June, 2017
Manual Simulations to Aid in Device Prototyping

Objective:
From the initial discussions of the NYUAD iGEM team, the project idea was finalized to create a device that can detect the presence of Shiga toxin in street food. To support the biological reactions for the successful detection of the toxin, the device was supposed to have three compartments. The first compartment was to be at a temperature of 95˚C for lysis, the second at a temperature of 65˚C for the reaction, and the third compartment was supposed to be a waste compartment. The objective of the experiment was to create a device prototype and run flow of sample simulations to help create the original device.

Experimental Conditions:
The materials used to construct the prototype included a rectangular plastic box, a cylindrical plastic vessel, and straws. A soldering iron was used to melt the plastic box in the appropriate place where the cylindrical vessel was added. The straws were then added vertically to the cylindrical vessel. The box represented the first compartment, the cylinder represented the second compartment, and the straws represented the third compartment (the waste compartment, Figure 1).

Figure 1. First prototype for the device.

This prototype was then used to simulate the flow of the sample solution using water as the sample. A cardboard cutting was used as a temporary valve, and the water was allowed to fill up most of the first compartment, with the cardboard separating the two compartments.

Results:
As the valve was removed, the water smoothly flowed to the end of the second compartment where the results were meant to be visualized. The excess water fell down from the straws, and was collected in a separate waste compartment.

Discussion:
The results showed that having an excess of the sample in the first compartment can reliably ensure the movement of the sample through the various tubes to the different compartments in the original device. The results also indicated the importance of a valve to maintain the flow of the sample. This experiment allowed for the focus of the engineering team to then be casted upon device prototyping and the heating mechanisms for the device.

July 2-13, 2017
Experimentation with Chemical Heating

Objective:
A series of experiments were performed to determine the viability of chemical heaters, in the form of chemical hand-warmers, as suitable choices for the heating system in the device. Different combinations of the chemical heaters were experimented with, and their temperatures were recorded using a thermocouple. The results of these experiments were then used to determine ways in which high temperature could be achieved and maintained for a long period of time.

Experimental Conditions:
Several experiments were conducted in the laboratory to accurately determine the best heating mechanism for the device. The temperature of the lab was relatively low. Amongst the various experiments that were conducted, we tried the following variables:

1. Thermocouple connected to a single hand-warmer
2. Thermocouple connected to two hand-warmers combined
3. Thermocouple exposed to the chemical inside the hand-warmer

The temperature from all these experiments was recorded over time. Figure 2 shows the set-up for the fourth experiment.

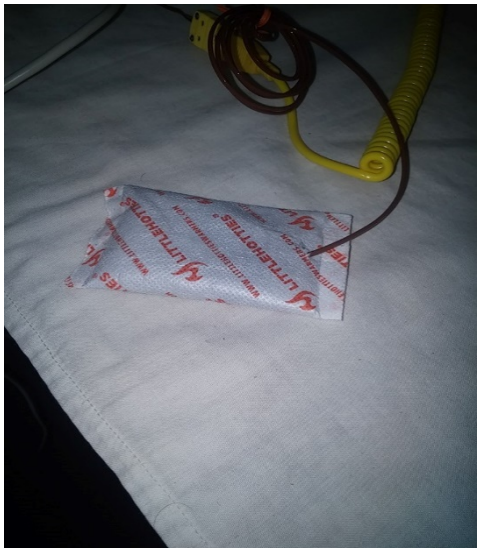

Figure 2. The thermocouple is exposed to the chemical inside the hand-warmer.

Results:
The highest temperature was recorded when the chemical was directly exposed to the thermocouple, and the lowest temperature was recorded when only one hand-warmer was used, and the chemical was not exposed. Increasing the number of hand-warmers used simultaneously also increased the resultant temperature. These results are shown in Figure 2. All the experiments were conducted for a period of one hour, and they showed that the temperature remains stable over time.

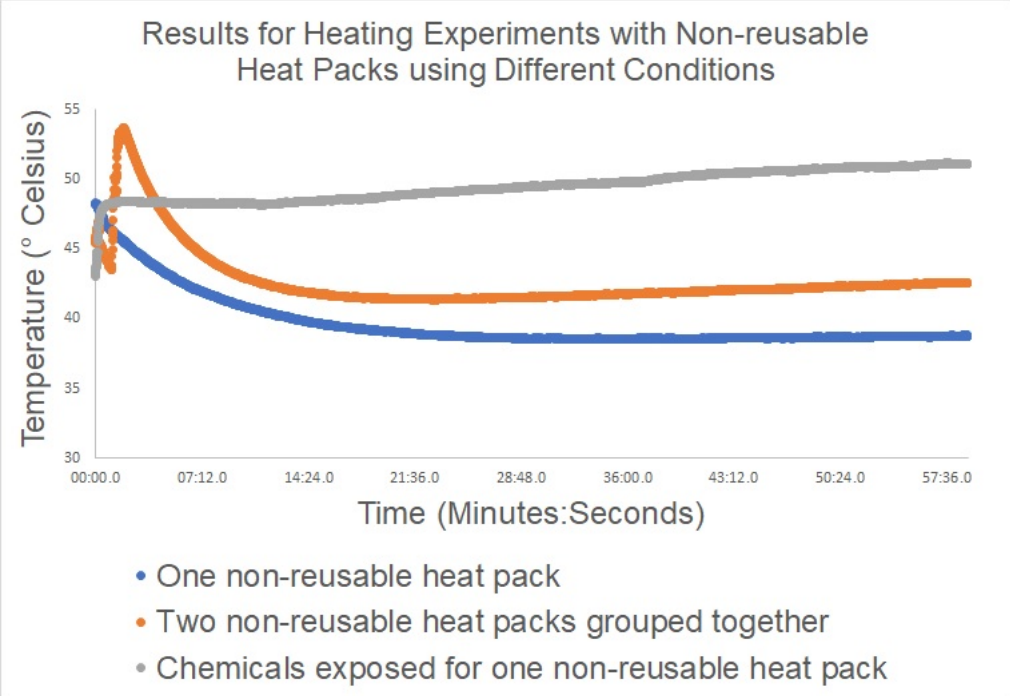

Figure 3. Temperatures over time for the experiments conducted. The blue line represents the first experiment which used one hand-warmer. The orange line represents the second experiment which used two hand-warmers grouped together. The grey line represents the third experiment which exposed the chemical inside to the thermocouple.

Discussion:

The temperature of 38˚C recorded before exposing the chemical and the average temperature of 48˚C after exposing the chemical were not adequate for the heating required for the reaction. However, the lack of any insulation and the cold environment of the laboratory affected the temperature, and thus, more experiments needed to be conducted with proper insulation. The idea of exposing the chemical was set aside, as it could potentially pose as a safety hazard. Since we discovered that different arrangements of hand-warmers together produced different results, we decided that more experiments should be done by testing other variables, such as putting holes in the hand-warmers, to achieve higher temperatures.

