DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Microscope Slide Scanner

BME 200/300 - Preliminary Report

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Abstract

Many cytology labs require the use of microscope slide scanners in order to digitize images of their microscope slides. There are many current slide scanners on the market that are able to provide fast, high resolution images of slides. However, these slide scanners are extremely expensive, and out of reach for those working in low budget cytology labs. Thus, there is a need for a new method of slide scanning that is cost effective and accessible to more labs. The design described in this report utilizes two stepper motors to turn existing x and y axis controls on a microscope stage and ImageJ software to stitch together individual photos of the cytology slide. The design was able to control the movement of the slide in order to produce overlapping pictures that were then stitched together. However, the stitching process has not been fully automated, and more testing is required to ensure image quality. The stepper motors will also need to be more permanently attached to the microscope stage in the future. The finalized product will eventually provide multiple labs on the UW Madison campus with an affordable option of producing high quality and efficient scans of microscope slides.

I. Introduction

Motivation

Cytology labs require the use of microscope slide scanners to create digital copies of the slides they scan. Digital microscopy has introduced novel ways to improve tissue-based research and imaging. They do this by allowing for faster and easier sharing of images via computers, improving storage methods, and allowing for annotations of features on the slides. Additionally, they provide many benefits when it comes to education. [1] However, many slide scanners are expensive, and hard to acquire for cytology labs, especially those at public universities. Therefore, there is a need for a method to create a low cost slide scanner to expand accessibility and availability of high quality slide scanners to a broader audience.

Existing Devices and Current Methods

The client's lab currently uses a Leica Aperio CS2 Slide Scanner. This slide scanner creates digital images of microscope slides from your desktop [2]. However, the client has found many issues with this scanner. Because the scanner is an older version, it is time consuming to scan slides, and thus the clients are unable to scan the number of slides they wish to in a day. Additionally, this slide scanner is unable to capture the high resolution images the client needs, especially of the nucleus of cells. This is in part due to the old camera on the scanner, but also due to the z-axis limitations that the scanner has.

There are currently several commercial microscope slide scanners on the market that meet all of the needs of our client. The Hologic Genius Digital Imager uses Volumetric imaging to capture high resolution, in-focus digital images of microscope slides. It also utilizes AI capabilities and images of multiple z-axes of the slide to address the issue of multiple z-axis. Additionally, this scanner has a 400 slide capacity, and can scan slides at a much higher rate [3]. Morphle Labs Inc also creates microscope slide scanners that offer live z-stacking, volume scanner, and whole slide imaging. These scanners offer high resolution images, can image around 120 slides a day, and have a short scanning time [4]. The Hamamatsu NanoZoomer S20 Digital Slide Scanner is also highly rated for pathology, and can scan approximately 20 slides in 15 minutes. They also offer desktop digital slide scanners such as the NanoZoomer-SQ, which allow for fast slide scanning, though they do not provide as high resolution of images [5]. Though these commercial slide scanners address the needs of our client, they are extremely high in cost, and thus not feasible for our clients to purchase and utilize.

Problem Statement

The team has been tasked with finding a more efficient way to scan microscope slides using digital scanning. The client’s department already has a scanner, but it is time consuming and provides low quality images. Therefore, the team must find a way to enhance the user quality of their digital scanner, as well as the images themselves. The department has also asked our

team to create software capable of housing the images. This project will benefit multiple labs who utilize slide scanning on campus, including the primate lab and SMPH.

II. Background

Relevant Biology and Physiology

Slide scanners are used to obtain digital images of a microscope slide that allow access to a virtual slide that can be easily shared and referenced in the future. They do this by taking individual images across the slide of a microscope and utilizing software to stitch the images together, allowing for a detailed digital image that can be used for a myriad of purposes. Having access to virtual slides is essential for cytologists, researchers, and educators alike. Automated slide imaging allows for an efficient way to analyze whole slides or large tissue sections [6]. However, many automated slide scanners are expensive and difficult to obtain for labs and educators. Thus, a need exists for a cheap and universal device that allows for simple automated slide imaging that can be utilized by research labs.

Client Information

The clients, Terri Stewart and Joshua Faulkes, are employees of a cytology lab at the University of Wisconsin-Madison. They require a microscope slide scanner that provides high quality images of microscope slides, especially the nucleus of cells, for educational purposes. If a final prototype is completed, multiple labs on campus may utilize the design to scan microscope slides.

Design Specifications

The prototype built for the client's lab must scan and take high quality images of slides until the client can receive the grant needed to purchase a new one. Due to the client's 20 year old scanner, many issues have been made clear. The images are blurry, dark, are time consuming to capture, and do not have good z axis resolution. It is essential to the lab to have a device that can scan slides in 10-15 minutes to maintain the needed pace for daily slide scanning. The final images must be clear with proper stitching. The slides must retain zero damage and be left completely intact during and after the scan. Additionally, the design must last the lab until they get a grant for a newer/better professional scanner. The prototype may not interfere with other equipment or operations in the lab. Our design must be small, built in, and compact to prevent interference with equipment. Lastly, any capital purchases must not exceed $5000. Finally, the design must be as universal as possible to be available for use by multiple labs on campus.

III. Preliminary Designs

Design 1: The Slide Glider

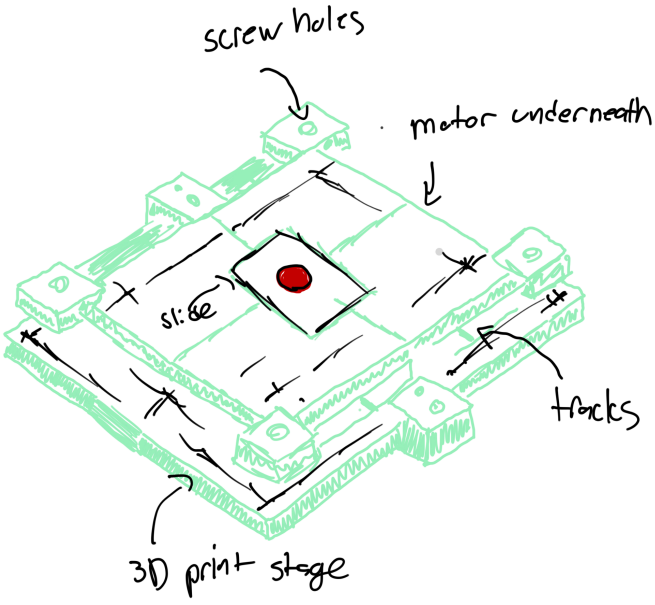

Figure [1]: The slide glider design drawing

The Slide Glider design features a 3D-printed microscope stage equipped with two motors for precise movement across the X-Y axis. The stage is supposed to replace the existing microscope stage and is secured in place with a bolt. Motors are mounted on each side of the stage. An integrated algorithm controls the motor movement and works in tandem with micromanager to automatically take microscopic pictures. Each image would be taken with a 20-25% overlap for more accurate image stitching and then instruct the motors to move to the next section of the slide. Once all images have been collected they are stitched together using ImageJ which is integrated in the micromanager software creating a high-resolution composite image. This process,"crop, stitch, save," sets the Slide Glider apart. While it shares some functionality with products like those from Zaber [7], the Slide Glider stands out with its two-motor system and its customizable, 3D-printed, replaceable stage.

Design 2: Deconvolution Software

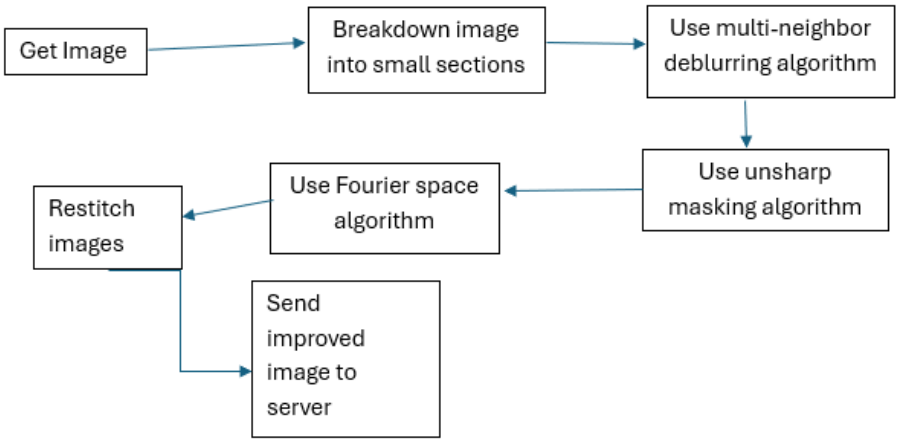

Figure [2]: Deconvolution software flowchart

The deconvolution software utilizes a set of algorithms to reduce blurriness in images that are currently produced by the existing microscope slide scanner. It processes the scanned slides by deconstructing them into their original components, which are then reassembled using ImageJ (a program for stitching images). The main algorithm used is the multi-neighbor deblurring algorithm, which uses additional image layers to estimate the locations and color of pixels within the image [8]. However, this method of deblurring (or Z-stacking) relies on predictions that can vary in accuracy especially with complex images such as our collection of microscopic slides [9]. To mitigate this margin of error and minimize the inaccuracies, we integrated unsharp masking and Fourier space algorithms [9]. The unsharp masking algorithm refines the image by aligning its resolution with the original, comparing both images, and reconstructing a sharper version based on the results of the comparison [10]. Complementary to this process the fourier space algorithm analyzes image traces of both the original and improved and applies improvements to keep their traces the same but with an improved image quality.

