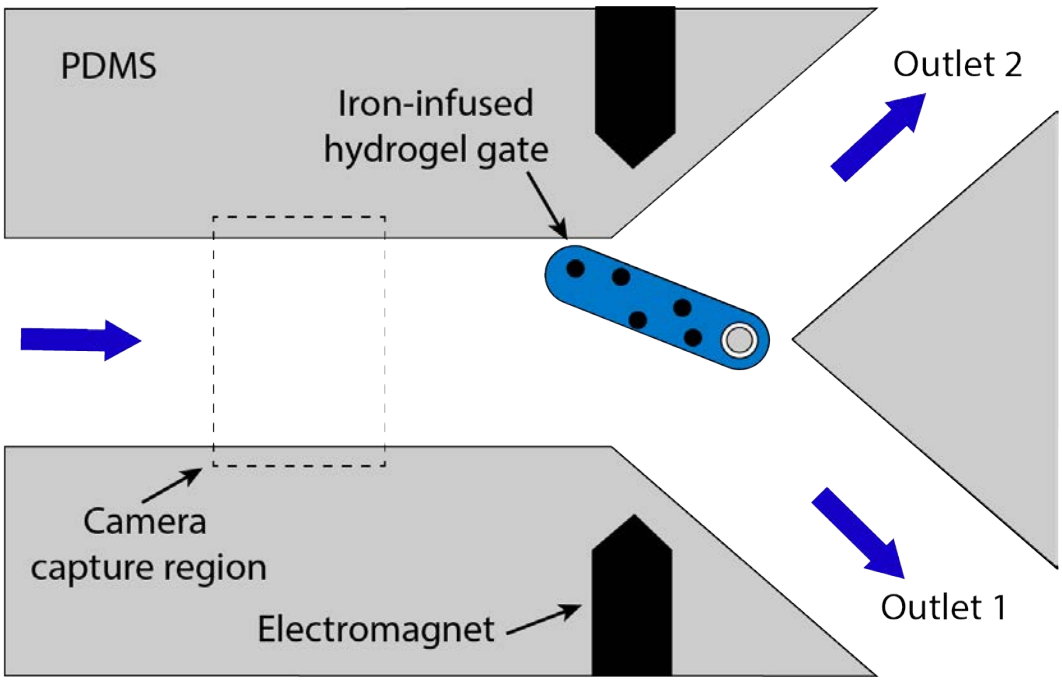
PennState

Harvesting Cells from Biological Fluids Final Report

December 18, 2019



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No - Non-Disclosure Agreement

Executive Summary

The goal of this project is to sort arbitrary cells in some arbitrary microfluid. In this paper, we will cover existing products on the market, why those products are not enough for our needs, background information about the challenges of cell sorting, our design goals, and our proposed solution.

Our sponsor needs a device that sorts cells in a biological fluid so that they can more easily analyze the contents of the solution. Many current diagnostic tests depend on fractionated blood components: plasma, red blood cells, white blood cells, and platelets. Finding an efficient, reliable, and affordable way to separate cells without the use of fluorescence or cell tagging can help retrieve information that can be used to treat and diagnose patients. As more advanced technology and resources become more available, cell separation methods have started to use machine learning based image recognition to control the device. Using a machine learning based solution allows us to retrain the software for many different types of cells and fluids. This allows us to re-purpose the same channel design for many different types of fluids. We have been tasked to build an affordable, no-tag device, made in-house, that can be programmed to sort different sized particles. As a proof of concept, we will build a device that can sort different sized microbeads in DI water.

The designed cell sorting devices consists of a polydimethylsiloxane (PDMS) channel containing a hydrogel solution gate infused with iron that is controlled using electromagnets. The PDMS channel is attached on top of a PDMS covered glass slide and contains a small pillar at the bifurcation to act as a hinge for the gate. The hydrogel solution was created by mixing 6% 2-hydroxy-2-methylpropiophenone photo initiator in poly(ethylene glycol) diacrylate oligomer by volume and roughly 0.2 grams of iron powder. The PDMS channel was filled with the solution and a lithography mask for the gate was positioned at the PDMS hinge pillar. UV light is flashed on the mask for ~1 second and the remaining solution is rinsed out, leaving the gate on the hinge.

In order to identify arbitrary kind of cells, a Deep Learning and Convolutional Neural Network based solution was chosen because of its flexibility. TensorFlow was chosen as our development platform due to its performance, ease of use, and developer support. Before training our network, a set of image preprocessing algorithms was developed to better identify edges, shapes, and the areas of the cells. With help of the OpenCV library, we implemented several algorithms to preprocess the time-sequenced images. We then used those images to train our network in order to recognize microbeads of different sizes, shapes, and fluorescence properties.

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1.0 Introduction

Microfluidic cell sorters have been around since the 1960s. Since then, there have been many methods used to separate different kinds of cells from biological fluids. Most of these methods can fall under two categories: tagged and non-tagged. Some cell sorting methods involve biologically tagging magnetic materials or fluorescent activators to cells of interest, while others find different ways to separate target cells. The following are some examples of non-tag cell sorting methods:

- Using flow to push cells into different channels
- Creating geometry to interact with fluid flow to push differently sized cells to different channels
- Targeting specific cells with optical and acoustic rays

As advanced technology becomes more available, image recognition software is being used more and more to control these cell sorting devices. Image recognition software allows us to build a reconfigurable cell sorting device. This is because we can reconfigure the software to recognize different types of cells in different fluids.

Important information can be obtained from cells in a biological fluid. Many current diagnostic tests depend on fractionated blood components: plasma, red blood cells, white blood cells, and platelets. Rare cells may also be found in blood. For example, circulating tumor cells (CTCs) may be useful for stratifying cancer patients and following up on a course of treatment noninvasively (Label-free cell separation and sorting in microfluidic systems). Finding an efficient, reliable, and affordable way to separate cells can help retrieve information that can be used for treatment and diagnostics of patients.

1.1 Initial Problem Statement

We propose to develop an intelligent microfluidic cell sorter that will harvest cells suspended in biological fluids. This tool can be potentially used as a diagnostic, or even as a therapeutic device. The instrument will be composed of a microfluidic channel that branches off into two sub channels. A gate will be placed at the bifurcation to control which channel the particles go into. A microscope camera will take pictures of the microfluid and feed it into our image recognition software. The software will then move the gate, thus deflecting the particle into the proper channel. The long-term goal is to make a device that can select specific types of cells, such as cancerous or virus-infected cells, making it a versatile tool for tackling problems involving different biological fluids. For this semester long project, this device will be able to select different sized plastic particles and sort them to two different channels.

1.2 Objectives

We expect our design to be able to identify different sized particles using machine learning based image recognition. We should then be able to use the software to deflect the particle into the proper channel. In order to effectively sort the particles, they must be diluted so that our software only needs to identify one at a time. The speed of the image processing will determine the speed of the input flow rate.

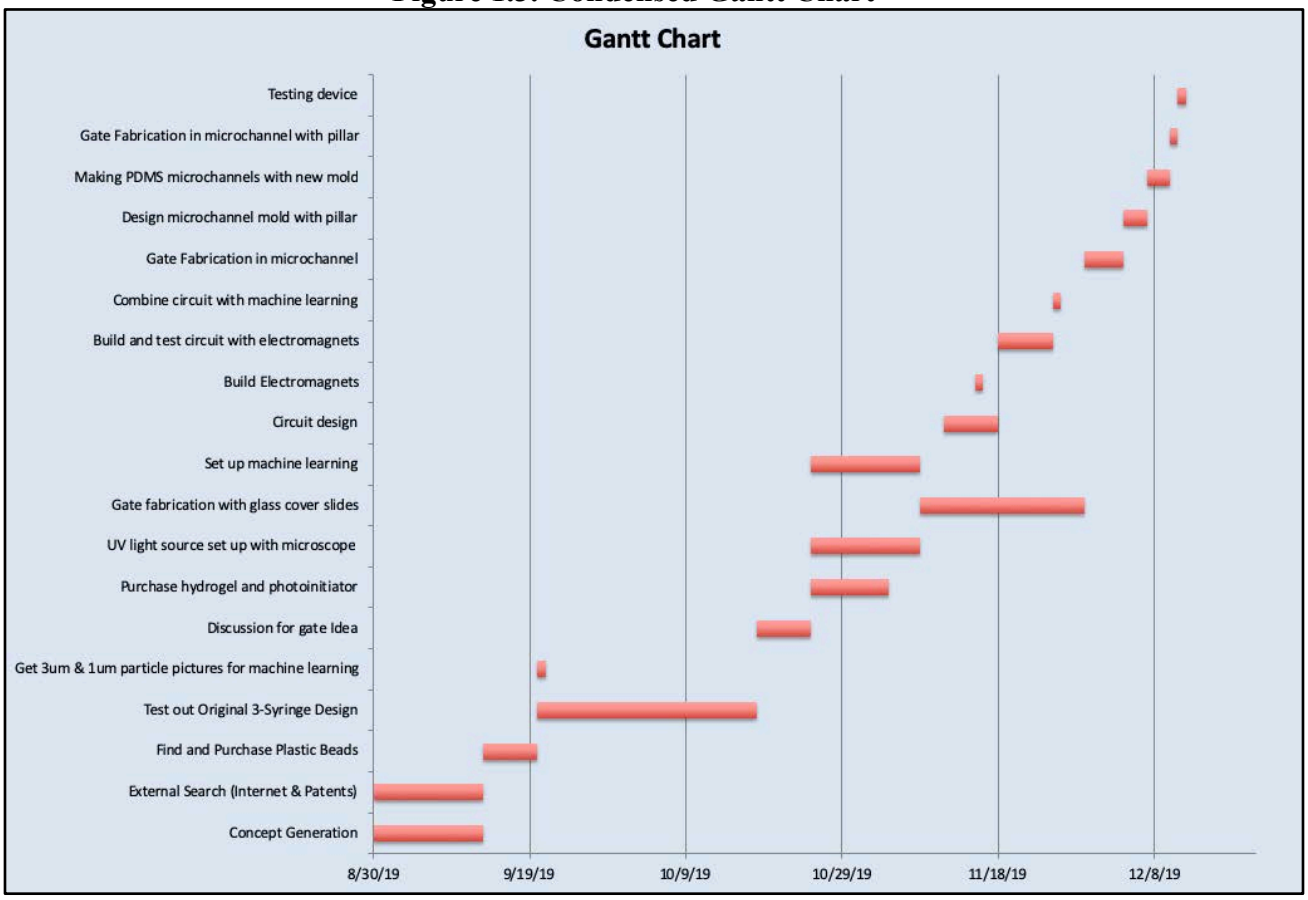
There are several constraints and assumptions for this project. We are limited to a $1,000 budget, so the of cost materials must be carefully considered. The lab that the device will be built only has one specific type of syringe pump. There are only three syringes that we can use to control the flow in the microchannel. Mechanically, we cannot make the channels in the device small enough to funnel the cells in a single file. That is because we assume that a passageway of that size will easily clog and stop the fluid flow. For the Machine Learning program, we do not have access to large-scale training sets and because of the arbitrary nature of the problem, it is difficult to build a system that fits for all types of biological cells while still maintaining a high accuracy.

1.3 Project Management

Our team consists of 5 students with various academic backgrounds, including computer science, computer engineering, biomedical engineering, and mechanical engineering. The computer science students will work on the machine learning based image recognition that will recognize the particles to be sorted. With the help of the computer engineering student, they will build a circuit that communicates with the image recognition software and controls the gate. The biomedical and mechanical engineering students will focus on the design and fabrication of the cell sorting device, including the microchannel, hydrogel gate, and a device that connects the microchannel and electromagnets.

In order to keep everyone updated and organize collaboration, a group chat is made where all communications are made. We also have a shared Box folder to collaborate on documents and reports. *Figure 1.3* shows a condensed Gantt chart that summarizes all the tasks and to keep a clear timeline, making sure proper progress is made toward the final goal. Refer to *Appendix A* for the full Gantt chart.

Figure 1.3. Condensed Gantt Chart



1.4 Communication and Coordination with Sponsor

Our communication from our sponsor consists of emails, lab visits, and periodic meetings to discuss designs and processes that we have researched online. During research stages, emails have been exchanged between students and the sponsor to establish the sponsor's expectation about the device.

Group meetings were held biweekly with our sponsor to discuss the progress of the project, to brainstorm ideas, and to discuss plans for the next two weeks. During the meetings, we discuss design

possibilities, research on cell sorting methods, findings and takeaways during the project, and future plans. Throughout the semester we worked closely and frequently with our client in and out of the lab.

2.0 External Search

There are many developed methods for cell sorting devices, and a lot of research has already been done on microfluidic devices. Our team researched different cell sorting methods that would potentially fit the sponsor's needs. There are techniques that involve tagging cells, but this project requires a non-tagging method.

One method that is commonly used is acoustophoresis. This method uses acoustic pressure waves to move cells depending on the size or density of cells. Different wavelengths and mediums can be used to control the amount displacement of the cells. Waves consist of nodes (points of minimum wave displacement) and antinodes (points of maximum wave displacement). These acoustic wave separates different cells towards pressure nodes or antinodes depending on their reaction to radiation forces. Like any method, there are pros and cons to using acoustophoresis. Using acoustic waves are less invasive for cells and it has a good success rate with sorting cells. It is also able to capture cells with a relatively high flow rate (roughly 86% with 4000 cells). However, smaller cells require a higher frequency, and high-power acoustics may cause increase in temperature. This method is also limited to sorting cells with different sizes and densities.

Another method is inertial separation. This technique takes advantage of migration of cells or particles across streamlines in laminar flow streams through curved tubes. Particles of different size and density will have different inertial forces in the curved microchannel. In curved tubes, centripetal forces at the bend will cause the fluid particles to change their main direction of motion. A pressure gradient develops and circular contrary motion (dean flow) develops in the tube. This dean flow is what helps to separate the particles. This sorting method has high cell viability and many cells can be separated at once; however, it is difficult to sort cells that are similar in size and density.