July 14-20, 2017
Experimentation with 3D printed Prototypes

Objective:
After our first experiment, two new prototypes were 3D printed to provide the framework for the device (Figures 4 and 5). Both prototypes had a large sample chamber where the sample would be placed in excess, and a reaction chamber where the sample would eventually travel to which would contain the reagents necessary for the reaction. One of the two prototypes also contained two waste chambers for the excess sample to flow into (Figure 4). The objective of the experiments was to test the flow of the sample (using water), and to make sure that gravity and excess water are sufficient to drive the sample so that no extra force needs to be provided.

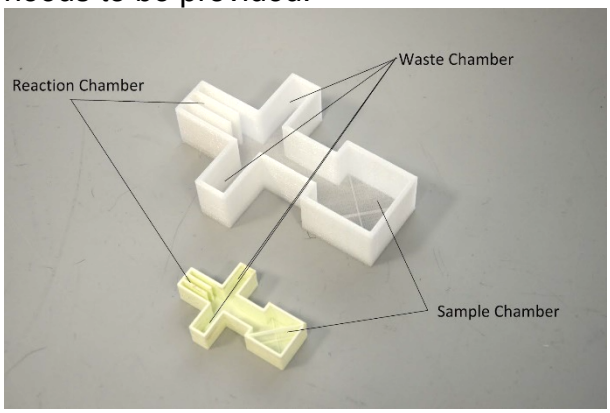

Figure 4. The first 3D printed prototype.

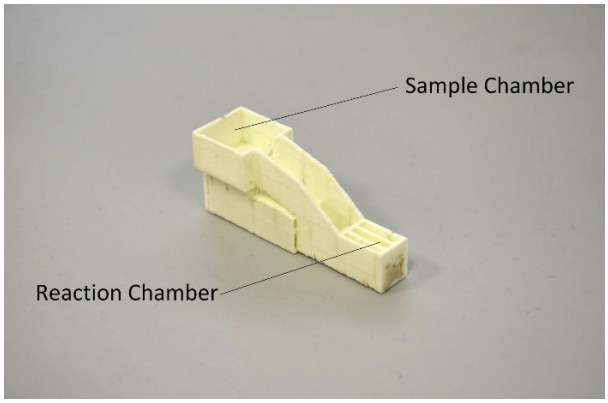

Figure 5. The second 3D printed prototype.

Experimental Conditions:
Small barriers were made with cardboard to prevent the water from flowing outside the sample chamber. These valves were adjusted as gates for the sample chamber, and then an excess volume of water was added. The valves were then removed, and the water was allowed to flow. The prototypes were placed on a smooth surface to ensure that gravity and excess of the water drives the water to the reaction chamber.

Results:
For the prototype with the waste chambers (Figure 4), we observed that the water first filled the reaction chamber, and then the excess went into the waste chambers. For the other prototype (Figure 5), the water went directly to the reaction chamber. There was no overflow of sample in either prototype.

Discussion:
The experiments conducted provided successful results in achieving the objectives. However, the reaction required smaller volumes, specifically in microliters. We decided to change the prototype for the device, and replace it with a new prototype that would have a sample chamber to contain microliter volumes of fluid. The sample chamber would be connected to a microfluidic channel, and all the flow of sample would take place through microfluidic channels. The microfluidic channel would connect the sample chamber to a reaction chamber and a waste chamber.

September 4-12, 2017
Comparing Reusable and Non-Reusable Chemical Heaters

Objective:
All experiments were previously conducted on non-reusable hand-warmers to provide a source of heat. We decided to experiment with reusable hand-warmers as well to test a more environmentally friendly option. The reusable hand-warmer uses the reaction of saturated pure sodium acetate with a metal, which releases energy as the sodium acetate solidifies. Thus, the objective of the following experiments was to test the temperature of the reusable hand-warmers over time. Experiments were also conducted on non-reusable hand-warmers with insulation.

Experimental Conditions:
Before resuming experimentation with thermocouples and hand-warmers, we recalibrated the thermocouples using a device called thermo-block and a thermometer. The temperatures recorded by these three instruments were approximately the same which showed that the calibration was correct. Experiments showed that both reusable and non-reusable heaters maintained their maximum temperatures for a reasonable amount of time. To ensure that the results were not affected by the outside temperatures, the system was insulated by placing it in a Styrofoam box and covering it up. For further accuracy, these experiments were conducted in a warmer laboratory. In some experiments, holes were made on the non-reusable hand-warmers to achieve greater temperatures with proper insulation (Figure 6).

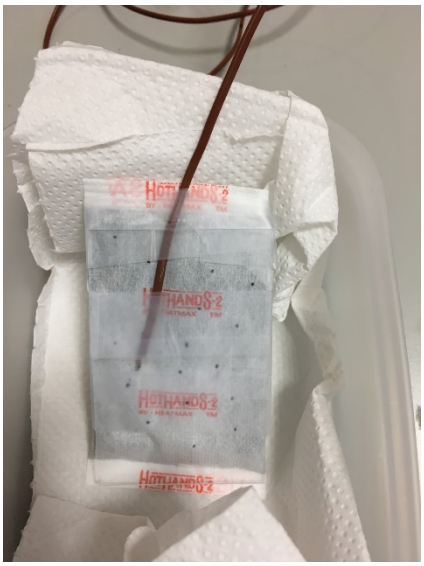

Figure 6. Non-reusable hand-warmer with holes.

Results:
The results showed that there was a positive correlation between the number of hand-warmers used and the recorded temperature. Experiments conducted using the non-reusable hand-warmer with holes also produced a higher temperature than previous experiments. However, two hand-warmers packed together reached an even higher temperature than the hand-warmer with holes, which reached 75˚C in 15

minutes and remained constant for the duration of the experiment (10 hours). The reusable hand-warmer only recorded was 30˚C for the 10 hours the experiment was conducted. These results are shown in Figure 7.