Design 3: AI Image Improvement

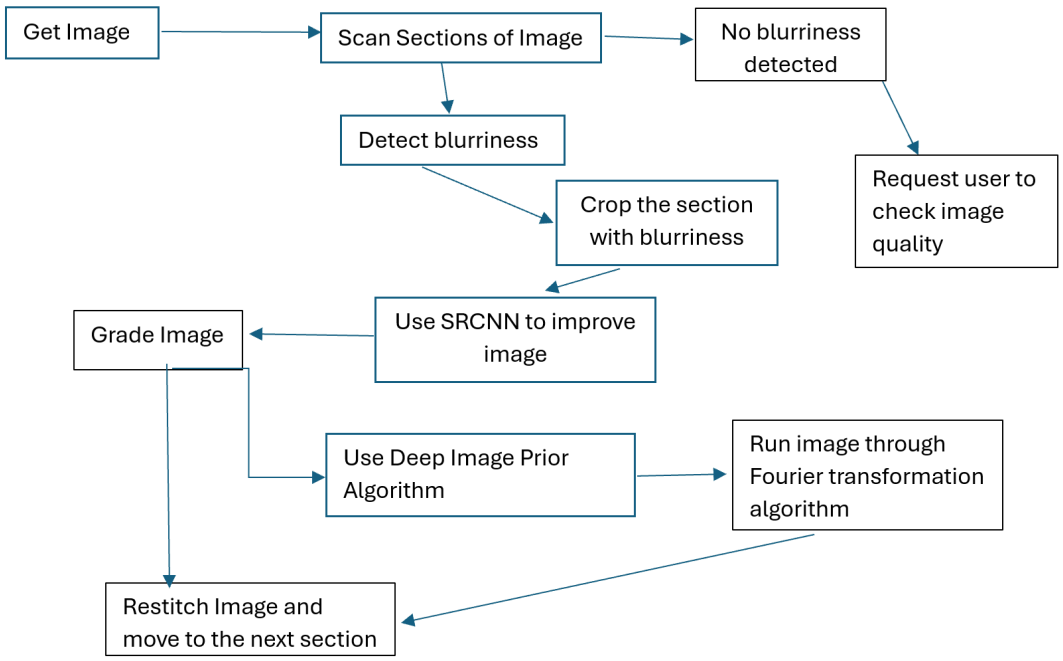

Figure [3]: AI Image Improvement flowchart

The application of AI-powered image enhancement offers significant image improvement that would meet our clients ideas. At the core of our design is the SRCNN (Super-Resolution Convolutional Neural Network. This neural network analyzes every part of the image to identify areas that require improvements based on the programmed criteria. SRCNN enhances the selected areas by applying the optimal adjustments to improve the image [11]. After the improvements the image undergoes a grading evaluation based on the predefined quality standards of our choice. If it does not meet our criteria then the Deep Image Prior (DIP) algorithm is applied. DIP calculates the image's depth by analyzing the image's color and complexity to identify and apply the most suitable improvements [10]. To further ensure the integrity of the enhanced image, it passes through a fourier transformation algorithm. This step verifies that the images structural traces remain consistent between the original image and the improved image and correct any issues detected [8]. Afterwards, the image is fully restitched and saved.

IV. Preliminary Design Evaluation

Design Matrix

Design Criteria	Design #1: Automatic Slide Glider		Design #2: Deconvolution		Design #3: AI Image Improvement	
Accuracy (30)	4/5	24	3/5	18	4/5	24
Feasibility (25)	3/5	15	3/5	15	2/5	10
Useability (20)	4/5	16	4/5	16	3/5	12
Speed (15)	4/5	12	2/5	6	3/5	9
Cost (5)	3/5	3	5/5	5	5/5	5
Manufacturability (5)	4/5	4	5/5	10	4/5	8
Total (100)	74		70		68	

Figure [4]: Preliminary Design Matrix

As shown above, the team evaluated three preliminary designs based on 6 criteria, each weighted based on our clients needs and requirements totalling up to 100 points. Accuracy, the highest-weighted criterion, measures how effectively our design improves the scanned slides image quality. This was prioritized as the client works in a cytology lab that focuses on teaching and diagnostics that requires accurate and clear images to avoid misdiagnosis. Our next criterion, Feasibility, assesses whether the design can be developed within the semester with our skills to ensure that the client has a usable device for teaching purposes. Usability is another important criteria, measuring how easy and accessible our design is for our client. Our client also mentioned a desire to improve the speed, however it was not our client's main issue, therefore it was not deemed as critical as the other criterias and was assigned a lower weight. Cost was also necessary to consider due to the project budget, but it was deemed the less significant due to the general low-cost of our designs compared to the available budget. The last criterion, manufacturability, or the ease of replication was also determined to be of low significance since it is not a primary concern for this semester.

The first design, Automatic Slide Glider, scored the highest overall, excelling in most key categories. It achieved top marks for accuracy, as the client's current microscope already meets the clients clarity standards, and the proposed design aims to automate and enhance the processes. The design is highly feasible to fabricate. Leveraging the team's existing skills and knowledge. Its fully automated process, from scanning slides to stitching images, with a press of a button ensures exceptional usability. Additionally, the mechanism proposed operates faster than the client's current microscope slide scanner. Despite the cost and manufacturability being rated moderately due to the need of expensive motors and specific materials, the design's strong performance in key categories solidifies the design as the top choice.

The second design, deconvolution design, received a lower accuracy score because it is uncertain whether this method can adequately address the blurriness issue in the scans. However, it scored high in feasibility and usability since it involves repurposing pre-existing software and developing a user-friendly interface for the client. The design scored poorly in speed, as it relies on capturing multiple Z-axis images, a computer-intensive process that our client's office level computer will struggle in running. On the other hand, its reliance on software makes it very cost-effective simply requiring only memory space. Additionally, because this design is software based it can be easily replicated and manufactured. Despite its strengths, the general mediocre scores in the critical criterias ultimately makes the design not the best choice.

The third design, AI Image Improvement, has scored very high in accuracy and cost. Advanced scanners utilizing AI have demonstrated excellent image quality fulfilling our clients needs. Furthermore, as it is a software-based design ensures low costs. Despite these advantages, the design has many drawbacks. Including processing speed, computer-intensity, and feasibility. These issues heavily affect the usability of this design. Additionally the team lacks the knowledge to develop AI software within the given time-frame. These drawbacks make this design not a viable choice despite its great potential.

Therefore, the team has decided that the Automatic slide glider is the most suitable option, scoring the highest in the categories that align with the client's needs. It offers a balance of accuracy, speed, usability, and feasibility making it our best design choice to improve the process of slide scanning and imaging.

Proposed Design

Based on our design criteria our team has chosen the Automatic Slide Glider as the final design, primarily due to its high accuracy and feasibility. This design leverages the client's microscope camera, which meets our client requirements regarding image clarity, to create an easy and user-friendly way to digital scan the slides. Additionally, the team's skill set aligns with the manufacturing requirements of the design, ensuring the design can be built within the limited timeframe. This design streamlines the scanning process, making it fully automated and much easier to use and utilize compared to the other designs. Comparing the other designs, the

Automatic slide glider was the only one not depending on the old scanner the client used. Despite the minor challenges of cost and manufacturability the cost remains within the allocated budget and the manufacturability is manageable within the scope of the semester. These factors solidify the automatic slide glider design to be the optimal choice.

V. Fabrication

Materials

This project budget consisted of no capital purchases over $5000. Our team purchased 2 Nema 17 Stepper motors with a microstep of 0.9 to precisely control the movement of our stage. Getting a stepper motor with the smallest available microstep allows us to move at smaller intervals which is essential since we are trying to take microscoping pictures. We got steel L beams to provide a strong bracket that could hold both the weight and tension of our design. The L beam or Bracket allows us to position the motors at any point in the vertical axis making one type of L-bracket usable for both sides of the base where the movement control rods are located. The team also got two brackets made of steel designed specifically to hold the Nema 17 motor with a hole for the timing belt. To connect the two steel brackets, we purchased sets of nylon bolts and nuts due to their strength, but also lightweight. Two A4988 stepper motor drivers and an Arduino Uno were also acquired for the purpose of controlling the movement of the stepper motors.

