An Automated System To Replace A Human User In Conventional Laboratory Well Plate Imaging

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Abstract

Well plate imaging is a pervasive yet incredibly tedious research procedure by which laboratory plates (ranging from 6-96 wells/plate) are filled with biological samples to be imaged, catalogued, and analyzed. Currently, researchers must manually move and visually center a two-dimensional stage over a microscope lens before switching to a software application to complete image acquisition. Though not an inherently difficult process, it is highly repetitive and monotonous. This task can consume the limited time of a scientist and can lead to mental fatigue, which in turn leads to human errors (skipped, duplicated, or mislabeled images). These errors reduce the reliability of data collection and impede biomedical research progress. We here developed an automated well plate imaging system to eliminate the requirement for continuous human operation, thus freeing up the valuable time of a scientific researcher and removing the possibility of fatigue-induced human error. Specifically, we created a prototype system with programmed two-dimensional movement, automated calibration, variable plate configuration compatibility, variable path feasibility, reliable well plate image capture, and an intuitive graphical user interface. Successful implementation of our device would immediately benefit laboratory scientists, giving them more time to pursue the next biomedical breakthroughs.

Introduction

Background

The standard well plate is a cheap, versatile, and extremely common piece of equipment that can be found in laboratories all around the world. The typical scientific well plate consists of a clear, 3-dimensional rectangular plate filled with hollowed-out cylindrical wells. The outer plate dimensions are fixed and the wells are aligned in pre-defined matrices according to the total well number, for example a 6 well plate consists of a 2x3 matrix and a 12 well plate consists of a 3x4 matrix (**Fig. 1**). A variety of biological or chemical assays can be performed in a well plate, but one of the easiest ways to collect data is by simply imaging the samples in each well. For example, time-dependent proliferation of cell clusters or solution-dependent swelling of hydrogel capsules can both be effectively observed via well plate imaging.

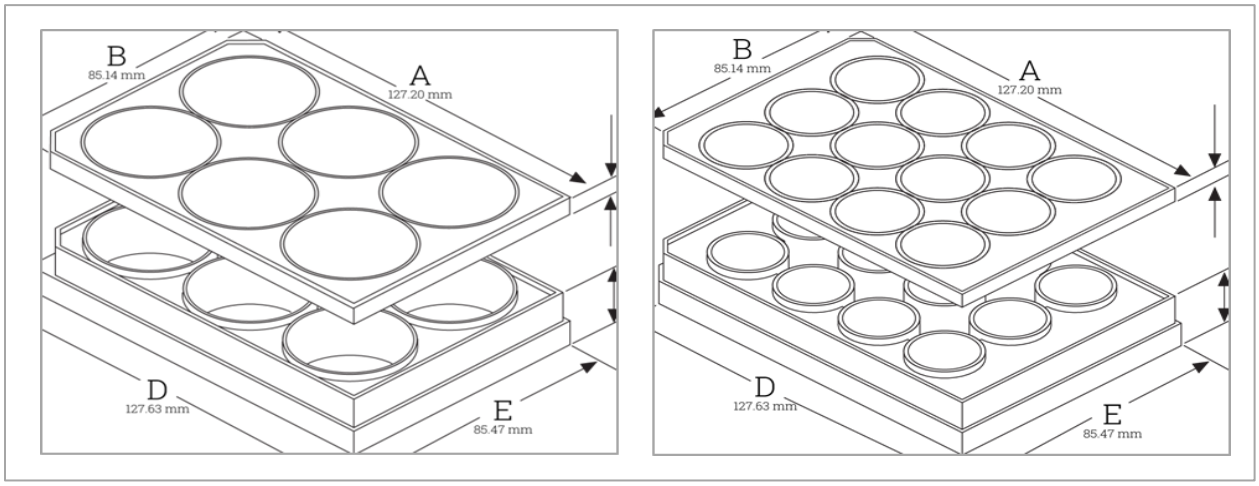


Figure 1. Schematic of a 6 Well Plate vs. a 12 Well Plate.

Procedure

Researchers deposit their samples within the various labeled wells within a plate, and then may choose to perturb or monitor the samples over time. To capture images of the samples, researchers place the well plate on an XY stage and then manually manipulate the stage in two dimensions to correctly position the well of interest over a camera lens (**Fig. 2**). Because of the need for a high degree of spatial accuracy and precision, manual positioning comprises the bulk of the time required for imaging execution. Once the stage is placed in the right location, researchers then utilize software to capture, label, and save the image in a desired digital location (**Fig. 2**).

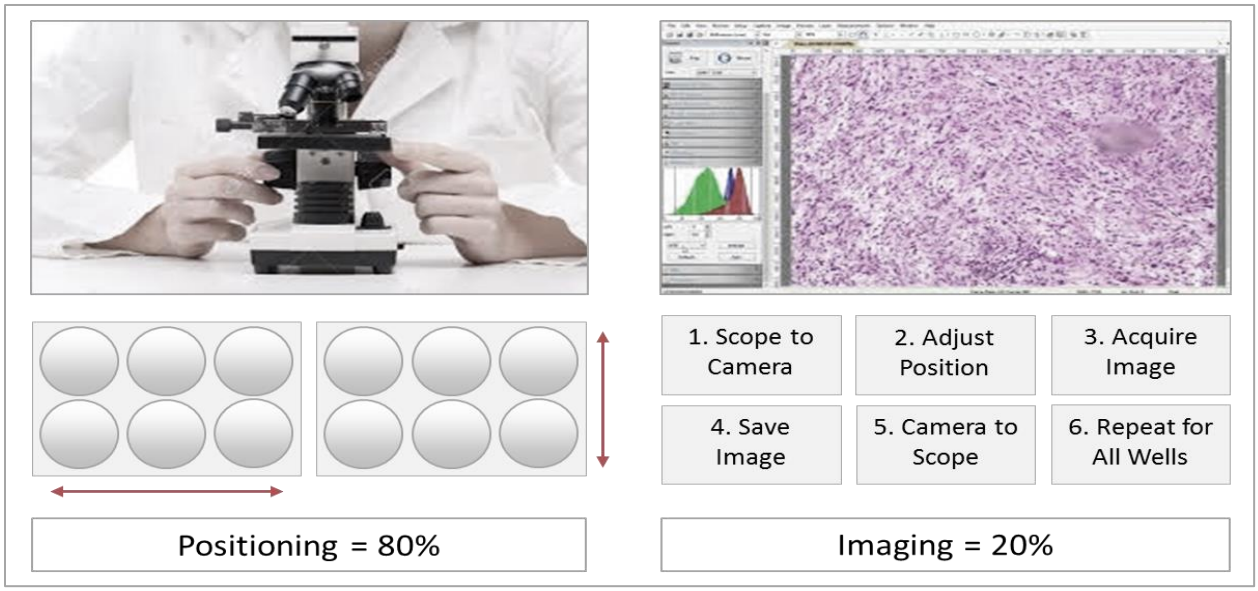


Figure 2. The Basic Procedure for Manual Well Plate Imaging.

Problem

Unfortunately, this manual procedure must be repeated many times to accumulate the required data. Since a researcher often has to analyze several well plates per day, and since each plate contains 6+ wells, well plate imaging can result in significant consumption of a researcher's valuable time (**Fig. 3**). To make matters worse, the dull and tiresome nature of the procedure can quickly lead to mental fatigue, which increases the probability of human error. Lapses in concentration can lead to skipped, duplicated, or mislabeled images, which decreases the reliability of the collected data (**Fig. 3**).