Deterministic lateral displacement (DLD) is a creative technique that uses elevated geometry arrays and laminar flow in order to separate cells. Particles or cells below a critical size follow streamlines through the array gaps with no net displacement from the original streamline. Particles above the critical size are displaced laterally to cross sequential streamlines with each row traveling at an angle predetermined by the post offset distance.

The method that most closely follows our project description is piezoelectric actuation. This technique uses a piezoelectric material to disrupt the flow to move cells into different channels. A piezoelectric material is one that deforms when a voltage is applied to it. This deformation affects the flow to displace cells as they pass by the actuator. Additionally, software is used to recognize different cells as they pass by a camera. The software controls the actuator, so it can move specific cells into the correct channel. Using software allows more adaptability to the microfluidic cell sorter. The software can be trained to recognize certain cells by giving it a set of example images. This may slow the rate the cells can be separated because particles have to pass by the actuator one at a time. However, the accuracy and flexibility of this system is greater than the methods previously discussed.

External search has also been done on the topic of Machine Learning software. More specifically, we evaluated several appropriate Image Processing and Deep Learning frameworks that fit into our project. Search results show four potential platforms, including TensorFlow, Caffe, Theano, and Torch. Each platform utilizes different approaches and has their own pros and cons. TensorFlow is developed by Google and supports the C++ and Python programming languages. Caffe is developed by UC Berkeley and supports C/C++, Python, and MATLAB. Corresponding medical plug-ins are also identified for these two platforms during the research – DLTK for TensorFlow and U-Net for Caffe. Theano is developed by University of Montreal and supports Python while Torch is developed

by Facebook and supports C/C++ and Lua. A detailed comparison between these four platforms will be conducted in later stages of this project, and the choice will be made through evaluations of their compatibility, performance, developer support, and usability (training difficulty).

2.1 Patents

Patent searches were performed on USPTO Patent database with various combinations of keywords surrounding the area of focus such as “microfluid”, “sorting”, “artificial intelligence”, “image processing”, “microfluidic”, “sorter”. Following patents cover microfluid devices and sorting them in various ways:

- Microfluidic sorting using high gradient magnetic fields (Ward & Kaduchak, 2019)
    - This patent is specific to magnetic cell separation in which a magnetic field is used to isolate cells within a fluid sample.
- Microfluidic device that separates cells (Esfandyarpour et al., 2019)
    - The patent covers a general device that separates cells using a microfluidic device. The general device mentions various methods to separate cells, however, there is no mention of the use of artificial intelligence or image processing to sort the cells based on flowing microfluids.
- Microfluidic devices and methods of their use (Davalos et al., 2018)
    - The patent shows different methods and uses of microfluidic devices which include manipulating drops of in microfluidic channels.
- Method and apparatus for bulk microparticle sorting using a microfluidic channel (Wagner, 2017)
    - This patent is specific for bulk sorting microparticles using a microfluidic channel into specific characteristics for individual cells.

2.2 Stakeholder and Market Analysis

This project is being commissioned by Dr. Nuris Figueroa-Morales of the Biomedical Engineering Department at Penn State University. She is our only stakeholder for this project, and thus we do not intend on mass producing our device. While yes there are existing products on the market, our client informed us they are not sufficient, and she wants us to custom build one. Specifically, we need to develop a reconfigurable machine learning program that can sort different types of cells using only one type of channel.

2.2.1 Primary Market

2.2.1.1 Existing Products

The CellRaft Air System utilizes a CytoSort Array system by releasing and sorting CellRafts. This also allows for the sorting of living cells such as bacteria due to its ability to maintain a standard cell culture environment. However, the image recognition of this device relies on 3-channel fluorescence and sorts cells at a rate of 96 cells per hour. The unmet needs of this devices are that it sorts too slowly and relies on fluorescence.

The WOLF Cell Sorter from NanoCellect Biomedical uses a piezoelectric actuator sorting system. This sorter uses a disposable microchip that contains the mechanisms to allow for analyzing and sorting cells that can be replaced to prevent contamination. However, this system also relies on fluorescence dependent flow cytometry to recognize cells. We cannot use this solution because it relies on fluorescence and therefore is more invasive to the cells.

The MoFlo XDP Cell Sorter from Beckman Coulter is a high-performance cell sorter that is able to sort 70,000 cells per second with >99% purity. This device is also able to do 4-way sorting for multiple

populations. An Aerosol Evacuation System (AES) is used to remove aerosols from the sort chamber to prevent safety hazards. This system uses flow cytometry of any wavelength to analyze cells. This solution is also unusable because it relies on fluorescence and therefore is more invasive to the cells.

2.2.1.2 Primary Market Analysis

This microfluidic cell sorter is a unique design exercise for our customer who works as a researcher for The Pennsylvania State University. This product is for their use only and will not be sold or used outside their lab. This device will likely see continued development after this Capstone period has been completed. This research lab will use the microfluidic cell sorter for different biological fluids (but mostly for blood) for diagnostic and possibly therapeutic purposes.

3.0 Needs Assessment

3.1 Gathering Customer/Stakeholder Input

During the group meetings during the research stages, stakeholders' inputs were collected, and discussions were established in person on multiple occasions. As a result, several users' needs were identified, and their corresponding weights evaluated to draw the needs weighting matrix.

3.2 Organization and Weighting of Needs

Table 3.2. Needs Weighting Matrix

		Safe	Ease of Use	Ease of Mfg	Cost	Eff.	Dur.	Accu.	Total	Weight
1	Safe	1.00	0.50	0.33	0.50	0.33	0.75	0.50	6.90	0.05
2	Ease of Use	2.00	1.00	1.00	2.00	1.00	2.00	0.75	12.80	0.20
3	Ease of Mfg	1.00	1.00	1.00	1.00	1.00	3.00	0.33	11.30	0.10
4	Cost	2.00	0.50	1.00	1.00	0.50	2.00	0.33	7.00	0.05
5	Efficient	3.03	1.00	1.00	1.00	1.00	2.00	1.00	11.00	0.20
6	Durable	1.33	0.50	0.33	0.50	0.50	1.00	0.33	5.50	0.15
7	Accuracy	2.00	1.33	3.03	3.03	1.00	3.03	1.00	17.40	0.25

4.0 Engineering Specifications

4.1 Relating Specifications to Needs

Table 4.1. Needs-Metric's Matrix

	Need \ Metric	1 Overall Cost	2 Size (mmt^3)	3 Weight (gram)	4 Time for Task (minute)	5 Number of Parts	6 Replacement Cost	7 Number of Uses	8 Number of People per Project	9 Production Time (hour)	10 Number of Hazardous Substances
1	Transportable	•	•			•					
2	Professional Appearance					•					
3	Cost-Effective	•				•	•	•		•	
4	Capable of Multiple Uses							•			
5	Efficient in Sorting Cells				•			•			
6	Safe										•
7	Durable							•			

4.2 Establishing Target Specifications

The table below shows our target specifications that we intended to meet by the end of the project. These specifications make sure that our product meets our sponsor's requirement and works for its intended purpose.

Table 4.2. Target Specifications Table

Metric No.	Need Nos.	Metric	Imp.	Units	Marginal Value	Ideal Value
1	2,5	Fluid Flow Rate	3	nL/min	$100 \leq Qf \leq 500$	500
2	2,5	Cell Sorting Rate	2	Cells/sec	$10 \leq C \leq 50$	50
3	3	Manufacturing Cost	5	US dollars	≤ 200	$\leq \$1000$
4	3	Manufacturing Time	4	days	≤ 3	< 21
5	5,7	Accuracy	1	%	$\geq 60\%$	$> 90\%$
6	6	Number of Uses	8	times	≥ 1	5

5.0 Concept Generation and Selection

5.1 Problem Clarification and Critical Subproblems

At the highest level, the input of our system will be some arbitrary microfluid, and the output will be particles separated into one of two outlets. More specifically, we have an inlet for the fluid that connects to a channel with a bifurcation at the end. The entire system is composed of the following parts:

- The microchannel
- A mechanical gate to close off one of the microchannels
- The machine learning software
- A circuit controlled by the machine learning software that actuates the gate

The main obstacles this project faces are as follows:

- Sort cells without the use of fluorescence or tagging
- Sort cells without introducing foreign fluids into the solution
- Fabricate the gate inside the microchannel in a cheap and repeatable fashion
- Calculate the timing of the system: machine learning speed, and the circuit's response time
- Programming a robust machine learning program that can be trained to sort many different types of cells

5.2 Concept Generation

In order to find the best idea, we all got together as a group and brainstormed solutions. All the team members performed their own research on possible solutions and brought their findings to the meeting. We documented all the ideas that could be a possible solution for our sponsor. Below are established methods for cell sorting designs and aligning cells in the microfluidic channel.

Sorting Designs

- Piezo Electric Actuator
- Magnetically controlled gate
- Ball valves
- Acoustophoresis
- Inflatable gates in bifurcation
- Introducing new channels that alter the flow in the microchannel

Designs for Cell Alignment

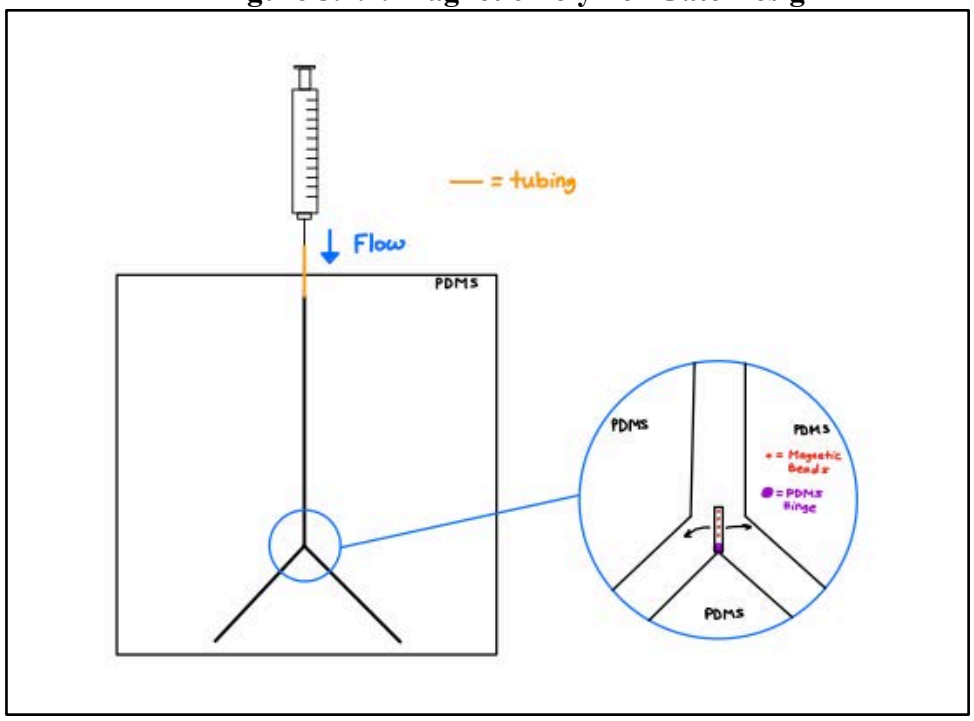
- Single cell width channel
- Acoustics
- Geometrical Obstacles8
- Introduce 3 input channels

A combination of these methods was put together to into one micro channel design idea. It is important to note that each design discussed below will have a microchannel made of PDMS. This material has been used in many documented micro channel designs. PDMS is not difficult to synthesize, and we will not have to buy the material since the sponsor we are working for has it ready to make in their lab.

The first idea (shown in *Figure 5.2.1*) is a simple bifurcation channel. There would be a lever made from a polymer on a hinge that would be crosslinked using UV light. Magnetic beads would be in solution of the polymer when the UV light crosslinks the material. This magnetic polymer lever with magnetic beads would act as a gate to deflect the cells into the desired channel at the bifurcation. The gate would be controlled with electromagnets. The electromagnet will create a magnetic field that can

move the gate into the correct position. A con of this method is the difficulty of adding the gate inside the bifurcation.

Figure 5.2.1. Magnetic Polymer Gate Design



The second concept (shown in *Figure 5.2.2*) is the same as the first idea, except instead of a lever for a gate, two ball valves would be placed downstream of the bifurcation to control the flow into each channel. The ball valves would open or close depending on whether a cell should go in that specific channel. The flow in the closed channel would stop, allowing the flow to continue with the open channel. Cons of this method include slow response time of flow, and the possibility of cells backtracking from the closed channel to the open channel.

The third idea (shown in *Figure 5.2.3*) is to use a piezoelectric actuator to alter the flow and deflect the cells into the desired channel. A piezoelectric material is one that displaces or deforms when current runs through it. This deformation affects the flow to displace cells as they pass by the actuator. The micro channel would also have a single inlet and two outlets. This method has been done before and was documented to have a reasonably high success rate with faster flow rates.

The fourth design concept (shown in *Figure 5.2.4*) is to have three syringes total for the two outlets and one inlet. The flow rate of the two outlets and the inlet would be the same to preserve continuity. Once each cell approaches the bifurcation, one of the outlets would increase the flow with a pulse. Once this happens, the inlet would have to simultaneously increase its flow with a pulse to preserve continuity. For clarity, both outlets and the inlet would be continuously flowing. This method could help prevent backflow of cells into the wrong channel if the flow were to stop completely in one of the outlets.

The fifth design (shown in *Figure 5.2.5*) would be to introduce two more inlets just before the bifurcation. These inlets would introduce flow to the system to deflect the cell into the desired channel. This is a similar concept to the piezoelectric actuator, but it would cost less money to build.

