as important but essential for creating a reliable solution.

Finally, as all our design concepts utilize electrical power sourced from the teaching lab's wall outlets, inherent safety concerns are associated with each potential prototype. Therefore, the safety category focuses on the potential risks posed to users during the operation of the device, as detailed in the *Safety* section of the PDS.

Design Evaluations

Regarding projected performance, the Linear Rail design stands out as the top performer among the three preliminary designs considered. This is because the Linear Rail design is directly connected to the stage. When interacting directly with the stage, it makes the device more accurate and precise, leaving less room for mistakes. On the other hand, both the Spur Gear and Worm Drive designs move the stage by interacting with the existing control knob. However, this indirect method means they may need a lot of calibration and testing to match the accuracy of the Linear Rail design. So, the straightforward connection between the Linear Rail design and the stage makes it perform better, providing more precise performance compared to the other designs.

The Spur Gear design has achieved the highest rating in the compatibility category due to the simplicity of its design and its relatively low interference with both the stage and the space beneath it. Spur gears are straightforward to work with and can be easily mounted on the cylindrical control knobs of the microscope. The Worm Drive design received a lower score because of the challenges associated with combining spur gears and worm gears. In contrast, the Linear Rail design scored the lowest due to potential compatibility issues with the stage and other microscope components. This is because the Linear Rail design bypasses the manual control knobs, moving the stage directly and potentially restricting its range of motion. While the Spur Gear design excels in compatibility, the Worm Drive design faces complexities, and the Linear Rail design raises concerns about potential interference.

For the cost category, both the Spur Gear and Worm Drive designs have received the highest score. Compared to the Linear Rail design, these two designs are much simpler and require fewer materials to fabricate. Furthermore, all the parts to fabricate are relatively simple and come from methods such as 3D printing and laser cutting, which are relatively inexpensive. The Linear Rail design has a lower score, primarily due to the higher expenses associated with its fabrication and materials. Due to the complex nature of the Linear Rail design, some components will need to be purchased instead of fabricated, which significantly raises the cost.

Considering the simplicity of the two designs, the Spur Gear and Worm Drive both score the highest in the ease of fabrication category. These two designs have fewer moving parts compared to the Linear Rail design, which is one of the reasons they scored higher in this category. Fewer steps are needed to fabricate the device for these two designs. Moreover, both the Worm Drive and Spur Gear designs have been fabricated by previous groups. This means that information on how to fabricate them can be gained by looking through previous groups' works and notebooks. Furthermore, it is certain that the Spur Gear and Worm Drive designs can be successfully fabricated because of the work done by previous groups. The Linear Rail design is entirely new, and it is uncertain what fabrication methods are needed in order to complete a physical product. There are no notes or experiences from previous groups that can be used as reference, making it a lot harder to fabricate compared to the Spur Gear and Worm Drive design.

The Linear Rail design is the top choice for longevity in the design matrix of the low-cost motorized microscope stage because it directly connects to the stage, reducing wear and tear on components. This direct link enhances the system's durability and lifespan by minimizing the chances of things breaking or wearing out over time, making it a durable and reliable solution for prolonged use. The Linear Rail design is a more robust and long-lasting option, ensuring the motorized microscope stage's overall reliability. On the other hand, the Spur Gear and Worm

Gear designs rely on indirect methods, interacting with the existing control knobs. This indirect approach introduces more points where things could wear down and require careful tuning to stay accurate, impacting their longevity. While the Spur Gear design is simple, it may face challenges in the long run due to its reliance on gears. The Worm Gear design, with its combination of spur gears and worm gears, is more complex, raising concerns about its ability to endure continuous use. The intricate nature of this design, along with potential wear in the gear assemblies, makes us question its long-term durability.

For safety considerations, the Linear Rail design was the most secure option. All three designs are reasonably safe, but safety does have to be considered because the device is powered by the lab's wall outlets. The Spur Gear and Worm Drive designs scored slightly lower on safety due to the positioning of the device. These two devices are attached to the manual control knob, where the person will have to come in close contact to operate the microscope. This orientation of these two designs increases the possibility of an injury, such as pinching, to the operator of the microscope.

V. <u>Fabrication/Development Process</u>

Materials

Overview: All materials necessary for the fabrication of this device have been documented and input into the BPAG Expense Table (Appendix B). For more information on part numbers, manufacturers, costs, and more, please refer to the materials and cost table (Appendix B).

Guide Rail Mounting Plate:

- 12" x 16" Cutting Board
    - Midnight Granite finish
- Epoxy
- Velcro
- Wood Screws

3D Printed Components:

- Black PLA
- Socket cap screws
- Heat-set inserts
- Set screws

Methods

Guide Rail Mounting Plate:

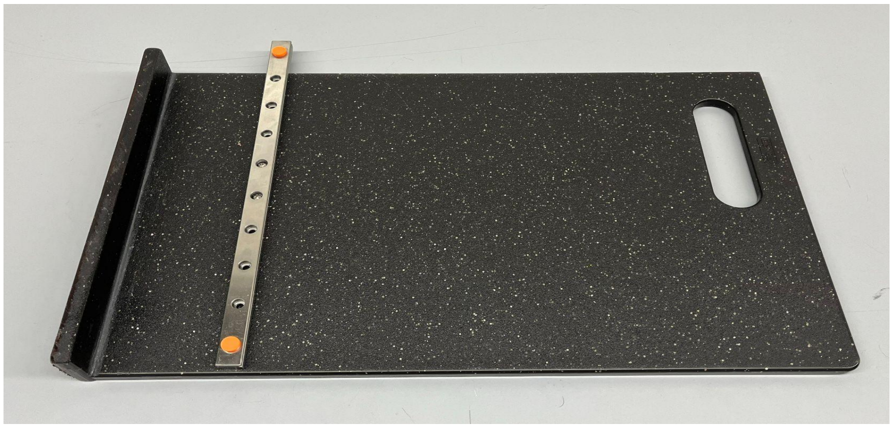


Figure 10: The GMP following the completion of fabrication.

The GMP is fabricated primarily from the aforementioned 12” x 16” cutting board. To begin, 2 inches will be cut off of the long edge of the board. This will leave a 12” x 2” piece and a 12” x 14” piece. After each cut in this process the edges will have to be cleaned up with a deburring tool and either a heat gun or blow torch to ensure mounting steps later in this process achieve a flush fit. After the cut has been cleaned up, the next step is to mount the smaller piece to the cut end of the board to create a lip that sits 2 inches off the ground. First, arrange the pieces as they will be mounted, and pre-drill and countersink holes for the wood screws to mount into. The drill bit used for pre-drilling should be smaller than the thread size of the wood screw that will be mounting the lip to the board. Prior to mounting the lip, mix and apply epoxy to all surfaces that will be mating. After this is all accomplished, the wood screws can be driven in. The extra epoxy can be cleaned up with a razor blade or by simply wiping it off with a glove on. This preliminary GMP now needs to be set for a day to allow the epoxy to cure. The GMP now needs to be correctly dimensioned to fit flush up to the microscope for mounting. Using TEAM Lab band and table saws, 0.5 inches must be taken off the top of the 2 inch lip, and the overall width of the plate must be reduced to 9 inches. Velcro is then applied to the mounting surface of the lip, which allows the GMP to secure itself to the microscope and resist movements during use. The final fabrication step involves the mounting of the linear rail to the plate. After using a marking pen to line up the microscope knob with the GMP, two wood screws are used to mount the linear rail directly under the path the knobs travel.