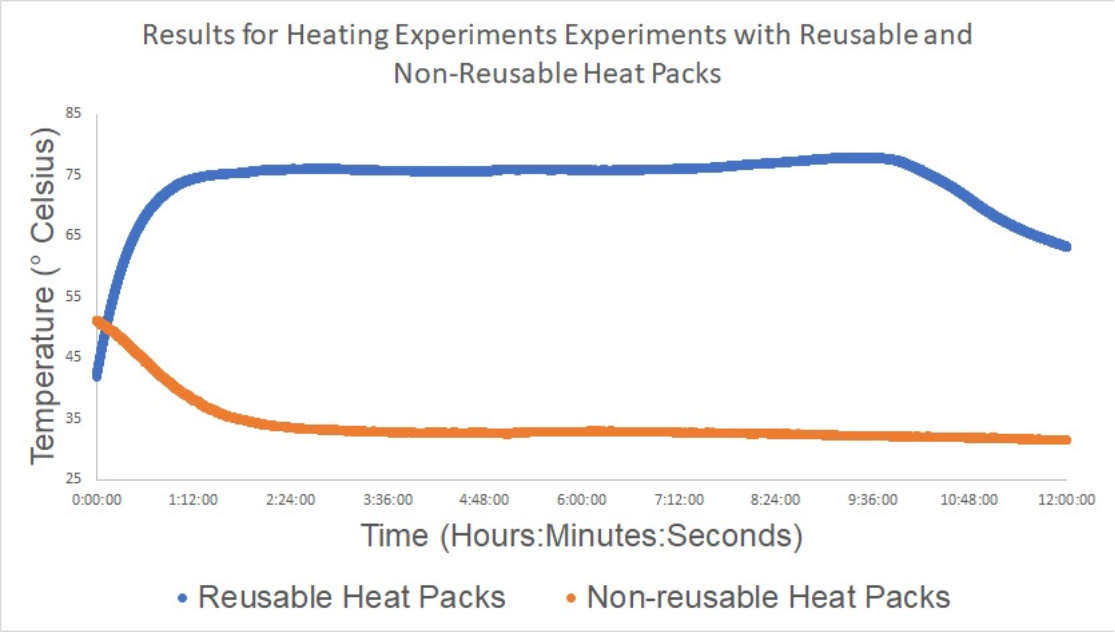

Figure 7. Graph showing the temperature over time for the reusable and non-reusable hand-warmers.

Discussion:

The results showed that when holes were poked at the area of the sensor/centre of the hand-warmer, it reached a higher temperature. This meant that using a single hand warmers with holes poked towards the centre was a more viable option instead of using multiple hand-warmers. Even though the reusable hand-warmer is a cheaper and eco-friendlier option, only the non-reusable one provided the necessary temperature. Insulating the system using the cloth and Styrofoam box allowed us to concentrate the heat in the appropriate direction and avoid heat loss. Thus, it was decided to use the non-reusable hand-warmers with the most necessary geometry of holes poked.

October 1-7, 2017
Testing the Microfluidic Channels

Objective:
Since the biological reactions were in microliter volumes, the device was redesigned using microfluidic channels and a chip. The new chip design had microfluidic channels which were embedded on a Polydimethylsiloxane (PDMS) plate using a silicone mould (Figure 8). The device that held the chip also contained screws to be used as valves, which allowed us to manipulate the sample flow (Figure 9). The objective of this experiment was to test the accuracy of the valves, and to observe the flow of dyed water through the microfluidic channels.

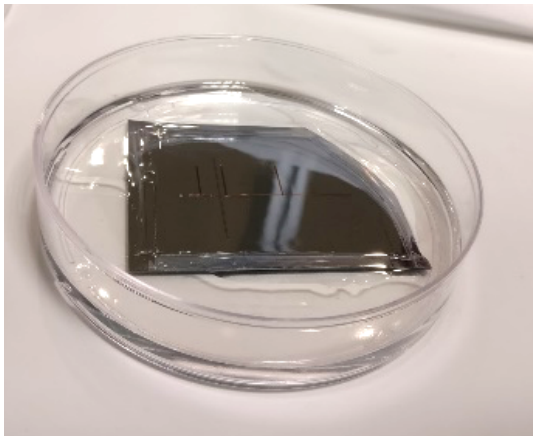

Figure 8. The silicon mould contains the design for the microfluidic chambers and channels. Polydimethylsiloxane is placed on it in liquid form and cured at 70˚C.

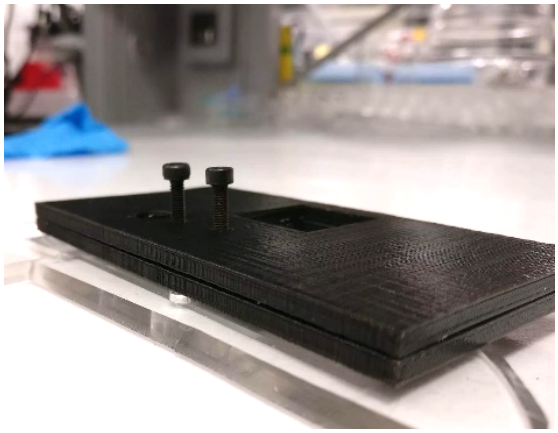

Figure 9. The device with the new chip design, and screw valves.

Experimental Conditions:
Blue dye was added to a small amount of water, and the solution was stirred until it was uniformly blue. The chip was then placed in the outer device containing the valves, and a pipette was used to transfer a few microliters of the dyed water to the sample chamber on the chip. The valve was initially closed and was opened a minute after the water was added. We checked for sample overflow from the sample chamber. The valve was closed before all the solution had passed through.

Results:
When the valve was closed, the solution stayed in the initial sample chamber. As the valve was slowly opened, the blue solution could be seen traveling along the microfluidic channel, and it began to fill up the reaction chamber and the waste chamber. When the valve was closed with some solution still in the sample chamber, the flow of the solution stopped.

Discussion:

These results showed that the valving mechanism worked for the microfluidic channels, and ensured that the flow of the sample in the microfluidic channels can be achieved by having an excess of the sample in the sample chamber from which the microfluidic channels originate. Upon finalizing the biological reactions, some final modifications were suggested for the device. The next steps will be to design a four-chamber device, so that it is possible to have duplicates and experimental controls. These chambers will be connected through microfluidic channels. Of the four chambers, one will be a sample chamber, one will be a reaction chamber, and two chambers will be closed off as positive and negative controls.

October 8-13, 2017
Testing the LAMP Reaction in the Newest Prototype

Objective:
A new prototype was made because of previously conducted meetings and experiments that consisted of four chambers, which were connected by microfluidic channels. The objective of this experiment was to conduct the LAMP reaction required for the detection of the Shiga toxin in the prototype, and observe and visualize the flow of the reagents and the reaction take place in real time using a microscopic camera device. This device zoomed into the microfluidic channels, making it easier for the movement of the fluids to be seen.

Experimental Conditions:
The new prototype consisted of the microfluidic channels and chambers moulded on a PDMS plate, which was then inserted into the device that had the valves to maintain the flow in the microfluidic channels (Figure 10).