The sixth design idea (shown in *Figure 5.2.6*) introduces three total inlets to either of the first five ideas. Since there will be laminar flow regime in the micro channel, particles will follow the streamline. The idea of this model would be to inject a cell/particle-free fluid into the outer inlets and

inject the solution with the cells/particles into the middle inlet. Introducing additional flow from two outer inlets would allow the cells/particles to follow only the streamline of the narrow laminar flow regime generated by the canter inlet. This concept allows the particles to align in a single file so it is easier for the sorting mechanism to do its job.

Figure 5.2.2. Ball Valve Design

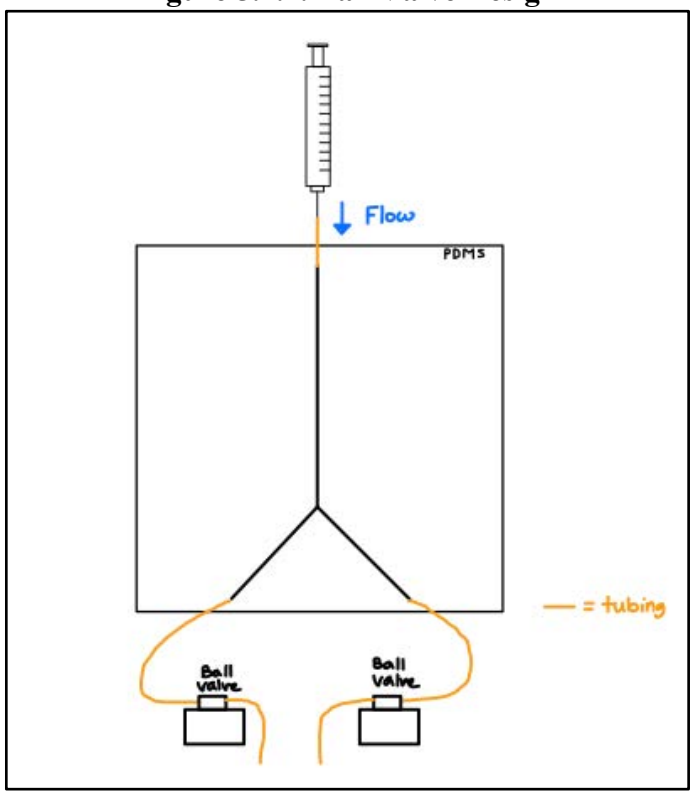


Figure 5.2.3. Piezoelectric Actuator Design

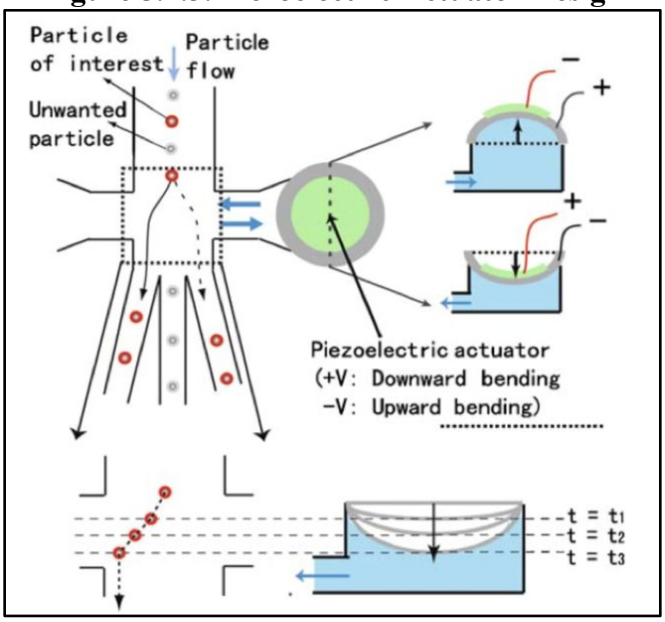


Figure 5.2.4. Output Syringe Flow Rate Design

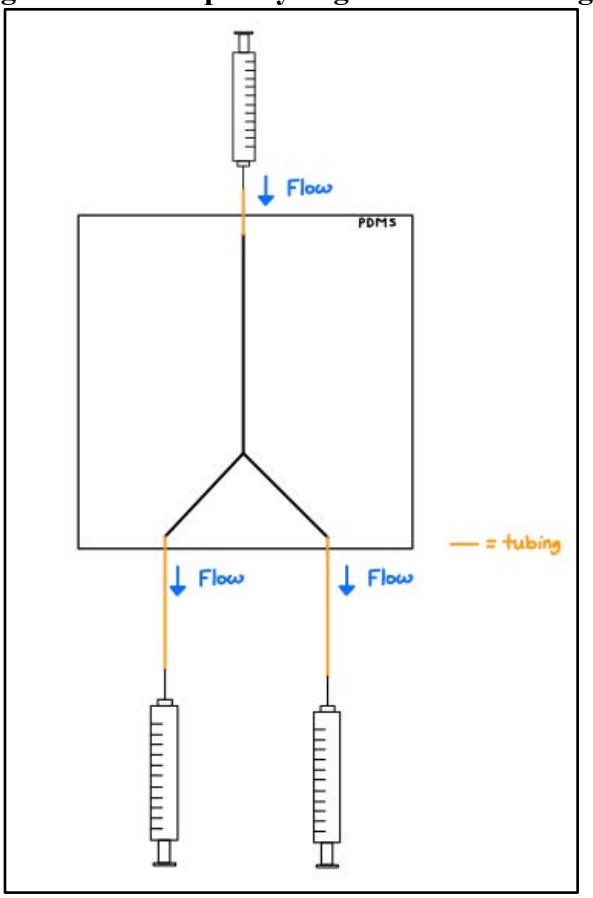


Figure 5.2.5. Flow Deflection Design

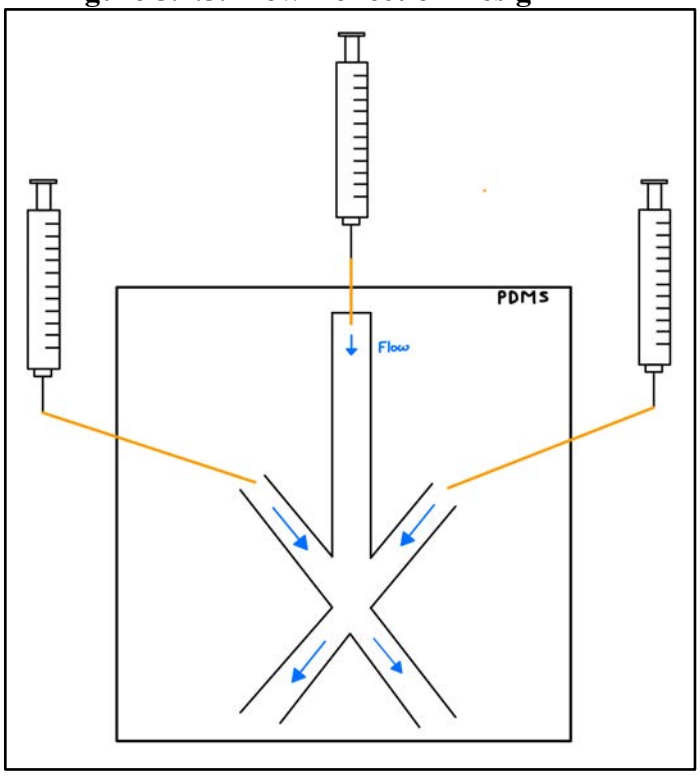
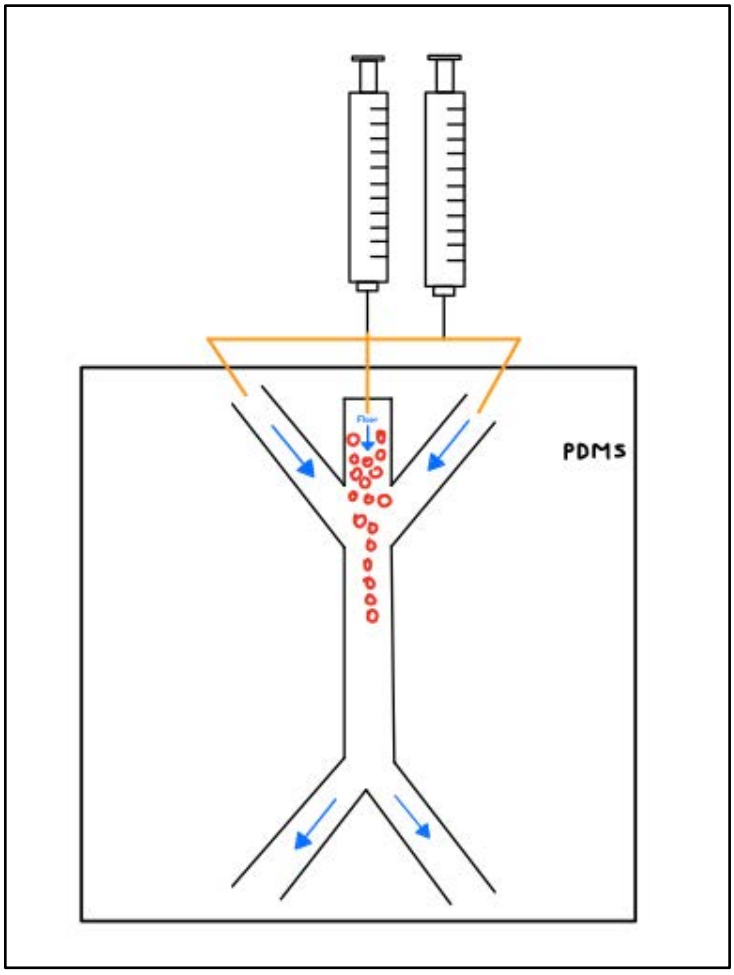


Figure 5.2.6. Single File Arrangement Design



5.3 Concept Selection

The pros of the 3 Flow Syringes design is the ease of manufacturing. All the materials are readily available. The only requirement would be to fabricate the channel and write the software to control the flow through the system. The major con being that this design ultimately did not work due to the unpredictable behavior of the fluid within the system.

Our second choice is the Polymer Gate design. The disadvantages of this design are that it is more difficult to manufacture. We would have to attempt to synthesize the gate, and then place it in the correct part of the channel. Next, we will need to make or buy electromagnets strong enough to move the gate within the channel. The pros for this design being we can force the fluid to flow down the channel we want by blocking on the channels with the gate. This should hopefully make it easier to control the fluid flow.

Table 5.3.1. Microchannel Concept Scoring

		Concepts									
		Polymer Gate		Ball Valve		Piezoelectric Actuator		Flow Syringes		Single File	
Selection Criteria	Weight	Rating	Wgtd. Score	Rating	Wgtd. Score	Rating	Wgtd. Score	Rating	Wgtd. Score	Rating	Wgtd. Score
Safe	0.05	5	0.25	5	0.25	5	0.25	5	0.25	5	0.25
Ease of Use	0.20	5	1.00	4	0.80	**3**	0.60	4	0.80	**3**	0.60
Ease of Mfg.	0.10	1	0.10	4	0.40	1	0.10	5	0.50	4	0.40
Cost	0.05	2	0.10	2	0.10	2	0.10	5	0.25	4	0.20
Efficient	0.20	**3**	0.60	**3**	0.60	4	0.80	4	0.80	4	0.80
Durable	0.15	**3**	0.45	4	0.60	4	0.60	4	0.60	4	0.60
Accuracy	0.15	**3**	0.75	**3**	0.75	4	1.00	**3**	0.75	**3**	0.75
	Total Score		3.25		3.5		3.45		**3.95**		3.60
	Rank		5		3		4		**1**		2
	Continue	No		No		No		Yes – Prim. Dsgn		Yes – Alt. Dsgn	

Table 5.3.2. Machine Learning Concept Scoring

		Concepts							
		1 TensorFlow		2 Caffe (ref)		3 Theano		4 Torch	
Selection Criteria	Weight	Rating	Wgtd. Score	Rating	Wgtd. Score	Rating	Wgtd. Score	Rating	Wgtd. Score
Compatibility	0.40	**3**	1.20	**3**	1.20	**3**	1.20	3	1.20
Ease of Use	0.05	4	0.20	**3**	0.15	**2**	0.10	2	0.10
Developer Support	0.05	4	0.20	**3**	0.15	2	0.10	2	0.10
Medical Image Library Support	0.10	**3**	0.30	**3**	0.30	2	0.20	1	0.10
Performance	0.40	4	1.60	**3**	1.20	2	0.80	2	0.80
	Total Score		3.5		3.0		2.4		2.3
	Rank	1		2		3		4	
	Continue	Yes- Prim. Dsgn		Yes - Alt. Dsgn		No		No	

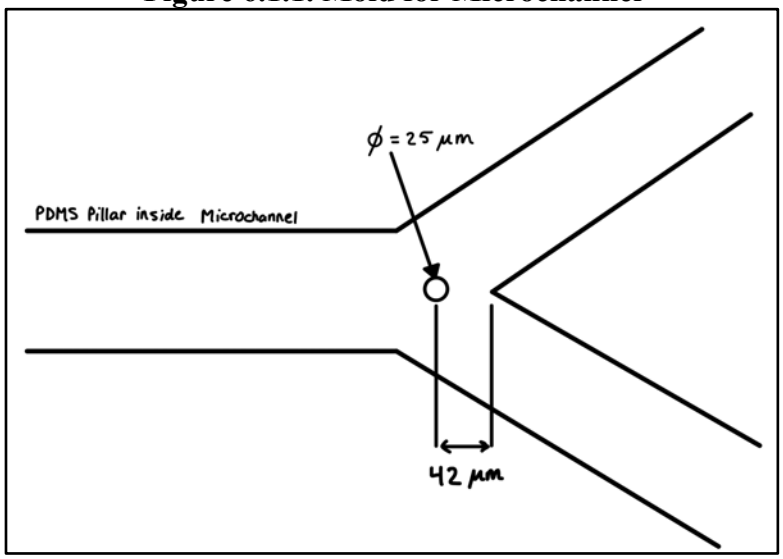
Relative Performance	**Rating**
Much worse than reference	1
Worse than reference	2
Same as reference	3
Better than reference	4
Much better than reference	5

6.0 Detail Design

6.1 Manufacturing Specifications and Process Plan

A mold must be fabricated to make the PDMS microchannel. This mold is made by cross-linking a polymer in the shape of the microchannel onto a mask. The mask and mold fabrication were made through the nanofabrication department at Penn State. The specifications for the mold design can be seen below in *Figure 6.1.1*.

