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Automated Portable Detection Platform for Point of Care Diagnostics

A thesis submitted in partial satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

in

ELECTRICAL AND COMPUTER ENGINEERING

by

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Abstract

Automated Portable Detection Platform for Point of Care Diagnostics

by

Ray Allen Jara

Biosensor technologies have enormous potential in the field of biomedical diagnostics. Despite this, certain barriers have prevented the widespread application of existing biodiagnostic technologies. The limitations include the requirements of high-end, expensive, and complicated laboratory instruments, as well as the lengthy laboratory procedures that may cause error on the part of the user. I have developed an Automated Portable Detection Platform for Point of Care Diagnostics to overcome these limitations. This platform includes a custom microfluidic Lab-On-Chip (LOC) device capable of performing point-of-care diagnostics, which will be demonstrated by performing enzyme-linked immunoassay, ELISA, tests. Using a low-cost pump and valves helped create a pneumatic system for a contactless flow between solutions in a microfluidic LOC device. The platform is relatively inexpensive and much more compact than other equipment that can be used to conduct similar tests. The automation is done via a user-friendly interface which could be accessed with a PC or Raspberry Pi microcomputer; here, a custom script may be entered to perform different functions and tests. This Automated Portable Detection Platform will be described in detail in this thesis, including its reliability and stability for precision

control over the fluidic flow. In addition, data was collected and will be presented to demonstrate the abilities of this platform. Overall, this thesis will cover a significant step forward toward the adaptation of widespread point-of-care testing platforms in different clinical settings.

Acknowledgments

I would like to thank my advisor, Prof. Ali Yanik, for giving me the opportunity to work in his research group as an undergraduate student, then motivating me to continue my education into graduate school. Without this continuous support and guidance, I would not have been able to accomplish what I have in the last four years.

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A very special thanks to Mustafa Mutlu for being a great role model, mentor, and friend. Thank you for always being there for me when I needed you the most, both in research and outside the lab.

Last but not least, my family. I would not be the person I am today without my parents always being my number one supporters. Thank you, Mom and Dad.

Chapter 1 Introduction

1.1 Motivation

Over the previous years, biosensing technologies have enormously impacted the biomedical field. These technologies include disease monitoring, drug discovery, and much more. However, current diagnostic technologies have been adapted to a limited extent due to their reliance on expensive and high-tech laboratory equipment as well as the high probability of human errors associated with time-consuming procedures.

To overcome these limitations and provide an effective and reliable solution, I have designed a new platform capable of full automation and the ability to perform enzyme-linked immunosorbent assay, better known as ELISA tests, using a microfluidic Lab-on-a-Chip (LOC) device. The objective of this autonomous platform is to help eliminate any human error that may occur executing long procedures. Automatization is done using a PC or Raspberry Pi with or a user interface. An Arduino Uno microcontroller is used to control a syringe pump and various valves for a contactless fluidic flow into a microfluidic LOC preventing contamination of the non-disposable components (such as valves and syringe pump). We have developed a microfluidic LOC device that allows us to convert complex procedures that require large laboratory equipment into a small 70 x 50 mm form factor platform, which eliminates the need for expensive equipment.

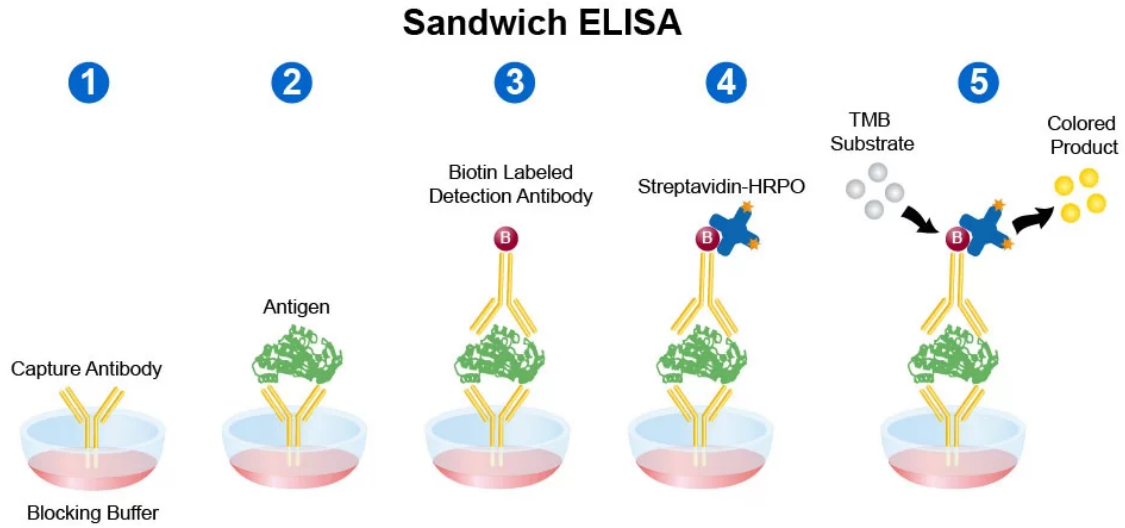

Figure 1.1: Sandwich ELISA requires the use of two antibodies, to then trap or 'sandwich' the antigen between them on the plate's surface. Once captured enzymatic labels are used to determine molecular recognition. [15]

To achieve such a platform, we must first understand the ELISA protocol. ELISA is a plate-based assay for detecting specific proteins in a complex mixture. In most common form, a sandwich ELISA protocol is executed using a highly specific antibody that is immobilized onto a microplate. This assay indirectly detects the presence of the target protein[13]. Using two different types of antibodies, capture and detection, the antigen is then sandwiched between the two, **Figure 1.1**. In our case, we use magnetic beads instead of a plate; by doing this, we could mix the reagents automatically via a linear motor with an attached 3D-printed mount that houses a permanent magnet. With this magnet, reagents can be mixed as we move a capturing substrate (magnetic beads) within the channel back and forth. This platform

is capable of performing an ELISA experiment with different concentrations of antigens. The results of these experiments will be shown in the final section, **Section 3.2**. This thesis will go over every part of implementation of such a platform, including the design and fabrication of a microfluidic LOC, the construction of a pneumatic contactless pumping of reagents, and the hardware and software needed to create an automated platform, as shown in **Figure 1.2**.

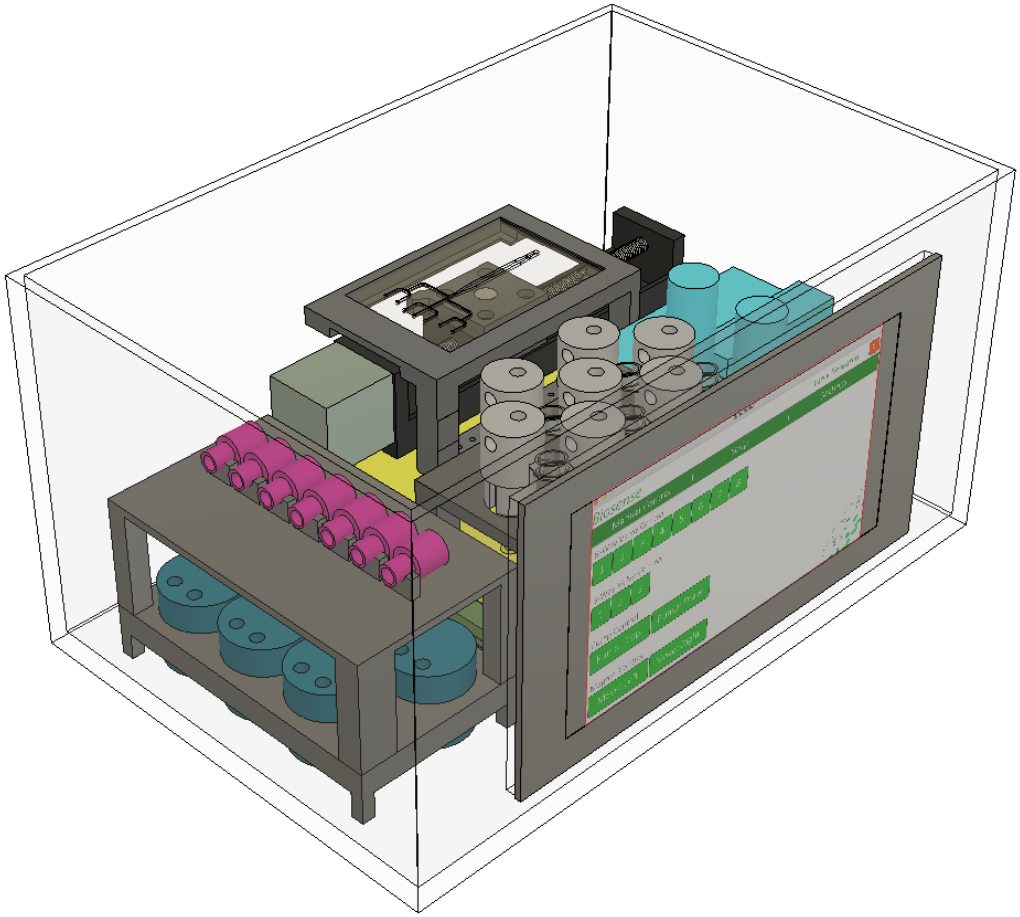

Figure 1.2: Render of Final Assembled Platform. Using various components, both purchased and 3D Printed, an Automated Portable Detection Platform is created for Point of Care Diagnostics.

Chapter 2 Materials and Methods

2.1 Microfluidic Lab-on-a-Chip Device

2.1.1 Design of PDMS Microfluidic Device

When designing our microfluidic Lab-on-a-Chip (LOC) device, the goal was to create a microfluidic device that would keep the reagents used separated until being pumped to mix in a mixing chamber designed at the center of the device. This layout would be challenging to develop as I faced several issues, most notably a backflow issue. This microfluidic LOC device will be central to creating an Automated Portable Detection Platform for Point of Care Diagnostics. The inlets were determined by the number of reagents used in the experiments. In our case, six different reagents and two of the same were used (for washing purposes). In total seven inlet ports are used while one single outlet is reserved for waste. The size of the mixing chamber was determined by the amount of volume needed to execute the assay protocol in an efficient manner. Earlier versions required a volume of around 150uL, which has been reduced to 30uL as improvements have been made.

As stated, there were issues when designing our microfluidic LOCs, such as bonding failures which can lead to leaks, nonspecific bonding of assays, and backflow. To overcome these issues, various solutions were implemented, and

different versions were developed. In earlier versions, the microfluidic LOCs were made from polycarbonate and double-sided tape, cut with an AutoDesk AutoCAD-designed Graphtec vinyl cutter. This approach proved to have many issues from leaks due to poor bonding, fabrication difficulties, and time consumption. I subsequently switched to a Polydimethylsiloxane (PDMS) based microfluidic LOC approach to address these issues. This was done by using AutoDesk Fusion 360 to design a master mold that will be used for PDMS fabrication. This route allowed us to fabricate precise device structures with stereolithography. Due to the varying height requirements for our channels in our LOC and unique patterns used to reduce backflow, we needed to quickly change design and update the channel structures within hours. There is also a significant cost saving between the two procedures, as shown in **Figure 2.1.**

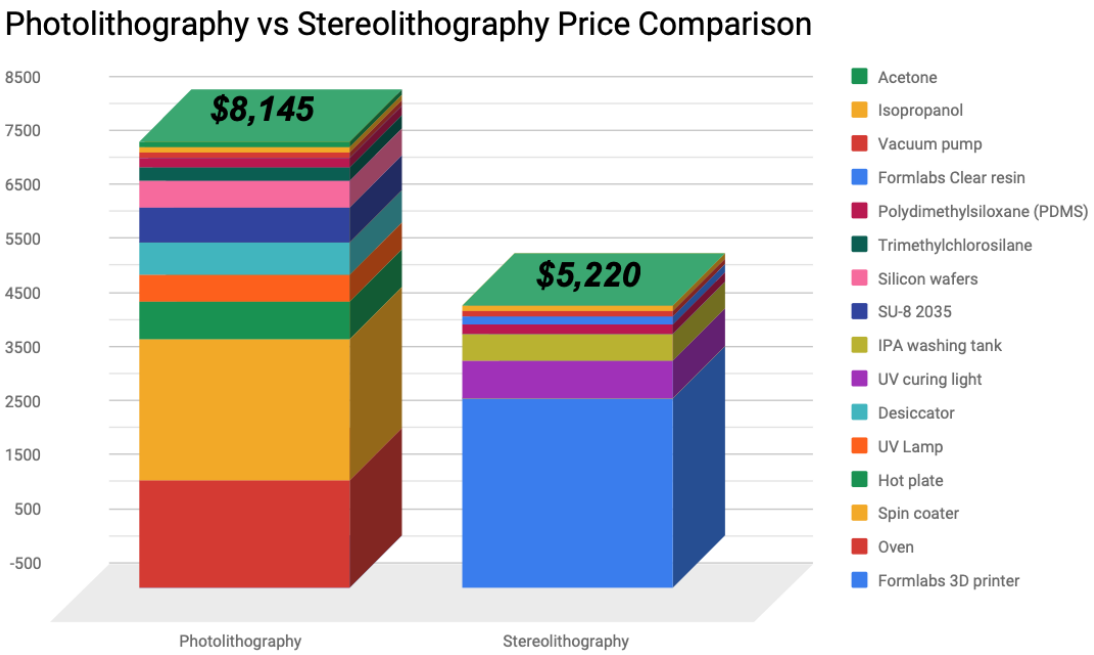

Figure 2.1: Photolithography vs. Stereolithography price comparison. Listed above are the main equipment and materials to perform each approach.