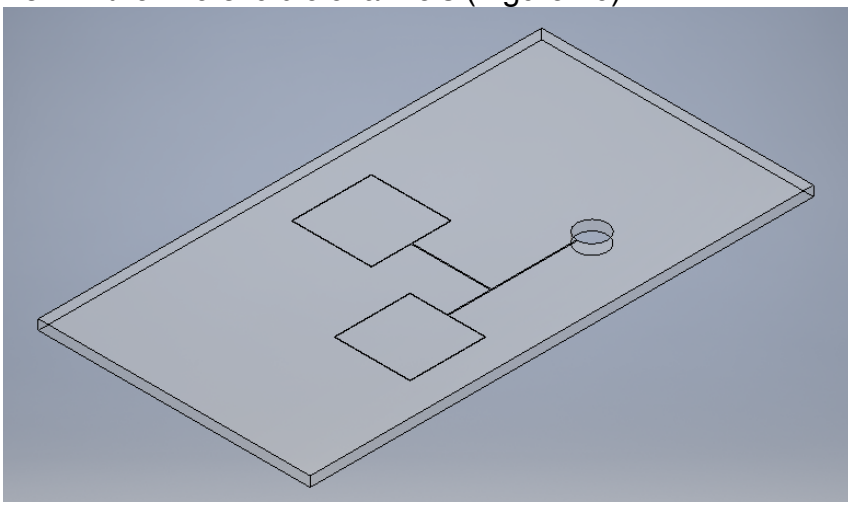

Figure 10. The new PDMS chip design, with two rectangular chambers for excess and reactions and a circular inlet chamber for sample addition.

An excess of the sample was added to the sample chamber, and the necessary reagents for the reaction to take place were added in the reaction chamber. The non-reusable hand-warmers were used as the heat source for this experiment. The hand-warmer was heated up, and the PDMS plate was then placed flush on the hand-warmer. The PDMS plate was then placed under a fluorescence microscope that allowed us to clearly visualize the flow of the fluids on a monitor.

Results:
The sample did flow through the microfluidic channel, but the flow rate was observed to be too slow. Additionally, the flow was uneven and stopped randomly without having reached the reaction chamber. The reagents from the reaction chamber also moved into the microfluidic channel, but achieved limited flow. Additionally, the lack of a proper heat sources meant that no reaction took place.

Discussion:

The lack of fluidic movement was attributed to the narrow width of the microfluidic channels, which were too constricted for any considerable movement to take place. Thus, we decided to increase the width of the microfluidic channels so that there is ample movement of the sample from one chamber to the other.

October 15-20, 2017
Testing the Microfluidic Flow with Wider Microfluidic Channels

Objective:
Based on our previous experiment, a new prototype was created. This prototype used double-sided tape to attach a PDMS plate to a glass slide. The double-sided tape was cut using a laser cutter in such a way that it formed a microscale well that would be equal to the necessary volumes needed for the reaction (Figure 11). The microfluidic channels were 35 times wider than the ones we worked with before. The objective of this experiment was to test the flow of dyed water to ensure proper flow and mixing in the reaction chambers.

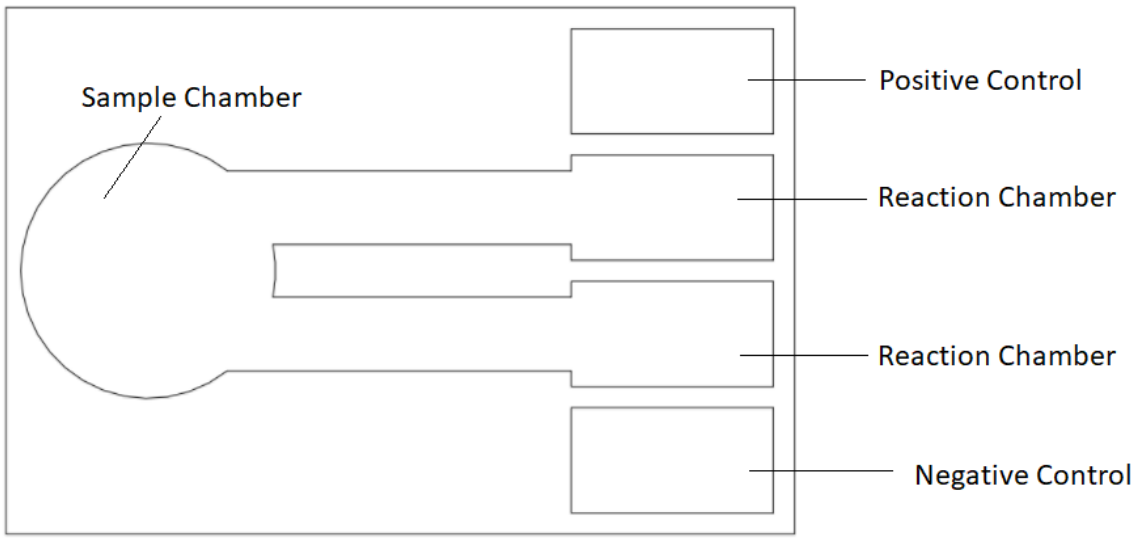

Figure 11. The design for double-sided tape for the microfluidic channels.

Experimental Conditions:
The double-sided tape with the design laser cut was placed on a glass slide. A blue dye was added to the reaction chamber and then fitted with the PDMS plate on the top. As the PDMS plate was added, there was some initial movement of the dye. We pressed at the juncture on the PDMS plate to replicate the effect of a valve to maintain the flow of the dye.

Results:
Even though an excess of dye was added, the microfluidic channels were too wide, and pressing the PDMS only transferred the dye up to the circular chamber. However, there was some initial movement of the dye on its own once the PDMS plate was added. The dye did not reach the reaction chamber, and the volume of the excess dye was not enough for it to be forced to the reaction chamber. Figure 12 shows what the microfluidic channels eventually looked like.