Figure 6.1.1. Mold for Microchannel



Because of the time constraints of this project, we were not able to receive the mold and make the PDMS channels with the pillar to hold the hydrogel gate within the bifurcation. This will be done as a next step as our sponsor continue the project. We had microchannel molds without the pillar, so the next step is to make the PDMS microchannels. The steps to fabricate the channels are the following:

1. Mix the PDMS solution with a PDMS curing agent ratio of 10:1 → mix for 1-3 mins
2. Centrifuge the solution to eliminate bubbles within the solution
3. Degas PDMS mixture by placing in a vacuum desiccator --> 15-60 mins
4. Put mold in specialized container --> pour PDMS onto mold
5. Place on top of specialized container on top of PDMS and to seal the enclosure. This allows the PDMS to solidify with a flat top surface
6. Place in oven with a temperature of 100C for 35 mins
7. Allow PDMS to cool and cut the PDMS channels into squares

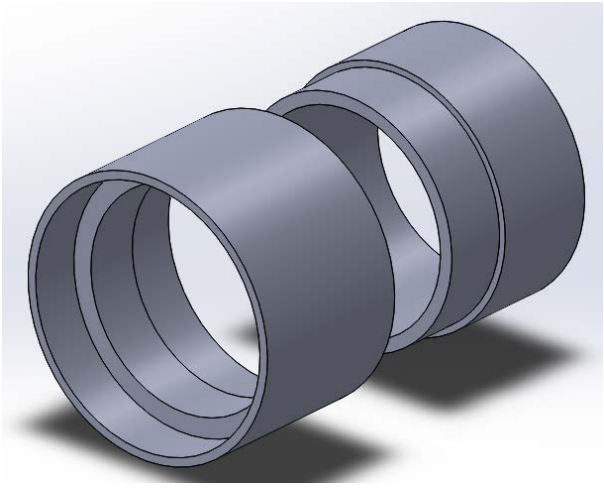
Once the microchannel is made, the hydrogel gate must be synthesized within the bifurcation around the pillar. To do this, we fill the channel with a solution of 94% hydrogel (PEGDA) and 6% photo-initiator (2-hydroxy-2-methylpropiophenone). Iron shavings are introduced into this solution before being injected into the microchannel. The solution can be crosslinked into the shape of a rectangular gate with the use of UV light. This UV light shines through the top of the microscope, through a mask that blocks out unwanted UV light, through a 2x magnification condensing lens, and into the microchannel. The mask is a glass plate with a chrome coating, which covers the whole glass plate except for a rectangular opening to allow UV light to pass through. The glass rectangle has length of 545 um and a width of 108 um. Because of the condensing lens magnification, the actual crosslinked

gate size has a length of 272.5 um and a width of 54 um. The mask can be seen in the following *Figure 6.1.2*.

Figure 6.1.2. UV Mask to Synthesize Hydrogel Gate

A custom part to connect the UV light source to the microscope also had to be machined using a lathe machine in the Learning Factory. Machining this part added no additional cost to the project because they were made from scrap metal tubing. This custom aluminum part consists of two pieces, which can be seen in the following *Figure 6.1.3*.

Figure 6.1.3. Aluminum Tubing Connection for UV Source and Microscope

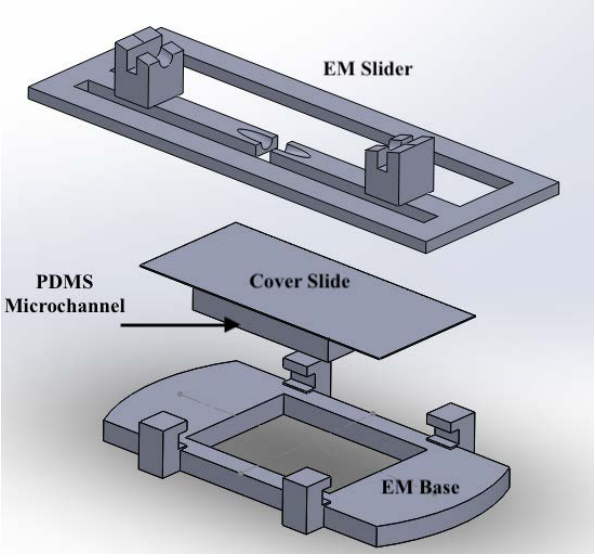


Our design involves electromagnets made of coated copper wire wrapped around a nail. The wire is wrapped 100 times around the nail. The ferromagnetic nail is made of steel, which is approximately 98% iron. The iron in the nail makes it more ferromagnetic. The nail acts as a ferromagnetic core for

the solenoid, meaning that the magnetic field strength is focused or increased. This makes the magnet more effective at moving the particles. Nails are also cheap, readily available, and they fit well within the budget. If the nails wear out, they can be easily replaced at little cost.

A device must be made to hold the electromagnets close to the hydrogel gate in the bifurcation. It is also important that the nail position can be adjusted because there are multiple channels within the PDMS square. The device shown below was designed to hold the PDMS channel and the nails in place under the microscope.

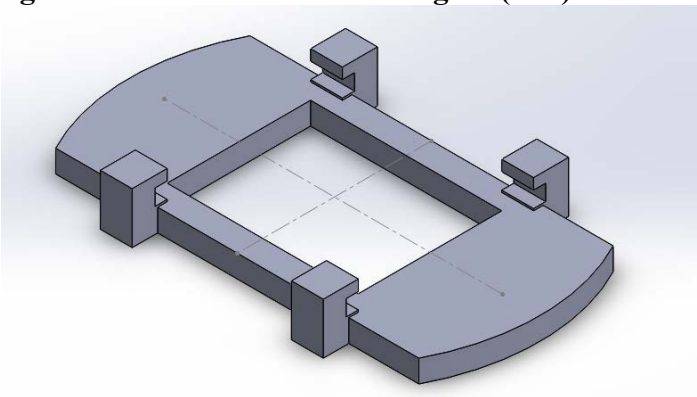
Figure 6.1.6. Exploded View of EM Holder & Microchannel



This assembly was designed with the PDMS microchannel facing down and supported by the PDMS coated glass cover slide. With this orientation, the magnetic field from the EM nails will more strongly affect iron-infused hydrogel gate in the bifurcation. As a result, the gate can switch back and forth with a faster response time. Refer to *Appendix B1* for all the Engineering Drawings.

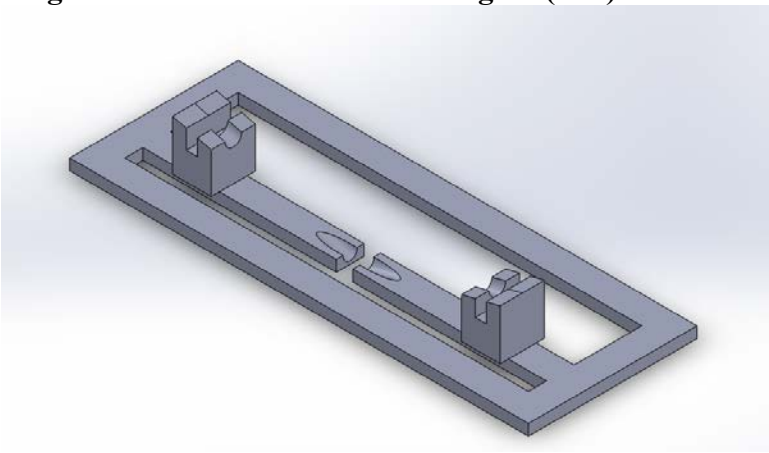
The base of the EM holder holds the PDMS microchannel by support of the PDMS coated class cover slip. The objective views the fluid flowing in the microchannel from above while the light source provides light from above. The structures on the sides allows the EM slider to attach to the EM base and allows the slider to move back and forth. The slider is also slightly lifted above the top surface of the base so that the slider does not interfere with the glass slide that lies on the top surface.

Figure 6.1.4. Base of Electro-Magnet (EM) Holder



The EM slider is the component that holds the electromagnets. The base of the nails rest on the elevated structures and the tips of the nails come towards the center of the structure and lie in the divots.

Figure 6.1.5. Slider of Electro-Magnet (EM) Holder



For the machine learning program that recognizes different cells, we used the pymba software package. It is an open-sourced python library and wrapper written for Vimba camera's C++ SDK, enabling our python program and Convolutional Neural Network to have direct access to the pictures of the microchannel captured by the camera installed on the microscope. Then we use OpenCV, a library of programming functions aimed at real-time computer vision. Specifically, we used it to apply Frame Differencing filters on the pictures and generate processed images that only track the moving objects in the microchannel. Then we implemented the LoG Blob Finding algorithm to identify the edges of the microbeads and the Hough Circle Transformation algorithm to calculate the area of the microbeads. We only focus on beads located in the bottom part of the image as they will be the ones going to be sorted by the gate next. Due to the position of the beads inside the microchannel and the nature of these vision algorithms, the calculated areas have a relatively big difference. So, we then feed these areas to our neural network to train the model. The trained model can give us an output of "1m" and "3m" to distinguish between microbeads of different sizes. Refer to *Appendix B2* for a detailed flowchart of our Machine Learning program.

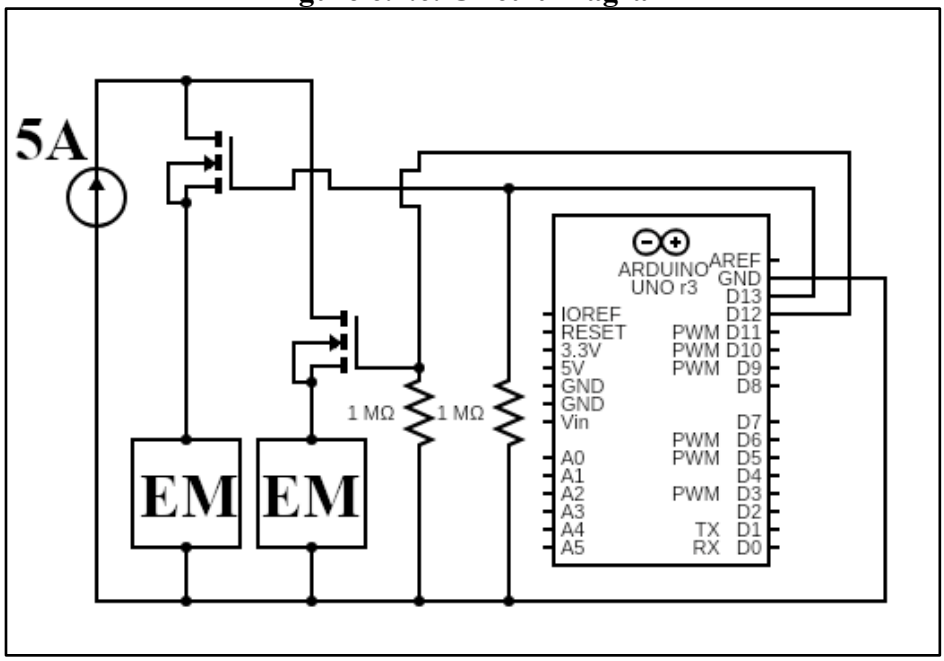
The design of the circuit and the electromagnet was based on the materials our sponsor had on hand. The circuit design process was simple. We need to move the gate from one position to another, therefore two electromagnets are needed. This is because the tips of the nails strongly attract the iron particles: reversing the current would not repel the particles. Refer to *Figure 6.1.5* to better visualize the placement of the nails.

Figure 6.1.6 below shows setup of the circuit. We only want one electromagnet active at a time, we need a circuit to switch the electromagnets on and off. A transistor is placed in series with the electromagnet. The transistor acts as an electrical switch that can be activated or deactivated via the Arduino Uno by applying 5V to the gate of the transistor. The Arduino Uno can be programmed to turn each transistor on or off based on commands received from its serial communications port. The machine learning software will send the commands over the serial port.

FQP30N06L MOSFETs were chosen because they have a low enough gate voltage to be activated by the Arduino Uno. The FQP30N06L MOSFETs also can have a sustained source-drain current of 5A running through them. 5A was the maximum output of the DC power supply our sponsor had, and

we wanted to create as strong of an electromagnet as possible. The number of turns for the electromagnets were found empirically.

