Low-cost Motorized Microscope Stage

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ABSTRACT

The use of microscopic serial imaging and stitching is a common practice used in laboratory settings to conduct research and contribute to academic experience. Said processes can be time consuming and tedious when done manually. Moreover, motorized microscope stages allow for a streamlining of this process and more efficient use of materials, but they are very expensive to purchase commercially. Creating a low-cost motorized microscope stage or mechatronic system for stage attachment would allow for more experimental throughput and expand on the potential of microscopy. The proposed device is a mechatronic system that will attach to the translational control knob of two UW-Madison BME Shared Lab microscopes via set screws. The design consists of a custom gear-grip system driven by two gear-reduced stepper motors. These will be mounted to the existing stage, with a control architecture implemented on an Arduino Uno. The Arduino microcontroller will interface with Micro-Manager imaging software to provide a contrived imaging experience with the microscopes of interest.

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I. INTRODUCTION

1.1: Motivation

In the advancing world of today's biomedicines, efficient research methods and technologies are at the forefront of progression and innovation. Integral to advancing an understanding of medical topics, is an improvement in the availability and accessibility to new research methods and cost effective improvements to current technologies. Such improvements allow for more accessible research as well as the prospect of more extensive research topics in labs and quicker development.

Microscopy is an expansive field of study that encompasses different technologies to allow for the study of microscopic organisms and contributions to the study of cell biology. Microscope use throughout the research and development process is a key precursor to preclinical and subsequent clinical development of new drugs and developments that advance the therapeutic standard. The ability for scientists to seamlessly realize all functionalities of these technologies, and to become well versed in their components, features, and user capabilities is crucial to harnessing the imaging potentials of microscopy. Moreover, tailoring the usability of these optical technologies can contribute to more laboratory throughput and quicker study completion.

Beyond the manual use of such powerful imaging technologies is the world of automated imaging - the application of computer software and motorized design to allow for quicker imaging and analysis of microscopic samples of interest. An automated platform for microscopy allows for environmental control, rotating filter wheels and high-speed wavelength selection, autofocusing, and motorized stage control - processes that mitigate worker intervention and

allow for more calculated sampling [1]. With the automation of culture observation and meta-data collection, scientists can broaden the scope of experimental biology, learn more efficiently, and in essence, progress modern research and development beyond its current potential [2].

1.2: Existing Devices

There are many examples of commercially available motorized microscopes from brands such as Nikon, Olympus, Ziess, and Leica. These devices range from around $7000 to $25,000, making them far out of our client's budget [3]. Other assemblies have been made to avoid the purchase of one of these expensive units. A team led by Dr. Robert Cambell created a microscope with x, y, and z motorization for under $1000 and obtained positional repeatability to the order of one micron [4]. While this is still way above our budget, it will be a guiding source of how to integrate the motors with translation as well as with the software to automate the imaging process. Their final product included many features that are not needed for our set of specifications, so we can trim the cost while maintaining the features we need.

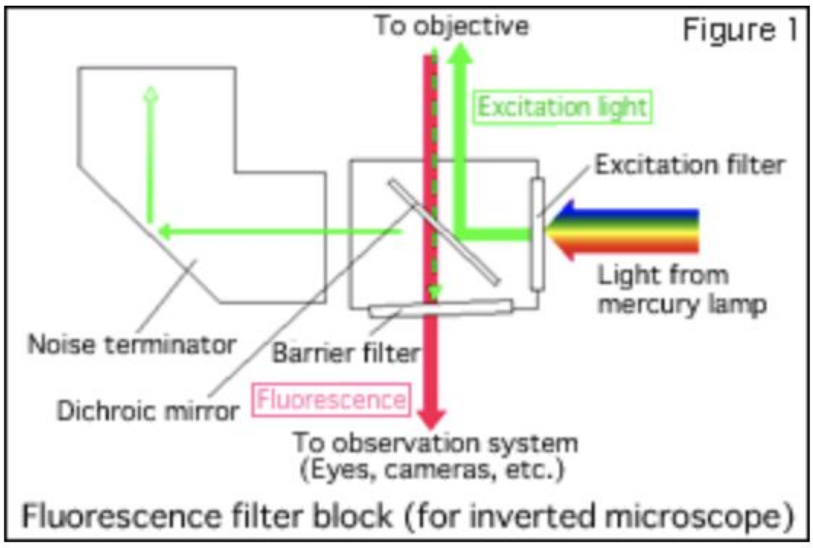
1.3: Problem Statement

The Biomedical Engineering Experimental Teaching Lab currently exhibits two inverted fluorescent microscopes that will be used for undergraduate student laboratory activities. These microscopes currently operate according to manual translational adjustment of their stages in order to visualize various positions across a specimen under study, which can be tedious and lacks uniformity. Thus, a cost-effective motorized system is desired to automatize translational adjustment of the stage throughout the xy plane in order to make analog control for users more intuitive and facilitate uniform serial imaging and image stitching processes.

II. BACKGROUND

2.1: Microscopy Methods

The microscopes in the Biomedical Experimental Teaching Lab are both inverted fluorescence microscopes made by Nikon and Olympus. Fluorescence is the ability of a specimen to absorb radiation and give off light of a shorter wavelength in the visible spectrum. [5] British scientist Stokes first observed fluorescence in the 1850s, and studies in the 19th century have shown certain specimens such as vitamins, minerals, crystals, and chlorophyll exhibit fluorescence when excited with ultraviolet light. Fluorochromes, or fluorophores are stains that can have highly specific targets or attachments, which are excited by only certain wavelengths and also emit known intensities of light. The development of new fluorophores is linked to the increased utilization of fluorescence microscopy. The aim of fluorescence microscope is to illuminate the specimen with a very intense light source (usually of a higher frequency) and then capture the emitted fluorescence which tends to be much lower intensity and a longer wavelength [6]. This filtering is done through the use of an optical or filter block. The light from the light source first passes through the excitation filter which selectively filters only the wavelengths necessary for excitation. The light then encounters a dichroic mirror positioned on a 45° angle to reflect light of longer wavelengths through the objective onto the specimen. Once the excitation light reaches the specimen, a combination of emitted light and excitation light reflect back toward the dichroic mirror which transmits longer wavelengths. The final separation of fluorescence from other light is done in the barrier filter which is positioned before the detector or eyepiece. [7]

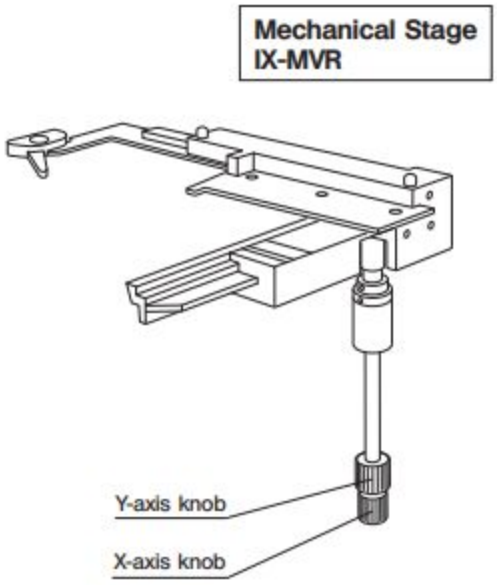


[Figure 1] Diagram of filter block for a fluorescence microscope. [7]

Figure 1 above outlines this process in a fluorescence microscope. The microscopes of interest are inverted fluorescence microscopes which are beneficial compared to upright microscopes because they allow for larger samples. In addition, samples typically sink to the bottom of solution, so an inverted microscope will look more directly at a specimen compared to an upright microscope. [8] Although the Biomedical Experimental Teaching Lab already has functioning microscopes, the team recognizes the importance of understanding the basic functionality of the microscopes with which our design will be designed to use. Ideally, the design will solely interact with the control knobs which position the stage in the xy plane, and it is important to ensure our design does not interfere with any of the existing functionality of the microscopes.

