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Liquitronics Final Project Report

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Interoffice Memorandum

TO: Dr. Eliseo Iglesias, Team Advisor
Dr. Darin George, Senior Design Administrator

FROM: Liquitronics: Drew Gray, Bryton Flecker, Matthew Roche, Benjamin Harter

SUBJECT: Final Project Report for the 96 well plate robotic liquid handler

DATE: April 24th, 2023

Team Liquitronics is pleased to present this proposal for a Senior Design Project that will bring affordable and efficient automation technology to Dr. Urbach and other academic chemistry labs.

This final report addresses the functional and design requirements to successfully operate the 96 well plate robotic liquid handler. In addition, the team has provided an overall design evaluation on the current prototype. Testing is outlined through an overview, objective, key features, scope, plan, and acceptance criteria. The results of each test are then analyzed and compared to the acceptance criteria. Lastly, the team expresses it's accomplishments with the current design and what can be done in the future.

Thank you for your time and consideration. Please feel free to contact Drew Gray (agray1@trinity.edu) with any questions or concerns.

Final Project Report

Liquitronics

Matthew Roche, Benjamin Harter, Drew Gray, Bryton Flecker

Sponsor: Dr. Adam Urbach,

Advisor: Dr. Eliseo Iglesias

April 24, 2023

Executive Summary/Abstract

This final project report details the design evaluation and tests the Liquitronics team conducted on the 96 well plate robotic liquid handler. The team was able to create a prototype that reflects the most important aspects the team set out to accomplish. The main focus of the semester was completing a functioning chassis and movement system along with the pipette mechanism.

The following tests were completed: z-axis positional accuracy, x/y-axis positional accuracy, tip discard test, plunger actuator test, fluid volume test, sustained power draw test, and a size and weight test.

Both positional accuracy tests passed without significant issues. The z-axis needed to be within 0.5 millimeters of the location for every trial, and the trial with the largest error had an error of 0.1 millimeters. Similarly, the x/y test needed each trial to be within 1 millimeter and the greatest error measured was only 0.6 millimeters. The tip discard test proved that the prototype could eject a pipette tip without fail. This test also gave the time a relationship between the voltage supplied to the linear actuator and the speed at which it moved. These results will aid in determining the working voltage for the prototype's actuators and electronics.

Unfortunately, there were two tests that did not meet their acceptance criteria. The final design is limited to a four foot wide and 2 foot deep space. The current prototype is currently 2.23 feet in both directions. However, after speaking with the project sponsor, it was agreed that the size limit was more flexible than originally stated and thus the current dimensions do not present any practical issues. Additionally, the prototype is well under the 500 pound weight limit measuring at 34 pounds.

The second unsuccessful test was the sustained power draw test. This test is meant to prove that the circuitry of the prototype can run for extended periods of time without any components failing. Without any of the motors running, the prototype was drawing just over 300 milliamps. This was lower than what was expected. Also, the voltage regulator began to burn out, and 2 of the 9 stepper motor drivers stopped working. The reason for these failures is not yet known, but the team is currently brainstorming ideas for how to pinpoint the solution, and ensure that it will be fixed.

Future improvements will be focused on getting a fully automated prototype. For this to happen, assembly of the mechanical parts must be completed, a full code must be written, and the power draw problems must be addressed.

Introduction

The Liquitronics team is tasked with creating an affordable robotic liquid handler that will precisely fill 96 well plates, with programmable variation in fluid, volume, and dilution for each well. The traditional process of filling a 96 well plate is slow and tedious, requiring a high level of manual labor to achieve various concentrations for different chemistry reactions. The use of autonomous machines has been used as an alternative. However, these systems tend to be expensive and potentially difficult for new users to learn how to use. These limitations can make it difficult for smaller laboratories to justify the purchase of such devices and introduces a need for a low-cost and easy-to-use system that can perform these reliably dispense liquid accurately.

A key requirement for the handler is a fast operation cycle, therefore one operation cycle of the device should be performed within thirty seconds. One operation cycle consists of the following: (1) installing a new tip, (2) setting volume to collect and dispense, (4) drawing liquid from a container, (5) depositing the liquid, and (6) discarding the tip. The source fluid, volume, and dilution level for each well should also be programmable for full use. Additionally the device should not allow cross contamination of samples, and work with a standard 120V wall outlet. Finally, the average amount of time it would take to teach a new user how to pipette fluid into a 96-well plate should not exceed the average amount of time it takes to teach a user how to use the automated liquid handler. All the tests performed serve to cover the full functionality and address the design requirements

The primary constraints of the final system involve adhering to specific dimensional and weight requirements. The handler should be no larger than two feet in length, three feet in width, and with a flexible height limit, fitting on a standard chemistry lab table. Additionally, the handler should be able to be transported on a service/utility cart to a desired location. There are several codes which apply to our prototype such as ASTM E3132-17 (Standard Practice for Evaluation Response Robot Logistics: System Configuration). However, these standards function as guidelines for building and best practices for documentation. Therefore, they were not addressed through test criteria. Future work will be done to ensure each code and standard are followed, including documentation and safety indicators.

Overview of the Final Design

The functionality of the handler consists of three main subsystems: the chassis and movement system, pipette control mechanism, and the user interface. To ensure a successful project, each subsystem will be built, assembled, and tested individually. From there the subsystems will be connected and tested again to ensure full functionality. Finally, the tests performed for each subsystem have the potential to be redesigned for the final test, based on feedback from any unforeseen issues. It is important to note that for this semester, the team did not plan to complete the user interface. Instead the team focused on the functionality of the two other subsystems as they were top priority, therefore this report will focus primarily on the chassis and movement system and pipette control mechanism.

Subsystem Designs

Chassis and Movement System

The chassis and movement subsystem is the basis for the entire device. This system determines how the pipettes, well plates, and other components fit and move within the robotic liquid handler. A successful chassis will smoothly transport the fluid, well plate, and tips to the correct position, remain sturdy, and stay consistent after many cycles while also being simple enough to operate and code. Several parts of the movement system were modeled after standard desktop 3D printers, due to their widely available hardware at an affordable price. The lower movement system utilizes belts and gears commonly found on 3D printers, providing fast movement and precise positioning that is required to hit each well plate.

The working criteria with their priority level for the chassis and movement subsystem are the following:

- Ease of Assembly → High Priority
    - The easier, the better as it allows the team to incorporate the other complex subsystems with relative ease.
- Simple to Control → High/Medium Priority
    - If the handler is simple to control, it will accomplish the goal of having a lower time to teach, thus making it a better option over manual pipetting.
- Sturdiness → High/Medium Priority
    - A sturdy machine ensures that the pipette can move fast, achieving an operation cycle of 10 seconds or less. This will also increase positional accuracy and avoid cross contamination.
- Positional Accuracy → High/Medium Priority
    - Positional accuracy ensures that the pipette will correctly dispense each fluid into the correct well, avoiding cross contamination.
- Cost → Medium Priority.
    - The use of low cost 3D printer hardware has created plenty of room in the team's budget so this is not a major concern.
- Repairability → Low Priority
    - As this is a prototype, the overall repairability is not a priority, but it is something to consider as repairs may need to be made during construction or in the future after use.

Pipette Control Mechanism

The pipette mechanism is the core of this project and easily the most important subsystem. This subsystem is responsible for operating the pipette, which includes adjusting the volume drawn and dispened, ejecting tips, and drawing/dispensing liquid. A successful subsystem will show high accuracy, high precision, and low operating time while remaining low cost.

The working criteria to realize this subsystem and their priority are the following:

- High Precision → High Priority
  - The actuation of the mechanism needs to be consistent enough where it can dispense the desired amount of liquid every time.
- High Accuracy → High Priority
  - Needs to be accurate to ensure each well plate has the correct amount of fluid that was originally programmed.
- Speed of Application → Medium Priority
  - Has to be fast to accommodate the cycle time requirements
    - A cycle consists of the pipette getting a new tip, getting the fluid from a stock container, dispensing the fluid into the well plate, and ejecting the tip
    - This cycle should be achieved within 10 seconds
- Low Cost → Medium Priority
  - Again the team has plenty of room in the budget
- Simplicity → Low Priority
  - Although helpful, not critical as this is a complex subsystem
- Easy Repairability/Maintenance → Low Priority
  - As this is a prototype, the repairability and maintenance are not a high priority, but since the team will be needing to make repairs during design and construction, it still needs to be considered

The most significant change the team decided on was priority on speed of application. After further research into the Opentron's own device, it was determined that the cycle time the team had instated was far from realistic [1]. Therefore, the cycle time was adjusted to be 30 seconds instead of the original 10 seconds.

Design Evaluation

The chassis and movement system tests in the x, y, and z-axes were all successful with error for all three being within tolerances. Features concerning the pipette mechanism subsystem such as the tip discard and plunger actuator functioned as intended. In one of the more critical tests, the fluid volume tests, the prototype succeeded with all errors falling within an acceptable range.

The sustained power draw test identified several issues in our electrical design. Several electrical components failed prematurely, thus making it difficult to test further. Testing further without these components renders the test irrelevant as the test is meant to demonstrate the maximum power draw from all electrical components. In addition, the prototype failed to fit the size constraints. However, after consulting with the sponsor it was determined that the size of the prototype was acceptable.

However, the team has addressed some of the issues identified in order to polish prototype functionality. Implementing features to make the prototype more presentable for the final presentation and demonstration such as a baseboard. The team primarily focused on reducing the cycle speed of the prototype. In addition, the team adjusted the Arduino controls so that powering and de-powering select components on the control circuit is possible. This will ensure the team does not turn on more components than existing power constraints of the breadboard.

Overall, the team was able to accomplish most of the design requirements that were made in the beginning of the semester.

Project Requirement/ Working Criterion

Applicable Constraints

- The dimensions of the final system shall be no deeper than 2 ft with a width of 3-4 ft while the height is flexible
    - It is expected that this device will fit on a standard chemistry lab table
- The prototype should be able to be transported on a dolly to desired location
- The prototype shall meet the codes and standards listed in the next section
- The prototype shall be able to be built for less than $1000

Applicable Codes and Standards

- IEC TC 44 (Safety of Machinery - Electrotechnical Aspects)
    - "Standardization of electrotechnical equipment and systems relating to the safeguarding of persons from hazards of the machinery, its associated equipment and the environment. To coordinate with the International Organization for Standardization (ISO) all matters concerning the safety of machinery."
- ASTM E3132-17 (Standard Practice for Evaluating Response Robot Logistics: System Configuration)
    - " 1.1 This practice, as a part of the response robot logistics test suite, specifies the requirements of identifying and documenting the configuration of a robot system under test as well as the associated processes for doing it. The aspects to be included in such a configuration practice are the key dimensions and weights, the existent subsystems and key components, as well as the key timing requirements for setting up and maintaining the system."

