MICROFLUIDIC CHIP PROJECT REPORT

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ENGR 7B: Introduction to Engineering

University of California, Irvine

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Executive Summary

Our goal for this project was to create a microfluidic system that could detect the concentration of any unknown solution with the use of an rgb sensor. For this project, we had to detect the concentration of fluorescein in the solution given to us. Our team created a chip made of Polydimethylsiloxane (PDMS) along with housing that would prevent light pollution to the chip and hold our electronic components. In order to fill the requirements of the project successfully, our team split up and focused on specialized tasks. This included learning how to laser cut, 3D print, code with arduino, circuit analysis, and learn chemical calibration. As we progressed throughout these ten weeks and learned these new skills, we proceeded with the construction of the system that would meet the design requirements.

We completed the system over a span of ten weeks. The first two weeks were spent brainstorming a design for a system, and deciding the size of the staging wells, detection well, waste well, and channels. After that, our mechanical and electrical hardware team spent the third and fourth week creating a preliminary design for the chip and the housing. Our design was then approved and we spent week five finalizing the details and modifying our design with the advice of our advisor. Once the design was finalized, we began the fabrication process by building the housing, cutting out the chip cover, creating the mold for the PDMS, and fabricating the PDMS chip. We also began the coding process during the sixth week, and this continued with the flowchart, troubleshooting, and debugging up until the final competition. We completed the fabrication of the system during week eight, which is also when we created the stock solutions and began calibration. This also continued until the final competition. We were able to meet our goal of detecting the amount of fluorescein in the solutions with the RGB sensor, though our results were not what we had expected. This could be due to an error in the fabrication of the system itself and the equipment, or some small errors in the code.

Throughout this process we did not only gain technical knowledge in the programs we used, but also the process of engineering. We learned that time management for this project was extremely important, as we had deadlines for several different aspects of the project, as well as the challenging and time consuming process of trial and error. Though we may have had brilliant ideas that should have worked on paper, reality presented us with several challenges. However, this also allowed us to focus on the importance of communication and teamwork, as well as working with others in the field of engineering.

Problem Definition

I. Introduction

We were tasked to build a microfluidic system that can detect the concentration of fluorescein in a solution. There are many applications to this system that we have studied in class, such as using other solutions with different colors for disease detection, or blood tests. These miniaturized labs help with portability and accessibility, as well as being a cheaper method of testing.

II. Technical Review / Background

The origin of microfluidics is mostly associated with the work done in the late 1960s at Stanford University on chromatography and IBM's ink jet printer nozzles. Both of these utilized micromechanics to create these microfluidic devices. There is also evidence that hundreds of years earlier, researchers such as Hippocrates (400 BC), Galen (200 AD), and Theophilus (700 AD) were already interested in subjects that pertained to microfluidic technology. Although they didn't have the devices and technology themselves, the idea of creating microfluidic devices has already been present for several centuries.

In recent years, the advancement of technology has brought on more sophisticated microfluidics. The development has allowed these systems to use less samples and reagents, increased portability, low energy consumption, rapid response, and several other advantages that would help with the analysis of these solutions. There is even potential for connecting these microfluidic devices to smartphones, and taking advantage of the features available in them; especially the optical detection system.[1]

III. Design Requirements

As part of our product specifications, we were given that the feature size should be at least 150 um and the channel width be between 150 um and 400 um. Detection well is designed to store 3 uL of fluorescein solution. As for our structure, we were allowed to use either PDMS and PMMA, and we decided to use both, one for the chip (PDMS) and the holders with PMMA. We made sure to use gloves during PDMS fabrication to avoid stains as suggested. We 3D printed components (specifically our darkness chamber) and also created a holder to house the LED and an enclosure for the entire system. We made sure that our wires and connectors are completely covered and insulated by threading them through the bottom of the housing platform. Our total cost to build the device was less than $350, as the budget sheet will later on show. Finally, our product deliverables were as follows: the lab-on-a-chip microfluidic device, a detection concentration of an unknown fluorescein solution, the final design report, and the oral presentation.

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[1] Castillo-León Jaime, and Winnie E. Svendsen. "Chapter 1 Microfluidics and Lab-on-a-Chip Devices: History and Challenges." *Lab-on-a-Chip Devices and Micro-Total Analysis Systems a Practical Guide*, Springer International Publishing, Cham, 2015.

Design Description

I. Summary of Design

Our design for our microfluidic system consists of a chip created by PDMS held together by two holders made of PMMA material. There is an RGB Sensor screwed into a holder made of wood and screwed into the chip, and the chip itself with the sensor is screwed into the housing, which is then covered by a darkness chamber that was 3D printed and painted black with acrylic paint. The top of the housing also holds the breadboard and arduino, which are also connected to the RGB sensor held on top of the chip. In order to prevent as much light entering the system as possible, the wires are fed through two holes that will go under the darkness chamber. The blue LED is held under the housing platform with a small piece of wood, and is connected to a resistor.

II. Design Details

A. Microfluidic Chip (refer to Appendix A. Page 14)
The microfluidic chip contains four staging wells; each with a diameter of 4mm. All four staging wells flow into 150µm channels that meet and flow into a 300µm channel to go into the detection well. The intention of making two different sizes is to prevent backflow with capillary action. To further ensure that there is no backflow, the chip has four capillary valves connected to the staging wells that each have a diameter of 1mm. The 300µm channel to the detection continues to the waste well that has a semi-minor radius of 2.50mm and semi-major radius of 6.40mm.

B. Chip Holder (refer to Appendix A. Page 15 & 16)
Two pieces of PMMA Acrylic were used to sandwich the PDMS chip. This prevented the PDMS material from getting soiled with any dirt from the outside environment. Aside from that, the chip holder was intended to prevent air bubbles that can allow liquid to flow outside of the system

C. RGB Sensor Holder (refer to Appendix A. Page 17)
The RGB sensor holder positions the RGB sensor in a position above the detection well of the PDMS chip to detect fluorescein concentration with the ambient light.

D. Detection Well (refer to Appendix A. Page 14)
The main channel is the 300µm channel, which leads to the elliptical detection well with a semi-minor radius of 1.59mm and semi-major radius of 3mm.

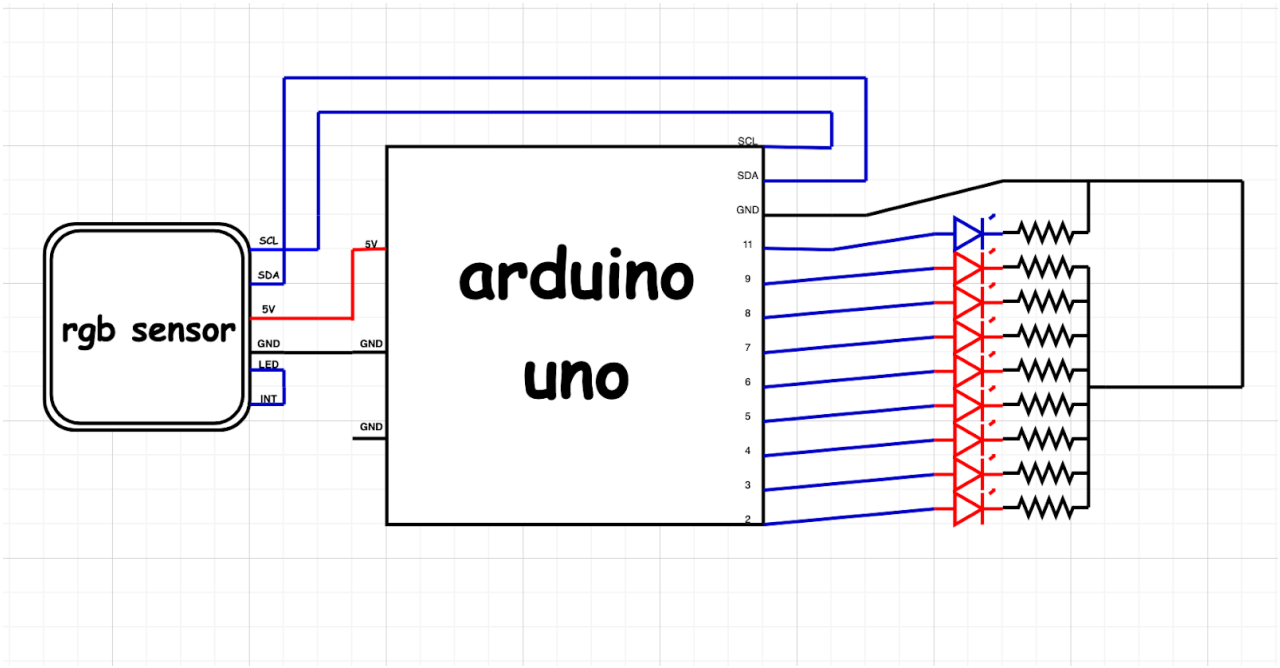
E. Darkness Chamber + Housing (refer to Appendix A. Page 18 & 19)
The darkness chamber (painted black) was 3-D printed and used to cover the RGB sensor to increase light detection for accurate results.