3D Printed Components:

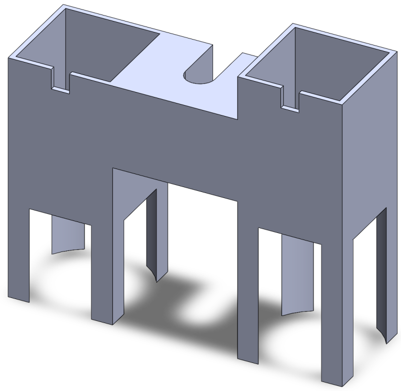


Figure 11: The dual stepper mount.

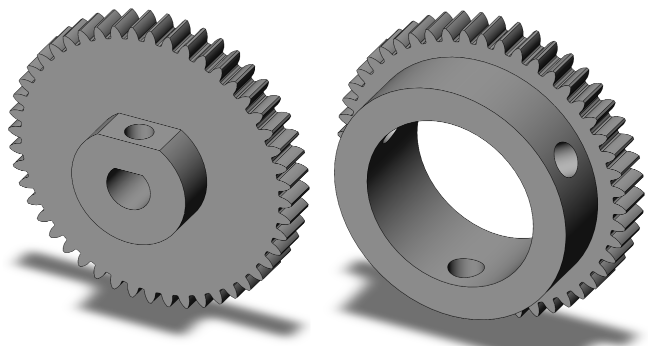


Figure 12: One of the knob gears (left) and one of the stepper gears (right).

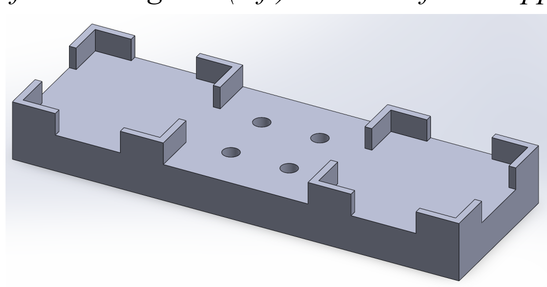


Figure 13: The sled mount.

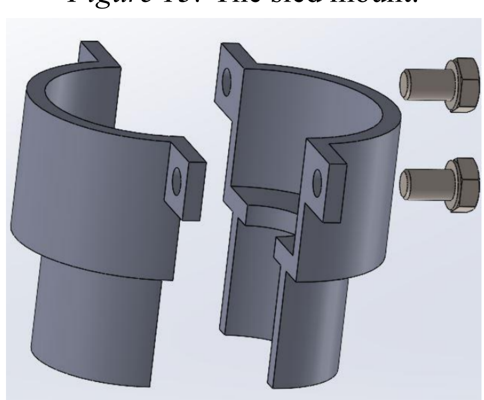


Figure 14: The stiffening cuff.

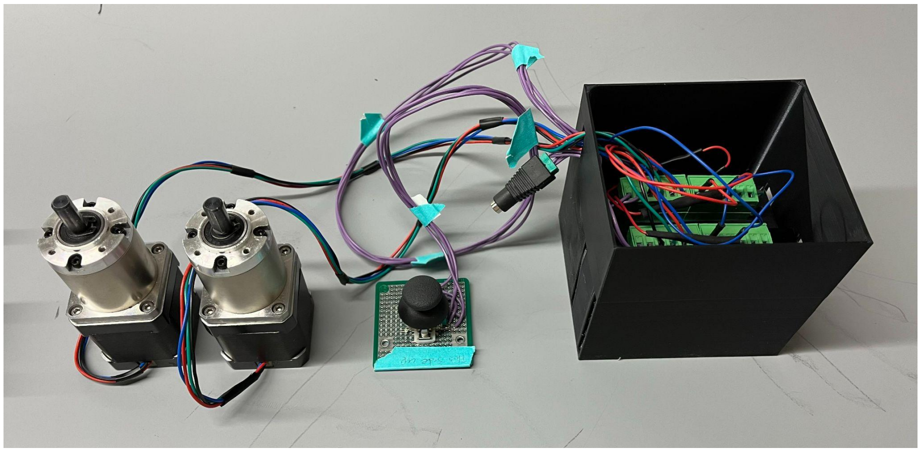


Figure 15: The Electronics Housing Unit and accompanying hardware.

The final, assembled design incorporates the GMP, as well as eight separate 3D printed parts. These parts are: the dual stepper mount (Figure 11), the two stepper gears (Figure 12, left), the two knob gears (Figure 12, right), the sled mount (Figure 13), the stiffening cuff (Figure 14), and the Electronics Housing Unit (Figure 15). With the exception of the stiffening cuff, which was made by a previous team, all of these parts were conceived of, designed, and fabricated by our team. Every part incorporated in the final design was printed on the Bambu Labs X-1 Carbon 3D printers in the UW-Madison MakerSpace using black PLA with 20% infill and supports on. Some parts required additional post-print modifications. CAD files and more detailed fabrication steps regarding each of these parts can be found under Fabrication Protocols and Computer Aided Design (CAD) Files (Appendix C, D).

Final Assembly

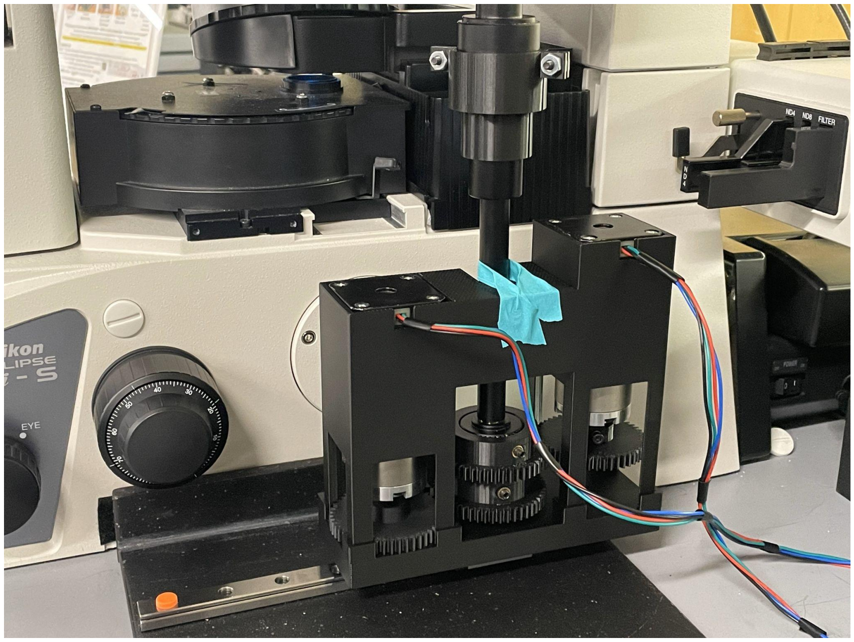


Figure 16: The final design of the Fall 2023 Low Cost Motorized Microscope Stage Project