Project Requirements

The objective of this project is to create a robotic liquid handler that will pipette well plates autonomously, with programmable variation in fluid, volume, and dilution for each individual well. The handler will seek to be efficient and easy to use, aiming for a goal of completing a well plate within three hours. By creating this affordable handler, university labs can eliminate the slow, inefficient process of manually pipetting. Listed below are several criteria that are required to create a successful handler.

1. One operation cycle of the device (attaching a new tip, setting the volume to collect and dispense, drawing liquid from a container, depositing the liquid, and discard the tip) should be performed in 30 seconds or less
2. Should work with standard 120 V AC power supply at 60 Hz
    - The robot should not require any power supply other than what can be provided by a standard United States wall socket
3. The device should be precise to within 1% of the specified volume and shall be within 2% of the specified volume
4. The accuracy of each linear actuator used for axial positioning should be within 0.5 millimeters

Tests

Z-Axis Positional Accuracy

Test Overview

The prototype robotic liquid handler shall be able to maneuver up and down accurately to collect and dispense aqueous solutions. The tests conducted in the previous semester indicated that the lower movement system accomplishes this goal.

Objectives

The purpose of this test is to determine if the pipette control mechanism can effectively maneuver from the source container into the well plate without any collisions in the Z-axis (height) direction. This is a necessary component which allows the pipette to both draw and dispense fluid.

Feature(s) Evaluated

The main feature being tested is the pipette control mechanism. Additionally, the programming control is being tested to determine the appropriate height which is required to draw and dispense aqueous solutions.

Test Scope

This test examines both the accuracy and repeatability. The variation in height will be recorded and collected. Additionally, a visual test will be conducted to see if there are any collisions present between the pipette tip and the containers.

Test Plan

This test will be repeated at least 15 times to demonstrate reliability. The pipette will start at the top of the Z-axis height (where the limit switch is located). The pipette will be lowered a defined distance so that the tip is at least 1 centimeters in the solution. The pipette will then be lifted up so that it is at least 1 centimeter above the container of the source solution (water). Finally, the device will lower again to be half a centimeter above a standard well plate.

Acceptance Criteria

This test will be considered successful if the mechanism can lower into the water, rise up above the height of the source fluid container, and lower into a well. The end of the pipette tip should be 1 cm into the water, 1 cm above the water, or a half centimeter above the well plate, with an error of 0.5 millimeters. The pipette tip should avoid colliding with the sides of both the fluid container and the well.

X/Y-Axis Positional Accuracy

Test Overview

The prototype robotic liquid handler shall be able to maneuver in the horizontal plane accurately to collect and dispense solutions. Tests conducted in the previous semester indicated that the lower movement system accomplishes this goal, but changes have been made to the code and circuit design.

Objectives

The purpose of this test is to determine if the lower and upper movement mechanism can effectively maneuver to any position it needs to. A secondary objective of this test is to build a calibration curve. This is a necessary component which ensures the correct fluid will be dispensed to the correct location.

Feature(s) Evaluated

This test determines if the team's design is able to be programmed to move to a location accurately. This will be part of evaluating whether any cross contamination may occur because of the lower movement system.

Test Scope

This test examines both the accuracy and repeatability. The variation in distance traveled will be measured with calipers. Additionally, a visual test will be conducted to ensure that there are no unplanned collisions or slipping.

Test Plan

This test will be conducted as a calibration test. The cart, which transports the well plate, fluids, tips, and pipette mechanism along the movement system, will begin touching the end of the rail. A diagram is provided in Figure 1 to help show how the test was conducted. An initial measurement was taken for the distance from the end of the rail and the start of the cart. The cart will then be moved 100 steps. A step is the measurement used for one predefined portion of a rotation of a stepper motor. The distance traveled will be recorded. It will then be moved an additional 100 steps and the distance will be recorded again. This will be repeated until the cart reaches 3000 steps from the start. The entire process will be repeated three times, with each time being referred to as a trial.

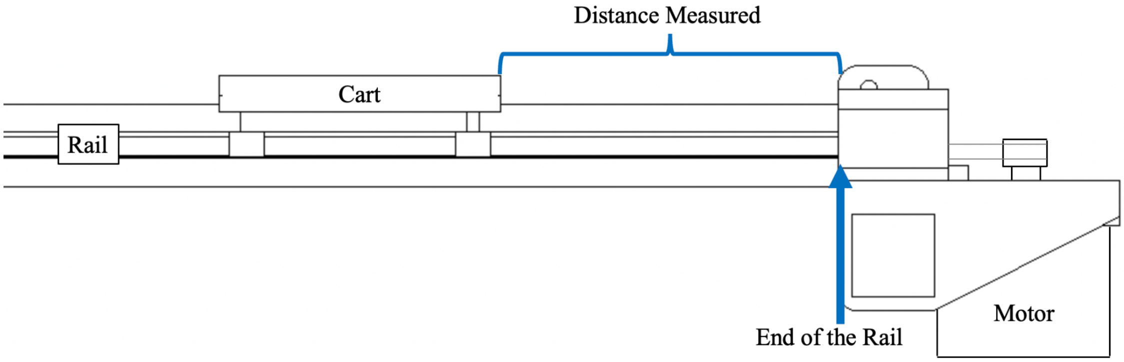


Figure 1. X/Y-axis positional test diagram

From this data collected, a calibration curve will be created. This will give the team an expected distance from the end based on a curtain number of steps. The data collected will be compared to the expected position based on the calibration curve.

Acceptance Criteria

This test will be accepted if the actual location is within 1 millimeter of the target location for all measurements. Additionally, any slipping of the cart will indicate a failed test. Cart slippage will cause inconsistent cart locations, requiring the cart-rail-stepper motor system to be zeroed.

Tip Discard Test

Test Overview

This test will confirm that the prototype can successfully release a pipette tip at the end of the usage/service cycle, ensuring the functionality of the tip ejector actuator.

Objectives

The purpose of this test is to determine if the pipette control mechanism can properly and cleanly discard tips without issues. This test will also evaluate the programming control of the actuator.

Feature(s) Evaluated

The main feature being tested is the pipette mechanism, with a focus on ensuring fast speed of application and high precision (consistency).

Test Scope

The test demonstrates whether the tip can be discarded effectively and in a timely manner by an ejector mechanism. It needs to consistently discard the pipette tip.

Test Plan

This test will be repeated twelve times (four times at three different voltages) to observe and record its reliability. The test will start with the pipette mechanism attaching a fresh tip and then an actuator mechanism will discard the tip. The test will be conducted by running five, ten, and fifteen volts with a current of 500 mA through the actuator mechanism. Each trial will be timed to determine how voltage has an effect on the speed of actuation (faster time indicates a faster actuation).

Acceptance Criteria

The test will be considered successful if the ejector can reliably discard the tip 100% of the time. This will be achieved by having the actuator completely remove the pipette tip, and drop into a discard bin positioned below the lower movement system. Failure to achieve this would result in a test failure.

Plunger Actuator Test

Test Overview

The prototype robotic liquid handler shall be able to collect fluid and then dispense the fluid using an actuator mechanism.

Objectives

The purpose of this test is to confirm that the pipette mechanism can properly collect and dispense fluid. This test determines whether the actuator can properly interact with the plunger inside the pipette mechanism.

Feature(s) Evaluated

The main feature being tested is the pipette control mechanism. This test also evaluates the programming control of the actuator.

Test Scope

This test identifies whether or not the plunger actuator functions reliably. Issues such as accuracy are not relevant to this test. The plunger shall be able to fully dispense the fluid collected, leaving no fluid left in the pipette tip.

Test Plan

This test will be repeated 10 times to observe its reliability. The plunger actuator will start by completely compressing the pipette plunger and then placing the pipette tip into an aqueous solution. The actuator will then lift the plunger to collect the fluid. The pipette tip will then leave the aqueous solution and be dispensed by completely compressing the plunger once again.

Acceptance Criteria

The test will be considered successful if the actuator can reliably dispense the entire fluid collected. There shall not be bubbles within the fluid or inconsistent fluid dispensing as the actuator operates.

Fluid Volume Test

Test Overview

The prototype robotic liquid handler shall be able to deposit a specified but modifiable volume of liquid. Initial tests were performed in the previous semester which proved the micropipette selected was appropriate for hitting the target volume goals.

Objectives

The purpose of this test is to determine if the pipette control mechanism can dispense a precise and accurate amount of liquid.

Feature(s) Evaluated

The main feature being tested is the pipette control mechanism. This is the mechanism that is used to adjust the position of the plunger used to draw and dispense fluid (see appendix D, Figure 7). Additionally, the programming control is being tested as it will be the program telling it how and when to draw liquid as well as determining the distance of plunger that relates to the specified volume.

Test Scope

This test examines the precision of the pipette mechanism. Furthermore, this test validates that the deposited amount by the mechanism falls within an acceptable margin of error, thereby ensuring that the precision criteria is satisfied. This is one of the design requirements and the project may be considered a failure if it does not meet this. The dispensed volume of fluid should be within 1% of the specified volume, and may be within 2% of the specified volume.

Test Plan

This test will be broken down into two parts. First, tests will be run with filling a single well to evaluate liquid dispensing. The amount of liquid will be measured by mass instead of volume. Dr. Urbach has his students employ this method to fill well-plates in his chemistry lab. We will be running this test with four target volumes of water. A high and low volume will be chosen for either the P100 or P200 pipette and the same will be done with the P1000 pipette.

Once the first trial is confirmed to be within the given requirements, we will test an entire well plate at once. The same method for measuring will be used, but for this test, All wells on the well plate will be full as opposed to only a single well.

Acceptance Criteria

This test will be considered successful if the mechanism can accurately dispense the four test volumes into single wells. In addition, the mechanism must also be able to consistently and accurately dispense liquid into 24 wells for each of the four volumes making a complete well plate with 96 wells filled in total. These dispensed volumes must also meet the percentage accuracy requirements specified in this test's scope.

Sustained Power Draw Test

Test Overview

The team will run the completed prototype under conditions which will induce the maximum expected power draw of the system. The power draw will be observed to ensure that the power supplies in use are capable of sustaining this demand.

Objectives

The goal of this test is to prove that the prototype can run off of the required supplied voltage, but also ensure it can run for an extended period of time without failing. This test shows whether or not such a failure is due to higher power demands than the power supply system can provide. Per ASTM 8.2.9, the power sources and expected operating time will be recorded for best practices [2].

From this test, we will be able to find if there is a maximum operational speed the robotic liquid handler can perform for an extended period of time. We currently assume it will be limited by the speed of the well plates. This test also will inform on whether passive cooling is sufficient, which will determine if an active cooling system is required. This could be observed if the motors are hot to the touch.

Feature(s) Evaluated

This test is intended to evaluate the power supply and electronics of the liquid handler's entire electrical system and all of its actuators. The only two components not included in this test was the Raspberry Pi and the user interface display as these are not in the scope of this year's project.

Test Scope

This test is intended to determine whether the operational limits of the movement system and power supply can be exceeded under high intensity workloads. Since this test is focused on the movement system and power supply, the performance metrics such as accuracy or cross contamination are not considered during this test.