F. Overview/final assembly (refer to Appendix A. Page 20 & 21)
The chip is covered by the Darkness Chamber to ensure that no ambient light interferes with the detection well. The box is 8in x 8in, made out of wood, and we painted the housing and chamber black to ensure the absorption of all light. The Arduino Uno circuit is screwed on next to the darkness chamber and the

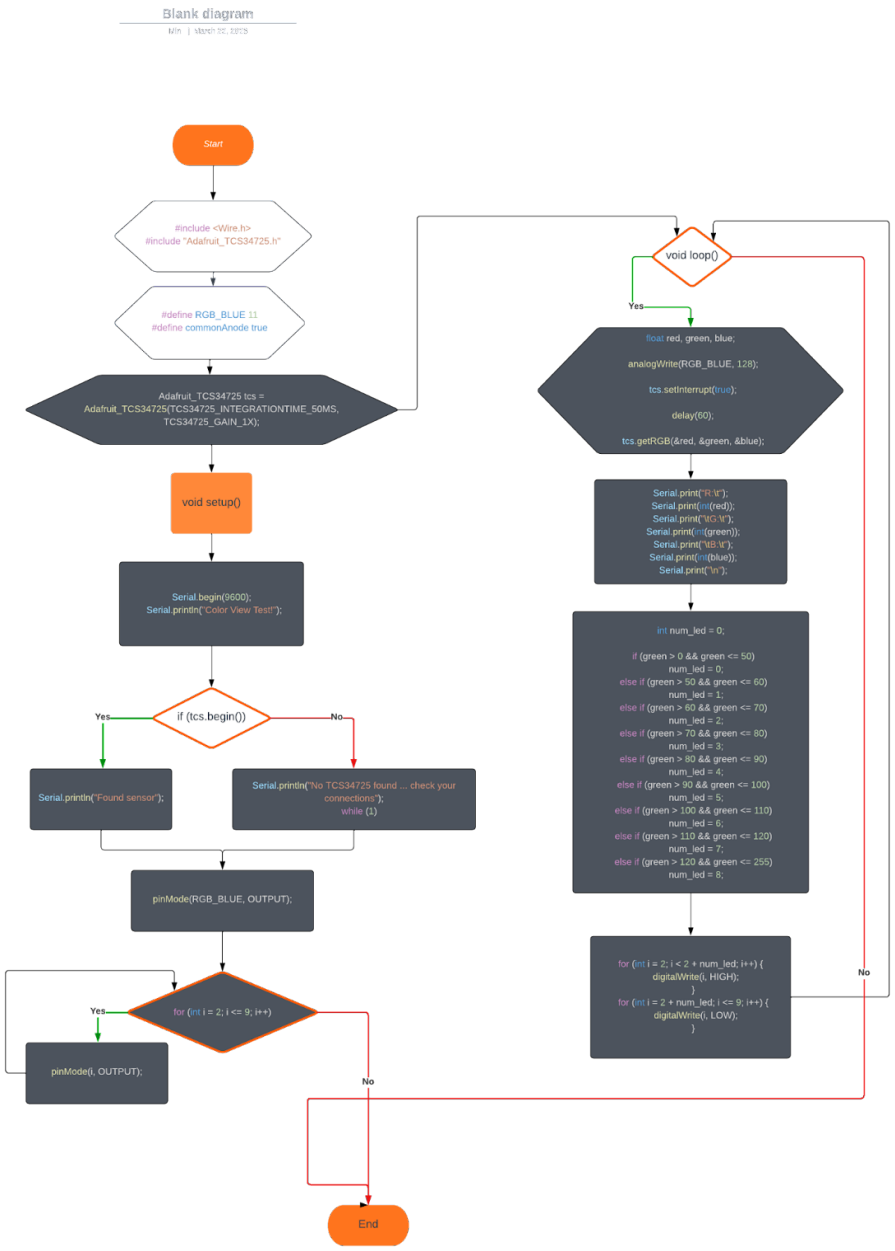
breadboard is next to it. The wiring from the Arduino circuit goes into the holes we drilled in the box and connects to the chip underneath. There are also multiple tubes connected to the chip which allows us to easily pump different concentrations of fluid into the chip for detection.

III. Wiring Diagram

Our electrical diagram contained an Arduino Uno and RGB sensor to detect the amount of fluorescein concentration in our chip. The way in which the amount of fluorescein could be detected was with how many red LEDs light up during the experiment. We had 8 red LEDs with resistors of the same resistance for all attached to them to demonstrate the amount of fluorescein solution, with one blue LED connected to the bottom of our housing platform. The blue wires signal anything not connected to ground (black) or voltage (red).



IV. Algorithm Design



- Overall very simple flowchart. Goes through initialization and into the two main methods, setup and loop. Loop iterates every 60 Hz. Setup is ran before loop.
- Code:
    - First off, the RGB sensor is initialized and turned on to detect RGB values in front of it. The light however that comes with the RGB sensor is not turned on.
    - Next, the blue LED is turned on as well. This will shine onto the fluorescein solution in order to allow for excitation. The solution will glow and will be picked up by the RGB sensor and is turned into numerical values.
    - The RGB value of green is compared to a set range that determines how many LEDS will be turned on, seen in the “for / else if” section of the code.
    - The brighter the fluorescein solution is, the more LEDS that will become activated.

- PSEUDOCODE:

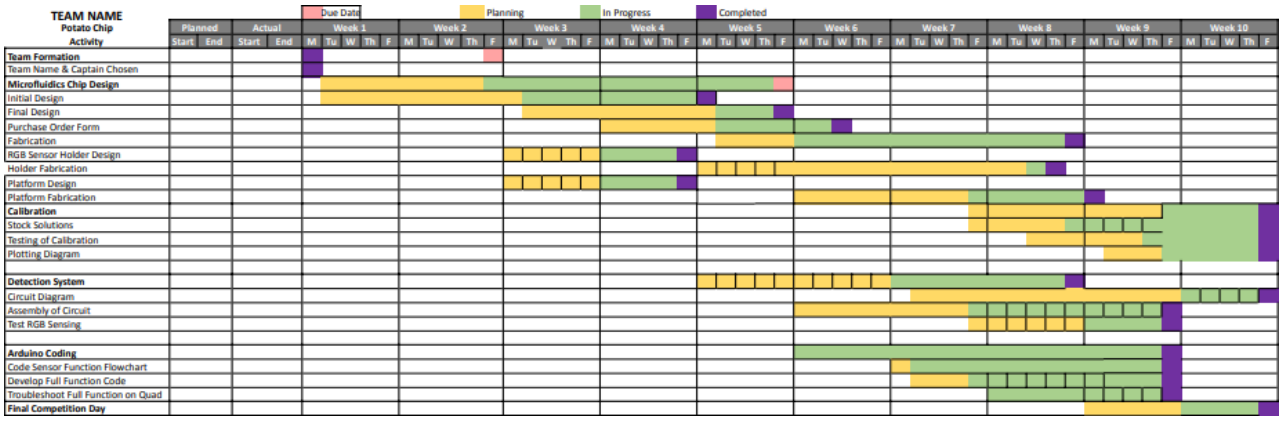
```
float g; // assume that it has already been gathered by the RGB sensor and converted into decimal

if ( g > 0 && g <= 31.875 )
{
turn on 1 LED
}


else if ( g > 31.875 && g <= 63.75 )
{
turn on 2 LEDs
}

else if ( g > 63.75 && g <= 95.625 )
{
turn on 3 LEDs
}

else if ( g > 95.625 && g <= 127.5 )
{
turn on 4 LEDs
}

else if ( g > 127.5 && g <= 159.375 )
{
turn on 5 LEDs
}

else if ( g > 159.375 && g <= 191.25 )
{
turn on 6 LEDs
}

else if ( g > 191.25 && g <= 223.125 )
{
turn on 7 LEDs
}

else if ( g > 223.125 && g <= 255 )
{
turn on all 8 LEDS
}
```