Stereolithography involves using a 3D printer, in our case, the FormLabs Form3 printer with an XY resolution of 25µm to define a PDMS master mold. Stereolithography neglects the initial step of wafer etching, which involves various toxic chemicals, complex equipment, and overall cost. In addition to cost, photolithography requires the use of a cleanroom and special training, while a 3D printer could be used in any location with proper ventilation. A stronger bond was achieved between PDMS and the 70x50mm glass slide upon switching to PDMS fabrication, and leaks were eliminated.

The last major issue I encountered was backflow. Backflow could be described as unwanted flow in the reverse direction. In this case, the unwanted flow moved in the reverse direction of the neighboring inlet, which caused a significant issue when trying to keep specific solutions from mixing. This would cause premature mixing of reagents before their intended mixture protocol. For this reason, I decided to create a path of high-to-low resistance toward the microfluidic chips outlet. In this way, the fluid will flow along the path of least resistance towards the waste outlet, preventing backflow. **Figure 2.2** shows the resistance of the final microfluidic LOC design. The resistance is controlled by varying the width of the channel.

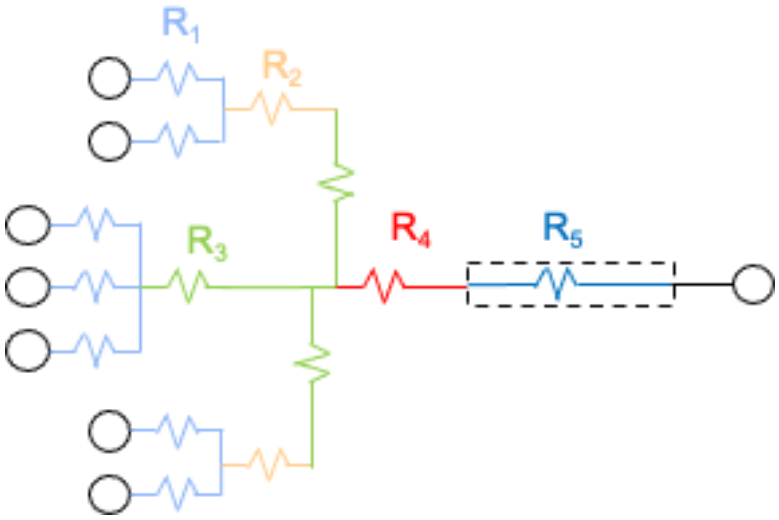

Figure 2.2: Microfluidic Lab-On-Chip Resistive Channels

The final microfluidic chip design, with a 30µL volume mixing chamber is shown in **Figure 2.3**. The channel height is 200µm, with the first inlet channels (R_1) having a

width of 250µm. This is then doubled to 500µm for the next set of channels (R_2 & R_3). The final channel (R_4) width before the mixing chamber is then stepped up to 750µm[2]. The mixing chamber has a maximum width of 2.5mm, leading to an outlet port of 2mm in diameter. In addition to having high to low-resistance channels, Elveflow One-way Microfluidic Check Valves were also added to the tubing before the inlets. These one-way microfluidic check valves help protect from any backflow surges, which adds a layer of protection, explained in more detail in **Section 2.2.4**. To help demonstrate that this high-to-low resistance design does indeed help prevent backflow in our application, a simple colored DI test was done. In this test, a microfluidic LOC with four inlets was used, each with a different color, as shown in **Figure 2.4**.

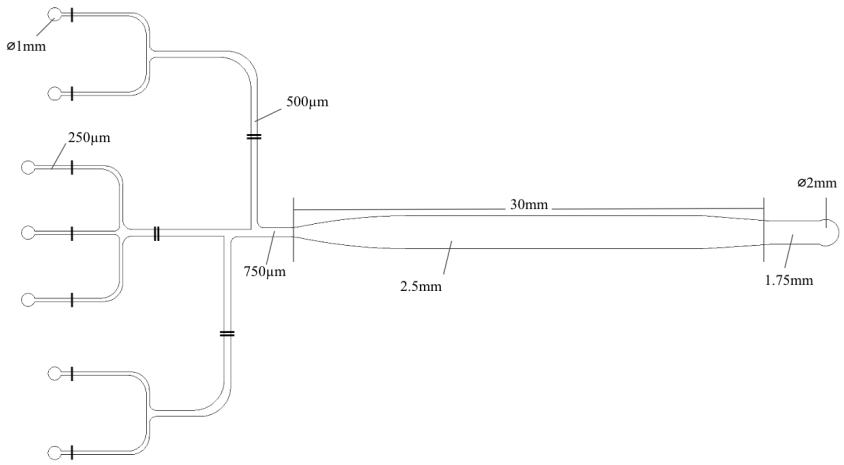

Figure 2.3: Dimensions of Microfluidic Lab-On-Chip

In this experiment, different color solutions were flown one at a time. Without a

high-to-low resistance channel, the fluid dispensed would mix or push the solution back to the neighboring inlet. **Figure 2.4** shows a junction point within the microfluidic LOC seen under a microscope. No green colored solution was pushed backward towards the red solution inlet. Only a limited mixing between the two color solutions is observed at the junctions.

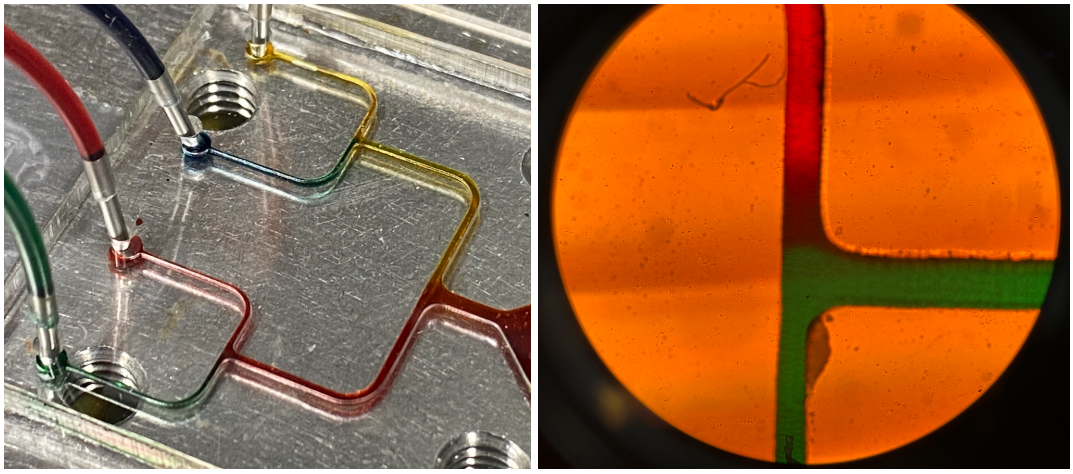

Figure 2.4: Colored DYE Test to Verify Function of Resistive Channels**A)** View of Microfluidic LOC. No backflow or major mixing of the different colored DI **B)** Microscopic view of the junction between red and green dyes

2.1.2 3D Printed PDMS Molds

After designing a microfluidic LOC with no bonding/leaks, or backflow issues, the AutoDesk Fusion 360 file is then ready to be exported for 3D printing.

As mentioned before, the traditional route of PDMS fabrication was avoided

and a rapid prototyping method was implemented. The route chosen to fabricate these devices was PDMS fabrication, which will require a mold to be designed and printed. This was created using stereolithography 3D printing, which uses a light source, a laser, or a projector, to cure liquid resin into hardened plastic, as shown in **Figure 2.5**. I used FormLabs' Form 3 3D printer to carry out this process.

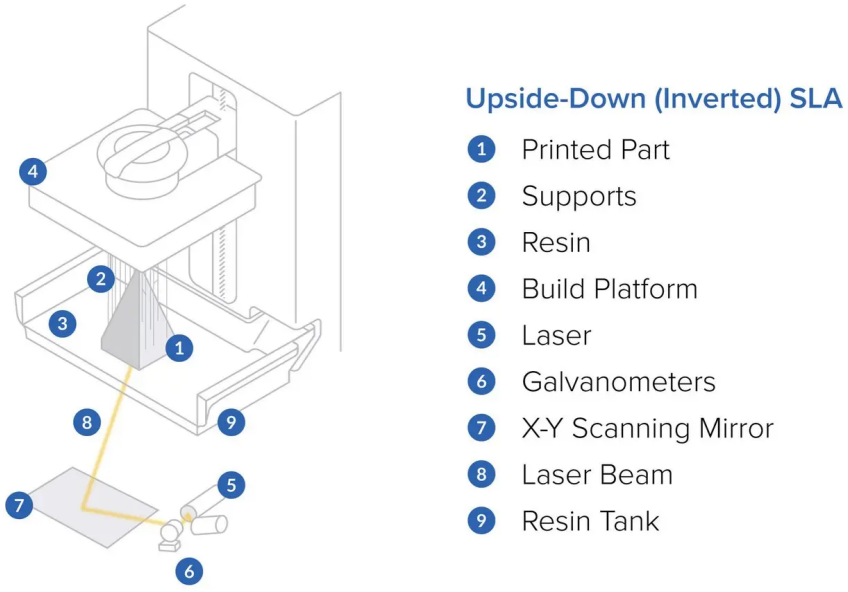

Figure 2.5: FormLabs Form3 Stereolithography Printer [18]

The FormLabs Form3 3D printer can achieve an XY resolution of 25µm, which is sufficient for the microfluidic mold fabrication purposes. A clear resin offered by the manufacturer is used. This resin worked great for the PDMS mold printing. This clean resin polishes to near optical transparency, making it ideal for working with light or showcasing internal features.

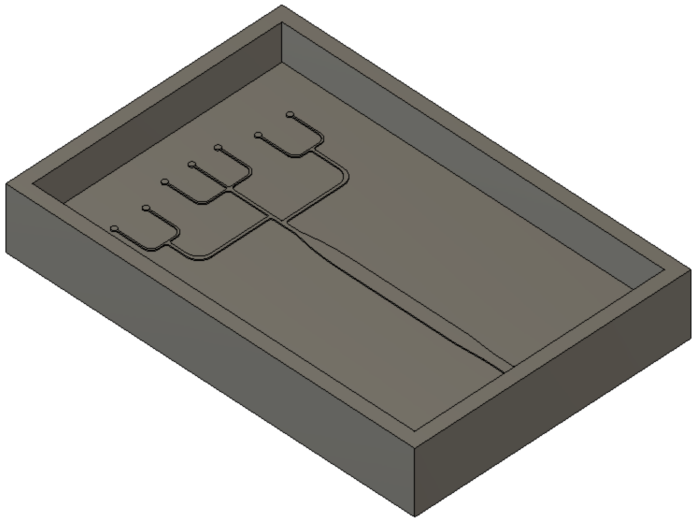

Figure 2.6: Microfluidic Lab-On-Chip PDMS 3D Printed Mold designed on AutoDesk Fusion 360

The process of fabricating a set of four molds took about ten hours and could be completed in six steps without any additional training. The first step is to create the 3D design in AutoDesk Fusion 360, then export the file to FormLabs Perform software. In this software, the user can select the proper printer, resin type, and layer resolution. After choosing the desired settings, support beams are added to realize more reliable fabrication. This could be done automatically or manually.The print can be started by exporting the file to the printer via USB cable or WiFi. Once the printing is completed, the tray that is attached to the printed mold is moved into an Isopropyl Alcohol (IPA) tank to be washed for 30 minutes. After the washing is completed, the prints could be removed from the tray and placed into a UV curing machine to harden for 30-60 minutes. The following is a summary of all the steps:

1) Create a 3D design in AutoDesk Fusion 360
2) Export .stl file to FormLabs Preform
3) Setup print
    a) Select proper printer
    b) Select clear resin
    c) Select layer resolution (25-100um)
4) Automatically or manually setup support beams
    a) If automatically, select “Auto-Generate All”
    b) If manually, go to “Support” menu and generate supports
5) Start Print
    a) Around 10 hours for printing molds
6) Once Print is done remove from tray from printer
7) IPA wash
    a) Place printer tray into the IPA wash unit
    b) Wash for 30 minutes
8) UV Cure
    a) Remove molds from tray
    b) Place into UV cure unit for 30-60 minutes

2.1.3 Fabrication of PDMS Microfluidic Device

Once the molds have been designed and printed the PDMS fabrication can be performed by pouring a PDMS mixture into the mold. This process is accomplished in the UC Santa Cruz Nano- and Microscale Fabrication and Characterization Cleanroom, **Figure 2.7**. This clean room houses equipment to perform various tasks, such as photolithography, PDMS work, metal deposition, and wet- and dry-etching. PDMS microfluidic chip fabrication is completed following standard procedures in this lab[19].

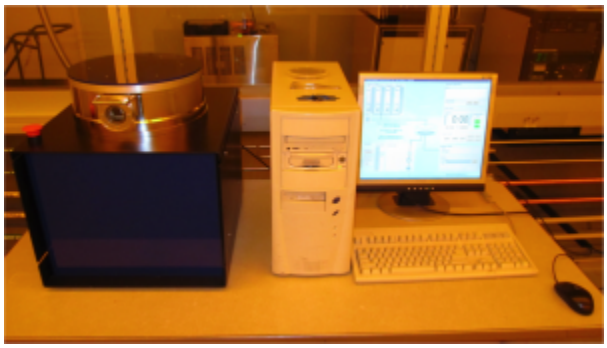

Figure 2.7: UC Santa Cruz Nano- and Micro-scale Fabrication and Characterization Cleanroom. Plasma Etch Reactive Ion Etcher used to bond PDMS to glass slide

Overall, there are six main steps to this fabrication process: Cleaning the molds, Preparation of PDMS, Pouring PDMS, Placing PDMS into the oven, removing hardened PDMS, and Bonding PDMS to a glass slide. As mentioned, although tedious the fabrication process is time-consuming, taking around 15 hours, with the oven hardening taking 12 hours.