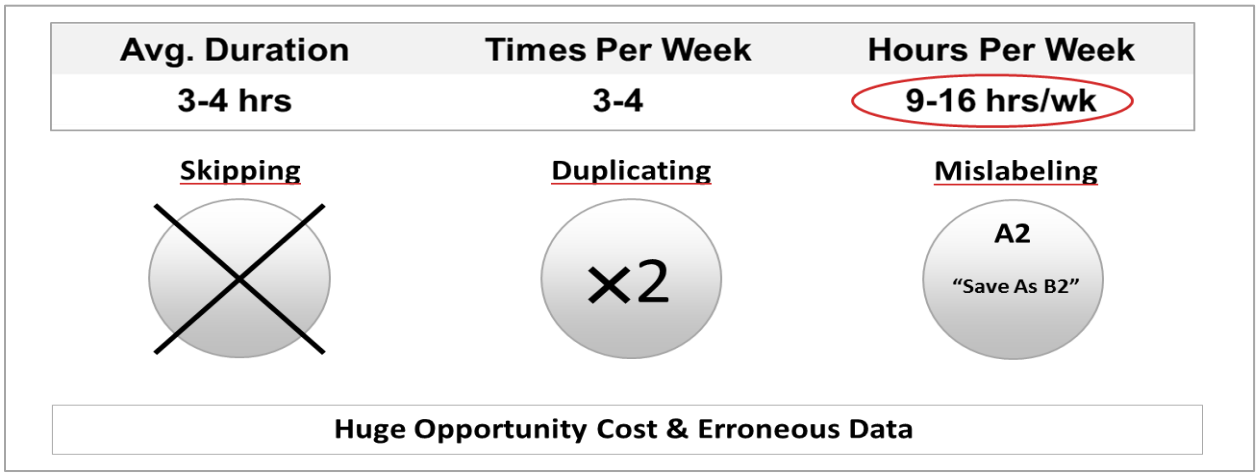


Figure 3. The Drawbacks of Manual Well Plate Image Collection.

Review

To summarize, manual well plate imaging is a ubiquitous and well-accepted method for the study of laboratory specimens (**Fig. 4**). It is easy to perform and provides crucial information about the state of the samples in question. However, it also places large demands on a scientist's limited time and the monotonous nature of the task can lead to fatigue-induced human errors (**Fig. 4**). Because of the highly repetitive nature and the propensity for manual errors, we identified well plate imaging as a technique particularly well-suited for automation.

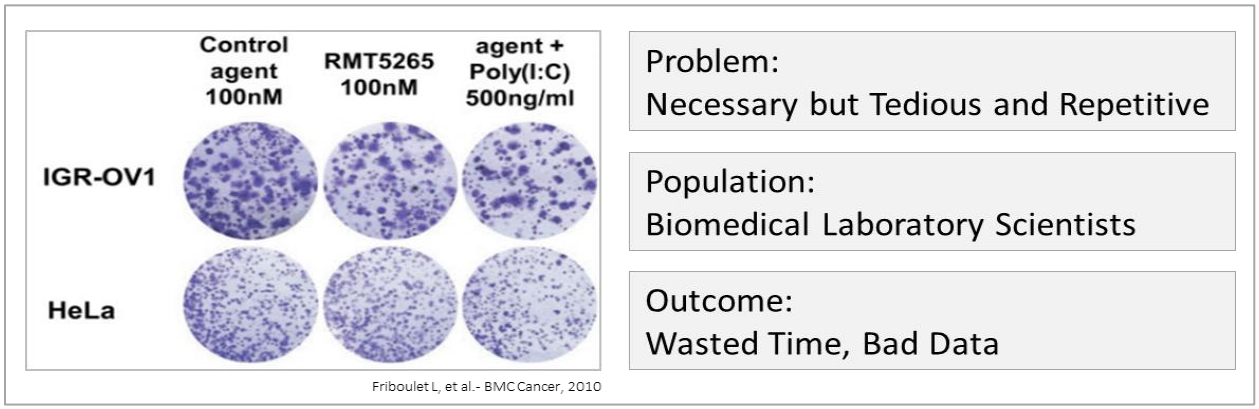


Figure 4. An Example of Published Images Acquired from a Well Plate and the Problems Associated with Manual Imaging.

Design Goals and Parameters

Project Vision

Well plate imaging is currently achieved through repetitive and inefficient human effort. An automated device would save hundreds of man-hours that should be better spent on mentally demanding tasks like experimental design or data analysis. A more efficient allocation of human scientific brainpower could lead to increased scientific output and ultimately better outcomes for patients who depend on biomedical research (**Fig. 5**).

Figure 5. Projected Benefits of Device Implementation in Biomedical Laboratories.

Design Constraints

We identified several design constraints to guide the development of our system. First and foremost is the mechanical constraint we faced by appropriating a decommissioned XY microscope stage. This forced us to creatively utilize the existing stage mechanics to achieve the positioning functionality. Because the device would replace a scientist we recognized both spatial and temporal constraints; the platform needs to comfortably fit within a standard laboratory setting and it should (at the very least) perform a task as quickly as a human user. Lastly the system must be low cost (to make it economically feasible to implement in a laboratory) and capable of robustly performing hundreds of positioning and imaging cycles (**Fig. 6**).

Constraint	Description
Mechanical	Device Commandeers Pre-Existing Stage Mechanics
Spatial	Platform Will Fit on Standard Laboratory Benchtop
Temporal	Imaging Cycle Performed in Reasonable Timeframe
Financial	Device Should Be Low Cost
Sustainability	System Should Be Capable of Thousands of Cycles

Figure 6. Constraints on Device Development and Implementation.

Specific Project Goals

To replace a human user, the most crucial device functions are reliable XY positioning and reliable well plate imaging. Without the ability to program and control stage motion in 2 dimensions, the device cannot accurately or precisely locate samples of interest. Without the ability to capture an image, the device cannot record samples for a scientist to analyze later. These two design goals formed the baseline for acceptable device functionality (**Fig. 7**).

Beyond that, we also looked to develop and implement a number of higher order functions. The system should be compatible with more than one type of well plate, allowing a researcher to utilize the plate configuration that best suits the current research need (i.e. the device should be capable of toggling between a 6 or a 12 well configuration) (**Fig. 7**). The system should also provide an option for variable, user-defined imaging routes (i.e. the scientist could choose exactly which locations should be imaged, rather than waiting for the device to image every location in the well plate during every cycle) (**Fig. 7**). Further, the platform should be capable of an auto-calibration sequence to detect and store the stage's zero point, which is the reference value for all future XY positioning calculations done by the system. This step would ensure precision over multiple imaging

cycles and diminishing the probability of system errors affecting the next set of measurements (**Fig. 7**). Lastly, the device should have a simple and easily utilized graphical user interface to allow a researcher to quickly calibrate and execute a plate imaging cycle (**Fig. 7**).