Methods

The construction of our prototype was created with the equipment from UW Madison's TeamLab as well as parts ordered online for component orders and mechanical pieces. The primary motor bracket and timing belt set up was constructed in SolidWorks. Multiple timing belts were attached and later modified to experiment with belt tension and distance from the stage. The brackets were modified with a drill press to allow two points of attachment through the premade adjustable slits for increased strength. Due to issues mounting multiple motors to just one stage control knob, we regeared the stage setup to permit a x and y axis knob on each side. In doing this, we allowed for one motor on each side with individual timing belts resulting in more reliability and simplicity. Since the team did not want any permanent alterations to the microscope, the mechanism was attached with duct tape on the top, as well as glued support beams that can be detached if needed. While the design ended up with a dual stepper motor/timing belt setup using the existing stage to save time and increase simplicity, the team started with a design of a movable stage, but this proved far too sophisticated and time consuming.

The proposed design originally required the use of a RAMPS 1.4 board to control the movement of the stepper motors. The ends of the stepper motor wires were then soldered to female connectors in order to connect to the board. However, problems occurred when uploading

the code for the RAMPS board due to possible complications with the board itself. Therefore, the team took two of the A4988 motor drivers off of the RAMPS board and connected them to a breadboard. A circuit was then set up with the stepper motors drivers, stepper motors, and an Arduino Uno board to control the movements of the motors. The final circuit board and arduino were then securely placed in the back of the microscope so the components wouldn't interfere with the movement of the microscope stage.

Mechanical Design

The final design utilizes a combination of two Nema stepper motors, L brackets, Nema motor brackets, two timing belts, and a rotating gearing system in the x and y direction respectively. The stage control knob already attached to the microscope allows for the movement of the stage in the xy direction. However, for the final design, the x axis was disconnected on the right and the gearing system was rerouted to the left so that there were independent controls on each side of the stage. Adding a second stage control knob, the team could now precisely position the stage along the xy coordinate plane without the inference of two directions on one stage control knob and how motor attachments would join them. With the independent control knobs, the final design consisted of mounted L brackets with a Nema 17 bracket and motor mounted to the end with nylon screws and nuts. As seen in figure [5] , the timing belt is attached to the stepper motor which then wraps around the x and y direction control knobs, allowing for the automation. Lastly, belt tension was a necessity to permit the knobs actual movement. When loose, the belt would refrain from turning or would fall off. To combat this issue, the project was finalized by adding support beams to both sides to keep the beams and motors timing belts under a constant tension capable of turning the mechanism.

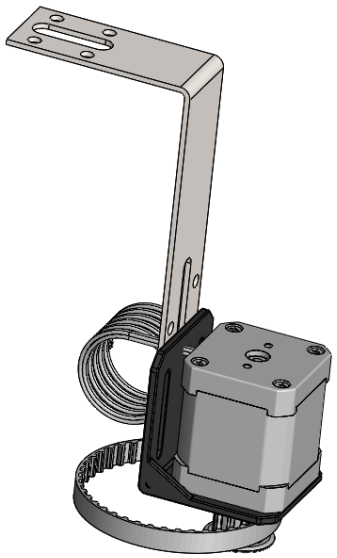

Figure [5]: CAD model of stepper motor, L bracket, and timing belt

Electronic Design

The goal of the electronic portion of the final design is to turn two stepper motors at different time intervals in order to move the entire slide through the microscope. Two A4988 stepper motor drivers were used to control each NEMA 17 stepper motor. The drivers were connected to a 12V power supply. Since the drivers are susceptible to LC voltage spikes, a 100 µF capacitor was added between the 12V power supply and ground [12]. Each driver was then connected to a stepper motor and the STEP and DIR pins were connected to digital pins on the Arduino UNO board. The RST pin was also shorted to the SLP pin on each driver in order to enable the movement of the motor.

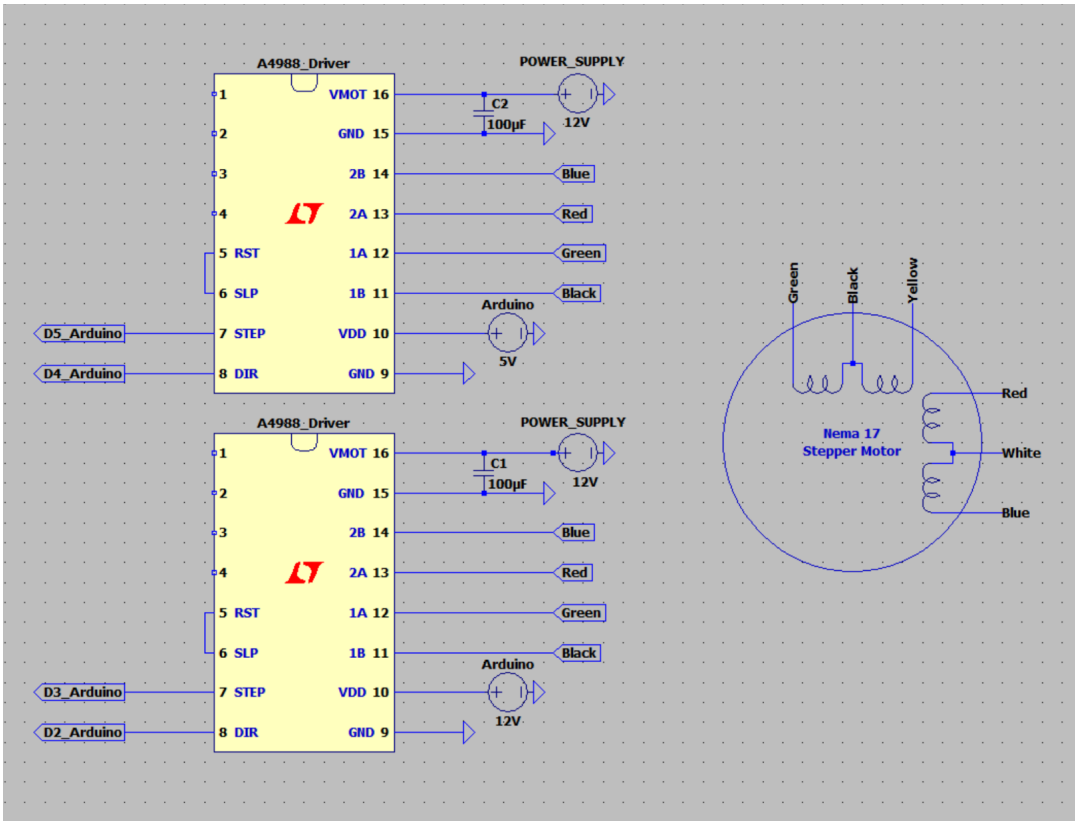

Figure [6]: Final circuit diagram showing the NEMA 17 stepper motor and A4988 driver connections

Code was then written in Arduino IDE with the goal of moving the motors in a snake like fashion (right, down, left, down, etc.). The code consists of one big loop that runs the entire program for 11 iterations before exiting. The program inside consists of 4 parts: moving the slide to the right for 25 iterations, moving it down once, moving it the left for 25 iterations, and moving it down again. In order to move the slide to the right, the direction pin (DIR) on the motor controlling the x-axis was set to low in order to spin the motor counterclockwise. The step pin (STEP) is then set to high then low with a delay of 1 second repeatedly for ⅛ of a revolution

in order to move the motor one step. This is done 25 times with a delay of 1 second between steps, however, this delay can be increased to allow for more time between steps so that pictures can be acquired for every portion of the slide. The direction pin on the motor controlling the y-axis is then set to low in order to move the motor clockwise thus moving the slide down. The step pin is then set to high for one iteration so the slide only moves ⅛ of a revolution once. The x-axis motor is then coded to go through the same process as above, except the direction pin is set to low so the motor moves clockwise, moving the slide to the left. The y-axis motor is then activated for one more step to move the slide down again before the same process continues for the required 11 iterations. The Arduino IDE script can be viewed in Appendix E.

ToupView was downloaded in order to view the microscope slide on a computer for the purpose of acquiring individual pictures [13]. Setting up ToupView requires downloading the required version from ToupTek and using a USB cable to connect a laptop to the AmScope camera located on the microscope. The camera can then be shown in a live view on ToupView. Micro-Manager was initially used to project the microscope slide on a laptop, however, many issues occurred with connecting Micro-Manger with the driver for the AmScope camera and the image quality of the microscope slide. Therefore, ToupView was used for testing/viewing purposes.