Action Item Report

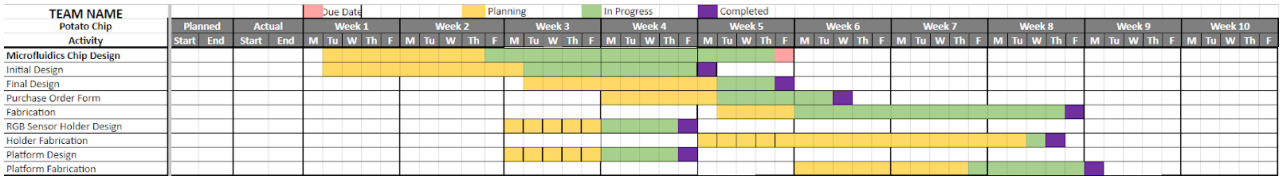
I. Task Assignments

Team Captain: Kaitlyn Nguyen
Chemical & Biomedical: Michelle Tran
Mechanical & Electrical Hardware: Emily Gao, Kaitlyn Nguyen
Electrical Software: Mharlo Borromeo, Min Kang, Ryan Luong

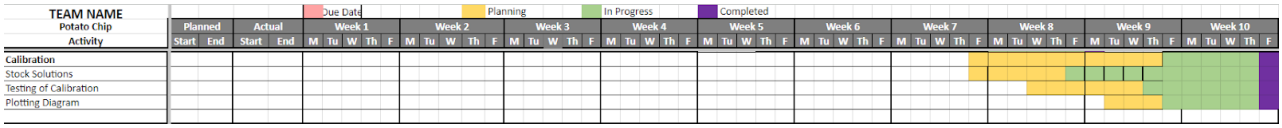
II. Gantt Chart



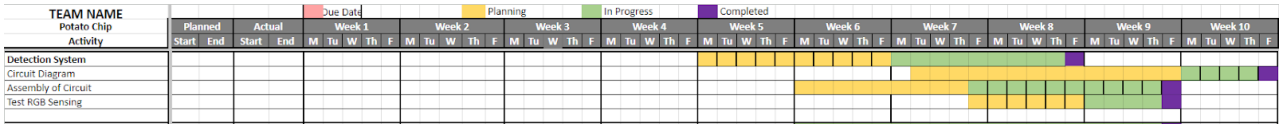
A. Microfluidic Chip Design and Fabrication



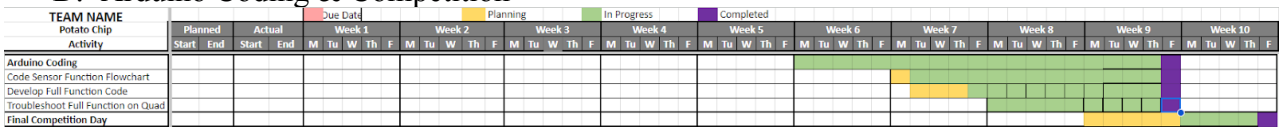
B. Calibration



C. Detection System



D. Arduino Coding & Competition



III. Timeline

Week 1:

- Formed “Team Potato Chip” and chose our group leader
- Downloaded Arduino and got familiar with it by completing coding assignments.

Week 2:

- Made subteams such as Electrical Team, Mechanical Team, Chemical Team, and

Biomedical Team.
- Familiarizing ourselves with Arduino more and the project as a whole.

Week 3:

- Learned how to clean and prepare a PDMS chip during the lab.
- Created our Action Item Report and planned our goals for the upcoming weeks

Week 4:

- Created the base design of the PDMS chip on solidworks with channels and wells
- Prepped the PDMS by cleaning, degassing, and baking it

Week 5:

- The Microfluidics Chip Design was modified with proper calculations and now has a straight channel, then finalized.
- The design for the RGB Sensor Holder as well as the Platform are modified again
- Learned 3D printing and laser cutting for the fabrication process

Week 6:

- Worked on and finished Preliminary Presentation, outlined specifics of PDMS chip and other following components.
- The design specification, calculations, as well as the budget / part list were all finalized.

Week 7:

- Flowchart for coding design and process is started and finished this week
- The housing for the chip was redesigned to take into account LED, screws and wires.
- The Business Plan was worked on, outlining the audience, purpose, and marketing
- Laser cut some components and 3D-printed the darkness chamber for fabrication, had to paint it to prevent light pollution.

Week 8:

- Actual coding process began and is mostly completed
- The circuit board design was refined to be more compact
- Further work on the business plan
- Finalized Design of housing

Week 9:

- Begin calibration process, ran into some difficulties regarding the LED and sensor
- Continued work on business plan, came up with company name
- Coding was refined

Week 10:

- Presented a finalized business plan.
- Finalized calibration
- Presented our findings with the unknown solutions

Evaluation

I. Calculations

A. Total volume (sample only, for more detail visit Appendix D. Page 27)

I. Total area of microfluidic chip:

$A_{small\ channel}$	$1.4393375mm^2 \times 4$	$5.75735mm^2$
$A_{large\ channel}$	$1.08825mm^2 \times 4$	$4.353mm^2$
$A_{capillary\ valve}$	$0.710114027mm^2 \times 4$	$2.840456108mm^2$
$A_{staging\ well}$	$12.56633544mm^2 \times 4$	$50.26798176mm^2$
$A_{detection\ well}$		$14.98539699mm^2$
$A_{waste\ well}$		$50.26548246mm^2$
A_{chip}	All areas added up together	$128.4696673mm^2$

II. Total volume of microfluidic chip:

$$V_{chip} = A_{chip} \times h_{chip}$$

$$V_{chip} = 128.4696673mm^2 \times 0.2mm$$

$$V_{chip} = 25.69393346mm^3$$

II. Calibration Curve

To prepare the 10mL solutions of 250, 500, 750, and 1000 PPM, 25.1 mg of fluorescein powder was measured with a scale. 25 mL of distilled (DI) water was collected with a microliter pipette into a 50 mL tube. The 1000 PPM solution was created by pouring the 25.1 mg of fluorescein powder into the tube with 25 mL of DI water and shaking the capped tube. This 1000 PPM solution was the stock solution used to be diluted into the remaining concentrations. Calculations of how much of the stock solution needed for each concentration are shown below and use the dilution equation $C_1V_1 = C_2V_2$. The amount of DI water to be added was found by subtracting the volume of 1000 PPM (V_2) from the desired volume (V_1), 10 mL.

Concentration (PPM)	$C_1V_1 = C_2V_2$	V_2 needed (mL)	DI water need (mL) (10 - V_2)
250	(250)(10)=(1000)(V_2)	2.5	7.5
500	(500)(10)=(1000)(V_2)	5	5
750	(750)(10)=(1000)(V_2)	7.5	2.5

C_1= desired concentration. V_1= desired volume. C_2=1000 PPM. V_2= mL of stock solution (1000 PPM).

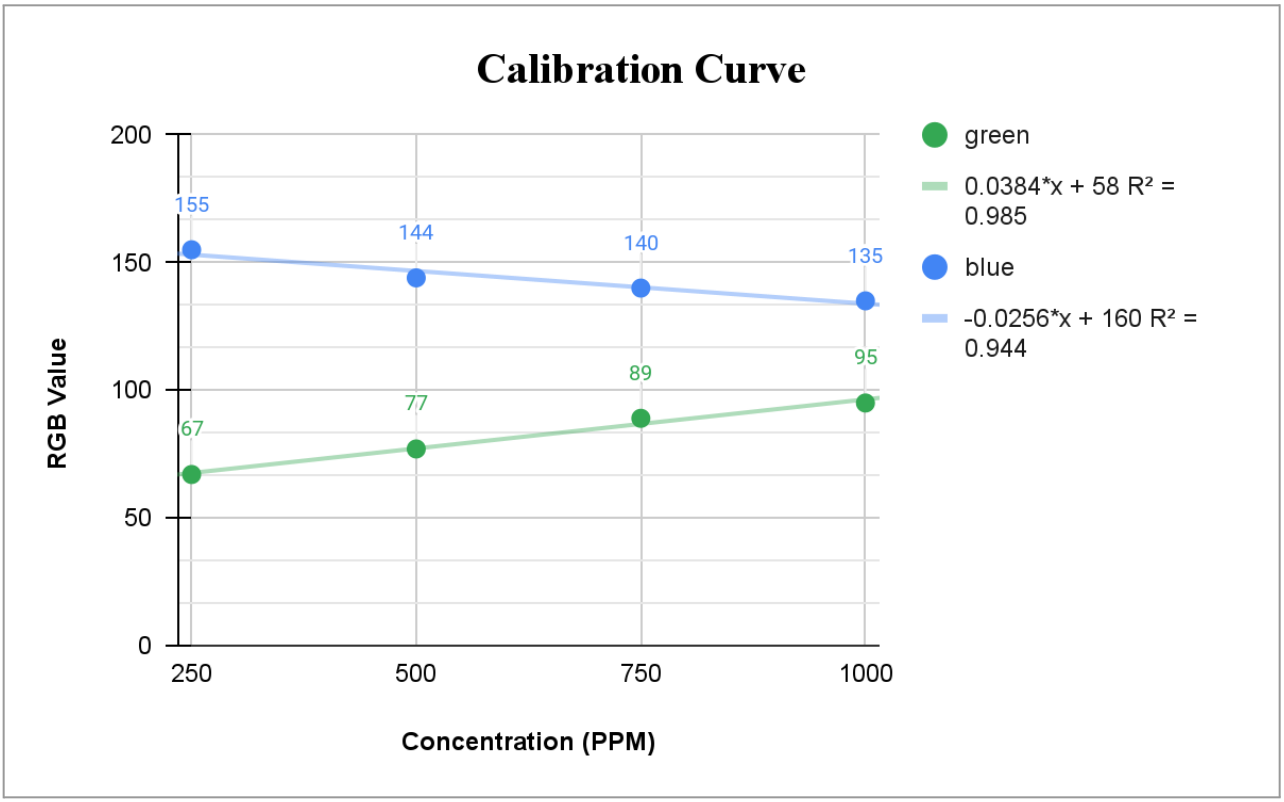
The correct volume of the stock solution needed (V_2) for the concentration was inserted into a new 50 mL tube with a microliter pipette. Its corresponding amount of DI water needed was added with a microliter. The tube was labeled with its concentration. This process was repeated for the three concentrations 250, 500, and 750 PPM. These were the solutions used for calibration.

