Microscope Low-Cost Motorized Stage

Preliminary Report

BME 200/300
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Abstract

The current two inverted fluorescent microscopes inside the biomedical engineering teaching lab have fixed stages and can only be moved through two manual translational control knobs each controlling the x-axis movement and y-axis movement. Because the manual movement prohibits automated imagings therefore causing undesirable motions and no reference as to the distance moved, the new designed motorized stage will be capable of automatic movements, self-corrected positioning, and better interface with softwares and joystick. The current competing designs of motorized microscope stages are too expensive to be supported by our experimental teaching lab thus a low cost motorized stage is crucial for educators and students utilizing the fluorescent microscope. Our goal is to integrate previous design, fabricate prototypes, and improve system capabilities while keeping the cost of the project under our one hundred dollar budget and resolution of movement to be around one micrometer.

Table of Contents

I. Introduction

A. Motivation/Global Impact

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

Motorized microscope stages are commonly used in bioimaging and other cell culture laboratories. They allow advanced imaging techniques, such as time lapse microscopy and image stitching, to be completed quickly and accurately. However microscopes with motorized stages are often much more expensive than their non-motorized counterparts. This creates a large barrier to accessibility, particularly for low-budget and teaching laboratories. Our design for a low-cost motorized microscope stage attempts to close that gap and make fluorescent microscopy more accessible.

B. Existing Devices & Current Methods

Currently, there exist two major solutions to motorized stage microscopy. The first is to purchase the motorized version of a fluorescent microscope. As mentioned earlier, this is often too large an expense for new labs. For example, the non-motorized fluorescent microscope that is currently used in the BME teaching lab is a Nikon TI-U, which retails at around $16,000 used [2]. The motorized version of this microscope is $19,000 used, or around $70,000 new [7]. To address the need for a more cost effective motorized microscope stage, other designs have been developed.

One of these such designs is the open-source project Openstage [8]. Openstage is a design for a low cost motorized microscope stage created for a multiphoton microscope. The project was able to achieve 1µm accuracy in the x and y axes. However the budget of the Openstage is significantly higher than this project, at $1000.

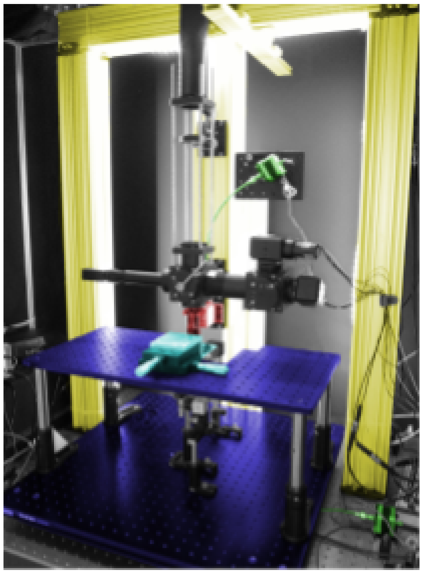


Figure 2: Openstage Motorized Stage [4]

C. Problem Statement

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

II. Background

A. Physiology and Biology

Fluorescent microscopes are an optical microscope that uses fluorescence that uses to study biological samples.They use high intensity light source to excite fluorescent species. The specimen reflects back a shorter wavelength that is then measured[1]. The fluorescent microscope uses filters to match the specific wavelength range of the specimen that is being measured[2]. The functions of the Fluorescent microscope include measuring cell metabolism. This is done by exciting the fluorescent ROS (reactive oxygen species) NADH and FAD which fluorescent molecules. Measuring the wavelength they readout allows for researchers to measure the metabolic rates of specific samples of cells[3]. At the teaching lab here there are 2 inverted fluorescent microscopes. The Nikon Ti- U and the Olympus IX71. The microscopes are currently controlled by manual translation control knobs. The knobs move in both the x and y directions so therefore 2 motors are required to move each individually[4]. Affordable solution, easily attached or removed and integrated with Nikon Elements. The impact of fluorescent microscopes is crucial to the development of technology and advancement of science[5]. Making the process of imaging and analyzing cells more accurate and time efficient would help advance the research field and make it more accessible for low funded labs to conduct important experiments.