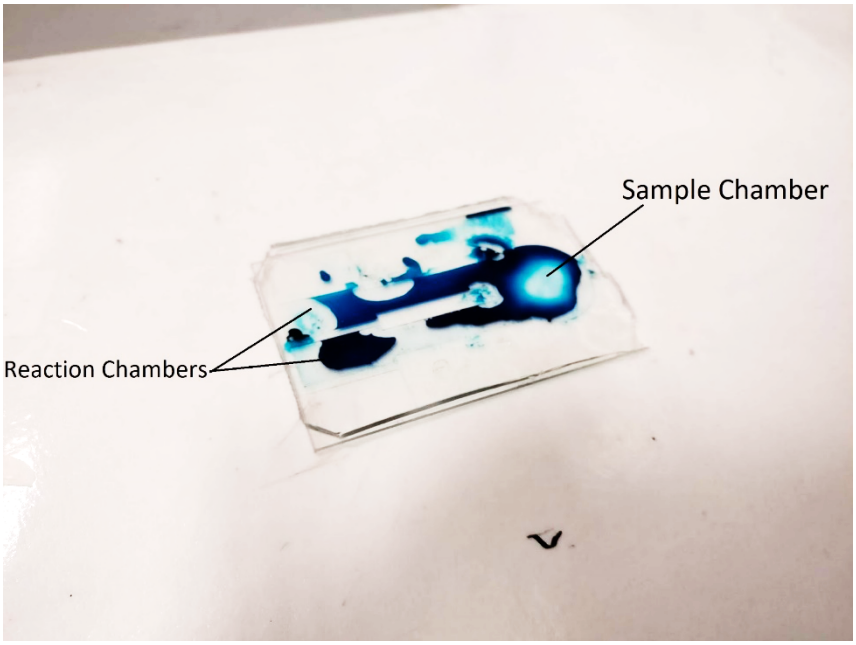

Figure 12. The final settlement of the dye in the channels.

Discussion:

The results showed that these newer microfluidic channels that were made without the use of a silicon mould did allow the movement of the dye, but were too wide in this case. Additionally, the diameter of sample chamber was too large, so most of the dye collected through and would not continue to flow. Hence, we decided to vary the width of these channels and to create a separate circular chamber for the addition of the sample closer to the reaction chamber.

October 23-24, 2017
Experimenting and Troubleshooting Fluid flow with varying channel sizes and Inlet Positions

Objectives:
The objective of this experiment was to determine two parameters for the chip design; 1-the optimal channel width and 2-the best position of the Inlet relative to the entrances of the reaction chamber. A total of nine chips were tested and the dimensions of the chip were altered based on the results of each test. All chip designs were cut using a laser cutter to ensure precision and rapid fabrication for the next iteration.

Experimental Conditions:
After each chip design was fabricated, it was assembled on a cleaned glass slide. After assembly, the test was conducted by inserting a sample fluid (dye) and a clear fluid (water) into the inlet and reaction chambers respectively using pipettes at 50 µl and 25 µl.

The chip designs are as follows:

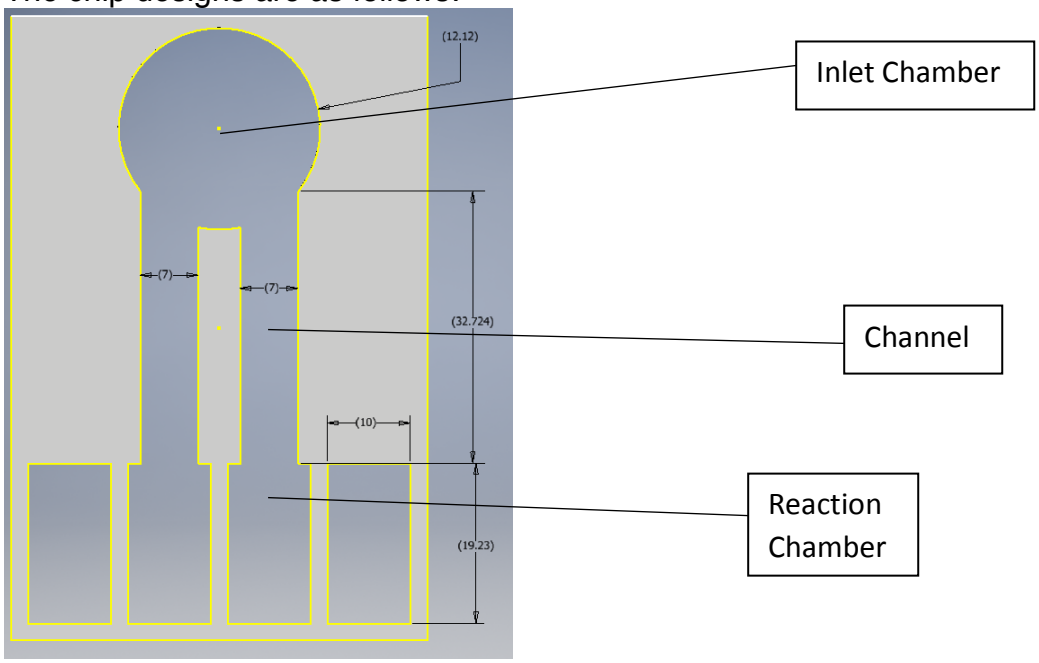

Figure 13. Chip design using a 12.12mm (radius) inlet chamber, 7mm wide channels and a 25 μl reaction chamber. The Chip is 0.130 mm thick.