The culmination of this semester's efforts led to the development of the final prototype (Figure 16). Similar to previous designs (*Design History*), this design manipulates the preexisting manual control knobs that are used to drive stage movements. This prototype utilizes a single linear rail to maintain a stable, consistent connection between the stepper gears and the control knob gears by ensuring that the device's motion is directly correlated with the movement of the control knob shaft. The linear rail system is mounted on the GMP (Guide Rail Mounting Plate), which supports a ball bearing carriage that rides on the rail. This carriage did not meet the mounting requirements of the entirety of the device as there was simply not enough room to mount all of the equipment onto the carriage that was only approximately 50mm long. To overcome this issue, an additional mounting plate that is referred to as the "sled mount" was created with a purpose of increasing the mountable surface area. The sled mount was mounted onto the top of the original carriage of the linear rail system. This allowed for larger 3D models to be created without needing to worry about mounting it to the smaller carriage. The sled mount acted as a support for the stepper motor housing unit that is referred to as the dual stepper mount. This dual mount housed each of the stepper motors in an inverted orientation, meaning that the drive shafts of the stepper motors would be pointed towards the GMP. They were positioned in this way to allow the stepper gears to be spatially arranged in a way such that they are capable of meshing with the gears fixed to the microscope knobs. The dual mount also acted to stabilize the manual control knob shaft in order to reduce inaccuracies imposed by unwanted deflections of the knob shaft.

This device is currently usable through two different modes of user input. The first of these two options revolves around the joystick that has been soldered into the electrical circuit.

After uploading the correct code (Appendix E) from the Arduino IDE to the Arduino board, the user can drive the stage by simply moving the knob in the direction they would like the stage to go. While the mechanisms enabling this movement still need some fine tuning, this panning feature is fully functional in nature. Coordinate or Step count based movements, the other mode of interaction with this device, are not quite as functional. The current design is fully capable of moving the stage a set number of steps, however, improvements in the driving mechanisms of this design will need to be made before this changes to a system where the user is able to input coordinates and have the stage center that point under the field of view. Loss of motion and shaft deflection related inaccuracies continue to be the center of motivation for future work, and the following testing protocols were created in order to assess where, specifically, improvements need to be made.

Testing

The testing phase of this project aimed to assess the performance and capabilities of the automated microscope positioning system. The primary components used for testing included a slide with fluorescent beads viewed using the Nikon Ti-U inverted fluorescence microscope, a universal joint connector, and the motorized positioning device. For direct connection testing, the universal connector was securely fastened to the X-axis manual control knob and the stepper motor, as seen below in Figure 17. For linear motion and loss of motion testing, the motorized device was secured to the microscope with the dual stepper motor mount placed on the carriage and lined up with the gears, as seen above in Figure 16.

Figure 17: Direct connection testing set up. The universal joint connector is securely fastened to the x-axis manual control knob and the selected stepper motor.

The team performed three distinct tests: Direct connection, loss of motion, and linear motion. The direct connection test was aimed at recording and quantifying the capabilities of the motors and the manual knobs. This test sought to determine the optimal gear ratio for the motorized positioning device to achieve the movement specifications outlined in the *Physical and Operational Characteristics: Accuracy and Reliability* section of the PDS (Appendix A). The loss of motion test was necessary to ascertain the quantity of movement lost when the device changes direction. Establishing a consistent loss of motion buffer will aid in a future calculation to convert an inputted distance to an associated number of required motor steps. Finally, linear motion testing aims to measure a consistent stage displacement per motor step while mitigating loss of motion error. The results of linear motion testing will also contribute to establishing a calculation to convert inputted stage displacement into the required amount of motor steps.

For each test, a fluorescent bead slide was placed on the stage, ensuring the slipcover face was positioned downward on the stage. The microscope was turned on and set to the predetermined settings for the distinct test. For all testing, the motors moved the device along one axis and direction until no loss of motion was ensured, and a starting image was captured. During direct connection and linear motion testing, the motors were automated to move between 150-400 steps along the same axis and direction. However, for loss of motion testing, the motor was automated to move between 400-600 steps along the same axis but in the opposite direction. A final image was captured, and this process was repeated until ten data points were collected. The linear motion and loss of motion procedures were repeated along the same axis but with a different number of steps and along the other axis with two different amounts of steps. For full written out steps refer to Testing Protocols (Appendix F).

The images were saved and sorted into different folders on a thumb drive. The photos were then uploaded to folders on personal devices. The images were then put through Fiji: ImageJ to overlay the initial and final photos for data collection. The data was then stored and combined into a large spreadsheet (Appendix G).

A possible source of error while testing could stem from the gears not meshing properly. The gear attached to the Y-axis stepper motor did not always have a tight fit against the gear attached to the associated manual control knob. The poor gear meshing may result in issues with gear catching or consistent and accurate movement. Additionally, the error may be due to the lack of a locking mechanism to hold the control knob shaft to the positioning device, which allowed for the knob to slide and move. To mitigate sliding, the team placed tape with a folded-over center around the shaft to hold the manual knobs in proper placement with minimal rotational friction, as pictured above in Figure 16.

VI. **<u>Results</u>**

The images that were collected in the testing phase were analyzed using Fiji ImageJ [9] software. The start and end images from a given trial were inputted into the Fiji software and overlaid with the start image being red and the end image being green, as seen in Figure 18. The

Fiji software was then used to measure the distance between the two images by drawing a line between a distinguishable point on both the start and end images. The software was used to calculate the number of pixels between the two chosen points and convert that number to a measurement in microns based on the microns per pixel scalar value included in the image files. The measurements were then compiled into an Excel spreadsheet for further analysis. The full spreadsheet of measurements, along with all of the images taken during testing and the ImageJ overlays, can be found under Raw Data (Appendix G).