2.2: Existing System in Use

The Biomedical Experimental Teaching Lab contains two inverted fluorescence microscopes; Olympus IX71 and the Nikon TI-U. Both microscopes have a monochromatic camera and objectives ranging from 2x to 60x. [9] Each microscope is equipped with the associated software to control all of the imaging specifics. This software allows the microscopes to interface with a computer in order to control visualization and actuation of picture taking. Although not currently in use with the two relevant microscopes in the Experimental Teaching Lab, a third-party open-source software known as MicroManager is available. MicroManager is a flexible and programmable software that can be used to interface with microscope systems, such as the Nikon TI-U and Olympus IX71, as well as supplemental systems, such as the one to be designed. This software is designed for serial image acquisition tasks such as those the team hopes to accomplish with its motorized design [10].



[Figure 2] Diagram of stage control knob system fundamentally similar to that implemented in the existing manual microscope stage system [11].

The stages currently used with the two inverted fluorescence microscopes of interest are controlled with two translational control knobs as depicted in Figure 2. These two knobs are located at the bottom of a shaft protruding downward from the stage itself, located to the side of each microscope. The lower knob, which can be rotated independent of the upper knob, translates the stage in the x-direction, while the upper knob translates the stage in the y-direction. Control of these two knobs allows for movement of the stage to any coordinates in the xy plane, according to the translation limits of the stage.

2.3: Client Information

Our client, Dr. John Puccinelli, is head of the undergraduate Biomedical Engineering design curriculum at the University of Wisconsin-Madison. He is seeking a low-cost mechanism to motorize the stages of each of the inverted fluorescence microscopes in the Experimental Teaching Lab at the university, used for undergraduate learning.

2.4: Product Design Specifications

The ultimate goal of this project is to streamline the serial imaging process often used for scientific research through motorizing and programming the microscope. Our client specified that he wants a motorization mechanism that works for the two microscopes in the undergraduate laboratory, the Olympus IX71 Inverted Fluorescent Microscope and Nikon TI-U Inverted Fluorescent Microscope. For this mechanism, our client wants bidirectional control in the x-y plane and a 1 micron resolution of translation. Additionally, our client has asked that it be

compatible and programmable through either Micro-Manager Software or NIS-Elements BR software. The final product we fabricate should cost a maximum of $100 dollars.

In researching other motorized microscope stages, most have cost anywhere from $1000-10,000. This indicates that the team will need to get creative in ways to save money throughout the design process. This solution should be a long term one as the anticipated life in service for this device is at least 10-15 years. Testing of the product should ensure that the serial imaging process is indeed more efficient and does not harm the user or the microscope in any way.

III. PRELIMINARY DESIGNS

3.1: Choosing a Starting Point

To best address the client's design needs, the team will be reviewing various system design solutions. Following an initial client meeting, the group began brainstorming ways of fulfilling the design requirements. The group came up with three different designs to evaluate with a design matrix, considering multiple ways of altering the two existing microscope systems to differing degrees.

The three preliminary designs we converged on were a replacement stage with incorporated motorization, a removable gear cap model, and a gear-fastened translational knob model. Each of these preliminary designs incorporates a different way to satisfy our client's product design specifications. A detailed description of each design is listed below.

3.2: New Replaceable Stage

[Figure 3] Image of a non-factory, motorized stage. The New Replaceable Stage would reflect this structure and install in the place of the commercial part.

The primary design alternative, termed the New Replaceable Stage, features a device that, just as its name describes, would be fitted to the microscopes themselves, replacing the existing stages that currently operate with manual control. The attachable, motorized stage would be similar to that depicted in Figure 3. This design would be made specific to each of the targeted microscopes in the Experimental Teaching Lab, tailored to fit specifically to the stage attachment compartments of the Nikon TI-U and Olympus IX71, respectively. The attachment of this motorized stage will be fixed, while a translating surface will move with respect to the attached frame. Operationally, this stage will translate via orthogonal rack and pinion mechanisms seated within the housing of the stage as a whole. The rack portions will be fixed to the translating surface, to which specimens could be mounted, while motors fixed to the immobole frame portion will facilitate linear sliding of the surface by driving pinions along these racks.

Advantages:

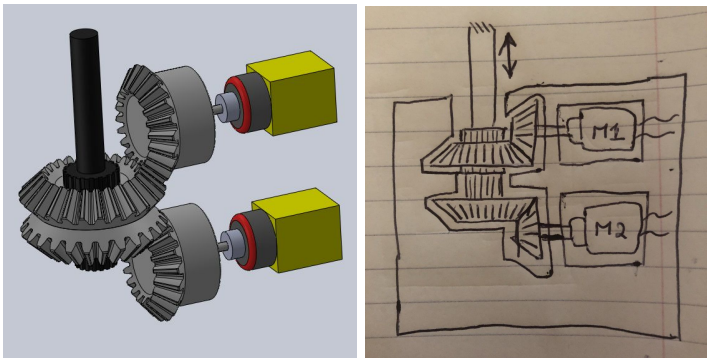
This design is the approach taken by existing motorized microscope stage products due to the fact that its approach provides a template for flexibility and customization of automatic stage mobility that other design approaches cannot. Namely, by directly creating a stage, rather than a controller that interfaces with an existing stage, designers cut out levels of control (i.e. system interfacing with control knobs and control knobs interfacing with the stage), which promotes high precision and lower error potential. Furthermore, implementing a stage that attaches to the microscopes in place of the existing manual stage has the potential to cut down the space occupied by the motorized system, leading to a sleeker implementation.

Disadvantages:

Though many existing motorized stages are designed in this fashion, they all come with a high price point, and this is for a reason. Designing and fabricating an entirely new stage with internal translating mechanisms that retains high precision and fine-tuned control requires precision-machined components and electronics that cannot compromise with a low budget. Due to the fact that one of the clients highest priority specifications is low cost, pursuing the designing of a replacement motorized stage that functions according to his operational specifications while maintaining a budget under $100 will be challenging. Additionally, this design alternative would be designed to attach to each of the two microscopes of interest in place of their existing stages. Although both microscopes are Inverted Fluorescence Microscopes, their dimensions and construction differ. Thus, this design would need to be custom tailored to each individual microscope, and to any other types of fluorescent microscopes to which this design

could apply. In this design project, and in many design projects, generating a design that is widely applicable is optimal, as opposed to generating multiple similar designs individually.

3.3: Removable Gear Cap



[Figures 4 & 5] SolidWorks drawing of the gear/motor interior of the removable cap. Hand drawing of the surrounding structure. The Cap will attach to the commercial translational knobs of the microscope as shown on the far right.