III. Test Plan

Prior to testing, the upper and lower levels were checked for bubbles. If bubbles were seen between the two PDMS layers, the chip was disassembled to be cleaned then reassembled, making sure the wells and holes for the screws lined up. If bubbles appeared between the top PMMA and PDMS layer, pressure was applied from the top to minimize bubbles. Next, all the unused wells were blocked by inserting a tube with a syringe into those holes. This ensures the liquid flows to the least resistant path towards the detection well.

The code was run to get a general understanding of the RGB values. Distilled (DI) water was inserted with a syringe and tube to test which well had the smoothest flow to the detection well. The far right (fourth from the left) well was determined to have the smoothest flow. It is suspected that this well was best aligned on the PDMS and PMMA layer, allowing the tube to connect better than the others. It was attempted to realign the two layers, though the fourth well continued to flow the easiest.

Starting testing, some of the 250 PPM solution was collected into an empty syringe. The solution was slowly inserted into the fourth well until it filled the detection well. The chip was covered with the darkness chamber to eliminate any outside light. Green and blue RGB values were recorded. The syringe with the solution was replaced with a syringe with DI water, which was used to flush out the solution. This process was repeated with the 500, 750, and 1000 PPM solution. The chip and RGB sensor were not adjusted between calibrations to keep consistency. Between testing of different concentrations, the chip was pushed down if any bubbles were visible on the top layer. Below is our calibration curve from collected data.



The points were plotted into a spreadsheet and graphed. The linear regression line fits the data well with an R^2 value of 0.985 and 0.944 for the green and blue curve, respectively. The green regression line was $\mathbf{y_g=0.0384x + 58}$, and the blue regression line was $\mathbf{y_b=-0.0256x + 160}$. After creating the curve, the chip was ready for testing. The same procedure as the previous solutions was followed. The channel was flushed with DI water, then Solution A was inserted until it filled the detection well. The chip was covered with the darkness chamber and its green and blue values were recorded. This process was repeated for Solution B.

IV. Results & Discussion

The averages of three trials were used in the calibration equations to determine unknown concentrations. The data and results are below.

Data Collected:

	Avg. Green Value (y_g)	Avg. Blue Value (y_b)
Solution A	88.67	135.67
Solution B	114	99

Data plugged into equations. $y_g=0.0384x + 58$, $y_b=-0.0256x + 160$ to solve for x, the PPM.

Curve	Green	Blue	Average (of Green & Blue)	Actual Concentration
A (PPM)	799	951	875	300
B (PPM)	1458	2370	1914	900

Percent Error

Equation: $\frac{value\ observed - value\ expected}{value\ expected} \times 100$

Solution A: $\frac{875-300}{300} \times 100 = 191.7\%$

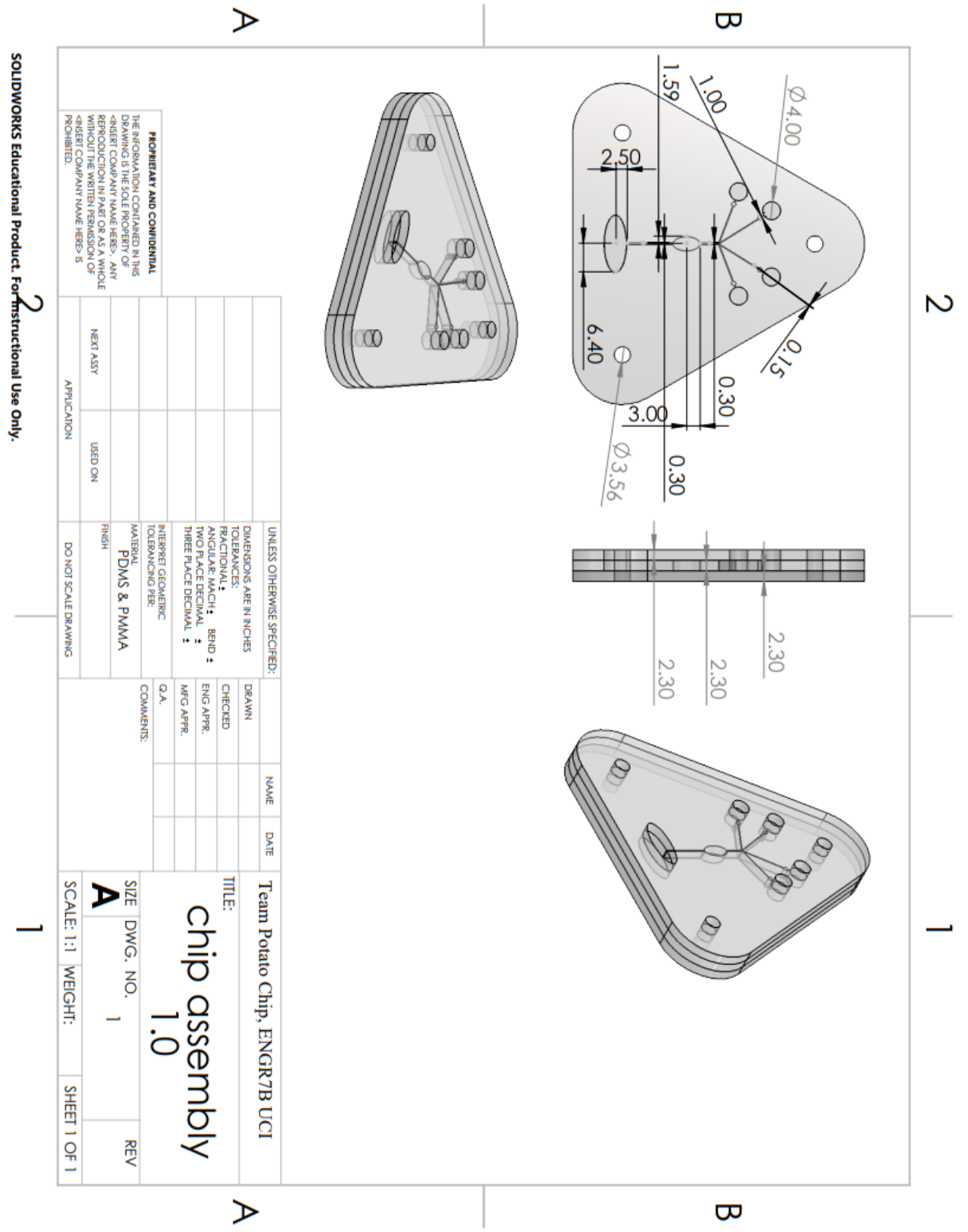
Solution B: $\frac{1914-900}{900} \times 100 = 112.7\%$