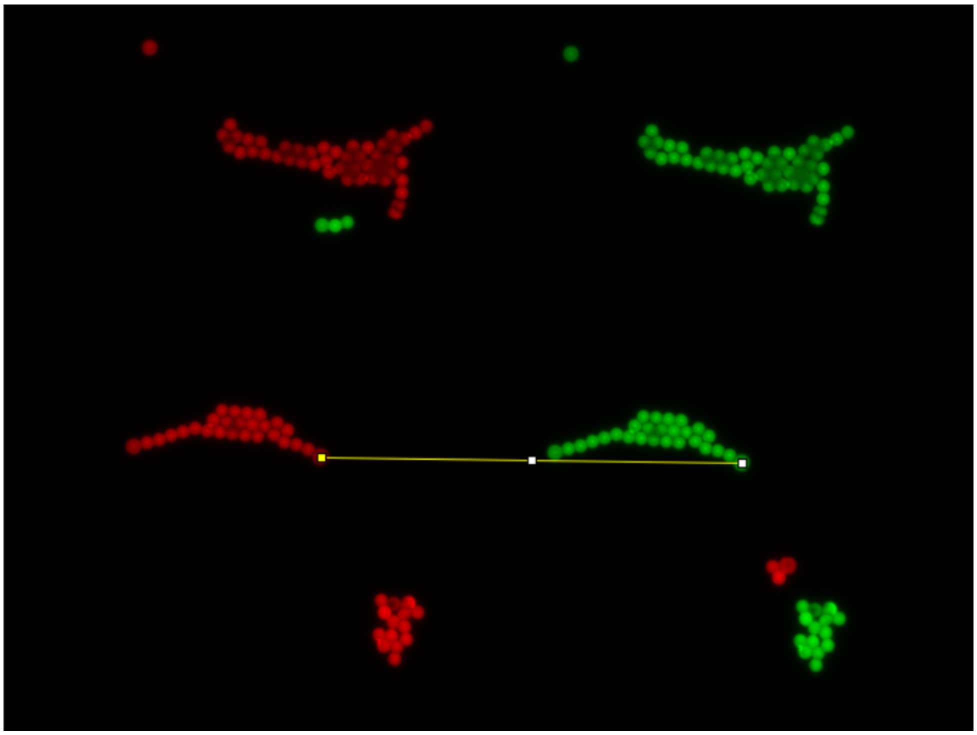


Figure 18: The start (red) and end (green) images from trial 8 of the 200 step X linear testing experiment. The yellow line is part of the Fiji ImageJ software and was used to measure the distance (in microns) between the start and end images.

The first calculation that needed to be made was the calculated microns per step (MPS), which is simply the distance (in microns) that the microscope stage moves for each step of the motor. This is an important value to calculate because it quantifies the smallest possible movement that can be made and is needed in other calculations as well. The testing procedure for the linear testing can be found in the testing section of *section V*. To find the MPS, the measured travel distance was divided by the number of steps that the motor had been told to move, as seen in Equation 1. In Equation 1, MPS is calculated microns per step, MT is measured travel of the stage (in microns), and S is the number of steps moved by the motor.

$$MPS = \frac{MT}{S} \tag{1}$$

The MPS calculation was used on the data from both the direct connection testing and linear testing. The final goal was to get a calculated average value of 1 MPS, which would mean that the stage could be consistently moved in single micron increments, which was our original goal outlined in the *Accuracy and Reliability* section of the PDS (Appendix A**)**. Graphs of the data for the direct connection and linear testing results can be found below in Figure 19 and Figure 20, respectively. The average MPS that was calculated for the direct connection was 1.41, which was very close to the original goal of 1 MPS. For the linear testing of the X and Y axes, the calculated MPS was .95 and 1.59, respectively. The standard deviations for the direct connection test, X axis linear test, and Y axis linear test were 0.146, 0.015, and 0.042, respectively, showing that the X axis was the most consistent.

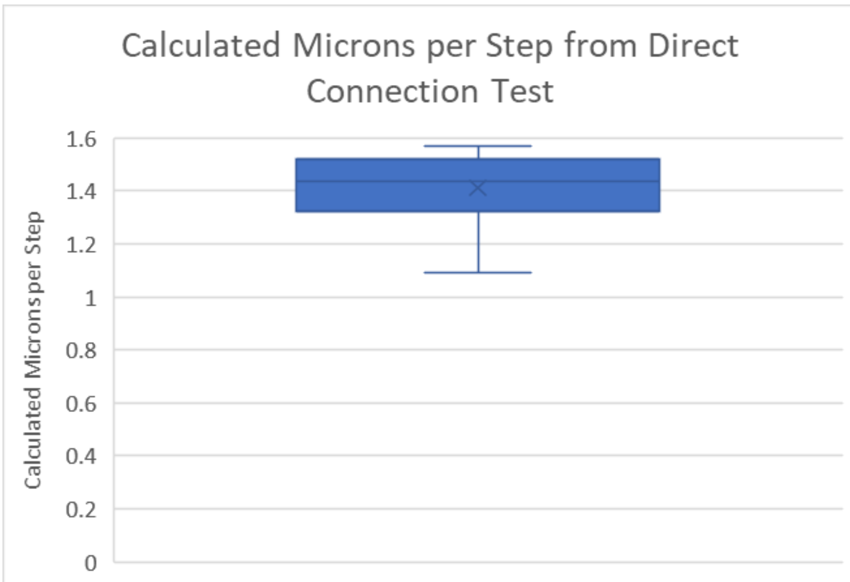


Figure 19: Box and whiskers plot representing the calculated microns moved per step of the motor, where the motor was moved in 500 step increments.

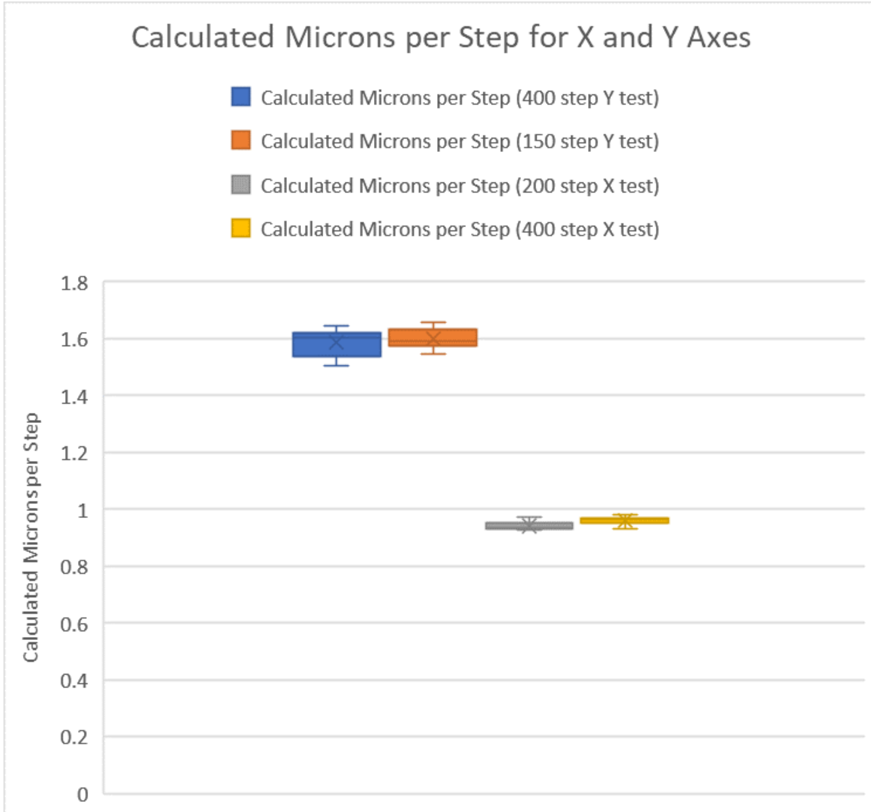


Figure 20: Box and whiskers plot representing the calculated microns moved per step of the motor using multiple different increments of steps.