The second proposed design alternative, the Removable Gear Cap, is the first of two that operates according to a second general approach: interfacing with the existing control architecture of the current microscope stages. As each existing stage features two control knobs - one for translating along the x direction and one for the y - positioned at the end of a shaft protruding downward from the stages themselves, a mechanism analogous to that shown in Figure 4 can be used to provide motorized control to each knob in order to automate the movement of the existing stage in the x-y plane. Operationally, two gears are independently fixed to the two knobs, such that when each gear is driven, the mated knob will be turned, thus translating the stage along the respective linear axis. These gears will be bevel gears mated to two respective pinions driven by two separate motors. Each motor would thus be controlled separately to turn the pinion mounted to its shaft, driving the mated bevel gear, turning the mated

control knob, translating the stage. This specific design implements this mechanism such that it can be easily attached or removed from the manual control knobs via a "cap" system. More specifically, mating of each of the gears to their respective control knobs would be accomplished by custom cutting the profile of the notched knobs through the center of the gears, such that the gears can slide over the knobs, and grip them as the gears turn. Furthermore, the gears, pinions, and motors will be enclosed within a housing specifically designed to prevent movement of the gears axially along the control knobs, such that all components of the motorized mechanism remain fixed in position relative to one another (Figure 5). This full housing can thus be added or removed from the microscopes as necessary.

Advantages

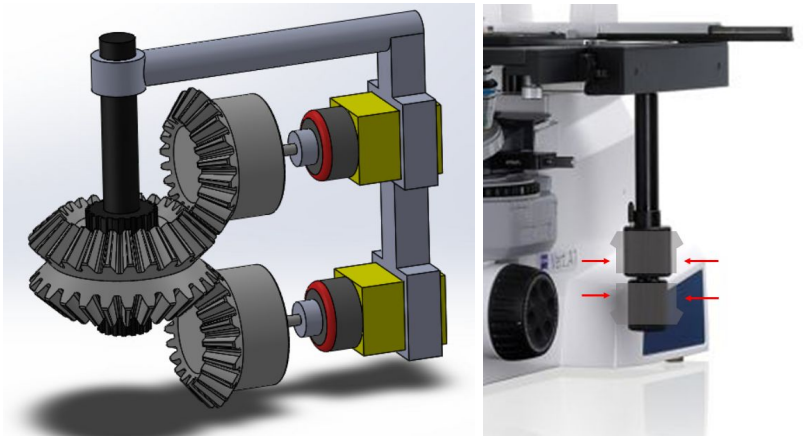
The primary draw to this Removable Gear Cap design is in its ease of detach/attachability to systematically alternate between manual and automatic translational control. Subsequently, this feature makes this motorized system noninvasive to the current operation of the microscopes. In other words, nothing about the microscopes in their current states must be altered to implement automatic control of the stage according to this design, and removal of automatic control without compromising the overall microscope's integrity is incredibly facile.

Disadvantages:

In order to facilitate "cap" operation, such that the housing and system can slide on and off of the existing control knobs, this design will likely sacrifice consistent, reliable gear mating while allowing for some slippage between gears and control knobs. In order to fix the gears in space, they will each be sandwiched between extrusions from the housing's interior, which will result in frictional wear to the housing and the gears over time, as well as potentially substantial

energy loss to friction here. Finally, the intricate features of the housing, as well as the custom-cut gear interior for mating to the control knobs will be challenging to fabricate with a high level of precision with the fabrication methods available to the team, especially under the cost requirements provided by the client.

3.4: Gear-Fastened Translational Knobs



[Figures 6 and 7] SolidWorks sketch of the Gear-Fastened Translational Knobs design which will surround the microscope's commercial translational knobs as shown on the right, with set screws fixing the gears to the control knobs at the red arrows.

The Gear-Fastened Translational Knobs is the final proposed design alternative. This design fundamentally operates similarly to the previous design (Removable Gear Cap), in that it utilized mated gears attached to two motors to independently adjust the linear translation of the microscope's existing stage by turning the current manual control knobs automatically. This design, however, forgoes the all-encapsulating housing unit and slide-on knob mating functionality. Instead, gears would be fixed to each control knob independently via set screws through the body of bevel gears, as depicted in Figure 7. Because this fastening method would ensure that the gears both reliably mate to the knobs while rotating and cannot move axially along the knobs, they do not need to be incorporated into a frame that fixes the gears, pinions,

and motors relative to one another. Instead, the motors, to which pinions that mate to the gears are fixed on the motor shafts, will be fixed in space by a frame that attaches to the control knob shaft, also via set screws, as shown in Figure 6.

Advantages:

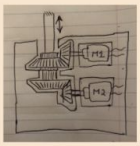
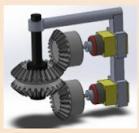
Similar to the Removable Gear Cap design alternative, this design minimizes fabrication and component cost by interfacing with the existing stages of the microscopes, as opposed to replacing them, as suggested by the New Replaceable Stage. Additionally, unlike the Removable Gear Cap design, this design has been optimized to simplify fabrication methods to be more attainable according to the resources available to this design team this semester. This design was also specifically chosen to fix its gears to the control knobs via set screws. Not only will this design choice present more reliable mating between gears and knobs, but it will also allow the team to consider purchasing gears for use in this system, rather than custom making them, as is necessary for the Removable Gear Cap gears.

Disadvantages:

The main drawback of this design, especially with respect to the Removable Gear Cap design, is in the inability to immediately attach or remove the system from the microscope. Although this device would not be permanently fixed to the existing knobs or shaft of the microscopes, it would not slide on and off as a full system; rather, attachment/removal would require manipulation of multiple set screws in each of the gears and motor positioning frame, and an installation procedure that is cognisant of specific positioning and mating of the pinions relative to the gears.

IV. PRELIMINARY DESIGN EVALUATION

4.1: Explanation of Design Matrix:

	Preliminary Design Matrix					
Criteria (weight)	 **New-Replaceable Stage**		 **Removable Gear Cap**		 **Gear Fastened Translational Knobs**	
	Score	*Weighted*	*Score*	*Weighted*	*Score*	*Weighted*
Cost (25)	*1/5*	5	*3/5*	15	*4/5*	20
Functionality (20)	*5/5*	20	*3/5*	12	*4/5*	16
Precision (20)	*5/5*	20	*3/5*	12	*4/5*	16
Fabrication (10)	*1/5*	2	*2/5*	4	*4/5*	8
Ease of Use (10)	*5/5*	10	*4/5*	8	*3/5*	6
Detachment (10)	*2/5*	4	*5/5*	10	*3/5*	6
Safety (5)	*3/5*	3	*5/5*	5	*5/5*	5
TOTAL (100)		64		66		77

[Figure 8] The Low-Cost Motorized Microscope Stage Design Matrix. Green indicates which design scored highest in each category. The final row indicates the overall score of each design. Scores were obtained by taking each design's individual score in a category out of 5 and then multiplying that by the weight of the respective category. Final scores were obtained by adding all the weighted scores for a given design.

Cost

Cost is one of the primary considerations for this project since the task involves a stringent budget. The total cost must stay below the threshold of $100 dollars and function to allow for motorized movement of the stage. With all of the different mechanical and electrical elements including gears, motors, a microcontroller, joystick, and circuit elements, our budget will have to be used cautiously and effectively in order to complete the project. The "New-Replaceable Stage" design scored the lowest in this category as the fabrication of an

entirely new stage would require a number of expensive parts. While the “Removable Gear Cap” and “Gear Fastened Knob” designs are similar, the need for a housing unit on the removable cap unit would require additional materials and an extra degree of precision during fabrication which would result in higher costs, so the “Gear Fastened Knob” design scored the highest in this category.