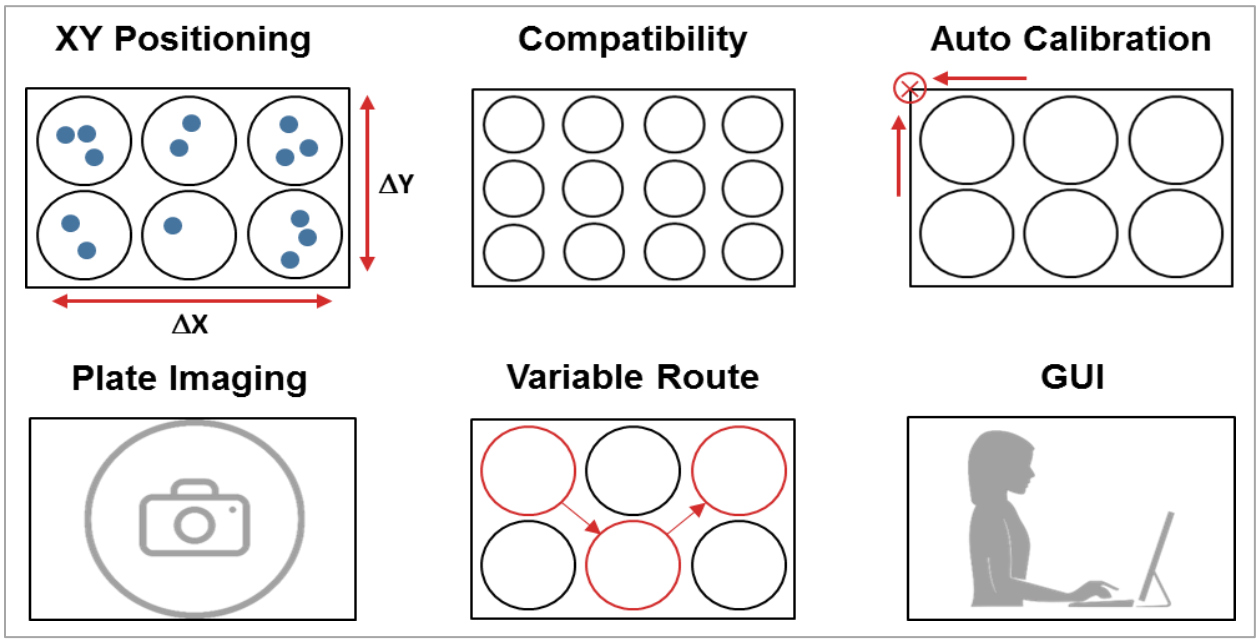


Figure 7. Project Goals for Core Device Functionality.

Design Concept

General Device Setup

To develop a fully automated system we first repurposed a decommissioned stage from an old microscope. The goal was to use stepper motors to robotically manipulate the manual X and Y control knobs of the stage, with a physical resolution comparable to (or exceeding) a human user. The motors were controlled via an Arduino and by altering the code a user could target specific locations within the workspace of the stage. At the first stage of the project, manipulating the microcontroller required hard coding via the Arduino Integrated Development Environment, which was not user friendly. To hard code we used a simple linear conversion between stepper motor steps and x-y stage translational distance. Because the stage was not custom built we experimentally determined the ratio between motor steps taken and stage distance moved. Contact switches were added to provide reference points for the X and Y axis calibration sequence (**Fig.8**).

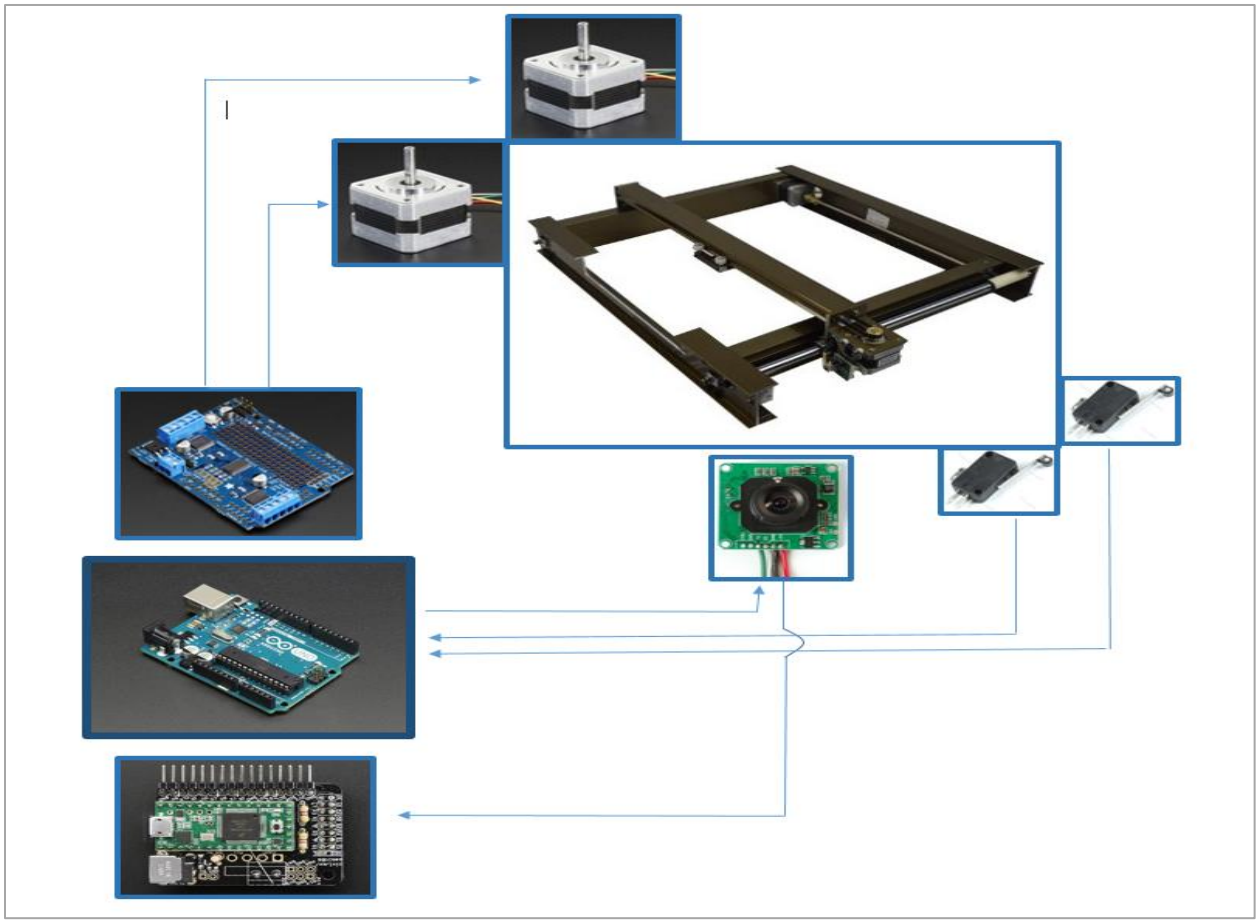


Figure 8. General Electro-Mechanical Arrangement for System.