Figure 6.1.6. Circuit Diagram



Note: EM stands for electromagnet

Table 6.1.7. Manufacturing Process Plan

ASSEMBLY NAME	MATERIAL TYPE	RAW STOCK SIZE	OPERATIONS
Microchannel Mold			• Crosslink polymer in the shape of microchannel on a plate using UV light • Crosslink an elevated pillar in the bifurcation
EM Holder Base		-	3D printed
EM Holder		-	3D printed
EM Holder with PDMS Microchannel	-	-	• Put microchannel face down on EM holder base • Put EM holder slider through the slots on the side of the EM holder base • Put EM nails on the slider
UV Light Source – Microscope Connector	Aluminium - Unknown		Machine to size on Lathe

6.2 Bill of Materials

Vendor	Product Number	Product Description	Unit Cost	Qty	Total Cost	Makes	Cost per Unit
Sigma-Aldrich	437441-100ML	POLY(ETHYLENE GLYCOL) DIACRYLATE	$44.60	1	$44.60	200	$0.22
Sigma-Aldrich	405655-50ML	2-HYDROXY-2-METHYLPROPIOPHENONE, 97%	$50.80	1	$50.80	100	$0.51
Sigma-Aldrich	267953-5G	IRON, POWDER, LESS THAN10 MICRON, >=99.&	$28.00	1	$28.00	250	$0.11
Electron Microscopy Sciences [Provided by sponsor]	71887-30	Platinum Line Cover Glass 22 x 50 #1	$4.00	1	$4.00	70	$0.06
VWR [Provided by sponsor]	700014-034	Polydimethylsiloxane, Technical Grade	$98.57	1	$98.57	20	$4.93
N/A [Provided by sponsor]	N/A	Enameled Copper Wire	$6.98	1	$6.98	100	$0.07
N/A [Provided by sponsor]	N/A	1" 18ga Steel Nail	$1.50	1	$1.50	400	$0.00
N/A [Provided by sponsor]	N/A	Arduino Uno R3 Development Board	$15.98	1	$15.98	1	$15.98
N/A [Provided by sponsor]	N/A	ELEGOO MB-102 Breadboard 830 Point Solderless Prototype PCB Board	$3.00	1	$3.00	1	$3.00
N/A [Provided by sponsor]	N/A	EDGELEC 100pcs 1K ohm Resistor 1/4w (0.25 Watt) ±1% Tolerance Metal Film Fixed Resistor	$5.69	1	$5.69	50	$0.11
Amazon	FQP30N06L	MOSFET (FQP30N06L)	$15.88	1	$15.88	5	$3.18
Amazon	ASD1005	BJT Transitor (TIP120)	$6.25	1	$6.25	1	$6.25
				Total	**$308.15**		**$34.50**

Poly(Ethylene Glycol) Diacrylate is a cross-linking agent that serves as the base materials of the gate. 2-Hydroxy-2-methylpropiophenone serves as the photo initiator for cross linking with UV light. Iron powder <10 μm is incorporated into the gate to allow for control via a magnetic field. Iron powder was infused into the gate to allow for movement control through a magnetic field. Cover glass was coated with PDMS and used as a base for the PDMS channel. Polydimethylsiloxane (PDMS) was used to create the microchannel. Enameled copper wire and 1” nails were used to create the electromagnets

used to control the gate. An Arduino Uno, breadboard, 1K ohm resistors, and MOSFETs were used to create a circuit to control electromagnets and interface with the machine learning system. See *Appendix C* for a full Bill of Materials.

6.3 Analytical and Physical Modeling

COMSOL was used to make simulations on the hydrogel gate that will be used for deflecting particles in the fluid. This gate is made of a hydrogel called polyethylenglycol diacrylate (PEGDA), which has a molecular weight of Mn=575 g/mol. This hydrogel requires the photo-initiator called Darocur 1173 (2-hydroxy-2-methylpropiophenone) to initiate crosslinking with UV light exposure. The article "Microfluidic in situ mechanical testing of photopolymerized gels" (Duprat el al., 2015) finds the Young's Modulus given different concentrations of the hydrogel-photo-initiator solution and different exposure times to UV light. This article finds that if the concentration of the photo-initiator [PI] > 1.5 %, the hydrogel will converge to a Young's Modulus of 12 MPa. The Young's Modulus converges faster with UV exposure if the concentration is higher. We decided to use [PI] = 6% for a faster crosslinking process. *Figure 6.3.1* shows the Youngs Modulus as a function of concentration and exposure time.

Our team needed further analysis to see if the material properties would suffice for the demands of our proposed system. In order to do this, Von Mises stress and displacement analysis was done on the gate with the flow rate acting normal to the beam (as a worst-case scenario for stress/displacement) with a flow rate of 2 um/s. We set the material properties to have a Youngs Modulus of 12 MPa. Under this condition, a beam with a length of 220 um displaces downward ~20um. These displacements and stresses are not concerning for the system. Figure 6.3.2 below shows COMSOL stress and displacement analysis of the gate.

Figure 6.3.1. Evolution of Youngs Modulus of PEGDA (Duprat el al., 2015)

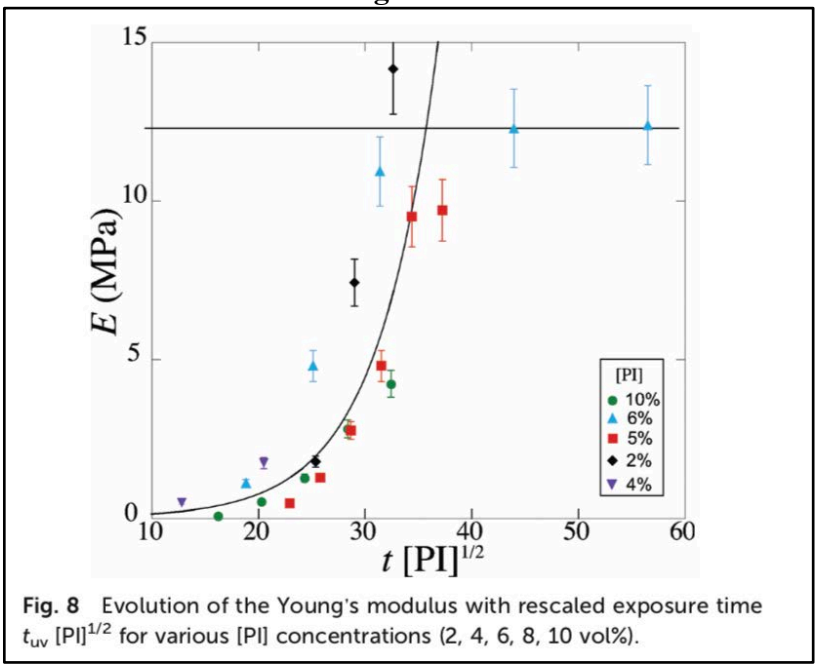
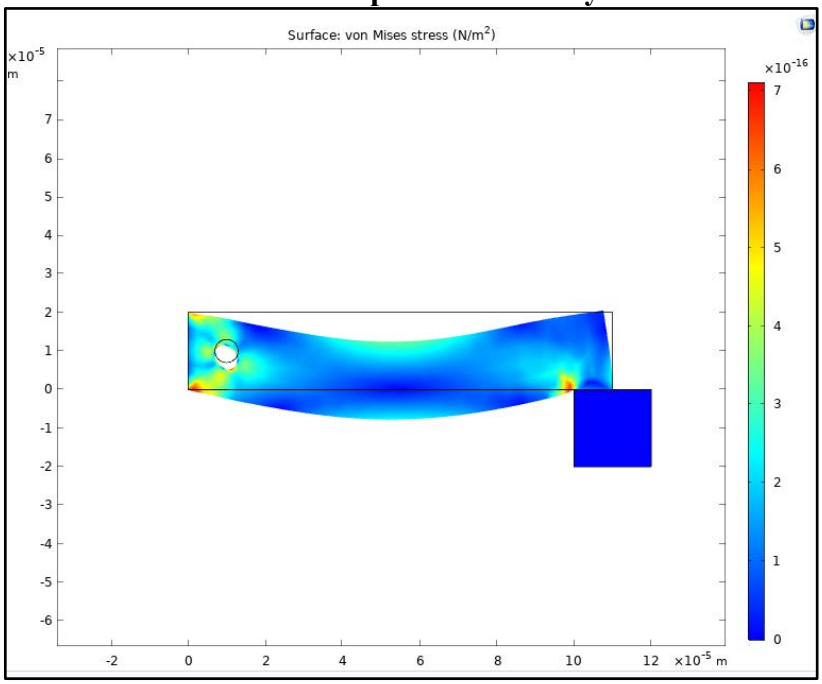


Fig. 8 Evolution of the Young's modulus with rescaled exposure time t_{uv} $[PI]^{1/2}$ for various [PI] concentrations (2, 4, 6, 8, 10 vol%).

Figure 6.3.2. Von Mises Stress and Displacement Analysis of $110 \times 20um$ channel



6.4 Risk Analysis

- Circuit Power Source: There is a risk that the user could hurt themselves when connecting the circuit's power source. This isn't due to a flaw in the design, but a fact that working with electricity can be dangerous. Additionally, providing too much current to the system could break it.

- Electromagnet Over Heating: If one of the electromagnets is left on for too long, it could overheat and melt its housing and the PDMS channel.

- UV Light Exposure: The user, when building the gate for the channel, could be exposed to UV light. Proper eye protection should be used to prevent eye damage.

6.5 Testing

Poly(ethylene glycol) diacrylate (940 mL) and $60mL$ were mixed to create 6% solution of hydrogel. A long glass cover slide and two short glass cover slides were used to create a shallow well in the middle of the slide. Another long glass cover slide was put on top of the well. Hydrogel solution (50 uL) was fed into onto the well via water tension.

A UV lamp was positioned at the light input of a ZEISS Axiophot Fluorescence microscope. A chrome lithography mask containing rectangular clear beams of various sizes was placed on top of the light source. The glass slide carrying the hydrogel was set on the platform under the objective lens. A picture of the set up can be seen below.

Figure 6.5.1. ZEISS Axiophot Fluorescence Microscope

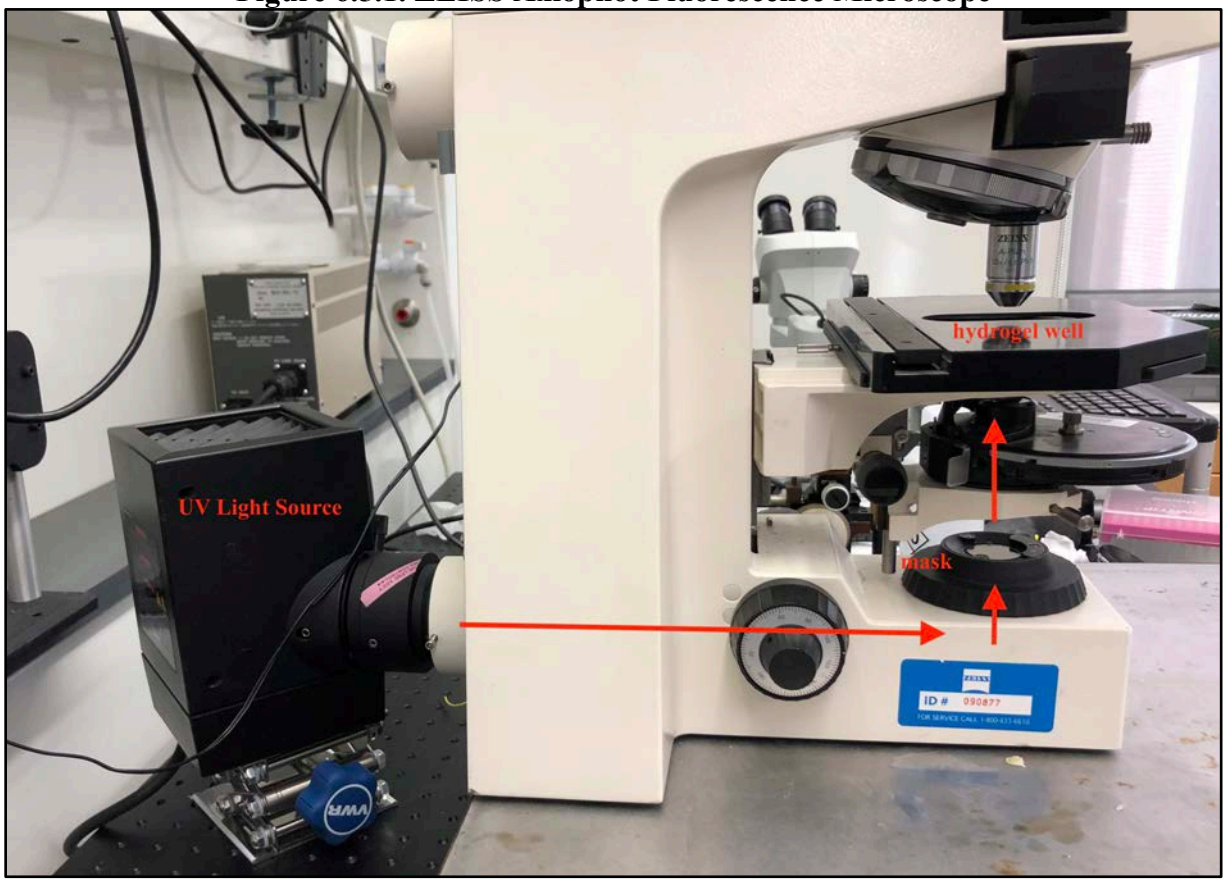
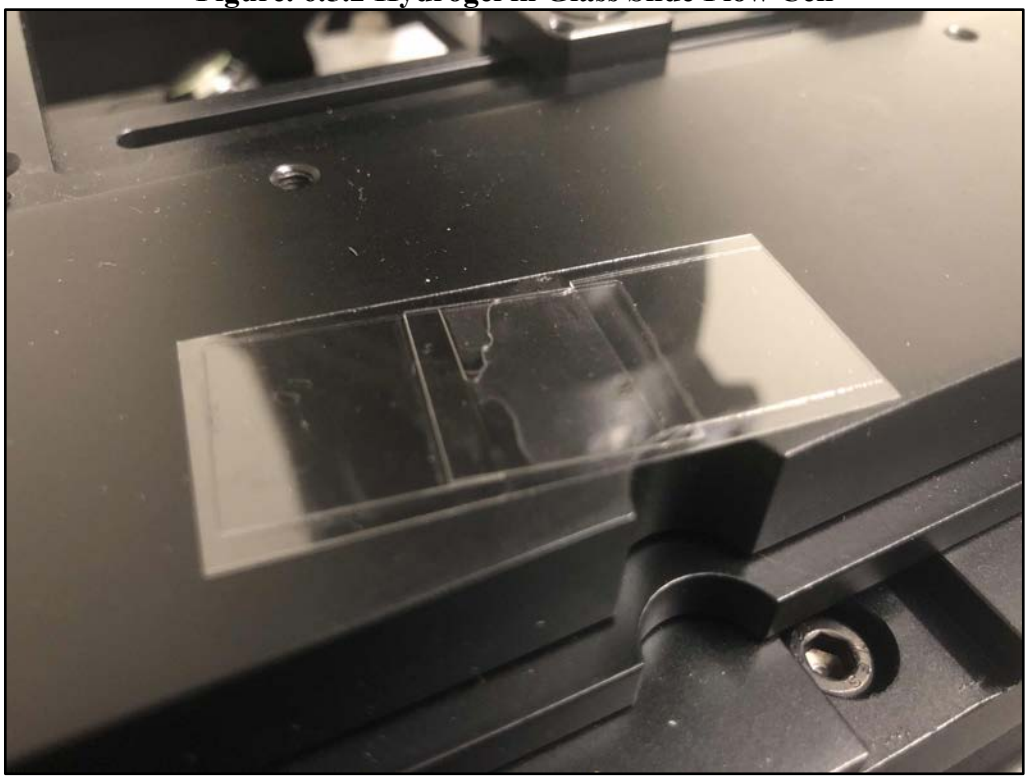