The first step is to clean the 3D-printed molds, which is self-explanatory. Both the new and used 3D-printed molds will require cleaning in this step. If the printed molds are new, IPA is used to rinse the surface of the mold, then dried with a nitrogen air gun. If the molds were previously used, an additional step is required. The mold may contain leftover PDMS from the previous use, a pick tool or razor blade is used to remove the excess PDMS before solution based cleaning. Once cleaned, PDMS mold is rinsed with IPA and dried with nitrogen.

Sylgard 184 kit is used for PDMS fabrication. Two components, a polymeric base, and a curing agent. Using a 10:1 ratio of the polymeric base and curing agent with a 10:1 ratio is needed for this. It is beneficial to use PDMS because it is almost transparent (with a refractive index of 1.42) at optical wavelengths. The viewer is therefore able to observe the flow of reagents during microfluidic for the future incorporation of an imaging system for the detection of the ELISA product. Each mold uses roughly around 20g of PDMS. A 0.25 - 1% of PEG additive is then added to this mixture. This is to address the issue of non-specific adsorption of proteins and other molecules while keeping the mechanical properties of PDMS unchanged. Once the PEG additive is mixed with the 10:1 PDMS mixture, this is then placed into a vacuum chamber for 30-60 minutes to remove any air bubbles.

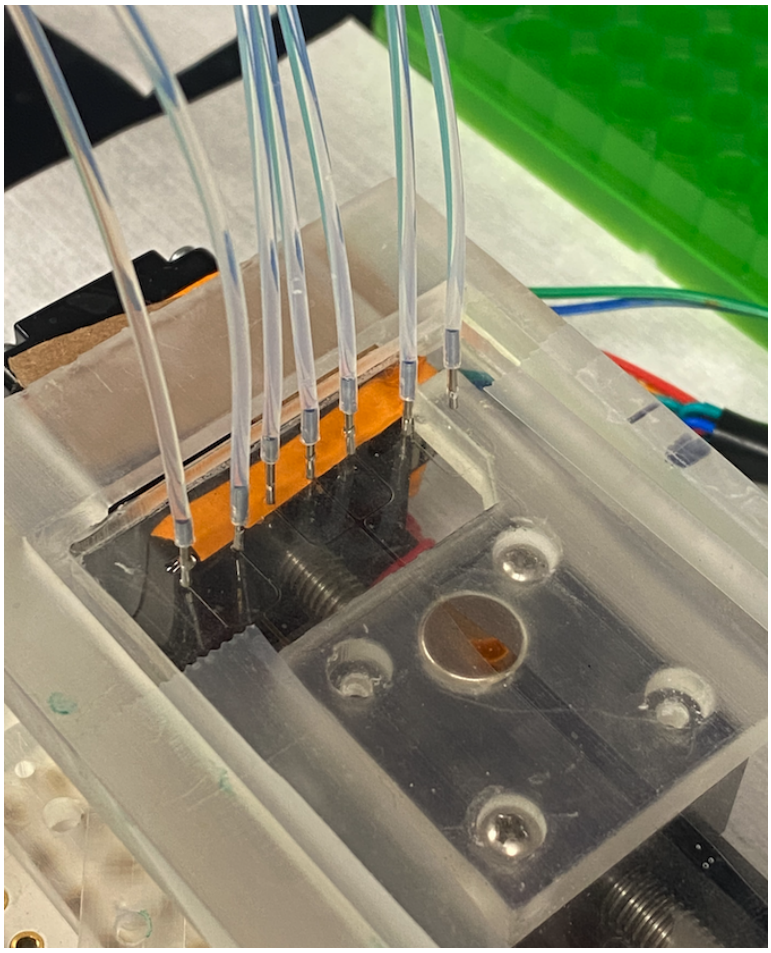

Figure 2.8: PDMS Microfluidic Lab-On-Chip

Once the mixture is free of any air bubbles, it can then be poured into the 3D-printed molds. The mixture was subsequently removed from the vacuum chamber, and poured into the mold. Once filled, the mold is placed back into the vacuum chamber for another 30 minutes to remove any air bubbles that may have been created during the pouring process.

After 30 minutes, the PDMS-filled molds may be placed into a 60°C oven for 10-12 hours; this is usually done overnight. There is an improvement in performance

when PDMS is cured for longer than 4 hours, which was employed initially.

Once the PDMS has hardened in the oven, the PDMS is now ready to be removed from the molds. The PDMS is carefully separated from the sides of the mold with a razor blade. Once removed, a 1 mm hole punching tool is used to create the holes for the inlets and outlet.

In the final step, the bonding of PDMS to a glass slide measuring 70 x 50 mm is completed. A microscope glass slide is first cleaned similar to step 1, using IPA and nitrogen. Once cleaned, a Plasma Etch Reactive Ion Etcher (RIE) is used to prepare the surfaces for bonding, as seen in **Figure 2.7**. The glass slide and PDMS are placed into the RIE equipment for 30 seconds; the PDMS microfluidic chip and glass are physically pressed together to create a sealed microfluidic device.

PDMS fabrication is more involved when compared to the 3D printing steps. PDMS will require additional training from the UC Santa Cruz staff as all the fabrication is done in a clean room. Although there are more complex steps, a researcher could still do the fabrication process with the proper training. The steps could be summarized as follows:

1. Clean 3D printed molds for PDMS
    a. Clean with IPA
    b. Dry with Nitrogen
2. Prepare PDMS
    a. Pour a 10:1 ratio of the following solutions
    b. Add PEG additive
    c. Mix for 5 minutes
    d. Place into a vacuum chamber for 30 minutes
3. Pour PDMS mixture into molds
    a. Place PDMS-filled molds into a vacuum chamber for 30 minutes
4. Place air bubble-free PDMS-filled molds into the oven for 12 hours
5. Remove PDMS from molds
    a. Use a razor blade to separate PDMS from molds
    b. Use a 1mm hole punch to make holes for the inlets/outlet
6. Bond PDMS to glass slide
    a. Clean 3x2” inch glass slide with IPA and dry with Nitrogen
    b. Use Reactive Ion Etcher
    c. Place PDMS onto a glass slide, bonding the two

2.2 Pumping Setup

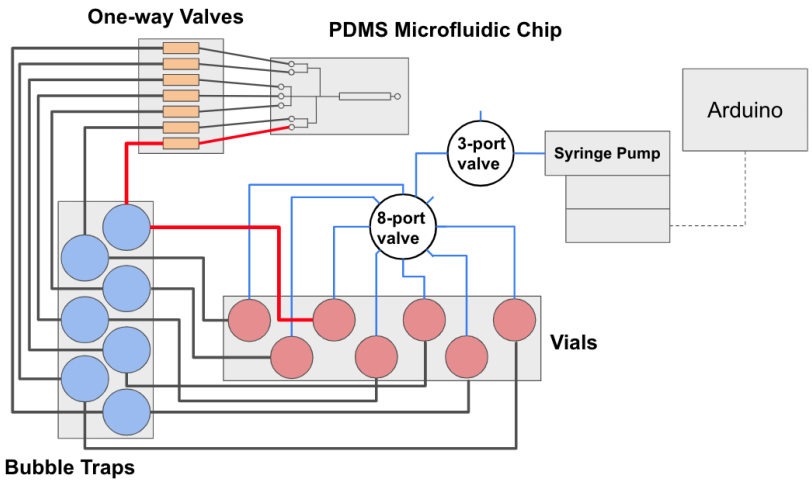

Figure 2.9: Layout of Components Used for Pneumatic Pumping System. Lines present 1/16” OD tubing used.

2.2.1 LabSmith pump and Valve Setup

For the fluidic flow of reagents into the microfluidic LOC device, a pneumatic system was developed. TThere are two types of fluidic flow, passive and active[11], each with their own advantages and disadvantages. For our applications, a pneumatic system is chosen. The goal was to create a system that delivers contamination-free, reliable, and continuous pumping. By employing an air-pressure driven flow, we keep the reagents separated from one another until the correct time to be mixed in the microfluidic LOC. A pneumatic system also reduces the amount of tubing that is

needed to be replaced after each experiment, as air will just be pumped up until the reagent reservoirs. This was achieved using a LabSmith uProcess Syringe Pump. This syringe pump met all of the requirements regarding flow rate, accuracy, and form factor. The micro syringe pump is employed with LabSmith's uProcess Automated 3-port Selector Valve AV201 and uProcess Automated 8-Position Selector Valve AV801 for automated fluidic handling of different reagents. 3- and 8-port valves are used to control the air flow that is to be pumped into the selected reservoir vials. As mentioned before, the goal is to use pneumatic flow, to push the desired volume of solution into the mixing channel. LabSmith was chosen due to its easy integration of parts. LabSmith's ecosystem can be controlled via its Windows-based software or with a customized user interface, which we chose to employ, as will be explained in the next section.

LabSmith has various syringe pumps with varying flow characteristics. A 100µL volume syringe pump with a minimum flow rate of 1 µL/min, a maximum flow rate of 2800 µL/min, and a max pressure of 200 psi is chosen for our purpose (see **Figure 2.10**). The accuracy for both volume and flow rate is rated at 1%. The overall dimensions of the pump are suitable for the development of our compact and portable platform. The syringe dimensions were roughly 100mm, 25mm in width, and 21mm in height. Connection to the 3-port valve is established using 1/16" OD tubing, which does not need to be replaced between different tests due to the contamination free fluidic handling approach we developed.

Figure 2.10: LabSmith uProcess Syringe Pump

LabSmith uProcess Automated 3-port Selector Valve and uProcess Automated 8-Position Selector Valve were chosen due to their accuracy, form factor size, and ease of integration through a single software. The use of two valves will be explained more in-depth in the next section. As shown in Figure 2.12, the 3-port valve serves the purpose of "redirecting flow."

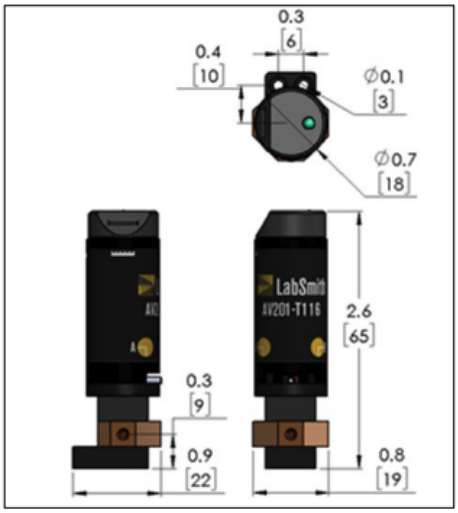

Figure 2.11: LabSmith uProcess Automated 3-port Selector Valve AV201

This first port will help in the aspiration of air to the syringe pump and redirect to the 8-port valve. The 8-port valve will be used to select from multiple reagent vials to

dispense the aspirated air, giving us a contactless flow into the microfluidic LOC. Both valves are similar, with a height of 65mm, a width of 13mm, and a length of 22mm. The switching time between ports is also critical, with a minimum time of 1 second and a maximum time of 2.75 seconds, depending on the port being selected next.

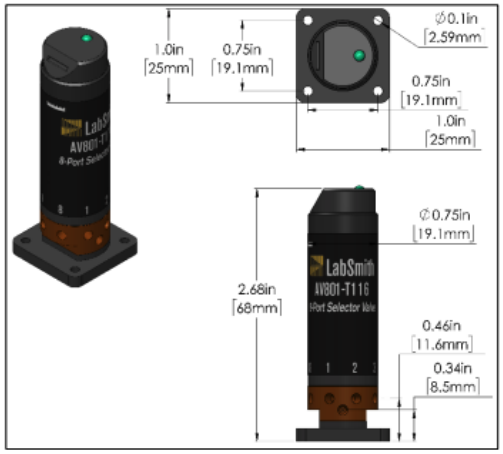

Figure 2.12: LabSmith uProcess Automated 8-Position Selector Valve AV801

A LabSmith breadboard with five ports is used for controlling different LabSmith components. UProcess Automation Interface EIB200 is added to establish a connection between LabSmith components and a computer. The entire fluidic handling equipment is powered using a 12v 2A input. A uProcess Manifold is also added to control the two valves. The breadboard also facilitates the use of the syringe pump through the port provided on it. Furthermore, we utilized one of the breadboard ports to connect to our Arduino Uno microcontroller, as explained below. The breadboard size was relatively compact with the overall dimensions being 133 x

185mm. Almost the entire system is fitted onto this small breadboard, as shown in **Figure 2.13**, with the LabSmith breadboard highlighted in yellow.