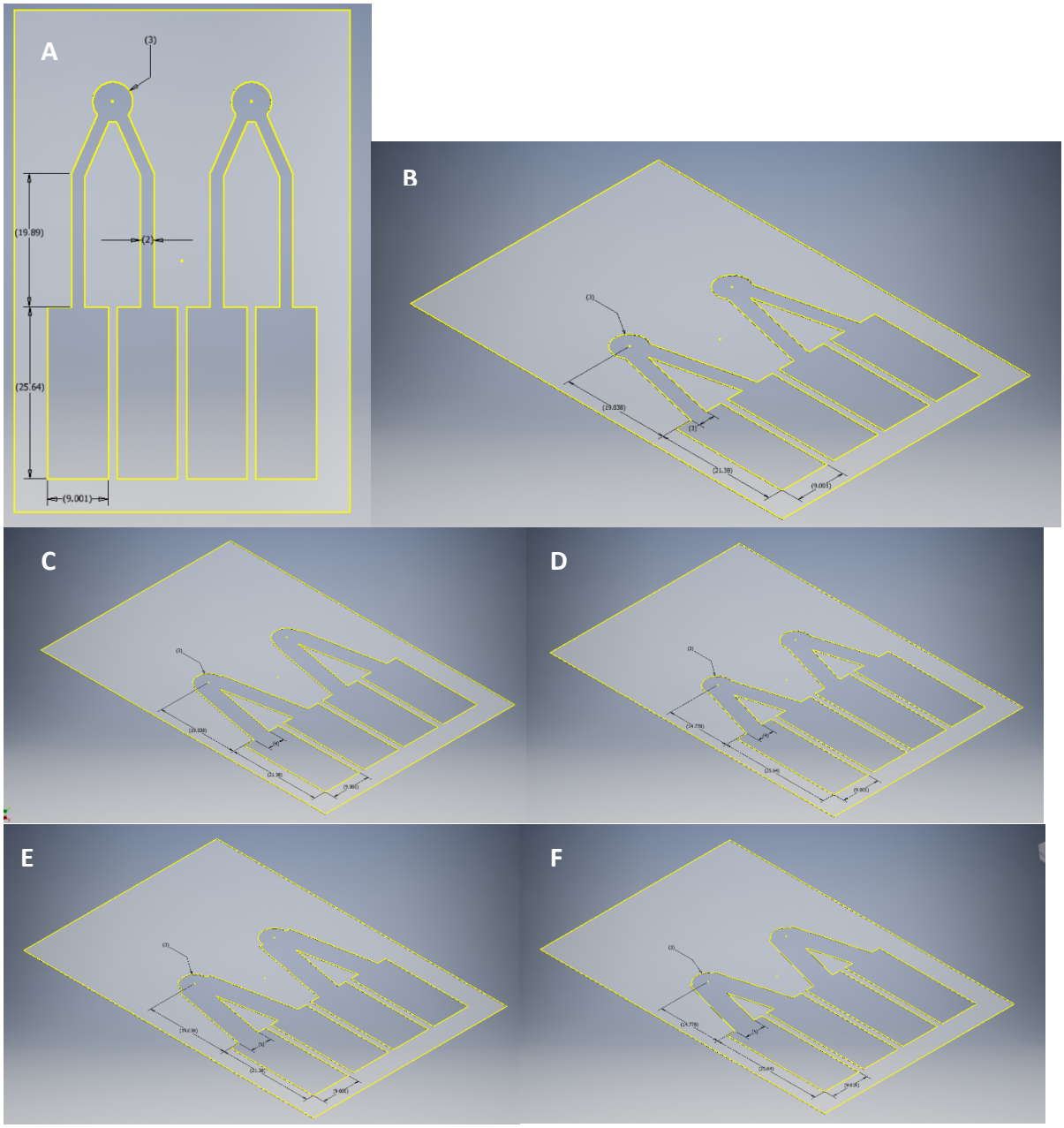

Figure 14. Chip design using **(A)** a 3mm (radius) inlet chamber, 2 mm wide channels and a 25 μl reaction chamber; **(B)** a 3 mm (radius) inlet chamber, 3 mm wide channels and a 25 μl reaction chamber; **(C)** a 3 mm (radius) inlet chamber, 4 mm wide channels and a 25 μl reaction chamber; **(D)** a 3 mm (radius) inlet chamber, 4 mm wide channels and a 30 μl reaction chamber; **(E)** a 3 mm (radius) inlet chamber, 5 mm wide channels and a 25 μl reaction chamber; **(F)** a 3 mm (radius) inlet chamber, 5 mm wide channels and a 30 μl reaction chamber.

Results:

It was observed after the first test using the 7mm wide channel design (Figure 13), fluid was greatly susceptible to backflow. This prevented it from entering the reaction chamber and instead remain in the inlet chamber. The next designs then reduced the width of the channels to 2mm and reduced the size of the inlet chamber by splitting it into two (Figure 14). The next tests had revealed that the fluid was still being trapped in the channels due to their length, so the length of the channels and thus the

distance of the inlet chamber from the entrance of the reaction chamber were reduced to minimise the volume of fluid being lost within the channels. The next designs from Figure 15 onwards varied the width of the channels between 3, 4 and 5 mm while the inlet hole was kept at 6mm then to 5 mm. From the test on these channels it was found that a channel width of 3mm gave the best results as fluid successfully flowed from the inlet into the reaction chamber and began to dilute the green dye, which simulated the mixing that would take place with the actual sample and reactants.

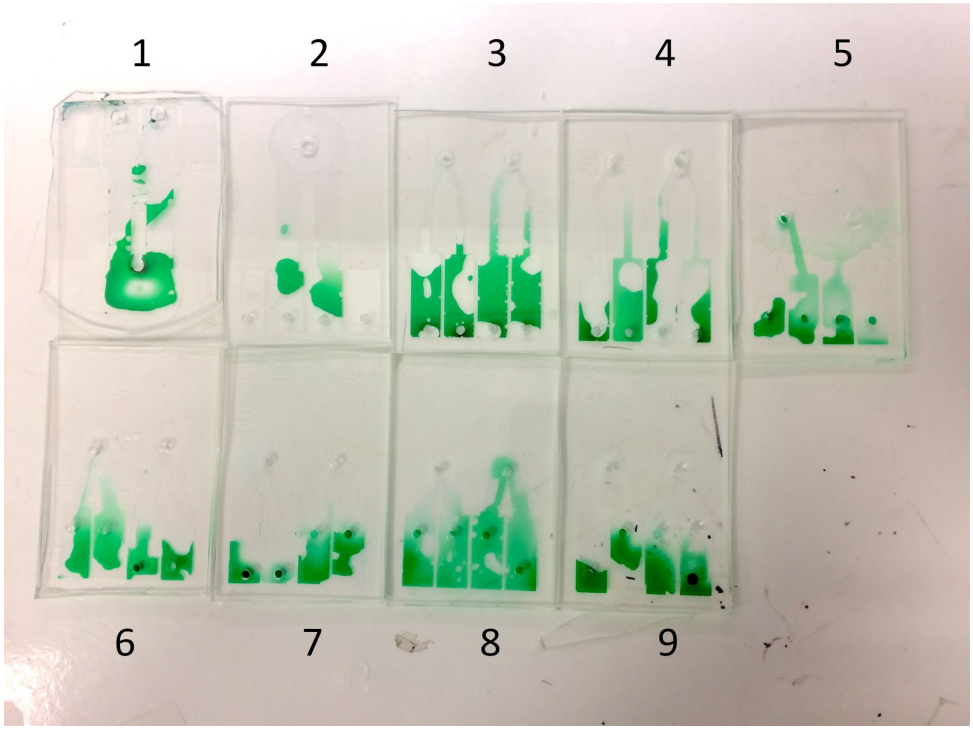

Figure 15. Results of testing the various chip designs. The numbers from 1-9 represent successive iterations of the chip design. Experiments 7, 8, and 9 gave the best results in terms of flow and mixing without any backflow or blockage.

Discussion:

With the optimal chip parameters being a 25 μl chamber volume, 3 mm channel width and an inlet to chamber distance of 14.778 mm, the next step is to create and test a prototype with these parameters and test them with the actual LAMP reactants and heating device.

October 22-24, 2017
Testing and troubleshooting final design and running LAMP reaction on chip

Objective:
The goal for today was to resolve new issues that had arose from the previous experimentation regarding two things, the fluid being absorbed into the paper backing of the chip and the fluid either escaping through caps during heating or evaporating out due to build-up of pressure.

Experimental Conditions:
We constructed various prototypes of different combinations to troubleshoot problems that had arisen in previous tests. After each chip design was fabricated and assembled, the test was conducted by inserting a sample fluid (Green Dye) and a clear fluid (Distilled water) into the inlet and reaction chambers respectively using pipettes at 50 µl and 25 µl. Evaporation tests were conducted on a hot plate calibrated to 65˚C for 20 minutes to mimic experimental conditions.