Device Communication Scheme

The stepper motors were electronically controlled via connection to a microcontroller. The Arduino interacted with the motors through an Adafruit V2 Shield that was placed on top of the female pins of the Arduino Uno (**Fig. 9**). The contact switches were connected directly to the unused pins of the aforementioned shield. We designed and uploaded a master code into the Arduino so that stage control could occur through Serial communication, removing the need to continuously hard code the device with the IDE. Controlling the stage required manually sending a particular string of predetermined characters that represented certain commands (**Fig. 9**). The next step was to build a GUI (through LabVIEW) to allow any user to easily interact with the device. The Arduino code ran on a continuous loop that waited for a string to come through the Serial port. When the Serial port became available and a string was sent, the incoming string was decomposed into characters and interpreted as commands (**Fig. 10**). These commands made the motors move the stage according to the user's choice. When the correct XY location was reached, the camera then received a signal to take the picture of the well at that position. The GUI constructed the string (that the Arduino code interprets) when the

user pressed certain buttons (**Fig. 11**). Those buttons were Boolean inputs that LabVIEW used to concatenate the final string that was sent to the Arduino.

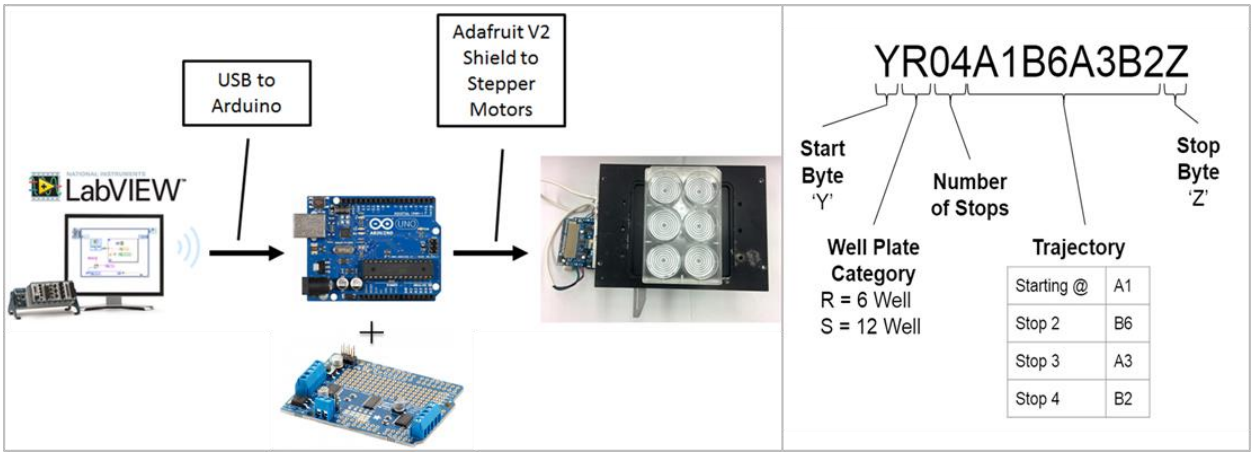


Figure 9. Overview of the Device Communications Scheme. Example of the Device Communication Protocol (Concatenated Character String).

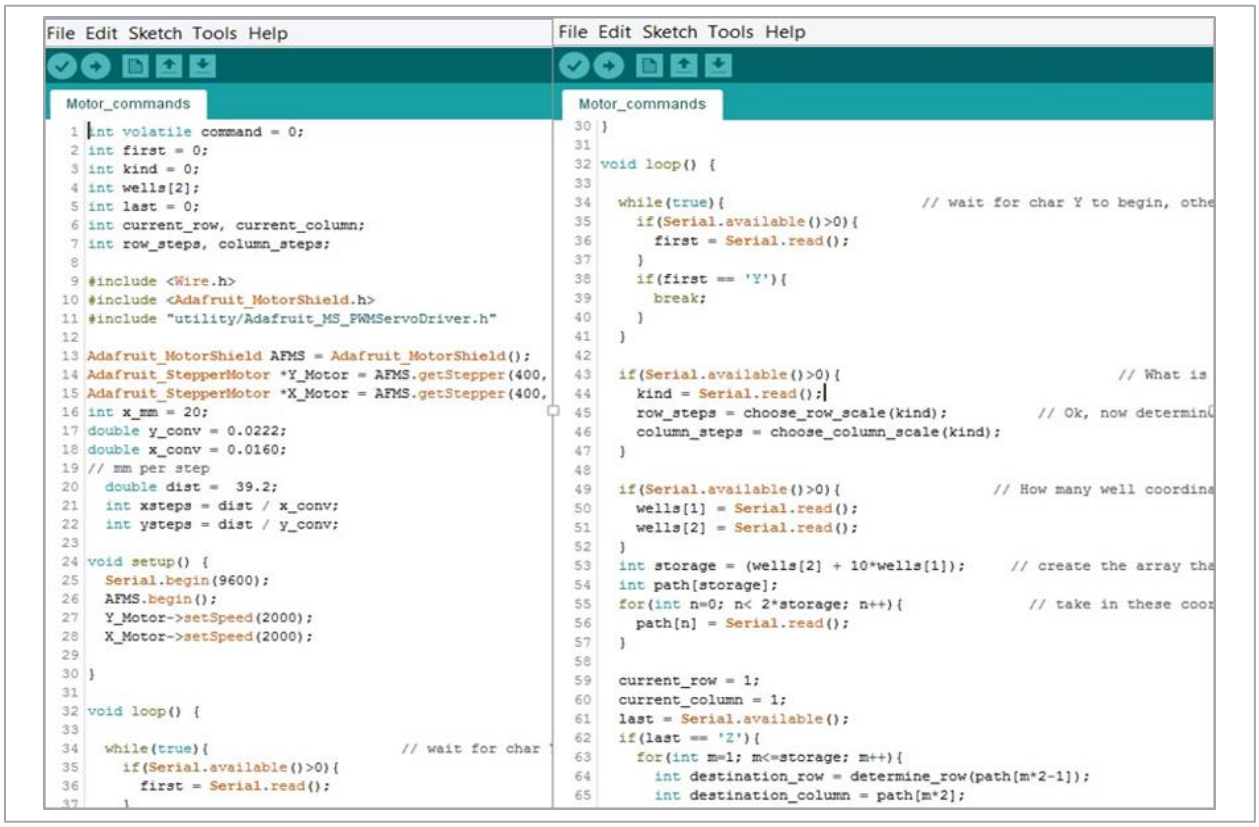


Figure 10. Arduino Coding for Converting GUI User Input into System Commands.