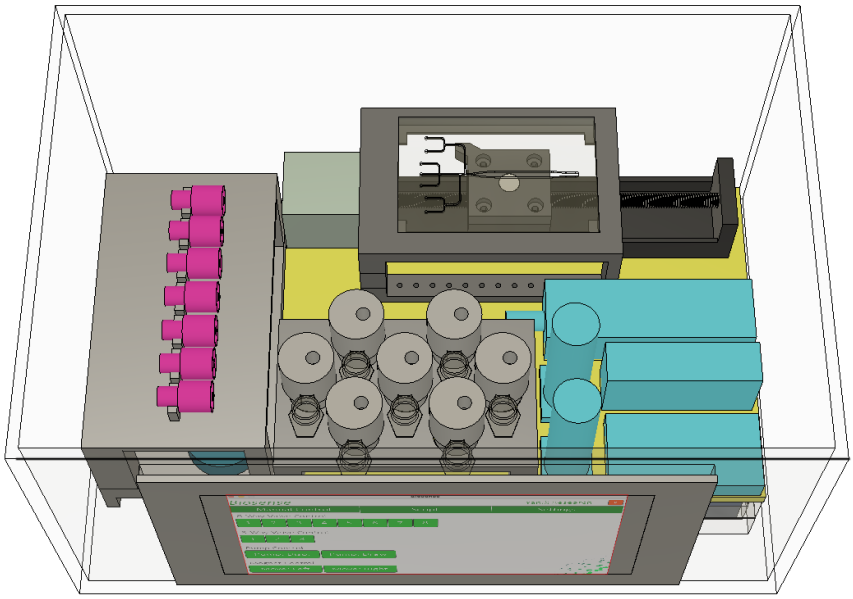

Figure 2.13: Top front view of the rendered platform. LabSmith components are highlighted in yellow, breadboard, and blue, pump, valves, manifold, and interface unit

The platform uses a pneumatic-based flow scheme, meaning pressured air is used to control the volume and flow rate of the reagents into the microfluidic LOC device. This is achieved through a carefully thought-out placement of LabSmith products. The 3-port valve serves the purpose of "redirecting flow." As can be seen in **Figure 2.14**, port one of the 3-port valves connects the syringe pump to the atmosphere to aspirate air. Switching to port three connects the syringe pump to the 8-port valve. Then the 8-port valve will be set to the desired reagent to dispense the

aspirated air, giving us a contactless flow into the microfluidic LOC. Traditionally, when using a single pump and multi-port valve, the valve will redirect the reagents into the syringe pump reservoir to then be dispensed through the valve into a newly selected port, contaminating both the syringe reservoir and the valve tubing. Using a pneumatic pumping system, we achieve a contamination free operation of the valves and the syringe. For the next set of tests, only the disposable reagent reservoir and tubing connecting them to the LOC device need to be replaced.

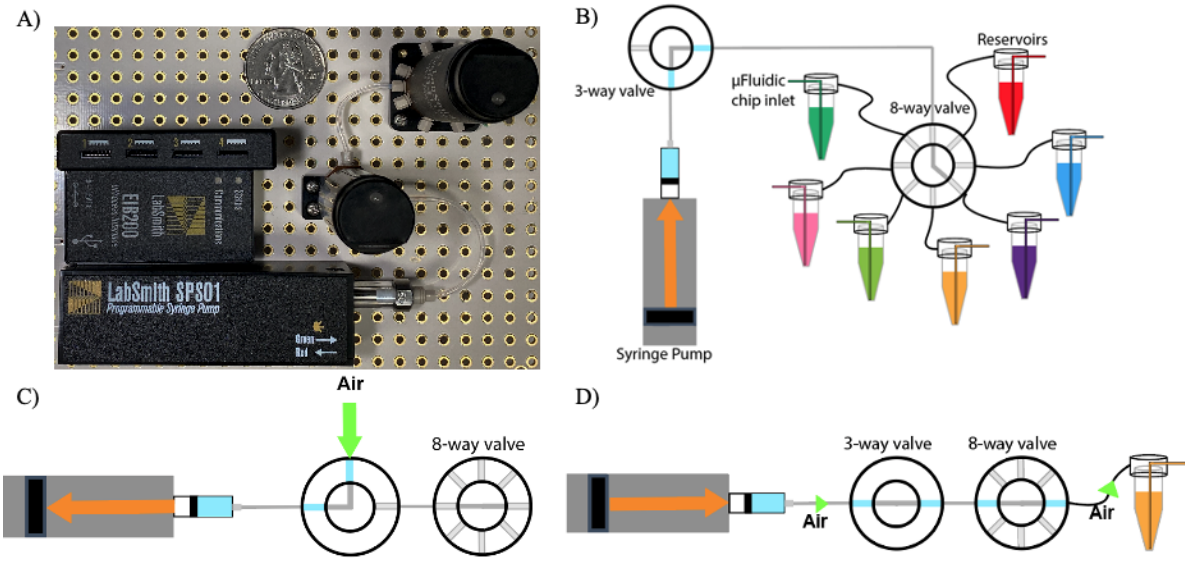

Figure 2.14: Pneumatic pumping setup. A) Demonstrates the aspiration of air B) 3-port valve switching positions to redirect collected air C) Syringe pump dispensing air into the 8-port valve to be directed into desired vial

2.2.2 Pressure Sealed Reservoirs

The air is aspirated into a LabSmith syringe pump and then dispensed into one of the Elveflow Microfluidic Reservoirs with either 1.5 mL or 15 mL volume through the 8-port valve. Upon dispensing of air, an equal volume of solution will be pushed out of the vial through the tubing that is located at the bottom of the vial, conveying the solution to the next component, as shown in **Figure 2.15.**

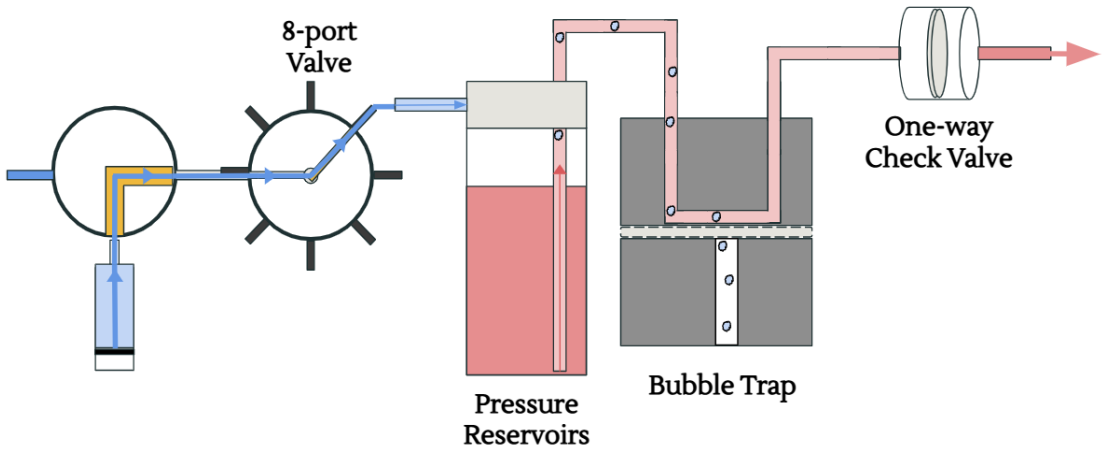

Figure 2.15: Air being dispensed from the syringe pump to be directed into a pressure-sealed vial via the 8-port valve. Pickup tube can be seen in red displacing the solution into the next components before entering the microfluidic LOC

2.2.3 Bubble Traps

A minor issue faced in the earlier stages of the development of the platform, was air bubbles coming from the Elveflow Microfluidic Reservoirs leading into the microfluidic LOC. To help eliminate this issue, Elveflow Bubble Traps were installed. These bubble traps use a membrane with a pore size of 10 µm to eliminate any unwanted air bubbles, as seen in **Figure 2.16**. Air bubbles in microfluidic applications are common issues. These bubble traps help eliminate these issues. The bubble traps are placed after the reservoirs to eliminate bubbles in the early stages. The air bubble traps measure 30mm in diameter and 30 mm in height.

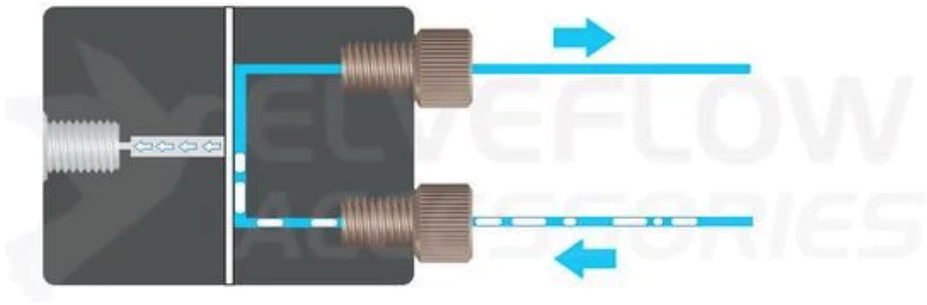

Figure 2.16: Elveflow Bubble Traps. "Liquid containing bubbles is pushed against a microporous Teflon membrane and bubbles are expelled through this membrane"[28]

2.2.4 One-way Valves

The issue of backflow was critical to us since this could cause a premature mixture of reagents before the intended mixture protocol, so to help combat this issue, the microfluidic lab on a chip device was made with channels that have lower resistance as it makes its way towards the outlet. This proved to help the backflow issue, but Elveflow One-way Microfluidic Check Valves were added to the platform as an additional safety precaution.

Figure 2.17: Elveflow One-way Microfluidic Check Valve

These one-way valves are placed between the bubble traps and the inlet for the microfluidic Lab-on-a-Chip device. The one-way valves have proven to be very beneficial, as the backflow issue has been completely resolved in our platform. The small form factor makes it a great addition to the platform, keeping the overall size as small as possible at 60mm long and 12mm in diameter. Maximum back pressure is also rated at 88 psi, making this ideal for our use.

2.3 Mixing Setup

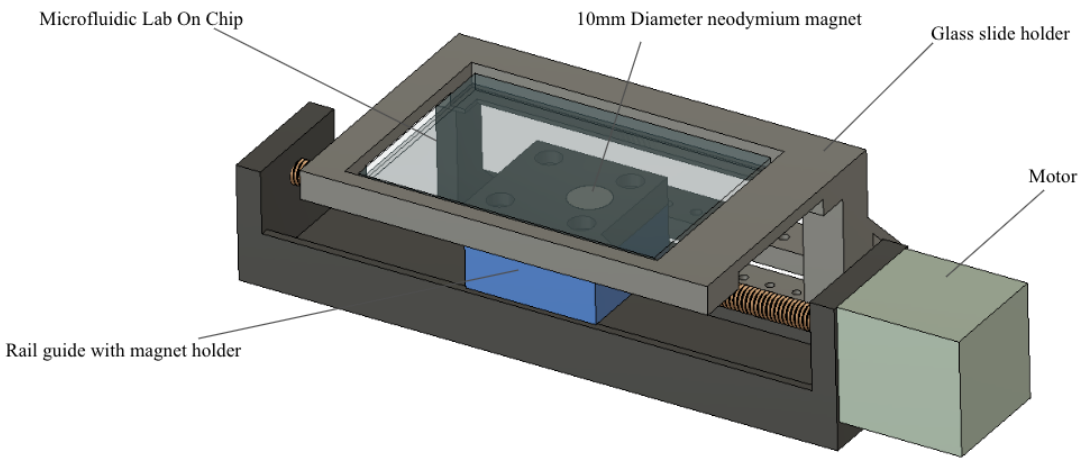

Figure 2.18: Render of Mixing System, components were both purchased and 3D printed.

2.3.1 Linear motor

This microfluidic LOC device contains a mixing chamber, however little has been described about how the mixing will be accomplished. This section describes the mixing process using a magnet, linear motor, and magnetic beads. The procedure for mixing antigen and detection antibodies will be explained in further detail.

As mentioned earlier, a linear motor was chosen to perform this mixing procedure. Early versions of this setup included a stepper motor that would drive a belt with a custom 3D-printed magnet holder. This version was replaced with a

smaller, more accurate linear motor with a built-in guide rail where a custom 3D-printed magnet holder is placed. A smaller step size was required for the type of mixing desired. A low-cost and relatively small linear motor was used. The linear motor has a physical dimensions of 150mm in length, 28mm in width, and 35mm in height. A built-in guide rail with a magnet hold was designed, and 3D printed to hold the magnet as close as possible to the underside of the microfluidic chip, as shown in **Figure 2.18**. The magnet used is a 10mm neodymium magnet from K&J Magnetics. It was chosen due to its small size and strength, with a pull focus of 1.41 lb and a surface field of 3661 gauss.

2.3.2 Mixing motion

To control the motion of the linear motor, an Arduino Uno and stepper motor driver were used. A bouncing motion proved to be the most effective mixing approach [9]. This motion could be described as the linear motor bouncing rapidly from one position to another, then moving a couple of millimeters forward and repeating this bouncing motion. The user interface will be explained in greater detail later in this thesis, however, a quick overview is presented here. The linear motor is controlled manually or fully automated by a custom script that is written by the user. The motor motion leading to solutions mixing and liquid handling through syringe pump and valves are controlled through a single script which is sent from the user interface to be run by the program. **Figure 2.19** displays a top view of the

microfluidic LOC used; within this LOC, magnetic beads can be seen in the mixing chamber being manipulated by the 10mm magnet placed directly underneath the glass slide.