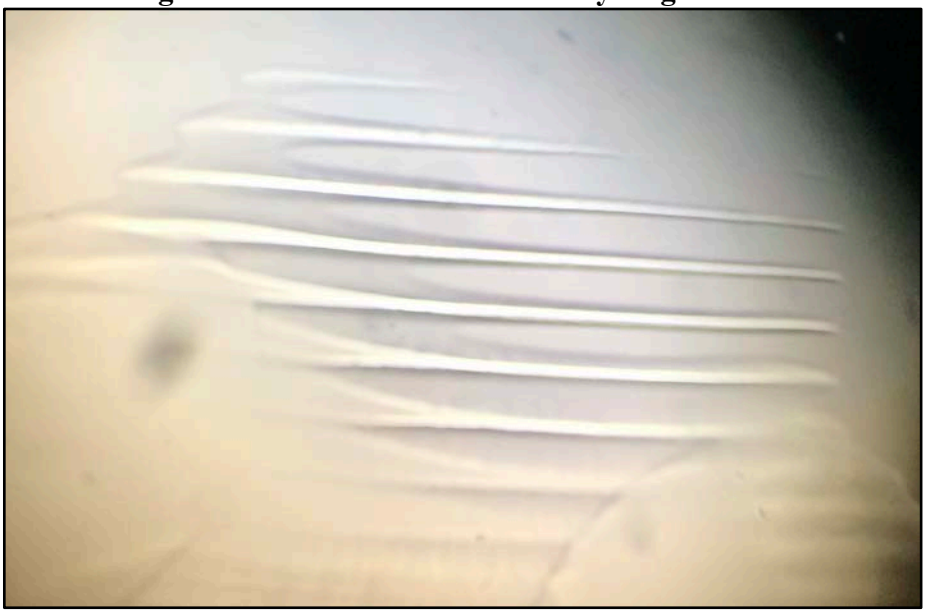
Figure. 6.5.2 Hydrogel in Glass Slide Flow Cell



UV light must be focused on the well filled with hydrogel in order to crosslink well defined rectangles. The heights of the condenser lens and stage were adjusted slightly if the beams were not clear. The process was repeated until beams were as clear as possible. UV light was flashed for a 1 second time period on the sample. The size of beams crosslinked were gradually decreased until the desired size able to fit within the $200um$ channel was reached. Beams were evaluated in the clearness of the edges and accuracy of shape. The test was repeated with hydrogel solution mixed with iron particles to see if iron affected ability to crosslink.

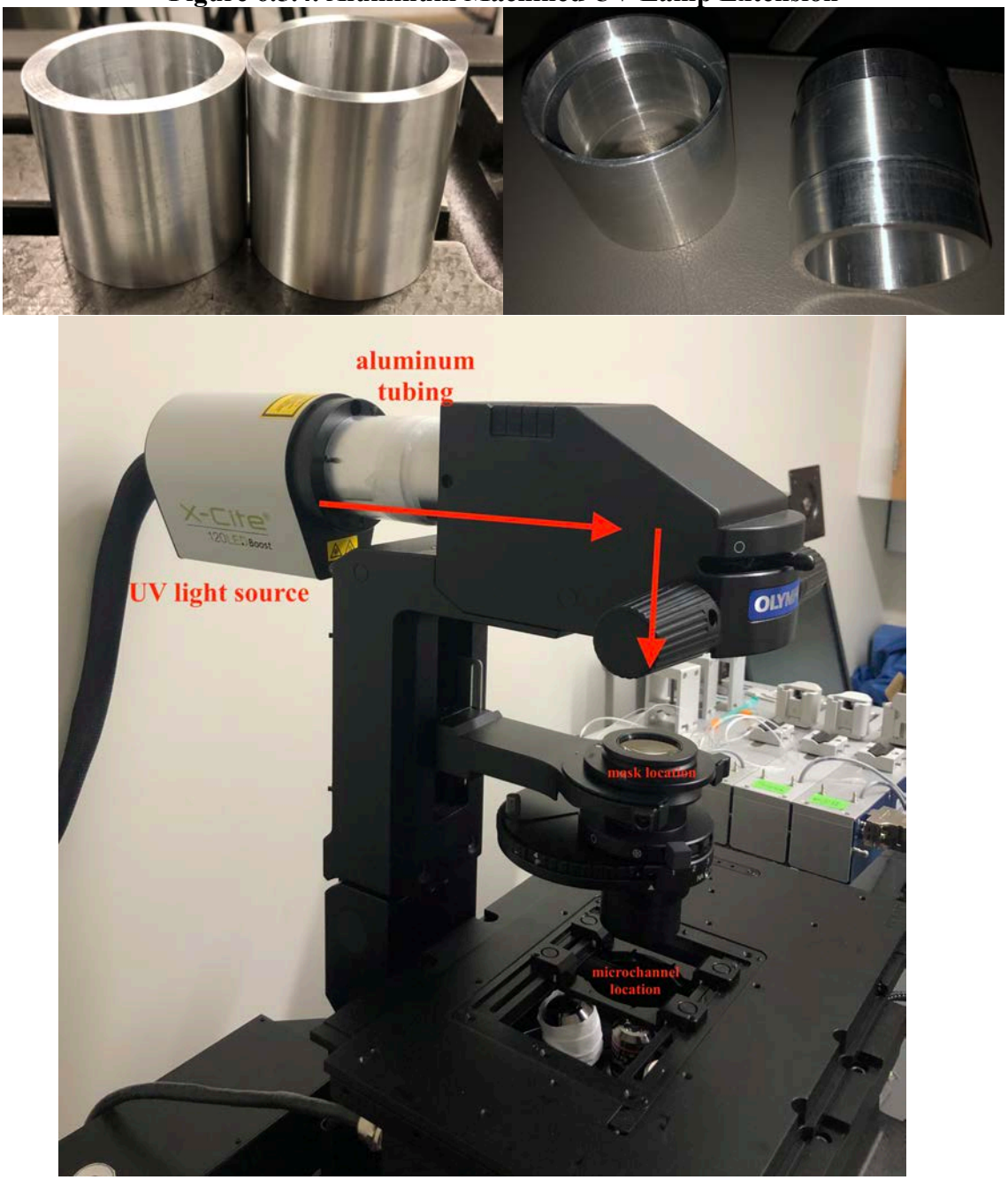
This set up was not good for crosslinking defined rectangles. The microscope is older and does not have solid attachments for the UV light source. It takes a long time to adjust the light source so that the light can focus through the chrome mask. In this microscope, the position of the platform holding the microchannel and the middle lens could both be moved up and down. This made it difficult and time consuming to find the focus point of the UV light, especially if someone else from the lab had to take apart this set up for another project. There came a point where we could not focus the UV light to have very defined rectangles that were small enough for our microchannel. The following figure shows some crosslinked rectangles obtained from this set up:

Figure 6.5.3. First Well-Defined Hydrogel Beams



We had a need for more defined and repeatable rectangles, so we decided to go with a new UV light set up. The second UV light set up idea used an X-Cite 120 LED Boost lamp that is attached to the back of an Olympus microscope. This set up initially posed a challenge because the UV lamp could not fit flush to the opening in the back. We custom machined two pieces of aluminum tubing to fit the UV lamp and the opening in the back of the microscope. This aluminum tubing allowed the light source to be fixed with respect to the microscope, which was not the case in the first set up. The aluminum tubing and microscope set up can be seen in *Figure 6.5.4*.

Figure 6.5.4. Aluminum Machined UV Lamp Extension



The light comes from the back of the microscope and shines through a middle condensing lens with a 2x magnification. This magnification makes it so the actual crosslinked area of the hydrogel is smaller than the mask size. The following figures show how the magnification effects the size of the crosslinking:

The hydrogel solution is exposed to 100% UV exposure for 1 second. The UV light was focused by moving the lens that supports the mask. Next ~0.02 g/mL iron shavings are introduced to the 6% hydrogel solution. The gate is crosslinked with the same height and UV exposure.

To test the accuracy of our machine learning program, basic statistics were calculated for the outputs generated from our test sets, including average and standard deviation of microbead areas detected with respect to the microbead sizes. The result shows that the average area for 3-micron bead

is 362.37 with a standard deviation of 104.38; the average area for 1-micon bead is 152.26 with a standard deviation of 61.26. The distinction is big enough to categorize two different sizes of microbeads. Our circle detection algorithm can be seen in *Figure 6.5.5* with the area for each bead above the circle. The sorting algorithm in *Figure 6.5.6* works by detecting the size of the bead through the neural network model in real-time and sends the signal to the gate based on the bead detected.

Figure 6.5.6. Microbeads sorting (3-micron detected)

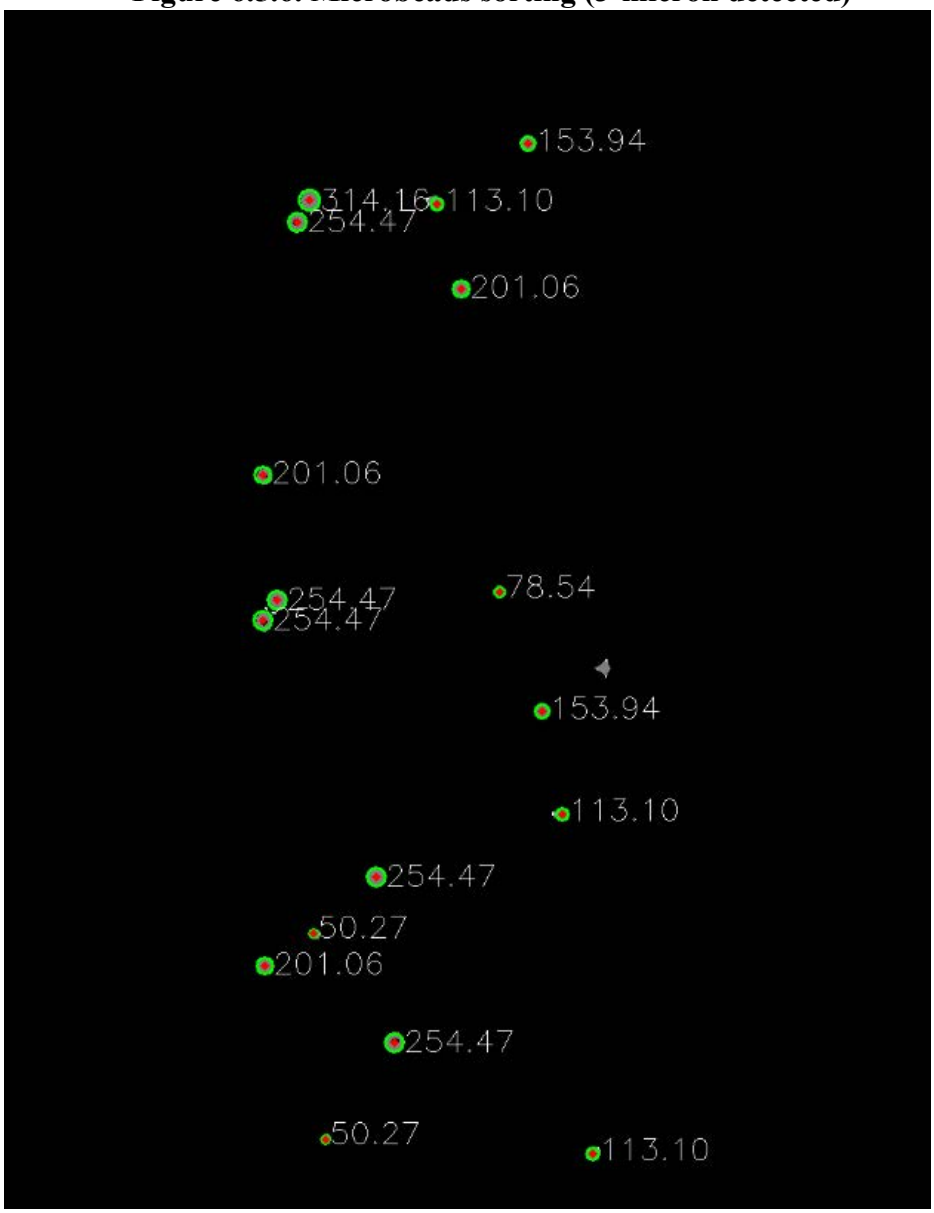
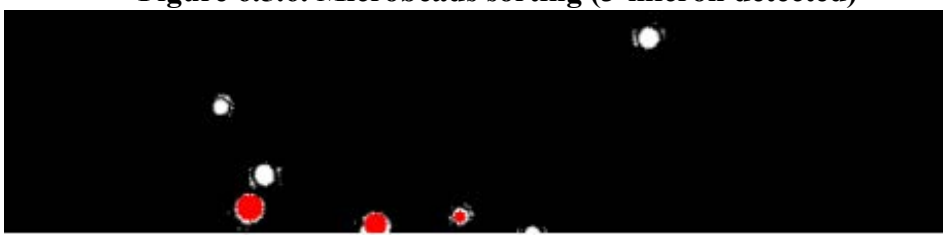