Results:
While attempting to improve fluid flow by increasing the spacing between the PDMS layer and the glass slide, so as not to prevent the PDMS from collapsing and blocking the flow, a layer of paper backing was still left on the chip to increase the thickness by another 100 microns. From initial fluid tests, this was sufficient enough to allow optimal fluid flow. However, when this setup was left after some time with the dye as fluid testing, the paper backing began to absorb the fluid from the reaction chambers;

Figure 16. Bifurcated chip design with 2-3mm inlet holes and a total of 4 rectangular reaction chambers. The chip had 3mm wide fluid channels linking the inlets and the reaction chambers. Notice the green dye being absorbed into the surrounding paper backing.

A proposed solution to this was to make the paper backing hydrophobic, to prevent it from absorbing water. This was done by coating the chip in a Teflon solution and retesting the device. However, the same issue erupted which meant that the fluid was being absorbed from the sides of the wall in the reaction chambers, where the Teflon could not maintain a good seal, even after 3 layers.

Another design was then proposed which removed the channel design and instead moved to create wells within the PDMS, which would serve as reaction chambers.

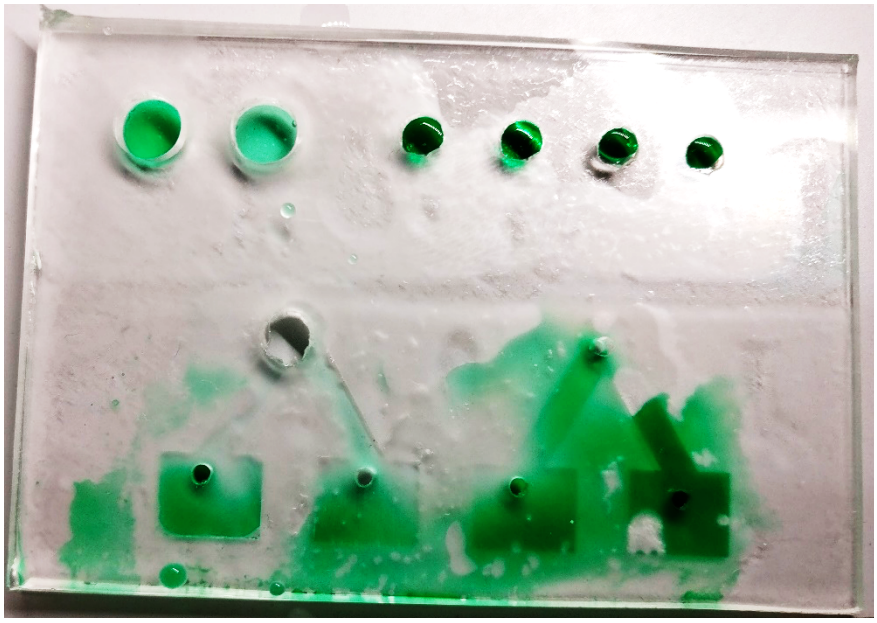

Figure 17. Same design with PDMS layer, with paper backing removed and 6 holes being punched on the top of the chip. The two holes on the left are 6mm wide and the other 4 are 4mm wide. Fluid within these wells remains intact without any leakage or spillage. The PDMS layer was 4mm thick.

To test heating, the evaporation needed to be contained. This was done by covering the exposed holes of the wells, after fluid insertion, with a glass slide that was wrapped in Parafilm, which is known to be hydrophobic.

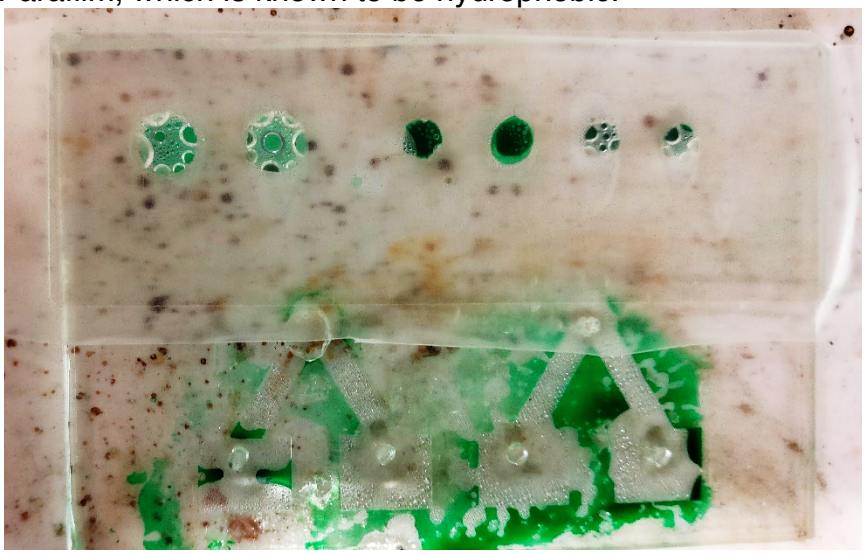

Figure 18. Testing the same PDMS configuration with parafilm covered glass slide placed on top to cover the holes. Heating was done at 65 degrees Celsius to simulate actual behaviour of fluid in LAMP scenario.

While the wells resulted in the least amount of evaporation and contained the liquid best, we could not get a good seal with parafilm. Instead, we sealed the PDMS chip with optical adhesive film and ran the test again. This provided the most optimal conditions to reduce loss of reagents due to evaporation and mixing between samples.

The final chip design (Figure 19) was made to maximize the distance between chambers to prevent any condensate from mixing.