VI. Testing and Results

Data Collection

The stitching process was originally going to be executed in Micro-Manager, but due to problems with connecting the camera's driver, this was not possible within the time allowed. However, since Micro-Manager uses ImageJ/Fiji software in order to stitch photos together, the team was able to test within ImageJ. In order to check the requirements for properly stitching together photos, two cytology photos were acquired and cropped into a 3 by 3 grid. Three tests were then performed based on the amount of overlap between photos: none, 10%, and 20%. The photos were then stitched within ImageJ using the gridwise stitching plugin and a snake-by-rows format [14]. Matlab code, seen in Appendix D, was then used to assess the similarity between the fused and original photos. The complete testing process can be found in Appendix C.

The required distance each slide had to move was then determined via altering the amount of a revolution the stepper motors turned for each step. The motors were first programmed to turn ⅛ of a revolution, then, as the slide was moved through the microscope, three pictures were taken in ToupView. The pictures acquired had shadows around the edges due to the circular nature of the lens, so each photo was cropped to 81.42% of its original size. A notable cell cluster was then marked throughout the three photos in order to approximate the amount of overlap between them. This process was repeated with the motors programmed to run at ⅛ of a revolution. Two photos from each test were then stitched together using the pairwise stitching plugin in ImageJ to check for any differences between the different overlaps.

One requirement that was important for the client was the scan time. Therefore, as the slide was repeatedly moved through the microscope, the team took note of the time it took for the slide to move across one time. This value was then used to assess whether the amount of overlap between sections would still produce a scan time between 10 and 15 minutes.

Data Analysis

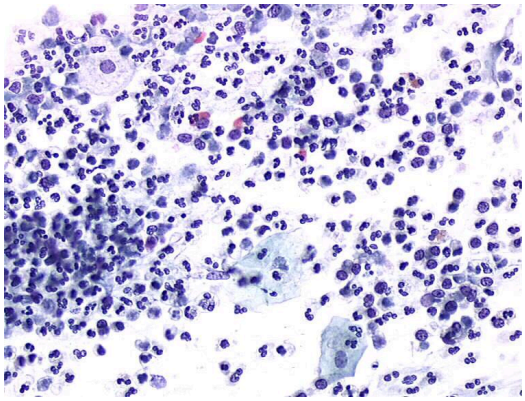

Figure [7]: Test 1 original photo [15]

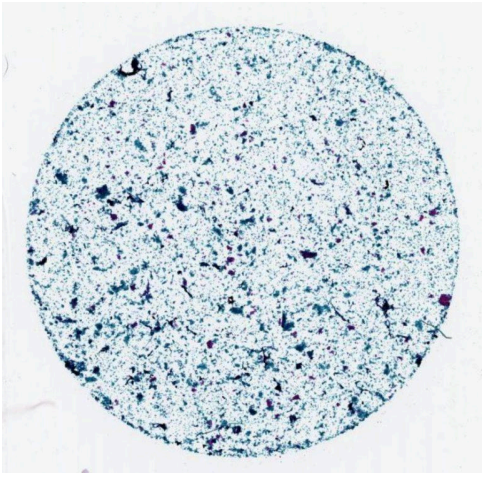

Figure [8]: Test 2 original photo [16]

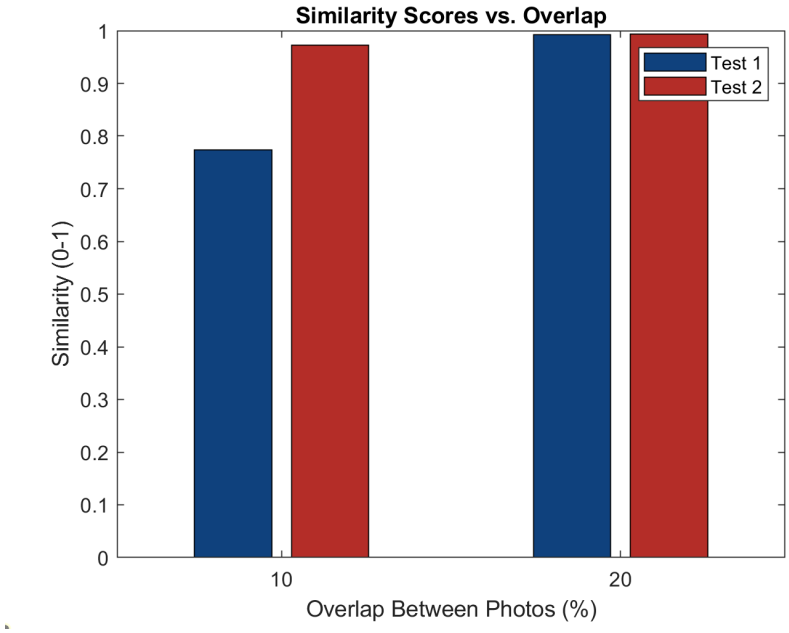

Figure [9]: Graph showing similarity scores per overlap for each test

Figures 7 and 8 show the original photos for tests 1 and 2 respectively which were used to test the ImageJ stitching software. Figure 9 summarizes the results by comparing the similarity scores for each test at 10% and 20% overlap. When no overlap was used between photo sections, the pictures were placed in the wrong spots with varying amounts of black space around the edges. Therefore, due to vast differences in size compared to the original photos, the pictures' similarity could not be assessed using the Matlab code. This shows that ImageJ relies on a certain amount of overlap in order to place the photos in the right order. The similarity scores above were on a scale of 0-1 with 1 being the exact same photo as the reference image. The scores varied by 0.2188 at 10% overlap and only 0.0209 at 20% overlap. This shows that at around 20% overlap, even more complex photos, such as test 1, are able to be fused at a level similar to photos that are easier to stitch. Test 2 already had a high score at 10% overlap, but this still increased by 0.75% at 20% overlap. This shows that increasing the overlap will continue to increase the quality of the fused photos. Therefore, the team decided that 20% was the minimum value of overlap needed between photo sections, but aimed to obtain a value higher than this threshold in order to increase the quality of the scanned slides.

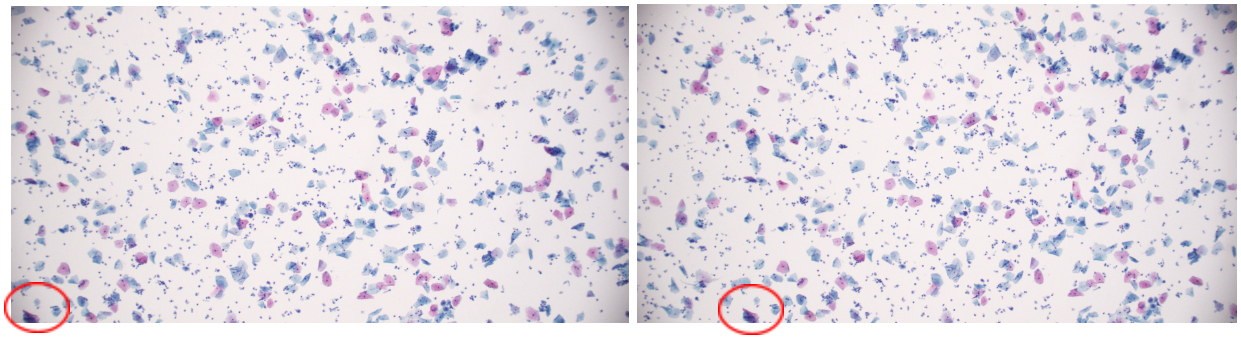

Figure [10]*:* Consecutive photos taken with motor set to ⅛ of a revolution

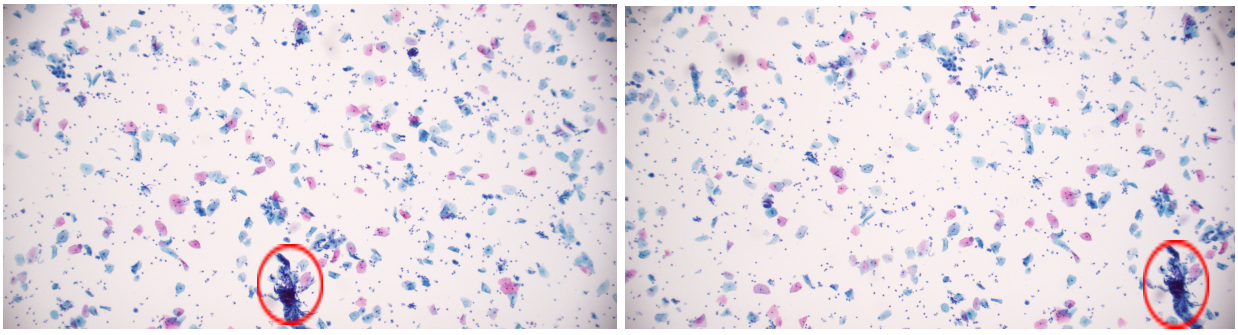

Figure [11]: Consecutive photos taken with motor set to ⅛ of a revolution

A notable cell cluster was marked in each set of photos seen above. When comparing the location of each, an approximate overlap of 70% can be seen in Figure 10, when the motors were turned ⅛ of a revolution. Figure 11, when the motors moved ⅛ of a revolution, produced around 40% of overlap. A minimum value of 20% was required between photo sections which both

movements achieved. However, when timing the scanning process, it took around 13 minutes for a slide to scanned when the motors moved ⅛ of a revolution which was in the target value of 10 to 15 minutes. Therefore, this value was chosen for the movement of the motors. The amount of steps required to cover the length and width of the slide was then calculated to be 25 across and 22 down and was used to determine the amount of iterations needed for each movement.