Test Plan

To complete this test, the power supply will be connected to standard wall power (120 V at 60 Hz). The entire circuit was assembled excluding the Raspberry Pi and display. First, no commands will be sent to the arduino and the circuit will be held in a constant state for 10 minutes. The current leaving the power supply will be recorded. Additionally, all motors and actuators will be checked to see if they are being powered. The wait time of

10 minutes is to check if all circuit components will be able to handle this initial load for an extended period of time.

If the electronics pass the initial power draw test, the test will proceed to the operating cycle and movement system. From minutes 10 to 20 of the test, one motor will be told to continuously move 2000 steps in each direction repeatedly. The current will be measured once again and all components will be checked to see if they are still functioning and not overheating. From minutes 20 to 30, another motor will begin to move. This process is repeated until all motors are moving. Once all motors are moving, the linear actuators will be given a command to fully move in and out on repeat. The entire system will be left in this state for 30 minutes and measurements and observations will take place.

Acceptance Criteria

For this test to be accepted as a success, there must be no damage to any components as indicated by smoke, melted hardware, or other such indicators. All circuit components must work without fail for the entirety of the test. The temperature of all components will be monitored by touch.

Prototype Size and Weight Testing

Test Overview

The final prototype, excluding peripherals such as the touch screen monitor, shall not exceed a size of 2 ft deep, and 4 ft wide and must be transportable by a standard service/utility cart. This test will confirm those goals.

Objectives

The goal of this test is to ensure that the prototype can both fit in a standard lab workstation and be transported to the location where it will be used. These two objectives are the only factors which will constrain the size and weight of the prototype as it is not expected to be a portable device. Furthermore, while performing this test, the physical dimensions of the prototype (including length, width, height, and weight) will be recorded as per ASTM standard 8.2.4.1 for best practice [2].

Feature(s) Evaluated

This test is primarily intended to evaluate the chassis and movement systems of this design since the test’s outcome will be dominated by these features, but it will include both the pipette and controller as well since they will still contribute to the weight of the prototype.

Test Scope

The scope of this test will cover both the size and weight of the final prototype as it pertains to the transport and final placement of the device. This test has no functionality requirements and is purely dependent on the physical dimensions of the liquid handler.

Test Plan

The test will require the usage of a tape measure, a scale, and a cart for transporting the prototype. Since the size of the prototype should only be limited by the size of a standard laboratory workspace, the cart should be able to transport an item with base dimensions of at least 2 ft by 4 ft.

The width and depth of the prototype should be measured at the base since there should be no part of the liquid handler that exceeds the size of the base. The prototype should then be weighed to ensure that it does not exceed the maximum load capacity of a standard utility cart. All physical dimensions will be recorded and presented with the final design.

Acceptance Criteria

This test will be considered successful if the size of the final prototype is determined to be less than or equal to 2 ft by 4 ft, and the weight of the prototype does not exceed the expected 500 lb capacity that a standard utility cart can carry [3].

Test Results

Z-Axis Positional Accuracy Test Results

The z-axis movement system was tested to ensure that it could be moved to a set position with a positional error of less than 0.5 mm. Based on the results shown in Table 1. This test shows the process of moving the pipette mechanism 20 millimeters over the course of 15 trials with a maximum error of no more than 0.09 millimeters which is well within the required margin of error of 0.5 millimeters.

Table 1. Z-Axis Positioning Test Results

Trial	Set Position (mm)	Measured Position (mm)	Error (mm)
1	20.00	20.05	0.05
2	20.00	20.02	0.02
3	20.00	20.07	0.07
4	20.00	20.03	0.03
5	20.00	20.08	0.08
6	20.00	20.04	0.04
7	20.00	20.06	0.06
8	20.00	20.09	0.09
9	20.00	20.06	0.06
10	20.00	20.05	0.05
11	20.00	20.03	0.03
12	20.00	20.07	0.07
13	20.00	20.06	0.06
14	20.00	20.08	0.08
15	20.00	20.05	0.05

X/Y-Axis Positional Accuracy Test Results

Table 2 shows the data collected from the X/Y-axis position test. Each trial began with a slight offset (0.4 to 0.7 mm), as this test is focusing on the distance traveled, this offset had no influence on the results. After each 100 steps, the cart would travel about 1 centimeter farther. The distances were then converted to distance traveled so they could be plotted on the same graph, shown in Figure 2. A linear curve fit was applied to this data and the corresponding equations can be found in Eq. 1. The slope of the line reveals the distance traveled in centimeters per step, which was calculated to be 0.01 cm per 1 step. Any slight offstep on the y-axis is negligible, as it is merely 0.03 mm. Utilizing this equation, our team was able to determine the distance between each measurement and the anticipated location, as presented on the right-hand side of Table 2. The greatest difference was 0.6 mm, while the average magnitude of the difference was 0.2 mm with a standard deviation of 0.1 mm.

Table 2. X/Y-axis position test data

Steps	**Distance from end (cm)**			**Offset from expected position (cm)**		
	Trial 1	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
0	0.07	0.04	0.06	-	-	-
100	1.08	1.06	1.08	0.01	0.02	0.02
200	2.09	2.00	2.09	0.02	-0.04	0.03
300	3.07	3.03	3.04	0.00	-0.01	-0.02
400	4.10	4.00	4.07	0.03	-0.04	0.01
500	5.05	5.05	5.06	-0.02	0.01	0.00
600	6.02	6.03	6.07	-0.05	-0.01	0.01
700	7.09	7.09	7.01	0.02	0.05	-0.05
800	8.09	8.06	8.08	0.02	0.02	0.02
900	9.07	9.09	9.06	0.00	0.05	0.00
1000	10.06	10.01	10.09	-0.01	-0.03	0.03
1100	11.09	11.00	11.07	0.02	-0.04	0.01
1200	12.08	12.06	12.07	0.01	0.02	0.01
1300	13.03	13.05	13.03	-0.04	0.01	-0.03
1400	14.07	14.02	14.08	0.00	-0.02	0.02
1500	15.07	15.07	15.05	0.00	0.03	-0.01
1600	16.06	16.05	16.02	-0.01	0.01	-0.04
1700	17.09	17.07	17.05	0.02	0.03	-0.01
1800	18.08	18.07	18.06	0.01	0.03	0.00
1900	19.06	19.08	19.09	-0.01	0.04	0.03
2000	20.04	20.10	20.07	-0.03	0.06	0.01
2100	21.05	21.07	21.03	-0.02	0.03	-0.03
2200	22.10	22.06	22.04	0.03	0.02	-0.02
2300	23.08	23.03	23.06	0.01	-0.01	0.00
2400	24.07	24.02	24.08	0.00	-0.02	0.02
2500	25.08	25.04	25.07	0.01	0.00	0.01
2600	26.09	26.06	26.07	0.02	0.02	0.01
2700	27.05	27.03	27.10	-0.02	-0.01	0.04
2800	28.04	28.02	28.04	-0.03	-0.02	-0.02
2900	29.03	29.08	29.05	-0.04	0.04	-0.01
3000	30.05	30.05	30.07	-0.02	0.01	0.01

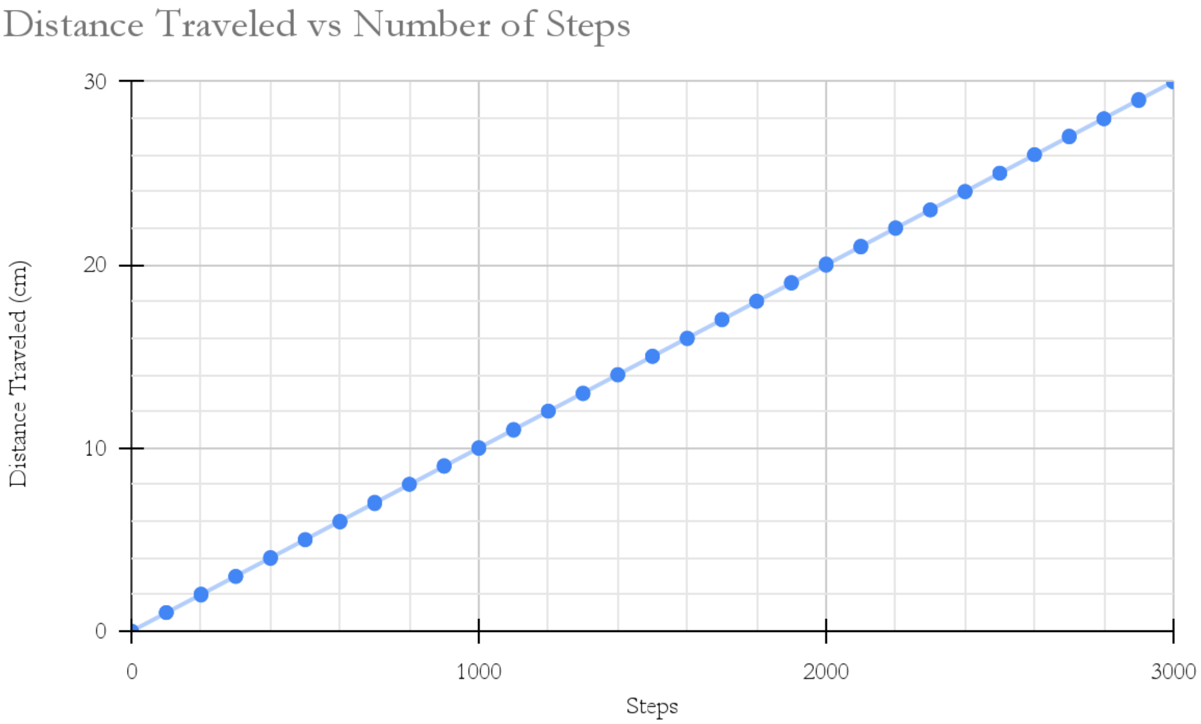


Figure 2: Distance traveled along X/Y-axis compared to the number of steps

Tip Discard Test Results

Table 3 shows the result of the tip discard test, where all trials were observed to have passed. Three different voltages were used to determine how voltage has an impact on extension and revert time, which will be useful in calculating the total cycle time. Extension time was recorded as the time it took the mechanism to fully reject the tip into the discard bin; while revert time was found to be the time it took to reset the tip ejector back to its original position.

Table 3. Tip Discard Test Results

Trial	Pass/Fail	Voltage (V)	Extension time (Seconds)	Revert time (Seconds)
1	Pass	5.22	2.2	3.73
2	Pass	5.22	2.21	3.62
3	Pass	5.35	1.96	3.52
4	Pass	5.25	2.05	3.13
5	Pass	9.82	1.11	1.75
6	Pass	9.8	1.05	1.87
7	Pass	9.8	1.05	1.61
8	Pass	9.8	1.01	1.38
9	Pass	14.92	0.71	1.11
10	Pass	14.92	0.73	1.18
11	Pass	14.92	0.8	1.2
12	Pass	14.92	0.72	1.2

Figure 3 shows the input voltage graphed against the extension time. The plot shows that as voltage increases, extension time decreases.