Figure 6.5.6. Microbeads sorting (3-micron detected)



6.6 Budget

Vendor	Product Number	Product Description	Unit Cost	Qty	Total Cost	URL
FISHER SCIENTIFIC	G0100	Thermo Scientific™ Fluoro-Max Dyed Green Aqueous Fluorescent Particles (1 um) (10 mL)	$257.00	1	$257.00	https://www.fishersci.com/shop/products/fluoro-max-dyed-green-aqueousfluorescent-particles/09980446#?keyword=G0300
FISHER SCIENTIFIC	G0300	Thermo Scientific™ Fluoro-Max Dyed Green Aqueous Fluorescent Particles (3 um) (10 mL)	$257.00	1	$257.00	https://www.fishersci.com/shop/products/fluoro-max-dyed-green-aqueousfluorescent-particles/09980440#?keyword=G0100
Sigma-Aldrich	437441-100ML	POLY(ETHYLENE GLYCOL) DIACRYLATE	$44.60	1	$44.60	https://www.sigmaaldrich.com/catalog/product/aldrich/437441?lang=en®ion=US
Sigma-Aldrich	405655-50ML	2-HYDROXY-2-METHYLPROPIOPHENONE, 97%	$50.80	1	$50.80	https://www.sigmaaldrich.com/catalog/product/aldrich/405655?lang=en®ion=US
Sigma-Aldrich	267953-5G	IRON, POWDER, LESS THAN10 MICRON, >=99.&	$28.00	1	$28.00	https://www.sigmaaldrich.com/catalog/product/aldrich/267953?lang=en®ion=US
Amazon	FQP30N06L	MOSFET (FQP30N06L)	$15.88	1	$15.88	https://www.amazon.com/FAIRCHILD-SEMICONDUCTOR-FQP30N06L-CHANNEL-MOSFET/dp/B00MMY28AM
Amazon	ASD1005	BJT Transitor (TIP120)	$6.25	1	$6.25	https://www.amazon.com/TIP120-Darlington-Transistor-Arduino-Diodes/dp/B00FVLGYEY
Penn State Nanofabrication Department	N/A	UV Lithography Mask	$50.00	1	$50.00	N/A

Penn State Nanofabrication Department	N/A	Nanofabrication Lab Cleanroom Time	$200.00	1	$200.00	N/A
Engineering Copy Center	NA	Printed Poster for Showcase	$62.24	1	$62.24	N/A
				Total	**$971.77**	

In hindsight, it may have been more effective to get a set of microbeads with a larger difference in size to make the machine learning easier and to better visualize and demonstrate the sorting during the presentations.

7.0 Special Topics

7.1 Ethical Considerations

The only ethical consideration for our device would be the waste generated when making or using up any of the channels made of PDMS. Other than that, there are no apparent ethical dilemmas for this given project.

7.2 Environmental Statement

For this project we will need to make microfluid channels out of PDMS. Ideally, we will be able to use those channels 4 or 5 times. While PDMS has no apparent downsides, it can still generate a considerable amount of waste if used often.

7.3 Regulatory Considerations

The device we developed is intended to be used for research purposes and not intended to be marketed for sale. Therefore, no considerations are necessary.

8.0 Final Discussion

8.1 Test Results and Discussion

Larger beams were successfully crosslinked with clear edges; however, beams with dimensions small enough to fit within the desired channel size were not able to be crosslinked with clear edges. Potential reasons for this may include diffraction of the UV light off the inner walls of the chrome mask leading to a blurry area around the beam being crosslinked. Another potential error may be that the ZEISS microscope used is not suited to focus UV light onto the slide. Ideally the beam would be created by shining UV light directly perpendicular to the mask; however, with the available equipment this was not possible. Addition of iron particles did not appear to influence the hydrogel solution's capacity to crosslink.

An Arduino Uno with LEDs was successfully connected to the sorting algorithm to test the detection in real-time with previously collected data. The LEDs accurately flickered based on the incoming signal for which direction the gate should open given a set of data. Although we did not have enough time to test the system with any real-time data, we have already built scripts to handle the camera's real-time frame collection for future testing.

8.2 Future Recommendations

Using particles with a more significant difference in size for testing is suggested for ease of training the machine learning software and for better visualization. Using a higher resolution microscope from the start would be recommended for making the hydrogel gate in order to ensure a better definition of the gate shape at smaller sizes.

For our machine learning program, currently all the training and testing is based on microbeads, and our model can categorize them based on sizes and shapes. For future developments, Transfer Learning concepts are recommended to speed up the training process and improve model performance. In addition, current training sets must be captured and cleaned manually by the group members. In the future, it would be ideal and recommended to get external open-sourced training sets to increase the model's accuracy. Training the model to recognize a variety of cells will require large amounts of data which can be acquired from open-source data sets, working with these open-source data sets will yield better results as they have been tested by various other sources as well.

8.3 Group Reflection

This group consists of five students that are studying Computer Science (2), Computer Engineering (1), Biomedical Engineering (2), and Mechanical Engineering (1). Each student had an important role in this project, and everyone did it well. Everybody spent a significant amount of time outside of class in order to make progress on their part of the project. Communication was consistent throughout the semester, and we were able to coordinate with our sponsors in an effective manner through emails and meetings. Once we had our final concept, we were able to work together as a unit to make our separate parts come together. Although we didn't get as far as we had hoped, are team had good chemistry, knowledge, and dedication to make this project come to life.

Acknowledgements

Alex Thomason	Exec. Sum, 1.0, 1.2, 1.3, 2.0, 3.2, 4.1, 4.2, 5.1, 5.2, 5.3.1, 6.1, 6.3, 6.5, 8.3
Andrew Kim	Exec. Sum, 1,1, 2.2.1, 3.2, 5,2, 5.3, 6.2, 6.5, 6.6, 7.3, 8.2
Tyler Reichard	Exec. Sum, 1.4, 2.2, 2.2.1.2, 5.1, 6.1, 6.4, 7.1, 7.2, Proof Reading/Editing (all sections)
Eric Zhewen Li	Exec. Sum, 1.3, 1.4, 2.0, 3.1, 5.3, 6.1, 6.5, 8.2, Formatting, Citation
Jay Vyas	Exec. Sum, 2.0, 2.1, 2.2, 5.3.2, 6.5, 8.1, 8.2

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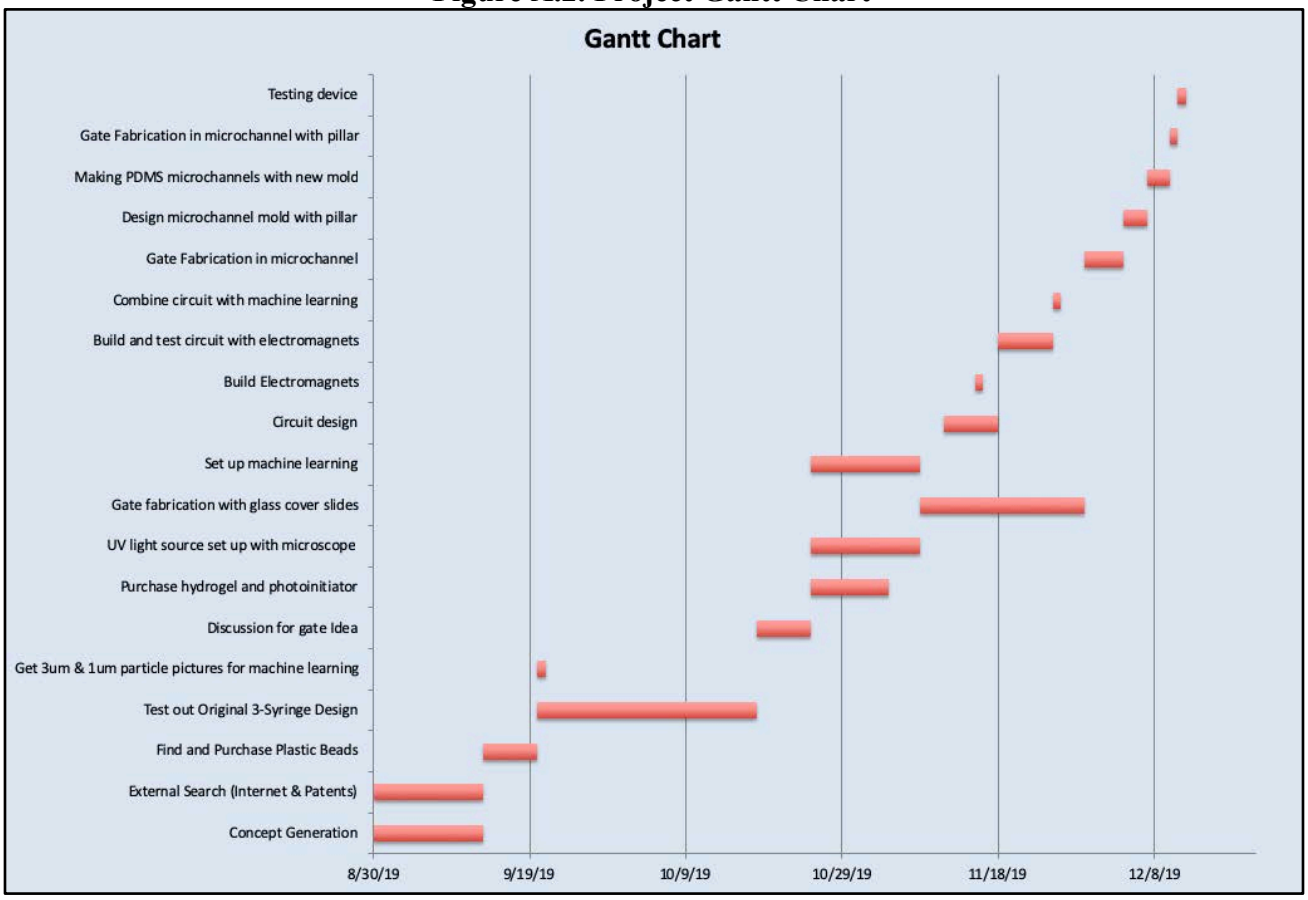
Appendix A: Project Gantt Chart

Table A.1. Project Timeline and Task Management

Task	Task Start	Days to Complete Task	Task End	Assignee
Concept Generation	8/30/19	14	9/13/19	All
External Search (Internet & Patents)	8/30/19	14	9/13/19	All
Find and Purchase Plastic Beads	9/13/19	7	9/20/19	Alex, Andrew
Test out Original 3-Syringe Design	9/20/19	28	10/18/19	Alex, Andrew, Tyler
Get 3um & 1um particle pictures for machine learning	9/20/19	1	9/21/19	Alex, Andrew, Eric, Jay
Discussion for gate Idea	10/18/19	7	10/25/19	All
Purchase hydrogel and photoinitiator	10/25/19	10	11/4/19	Alex and Andrew
UV light source set up with microscope	10/25/19	14	11/8/19	Alex, Andrew
Gate fabrication with glass cover slides	11/8/19	21	11/29/19	Alex, Andrew
Set up machine learning	10/25/19	14	11/8/19	Eric, Jay
Circuit design	11/11/19	7	11/18/19	Tyler
Build Electromagnets	11/15/19	1	11/16/19	Tyler
Build and test circuit with electromagnets	11/18/19	7	11/25/19	Tyler
Combine circuit with machine learning	11/25/19	1	11/26/19	Tyler, Eric, Jay
Gate Fabrication in microchannel	11/29/19	5	12/4/19	Alex, Andrew
Design microchannel mold with pillar	12/4/19	3	12/7/19	Alex, Andrew
Making PDMS microchannels with new mold	12/7/19	3	12/10/19	Alex, Andrew
Gate Fabrication in microchannel with pillar	12/10/19	1	12/11/19	Alex, Andrew
Testing device	12/11/19	1	12/12/19	All

Figure A.2. Project Gantt Chart



Appendix B1: Engineering Drawings

The following engineering drawings are the specifications for the SolidWorks drawings shown in Section 6.1.