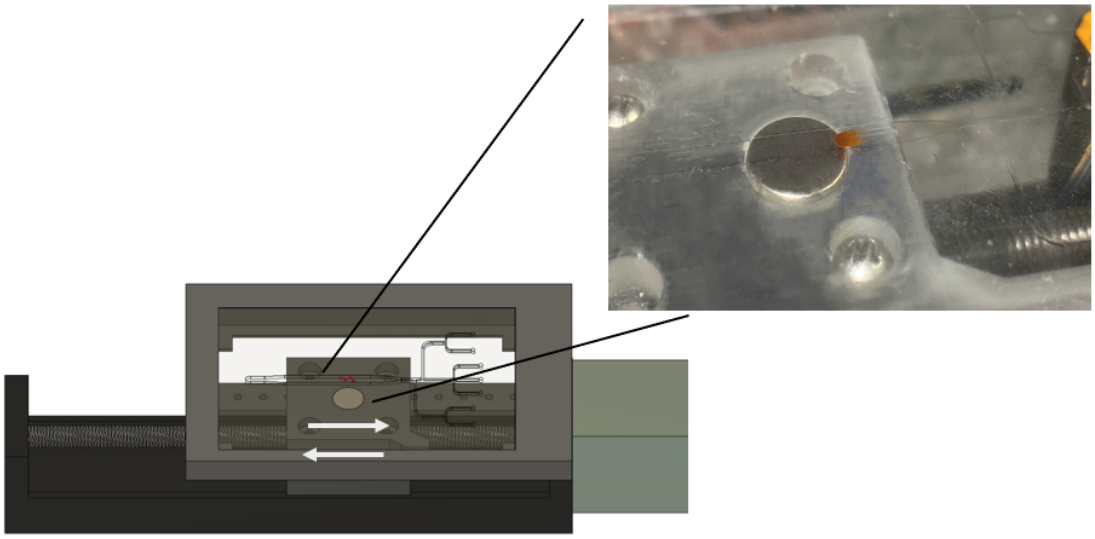

Figure 2.19: Top View of the Mixing of Magnetic Beads. The linear motor is used with a 3D printed mount to house the 10mm neodymium magnet

2.4 Hardware

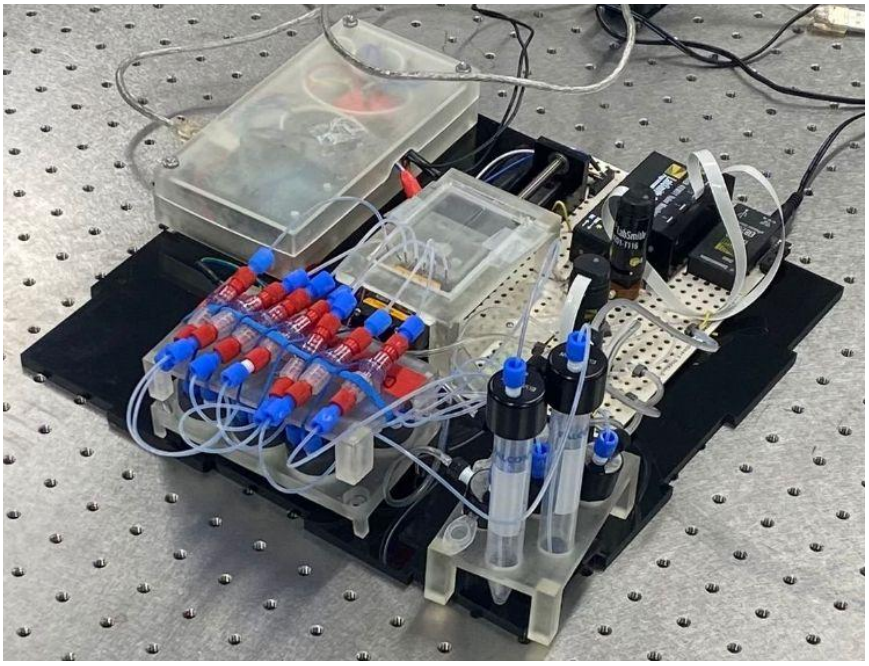

Figure 2.20: Current Status of Lab-On-Chip Platform

2.4.1 Electronics

The platform uses two main breadboards, the 133x185mm LabSmith breadboard and a basic 55x178mm breadboard. In addition to these breadboards, a PC or Raspberry Pi is used to communicate with the Arduino Uno which controls the rest of the board's components. **Figure 2.21** demonstrates the overview of the electronics in this system. The user interface will be explained in the next section; here I will focus on the breadboard and other hardware components.

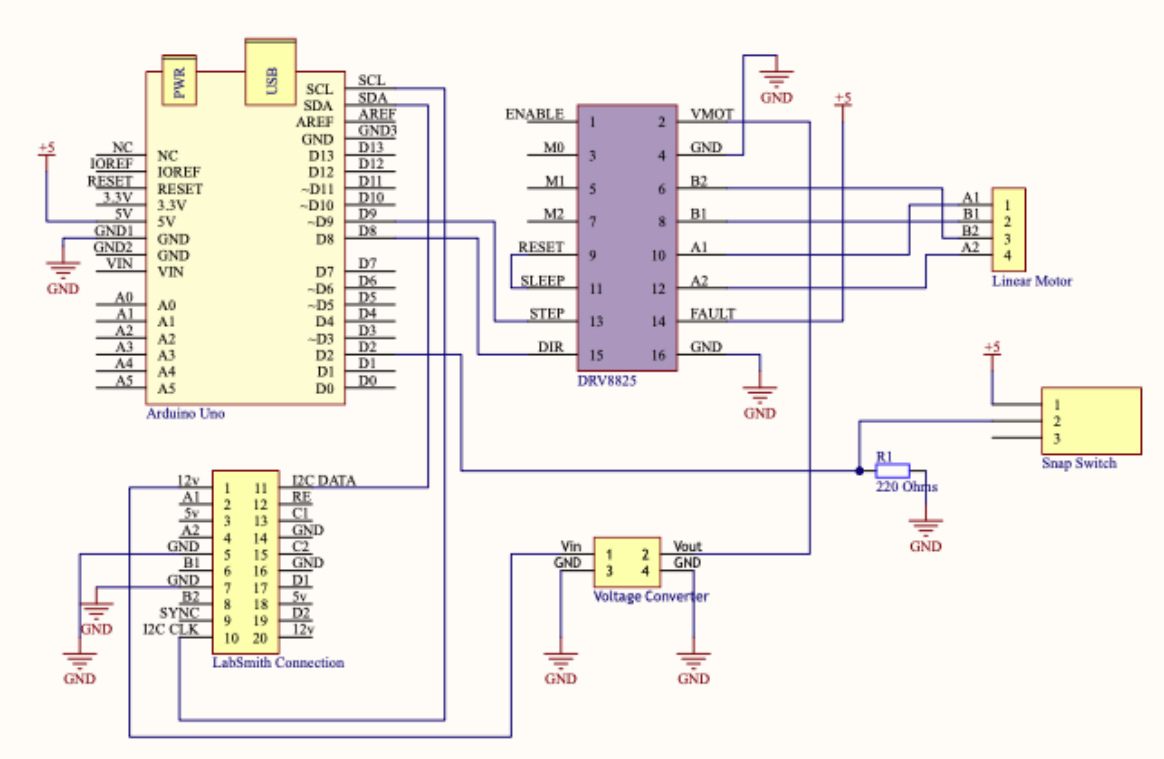

Figure 2.21: Wiring Diagram of Electronics

The LabSmith uProcess breadboard connects all LabSmith products, such as the EIB 200 Automation Interface, Syringe Pump, and Valve Manifold. With 5 available slots for device connections, we use one of the remaining slots to connect the LabSmith I2C clock and data pins to the Arduino Uno, giving us control over the LabSmith pump and valves. Using a 12v pin from the LabSmith board to be converted into 24v for a DRV8825 high current stepper motor driver.

The second board contains other components, including a stepper motor driver, a step-up voltage converter, and a simple switch mechanism to home of the linear motor rail. A DRV8825 stepper motor is used to control the linear motor. As

shown in **Figure 2.22**, this is a high-current stepper motor driver meaning it requires a high voltage input. The stepper motor used in this system requires 24v, to supply the motor with the correct voltage, a voltage step up converter was used. It is necessary to use a step-up voltage converter for this stepper motor, which requires 24 volts as an input voltage. In addition to the stepper motor driver and voltage converter, a simple switch mechanism is used to set a home point for the linear motor rail guide. This is useful to set a home point for the rail guide, which houses the magnet used for mixing. By setting a home point, the user can easily tell the motor to move any given amount in the +X direction starting from 0.

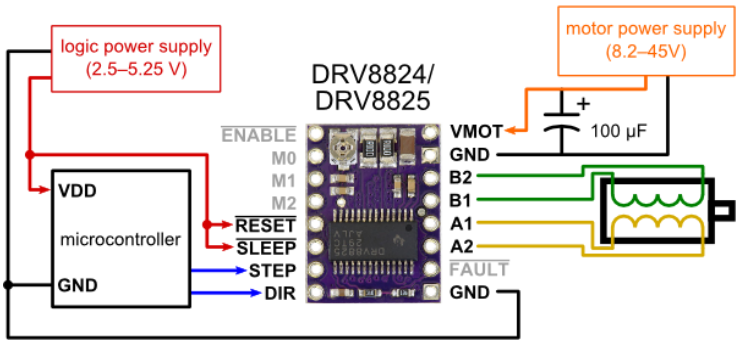

Figure 2.22: DRV8825 High Current Stepper Motor Driver

2.4.2 3D Printed Parts

Aside from the microfluidic molds, this platform also contains other parts that are 3D printed. For example, vial holders were created for the Elveflow pressure reservoirs, bubble traps, and one-way valves. Other 3D printed parts include a case for the breadboard and circuits and a stand for the microfluidic chip, which places it directly above the magnet for mixing.

Vial holders were designed to hold up to seven Elveflow Microfluidics reservoirs. It was designed to be extremely compact and small as it measures 32mm in height, 60mm in width, and 96mm in length.

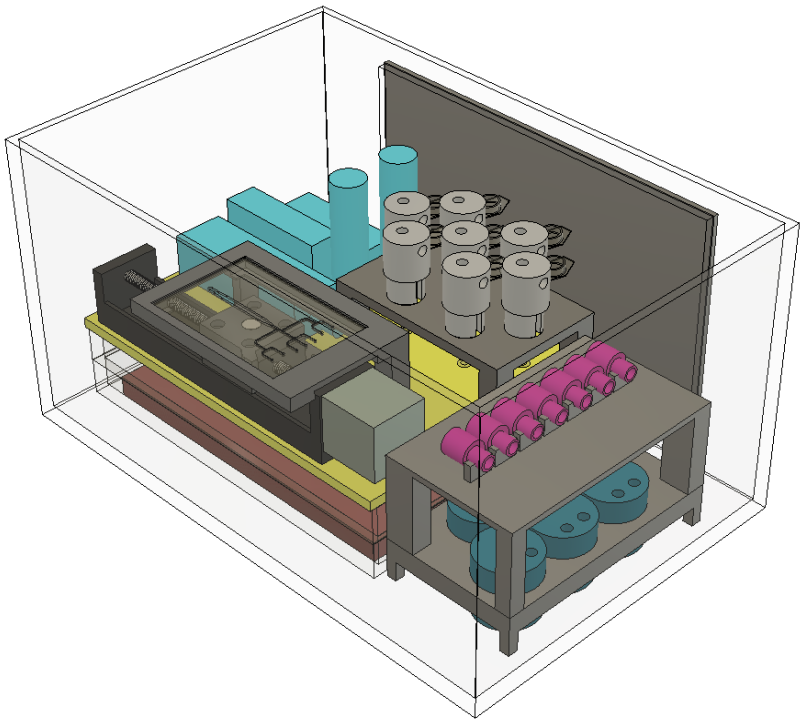

Figure 2.23: Render of Platform Highlighting 3D Printed Parts. Components in gray were designed and printed to fit our applications

A similar holder was made for the Elveflow bubble traps. This has a size of 20mm in height, a width of 68mm, and a length of 117mm, with six slots for the bubble traps to be held. A mount for seven Elveflow one-way valves was designed to sit on top of the bubble trap holder to save space and tubing; by having the two 3D printed parts close to each other, the use of tubing is reduced to 15mm. The height of the mount is 23mm for a combined height of 43mm for both bubble trap holders and one-way valve mounts. Both width and length are the same as the bubble trap holders.

A case was designed and printed to house the breadboard and Arduino Uno. Currently, a custom PCB has yet to be designed, as the circuit design may be modified in future versions. The case is one of the larger items in this platform; as mentioned, it has to enclose a breadboard, various circuits attached to the platform, and an Arduino Uno. The case is built as two halves with a total height of 35mm, a width of 87mm, and a length of 171mm. Another part printed is the stand for the microfluidic LOC device. This stand holds the LOC device above the linear motor, which centers the middle of the mixing chamber with the magnet. This stand was designed to be bolted to the LabSmith breadboard and could easily be removed or moved to a new position. This stand has a height of 45mm, 82mm in width, and 100 mm in length. The 3D printing procedure for these parts is the same as in **Section 2.1.2**.