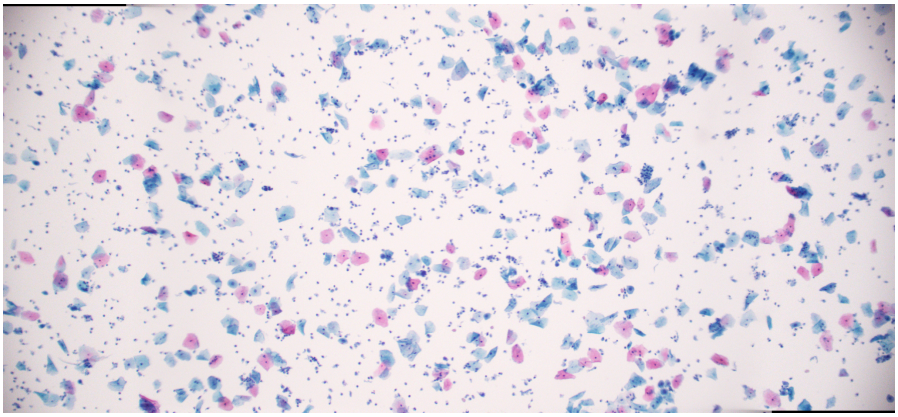

Figure [12]: Fused photos with motor set to ⅛ of a revolution

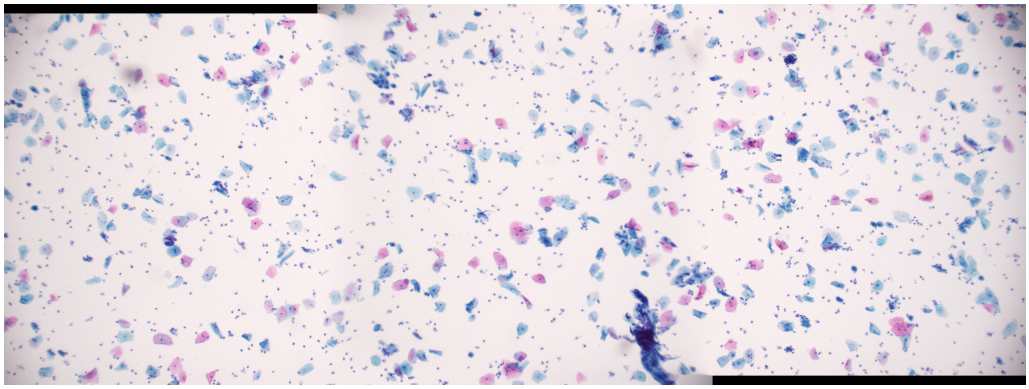

Figure [13]: Fused photos with motor set to ⅛ of a revolution

The two photos seen in Figures 10 and 11 were stitched together and produced the images shown in Figures 12 and 13 respectively. Black spaces can be seen at the corners for each but this effect was reduced when there was 70% overlap between photo sections. This could be due to the way the slide moved through the scanner, and since the scan time was too slow for 70% overlap, it was determined the motors should continue to produce photo sections with 40% overlap. More testing is needed to determine if this problem is still present when the entire slide is scanned. However, it is very likely that including the next row in the fused photo will fill in the black sections so that every part of the slide is included in the scanned photo.

VII. Discussion

The results implicate that it is integral to manufacture stage movement that allows for image overlap, to provide the most accurate image stitching possible. It was determined that images with 40% overlap should allow for proper stitching after more testing is completed. There is an ethical consideration to ensure image stitching is as accurate as possible, as cytology microscope slides can be used for diagnostic purposes, as well as educational purposes. Ensuring proper stitching will guarantee cytologists and students looking at slides are able to properly investigate the slides and no part of the slide is missing.

After evaluation, it is clear that more work will need to be done to ensure proper image stitching. More testing will need to be done with different image overlap values to ensure the value chosen is as accurate as possible. Additionally, changes may need to be made to the speed of stage movement to ensure that the system is able to properly capture images of each section of the slide. This will help to ensure proper stitching and image accuracy. There are potential sources of error for image stitching due to inconsistent stage movement. The team was unable to attach the stepper motors to the stage in a permanent manner, meaning the stepper motors may have moved during stage movement, thus leading to innacture stage movements. Additionally, it is possible for the belts attached to the stepper motors to ‘slip’ when rotating the stage handles, leading to inaccurate stage movements.

VIII. Conclusions

The team was tasked with developing an automated device to improve the scanning and stitching of microscope slides, addressing the client’s need for accuracy and usability in a cytology lab. Initially, the Automatic Slide Glider was selected as the preferred design due to its strong performance in key evaluation criteria. However, as the project progressed, the team shifted to a dual-mounted motor setup, realizing time constraints. This updated design provided greater precision and reliability within the constraints of the project timeline and available resources/backgrounds.

The final design uses two NEMA 17 stepper motors mounted on custom steel brackets, with timing belts transferring motion to the microscope’s stage control knobs for independent movement along the x and y axes. Careful attention was paid to maintaining consistent belt tension and minimizing interference with the microscope’s original structure, ensuring precise stage movement without permanent alterations. An Arduino UNO, paired with A4988 stepper motor drivers, controls the motors in a snake-like pattern to achieve consistent overlap between images. This setup balances automation and adaptability, leveraging existing microscope components while significantly enhancing its functionality.

While the prototype showed promising results—such as achieving adequate image overlap and maintaining scan times within 10–15 minutes—key features remain incomplete. The integration of mechanical and software components is still in progress, and image stitching is not yet fully automated. Additionally, the motor mounting system, though functional, would benefit from redesigns to improve durability and alignment for long-term use.

Future work is essential to realize the full potential of the device. Priorities include fully integrating ToupView with μManager for automated control, customizing the RAMPS board for compatibility, and further refining the Arduino code for smoother operation. Mechanical improvements, such as adding end stops for precise stage control and optimizing the motor mounting system, will enhance reliability. Testing will also need to expand to ensure compatibility across different labs and with multiple users.

Although the project remains unfinished, the dual-mounted motor prototype establishes a strong foundation for continued development. With further refinements and testing, this design has the potential to revolutionize slide scanning workflows, offering a precise, automated, and user-friendly solution for cytology labs focused on diagnostics and education.

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X. Appendix

A. Product Design Specifications

Product Design Specification

Microscope Slide Scanner

Team Members:
Lia Lejonvarn (Leader)
Amanda Kothe (Communicator and BSAC)
Xavier Snider (BWIG)
Hamad AlDhaheri (BPAG)

Client: Teri Stewart

Advisor: Dr. James Trevathan

September 14, 2024

Function

The team has been tasked with finding a more efficient way to scan microscope slides using digital scanning. The client's department already has a scanner but it takes a while to scan one slide and the images are not of the best quality. Therefore, we must find a way to enhance the user quality of their digital scanner as well as the images themselves. The department has also asked our team to create software capable of housing the images. This project will benefit multiple labs who send in slides for processing including the primate lab and SMPH.