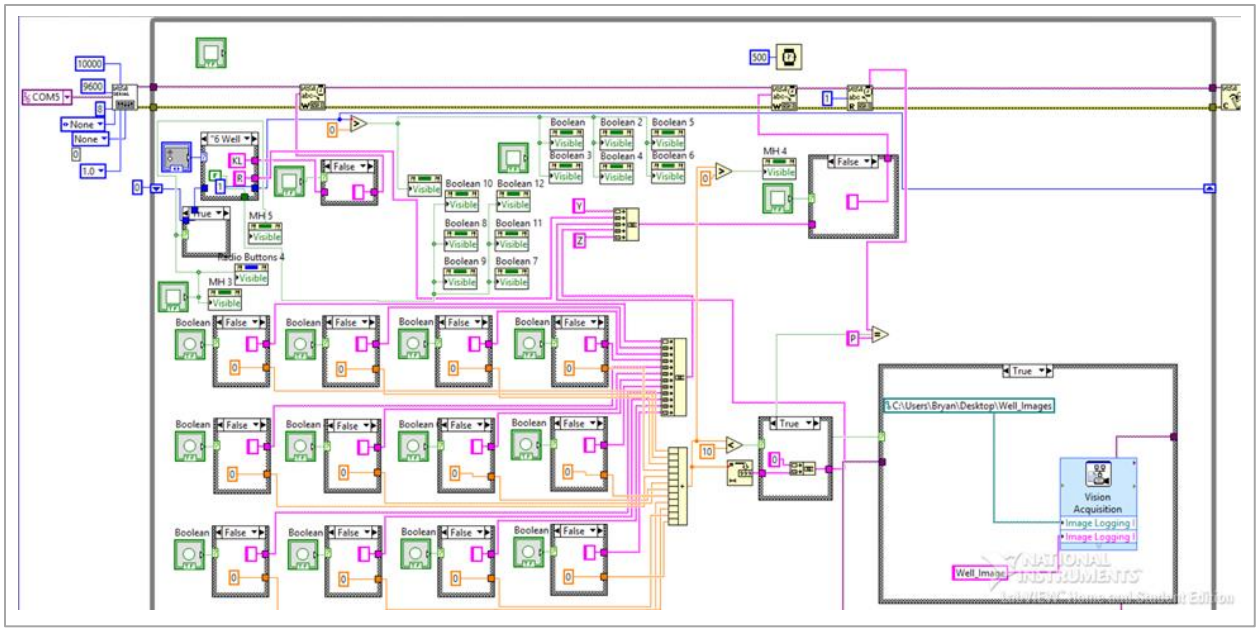


Figure 11. Labview Programming for Device Graphical User Interface.

Design Execution

Motor Attachment to Stage

First the microscope stage had to be retrofitted with a metal anchor plate that was used to attach the stepper motors to the stage (**Fig. 12**). This anchor plate was parallel with the two-dimensional plane of the stage and the stepper motors were fitted perpendicularly onto the bottom face of the anchor plate (such that the motors would be suspended vertically once the stage was flipped upright).

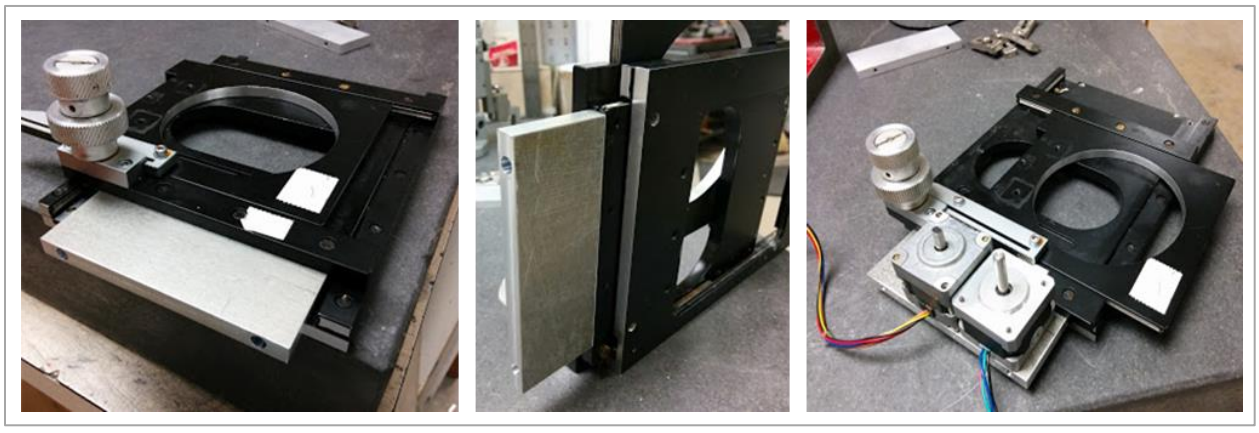


Figure 12. Attachment of Motor Anchor to Stage. Attachment of X and Y Axis Stepper Motors to Anchor Plate.

Motor Attachment to Microcontroller

After anchoring the motors to the stage they were subsequently connected to the existing manual control knobs of the stage via the belt (**Fig. 13**). As the stepper motor turned it subsequently turned the corresponding control dial, thus allowing for precisely programmed stage motion in the XY plane. The stepper motors were in turn controlled by an Arduino Uno. The Arduino interacted with the motors through an Adafruit V2 Shield (placed on the female pins of the Arduino Uno) (**Fig. 13**).The initial prototype of the device was controlled by tedious hard-coding but the finalized version used a clean graphical user interface to allow for user-programmed XY stage motion.

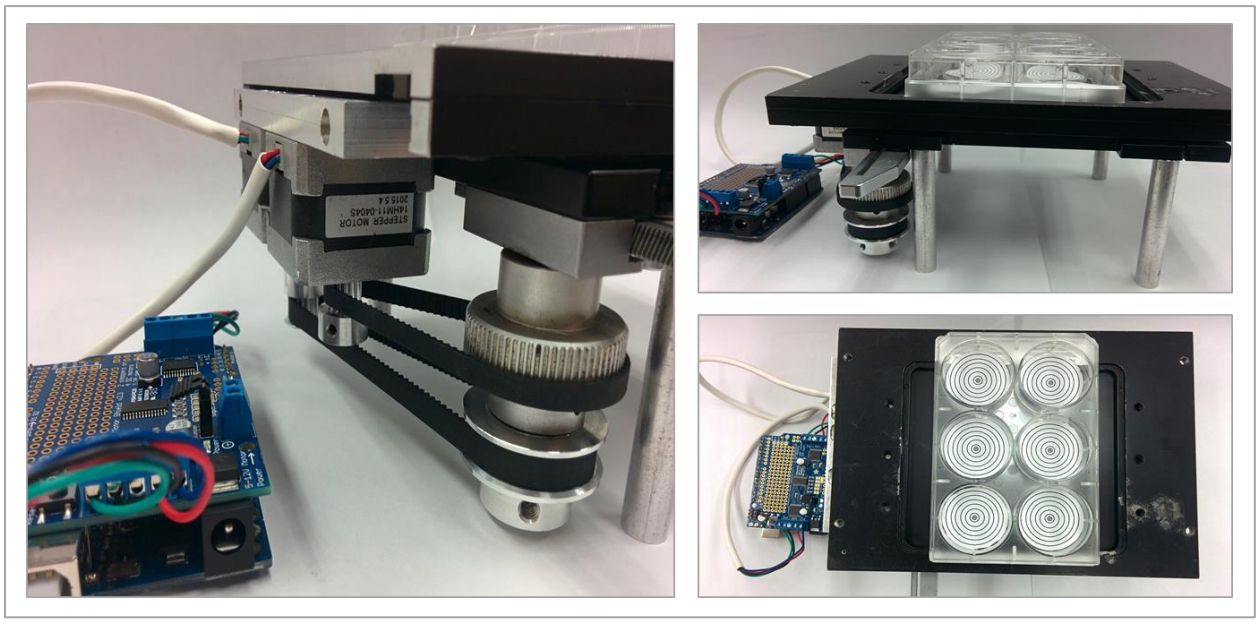


Figure 13. Close-Up of Belt Attachment to Stage Mechanics and Stepper Motor Electrical Connection to Arduino. Side View and Top View of Motorized Stage.