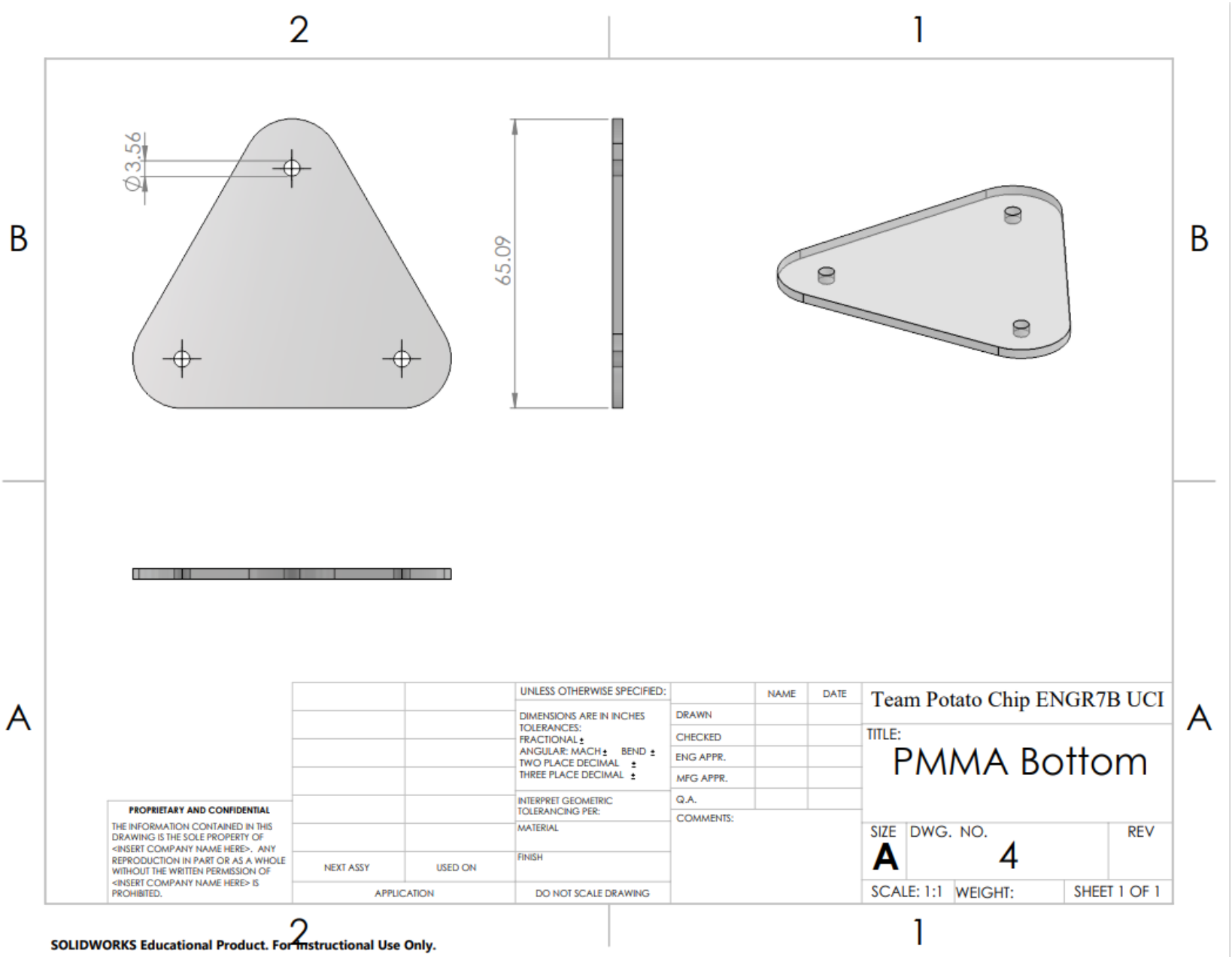
This was quite off from the actual concentrations of the solutions, which is suspected to be because of flooding between the top PMMA and PDMS layer. Despite efforts of recleaning and reassembling the chip, bubbles on the top layer remained. With limited time, the unknown concentrations A and B were tested for their green and blue values. There was no flooding during calibration, which is why that curve is consistent. It is probable that the flooding impacted recorded RGB values because the solution(s) covered a larger surface area, increasing the green ambient light. This could explain why the recorded green values are high. Also, the DI water may have mixed with the solution(s) and diluted it. The potentially diluted solution covered the actual solution in the detection well. Therefore, the sensor was not able to detect the true concentration.

Some lessons learned were to double check everything, and do extra trials if time allows to catch any mistakes such as using the wrong solution. Problem solving and perseverance was also important for this project.

Potential changes considered are to use a material that would adhere better to eliminate bubbles and ensure all liquid goes through the channels to the detection well. Also, the chip would be redesigned to have a smaller ratio between the well and channel width. Possible solutions could include having a smaller detection well with the current values, or have a larger channel width for the smaller channels. This would ensure that the ratio was even and ensure that the pressure applied would not cause overflow, and the liquid would flow smoothly through the channels.

Appendix A: SOLIDWORKS Drawings & Structure

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Team Potato Chip ENGR7B UCI
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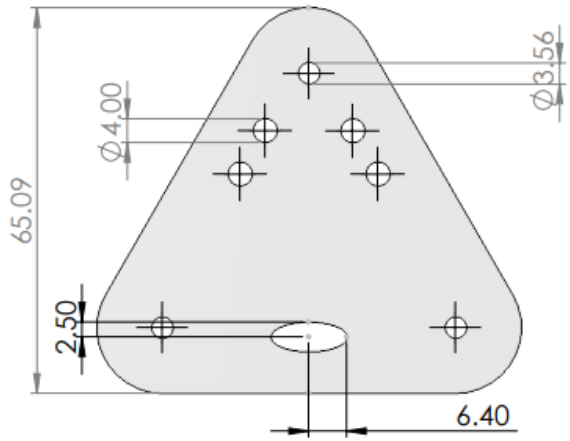

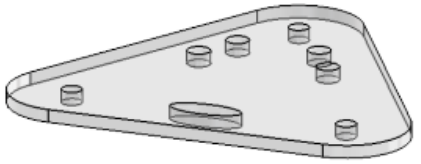

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Team Potato Chip ENGR7B UCI

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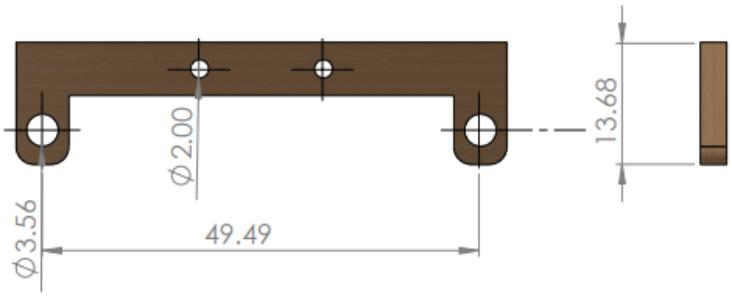

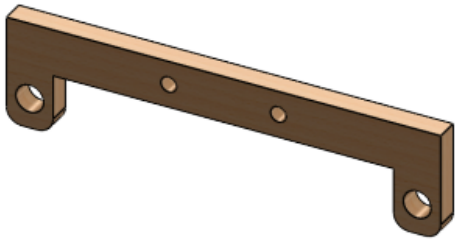

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Team Potato Chip ENGR7B UCI

TITLE:

RGB Sensor Holder

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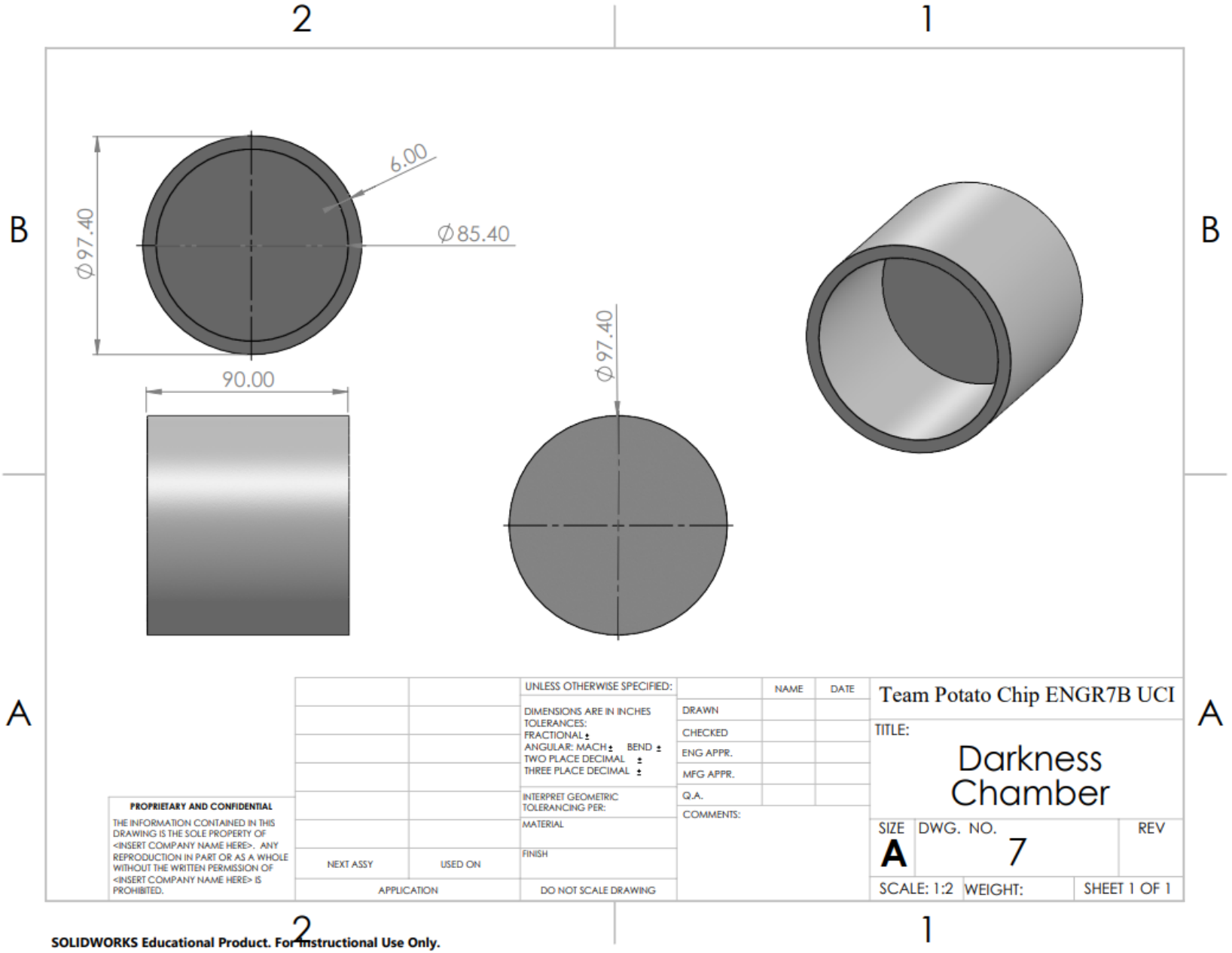
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Darkness Chamber
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SHEET 1 OF 1
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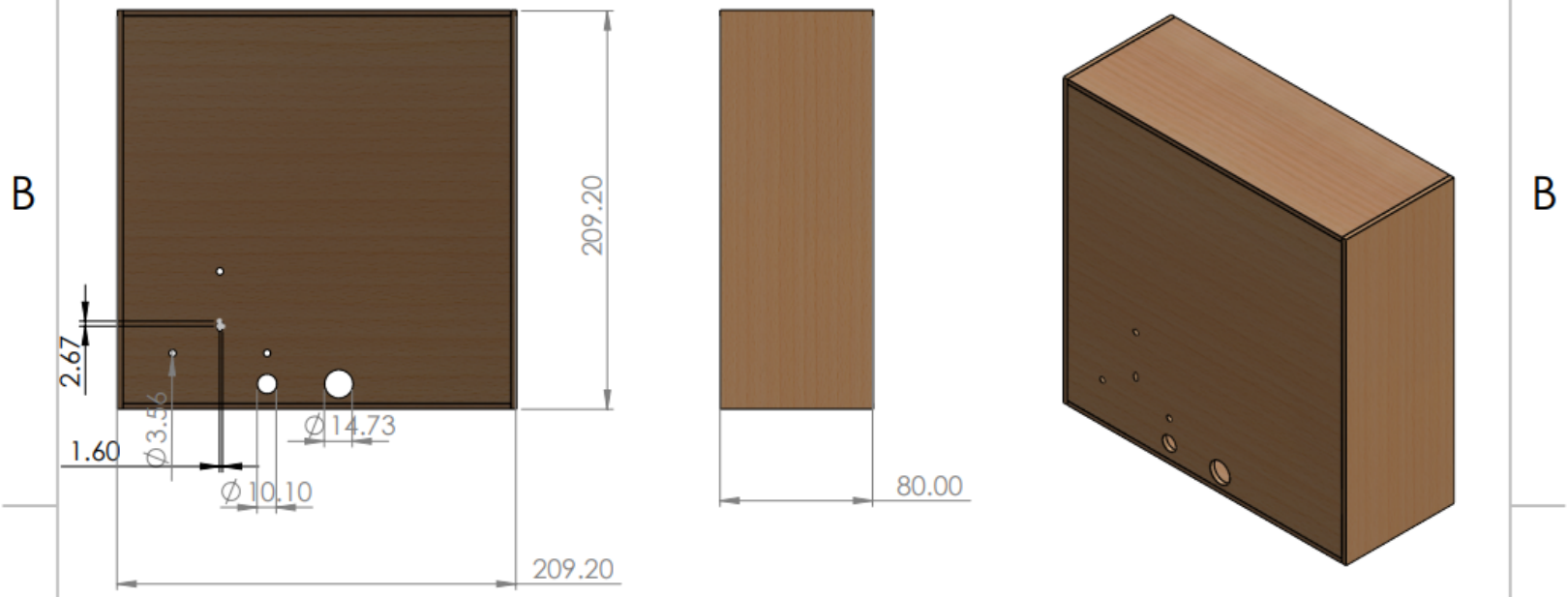


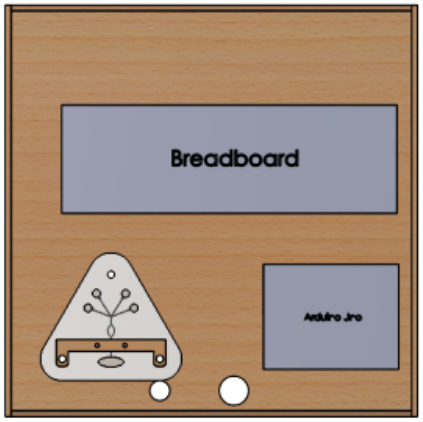
		UNLESS OTHERWISE SPECIFIED:		NAME	DATE
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN		
			CHECKED		
			ENG APPR.		
			MFG APPR.		
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.		
		MATERIAL Italian Poplar Wood	COMMENTS:		
NEXT ASSY	USED ON	FINISH			
APPLICATION		DO NOT SCALE DRAWING			

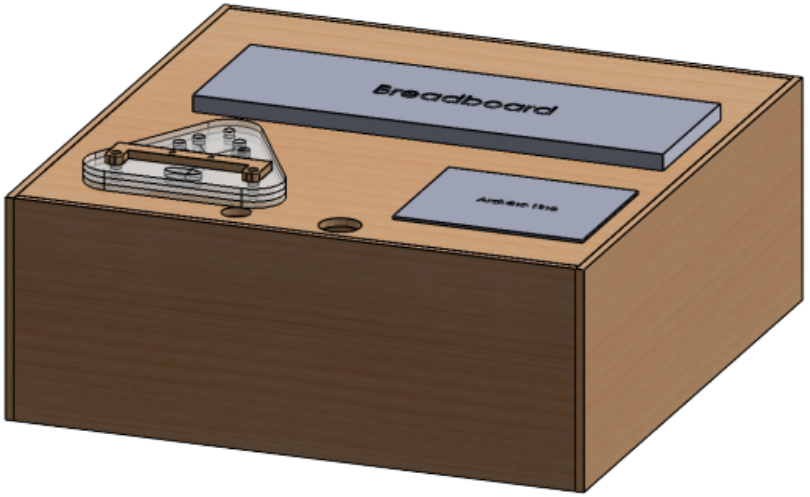
Team Potato Chip ENGR7B UCI

TITLE:

System Housing 1.0

SIZE	DWG. NO.	REV
A	2	
SCALE: 1:5	WEIGHT:	SHEET 1 OF 1





		UNLESS OTHERWISE SPECIFIED:		NAME	DATE
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN		
			CHECKED		
			ENG APPR.		
			MFG APPR.		
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.		
		MATERIAL	COMMENTS:		
NEXT ASSY	USED ON	FINISH			
APPLICATION		DO NOT SCALE DRAWING			