Functionality

The design’s intended use is to automatically control the position of an inverted microscope stage in two dimensions. The functionality category rates each design’s capability in performing this function. A design which merits a high score would be able to be controlled by a joystick as well as the existing software. This design would also improve the efficiency of stitching together adjacent images within the microscope field - without sacrificing image quality or position accuracy and reproducibility. The “Removable Gear Cap” design would have a secure attachment to the translation knobs on the microscope, resulting in a lower functionality score. Due to the secure nature of the “Gear Fastened” design, there would be improved control of stage movement and image quality, so this design scored higher. Finally, a completely new stage would allow for the most control over stage movement and image quality, so this design scored the highest in this category.

Precision

The client would like to have a 1 micron resolution of translation. This is important to the function that the system will provide to users, automating the microscopy process with reliable precision. In addition, the user should be able to move away from and return to a desired position accurately. Similarly to the functionality category, a brand new stage would allow for greater

precision of movement, as it can directly be controlled through Micro-Manager software and a joystick, so this design scored the highest. Additionally, the "Gear Fastened Knob" design allows for more precise movement than the "Removable Gear Cap" design.

Fabrication

The methods of fabrication should be in the scope of the resources available to the team. Additionally, limitations such as the resolution of 3D printers could cause problems with gear manufacturing down the road. The fabrication of a new stage for each microscope would be very difficult to construct and integrate with an existing microscope stage, so this design scored very low. The housing for a removable gear cap would also be very challenging to construct, as it would require extremely precise 3D printing and any small error would result in a complete loss of functionality for the device. Finally, the "Gear Fastened Knob" design uses screws to hold the gear in place, which is a simpler mechanism for the attachment of the device to the microscope and would be the easiest to fabricate.

Ease of Use

This category relates to the integration of the translational motion to the MicroManager software. The user should be able to easily and quickly set up a sequence of images and be able to leave the microscope as it runs a specified program. Due to the integration of the replaceable stage with the microscope, the team determined that it would be the easiest to use. Both gear designs were similar in this category, however the removable nature of the gear cap would make it overall more user-friendly.

Detachment

This criteria describes the ability of a design to quickly and easily detach from the existing microscope system in such a case when the user would like to revert back to manual control of the stage using existing hand knobs. The removable gear cap design scored the highest in this category, as the user could easily clip the device on and off of the microscope. Meanwhile, the screws holding the "Gear Fastened Knob" design would be more difficult to remove, and a completely new stage would also require a lot of effort in order to set up and remove.

Safety

Safety is not a major consideration since there are not many elements of the design that could physically harm the user. One element of safety is ensuring the circuit is grounded and all elements are correctly wired. Additionally, the elements should be contained so that they cannot be tampered with or cause accidental injury. Both gear designs scored equally in the safety category, and the replaceable stage scored lower due to the potential for accidents with an entirely new stage on the microscope.

4.2: Proposed Final Design:

The design ultimately chosen for development is the "Gear Fastened Translational Knob" concept. This design was chosen in large part due to feasibility of fabrication within the budget constraints set for the project, as well as the improved functionality and precision compared to the other gear design. The screws which hold the gears in place result in a smaller chance of slipping when attached to the knobs, which will give the user greater control over movement.

This is essential on a fluorescent microscope where the desired precision is 1 micron, and any improper connection to microscope knobs could result in error. Additionally, the materials required for this design are estimated to be much less expensive than an entirely new microscope stage, ensuring that the team spends less than the $100 budget. Each motor will control one of the translational knobs on the microscope, and microcontroller code (expected to be an Arduino) will allow for the integration of a joystick and Nikon Elements software in order to control the motorized movement. Due to this design's score on the design matrix accounting for all of the above-mentioned criteria, the team will move forward with the "Gear-Fastened Translational Knob" design.

4.3: Fabrication and Testing Research

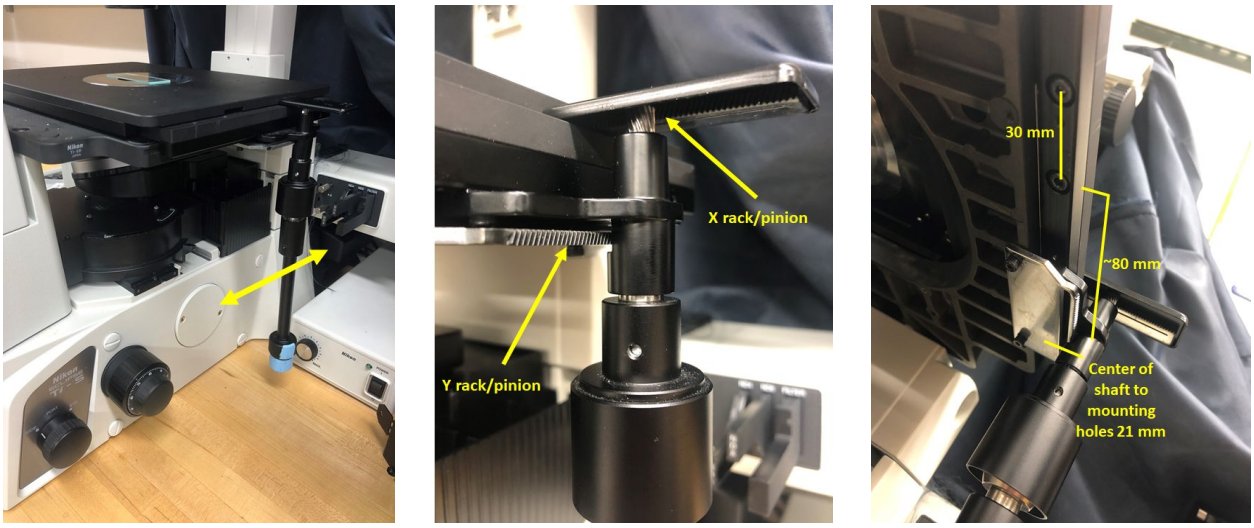
Materials

The materials that we use will be instrumental in maintaining the stringent budget of the project. We will look into obtaining commercial bevel gears for the translation of motor rotation to that of the translational knobs. Such gears, as available from vendors such as McMaster Carr, are machined as highly durable metals, including various steels (i.e 1045 Carbon Steel). Such materials will prevent wear between the gears and pinions over extensive operation. The price of these gears will determine whether it is feasible to include them in our final product. If the commercial gears prove to be out of our budget, we plan to use CAD and 3D printing as available in the MakerSpace, which will result in gears made of plastic-like resin material, as fabricated by FormLabs SLA Resin printers. This will certainly be a cheaper alternative, but wearing of the gears will be a major concern for the team. Additionally, the frame to which the

motors and attached pinions will be fixed will also be printed in the resin material available with the SLA printers.