Device Finalization and Mounting

Following the electrical connection of the motors, we added contact switches in order to detect the extremities of X and Y axes so we could develop the calibration sequence. The camera was then linked to the microcontroller so it could receive the imaging signal when the stage had reached the programmed imaging locations. We then mounted the entire device on an acrylic base to ensure stability, fix the camera location, and decrease the probability of device migration due to vibration from the motors (**Fig. 14**).

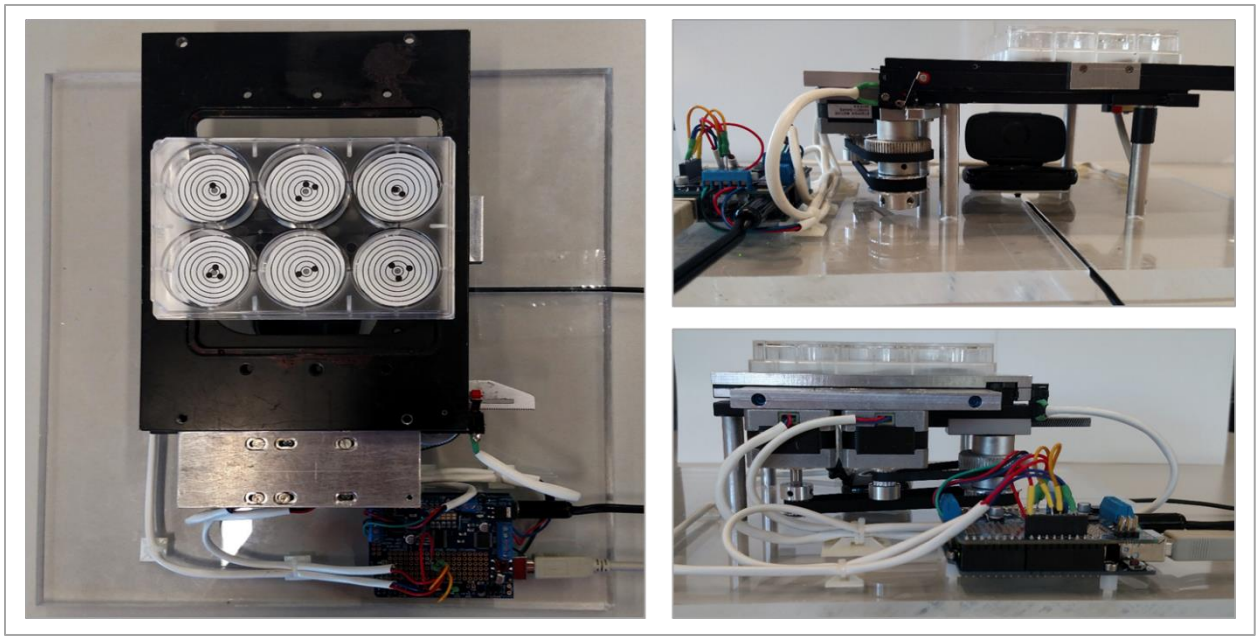


Figure 14. Top View of Completed and Mounted Device. Side View 1 and Side View 2 of Completed and Mounted Device.

GUI Implementation

We developed a simple Graphical User Interface to allow a researcher to easily control the device (**Fig. 15**) The scientist begins the program by pressing the "Start" button, at which point they will then be permitted to "Calibrate" the system. This sequence prompts the device to automatically find and record the location of the X and Y axis zero points, which allows the system to accurately calculate the XY coordinates of the programmed imaging locations. Following calibration, the user is then allowed to "Select Well Plate Type." For this prototype we gave the researcher the opportunity of using either a 6 well plate or a 12 well plate. Selection of the plate type delineates whether the system should utilize the 2x3 matrix or the 3x4 matrix for stage positioning. After selecting the well plate type, the user is then prompted to "Select Wells." This option was added in case the scientist does not want the system to image all of the wells in a given plate (a common occurrence in the lab). To select the wells, a grid of buttons will be revealed, at which point the user can click on the desired wells. Selected wells (to be imaged) appear in black, whereas unselected wells (to be passed over) appear in white. Once the researcher has selected the desired wells, he or she may press "Run" to authorize the device to complete the imaging request. Lastly, we also incorporated a live-stream of the system's camera (the large box on the bottom right, showing the concentric circles of an empty well), as well as a "Cancel" button to terminate the imaging cycle should a problem arise.