B. <u>Client Information</u>

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

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

C. <u>Design Specifications</u>

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

The motors must be calibrated to accurately and precisely translate to an inputted location of the sample. Specifically, the stage system should have a 1 um resolution when inputting locations, with a +-0.1 um error. The stage must also maintain the distance between the sample and the lens to ensure that the focus of the microscope remains the same and all effects of gravity are negated. To achieve this accuracy and precision, in addition to effectively calibrating the motors, that design that holds the motors must also be stable and its resistance to movement will prevent the buildup of error throughout its use. The joystick must also be reliable where the stage translations should correspond to its use, and this error should also fall within the +-0.1 um range. The budget for this project should be within $100.

III. Preliminary Designs

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

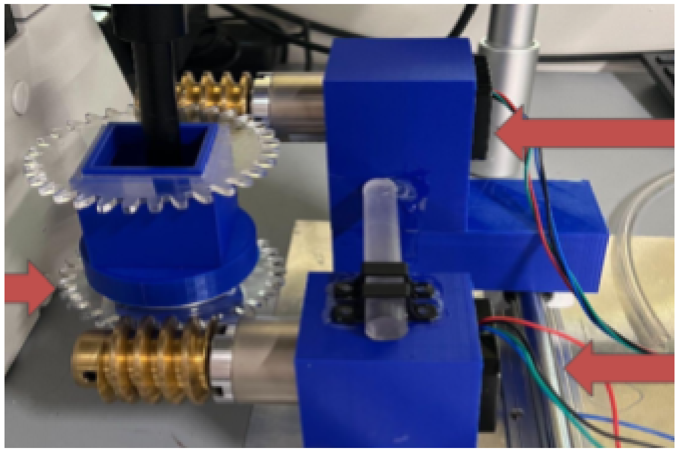


Figure 3: Preliminary Design 1 - Worm Drive Gear System

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

B. Design 2 - Chain and Sprocket:

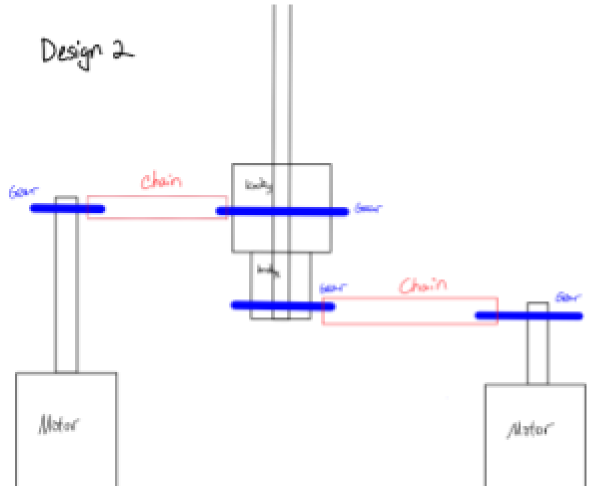


Figure 4: Preliminary Design 2 - Chain and Sprocket

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

C. <u>Design 3 - Meshed Spur Gears Design:</u>

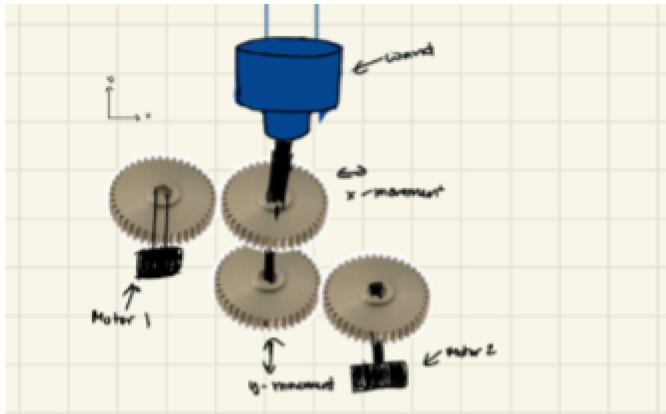
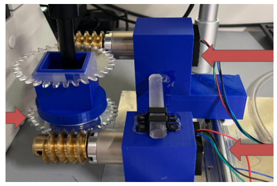
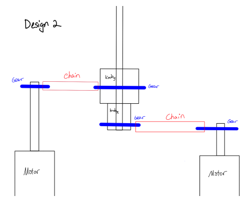
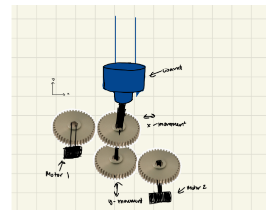


Figure 5: Preliminary Design 3 - Meshed Spur Gears

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

IV. Preliminary Design Evaluation

A. Design Matrix

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

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

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

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

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

B. Proposed Final Design

Based on the design matrix, the proposed final design was the Gear to Gear design. This design scored higher in comparison to the others and was decided to move forward with as it best meets the clients needs. With winning scores in Functionality and Size, the criterias weighed the heaviest, the Gear to Gear's simplistic design was found to be the best match for the team to construct and appease the client.