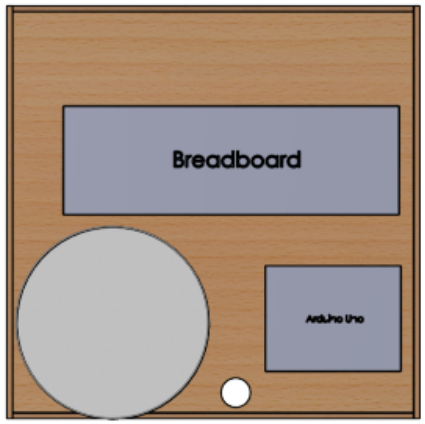
Team Potato Chip ENGR7B UCI

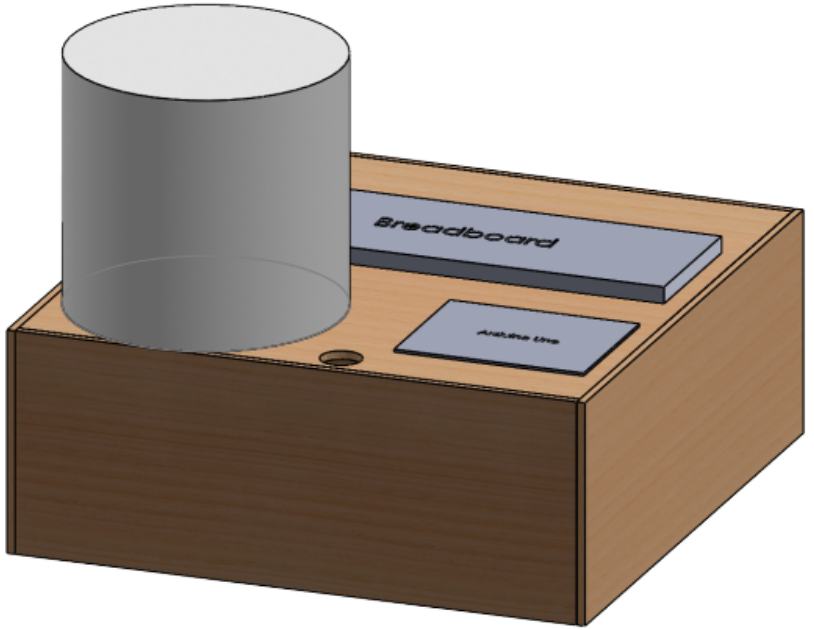
TITLE:

System Assembly w/o Darkness Chamber

SIZE	DWG. NO.	REV
A	6	

SCALE: 1:5 | WEIGHT: | SHEET 1 OF 1





		UNLESS OTHERWISE SPECIFIED:		NAME	DATE
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN		
			CHECKED		
			ENG APPR.		
			MFG APPR.		
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.		
		MATERIAL	COMMENTS:		
NEXT ASSY	USED ON	FINISH			
APPLICATION		DO NOT SCALE DRAWING			

Team Potato Chip ENGR7B UCI

TITLE:

System assembly

SIZE	DWG. NO.	REV
A	5	

SCALE: 1:5 | WEIGHT: | SHEET 1 OF 1

Appendix B: Bill of Materials

PURCHASE ORDER FORM

The Henry Samueli School of Engineering

One PO per team. Parts will be given to you after submitting the PO.

Requested By:*	Team Potato Chip	*Captain:	Kaitlyn Khanhlam Nguyen	*E-Mail:	kaitlkn2@uci.edu
Delivery Address:*	Engineering Tower (*Building Name for Abbreviation)		ET 409 (*Lab Room #)	Date of Request:	02/15/23
Lab Instructor Name:*	Lily Wu	Lab Section:*	Friday 11:00 AM - 12:50 PM		
Account Name:*	ENGR 7B				

Account*	Fund*	Sub	Project	% (If split funding)	Amount	Accounting Review
ENGR 7B	56123	03	7B	100%	$302.28	
				0%	$0.00	

Quantity*	Unit	Company*	Item Description*	Catalog #*	Unit Price*	Estimated Extended Price*
1		Sygiard	PDMS kit (base + curing agent)	184	$0.40	$16.00
2		Robbins Instruments	4mm Hole Punchers	B007TVKSPU	$1.60	$3.20
1		AZM Displays	PMMA (Acrylic)	B07HDYRTQY	$12.99	$12.99
1		Sigma-Aldrich	Fluorescein sodium salt	F6377	$0.41	$0.41
5		BD Oral	Syringes	B001BBSQQC	$0.49	$2.45
6		Jaquard	Tapered tip syringe	B0027A3H4M	$4.69	$28.14
2 ft		siny	Tubing 5/32" ID x 7/32" OD per foot	B00JQFAQ3Q	$0.52	$1.04
7		QTEATAK	plastic screws	B0B17JHRKV	$0.02	$0.14
1		sparkfun	Arduino	DEV-11021	$27.95	$27.95
1		sparkfun	breadboard	PRT-12002	$5.50	$5.50
8		sparkfun	LED (red)	COM-09590	$0.45	$3.60
2		chanzon	LED (blue)	B01AUI4VYW	$0.07	$0.14
9		sparkfun	resistors	COM-08377	$0.30	$2.70
1		adafruit	RGB Sensor	TCS34725	$7.95	$7.95
24		sparkfun	Wires (jumper cables)	PRT-13870	$0.65	$15.60
1		Clean room	Equipment cost (spinner)		$10.00	$10.00
2		Clean room	Clean room user cost		$38.00	$76.00
1		Clean room	Silicon Wafer		$23.00	$23.00
8.5		3D Printing	Darkness Chamber		$4.00	$34.00
0.1733		Laser Cutting	RGB Sensor Holder + Top Platform		$8.00	$1.39
3			Italian Poplar 16" x 16"		$2.78	$8.34
					Subtotal	$280.54
I Need My Order Delivered By This Date:*		02/25/23				
					*Tax rate: 7.75%	$21.74
					TOTAL ORDER PRICE	$302.28

Appendix C: Arduino Code

```
#include <Wire.h>               // sensor communication
#include "Adafruit_TCS34725.h"  // RGB sensor library


#define RGB_BLUE 11
#define commonAnode true


Adafruit_TCS34725 tcs = Adafruit_TCS34725(TCS34725_INTEGRATIONTIME_50MS,
TCS34725_GAIN_1X);

void setup() {
  // Serial monitor setup
  Serial.begin(9600);
  Serial.println("Color View Test!");

  if (tcs.begin()) {
    Serial.println("Found sensor");
  } else {
    Serial.println("No TCS34725 found ... check your connections");
    while (1)
      ;
  }

  pinMode(RGB_BLUE, OUTPUT);

  for (int i = 2; i <= 9; i++) {
    pinMode(i, OUTPUT);
  }

}

void loop() {
  float red, green, blue;

  analogWrite(RGB_BLUE, 128);

  tcs.setInterrupt(true);

  delay(60);

  tcs.getRGB(&red, &green, &blue);

  Serial.print("R:\t");
```

```
  Serial.print(int(red));
  Serial.print("\tG:\t");
  Serial.print(int(green));
  Serial.print("\tB:\t");
  Serial.print(int(blue));
  Serial.print("\n");


  int num_led = 0;

  if (green > 0 && green <= 28.33)
    num_led = 0;
  else if (green > 28.33 && green <= 56.66)
    num_led = 1;
  else if (green > 56.55 && green <= 84.99)
    num_led = 2;
  else if (green > 84.99 && green <= 113.32)
    num_led = 3;
  else if (green > 113.32 && green <= 141.65)
    num_led = 4;
  else if (green > 141.65 && green <= 169.98)
    num_led = 5;
  else if (green > 169.98 && green <= 198.31)
    num_led = 6;
  else if (green > 198.31 && green <= 226.64)
    num_led = 7;
  else if (green > 226.64 && green <= 255)
    num_led = 8;

  for (int i = 2; i < 2 + num_led; i++) {
    digitalWrite(i, HIGH);
  }
  for (int i = 2 + num_led; i <= 9; i++) {
    digitalWrite(i, LOW);
  }
}
```

TeamPotatoChipCode2.ino

```ino
#include <Wire.h>               // sensor communication
#include "Adafruit_TCS34725.h"  // RGB sensor library


#define RGB_BLUE 11
#define commonAnode true


Adafruit_TCS34725 tcs = Adafruit_TCS34725(TCS34725_INTEGRATIONTIME_50MS, TCS34725_GAIN_1X);

void setup() {
  // Serial monitor setup
  Serial.begin(9600);
  Serial.println("Color View Test!");

  if (tcs.begin()) {
    Serial.println("Found sensor");
  } else {
    Serial.println("No TCS34725 found ... check your connections");
    while (1)
      ;
  }

  pinMode(RGB_BLUE, OUTPUT);

  for (int i = 2; i <= 9; i++) {
    pinMode(i, OUTPUT);
  }

}
void loop() {
  float red, green, blue;

  analogWrite(RGB_BLUE, 128);

  tcs.setInterrupt(true);

  delay(60);

  tcs.getRGB(&red, &green, &blue);

  Serial.print("R:\t");
  Serial.print(int(red));
  Serial.print("\tG:\t");
  Serial.print(int(green));
  Serial.print("\tB:\t");
  Serial.print(int(blue));
  Serial.print("\n");

  int num_led = 0;

  if (green > 0 && green <= 50)
    num_led = 0;
  else if (green > 50 && green <= 60)
    num_led = 1;
  else if (green > 60 && green <= 70)
    num_led = 2;
  else if (green > 70 && green <= 80)
    num_led = 3;
  else if (green > 80 && green <= 90)
    num_led = 4;
  else if (green > 90 && green <= 100)
    num_led = 5;
  else if (green > 100 && green <= 110)
    num_led = 6;
  else if (green > 110 && green <= 120)
    num_led = 7;
  else if (green > 120 && green <= 255)
    num_led = 8;

  for (int i = 2; i < 2 + num_led; i++) {
    digitalWrite(i, HIGH);
  }
  for (int i = 2 + num_led; i <= 9; i++) {
    digitalWrite(i, LOW);
  }
}
```