Figure B1.1. Aluminum Tubing Connection for UV Source and Microscope

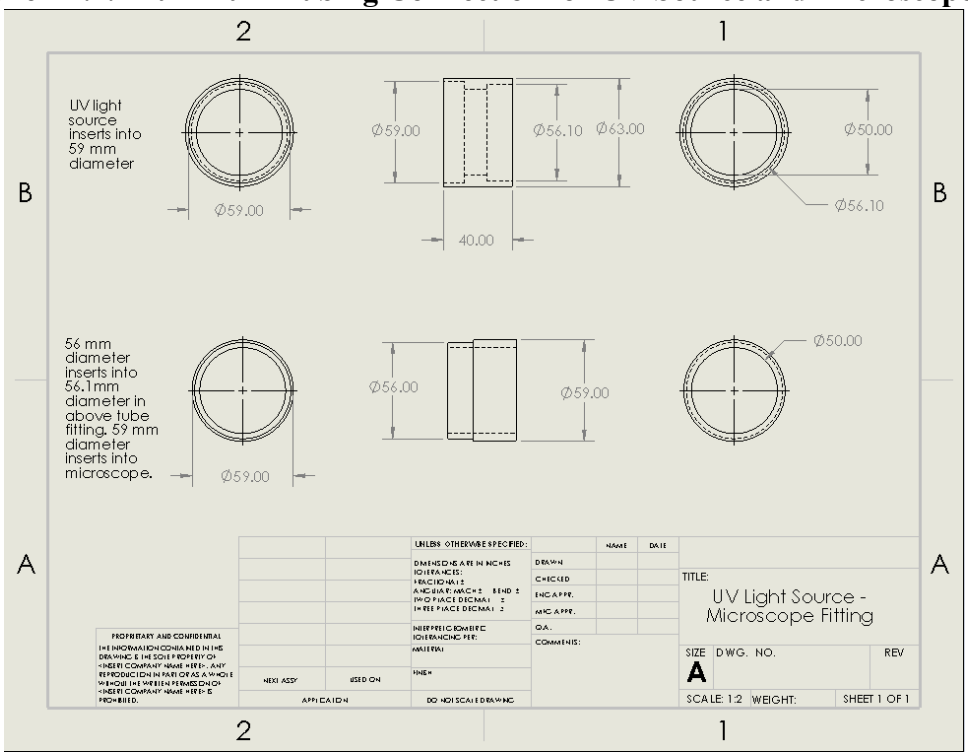


Figure B1.2. Base of Electro-Magnet (EM) Holder

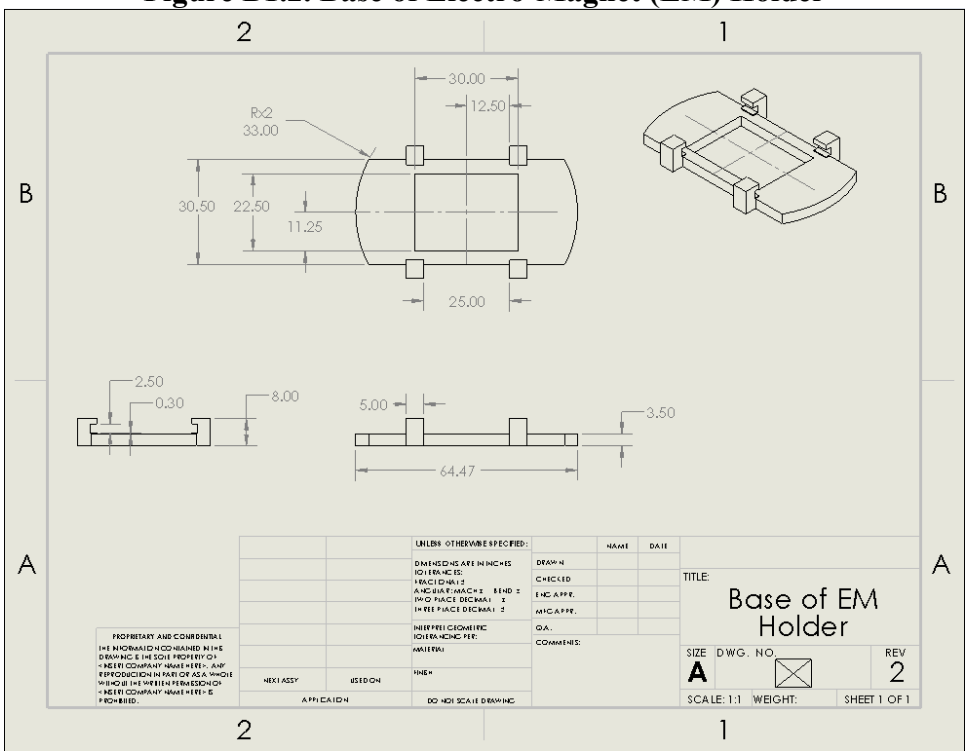
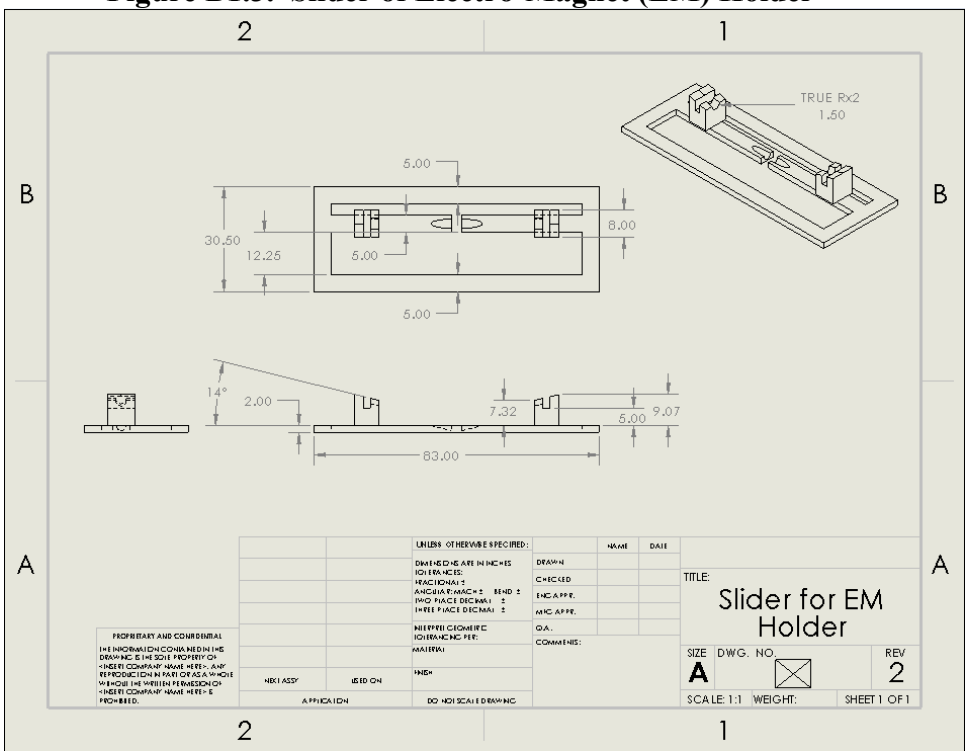


Figure B1.3. Slider of Electro-Magnet (EM) Holder



Appendix B2: Machine Learning Program Flowchart

Figure B2.1. Program Flowchart

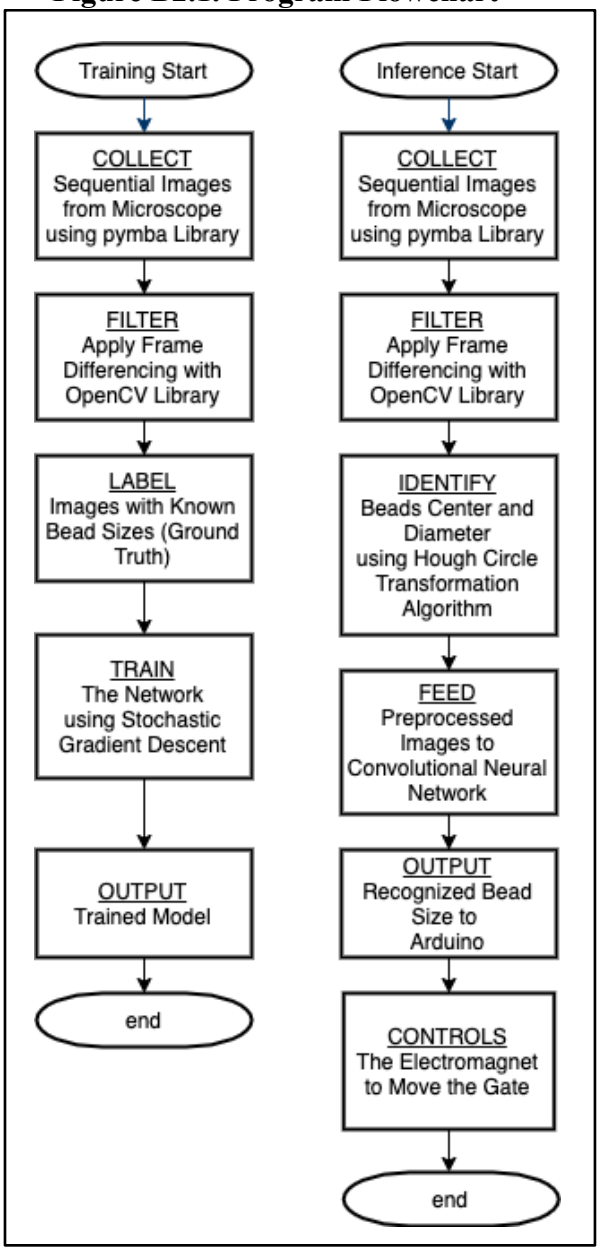
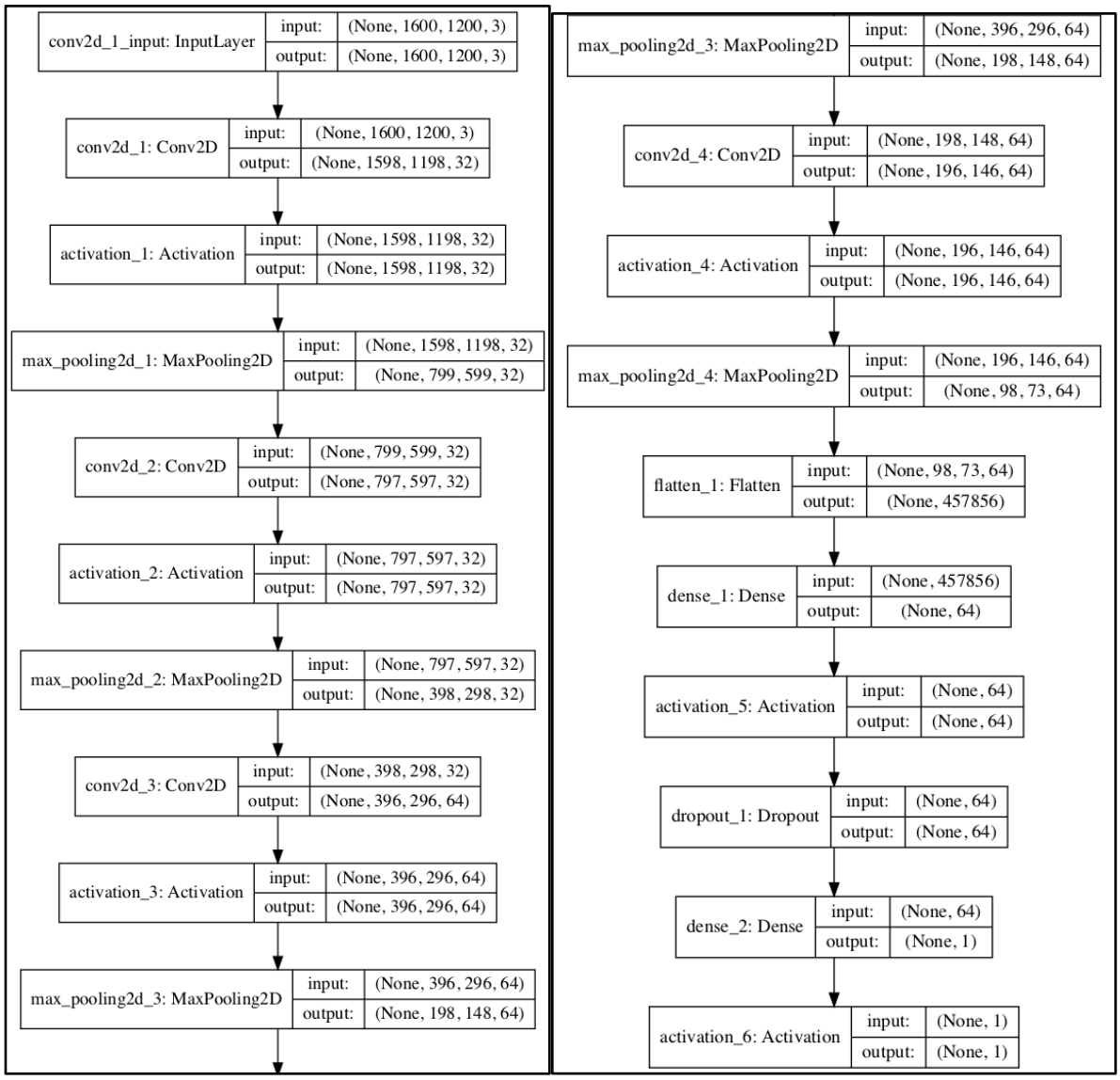


Figure B2.2. Neural Network Configuration



Appendix C: Bill of Materials

Table C.1. Full Bill of Materials

Vendor	Product Number	Product Description	Unit Cost	Qty	Total Cost	Makes	Cost per Unit
Sigma-Aldrich	437441-100ML	POLY(ETHYLENE GLYCOL) DIACRYLATE	$44.60	1	$44.60	200	$0.22
Sigma-Aldrich	405655-50ML	2-HYDROXY-2-METHYLPROPIOPHENONE, 97%	$50.80	1	$50.80	100	$0.51
Sigma-Aldrich	267953-5G	IRON, POWDER, LESS THAN10 MICRON, >=99.&	$28.00	1	$28.00	250	$0.11
Electron Microscopy Sciences [Provided by sponsor]	71887-30	Platinum Line Cover Glass 22 x 50 #1	$4.00	1	$4.00	70	$0.06
VWR [Provided by sponsor]	700014-034	Polydimethylsiloxane, Technical Grade	$98.57	1	$98.57	20	$4.93
N/A [Provided by sponsor]	N/A	Enameled Copper Wire	$6.98	1	$6.98	100	$0.07
N/A [Provided by sponsor]	N/A	1" 18ga Steel Nail	$1.50	1	$1.50	400	$0.00
N/A [Provided by sponsor]	N/A	Arduino Uno R3 Development Board	$15.98	1	$15.98	1	$15.98
N/A [Provided by sponsor]	N/A	ELEGOO MB-102 Breadboard 830 Point Solderless Prototype PCB Board	$3.00	1	$3.00	1	$3.00
N/A [Provided by sponsor]	N/A	EDGELEC 100pcs 1K ohm Resistor 1/4w (0.25 Watt) ±1% Tolerance Metal Film Fixed Resistor	$5.69	1	$5.69	50	$0.11
Amazon	FQP30N06L	MOSFET (FQP30N06L)	$15.88	1	$15.88	5	$3.18
Amazon	ASD1005	BJT Transitor (TIP120)	$6.25	1	$6.25	1	$6.25
				Total	**$308.15**		**$34.50**