2.5 Biosense

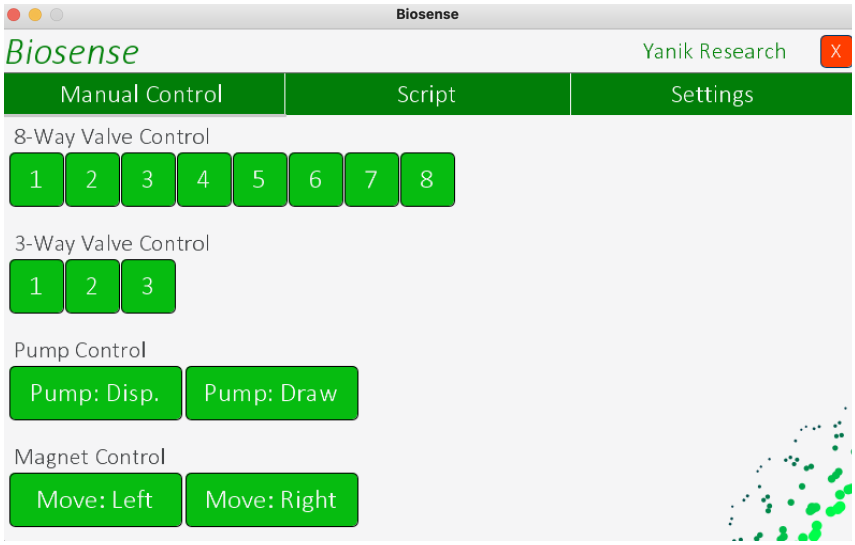

Figure 2.24: Biosense User Interface Home screen. Three menu options, the main page is “Manual Control”, “Script” page will allow the user to upload the desired script. Final menu will be a “Settings” page

2.5.1 Overview

Biosense user interface is developed to manually or automatically control the platform. The manual controls allow the user to set any port in the 3- and 8-port valves, aspirate or dispense the syringe pump, or move the magnet via the linear motor, see **Figure 2.24**. There is also an automated setting page. On this automated page, the user can upload a script executing a complex operation involving many steps.

I currently use a PC to control the platform; this allows us to adjust and change the script during experiments much easier than using a Raspberry Pi. A Raspberry Pi and touch screen have already been installed and will likely be our final version, with a finalized script (protocol) that could be used in different tests. The Raspberry Pi will allow us to keep the overall size very small since it measures only 2.2 inches by 3.4 inches and features a 7-inch touch screen. Components of the system with the Raspberry Pi is shown in **Figure 2.25**.

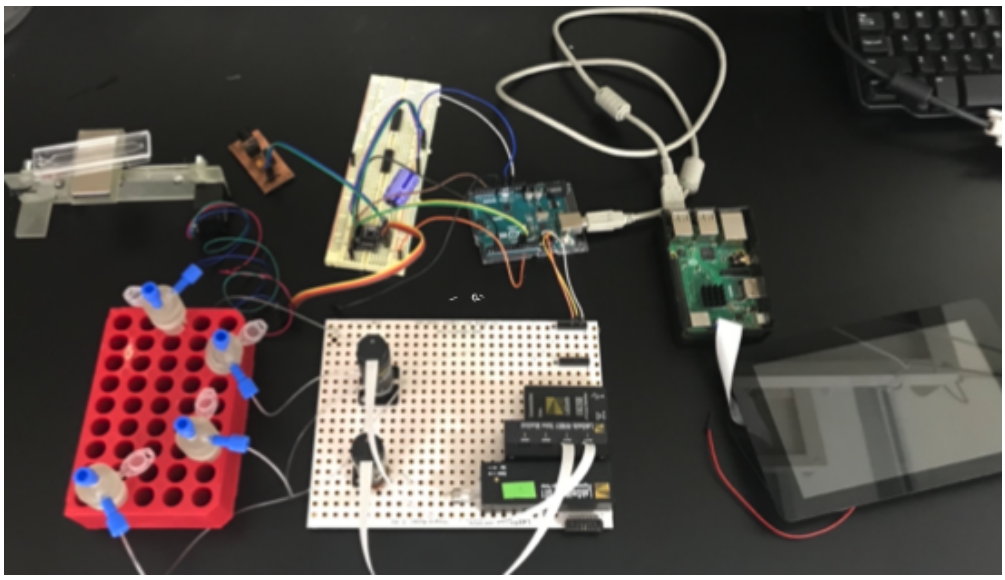

Figure 2.25: Early Version of Platform Using Raspberry Pi & Touchscreen

2.5.2 User Interface / Controls

There are two different user interfaces written in two different programming languages. There are two sections to the program created for this platform, a PC / Raspberry Pi system and an Arduino system. In the PC system, the PC or Raspberry Pi controls the GUI, graphical user interface, and the back end of the

program; this can be seen in **Figure 2.26**. The Arduino Uno section controls the entire LabSmith uProcess system, EIB200 Automation Interface, the DRV8825 stepper motor driver, and a homing switch for the linear motor rail.

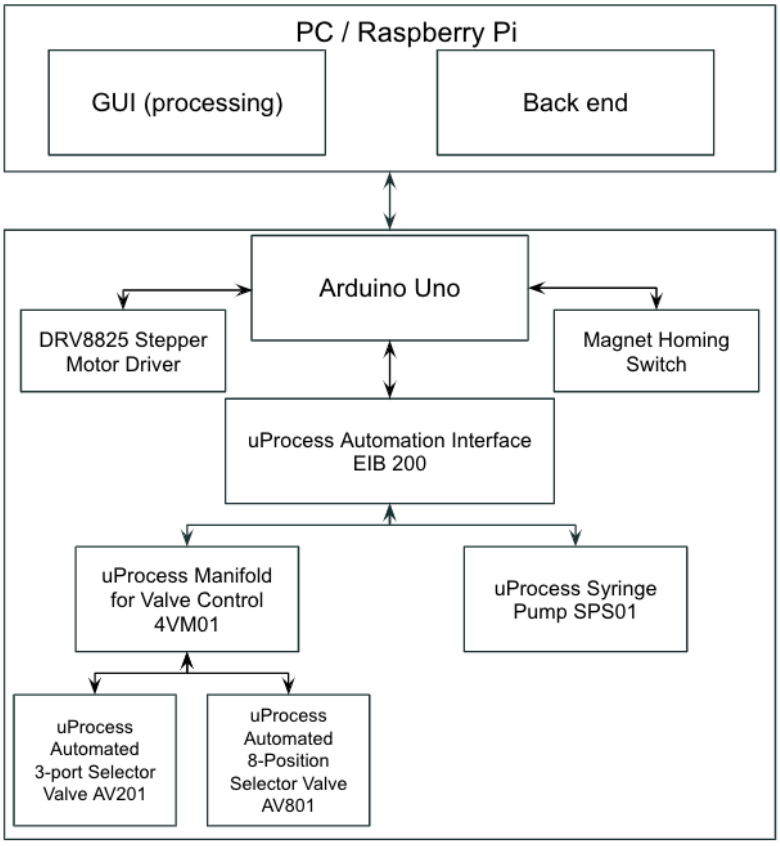

Figure 2.26: Overview of the system showing the communication pathways

In the first section, the GUI and the back end were written in Processing 3, primarily based on JavaScript. This was done to give the user an easy-to-use interface, as Processing is well known for its graphical abilities. The GUI includes icons, menus, and any clicking performed by the user. The back end handles everything not done by the user, such as reading and storing data, and is also primarily written in Processing 3, with some communication to the Arduino Uno.

The Arduino Uno section was written using the Arduino IDE, an Integrated development environment mostly based on C/C++. Using an Arduino Uno for this section, I could easily communicate with the stepper motor driver, homing switch, and the LabSmith uProcessing system. As mentioned in the Electronics section, all of the LabSmith components are connected via the LabSmith breadboard, which the Arduino connects to via an unused port, using two I2C pins, the Arduino Uno is then able to communicate with the LabSmith pump and valves. Another benefit of using the Arduino Uno, the code does have to be uploaded every time, as long as I use the same PC / Raspberry Pi.

2.5.3 Scripts

My objective in this section is to provide an example of one of the scripts that can be uploaded by a user, as well as the different adjustments that can be made.

To control the syringe pump to aspirate and dispense air into the pressure-sealed vials to dispense fluid into the microfluidic LOC, I use two commands, “VALVE” and “PUMP.” In **Figure 2.27**, a screenshot of the code can be seen.

```
LOOP (5) {
        VALVE 12 5 1 0 0   # Move to air location
        PUMP  15 2300 30   # Fill pump with air
        VALVE 12 5 3 0 0   # Move to dispense location
        PUMP  15 50 0      # Dispense fluid
}
```

Figure 2.27: Screenshot of a sample script. Demonstrating the use of "VALVE" and "PUMP" commands

Here, the "VALVE" command is followed by five numbers; the first number is the device's address, here the valve manifold is addressed as 12, and the syringe pump is addressed as 15. The following four numbers for the "VALVE" command are the valves connected to the valve manifold. It is possible to control up to four valves with the valve manifold, but for the ELISA protocol, we only require two valves. The second number will tell the 8-port valve to move to position 5 which is connected to a 1.5mL pressured sealed reservoir. The third number will tell the 3-port valve to switch to position 1, which is the position to redirect air into the syringe pump, as illustrated in **Figure 2.14**. The following two numbers will be left as "0" as no valves are being used here. The second line of code will tell the pump to aspirate air. As mentioned, the LabSmith syringe pump has an address of 15, the number "2300" will denote the flow rate in uL/min of the aspiration, and the third number will indicate the volume of air being collected. The third line of code will now switch the 3-port valve to connect the syringe pump and the 8-port valve. Hence the only change that

occurred was switching “1” to “3” being the third position in the 3-port valve. The last line of code will execute air dispense into the system, done by changing the flow rate from “2300” to “50” uL/min as we desire a quick aspiration time and slower dispense flow rate, and changing the volume of the syringe from “30” µL to “0” as we wish to dispense all the air into the pressure sealed 1.5mL reservoirs. Repeating this operation five times by enclosing this code with a loop function gives us 250µL of solution to be dispensed into the microfluidic LOC.

```
SWEEP 5 100 15 0  # Bounce btw 0 & 15mm
WAIT 5            # pause for 5s
SWEEP 5 100 35 25 # Bounce btw 25mm & 35mm
WAIT 5            # pause for 5s
SWEEP 5 100 45 35 # Bounce btw 35mm & 45mm
WAIT 5            # pause for 5s
SWEEP 5 100 55 45 # Bounce btw 45mm & 55mm
WAIT 5            # pause for 5s
SWEEP 5 100 65 55 # Bounce btw 55mm & 65mm
WAIT 5            # pause for 5s
SWEEP 5 100 75 65 # Bounce btw 65mm & 75mm
WAIT 5
MOVETO            # Move to switch
```

Figure 2.28: Screenshot of sample code. Demonstrating the bouncing motion performed during mixing using the “SWEEP”, “WAIT” and “MOVETO” commands

Linear motor is controlled in a similar manner. In **Figure 2.28**, a sample code is shown for this purpose. Here, I use three commands, “SWEEP”, “WAIT” and “MOVETO”. “SWEEP” controls the linear motor with four numbers; the first number tells the motor how long to run. The second number is the motor's speed; 100 is the

fastest the motor can move at a speed of 10 mm/s. The following numbers will be positions A and B for the motor to move to. To achieve the bouncing motion discussed in the early mixing section, we can perform the following code found in **Figure 2.27**. Here, we use “5” seconds of bouncing between positions “0” and “15” mm at 100% of the motor's speed. Since this program reads every line after the other, we need to include a pause of five seconds or matching time in the “SWEEP” command so the program does not continue to the following line of code. This can be done using the “WAIT” command, telling the program to wait for the following line of code. Using the “SWEEP” and “WAIT” commands, we can make it back to the end of the mixing chamber, which is around 75mm long. To go back to the start, the reverse of positions A and B can be written, or the “MOVETO” command can be used. This command moves the magnet to the home point set by the switch. This switch is pressed when the linear guide rail moves towards the motor, causing an arm printed onto the magnet holder to push the switch setting the home point.

Chapter 3 Results

3.1 Final Platform

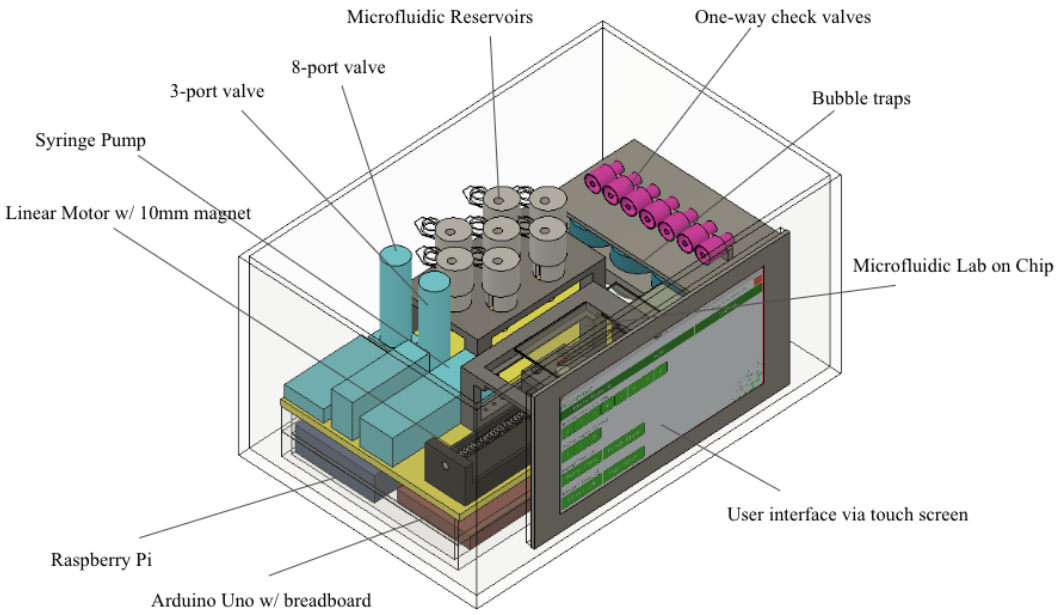

Figure 3.1: Render of the Final Platform with labels

The final setup is an automated portable platform that can execute complex protocols. This platform can perform ELISA tests in a very compact form factor, 255x255x140mm, eliminating the need for large-scale laboratory equipment. **Figure 3.1** shows a render of this platform using a Raspberry Pi and a 7" touchscreen display instead of a PC. All of the previous parts discussed in this thesis can be seen together in this render.