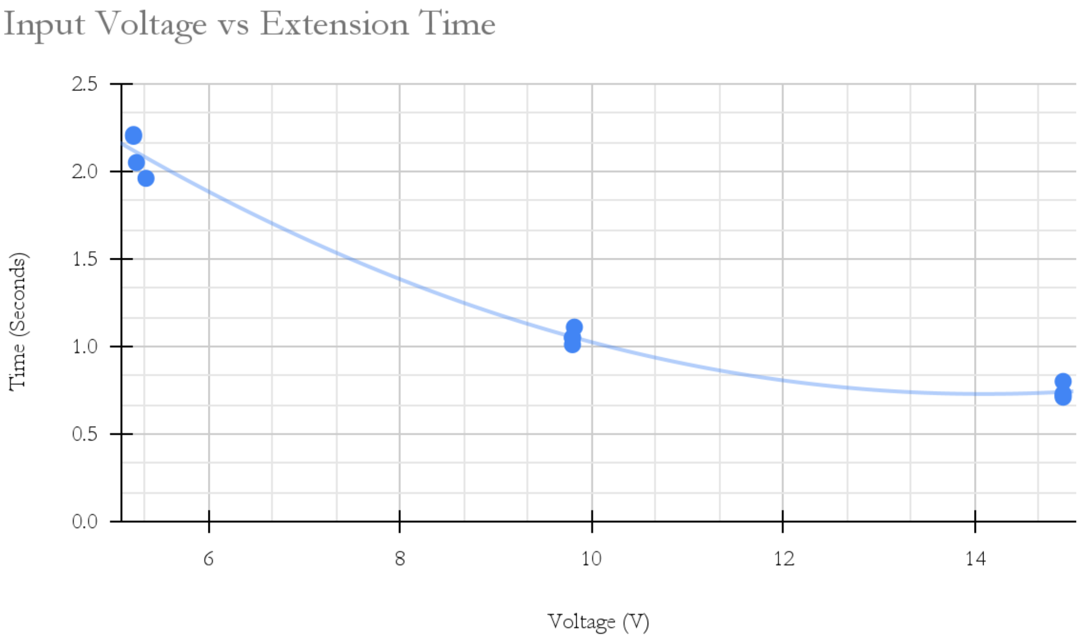


Figure 3. Input voltage of the tip ejector actuator versus the extension time. A trendline for best fit was used.

By using the data plotted in Figure 3, the team can roughly predict the expected ejection time based on an input voltage given, which will be useful for determining the total cycle time for the pipette.

Plunger Actuator Test Results

The test was conducted using components from a Gilson P1000 micropipette which is designed to accurately draw and dispense volumes between 200 and 1000 µL. Before conducting this test, we realized that it no longer reflected the way in which we expected the pipette actuator to operate. Originally, it was expected that the plunger would be actuated as shown in Figure 4, moving up from a fully depressed state to draw fluid into the pipette tip, then the plunger would be fully depressed again to dispense the drawn fluid. After some consideration, it was decided that the actuator should instead operate as shown in Figure 5. By not fully depressing the plunger before starting to draw the liquid, a small amount of air would be trapped behind the liquid that could be expelled to help ensure all fluid was dispensed from the plunger.

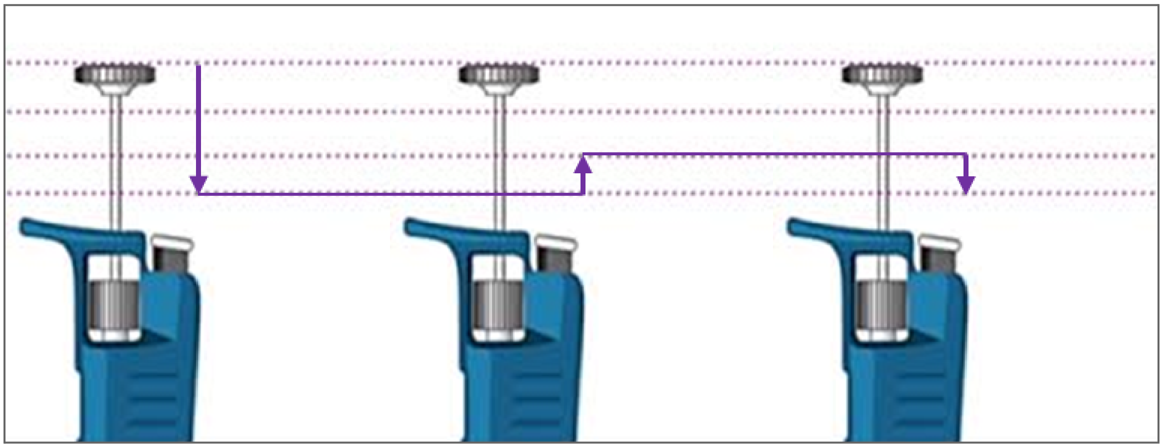


Figure 4. Original planned plunger actuation path

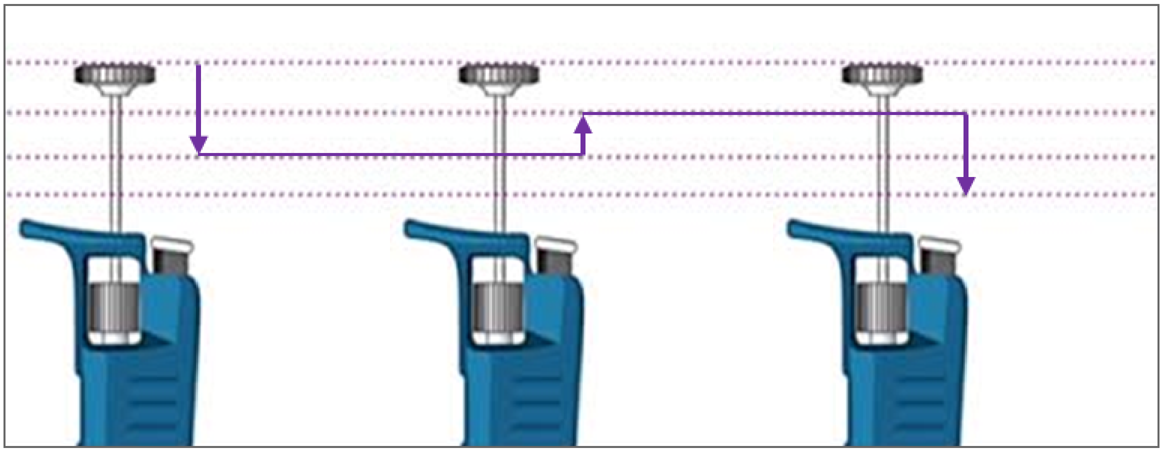


Figure 5. Revised plunger actuation path

As expected, when the test was run as written and the plunger was started from a position where it was fully depressed, the pipette was unable to expel all of the fluid that was drawn into the tip. Following this, the pipette actuator was programmed to draw roughly 1000 µL of water into the tip using the updated motions outlined in Figure 5. After each visual inspection, it was clear that in each of these 10 trials, the water was successfully expelled from the tip and that no meaningful amount was ever left inside the pipette tip.

Fluid Volume Test Results

This test was performed by drawing liquid by moving the pipette actuator at four different fixed amounts. Data was recorded by plotting the correlation between the number of steps taken and the volume of water dispensed. This test was performed 12 times for each of these volume setpoints instead of the 24 specified as the results were highly consistent.Since the original plan for 24 measurements at each volume was decided simply to fill a complete well plate, this modification should not affect the validity of the results.

Based on the data shown in Table 4, all measurements fell within the 2% required error range specified by the test criteria with three tests resulting in an error below 1%. This result is considered excellent since the only test case that resulted in error above 1% occurred when drawing a smaller volume of liquid. The P1000 is known to become less accurate towards the lower end of its volume range however and as such this slight increase in error was expected [5]. Based on these results, it was possible to plot a relationship between the volume and step count as shown in Figure 6 and come up with a well-fitting trend line as shown in Eq. 4.

Note that in Table 4, the plus or minus range on the average volumes indicates the maximum and minimum values measured during the tests; the error for each of these tests was calculated by dividing the plus or minus value by the mean volume measured.

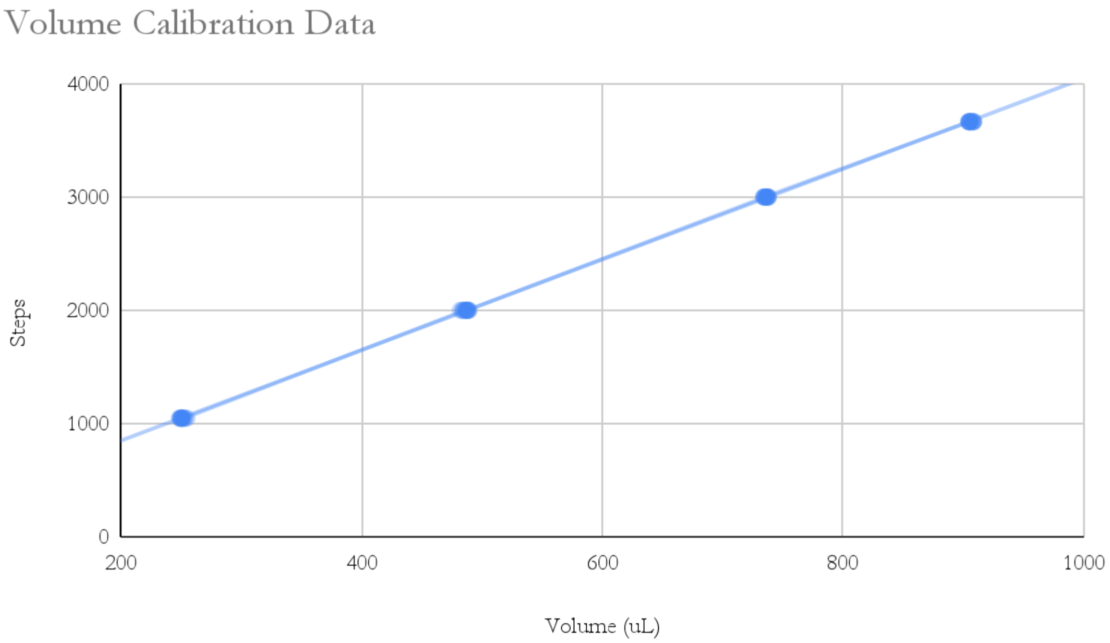


Figure 6. Calibration data for translating volume to stepper motor step count

Table 4. Fluid Volume Statistics

Steps	Mean Volume	Error
1047	251.1 ± 3.1 µL	1.25%
2000	486.6 ± 3.6 µL	0.74%
3000	736.0 ± 2.1 µL	0.29%
3665	906.1 ± 2.1 µL	0.24%

Sustained Power Draw Test Results

This test was not completed successfully due to component failures during the testing process. With all components connected, the measured current draw from the power supply fluctuated from 0.301 to 0.319 amps. However, a burning smell from the voltage regulator began after around two minutes. When touched, the regulator burned one of the team member's fingers. No errors in the circuit were found. Yet, two of the stepper motor drivers stopped working. The results can be seen in Table 5.