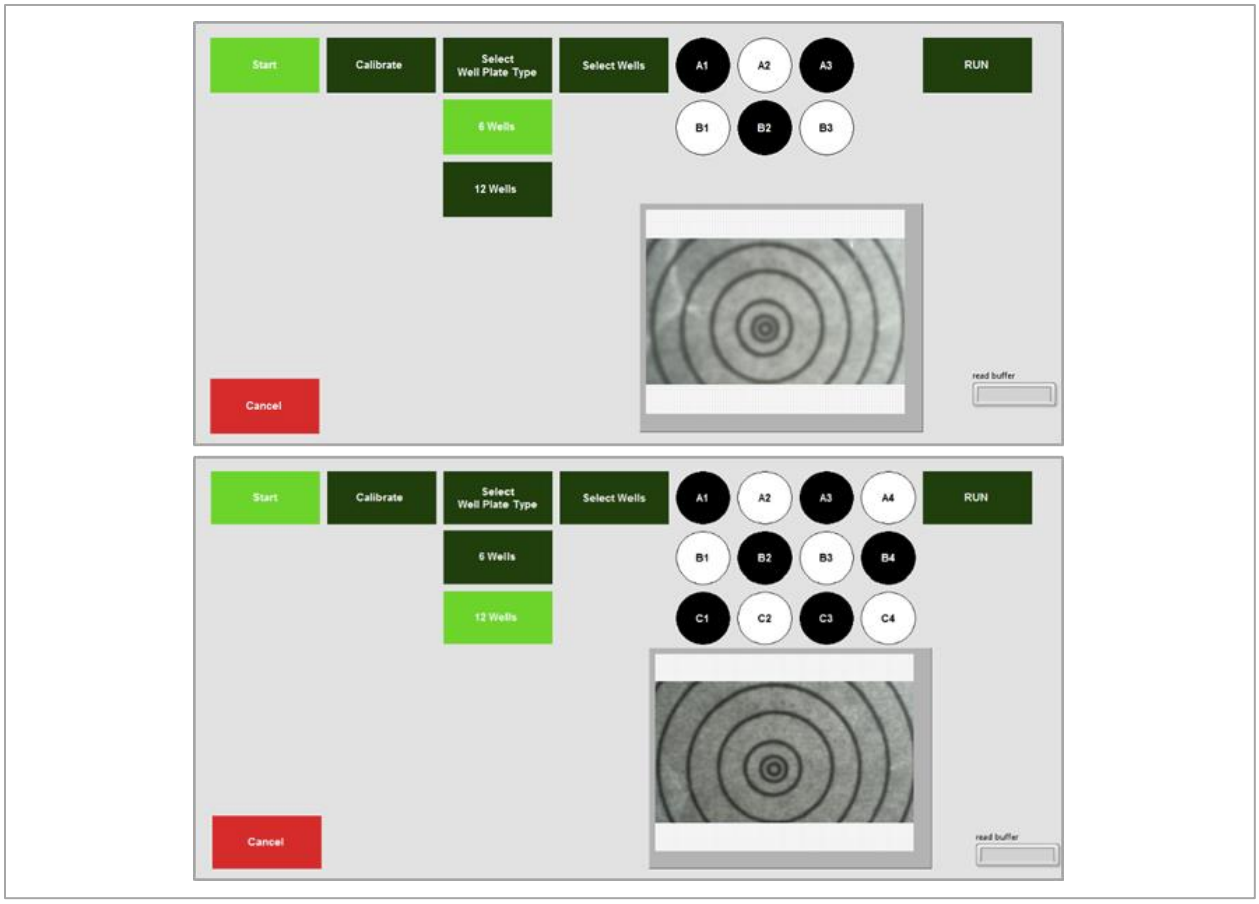


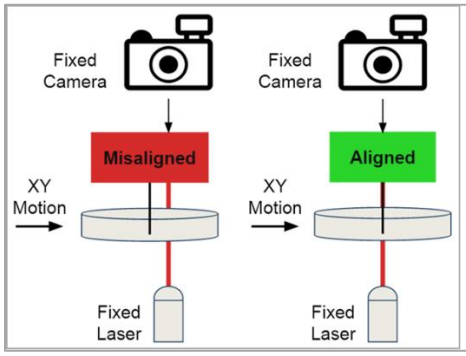
Figure 15. Graphical User Interface for 6 and 12 Well Plate Configurations.

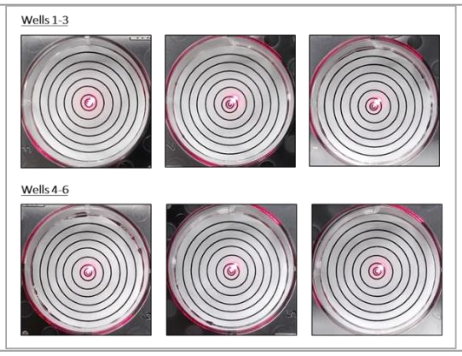
Design Validation

Proof of Concept

We first needed to show that our device was capable of accurate and highly repeatable positioning in the XY plane. To validate this functionality, we designed a test to mimic an imaging session to assess our device's programmable stage control. We positioned a fixed laser below the stage to simulate a fixed camera lens, then placed phantom targets (containing concentric circles) within each of the wells of a 6 well plate (**Fig. 16**). As the stage moved in the XY plane the laser would show up on the phantom targets, allowing us to determine the accuracy and precision of our programmed stage motion. A camera was placed overhead and images were taken at each well (**Fig. 16**). The images were then analyzed independently by each of the 4 team members on a binary Pass/Fail scale. If all 6 wells were considered a "Pass," the team member would then consider the entire trail a "Pass." We performed 6 independent trials and observed that our device was able

to consistently perform automated XY stage positioning (**Fig. 16**). With this important hurdle cleared, we could then proceed to build and validate additional device functionality.





Trial No.	Pass	Fail
1.	✓✓✓✓	
2.	✓✓✓✓	
3.	✓✓✓✓	
4.	✓✓✓✓	
5.	✓✓✓✓	
6.	✓✓✓✓	

Figure 16. Proof of Concept Design, Raw Data, and Final Output.

Higher Order Function Validation

After implementing higher order design features we needed to then robustly validate each one. First we wanted to see whether our system was capable of working with more than one type of well plate configuration. Previously trials were all done with a 6-well plate, so we chose a 12-well plate configuration to test our system's compatibility with more than one type of well plate. We repurposed the previous experimental setup (**Fig. 16**) to analyze our positional accuracy with a new well plate configuration. We placed targets with concentric circles in each well and looked to see which circle the fixed laser appeared within. For our test we defined the laser appearing in either Ring 1 (0.5 mm radius) or Ring 2 (1.25 mm radius) as a Pass, and conversely the laser's appearance between Rings 3 and 9 was considered a Fail. After performing the setup sequence and running 5 trials, we observed that 97.7% of the wells showed a Pass within Ring 1, and 100% of the time the laser appeared at least within Ring 2 (**Fig. 17**). This validated that the system could be easily adapted to multiple different well plate configurations. We then determined if our system could execute a variable, user-defined imaging route, rather than the standard imaging of each well in the plate. For this we utilized the same experimental setup, but rather than selecting all wells we chose to have the device image only half of the wells in the plate (every other one). We saw that 80% of the wells showed a Pass within Ring 1, while 100% of the wells showed a pass at least within Ring 2 (**Fig. 17**). We were therefore pleased with the system's ability to execute a user-defined imaging route. Next we performed the device calibration validation by repeatedly setting up and executing our automated zeroing function. We checked to see whether the X and Y axis zero points had been correctly identified and stored within the system after completion. Every single trial (N=5) passed with 100% for both the X and Y axis, indicating perfect system calibration (**Fig. 17**). Lastly we sought to determine whether the Graphical User Interface could be easily interpreted and utilized by other researchers. We recruited ten biomedical engineers (unaffiliated with our project) to try out the GUI and then surveyed the

participants on four questions: 1) Could you successfully calibrate the stage? 2) Could you successfully select the desired well plate configuration? 3) Could you successfully select the desired wells for imaging? 4) Overall, did you find this GUI useful and preferable to hard-coding character strings? We were happy to see that 100% of respondents were able to utilize our GUI to set up and run the device, and all participants were satisfied with the usefulness of the GUI (**Fig. 17**).