After many versions of developing a microfluidic LOC device, our final version is a 30uL LOC capable of performing enzyme-linked immunoassay, ELISA,

tests. This microfluidic LOC device was made for our research group's specific needs with seven inlets, one outlet, and a 20uL mixing chamber. The overall design can be easily changed or modified in AutoDesk Fusion 360. As stated before, the main issues when designing a microfluidic LOC device were addressed with months of research and testing. Using PDMS instead of polycarbonate and double-sided tape eliminated the issue of bonding, which led to leaks. Using a 3D printed mold for PDMS fabrication, custom designs, and changes could be made and tested within the same day, compared to days to a week for traditional methods of PDMS fabrication, photolithography. To help reduce the number of non-specific absorption, a PEG additive was added to the PDMS mixture. This has been proven to be successful with as little as 0.25% added in our experiments by reducing the number of antibodies attached to the surface of the PDMS channel walls. By increasing the channel width along the microfluidic device, a path of least resistance was created to avoid any backflow in the opposite direction. Overall, our microfluidic device could be fabricated within days and provide large laboratory equipment capability, all on a 70x50mm glass slide; see **Figure 3.2** for the final version of microfluidic LOC.

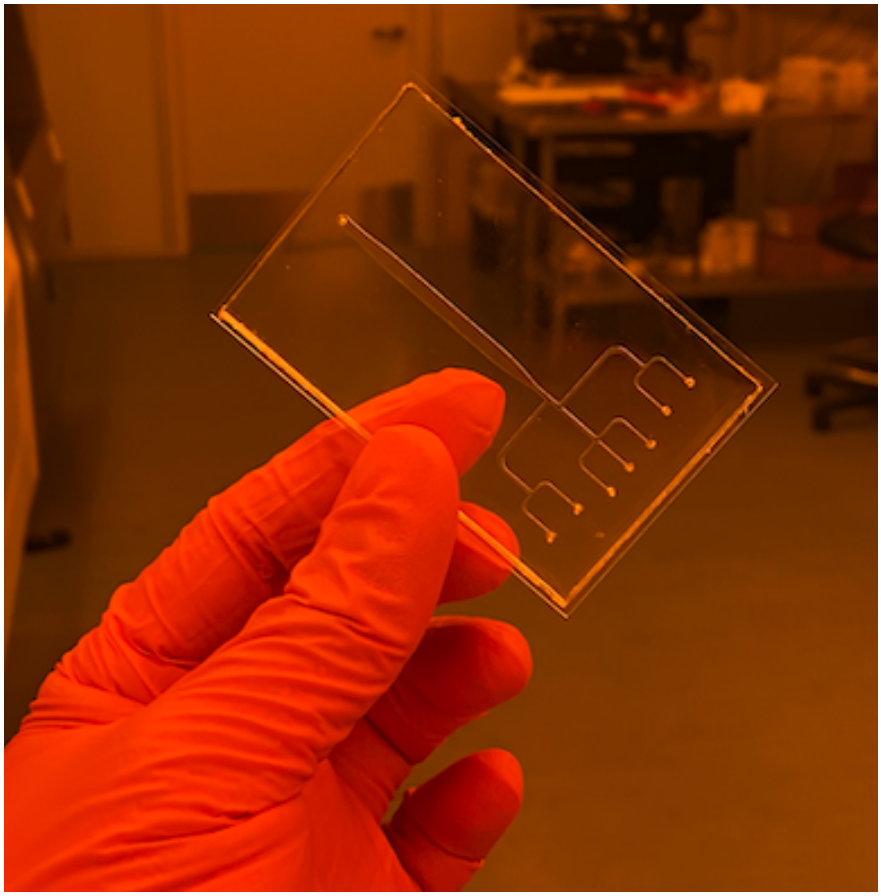

Figure 3.2: Photo of our Microfluidic Lab-on-a-Chip device

A LabSmith syringe pump, two valves, a 3- and 8-port, and additional accessories contribute to the creation of an automated contamination-free pneumatic system for handling microfluidic flow. This system can deliver an air bubble-free fluidic flow as low as 1 µl/min and as high as 2800 µl/min, with no backflow issue. This is achieved by using a bubble trap and one-way check valve after the 1.5mL pressure-sealed reservoirs.

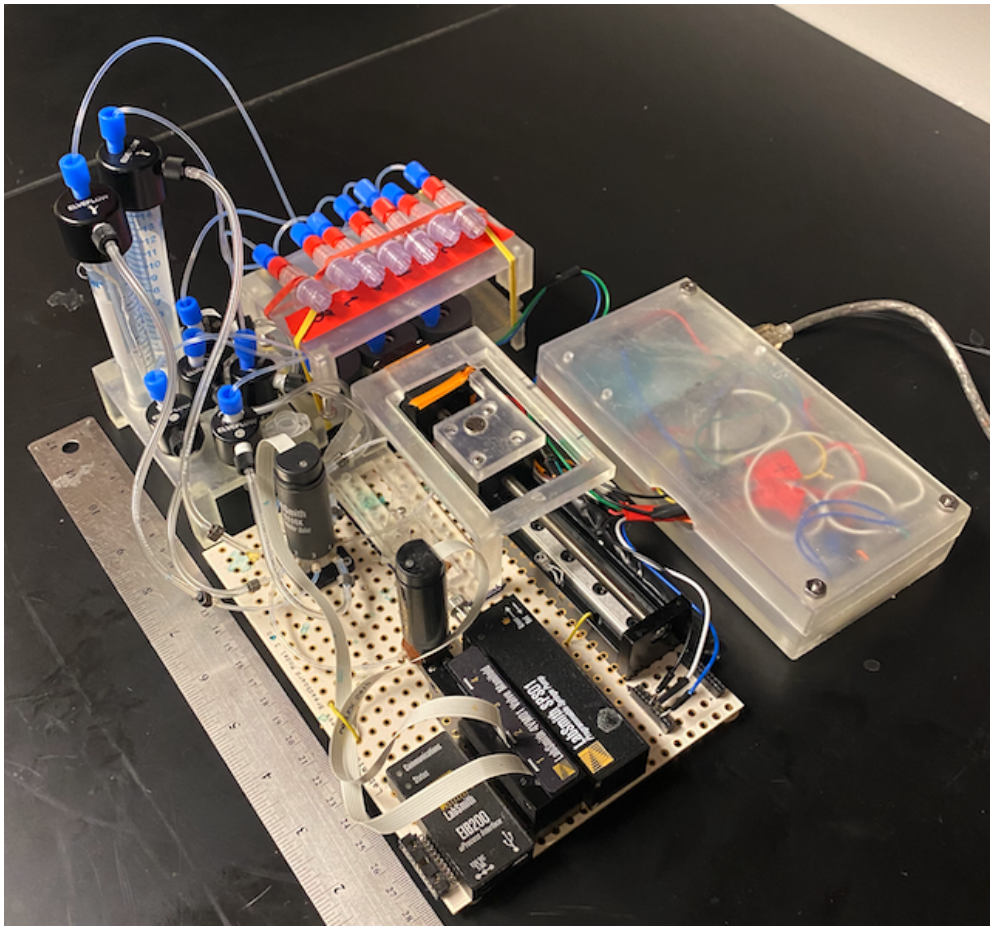

Figure 3.3: Final Assembled Platform

3.2 ELISA

3.2.1 Motivation

Enzyme-linked immunosorbent assay, better known as ELISA, tests are performed using the developed compact platform. There are four different types of ELISA types, Direct, Indirect, Sandwich, and Competitive. For our experiments, I performed magnetic bead–based sandwich ELISA tests[13][16]. Sandwich ELISA uses two antibodies, a capture and detection antibody, to sandwich the antigen in between the two. In typical sandwich ELISA experiments, the capture antibodies are immobilized onto a microplate, with the antigen then binding to the first antibody. Once the detection antibody with a HRP conjugate is added, the sandwich ELISA process is completed. **Figure 3.4**, a magnetic bead–based sandwich ELISA is illustrated.

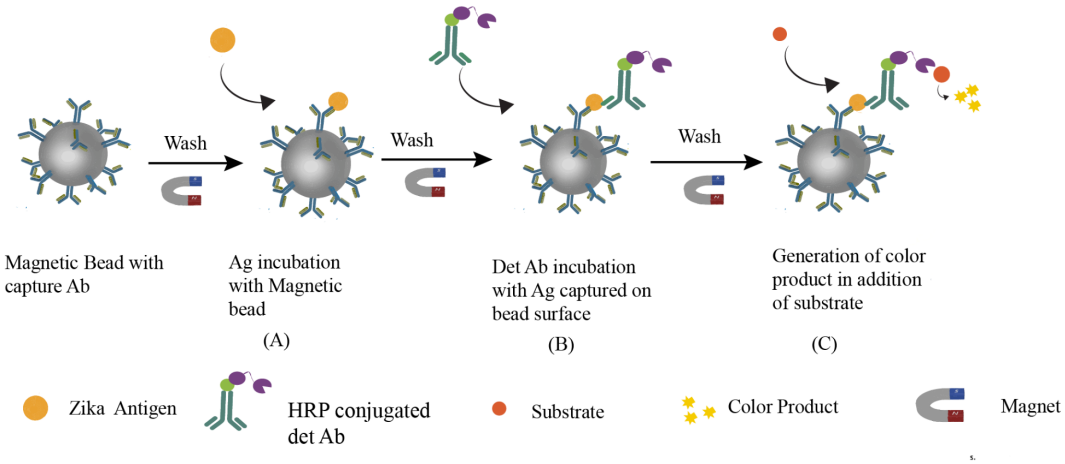

Figure 3.4: Magnetic Bead basedSandwich ELISA Protocol[22]

In order to make this platform work, I had to execute the assay using magnetic beads. This bead–based sandwich ELISA makes this entire process possible. By using magnetic beads, we can easily manipulate solution and substrate (capture surface) mixing and give the user complete control over the mixing of the reagents. Using solution based sandwich ELISA also gives the user a larger surface area while requiring less volume compared to well plate ELISA. As mentioned before, the microfluidic LOC has a volume of 40μL, but a length of 30mm. Overall, the use of a bead based sandwich ELISA has clear benefits over the use of a traditional well plate sandwich ELISA.

Our goal is to exemplify the abilities of this platform by performing several sandwich ELISA tests, to be then compared to results from other publications[21]. This is done by using magnetic beads instead of a microplate to immobilize the capture antibodies and automatically performing all mixing and incubation via the microfluidic LOC with a written script. A similar version done without using our platform would take around 4-5.5 hours to complete, ~1.5 hours of hands-on time, and ~2.5-4 hours of incubation time[23]. I demonstrated that I can execute ELISA protocol with our automated platform within 2 hours.

3.2.2 Methods and Materials

Our ELISA reagents were purchased from BioFront Technologies. This kit provides all the proper reagents and well plates needed for an ELISA sandwich

experiments[24]. Magnetic beads were purchased from Invitrogen Dynabeads Antibody Coupling Kit[25]. This experiment is done in 7 easy steps; preparation of reagents, priming microfluidic LOC, preparing the platform, priming the tubing with reagents, uploading a script, collecting the magnetic beads, and finally, taking the absorbance reading. A summary of these steps can be seen below.

To begin the experiment, I first have to prepare the reagents used. The assay for Zika NS1 will require four reagents; Couple superparamagnetic beads, Antigen, Detection Antibodies, and 1% BSA. The antibodies are immobilized onto the superparamagnetic beads, before using them on the platform. The antibody coupling protocol is split into two days with a total of twelve steps[26]. The first step is to calculate and weigh out the number of beads desired. Next, the beads have to be washed and collected with an external magnet, removing unwanted solutions. The antibodies are then added to the vial and incubated overnight. On day two, the beads are again collected using a magnet and the unwanted solution is removed. The antigen and detection antibodies are included in the BioFront Technologies Zika Virus NS1 ELISA kit. 1% BSA is prepared by diluting 10% BSA with Phosphate buffered saline (PBS) to get a product of 1% BSA.

The next step is to prime the microfluidic LOC with 1% BSA. This is done to reduce the level of nonspecific binding within the channel walls[27]. To reduce the chance of developing air bubbles within the microfluidic LOC, this is done in a backward flow direction. To prime the device, I first close all of the inlets with tape, but one, and use a syringe pump to dispense 1% BSA into the LOC device flowing

from the outlet to the opened inlet port. Once the 1% BSA has reached the open inlet, I cover this inlet with tape and open the next inlet to be primed. This is done until the entire LOC device is filled with 1% BSA. After filling the channels with BSA, the device is then placed into the 3D-printed mount right above the magnet.