Client requirements

- Develop a method of digitally scanning slides that is more efficient
- Find a way to increase the quality of the scanned images
- Develop software capable of housing the slides once scanned
- Reduce the time it takes to scan one slide

Design requirements

1. **Physical and Operational Characteristics**
    a. **Performance requirements**
        i. The method of scanning should produce images that are of notable quality when compared to the current method of scanning including a reduction in the blurriness seen around edges of cell clusters. This method should take less than 20 minutes, the current time it takes to scan one slide, with a goal of around 5 to 10 minutes. There should also be a software based method of housing the scanned slides.
    b. **Safety**
        i. The scanner should follow basic FDA safety guidelines regarding medical related devices [11].
        ii. The scanner should not interfere, contaminate, or alter the slides.
        iii. The scanner should be properly insulated with no short circuit that could possibly lead to fires in the lab [12].
        iv. The scanner should have an emergency response in case of broken slides within the slider [10].
    c. **Accuracy and Reliability**
        i. The method of scanning should produce consistent results across all slides and take less than 20 minutes to scan.
    d. **Life in Service**
        i. The product will be used to scan five or more slides for around five days a week and must last for at least a year.

e. **Shelf Life**
    i. The product must be able to hold up for at least a year or until an updated scanner can be obtained by the lab.
f. **Operating Environment**
    i. The scanner will be operating within a traditional lab.
    ii. The scanner would be required to work efficiently indoors in a well ventilated area with an electrical outlet.
    iii. The scanner must be able to operate remotely so that the user can access relevant scans from any location they may be in.
g. **Ergonomics**
    i. The scanner must not interfere with other equipment in the lab either due to size or power surging.
    ii. The scanner must be able to effectively scan the slides and notify the user once it finishes scanning a batch of slides.
    iii. The scanner should be equipped with handles or a mechanism to move/transport between areas or buildings.
h. **Size**
    i. The scanner must be able to fit into the clients lab without problem.
    ii. The scanner must be able to hold the preferred amount of slides for scanning.
i. **Weight**
    i. The scanner weight should not exceed 5 Ibs, since the typical weight of the scanner is between 2-4Ibs depending on existing models [9].
j. **Materials**
    i. A scanner is usually made of a combination of sensors, specifically CCD, CMOS, and sCMOS sensors[9].
    ii. Scanners also typically use LEDs and halogen lamps to properly maintain the illumination within the scanner[9].
    iii. Scanners also use sensitive cameras, a possible improvement over the current camera is an Amscope digital camera or an Omax digital camera[7].
k. **Aesthetics, Appearance, and Finish**
    i. The aesthetics of the scanner should not reduce efficiency of the scanner.
    ii. The aesthetics of the scanner should not impede or interfere with the surrounding environment and maintain its safety standards
    iii. The finished product's appearance and aesthetics are not as relevant provided that the scanner is effective and efficient meeting the clients needs and other requirements documented in this document.

2. **Production Characteristics**
    a. **Quantity**

i. Only one method of scanning is needed at this time as well as one method of storage.

b. **Target Product Cost**

i. The client would prefer that edits are made to existing devices, however, if new devices or technology need to be purchased, it must be less than $5000.

3. **Miscellaneous**

a. **Standards and Specifications**

i. eCFR 493.1274 Standard: Cytology clarifies that when cytology labs are using automated and semi-automated screening or scanning devices, the laboratory must follow the manufacturer's instructions for the machine [4]. Additionally, eCFR Standard 1254.80 specifies that when using a slide scanner, slides must be checked after scanning to ensure that no damage occurs when the slide is in the scanner. Automatic feeder devices on flatbed scanners are prohibited. Light sources in the scanner must not raise the surface temperature of the slide being scanned. Finally, no part of the equipment may come in contact with the slides in a way that will cause friction, abrasion, or any damage to the slides [5].

b. **Customer**

i. The customer for the slide scanner is Mrs. Teri Stewert. Working in the Cytotechnology program in the Wisconsin State Laboratory of Hygiene, she needs to digitally scan and upload slides for research and student purposes. Scanners on the market are either far too costly, or not permitting access to their resources. Her scanner is slow, outdated, and takes a long time with dated software.

ii. This product is being made with our client in mind, however, there is a large market for slide scanners and could be utilized by many researchers and or labs.

c. **Patient-related concerns**

i. The software used to store data must be able to keep the scanned slides confidential to those with access. Thus, it is important to ensure that there is a secure login feature in place to ensure confidentiality and protect sensitive information found in the scans.

d. **Competition**

i. There is a fast array of slide scanners currently being utilized in labs and on the market today. Some examples of which are, Motic Digital Pathology, Hologenic (genius system), Grundium, and others.

ii. Prices can reach upward of 250,000 making for unrealistic tools for lower budget labs.

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B. Material Cost Table

item	description	manufacturer	Vendor	Date	Cost	#	total	link
Motor	Stepper motor with full step increment of 0.9 degrees and shaft radius of 4.994mm	Nema	Mcmaster	11/18/2024	$87.41	2	$174.82	Link
Pulley	Corrosion-resistant Timing Belt Pulley with a trapezoidal teeth shape and a diameter of 25mm as well as shaft diameter of 6mm	lily-bearing	Mcmaster	11/18/2024	$13.67	2	$27.34	Link
Belt	A belt with trapezoidal teeth big enough to encase the microscope moving mechanism as well as the belt pulley	Mcmaster	Mcmaster	11/18/2024	$8.40	2	$16.80	Link
Duct Tape	General tape	Rugged Blue	Walmart	11/25/2024	$4.45	1	$4.45	Link
L-shape Brackets	An L shape bracket with dimensions of 101x60x30mm	Biaungdo	Amazon	11/25/2024	$11.85	3	$35.55	Link

	(Corner base)							
motor Brackets	Motor Brackets specifically made for our nema stepper motor made of alloy steel	OSM Technology Co.Ltd	Amazon	11/25/2024	$7.26	2	$14.52	Link
Bolts	Nylon bolts used to attach the brackets together 10x30mm thread size	Biaungdo	Amazon	11/25/2024	$8.49	4	$8.49	Link
Endstops	Endstops with operating V=300 Volts and current rating of 2 Amps plugs in with our board	AIMOCN	Amazon	11/25/2024	$8.99	6	$8.99	Link
Stepper motor drivers	The A4988 stepper motor driver carrier is a breakout board for Allegro's A4988 microstepping bipolar stepper motor driver. It has an adjustable current limit and five different microstep (1/16-step) operates with 8-35V up to 1A.	Pololu	Pololu Robotics and electronics	11/25/2024	$4.49	2	$8.98	Link
Arduino Uno Board	Arduino Uno REV3 CPU speed 16MHz Memory storage 32KB Ram Memory 0.2MB	Arduino	Amazon	11/25/2024	$27.60	1	$27.60	Link
						total	$327.54	

C. ImageJ Testing Protocol

Test 1: Stitching with 10% overlap

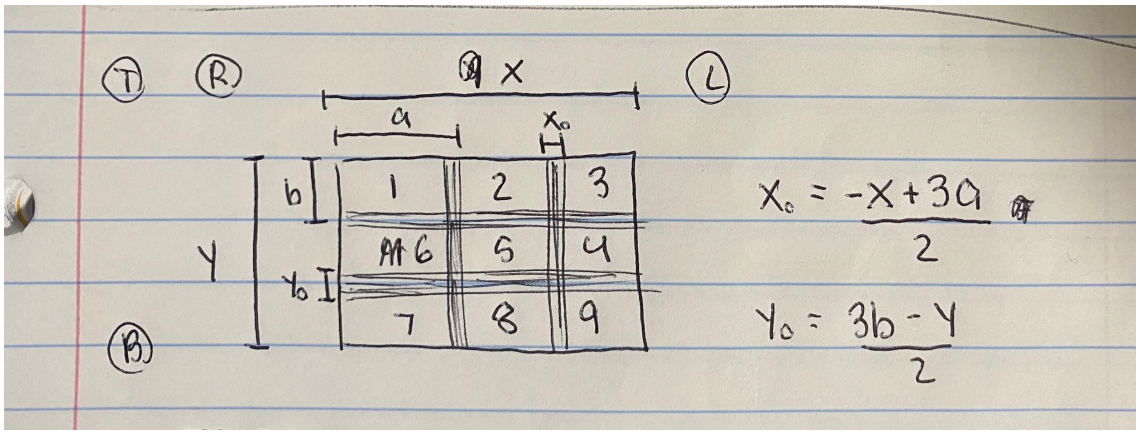
Photo 1

Process:

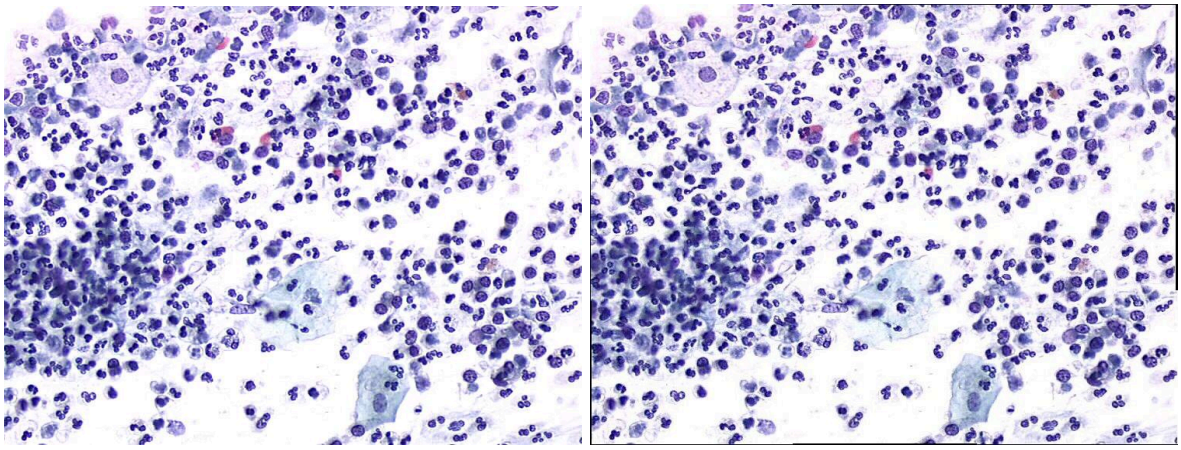
1. Download Fiji (an updated version of ImageJ) [1]
2. Find a picture of a cytology slide online, then crop it into 9 photos using the layout and pixel dimensions as a guide

3. Save each cropped photo using the same name with a different number according to where it lies in layout 1
4. In Fiji, go to Plugins -> Stitching -> Grid/Collection stitching
5. Set type as Grid: snake by rows and order as Right & Down then press ok
6. Set Grid size x and Grid size y as 3, select fusion method as linear blending and select compute overlap then press ok
7. Fused image should be displayed, go back to Fiji and save image
8. Run matlab similarity code to evaluate effectiveness of stitching (1 = greatest similarity)

Layout 1:



Original [2] **vs. Fused photo:**



Similarity Score: 0.7740
Overlap: 12 pixels in the x dimension and 15 pixels in the y dimension

Discussion:
When comparing photos, the code requires they be the same size. The stitched photo was one pixel larger in the x dimension and was cropped on the left hand side before calculating similarity. The overlapped photos also lined up too far in some parts causing the images to be

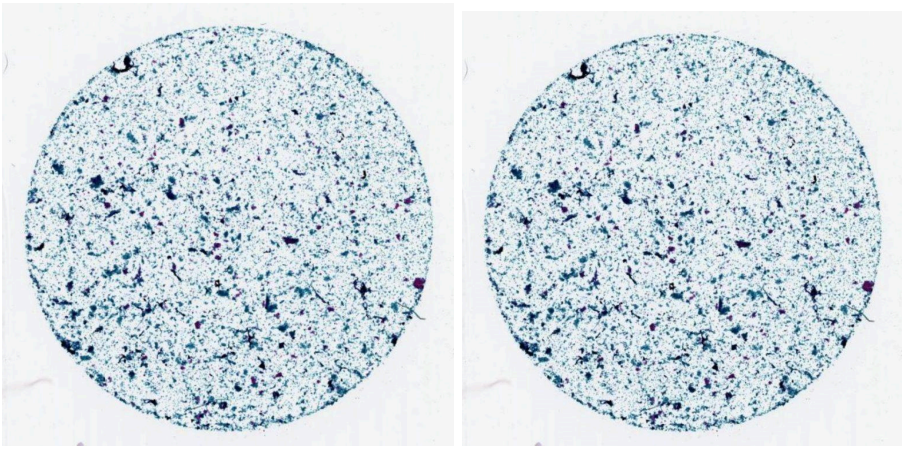
shifted slightly off center. This is what's causing the disjointed outline of the picture.This could possibly be fixed with either different settings or smaller overlap. One option might be to use pairwise stitching on the photos one at a time which might negate any errors or show where errors are occurring. However, this would take too much time for the actual project application.

Photo 2

Process:

**Same as above using different photo

Original [3] **vs. fused photo:**



Similarity Score: 0.9928

Overlap: 21 pixels in the x dimension and 14 pixels in the y dimension

Discussion:

Since there were problems with the edges of the previous photo, I attempted the same process using a cytology scan typical of what the client's current scanner outputs with the slide shown as a circle with a white outline. The similarity score was much higher than the previous one. This could be due to the change in outline or possible errors in the first cropping process. To further analyze the differences seen, both photos will continue to be used for the other tests.

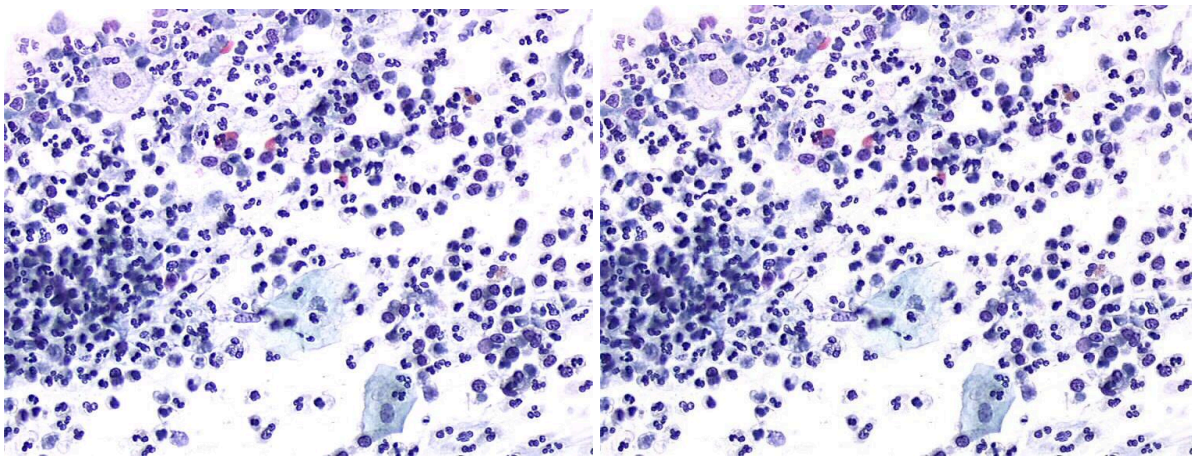
Test 2: Stitching with 20% overlap

Photo 1

Process:

**Same as above with greater Xo and Yo

Original vs. fused photo:

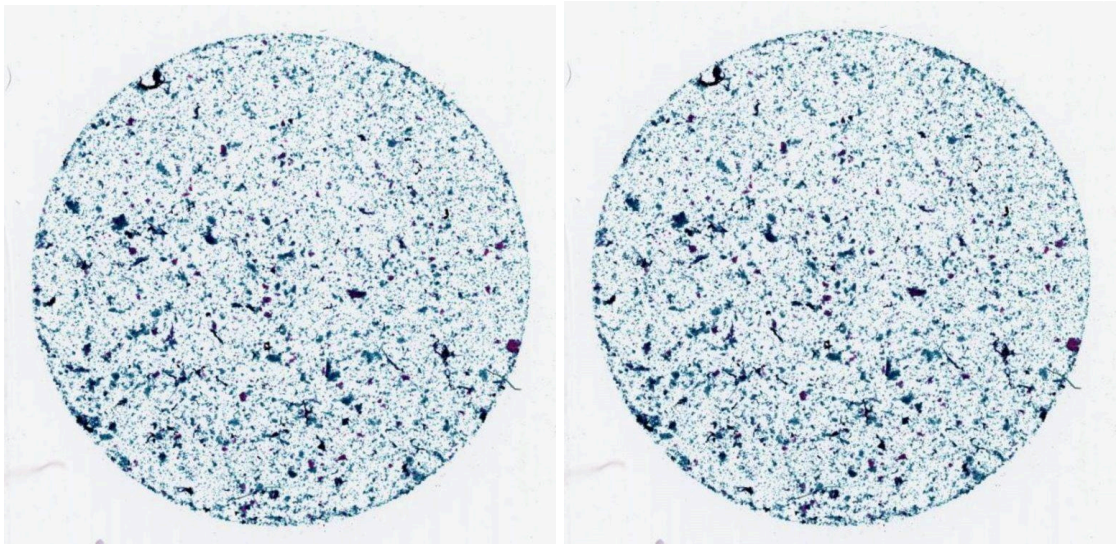


Similarity Score: 0.9722
Overlap: 42 pixels in the x dimension and 45 pixels in the y dimension

Discussion:
The similarity was greatly increased from trial 1. This seems to be due to the increase in overlap, however, performing this trial with the second photo will show whether it is the overlap itself or a lack of errors in the cropping process compared to the first trial.