Table 5: Sustained power draw test results

Working components						**Failed Components**	
Drivers	Switches	Stepper Motors	Arduino	Solenoids	Power Supply	Drivers	Voltage Regulator
7	9	9	1	2	1	2	1

Although this test failed, the circuit has been modified to provide greater control over the amount of power that the circuit will consume during operations. The Arduino controller can enable and disable individual driver circuits so that the stepper motors will not consume power unless in operation. This change should help reduce the load on the circuit and help prevent power draw issues going forward.

Prototype Size and Weight Test Results

The dimensions and weight of the current prototype are presented in Table 6. The prototype did not meet the size and weight requirements as of the testing date. Specifically, while the width of the prototype is well within the prescribed limit of 4 feet, the depth measuring at 2.23 feet, slightly exceeded the 2 foot limit.

Specifically, while the width of the prototype is well within the prescribed limit of 4 feet, the depth measuring at 2.23 feet, slightly exceeded the 2 foot limit. This outcome was not unexpected, however, the aluminum bars have yet to be cut to their intended length. Since this deviation does not compromise the functionality of the prototype, shortening the depth has not been prioritized. After talking with the project sponsor, it was noted that 2.23 feet was an acceptable length for the prototype, and it would still be able to fit on a laboratory table, fulfilling the applicable constraint.

Furthermore, it should be noted that the weight recorded in Table 6 is an approximation. The circuitry is still only functioning on a breadboard and not all components have been assembled. The estimated 34 lbs was obtained by placing the assembled parts on a scale along with all the unincorporated components. Notably, circuit components such as the Arduino, Raspberry Pi, and stepper motor drivers had a minimal impact on the overall weight, yet they were still placed on the scale. The team anticipates that the weight of the final prototype will still be around 34 pounds, which is significantly lower than the limit of 500 pounds.

Table 6: Prototype dimensions and weight

Width	Depth	Height	Weight
2’ 2.75”	2’ 2.75”	2’ 5.25”	~34 lbs

Evaluation

The Z-Axis Positional Accuracy test revealed that the margin of error did not increase meaningfully when the z-axis movement system was re-zeroed after each run. The last ten trials increased the number of movements from one to five and despite this, the error remained less than 0.1 millimeters and thus well within the set operational requirements.

The X/Y Axis Positional Accuracy Test also met our acceptance criteria, as all data points were within 1 mm of the expected location. Therefore, we can confidently conclude that our testing was successful and yielded highly accurate and precise results.

During the Tip Discard Test, it was observed that past 10 volts, the trendline in Figure 3 began to flatten, indicating a dropoff in increased speed per volt. This will be useful in determining the best voltage for the actuator, as increasing it too much may be excessive and energy inefficient. Overall, the test was successful in achieving a 100% pass rate, and more data was collected in order to fine tune the tip ejector actuator.

The Plunger Actuator Test was also considered to be a successful test since it proved that the mechanism could draw and expel the maximum amount of fluid that the P1000 can accurately measure. The Python script used to execute this test can be found in Appendix B and the Arduino code used can be found in Appendix C.

The Fluid Volume Test exceeded the team's expectations. As stated before, all measurements fell within the 2 % required error. However it is important to note that the one volume case that exceeded 1 % error was at a volume of the pipette that the team understood would not have done as well. Therefore, the rest of the data is more representative of how well the plunger actuation mechanism performed. The low error in the measurement data and the excellent correlation between step count and volume thus made this test a success.

The Sustained Power Draw Test was one of the tests that failed. However, the team was unable to compare the results with the project requirement listed for power. From this outcome, the team decided to make one major change. Moving forward, the Arduino will not be powered by the power supply and the voltage regulator. Instead, the Arduino will be connected directly to the Raspberry Pi via USB. This will provide several benefits. The first being that there will no longer have to be a concern over any spikes in voltages that would damage the Arduino. Also, a connection via USB may allow for more simple communication between the Raspberry Pi and Arduino. Lastly, the overall circuit will be able to be simplified and be significantly smaller considering the voltage regulator is a large component.

Prototype Size and Weight Testing also resulted in failure according to the prototype test plan. Yet, the team has deliberated over the dimensions of the prototype with the sponsor and found the current dimensions to be suitable. Therefore, the team has determined on maintaining the current dimensions making this test a success.

Finally, after full configuration was achieved, the operation cycle was measured to be around 54 seconds for a total cycle. While this was over the desired time of 30 seconds, the team met with the sponsor and determined this was an acceptable time for the first demonstration. The initial 30 second cycle was the fastest time recorded by Opentrons, an established engineering company. It is believed that the cycle time could be further improved with optimized code and better motor control.

Conclusions

Overall, the current prototype design demonstrates the team has achieved all its goals according to the most recent project plan. The prototype has functioning movement systems, pipette mechanism, and controlling implementations. The prototype is complete as the team intended from the onset of the semester and is a working prototype. In addition, the testing procedure proved the prototype achieved all project requirements and applicable size and weight constraints. Even though power concerns persist, the prototype still functions with a breadboard powered by wall-power. Although a 30 second cycle time was unable to be achieved, it is expected that further code optimization and better motor control can significantly reduce the current time of 54 seconds. Overall, one prototype was designed for $516.92 (see appendix C), achieving the applicable constraint of being built for less than $1000.

Additional improvements to the design would include adding another pipette, improve the cycle time, and further automate the processes. Furthermore, developing the User Interface and polishing the aesthetics would complete the prototype as envisioned when the project began in the previous fall semester. This would allow for additional tests that concern the User Interface that were not included in the project plan.

Appendix

Appendix A. Equations

$$Distance\ Traveled = 0.01 * Steps + 0.003 \quad (R^2 = 1) \tag{1}$$

$$0.0177x^2 - 0.498x + 4.24 \tag{2}$$

$$R^2 = 0.99 \tag{3}$$

$$Steps = 4.00 * Volume + 48.06 \quad (R^2 = 0.9999) \tag{4}$$

Appendix B. Additional data from Fluid Volume Test

Table 7. 1047 Step Volume Test

Mass (g)	Volume (uL)
0.2550	255.0
0.2507	250.7
0.2487	248.7
0.2501	250.1
0.2494	249.4
0.2527	252.7
0.2515	251.5
0.2499	249.9
0.2510	251.0
0.2507	250.7
0.2507	250.7
0.2533	253.3

Table 8. 2000 Step Volume Test

Mass (g)	Volume (uL)
0.4856	485.6
0.4879	487.9
0.4865	486.5
0.4873	487.3
0.4843	484.3
0.4890	489.0
0.4841	484.1
0.4821	482.1
0.4893	489.3
0.4878	487.8
0.4873	487.3
0.4876	487.6

Table 9. 3000 Step volume Test

Mass (g)	Volume (uL)
0.7349	734.9
0.7380	738.0
0.7346	734.6
0.7367	736.7
0.7363	736.3
0.7357	735.7
0.7338	733.8
0.7364	736.4
0.7364	736.4
0.7375	737.5
0.7378	737.8
0.7342	734.2

Table 10. 3665 Step Volume Test

Mass (g)	Volume (uL)
0.9052	905.2
0.9049	904.9
0.9042	904.2
0.9048	904.8
0.9045	904.5
0.9081	908.1
0.9059	905.9
0.9077	907.7
0.9085	908.5
0.9047	904.7
0.9083	908.3
0.9066	906.6

Appendix C. User Guide

Purpose

The objective of this user guide is to provide instructions for the operation and continued development of the robotic liquid handler. The guide will generally include instructions based on the currently assembled mechanical components, but it will also include details on the process of adding additional hardware controls.

Hardware Components Setup

To begin controlling the robotic liquid handler, the following hardware components must be connected to the Arduino Mega circuit: the pipette tip holder, the liquid holder, the well plate holder, the pipette y-movement stepper, the pipette z-movement stepper, the pipette plunger actuator, and the pipette tip ejector. Note that all listed components aside from the pipette tip ejector are composed of a stepper motor and limit switch.

Refer to the wiring diagram in Appendix C to connect the stepper motors, limit switches, and DC motor. It may be necessary to modify the pins used by the liquitron_controller sketch as the pinout has changed multiple times to accommodate broken pins on Arduino Megas used in this project. The stepper motors used in the liquid handler should be wired so that movement towards the limit switch is negative. To protect the stepper drivers, make sure to depower the stepper driver circuits before reversing the orientation of the stepper driver connection if the need to do so arises.

Software Configuration

To test and ensure that the hardware is connected correctly, clone the robotic-liquid-handler and liquitron repositories onto your computer. Refer to the GitHub documentation on cloning a repository if you need help with this process. In the robotic-liquid-handler repository, open and upload the liquitron_controller sketch to an Arduino Mega with the Arduino IDE or the Arduino Extension for Visual Studio Code.

There is a Python test script in the liquitron repository to help determine whether the liquid handler has been wired correctly. Instructions for running this script can be found in the repository's README file (it is recommended to read the file on GitHub to view a formatted version instead of raw markdown formatting). The README will also provide instructions for installing the liquitron Python library into a project for use in further feature development like a user interface. The liquitron library usage instructions can be found in the repository's README as well, and for details on the serial data packet format refer to the repository's SERIAL file.

Required Maintenance and Prevention

Certain parts of the prototype need to be regularly lubricated/maintained to ensure optimal use. Both the linear rail and threaded screw should be greased with GreaseCo automotive lubricant (or any available grease in the makerspace) after 30 hours of continual use. Lubrication should be applied along the thread or point of contact of the rail. Avoid excessive lubrication.

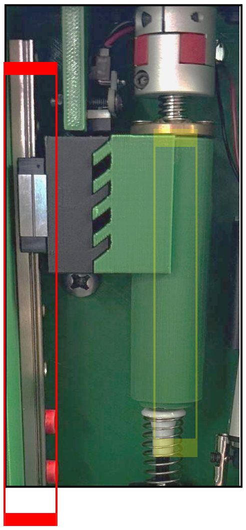


Figure 7: Picture of the linear rail (red) and threaded screw (yellow) that require maintenance.

The machine should be inspected before each use for any loose parts, bent limit switches, improper electrical connections or frayed/tangled wires to avoid any mechanical problems, or electrical hazards. The original micropipette system (spring, solenoid, and pipette shaft) should be visually inspected to ensure no cracks or damaged springs. It is recommended to remove the white pipette cylinder and clean bimonthly, or sooner depending on use. Gilson micropipette cleaning procedure found at the link below:

https://www.gilson.com/pub/media/docs/PIPETMANG_UG_LT801122-C.pdf.

If any parts become damaged or require replacement due to ineffective use, they can be found and repurchased from the parts order list. Any custom made parts can be 3D printed again.