Methods

In order to fabricate the "Gear Fastened Translational Knob" design, the team will utilize Makerspace 3D printing to make the gears, and purchase circuit elements such as motors and a microcontroller to construct the remainder of the device. The two main 3D printing methods available at the makerspace include fused filament fabrication (FFF) via Ultimakers and SLA Form printers [12]. While FFF is more cost effective, SLA allows for much greater detail, which could be crucial for fabricating the gears [13]. The gears can then be fastened to the motor system, controlled by the Arduino microcontroller, in order to provide translational movement of the microscope, via set screws, that are commonly available for purchase from hardware vendors. The frame that positions the motors and pinions in space will also be 3D printed, at least preliminarily. If 3D printing materials are deemed to be insufficient in strength to oppose internal forces such as moments, alternate fabrication methods will be evaluated, such as milling or laser-cutting, in order to implement more durable materials. Finally, the entire mechatronic system will be mounted to the underbelly of the microscope stage via an 80/20 aluminum apparatus and threaded anchor points that translate with the control knob shaft along the rack and pinion system (as shown in *Figures 9-11)*.



[Figure 9-11] The above three images are of the Nikon TI-U microscope in the Biomedical Experimental Teaching Lab. Highlighted with annotations in all three images (proceeding from left to right) is the translational motion of the control knob shaft, the rack/pinion system responsible for the translation, and suggested dimensioning for the mounting of the mechatronic system on the underbelly of the microscope.

System Components and Software:

Each of the proposed designs, and specifically the chosen final design, will operate according to an electronic system. As shown in the depiction of the Gear Fastened Knob design, two separate motors will be used to control the turning of the existing manual control knobs according to the gear system specified; these motors will be stepper motors. The system as a whole will be rooted in programs written onto an Arduino Uno that outputs speed and direction commands to the motors via pulses. Within the software written to the Arduino, the rotation of each motor will be mapped to specific values of linear translation of the stage mathematically. Thus, coordinates or vector values along the x- or y-directions can be inputted to determine the direction and extent to which each motor is driven to turn the control knobs and move the stage.

This operation will be extrapolated to interface with the control of the microscope as a whole via Micro-Manager (a microscopy programming aid with many hardware and software compatibilities). Programs will then be written to dictate specific translational movements, according to the field of view dimensions, to move the stage during serial imaging procedures. Furthermore, a joystick-type controller will also interface to the Arduino to allow for facile adjustment of the stage position, independently of the Micro-Manager programs.

Testing

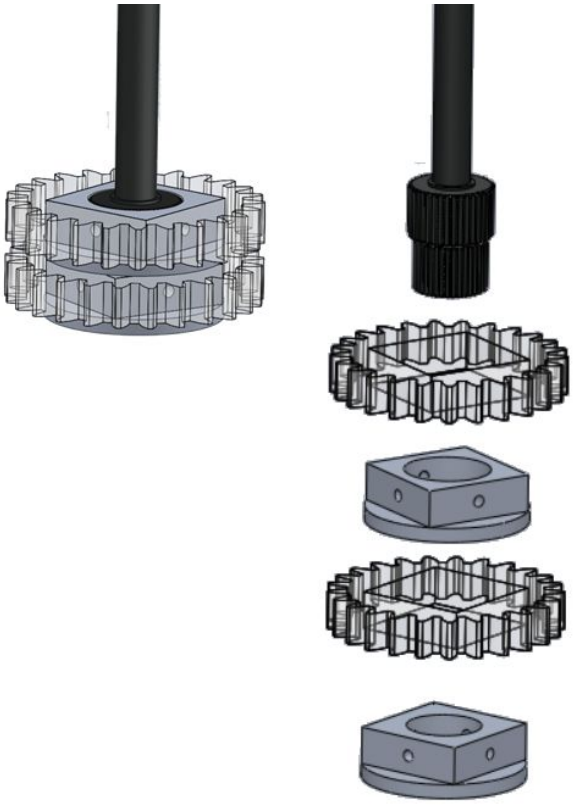
Once a prototype has been fabricated for the "Gear Fastened Knob" design, it will undergo testing procedures to determine its performance in practice and how well it meets the needs of the client. Two aspects of the design will be tested in particular: the precise movement of the device and the effectiveness of the image stitching using Micro-Manager software.

The team could use direct measurement to calculate the accuracy of motorized movement due to the prototype. This could be conducted by setting the program to move the stage a predetermined distance in a desired direction, and then directly measuring the distance that the stage has moved. However, this would only be effective for about 1 mm of movement, and not to the precision of 1 micron that the client has requested. The extremely precise movement of the stage could be monitored using images of cells, and determining how far the stage moved based on successive images. This also would test the integration of the device with the Micro-Manager software.

V. DEVELOPMENT PROCESS

5.1: Final Prototype

Following several design iterations, the Gear Fastened Translational Knobs design was updated and tuned in order to facilitate realistic fabrication with materials readily available to the team. This final prototype still operates based on semi-fixation to the existing control knobs, but does not require purchase of pre-fabricated metal gears. Instead, a custom gear system has been designed, featuring a gear holder component and fixed gear component to be attached to each independent control knob (*Figure 12*).

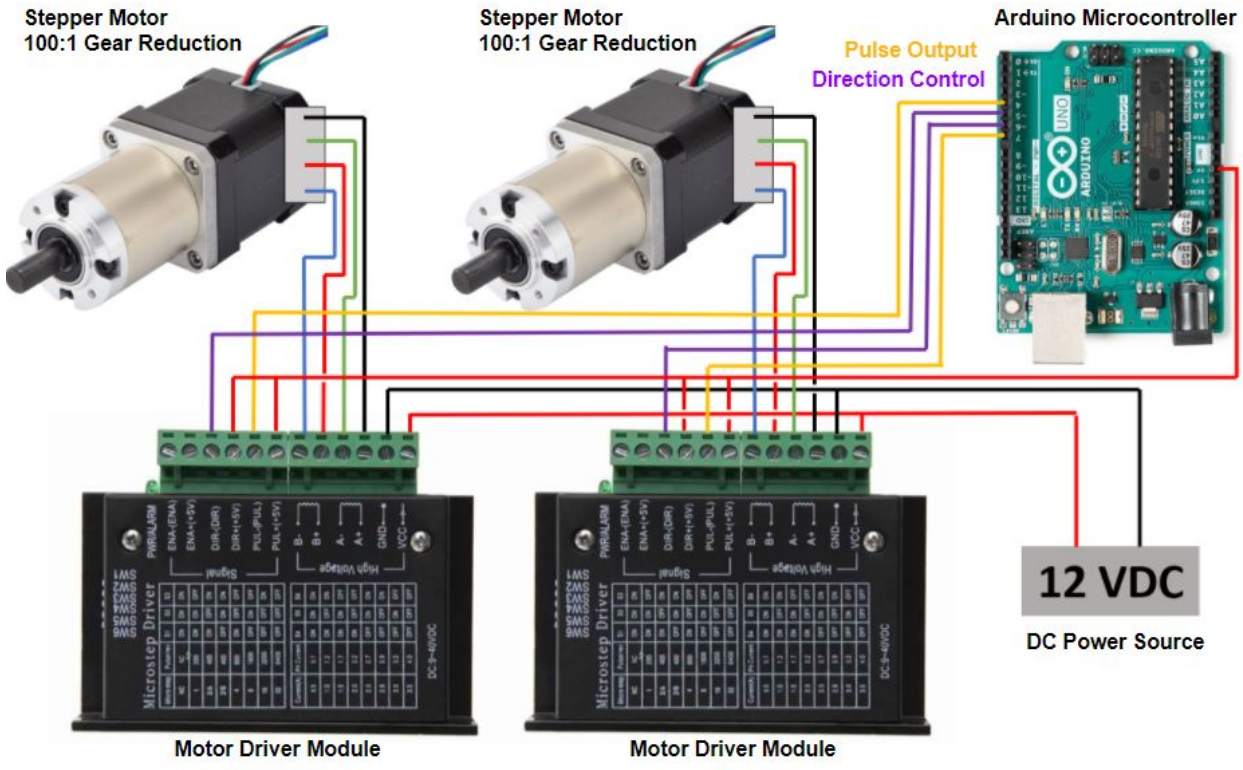


[Figure 12] The updated gear fastened translational knobs mechanical design. The assembled system on the knobs/shaft (left) and the exploded drawing displaying each individual component (right). Gear holder components (gray) fix to the knobs via lateral set screws while the gears themselves (transparent) fit over the square profile of the holders to transmit torque.