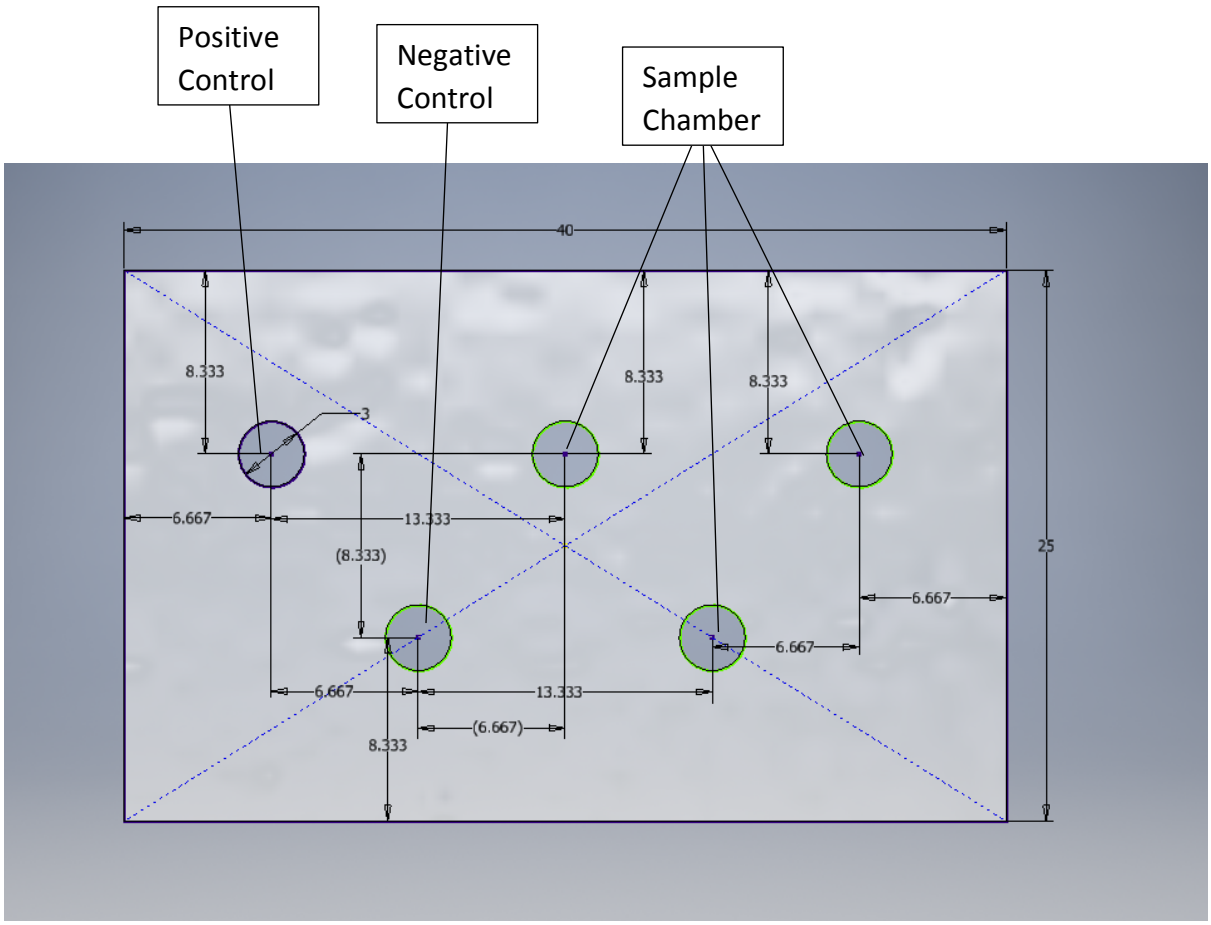

Figure 19. Final chip design using 5 wells for 3 samples, one positive control and one negative control. The design was made as such to equally space each well apart to prevent any loss of sealing due to such proximity. Each well is 3mm wide and the chip is 4mm thick.

Discussion:

With the final design made and tested, we were ready to finalize the heating model and test with the biological reactants.

October 25, 2017
Developing a controlled Rapid Heater

Objective:
To develop a cheaper version of the ITO heater in order to reduce the cost of the heating device while introducing feedback control, which is lacking from the heat pack method.

Experimental Conditions:
The heater needed to satisfy the following properties:

1. Sustain a temperature of 65˚C
2. Feedback control of maintain the temperature
3. Affordable

A Peltier Cooler Module was used because it could reach the desired temperature, was easy-to-use and customize, and was reasonably priced compared to the ITO heater. The Peltier can be used with 5V to 7V voltage input and 1A to 2A current input. This property was ideal, considering that most portable power banks have a DC output of 5V and 2.1A. Hence, the heater was developed such that a portable power bank can act as the power supply.

To control the Peltier Cooler Module, we used the tmp36 temperature sensor because it is cheap and easy-to-use. Additionally, an Arduino nano was used as the microchip controller due to its simplicity, a compact size, and its ability to do a proportional-integral-derivative (PID) control using tmp36. Finally, an N-Mosfet transistor was used to act as a switch control for the Peltier heater.

The schematic of the heater is shown in Figure 20.

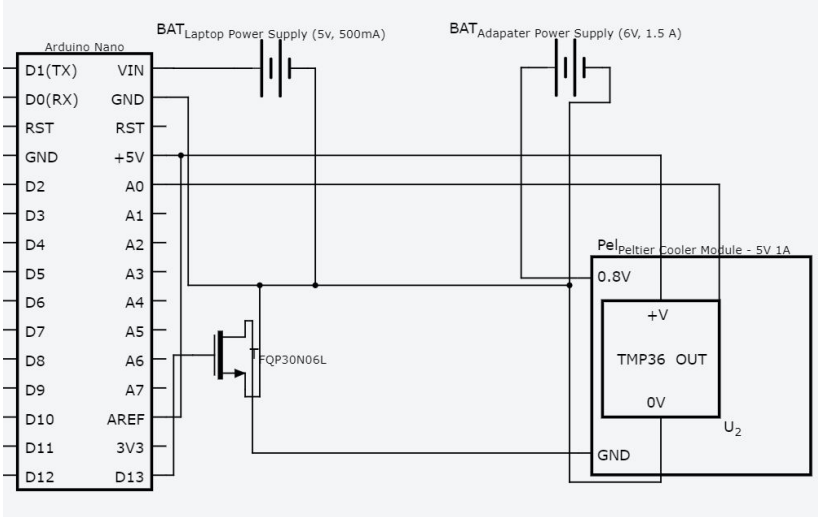

Figure 20. The schematic of the heater using an Arduino nano, N-MOSFET transistor, tmp35 and Peltier Cooler Module with external power supply adapter (represented as a battery).

An external power supply was added using a power adapter that allows for 6V and 1.5A. The Arduino nano was powered by Laptop USB, which only allows 500mA of current.

The tmp36 uses the 5V voltage as a reference to measure the temperature on the Peltier heating plate. This sensor controls the N-Mosfet transistor that works with a low-side switch setup, allowing the current to flow whenever the temperature of the Peltier registers below 65˚C.

Results:
To develop the algorithm to control the heater, a thermocouple using OMEGA data acquisition was used to measure the temperature on the top of the glass slide. We found that the temperature sensor on the Peltier and on the glass gave a constant temperature of 65˚C.

Discussion:
Since we obtained adequate heating and control using the Peltier, we redesigned our 3D device to house the electronic components needed. This heating method replaced the disposable heat packs, because it is more eco-friendly, and the ITO heater, because it provided the same properties at a lower cost. Our next steps will be to combine the Peltier heating with the chip and biological reagents for the first prototype test.