Complete List of Parts for Assembly

In order to fully assemble the prototype, the following parts are required:

List of Prototype Materials

Material	Quantity	Estimated Price
2020 Extruded Aluminum 21.25”	8x	$67.20
2020 Extruded Aluminum 23.5”	3x	$25.20
2020 Extruded Aluminum 11.375”	4x	$16.8
2020 Extruded Aluminum 2.125”	4x	$5.6
2040 Extruded Aluminum 23.75”	1x	$10.00
24” by 24” wood baseboard	1x	$7.40
Metric screw set	1x	$27.99
m3 screws	41x	N/A
m3 washers	8x	N/A
m3 nuts	8x	N/A
m5 screws	72x	N/A
m5 washers	72x	N/A
m5 t-nuts	72x	N/A
m6 screws	4x	N/A
m6 washers	8x	N/A
m6 nuts	4x	N/A

3D printer timing belt (5mm)	112 inches	$7.99
Assembled Big V Slot Gantry Plate for 2020 Aluminum Extrusion	3x	$30.00
Assembled 2040 Aluminum Extrusion V Gantry Plate Kit	1x	$15.00
Zeberoxyz 20pcs Copper Buckle	8x	$8.00
3D printer pulley set	1x	$14.99
3D printer idler pulley	4x	N/A
3D printer motor bore pulley	4x	N/A
ReliaBot 150mm MGN9 Linear Rail Guide	1x	$14.00
Epindorf P1000 Tip Holder	1x	N/A (Provided)
Gilson P1000 Micropipette	1x	N/A (Provided)
96 well plate	1x	N/A (Provided)
100mm long threaded screw 8mm	1x	$9.99
6mm to 3.5mm motor mount	1x	$2.00
Befenybay 200mm Travel Length Linear Rail Guide Ballscrew SFU1605	1x	$72.00
BIGTREETECH Direct 5*TMC2208 V3.0 Stepper Motore Driver	7x	$42.00
Stroke 25mm 40N IP54 Stroke 0.98" Force 8.81 lbs	1x	$19.99
Ardunio Uno Rev 3	1x	$27.60
Limit Switches	6x	$6.00
Neema 17 stepper motors	5x	$55.00
1691g SUNLU 3D printer filament	1x	$32.13

3D printed PLA corner joints (208g total)	4x	N/A
3D printed PLA T-joint (260g total)	4x	N/A
3D printed PLA pipette mechanism pieces (423g total)	1x	N/A
3D printed PLA lower movement system motor mounts (198g total)	3x	N/A
3D printed PLA lower movement system opposite side motor mounts (162g total)	3x	N/A
3D printed PLA upper movement system wide beam motor mount (97g total)	1x	N/A
3D printed PLA upper movement system opposite side wide beam motor mount (101g total)	1x	N/A
3D PLA printed stock container mount (242g total)	1x	N/A
	Total Price:	$516.92

Appendix D. Terminology & Setup

- **Step** - In the context of a stepper motor a step refers to a discrete rotational motion of the stepper motor.
- **Accuracy** - A metric of how close measurements are to the target value.
- **Precision** - A metric of how close measurements of the same target value are to each other.

Setup: Pictured in Figure 8 is the pipette mechanism setup used for the tip discard test, plunger actuator, and max volume test. The tip actuator (located to the right of the green cylinder) was used for the tip discard test. The volume mechanism (threaded screw and green cylinder) was used for the plunger actuator and max volume test.

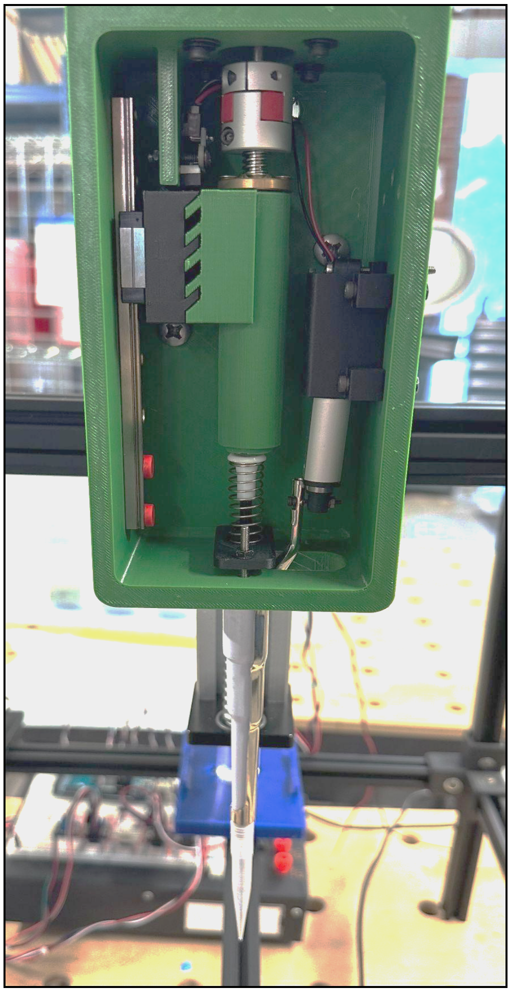
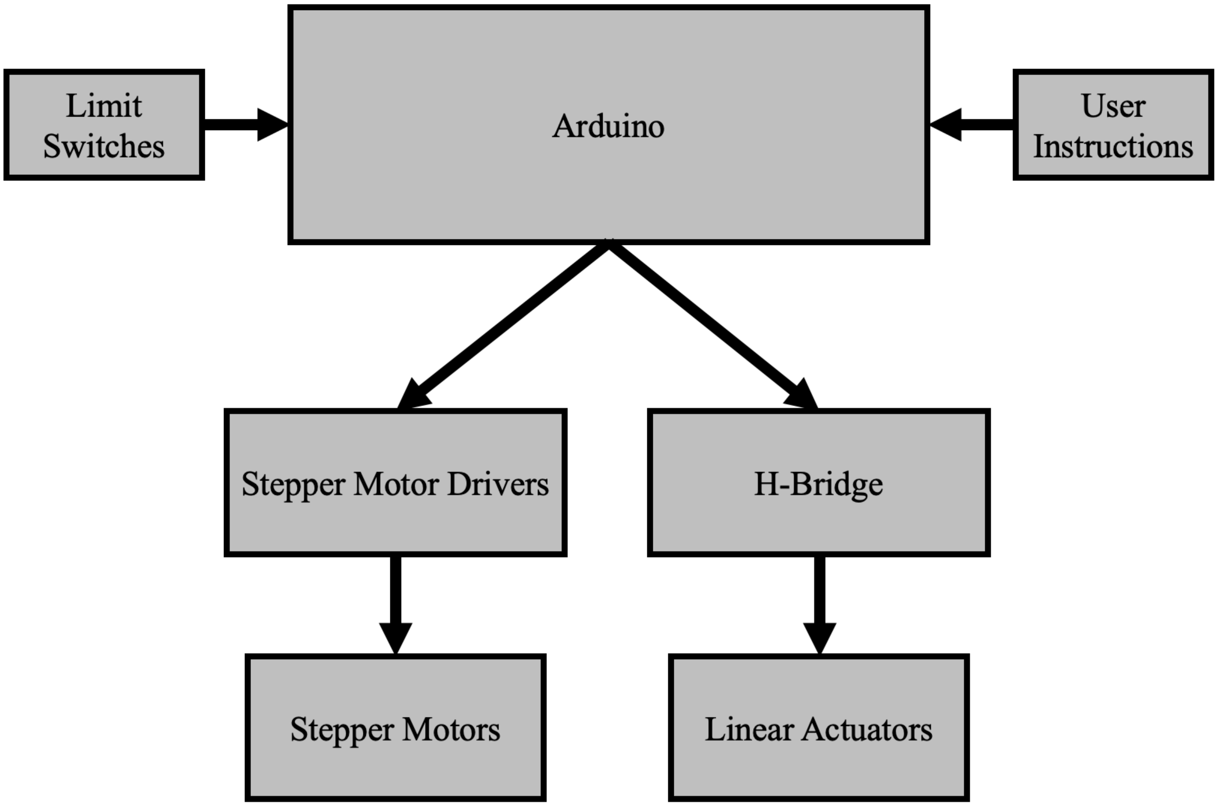
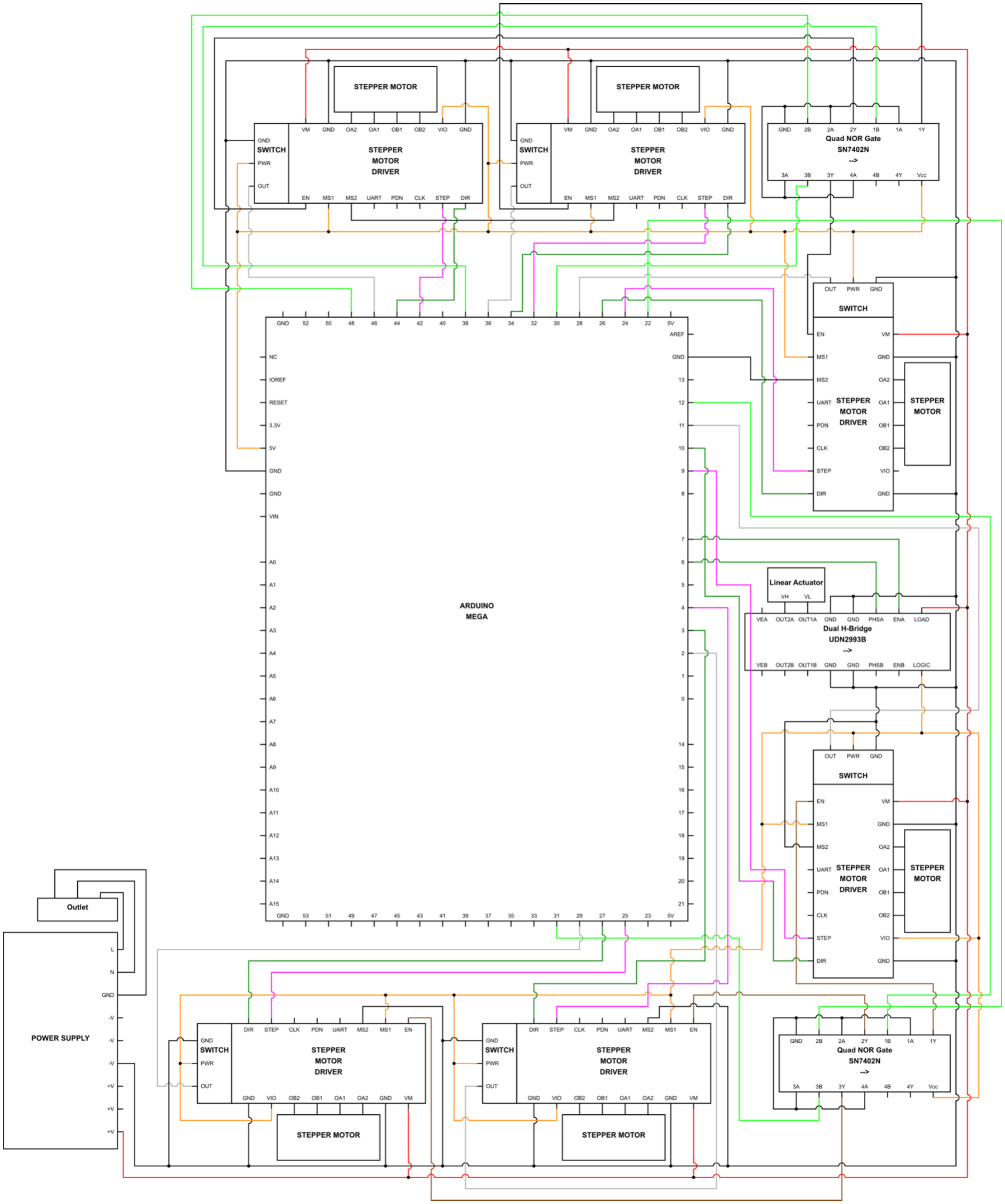