Another important data set that was analyzed was the loss of motion when the direction of travel was changed. As mentioned in the design research section of *section II*, having space between the teeth of the gears causes backlash, which then causes the stage to not move when the motors first start turning. This becomes a serious problem when a user is trying to input specific values of travel into the arduino, but the stage doesn't move as far as it is meant to. If the value of this loss of motion were consistent, however, the extra amount of motor movement needed to overcome the loss of motion during a direction change could be added directly into the Arduino code. To find the calculated loss of motion, the number of steps moved by the motor is multiplied by the calculated MPS for the respective axis, which gives the theoretical travel of the stage. The measured travel of the stage is then subtracted from the theoretical travel to get the travel distance that was lost (in microns). This process can be seen in Equation 2, where LoM is the calculated loss of motion, S is the number of steps traveled by the motor, MPS is the calculated microns per step for the respective axis that is being tested, and MT is the measured travel of the stage. The measurements for the loss of motion testing were found in the same way as the measurements for the previous tests.

$$LoM = (S * MPS) - MT \tag{2}$$

The final results that were found for the loss of motion testing can be found below in Figure 21. The data is displayed as the number of microns that were lost compared to the theoretical travel. When translated to the number of steps that the motors would have to move to compensate for this loss, the X motor would have to move an extra 48.5 steps on average (with a standard deviation of 3.48 steps) and the Y motor would have to move between 290 and 340 steps on average (with standard deviations of 54.3 and 75.3 depending on the number of steps used in the test). The large discrepancy between the X and Y axes here will be discussed in the sources of error section of *section VII*.

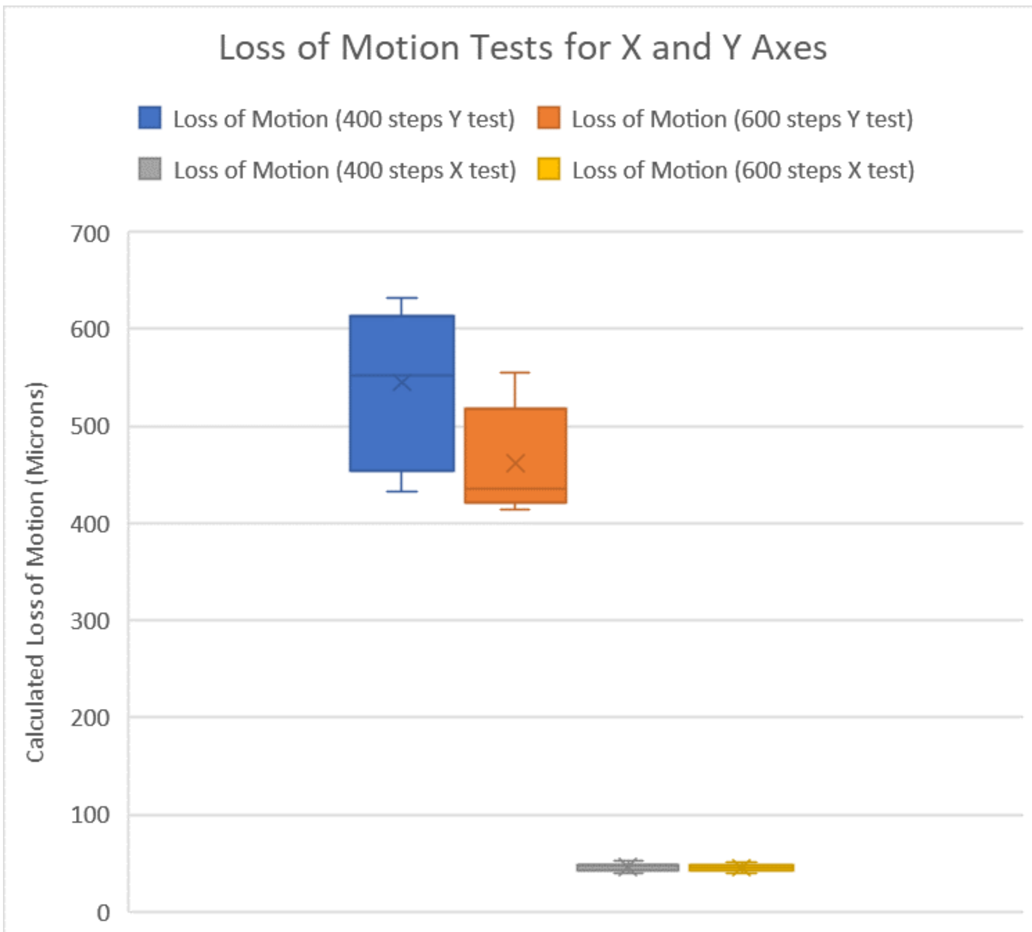


Figure 21: Box and whiskers plot representing the calculated loss of motion (in microns) for the X and Y axes using 400 and 600 step increments for each.

VII. **Discussion**

Implications of Results

After all of the testing and calculations performed by the team, several conclusions were able to be made. The first, which greatly affected the final fabrication process, was that near-micron resolution was possible by using the direct connection device. This proved that a 1:1 gear ratio would be able to be used in the final design between each of the motors and the manual control knobs. When looking at the linear testing of the final design, It is clear that the X axis was able to very consistently meet the team's goal of 1 MPS, and while the Y axis was slightly further away from this, it was still more consistent than the direct connection device. Consistency, in this case, is more important than having a precise value of 1 MPS. This is because the measured MPS value can be factored into the Arduino movement code so that the user can input a travel distance in microns (instead of the number of steps), and the motors will accurately and reliably move that distance.

A similar conclusion was also able to be made from the loss of motion calculation. For the loss of motion experiment, the X movement was again very consistent, while the Y movement had a lot more variation. The loss of motion value can again be factored into the Arduino movement code as long as it is consistent. This would have to be implemented in a way that the motors would only move the extra distance when they are switching directions, because when they are going in one continuous direction, there isn't any loss of motion. The Y axis results are most likely too spread out to implement this software solution at this point, but after some more fine-tuning of the dimensions, the Y gears should mesh better and have a more consistent loss of motion calculation that can be implemented into the Arduino code.

Ethical Considerations

When developing a device that functions in a regulated environment, ethical considerations must be addressed to ensure the safety and inclusion of all. Ethics surrounding research while designing a new device with possible hazards must take sufficient information and understanding, well-being, and safety into consideration. The research should consist of excellent quantity and quality to provide a complete understanding of appropriate design parameters to aid in choosing the correct design path. Additionally, honesty and providing proper credit is a substantial focus of research ethics.

Finally, inclusivity is a noteworthy ethical consideration during the design process because it allows the device to be available to all people of different backgrounds. Thus, the motorized positioning device considered economics, usability, and adaptability throughout prototype development. The chosen design allows for restricted and lower-budget projects to gain access to the necessary technology. The devices' usability makes for an easy transition between motorized and manual microscope use. Easy usability also allows for a simple learning curve of how to operate the device, which can help learners understand the functions of the microscope faster. Finally, the adaptability of the motorized device allows for control placement adjustment to aid in accommodating those with lower or limited mobility to participate in using the microscope.