This design builds upon the principle of the originally proposed design that allows the knobs to be driven with mated gears. Custom gear holders will fit over each of the translational

control knobs independently, fixed semi-permanently in space via lateral set screws. The gears themselves subsequently fit over the gear holders such that the square cutout of the gear components fit over the square profile of the holder components. This square geometry will fix the rotation of the gear relative to the holders, while the set screws will fix the rotation of both components relative to the knobs themselves. Thus, driving of the fixed gears will turn the knobs to drive linear translation of the stage. Additionally, the circular profile at the bottom of the gear holders employ gravity to prevent the gears from moving along the length of the knob shaft.

In addition to the mechanical gear components designed, stepper motors and relevant circuitry components were selected. A circuit was designed and implemented to allow for control of two simultaneous stepper motors independently that will ultimately drive pinions mated with the gear-fixed knobs for translation of the stage (*Figure 13*).



[Figure 13] Circuit connectivity diagram implementing control of two gear-reduced stepper

motors with motor driver modules, a power source, and a microcontroller (Arduino Uno).

This mechatronic system includes two (gear-reduced) stepper motors, each driven by a motor driving module, both controlled by an Arduino Uno microcontroller and powered by a 12VDC power source. The motor driver modules each modulate the 12VDC power source according to 5V digital output signals from the Arduino that control the direction and stepping of the motors. These output signals include a logic directional signal (purple wire) and a pulse signal (yellow wire) for control over each stepper motor.

Ultimately, the stepper motors will be mounted to the stage with pinions on their output shafts that mate with the gear-fixed knobs in order to drive the knobs to translate the stage. Software will be written and integrated with Micro-Manager software on the Arduino for positional control of the stage via pulse (step) commands to each of the motors independently.

5.2: Materials

This semester we acquired all of the required materials to formulate a mechatronic system. This system is based around two Planetary 100:1 Gearbox Nema 17 Stepper Motors from Stepperonline. Additionally, we acquired two Stepper Motor Driver Nema TB6600 boards to control the purchased motors. We then found appropriate structural components to use to mount our system, including motor brackets along with 8020 components. Next, we found an arduino, breadboard, jumper wires and power source to be able to complete our mechatronic system's set up. One item we will still need to fabricate is the motor-interfacing gear that will be made from acrylic stock. This gear will fit into a holder made from aluminum stock. Upon completion of this element, the system should be ready to assemble completely.

5.3: Fabrication

Many of the design components will be purchased and assembled, rather than directly fabricated. The mechanical gear components, however, will be fabricated from stock material. This includes the gear holders and the gears themselves.

The gear holders will be fabricated from cylindrical aluminum stock. This stock will first be turned down to the diameter of the cylindrical (bottom portion) of the holders on a lathe and subsequently cut to length. Then, on a mill, the top portion of these holders will be cut to a square profile as designed. A drill will be used on the mill to create a hole through which the knobs will fit (because the knobs are of two different sizes, this hole will be different for each of the two gear holders). Holes will also be drilled laterally on each of the four sizes of the square profile with a drill on the mill. These lateral holes will then be tapped to accommodate set screws for fixing the gear holders to the knobs.

The custom gears will be fabricated via laser cutting of an acrylic sheet. This laser cutting will be conducted based on 2D drawings of the gear profiles, which will include the gear teeth as well as the square cutout. The pinions that will be mated to the gear-fixed knobs and driven by the stepper motors will also be laser cut from the acrylic sheet.

5.4 Code Integration Plan

An Arduino microcontroller will be the primary link between the mechanical elements of the design and the image stitching software. Currently, the team is working on further development of an existing Arduino code used for a mechatronic system. This code utilizes a microstep module to control a stepper motor in the form of three commands: pulse, direction,

and enable [14]. These individual microstep modules will be applied to the two different stepper motor systems, each directing either the X or Y axis microscope translation. By changing the pulses per rotation values in the code, the team should be able to direct the movement of the motors with the precision required to maintain the desired resolution of one micron.

Further development of this code will be necessary in order to accommodate for the use of a joystick as well as automated serial imaging through ImageJ and Micro-Manager. Due to the open-source nature of Micro-Manager, the team will be able to write code to direct the actions of the stepper motors based on directions inputted by the user. Integration of a joystick with the mechatronic code will be a challenge, however some existing code found online will serve as a good starting point for this process. A joystick can be set as the input to our current microstep Arduino code, with different intensities of the joystick influencing how much the stepper motor is directed to move [15]. Further research and testing in these specific areas next semester will allow for both automation of the imaging and movement process, as well as user-controlled movement via joystick.

5.5: Testing

Motor speed testing: We performed rudimentary source code for speed testing to get an estimate of the time it will take to perform different functions on the microscope using our motors. By visually identifying one complete revolution, we calculated the translation speed that our motors will reach.

Torque Testing: We performed torque testing to determine if our motor would be powerful enough to turn the knobs of the microscope. We modeled an elastic coil as a spring attached to

fishing wire wrapped around the knob, then calculated its spring constant and used the displacement to calculate an approximate torque required to turn the knob.

Translation Resolution Testing: To test the estimated resolution of our motors, we measured the distance of translation of the stage that occurred for one complete revolution. Using this, we used the step size of our motor to calculate the approximate resolutions of translation.

The testing and calculations made help to better define our system and to ensure our client's design criteria are being met. As we continue with the fabrication process of our design, we will continue to use our measurements made to define the uses of the system. Additionally, we plan to do further testing including but not limited to final confirmation of the resolution and speed of our motor with translation of the stage, confirmation of accuracy within the serial imaging process, and successful integration with Micromanager software as well as with a joystick to manually control the stage. These tests will help to define the success we have in creating a device that will serve the purposes of our client and meet his design criteria.

VI: RESULTS

For our torque testing we measured a necessary applied torque of 0.0292 Nm to rotate the knob. Compared to the output that our motor is capable of, which is 4 Nm, we will have plenty of ability to move the knobs of the microscope. This eliminates the concern that our motor may not be strong enough to rotate our gears. From our speed testing, we calculated the maximum translation speed will be approximately 0.6 mm/s and 1.0 mm/s in the x and y directions respectively. While this will not be very fast. it should provide a speed that will not hinder the progress of scientific procedures like the serial imaging process. Faster speeds may lead to

difficulties controlling the field of view when controlling the stage with our proposed joystick. Using the step size for our motor of 0.018°/step, the calculated resolution was 1.6 μm/step and 0.9 μm/step in the x and y directions respectively. With our 2:1 gear reduction incorporated into our design, the final product should achieve sub-micron resolution of translation on our final product.