Figure 8: Pipette Mechanism setup for the prototype tests

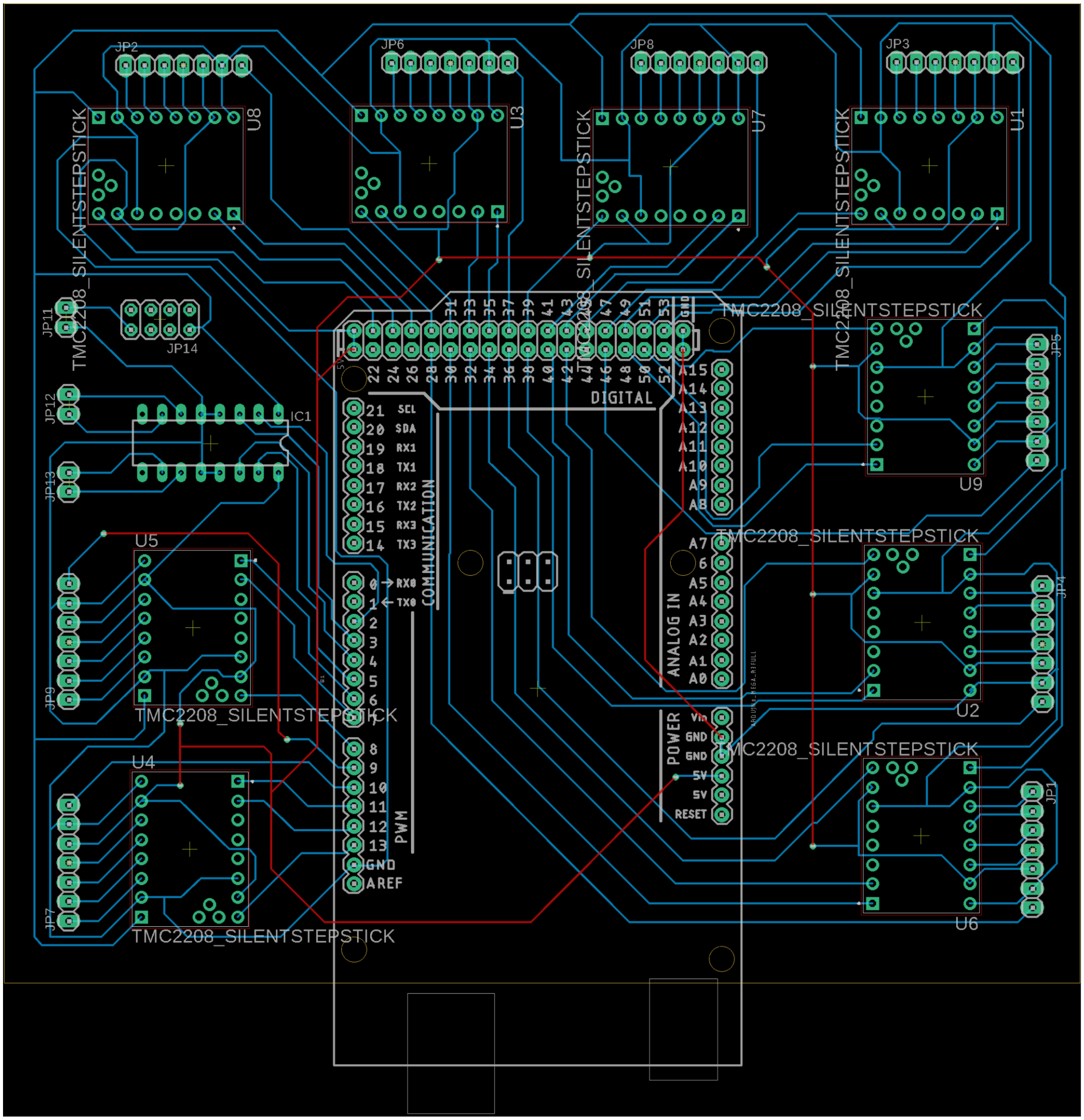
Appendix E. Flow chart of the electrical system



Appendix F. Arduino Controller Wiring Diagram



Appendix G. Arduino Controller EAGLE Schematic



Appendix H. Full Prototype Design and Drawing Schematics (units in millimeters)