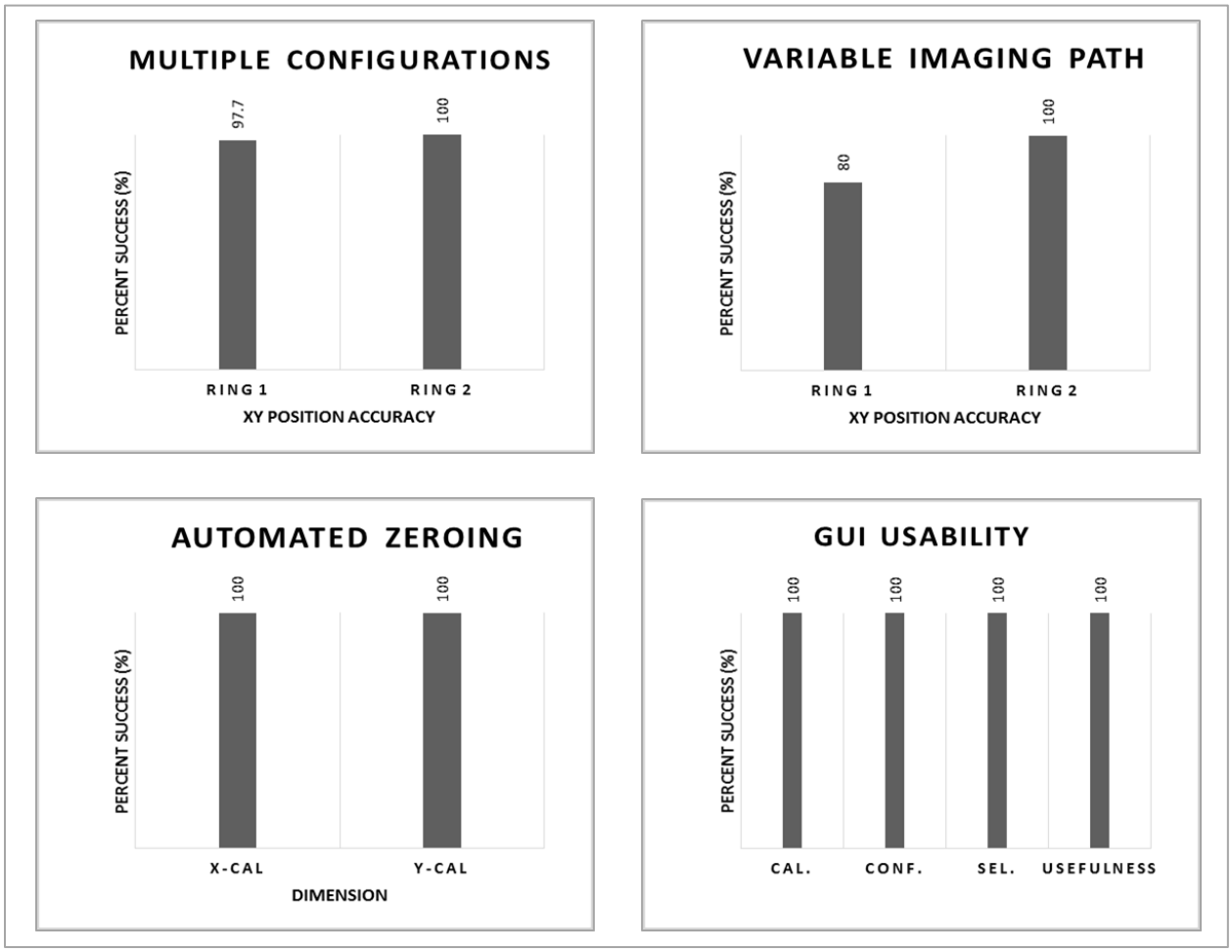
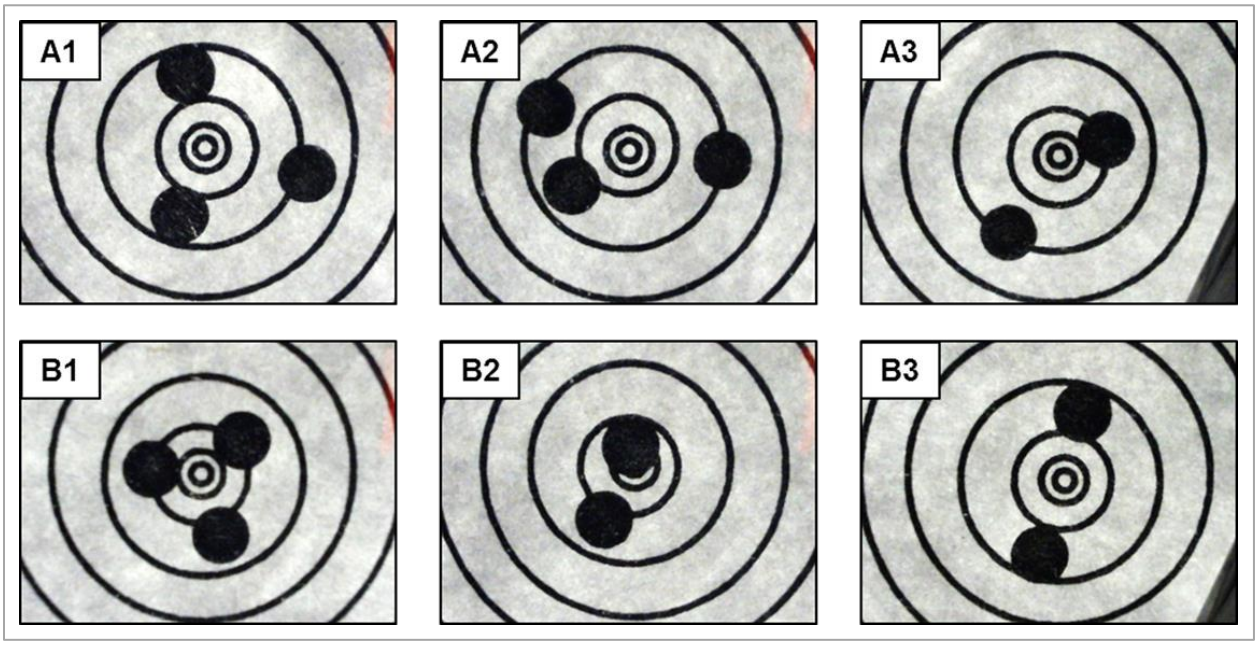


Figure 17. System Compatibility with Multiple Well Plate Configurations and System Compatibility with Variable Imaging Paths. Automated Stage Calibration Results and GUI Usability Survey Results.

Finalized Device Validation

After validating all of the individual functions, we then looked to validate the system as a whole. Since the entire purpose of the device was to replace a human researcher in well plate imaging, we designed an experiment to mimic this process. We created well plate phantoms containing dark circles to represent biological samples floating in a well. Then we performed the initial device calibration, well plate configuration, and desired well

selection. Following the setup we allowed the system to execute an imaging cycle by itself. We were very happy to see that the device automatically moved to the correct XY locations while acquiring and saving images of each of the wells with their respective "biological samples" (**Fig. 18**). We repeated the full imaging cycle 5 times and saw that the device was capable of completing an entire cycle of well plate imaging in 79.3 +/- 3.5 seconds. This final experiment fully demonstrated that our device was capable of accurately and precisely replacing a human researcher in well plate imaging tasks.



Conclusion and Future Steps

We successfully designed, built, and validated an automated system to replace a human user in conventional laboratory well plate imaging procedures. We first proved that our device is capable of precisely and accurately positioning a stage in the XY plane. We then showed that our device infrastructure is capable of hosting and imaging multiple different types of well plates (we tested 6-well and 12-well plates, and this experiment proved that the design can theoretically be scaled to any of the commercially available well plate matrices). We further confirmed that the device is capable of accurately following a variable, user-defined imaging route, rather than a constant imaging pattern throughout the entire well plate. We then demonstrated that the system can perform its own automatic calibration sequence to detect and store the zero points of the X and Y axes, thereby ensuring the accuracy of future positional calculations. We also collected user experience data to analyze the usability and efficacy of our device GUI. Lastly, and most importantly, we showed that our automated system was capable of executing and saving a complete

imaging cycle, effectively removing the need for a human researcher in laboratory well plate imaging.

In the future, we would like to add two additional functions to further improve the usefulness of our device. The first desired feature is Image Tiling, whereby the system would stop and image multiple sub-locations within a given well, rather than taking one broader image of the entire well. Image tiling would be particularly useful as the magnification of the camera lens increases (which would naturally decrease the amount of well plate surface area that could be captured in a single image). The second feature would be built-in image processing. Such an improvement would mean the device could not only perform all of the image acquisition, but also could possibly perform all of the image analysis for the researcher. This development would free up even more of the scientist's time, allowing them to pursue other experiments in the meantime.

Acknowledgements

We would like to thank the University of Miami Department of Biomedical Engineering for funding the execution of the senior design project. We would like to thank all of the faculty for their guidance, and especially Dr. Montero for his helpful advice, assistance, and mentorship throughout the duration of our project.

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