Photo 2
Process:
**Same as above with greater Xo and Yo

Original vs. fused photo:



Similarity Score: 0.9931

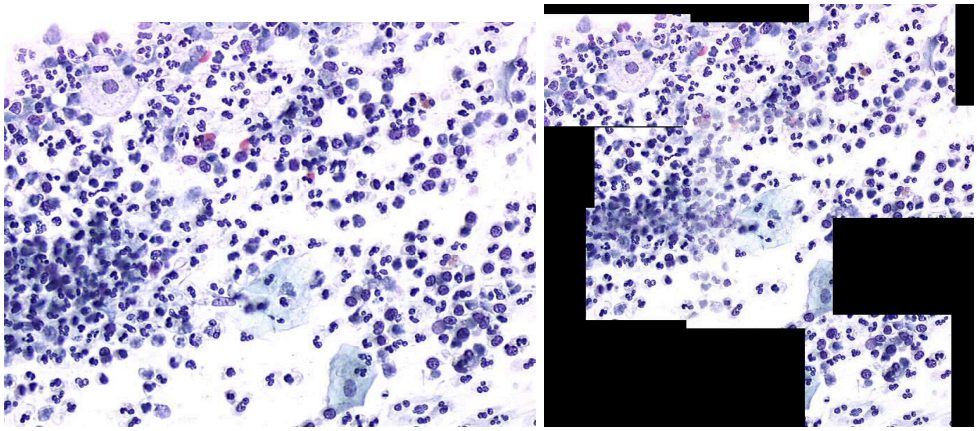
Overlap: 51 pixels in the x dimension and 43.5 pixels in the y dimension

Discussion:
The similarity score was increased by only 0.03% when cropped with greater overlap. It seems that once the right amount of overlap is achieved to stitch the photos together properly, increasing this value does not increase the accuracy by much. However, it seems large overlaps will help with accuracy instead of impeding it. This means our automated stage does not have to achieve high accuracy in terms of where pictures are taken as long as there is enough overlap to stitch them together.

Test 3: Stitching with no overlap

Photo 1
Process:
**Same as above with no overlap (Xo and Yo are 0)

Original vs. fused photo:

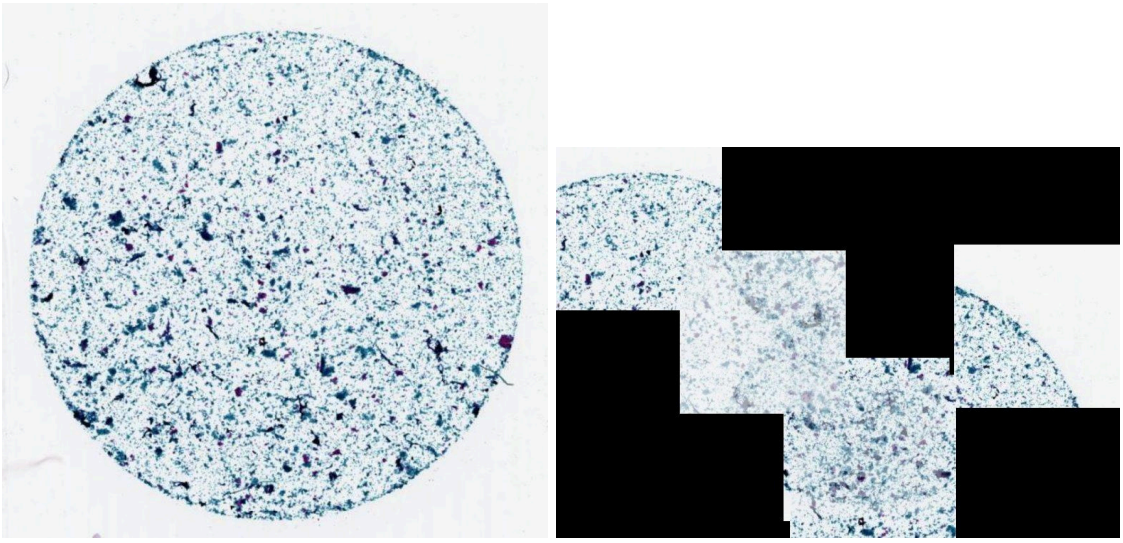


Similarity Score: N/A
Overlap: 0

Discussion:
A similarity score was not calculated for this trial due to the vast difference in the picture sizes. It is also very clear from looking at the photos that the pictures were not stitched together with any accuracy. It seems that some level of overlap is needed in order to know where the pictures place relative to each other. Performing this same trial with the second photo will make it clear whether the lack of an outline on this photo is causing less accuracy in the stitching process.

Photo 2
Process:
**Same as above with no overlap (Xo and Yo are 0)

Original vs. fused photo:



Similarity Score: N/A
Overlap: 0

Discussion:
This outcome matches that of the first photo. Once again finding the similarity between photos is not possible due to the difference in sizes, however, you can clearly see that the pictures did not stitch together correctly. This further shows that overlap is necessary for the algorithm to correctly place where the photos are supposed to be stitched together. This will affect how our automated stage moves and where the photos are cropped.

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D. Matlab Code

```
%Cyto Test Simularity Analysis
%References:https://www.mathworks.com/matlabcentral/answers/584714-how-do-i-compare-two-images-and-find-the-similarity-percentage
%Load in images with A being the fused photo and ref being the original
%Test1
ref1 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test1\cyto_test_original.jpg");
A1_10 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test1\cyto_test_fused_cropped.jpg");
A1_20 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test2\cyto2_fused.jpg");
%Test2
ref2 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test1.5\pap_original.jpg");
A2_10 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test1.5\pap_fused.jpg");
A2_20 =
imread("C:\Users\liale\OneDrive\Desktop\BME300\cyto_test2.5\pap2_fused.jpg");
%Calculate similarity score
ssimval1 = [ssim(A1_10, ref1) ssim(A1_20, ref1)];
ssimval2 = [ssim(A2_10, ref2) ssim(A2_20, ref2)];
ssimval = [ssimval1 ; ssimval2];
figure;
bar([10 20], ssimval);
legend('Test 1', 'Test 2');
title("Similarity Scores vs. Overlap");
ylabel("Similarity (0-1)");
xlabel("Overlap Between Photos (%)");
colororder("earth");
```

E. Arduino IDE Code

```
// Adapted from
https://www.makerguides.com/a4988-stepper-motor-driver-arduino-tutorial/
// Define stepper motor connections and steps per revolution:
#define dirPin_x 2
#define stepPin_x 3
#define stepsPerRevolution 200
#define dirPin_y 4
#define stepPin_y 5

void setup() {
```

```
  // Declare pins as output:
  pinMode(stepPin_x, OUTPUT);
  pinMode(dirPin_x, OUTPUT);
  pinMode(stepPin_y, OUTPUT);
  pinMode(dirPin_y, OUTPUT);
}


void loop() {
//Complete cycle 11 times
for (int i=0; i<11; i++) {

//Move microscope slide across 25 times

for (int i=0; i < 25; i++) {
   // Set the spinning direction counterclockwise:
  digitalWrite(dirPin_x, LOW);

  // Spin the stepper motor 1/6 revolution quickly:
  for (int i = 0; i < stepsPerRevolution/6; i++) {
    // These four lines result in 1 step:
    digitalWrite(stepPin_x, HIGH);
    delayMicroseconds(1000);
    digitalWrite(stepPin_x, LOW);
    delayMicroseconds(1000);
  }

  delay(1000);

}
delay (1000);

//Move microscope slide down 1 time

//Set the spinning direction clockwise:
digitalWrite(dirPin_y, HIGH);

// Spin the stepper motor 1/6 revolution quickly:
for (int i=0; i < stepsPerRevolution/6; i++) {
   digitalWrite(stepPin_y, HIGH);
   delayMicroseconds(1000);
   digitalWrite(stepPin_y, LOW);
   delayMicroseconds(1000);
  }

delay(1000);
```

```
//Move microscope slide across 25 times

for (int i=0; i < 25; i++) {
   // Set the spinning direction clockwise:
  digitalWrite(dirPin_x, HIGH);

  // Spin the stepper motor 1/6 revolution quickly:
  for (int i = 0; i < stepsPerRevolution/6; i++) {
    // These four lines result in 1 step:
    digitalWrite(stepPin_x, HIGH);
    delayMicroseconds(1000);
    digitalWrite(stepPin_x, LOW);
    delayMicroseconds(1000);
  }
  delay(1000);
}

delay (1000);

//Move microscope slide down 1 time

//Set the spinning direction clockwise:
digitalWrite(dirPin_y, HIGH);

// Spin the stepper motor 1/6 revolution quickly:
for (int i=0; i < stepsPerRevolution/6; i++) {
   digitalWrite(stepPin_y, HIGH);
   delayMicroseconds(1000);
   digitalWrite(stepPin_y, LOW);
   delayMicroseconds(1000);
  }

delay(1000);
}
exit(0);
}
```