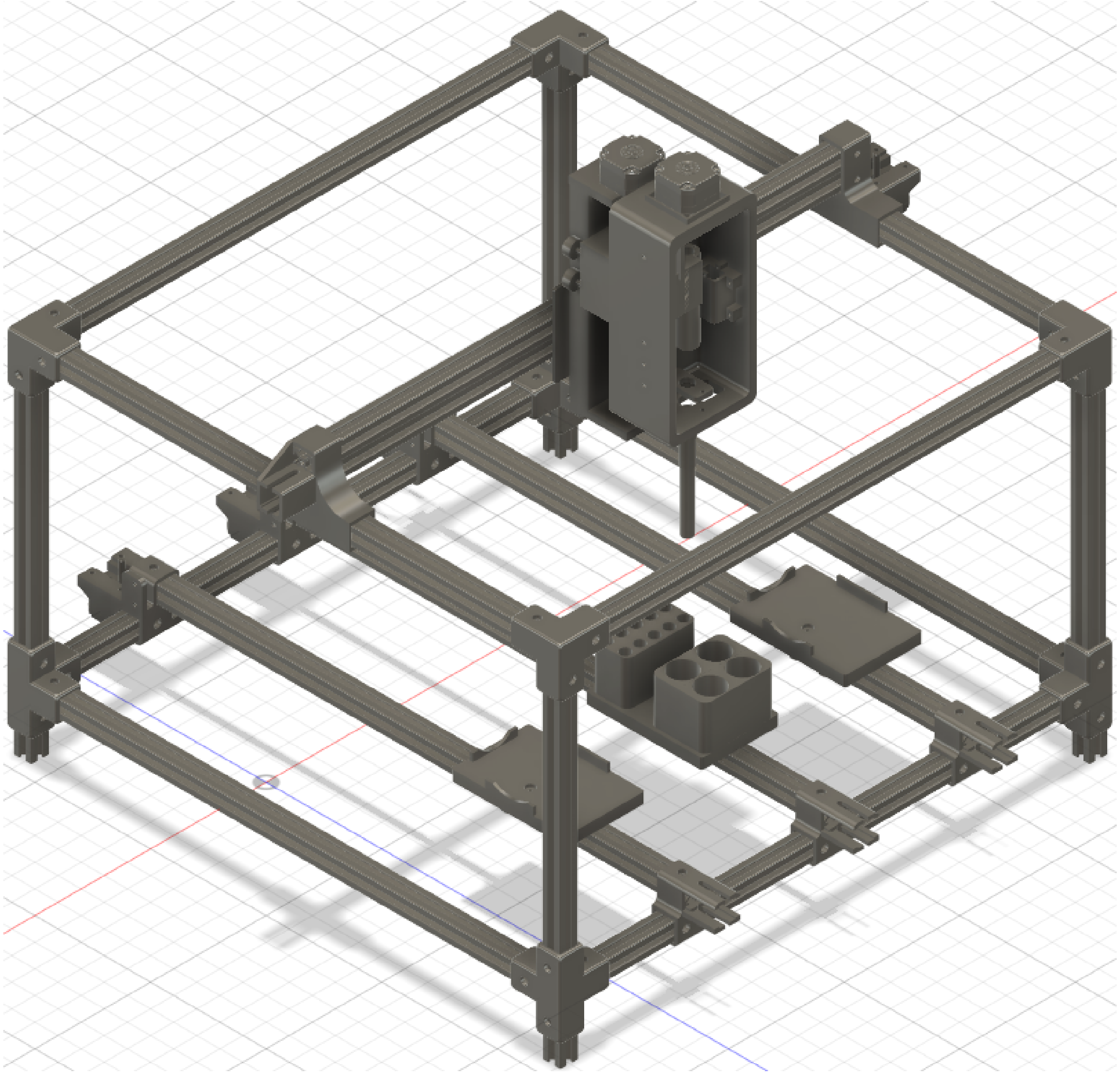


Figure 9: Final prototype fusion model

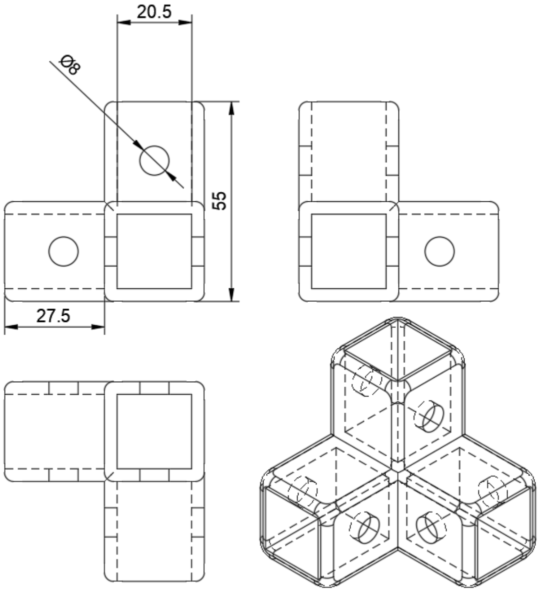


Figure 10: Corner Joint fusion drawing schematic

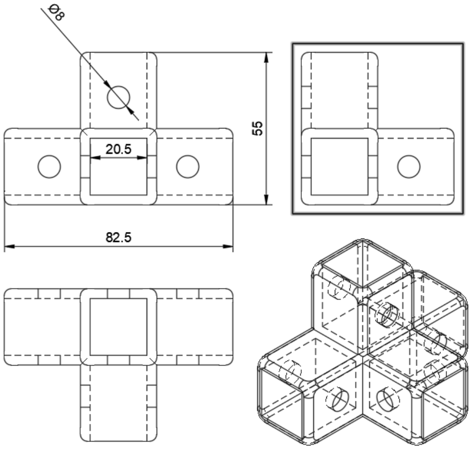


Figure 11: T-joint fusion drawing schematic

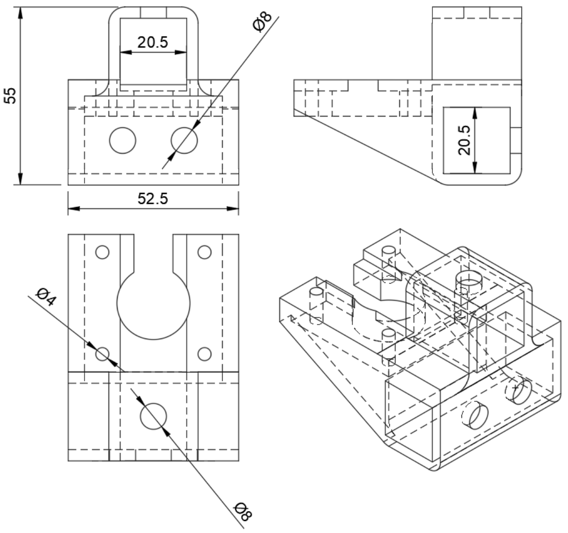


Figure 12: Lower movement system motor mount schematic

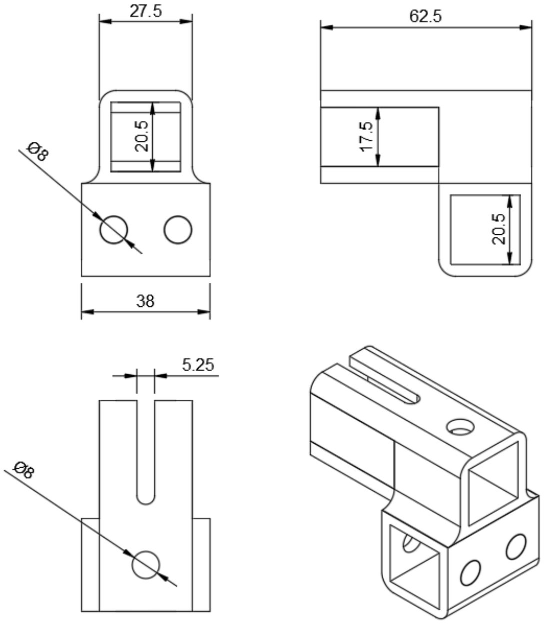


Figure 13: Lower movement system idler motor mount schematic

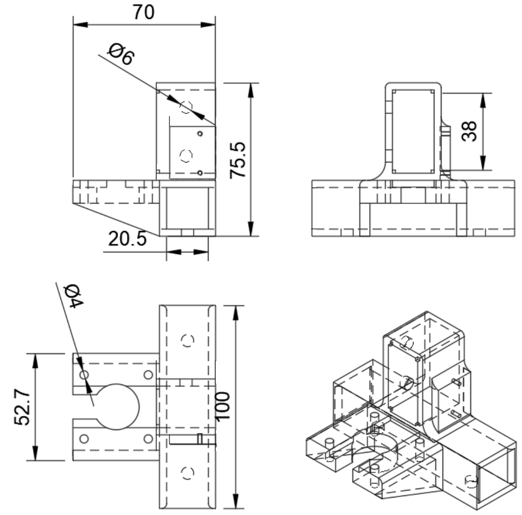


Figure 14: Upper movement system motor mount schematic

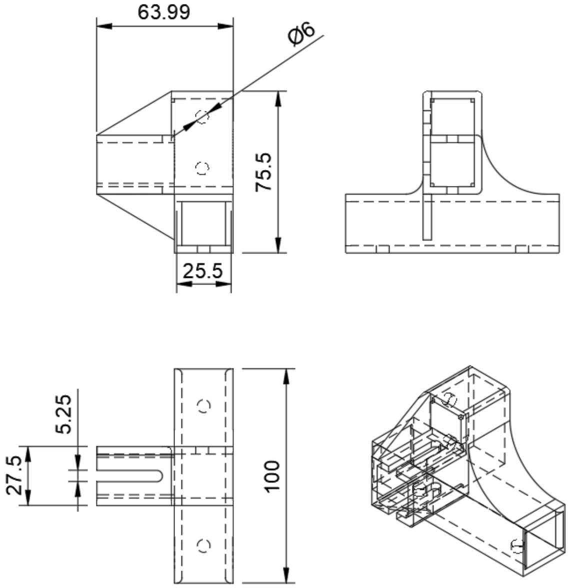


Figure 15: Upper movement system idler motor mount schematic

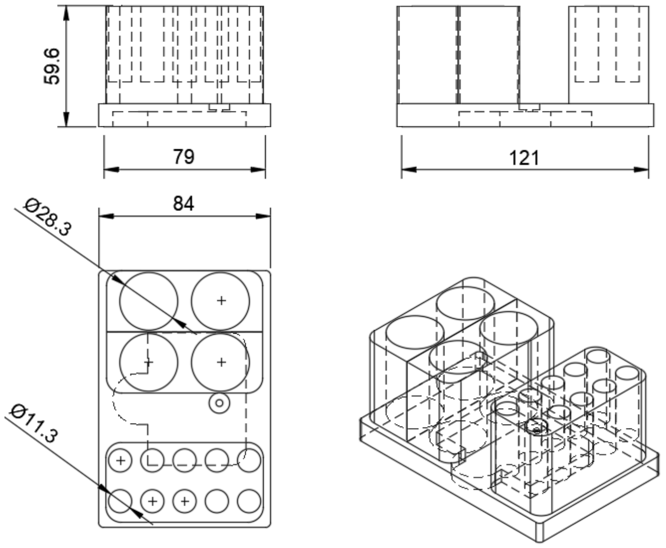


Figure 16: Stock container holder drawing schematic

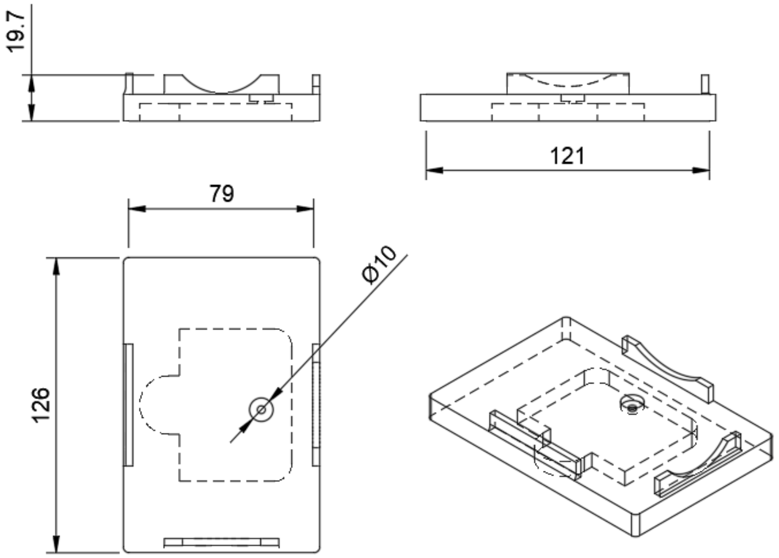


Figure 17: Tip holder and 96 well plate mount drawing schematic

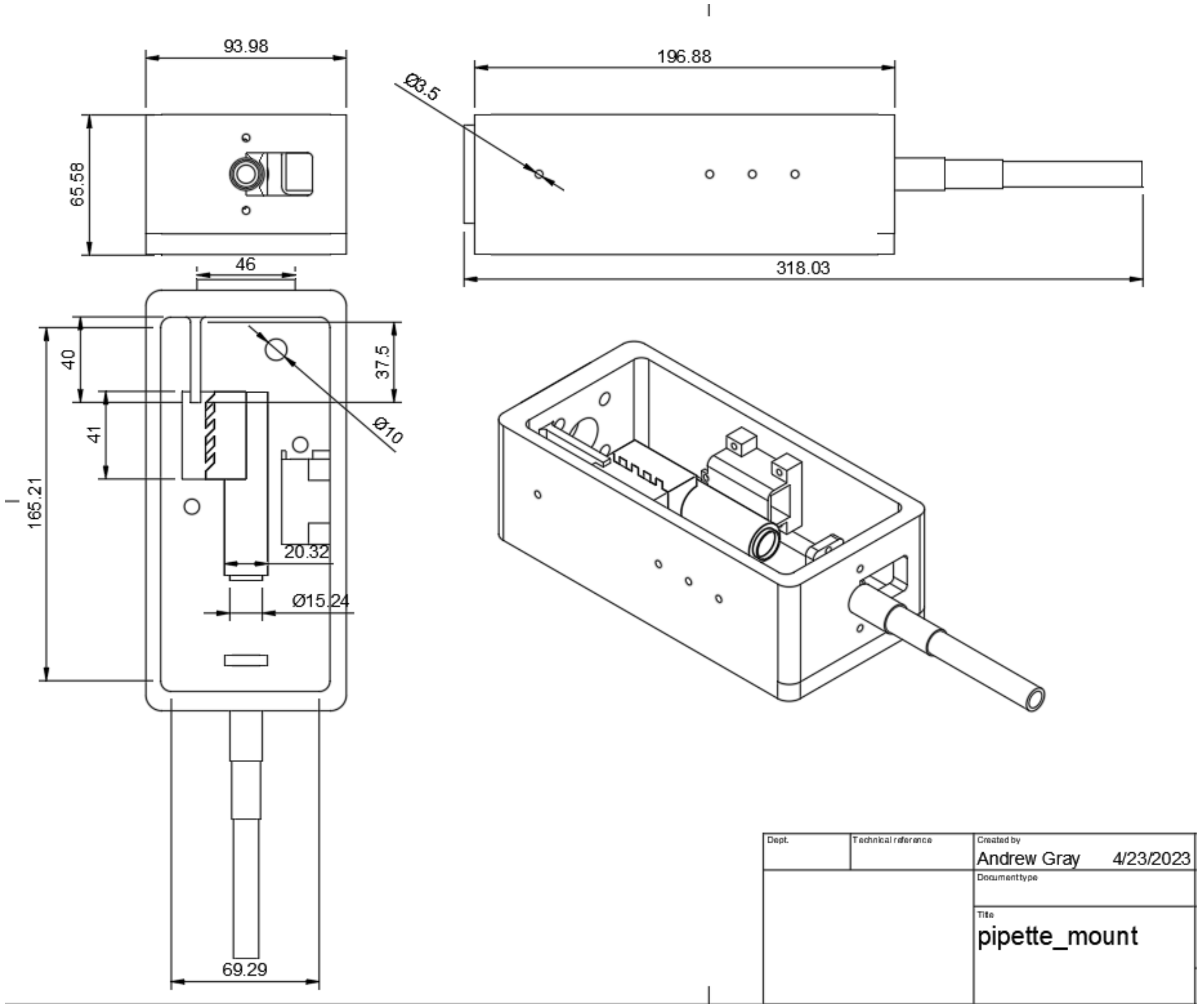


Figure 18: Complete Pipette Mechanism drawing schematic

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