VII: DISCUSSION

The results of the preliminary testing done this semester seem promising. In designing our device, the team was tasked with finding a motor and complimentary gearing strategy suitable for controlling the translation of a microscope stage for use in serial imaging. The team was able to find a balance between precision, speed, and torque which best fit our device's application by pairing a 0.018°/step stepper motor with a 2:1 gear reduction. Using the relationship between control knob rotation and stage translation measured by the team, we estimate the overall resolutions of our system to be 0.45 microns and 0.8 microns in the x and y directions, respectively. In addition, the motor's maximum speed of 4 rpm corresponds to translational speeds of 0.6 and 1.0 mm/s. Lastly, the measured torque required to rotate the control knob was much less than the capabilities of the stepper motor. The team did not have access to the most sophisticated methods of torque testing and resorted to using a spring assumed to have a linear spring constant. However, this slight inaccuracy isn't likely a source of serious concern because the measured torque is significantly lower than the motor's capability. On the other hand, the team might consider implementing strategies for ensuring the torque output by the device never strains the microscope in any capacity. The calculations for translational

resolution do not account for the interactions between components in our system such as the mating between gears. For this reason, the team plans to perform additional testing once the entire system is constructed. Since our target resolution is on the scale of a micron, this testing will likely involve the use of imaging software.

V. CONCLUSION

The team's efforts this semester focused on creating a device for use in the Biomedical Experimental Teaching Lab which would motorize translation of a microscope stage to allow for serial imaging. The team has progressed towards a design which features a custom gear system to interface with the existing microscope control knobs, a mechatronic system consisting of an arduino microcontroller as well as a pair of stepper motors and motor drivers, and an aluminum frame mounted to the underside of the microscope stage. Thus far, the team has managed to demonstrate the functionality of the mechatronic system by driving the stepper motor using very basic Arduino code. Moving forward, the team hopes to further fabrication by machining gears and developing a mounting apparatus to position all of our components relative to the microscope. Once this is complete, the team will be able to conduct more robust testing of translational speed and resolution. On the software side, more sophisticated Arduino code will be developed to take a position or direction input and map it to the proper motor function necessary to achieve the corresponding stage translation. This will then be incorporated into the existing Micro Manager software to allow the user to control all aspects of the serial imaging process with one interface. In addition, the team aims to incorporate autonomous image stitching with Image J and joystick control of the device which will both require more complex software

development. In the end, our resulting device should serve our client well as a low cost motorized stage with movement at sub micron resolution in the x and y directions.

VI. REFERENCES

[1] Vision Online. 2020. *What Is Automated Microscopy*. [online] Available at: <https://www.visiononline.org/blog-article.cfm/What-is-Automated-Microscopy-and-How-Does-it-Work/157#:~:text=The%20automation%20of%20how%20a,a%20long%20period%20of%20time.> [Accessed 7 October 2020].

[2] W. by Z. E. I. S. S. Microscopy, "Automated Microscopy," *Bitesize Bio*, 31-Mar-2020. [Online]. Available: https://bitesizebio.com/34349/automated-microscopy/. [Accessed: 07-Oct-2020].

[3] "Motorized Microscope Stage," *Labx*. [Online]. Available: https://www.labx.com/product/motorized-microscope-stage. [Accessed: 07-Oct-2020].

[4] Campbell RAA, Eifert RW, Turner GC (2014) Openstage: A Low-Cost Motorized Microscope Stage with Sub-Micron Positioning Accuracy. PLoS ONE 9(2): e88977.

[5] "What is Fluorescence?," *Office for Science and Society*, 24-Jul-2017. [Online]. Available: https://www.mcgill.ca/oss/article/you-asked/what-fluorescence. [Accessed: 07-Oct-2020].

[6] "Introduction to Fluorescence Microscopy," *Nikon's MicroscopyU*. [Online]. Available: https://www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescence-microscopy. [Accessed: 07-Oct-2020].

[7] "Filters for Fluorescence Microscopy," *Nikon*. [Online]. Available: https://www.nikon.com/products/microscope-solutions/explore/microscope-abc/learn-mo

re-microscope/filters/index.htm. [Accessed: 07-Oct-2020].

[8] K. Scheffler and C. Müller, “Five Advantages of Inverted Over Upright Microscopes in Industrial Applications,” *Learn & Share | Leica Microsystems*, 16-Mar-2015. [Online]. Available:https://www.leica-microsystems.com/science-lab/five-advantages-of-inverted-over-upright-microscopes-in-industrial-applications/. [Accessed: 07-Oct-2020].

[9] “BME Shared Lab Resources,” *BME Lab - 2005 ECB - Biomedical Experimental Teaching Lab*. [Online]. Available: https://sharedlab.bme.wisc.edu/labs/2005/. [Accessed: 07-Oct-2020].

[10] “Manager Open Source Microscopy Software,” *Micro*. [Online]. Available: https://micro-manager.org/. [Accessed: 07-Oct-2020].

[11] *Olympus IX81 Motorized Inverted Research Microscope Instructions Manual*. Olympus.

[12] “3D Printers – Pre-Pandemic,” *UW Makerspace*. [Online]. Available: https://making.engr.wisc.edu/3d-printers/. [Accessed: 07-Oct-2020].

[13] “SLA vs FFF / FDM workflow and space requirements,” *CEL*, 15-Mar-2019. [Online]. Available: https://cel-uk.com/sla-vs-fff-fdm-workflow-and-space-requirements/. [Accessed: 07-Oct-2020].

[14] DroneBot Workshop. (2019, May 25). Using BIG stepper motors with Arduino. Retrieved December 3, 2020, from Dronebotworkshop.com website: https://dronebotworkshop.com/big-stepper-motors/

[15] “How to Control Servo Motors With an Arduino and Joystick;: Arduino,” Maker Pro, 22-Mar-2018. [Online]. Available: https://maker.pro/arduino/tutorial/how-to-control-servo-motors-with-an-arduino-and-joys

tick. [Accessed: 13-Dec-2020].

VII. APPENDIX

7.1: Product Design Specifications

Low-Cost Motorized Microscope Stage

Product Design Specifications

September 17, 2020

Client: Dr. John Puccinelli
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Function:

Dr. John Puccinelli, faculty associate chair of the undergraduate program in the Department of Biomedical Engineering, presents the task of motorizing the control of a microscope stage for use in the new Biomedical Experimental Teaching Lab for undergraduate learning. The lab is equipped with two Inverted Fluorescence Microscopes - Nikon TI-U and Olympus IX71 - that have standard fixed stages with movement controlled by manual translational control knobs. With the current configuration, users do not have the ability to automatically conduct imaging with subsequent stitching of images because of the fact that distance of translation conducted by the control knobs is arbitrary. This capability is necessary for obtaining high resolution images at high levels of magnification while maintaining a holistic perspective on the specimen being imaged. Motorized stages that accomplish this task are available. One benefit of these commercially available stages is their ability to interface with imaging software for synergistic control alongside the microscope itself. However, these come with costs outside of the budget available to Dr. Puccinelli and the Biomedical Experimental Teaching Lab, namely in the range of several thousands of dollars [1,2]. Therefore, this team aims to design and implement a cost-effective mechatronic system that allows for motorized

control of a stage of the Inverted Fluorescent Microscopes, capable of moving linearly along the two axes orthogonal to the lens. With a budget of $100, this system will be controllable both with a joystick-type adjustment module and via interfacing with microscope software for serial imaging and image stitching.