As soon as the reagents have been prepared and the microfluidic LOC device has been primed with 1% BSA, the platform is readied for testing. I first need to replace the 1/16" OD tubing, if used. I currently replace any tubing which has fluid flow through it, since this is a pneumatic system, the tubing needing to be replaced will be minimal. In addition to replacing the tubing, the Elveflow

Bubble Traps also have membranes that will need to be replaced after 5-10 uses. Cleaning with DI increases their longevity. Once the tubing and membranes have been replaced, Elveflow 1/16" OD fittings will be used to ensure a leak-free system. As illustrated in Figure 2.10, the order of the components can be listed as, Elveflow pressure reservoirs, bubble traps, and one-way check valves.

Similar to the second step, the tubing will also need to be primed to provide an air bubble-free fluidic flow. This is done by using the manual controls of the user interface to dispense enough fluid to reach the edge of the tubing before being connected to the microfluidic LOC. The prepared vials from step 1 will now be inserted into the Elveflow pressure-sealed vials. The order of the placement is not important, as seen in the script below; vials are labeled by their port number on the 8-port valve. The Couple Magnetic Beads will be in port 8, Antigen in port 6, Detection Antibodies in port 4, and 1% BSA in port 3. Upon installing the vials into

their reservoirs, the PC program is opened. I then use the manual controls to carefully dispense enough fluid to reach the end of the tubing before it will be inserted into the microfluidic LOC. Once all tubings are primed to ensure no air bubbles will form, the tubing is then connected to the microfluidic LOC with a metal tube between the PDMS and Inner Diameter of the tubing, as seen in **Figures 2.5A** and **2.9**.

The last step before running the platform is to upload the script. The script was prepared to execute the Sandwich ELISA experiment, as seen below, but different scripts can be used to perform various procedures. Once written, the script can easily be uploaded by entering the procedure into a main text file and uploading it to the software via an "Upload" button. Using the LabSmith syringe pump and valves, I dispense around 1mL of the magnetic bead solution –using port eight of the 8-port valve, at 50uL/min. This flow rate will be used for all pumping steps. Once all of the magnetic beads are dispensed, 100uL of Antigen, port six of the 8-port valve, will be dispensed next, followed by a bouncing motion from the linear motor for 1 minute, mixing the magnetic beads and antigen. This is done ten times to get a total of 1mL of antigen pumped and an incubation time of 10 minutes. I then perform a washing protocol using 1% Bovine Serum Albumin BSA through port 3 of the 8-port valve and remove any excess antigen within the microfluidic LOC. This procedure is repeated ten times for a total of 1mL of washing solution and a mixing time of 10 minutes. The next step is to pump the detection antibodies, port four of the 8-port valve. This process overall repeated five times, giving us a total volume of 500uL and

a mixing time of 10 minutes. I tested five different concentrations, including a negative control, to obtain consistent data, ranging from 1 pM to 2.5 nM. Finally, one last BSA washing is performed, giving us a total procedure time of around 2 hours.

Once the script has been executed, the magnetic beads are ready to be removed from the microfluidic LOC to be analyzed. This is done by removing the LOC device from the magnet and using manual controls to dispense BSA. The LOC can easily be removed by simply lifting it. I remove the magnet to lose the magnetic field holding the magnetic beads down. Once the magnetic beads are free from any magnetic field, the manual controls are then used for flushing away the beads using 1% BSA. The beads are then collected in a 1.5 mL vial for the final step.

The final step is to measure the absorbance of the final sample. This is done using an Enspire Multimode Plate Reader to measure the absorbance @450nm.

1. Preparation of Reagents
    a. Perform Antibody Coupling
    b. Prepare Antigen
    c. Prepare Detection Antibodies
2. Prime Microfluidic Lab-On-Chip
    a. Prime LOC with 1% BSA
3. Setup Automated Platform
    a. Replace tubing
    b. Replace air bubble membranes
    c. Connect fittings and one-way valves
4. Prime Tubing
    a. Place vials of reagents into pressure-sealed reservoirs
    b. Use manual controls to prime tubing before collecting to LOC
5. Upload script
    a. Write a script
    b. Upload a script to the user interface
6. Collect beads
    a. Collect magnetic beads by removing the magnet and pumping BSA
7. Measure absorbance
    a. Take the final vial to be measured

```
#vial 8 (Magnetic Beads)
LOOP (45) {
        VALVE 12 8 1 0 0   # Move to air location.
        PUMP  15 2300 25   # Fill pump with air.
        VALVE 12 8 3 0 0   # Move to dispense location.
        PUMP  15 50 0      # Dispense fluid.
}
```

```
LOOP (10) {
#vial 6 (Antigen)
   LOOP (5) {
        VALVE 12 6 1 0 0   # Move to air location.
        PUMP  15 2300 20   # Fill pump with air.
        VALVE 12 6 3 0 0   # Move to dispense location.
        PUMP  15 50 0      # Dispense fluid.
        }
##Move Magnet to Mix
        SWEEP 5 100 15 0
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 45 35
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 15 0
WAIT 5
#Move to home position
        MOVETO
#Wait for magnetic beads to settle
        WAIT 60
}
```

```
LOOP(10){
#vial 3 (BSA)
   LOOP (5) {
        VALVE 12 3 1 0 0   # Move to air location.
        PUMP  15 2300 20   # Fill pump with air.
        VALVE 12 3 3 0 0   # Move to dispense location.
        PUMP  15 50 0      # Dispense fluid.
        }
#Move Magnet to Mix
        SWEEP 5 100 15 0
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 45 35
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 15 0
WAIT 5
#Move to home position
        MOVETO
#Wait for magnetic beads to settle
        WAIT 60
```

```
LOOP (5) {
#vial 4 (Detection Antibodies)
   LOOP (5) {
        VALVE 12 4 1 0 0   # Move to air location.
        PUMP  15 2300 20   # Fill pump with air.
        VALVE 12 4 3 0 0   # Move to dispense location.
        PUMP  15 50 0      # Dispense fluid.
        }
#Move Magnet to Mix
        SWEEP 10 100 15 0
WAIT 10
        SWEEP 10 100 35 25
WAIT 10
        SWEEP 10 100 45 35
WAIT 10
        SWEEP 10 100 55 45
WAIT 10
        SWEEP 10 100 65 55
WAIT 10
        SWEEP 10 100 75 65
WAIT 10
        SWEEP 10 100 75 65
WAIT 10
        SWEEP 10 100 65 55
WAIT 10
        SWEEP 10 100 55 45
WAIT 10
        SWEEP 10 100 35 25
WAIT 10
        SWEEP 10 100 15 0
WAIT 10
#Move to home position
        MOVETO
#Wait for magnetic beads to settle
        WAIT 60
}
```

```
LOOP(10){
#vial 3 (BSA)
   LOOP (5) {
        VALVE 12 3 1 0 0   # Move to air location.
        PUMP  15 2300 20   # Fill pump with air.
        VALVE 12 3 3 0 0   # Move to dispense location.
        PUMP  15 50 0      # Dispense fluid.
        }
#Move Magnet to Mix
        SWEEP 5 100 15 0
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 45 35
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 75 65
WAIT 5
        SWEEP 5 100 65 55
WAIT 5
        SWEEP 5 100 55 45
WAIT 5
        SWEEP 5 100 35 25
WAIT 5
        SWEEP 5 100 15 0
WAIT 5
#Move to home position
        MOVETO
#Wait for magnetic beads to settle
        WAIT 60
}
```

3.2.3 Results

As stated, the goal of these ELISA tests is to establish an automated portable platform that could be used for a variety of point of care diagnostic applications, including ELISA assays as can be seen in the data below.

Concentration	Neg	100pM	300pM	1nM	10nM	100nM
Optical Density	0.241	0.361	1.25	1.66	2.99	4.05

In **Figure 3.5**, the data acquired from the EnSpire Plate Reader is shown for absorbance @450mM vs. ng/mL. A detection capability down to 100pM concentrations and an analytical limit of detection (LOD) of 4.28 pM and lower limit of quantitative detection (LLOQ) of 12.96pM is shown. LOD and LLOQ of the assay was comparable to similar manually executed tests[29]. Additionally, we were able to detect three log ranges of concentrations, 100pM to 100nM, as opposed to two log ranges reported in literature.

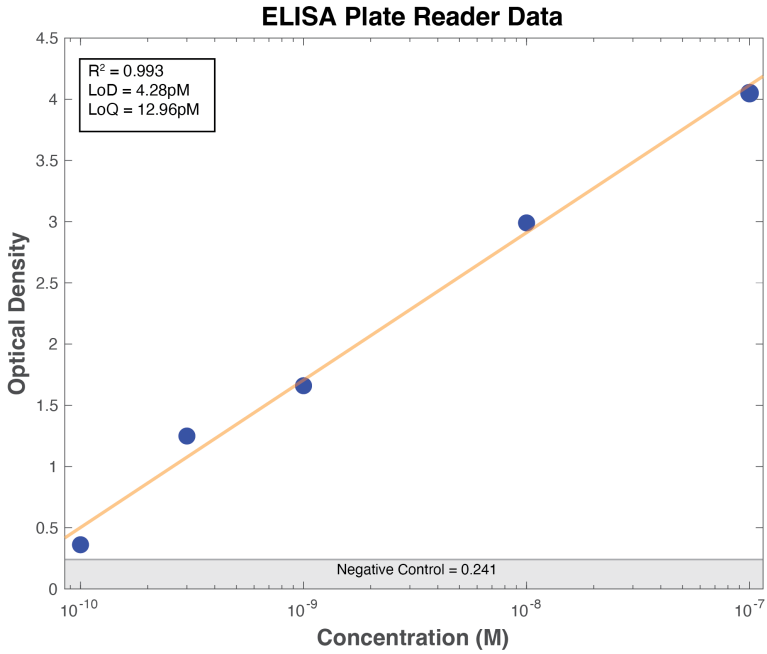

Figure 3.5: Absorbance (@450nm) vs ng/mL. After converting concentrations from M to ng/mL and plotting, a linear curve can be seen

Chapter 4 Conclusion

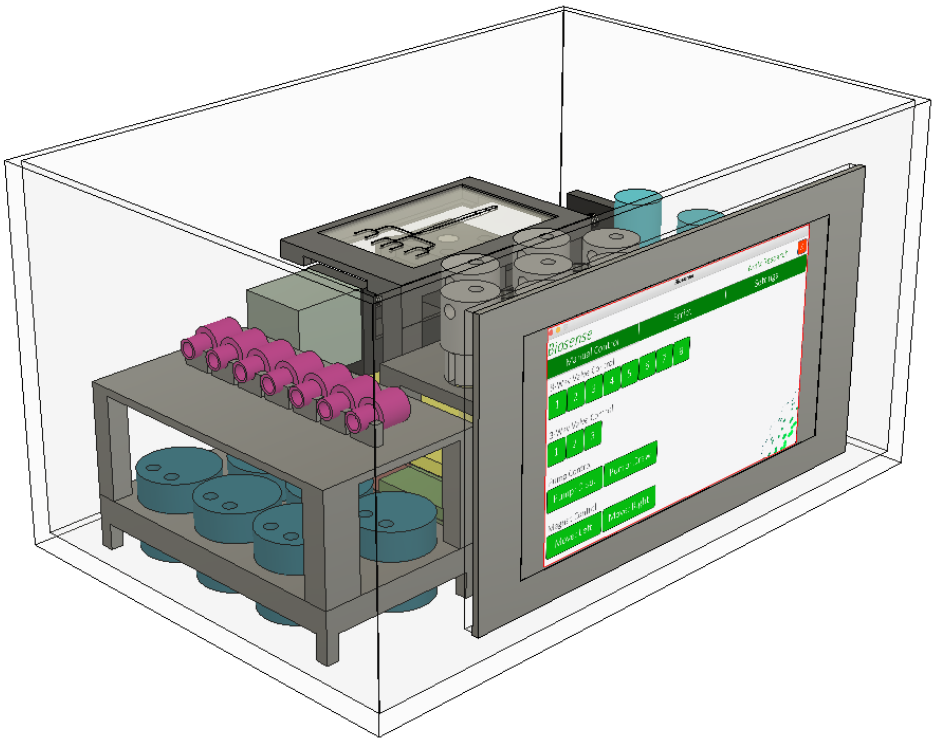

Figure 4.1: Render of Final Platform using Raspberry Pi & Touchscreen

4.1 Overview

Overall, this platform provides users with a compact, reliable, and autonomous system to run point-of-care diagnostic testing. By developing such a platform, the user no longer has to be concerned with large-scale, expensive laboratory equipment and/or user error when conducting time-consuming

experiments. I have designed a system that could easily be adapted as an alternative to similar high-cost platforms. An overall cost of developing a similar platform is approximately five thousand dollars, which is significantly lower than that of larger laboratory equipment. As shown, this platform has the ability to perform ELISA tests in a consistent manner, proving that it could easily be a reliable solution for point-of-care diagnostics applications.

4.2 Future Research

There is still room for improvement and further development on this platform. For example, the layout could be optimized for a small form factor; more parts could be designed in the lab and 3D printed to save on cost, and much more. This platform will continue to be further developed in the Yanik Labs: UC Santa Cruz NanoEngineering Group. Future improvements could include a sensing system alongside the microfluidic lab-on-a-chip and pneumatic system. A built-in sensor platform would not be too difficult to implement; the sensing platform could efficiently be coded within the existing software and display the results on the user interface. This sensing system could be optical or fluorescence-based. Besides a sensing platform, the microfluidic lab on a chip could also be optimized for other applications. As stated above, this platform has room for improvement and growth. I hope this platform can be taken to the next level and adapted for real world applications.

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