V. Fabrication/Development Process

A. Materials

Specific material choices have not yet been determined. The cams will either be 3D printed or laser cut, whichever is determined to be more conducive to the required shapes. The other materials will be chosen over the coming weeks.

B. Methods

The fabrication methods of the device have yet to be determined, however the process will involve 3-D printing and or possibly laser-cutting. The gears will either be made or procured depending on the requirements and further calculations.

C. Final Prototype

The final prototype has not been assembled at this time, however the goal is to follow the specified timeline for the fabrication.

D. Testing

At this time, no testing has been conducted. However, the team plans to use software to test cams of different shapes in order to determine the waveforms that they produce. Additionally, the team will work with the client's equipment to perform testing of the fabricated device and observe the waveforms on the monitor.

VI. Results

Since there has been no testing at this point, there are no results to discuss. However the team plans to use the results of the cam testing to make any necessary adjustments to the shape of the cams. These adjustments will provide more accurate results for the three desired waveforms.

VII. Discussion

The device will need to produce accurate waveforms so that healthcare professionals using the device can receive proper training. This is necessary because those being trained will need to be able to accurately read the blood pressure of patients. In order for the design to be ethical the team must ensure that the waveform readings are consistent, accurate, and precise.

VIII. Conclusions

The current inverted fluorescent microscope in the teaching lab has fixed stages with manual movements through knobs located on the side of the stage connected through a wand sticking out. The current method lacks precision and ease of control. The team's client, Dr. John Puccinelli, wants to integrate the stage system with an automatic motorized system capable of automatic positioning and movements through translational control knobs while keeping the cost low. The design could potentially influence future microscope developments and be used in future teaching classes and labs.

The final design the team chose is the gear-to-gear design which consists of two systems of gears each having two gears attached to both the motor, the manual knob on the microscope, and to each other. This method of gear automatic movements will be controlled through the central system program by arduino to interface with software and be used through the joystick.

This gear-to-gear system will prevent displacement of gears compared to other designs and achieve higher accuracy and maintain the low cost.

Next, the team will begin to work on CAD modeling and preliminary design building to determine any underlying issues the team might encounter. Throughout the process, the team will monitor any updates on requirements and make adjustments to the design if necessary. After fabrication is complete, the team will interact with the client to test on the microscope and record data to be analyzed and make more changes if necessary.

IX. References

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Appendices

A. Product Design Specifications

Function:

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

Client Requirements:

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

Design Requirements:

1. Physical and Operational Characteristics

   a. *Performance requirements:*

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

   b. *Safety:*

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

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

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

e. *Shelf Life:* Power off when not in use and store properly. If using batteries, store in a dry environment to prevent from heat source to secure a higher lifespan.

f. *Operating Environment:* The microscope will be used in a lab class environment for experiments. Presumably under room temperature and normal conditions. No direct exposure to sunlight and limited shakes and tilts followed by proper microscope use guidelines. Cover the device with a plastic cover to ensure no dust or corrosion gets in contact.

g. *Ergonomics:* The entire equipment used must be small and not bulky to ensure the comfortable usage of the microscope. The mechanism of the stage must not interfere with the actual functioning of the microscope and must integrate with the software used. The equipment should be easy to carry and fit for storage.

h. *Size:* The product should be as small and compact as possible. It should not get in the way of microscope operation. The components must be enclosed, but still able to access for maintenance.

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

j. *Materials:* A 3D printer will primarily be used from UW's makerspace to print plastic prototypes. The printing method chosen will most likely be FDM/FFF methods [9]. In addition, a laser cutter from Makerspace will be needed. The model included is the Universal ILS9.150D [6]. An analog 2-axis thumb joystick for operating the device will be purchased. A low cost option is a joystick with select button and breakout board, used by the 2022 Spring BME students. Any materials that cost over a total of $100 should not be used.

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

2. Production Characteristics

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

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

3. Miscellaneous

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

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

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

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

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

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

PDS References:

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