Sources of Error

Several sources of error must be scrutinized to identify and potentially resolve inconsistencies, limitations, and inaccuracies. Fabrication tolerances, such as those acquired during 3D printing, must be considered. Tolerances are especially prominent in accuracy-dependent areas of design, such as the gears, because the error may compound. Incorporating tolerances of fabrication methods into the design allows for error mitigation in aspects of the device, such as gear meshing.

A second source of error is poor gear meshing. Occasionally, the device would function inconsistently with previous movements. While improvement may always be achieved, gear meshing with the X-axis gears was smooth with little inconsistencies. However, the Y-axis gears had trouble interlocking as the gears were not as close together as they should have been. Poor gear engagement caused significant inconsistencies when measuring the movement per motor step and loss of motion.

Additionally, a third source of error, which results in testing variability, could stem from leeway in the motor and control knob. During testing, there were some issues with the control knob deflecting away from the gears. While the accuracy of the motors and control knobs is of substantial quality, no design is ever one hundred percent perfect. Considering errors in the pre-built aspects of the design allows for realistic expectations that aid in improving the design to reduce the resulting errors.

Finally, while it is not a source of error, it is essential to consider flaws in small-scale positioning. Small-scale positioning may have more error in accuracy compared to large-scale movement positioning. This error can be from many aspects, such as loss of motion, gear meshing, and tolerances. Due to the stages' small scale of movement, inconsistencies are highly showcased, while grander movements allow for less error. For example, with loss of motion, should the motors move an average buffer, the loss of motion will not be exact every time. Small distances may cause no movement or a relatively sizable overshoot of movement. With larger-scale movements, even when the loss of motion buffer is inaccurate, the sizable movement allows for the loss or gain of only a tiny distance.

Future Work

This semester’s final prototype was accomplished through organized brainstorming, structured evaluation of ideas, and collaborative fabrication efforts. The culmination of these factors led to not only a working prototype, but an established design for future students and groups to build off of. With this in mind, there are specific aspects of this design, as well as unmet design criteria, that need to be addressed in future iterations to create a more functional, lab-ready prototype for the client. To narrow the subject, aside from labeling (Table 2), the improvements needed can be broken down into categories of fine tuning precision and accuracy and integration of automating software.

Table 2: PDS-outlined safety labels needed to comply with ISO and IEC standards (Appendix A).

	Signifies that the main plug must be disconnected from the wall outlet prior to maintenance [10].
	Indicates that the equipment labeled is suitable for the use of alternating current only [11].

While the final created design was functional in nature, there were some limitations of its capabilities that were imparted due to loss-of-motion related issues. To improve the functionality of this device, several improvements have been proposed for future groups to address. First, the heights of the stepper mounts that are integrated into the dual stepper mount need to be very slightly adjusted to make the gears align perfectly vertically. This does not currently affect their ability to mesh, but the team believes that it will improve the design nonetheless. The gears also need to be addressed. As mentioned in Fabrication Protocol (Appendix C), all of the set screws utilize an insert to allow the mounting of the gears through set screws. In the current design, the inserts have proven to be very prone to becoming dislodged or popping out, leading to a loose fit and subsequent loss of motion during operation. The inserts used (Figure 22, left) were the inserts available to the team at the MakerSpace, however, the team believes that a heat-set insert more suited for this application (Figure 22, right) may be a more solid fit and resist popping out of place.

Figure 22*:* Knurled threaded inserts used by our team (left) [12] and proposed alternative heat-set insert option for future teams (right) [13].

Regarding automation, the team has one main goal in mind. Successful integration of software to produce image stitching and sequencing capabilities. As proposed by the client, Dr. John Puccinelli, this would include the use of various free-to-download plugins, such as the Fiji ImageJ Image Stitching Plugin [14]. Ideally, programming done would make these plugins compatible with both the existing Nikon software [15] and the prototype created to motorize the stage. All new steps taken in fabrication will require rigorous testing to prove the levels of competency of the design. Thus, with the successful integration of these plugins, future teams will look to create methods for testing this design through independent brainstorming or by collaborating with BME faculty.

VIII. **Conclusions**

The efforts of this semester's Motorized Microscope BME design team sought to build off of that of past teams in an attempt to accomplish all client criteria. Unfortunately, as seen in multiple previous teams, the majority of the previous work on this project was scrapped in an attempt to explore new design avenues. Through collaboration with faculty of the UW-Madison Departments of Biomedical and Mechanical engineering and strict discipline to the principles of engineering design, this semester's team was to successfully create a proof of concept level functional prototype. This design utilized two stepper motors and multiple mountable gears to drive microscope stage movements in both the X and Y directions. These movements are commandable by both joystick and IDE input from the user. While currently effective as a stage panning tool, the design still needs to be iterated upon and improved in order to reach levels of precision needed to create a coordinate-based movement system. As revealed by statistical findings and qualitative observations of all prototypes, this new working prototype is the most competent solution to the client proposal to date. This is not to say that the job is done, but to emphasize the point that progress truly has been made, and future groups should look to improve and expand upon the current prototype rather than looking to starting from the beginning of the design process in hopes of creating a more optimal stage driving design.

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X. **Appendix**

Appendix A: Product Design Specifications

Product Design Specifications: Microscope Low Cost Motorized Stage

September 22, 2023

BME 300/200

Clients: Dr. John Puccinelli

Advisor: Dr. Joshua Brockman

Team Members:

Team Leader: Tyler Haupert

Communicator: Nicholas Symons

BWIG: Sawyer Bussey

BPAG: Jerry Zhaoyun

BSAC: Julia Salita

Function:

The inverted fluorescence microscopes of the UW-Madison BME Teaching Lab are manually controlled microscopes that allow users to observe and collect data on cell and tissue culture. While the technology exists to convert these microscopes into motorized and automated devices, it is very expensive and beyond the budget of the school. The client, Dr. John Puccinelli, has requested the fabrication of a device to serve as a solution to this problem. The final working prototype should include mechanical, software, and hardware components to allow for the automation of manual inverted fluorescence microscopes. The physical structures of this device will interface with the manual adjustment knob on the right-hand side of the microscope. It will be capable of moving the stage in a coordinate-based fashion in both the X and Y directions. Software elements will be created to direct the movement of the stage. Finally, the client has also requested the development of software to process and stitch input images to add a scanning feature to the microscopes.

Client Requirements:

1. The device must be a motorized mechanism that controls the stage movement through the manual stage adjustment knobs.
2. The stage should be movable by using a joystick, computer keys, or by inputting values into the user interface.
3. The movements of the stage should be precise down to the micron range, with acceptable deviations within one order of magnitude.
4. The software created must be integrated into the existing NIS-elements software and assist in both taking images of the field of view and stitching them together.
5. The device will be powered by the wall outlets in the lab.
6. The project must remain under a final cost of 100 US dollars.