Client Requirements:

- Design a motorized mechanism to move a microscope stage.
- Must be used in conjunction with the microscopes in the BME teaching lab (Nikon TI-U Inverted Fluorescence Microscope and Olympus IX71 Inverted Fluorescence Microscope) and existing software (NIS-Elements BR or Micro-Manager).
- Design cannot exceed $100.
- Should be able to control stage movement with a resolution of 1 micron.
- The design must apply to move either the fixed stage of the microscope or the currently used translational control knobs.

Design Requirements:

1. **Physical and Operational Characteristics**

    a. *Performance requirements:*

The stage of the microscope must function by using a joystick as well as using the NIS-Elements BR Software or Micro-Manager Software to program distances to move between images. The latter function would allow for the automation of the imaging process, leaving stitching of the images being the only remaining manual part of the process.

    b. *Safety:*

The main safety concern with this device will be to safely store the motors involved in moving the stage. Additionally, the heat output must be monitored and effectively mitigated to maintain a safe working environment. The device also must not induce any damaging problems with the specimens being studied, the microscope, the computer, or the software including but not limited to overexposure of tissue, burning out light bulbs, or damaging the stage of the microscope.

    c. *Accuracy and Reliability:*

The motorized stage should be able to consistently respond to software as well as a user-controlled joystick and have resolution of movement of around 1 μm to accommodate for a cell diameter of 10 μm. It is crucial for the device to maintain this accuracy for image stitching.

d. *Life in Service:*

The motorized stage should be able to function with consistent performance and accuracy for the duration of the warranty period for the inverted fluorescent microscopes. For this reason, the device should function properly for a minimum period of 5 years, matching the warranty period of the Nikon TI-U, for example [3]. Beyond this, the client has specified that the device should be designed for a service life of 15 years.

e. *Shelf Life:*

This device will be implemented in the lab space immediately and will remain in use for the duration of its life, whenever the microscopes of interest are being use. For this reason, a specific shelf life does not apply to the design considerations of this system.

f. Operating Environment:

The device will be used in an indoor undergraduate educational lab for learning purposes and student projects. The room will be kept around room temperature, so the device won't be subjected to extreme temperature or pressures.

g. Ergonomics:

The joystick-type stage-adjustment module will be used to intuitively control the position of the stage. Thus, this controller component must act to move the stage at a reasonably viewable speed, dictated by traversing one field of view per second, with the field of view moving in the same direction that the joystick is actuated. This will be inherently more efficient and intuitive for users, as compared to the multiple control knobs that must be spun in order to translate the stage in each direction.

h. Size:

Currently, the team envisions two potential design routes for automating the stage's movement. The first of which consists of making a replacement stage which is under motor control. In this scenario, the motorized stage created would have to be nearly identical in size to that of the existing stage. More definitive measurements will come once the team is allowed to see the microscope in person. Second, the team has proposed controlling the existing manual stage knobs. In this case, the device would have to attach to the existing knobs without adding excessive bulk.

In general, size must not impede normal functions of the microscope, including but not limited to changing of the microscope filter or objective or visualizing specimens along the optical path. For this reason, a size no larger than 15cm x 15cm is desired for a control system.

i. Weight:

If the device and system are designed to reside on the bench alongside the microscopes, a maximum weight of 5kg is reasonable, allowing for easy transport or movement if necessary. If the device is to be attached to and supported by the microscopes themselves, the weight of the device is not to exceed the maximum load allowances specified by the individual microscopes.

j. Materials:

The materials that make up the motorized stage system must be sturdy, such that unindoctrinated undergraduate students that use it for the first time will not incidentally tamper with or impair its functionality. Thus, electrical components that are to sit separate from the microscope itself should be protected by a plastic or metal case.

Furthermore, if the team's design direction involves developing a stage that replaces the existing stage (rather than controlling the existing stage), the stage must be composed of a sterilizable material, as it may come into contact with various biological materials under study.

k. Aesthetics, Appearance, and Finish:

Due to budgetary constraints and the intended purpose of the stage for educational purposes, aesthetic and appearance are a lower priority than function. The stage should have a finish which prevents breakdown of the materials due to repeated use.

2. Production Characteristics

a. Quantity:

For this project, the client has requested a motorized stage for each model of the microscope present in the Biomedical Experimental Teaching Lab, the Nikon TI-U inverted fluorescent microscope and the Olympus IX71 inverted fluorescent microscope. The team will design and construct two total stage devices, one for each microscope.

b. Target Product Cost:

Our client has set a budget of $100 for this project, as it will be used largely for educational purposes. Current motorized stages on the market could cost several thousands of dollars, so the team must be conscious of all material choices and design options.

3. Miscellaneous

a. *Standards and Specifications:*

Microscopes and accessories fall under the Class I General Controls FDA regulations and therefore do not require premarket approval, as long as the device is not "labeled or otherwise represented as sterile [1]." Additionally, the American National Standard does not apply to microscope accessories.

b. Customer:

The client, Dr. John Puccinelli, is an undergraduate advisor and associate chair of the undergraduate Biomedical Engineering program at the University of Wisconsin-Madison. He has proposed this project with the purpose of implementing motorized microscope stages for educating students on automated imaging and image stitching using Nikon Elements software. Completion of this project within the budget constraints would allow for students to gain experience in fluorescence microscopy without the university spending thousands of dollars for a research-oriented motorized stage.

c. User-related concerns:

The main purpose of this project is to automate the imaging process. The user should be able to more quickly and ergonomically find their place on the microscope. Additionally, the user should be able to automate the imaging process by using a programmed system for imaging the extent of a tissue in predetermined steps. For this process, the user should remain near the microscope to handle any technical difficulties that may occur.

d. Competition:

While motorized fluorescent microscope stages are currently available on the market, the cost of these devices ranges from $1,000-10,000, which is significantly larger than the client's budget. The lowest cost found for a functioning motorized stage was called OpenStage designed by Robert Campbell for right around $1,000 [4]. A design allowing for automated imaging for the price of $100 would be extremely useful to the University of Wisconsin-Madison, as well as other universities aiming to provide students with automated imaging experience for a low cost.

4. PDS References

[1] Thorlabs.com. 2020. High-Speed Motorized XY Scanning Stages. [online] Available at: <https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=5360> [Accessed 17 September 2020].

[2] Edmundoptics.com. 2020. Zaber™ Motorized XY Microscope Stage | Edmund Optics. [online] Available at: <https://www.edmundoptics.com/f/zabertrade-motorized-xy-microscope-stage/14922/#:~:text=The%20Zaber%E2%84%A2%20Motorized%20XY,alone%20operation%20as%20scanning%20stages.&text=This%20stage%20is%20fully%20supported,automated%20microscopes%20and%20imaging%20systems> [Accessed 17 September 2020].

[3] Nikon Instruments Inc. 2020. Service After The Sale. [online] Available at: <https://www.microscope.healthcare.nikon.com/service/service-after-the-sale> [Accessed 17 September 2020].

[4] Campbell RAA, Eifert RW, Turner GC (2014) Openstage: A Low-Cost Motorized Microscope Stage with Sub-Micron Positioning Accuracy. PLoS ONE 9(2): e88977.