Appendix D: Volume Calculations

Volume calculations

1. Staging wells

$d = 4.00\ mm$

$r = 2.00\ mm$

$A_{circle} = \pi r^2$

$A_{circle} = \pi\,(2.00\ mm)^2$

$A_{circle} = 12.5664\ mm^2$

$b = 0.15\ mm$

$r = 2.00 mm$

$\sin\theta = \frac{0.075\ mm}{2\ mm}$

$\sin^{-1}\left(\frac{0.075\ mm}{2\ mm}\right) = 2.15°$

$r^2 = \left(\frac{b}{2}\right)^2 + h^2$

$4\ mm^2 = 0.005625\ mm^2 + h^2$

$h = 1.9986\ mm$

$A_{triangle} = \frac{1}{2}bh$

$A_{triangle} = \frac{1}{2}(0.15\ mm)(1.9986\ mm)$

$A_{triangle} = 0.1498\ mm^2$

$\theta' = 2\theta = 4.30°$

$r = 2.00\ m$

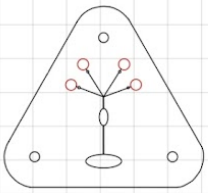
$A_{sector} = \frac{\theta'}{360°}(\pi r^2)$

$A_{sector} = \frac{4.30°}{360°}(\pi(2.00\ mm)^2)$

$A_{sector} = 0.1500963157\ mm^2$

$A_{sector} - A_{triangle} = 0.1501\ mm^2 - 0.1498\ mm^2$

$A_{sector}' = 0.0012\ mm^2$



$A_{staging} = A_{circle} - A_{sector}'$

$A_{staging} = 12.5664\ mm^2 - 0.0012\ mm^2$

$A_{staging} = 12.5651\ mm^2$

$V_{staging} = 4\,[h \cdot A_{staging}]$

$V_{staging} = 4\,[0.2\ mm \cdot 12.5651\ mm^2]$

$V_{staging} = 10.0521\ mm^3$

2. Capillary valves

$d = 1.00\ mm$

$r = 0.5\ mm$

$A_{circle} = \pi r^2 = \pi\,(0.5\ mm)^2$

$A_{circle} = 0.7854\ mm^2$

$r = 0.5\ mm$

$b = 0.15\ mm$

$h = \sqrt{r^2 - \left(\frac{b}{2}\right)^2} = \sqrt{(0.25) - (0.005625)}$

$h = 0.4943\ mm$

$A_{triangle} = \frac{1}{2}bh = \frac{1}{2}(0.4943\ mm)(0.15mm)$

$A_{triangle} = 0.0371\ mm^2$

$\sin\theta = \frac{0.075\ mm}{0.5mm}$

$\theta = 8.6269°$

$\theta' = 2\theta = 17.2539°$

$A_{sector} = \frac{\theta'}{360}\pi r^2$

$A_{sector} = \frac{17.2539°}{360°}\pi(0.5\ mm)^2$

$A_{sector} = 0.0376\ mm^2$

$A_{sector}' = A_{sector} - A_{triangle}$

$A_{sector}' = 5 \times 10^{-4}\ mm^2$

$A_{cv} = A_{circle} - 2A_{sector}'$

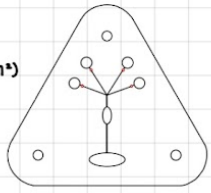
$A_{cv} = 0.7854\ mm^2 - 2(5 \times 10^{-4}\ mm^2)$

$A_{cv} = 0.7844\ mm^2$

$V_{cv} = 4\,[h \cdot A_{cv}]$

$V_{cv} = 4\,[0.2mm \cdot 0.7844\ mm^2]$

$V_{cv} = 0.62752\ mm^3$



3. Detection Well

Note: Area of sector with rectangle was negligible so I eluded it, it is still accurate to 4 decimal places

semi-major r: 3 mm

semi-minor r: 1.59 mm

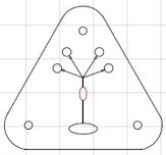
$A_{ellipse} = \pi ab$

$A_{ellipse} = \pi(1.59\ mm)(3mm) = 14.9854\ mm^2$

$V_{det} = A_{ellipse} \cdot h$

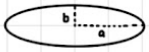
$V_{det} = 14.9854\ mm^2 \cdot 0.2mm$

$V_{det} = 2.9971 mm^3$



4. Outlet Well

Note: Area of sector with rectangle was negligible so I eluded it, it is still accurate to 4 decimal places



semi-major radius = 6.4 mm

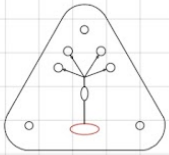
Semi-minor radius = 2.5 mm

$A_{ellipse} = \pi ab = \pi(6.4mm)(2.5mm) = 50.2655mm^2$

$V_{det} = h \cdot A_{ellipse}$

$V_{det} = 0.2\ mm \cdot 50.2655\ mm^2$

$V_{det} = 10.0531\ mm^3$



5. Small Channel

$C_1 = (\ell_1 \times \omega)\,4$

$C_1 = (0.51\ m \times 0.15\ m)\,4$

$C_1 = 0.306\ mm^2$

$C_2 = \frac{a+b}{2}h$

$C_2 = \frac{6.74\ mm + 8.83mm}{2}(0.15\ mm)$

$C_2 = 1.1778\ mm^2$

$C_3 = \frac{a+b}{2}h$

$C_3 = \frac{10.31mm + 10.20mm}{2}(0.15\ mm)$

$C_3 = 1.5383\ mm^2$

$C_4 = \frac{a+b}{2}h$

$C_4 = \frac{10.25\ mm + 10.15mm}{2}(0.15\ mm)$

$C_4 = 1.53\ mm^2$

$t_1 = \frac{a+b}{2}h$

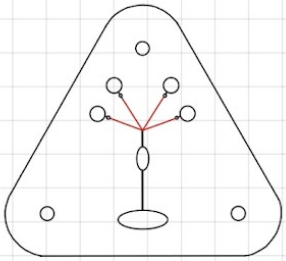
$t_1 = \frac{0.37\ mm + 0.3mm}{2}(0.16mm)$

$t_1 = 0.2696\ mm^2$

$V_{sc} = h\,(C_1 + 2C_2 + C_3 + C_4 + t_1)$

$V_{sc} = 0.2\ mm\,(0.306\ mm^2 + 2(1.1778\ mm^2) + 1.5383\ mm^2 + 1.53\ mm^2 + 0.2696 mm^2)$

$V_{sc} = 1.2559\ mm^3$

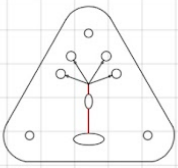


6. Large Channel

$C_{L1} = \ell_1 \times \omega$ $\quad C_{L2} = \ell_2 \times \omega$ $\quad V_{Lc} = h(C_{L1} + C_{L2})$

$C_{L1} = 4.13\ mm \times 0.30\ mm$ $\quad C_{L2} = 10.38\ mm \times 0.30\ mm$ $\quad V_{Lc} = 0.2\ mm\,(1.239\ mm^2 + 3.114\ mm^2)$

$C_{L1} = 1.239\ mm^2$ $\quad C_{L2} = 3.114\ mm^2$ $\quad V_{Lc} = 0.8706\ mm^3$

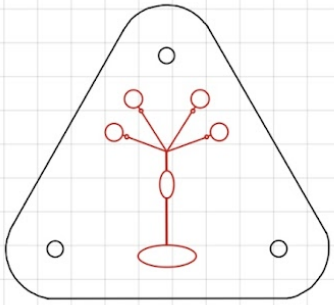


7. Whole System

$V_{total} = V_1 + V_2 + V_3 + V_4 + V_5 + V_6$

$V_{total} = 10.0521\ mm^3 + 0.62752\ mm^3 + 2.9971\ mm^3 + 10.0531\ mm^3 + 1.2559\ mm^3 + 0.8706\ mm^3$

$V_{total} = 25.8563\ mm^3$



Appendix E: References

References

Castillo-León Jaime, and Winnie E. Svendsen. “Chapter 1 Microfluidics and Lab-on-a-Chip Devices: History and Challenges.” *Lab-on-a-Chip Devices and Micro-Total Analysis Systems a Practical Guide*, Springer International Publishing, Cham, 2015.