Design Requirements:

1. Physical and Operational Characteristics:

a. *Performance Requirements:*

i. The device should be able to adjust the microscope stage in both the x-axis and the y-axis using the manual adjustment knobs

ii. The software must be able to participate in image sequencing and be easy to use

iii. The field of view should be movable by using an interfaced joystick or computer keys such as the keypad arrows

b. *Safety*

i. The device should not be hazardous.

ii. The device should not harm the user in any way, including electrical shock [1], physical pinching, or loud sound levels.

iii. The device should not damage the microscope while ensuring smooth operation.

iv. The cords of the device should be safely arranged so that it does not block off the user's workspace. Moreover, the cords should not be placed near or be in contact with any liquid.

v. Labeling

	Signifies that the main plug must be disconnected from the wall outlet prior to maintenance [2].
	Indicates that the equipment labeled is suitable for the use of alternating current only [3].
	Specifies that the operator's manual should be referenced and used during use of device [4].

c. *Accuracy and Reliability*

i. The device should have a movement resolution of 1µm.

ii. The movements should be repeatable without having to recalibrate the device.

d. *Life in Service*

i. The device should be reliable and last at least 10 to 15 years [5].

ii. The stepper motors have a lifetime of 20,000 hours[6], which translates to 2,500 8 hour work days or 500 5 day work weeks. This matches our requirement of a 10-15 year lifetime.

iii. The device should not break easily and withstand daily wear and tear.

iv. The device should be capable of undergoing regular sanitation via autoclaving and/or harsh cleaning chemicals.

e. *Shelf Life*

i. To ensure the longevity of the device while stored, the device should be kept dry and at regular room temperature and pressure.

ii. All materials used for the device should be stable at standard lab temperature and humidity conditions.

iii. The device should be able to be stored either attached or detached from the microscope at normal room conditions.

f. *Operating Environment*
   i. The laboratory will be kept within a temperature range of 20-25 degrees celsius with a relative humidity of 35-50 percent [7].
   ii. The device will be kept in the BME teaching lab and should be able to operate in the above conditions.

g. *Ergonomics*
   i. The device must be small and not disrupt the normal operation of the Nikon TI-U and Olympus IX71 microscopes.
   ii. The software must be operable with the nikon elements software and must have a user-friendly interface.

h. *Size*
   i. The device should take little to no table space next to the microscope.
   ii. Ideally, the entire device should be encased to minimize device interference and malfunction due to exposure.

i. *Weight*
   i. The device should be lightweight to minimize interference and damage to the function of the microscope.
   ii. Weight should not limit usage and accessibility of the microscopes for all users.

j. *Materials*
   i. All materials purchased, altered, and used must comply with the guidelines for a biosafety level 1 laboratory listed in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition [8] by the Centers for Disease Control and Prevention (CDC) [9].
   ii. 3D printing should be utilized to print most plastic prototypes using the FDM and FFF printing methods [10], [11].
   iii. The Universal ILS9.150D [12] laser cutter will be used to accurately cut precise pieces necessary for maintaining accuracy of movement of the microscope stage.
   iv. Soldering [13] may be used to stabilize the electronic connections within the necessary circuitry.
   v. All included materials must be resistant to the degradative effects of harsh chemicals used for regular sanitization of lab equipment.
   vi. Plastic gears should be used rather than metal gears to eliminate the need for lubricant, this would decrease the amount of maintenance needed in order to keep the device operational [14]. Additionally, plastic materials will also help to lower cost.

vii. Plastic materials expand and lose structure at increasing temperatures. Thus, material choice should consider the rate at which different plastic materials heat or cool.

viii. Materials used for gears should be made out of a plastic with a low thermal diffusivity, which is defined as the thermal conductivity ratio to the specific heat capacity of the material. Materials of large Thermal Diffusivity will respond quickly to changes in heat and Materials of low Thermal Diffusivity will respond slowly [15]. Thermal Diffusivity of PVC (7.8E-6 m^2/s) [16], this is a low Thermal Diffusivity meaning the material heats up slowly. The equation for this value is: $\boldsymbol{a}$=k/pCp

1. a= thermal diffusivity
2. k= material conductivity
3. p=density of material
4. Cp=specific heat of material

k. Aesthetics, Appearance, and Finish

i. The device should not be distracting to the user.
ii. The final product should be neat and blend in with surrounding equipment.
iii. All edges should be smooth and not pose a threat to users operating the device.

l. Spatial Configuration

i. The device should not hinder or block movement of the existing mechanical components of the microscope
ii. The device should be secure and stable on the points that it mounts to the microscope.
   1. Ex. screw holes, clamp points, adhesion points.

2. Product Characteristics

a. Quantity:

i. Only one device will be manufactured for the client
ii. The device should be replicable in order to produce additional products if necessary or to potentially be mass produced.

b. Target Product Cost:

i. The target budget is to remain under a total of $100 for the final cost of the device.
ii. The allocated budget for development is $300.
iii. The team should use previously purchased materials to keep costs low and reduce waste, however, the team should present the final total price to reflect the total cost if the device is to be replicated.

3. Miscellaneous

a. Standards and Specifications

i. All aspects of the device's design must comply with the many guidelines provided by the CDC for biosafety level 1 laboratories [8].

ii. The device should follow all guidance outlined in the FDA's "Chemical, Metals, Natural Toxins & Pesticides Guidance Documents & Regulations" to ensure safety and producibility should the device be reproduced [17].
iii. The following standards are to be referenced and used as guidelines throughout the development and implementation electrical systems designs [18].
    1. ISO 9001 Standard: Quality Management System.
    2. ISO 14001 Standard: Environmental Management System.
    3. ISO SOC Standard: System and Organisation Controls
    4. ISO 27001 Standard: Information Security Management.
    5. ISO 45001 Standard: Occupational Health and Safety Management System.
    6. ISO 10002 Standard: Complaint Management Systems
    7. ISO GDPR Standard: General Data Protection Regulation

b. *Customer*
i. While the team's client has a sole interest in the design, there is a potential for a more broad potential application of our design. If fabrication methods can be simplified and streamlined, it is likely that many universities and budget-aware labs would be keen to utilize our low-cost solution to the problem of motorizing and automating manual microscopes.

c. *Patient-Related Concerns*
i. The device should not inflict any danger to the surrounding users and equipment
ii. This device should be capable of undergoing regular maintenance and cleaning with harsh chemicals [8].
iii. No paper, cardboard, or other organic materials should be utilized in the final design.

d. *Competition*
i. The OpenFlexture project is an open-sourced, 3-D printable microscope that can be created and constructed for approximately $200, with multiple stages available to add motorized and automated functionality [19].
ii. One example of market-available motorized stages is Zaber's ASR series motorized XY microscope stages [20].
    1. While these are functional, accurate to within 12 µm, and available, they do not meet client criteria due to cost and the way they interact with the microscope.
iii. Detailed in a 2017 article, a group of German-based scientists created an automated, motorized, 3-D printed inverted fluorescence microscope. The article includes all necessary CAD and software files for construction, as well as a step-by-step instruction manual to aid users in building their device [21].

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Appendix B: Materials and Costs

Table 1: Total cost to re-fabricate a new mechanical positioning device.

Description	Manufacturer	Mft Pt#	Vendor	Vendor Cat #	Date	QTY	Cost Each	Total
12V 4A Power Supply for the motors	COOLM	P2-12V4A	Amazon/Makerspace	B07H493GHX	10/23/2023	1	$12.99	$12.99
3D-printed components of the device			Makerspace			347g of PLA	$0.08/gram	$27.76
MGN12H Mini Linear Rail Guide,200mm Linear Sliding Gide with Carriage Block			Amazon			1	$15.99	$15.99
two Nema 17 Stepper Motor Bipolar from previous group	Nema	17HS19-1684S-PG51	StepperOnline.com			2	$31.38	$62.76
Screws used to secure components in places and wires used for the Arduino			Makerspace			N/A	$7.00	$7.00
DEXAS 12 x 16 Poly Granite Cutting Board in Midnight Granite Used to fabricate the GMP	Dexas	452-50W	Walmart			1	$12.44	$12.44
Arduino used to program the stepper motors	Arduino		Makerspace			1	$10.00	$10.00
Usongshine TB6600 4A 9-42V Nema 17 Stepper Motor Driver CNC Controller Single Axes Phase Hybrid Stepper Motor for CNC/42 57 86 Stepper Motor（1 pcs)	Usongshine		Amazon			2	$9.98	$19.96
							TOTAL:	**$168.90**

Appendix C: Fabrication Protocols

3D Printed Components:

<u>Steps to 3D print components of the final design:</u>

1. Open part file in SolidWorks
2. Hit "Save As" and select the ".STL" file type option
3. Grab a flash drive from the bins by the MakerSpace 3D printing PCs
4. Load all .STL files you would like to print onto the flash drive
5. With assistance from MakerSpace staff, load all .STL files on to a Bambu Labs plate and prepare using the following settings
    a. Infill: 20%
    b. Resolution: Fine (0.8mm)
        i. Note: not all components need this resolution, but the gears absolutely do.
    c. Supports: Enabled
        i. Support Type: Standard
    d. Filament: Black PLA
6. Once all settings are selected, hit slice, export the .3mf file, and fill out the 3D printing form to have staff start the print
    a. Note: It is advised that as many parts are printed on the Bambu Labs printer at once as possible, due to the flat rate fee of $2 per print when using these printers. The Ulitimakers can be used to print without this flat fee, however, it is worth noting that they are much slower and have much lower resolution than the Bambu Labs printers.

<u>Steps to melt inserts into the stepper and knob gears:</u>

1. Remove the gears from the printer.
2. Using needle nose pliers, X-Acto knives, and tweezers, remove all supports from the 3D prints.
3. Select necessary insert for the application. Using current files, this would mean
    a. Stepper gears: M3 x 6mm x 5mm
    b. Knob Gears: M4 x 6mm x 6mm
4. Turn a soldering iron on.
5. Place the gear in a clamp with the whole you plan on melting the insert into facing up.
6. Using a rubber-coated tweezers, hold the insert up to the tip of the soldering iron and as it heats, gently apply downward pressure with the iron to slowly melt the insert into the hole.
    a. Note: if inserts are filling with plastic, try repeating this process with a screw already in the insert to stop melted plastic from entering the threads.

7. After the plastic has cooled, use the tweezers to clean up the excess on the inside of the print to ensure the knob/shaft will be able to fit flush into the inner hole of the gear.

Steps to mount sled mount to rail carriage:

1. Remove the sled mount from the printer.
2. Using needle nose pliers, X-Acto knives, and tweezers, remove all supports from the 3D prints.
3. Using the provided screws (M3 x 10mm), screw the sled mount into the threaded holes of the carriage.
4. If the print is completed using current files, the holes will be slightly too small for the supplied screws. In this case, the user will need to use a drill to make the holes slightly larger to allow the screw to pass through the sled mount and pin it to the carriage below.

Appendix D: Computer-Aided Design (CAD) Files

Located in this folder is a combination of part, assembly, and STL files created and organized for future groups. All future groups have full permission to download, modify, and reuse these files as they see fit.

Appendix E: Arduino Code

Located in this folder are the Arduino files that the team used for initial testing of the electronics and testing of the final design.

Appendix F: Testing Protocols

Direct Connection Testing Protocol:

1. Place a slide with fluorescent bead on the stage, with the coverslip facing down.
2. Turn on and set microscope settings to achieve the best view for moving approximately 500 µm, while overlapping a landmark with the initial position view. (Allow about a 550-600 µm field of view in the x direction)
3. Connect the universal joint connector to the x-axis manual knob (bottom knob) of the microscope, ensuring a secure fit.
4. Connect the universal joint connector to the stepper motor ensuring a secure fit.
5. Ideally, the universal joint should encounter no interference when operating, however, should the joint encounter interference set up in the least interference position as possible. If interference occurs, determine when interference occurs. Position the joint in a manner so that there is no interference for at least two movements of the device.
6. Automate the device to move in one direction until loss of motion is eliminated.
7. Capture and save a starting image.
8. Deploy the code to the electronics. The motor should then turn 500 steps.
9. Capture and save a final image.
10. Repeat steps 5-9 until ten or more trials are recorded.

Note you should end with at least 10 data points and at least 20 images before analysis

Loss of Motion Testing Protocol:

1. Place a slide with fluorescent bead on the stage, with the coverslip facing down.
2. Turn on and set microscope settings to achieve the best view for moving up to 600 µm, while overlapping a landmark with the initial position view. (Allow about 650 µm field of view along the desired axis).
3. Connect the motorized positioning device to the manual control knobs.
4. Automate the device to move in one direction along the X-axis until loss of motion is eliminated.
5. Capture and save a starting image.
6. Automate the motor to turn 400 steps in the opposite direction.
7. Capture and save a final image.
8. Repeat steps 4-7 until ten or more trials are recorded.
9. Repeat steps 4-8 for:
    a. Along the X-axis but turn 600 steps
    b. Along the Y-axis and turn 400 steps
    c. Along the Y-axis but turn 600 steps

Note you should end with at least 40 data points and at least 80 images before analysis

Loss of Motion Testing Protocol:

1. Place a slide with fluorescent bead on the stage, with the coverslip facing down.
2. Turn on and set microscope settings to achieve the best view for moving approximately 400 µm, while overlapping a landmark with the initial position view. (Allow about a 500 µm field of view along the desired axis)
3. Connect the motorized positioning device to the manual control knobs.
4. Automate the device to move in one direction along the X-axis until loss of motion is eliminated.
5. Capture and save a starting image.
6. Automate the motor to turn 200 steps in the same direction.
7. Capture and save a final image.
8. Repeat steps 4-7 until ten or more trials are recorded.
9. Repeat steps 4-8 for:
    a. Along the X-axis but turn 400 steps
    b. Along the Y-axis but turn 150 steps
    c. Along the Y-axis but turn 400 steps

Note you should end with at least 40 data points and at least 80 images before analysis

Appendix G: Raw Data

Located in this folder is all of our testing data, including the start and end images, the Fiji:ImageJ overlays, and the combined data spreadsheet.