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Study of Microfluidic Devices for Dielectrophoresis Separation of Cells in Liquid

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Study of Microfluidic Devices for Dielectrophoresis Separation of Cells in Liquid

THESIS

Submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Maryam Shakib

Thesis Committee:
Professor William Tang, Chair
Associate Professor Elliot Hui
Assistant Professor Jared Haun

2017

DEDICATION

For all my family and friends,

and my doctors at Cedars Sinai

TABLE OF CONTENTS

Page

LIST OF FIGURES

ACKNOWLEDGMENTS

I met my advisor, Professor Dr. William Tang, at the end of the first year of my Electrical Engineering PhD program. I had previously rotated in one other lab but hadn't been able to find anything that spiked my interest. At the first meeting with Dr. Tang, he was very kind and very understanding of my need of traveling back and forth between Los Angeles and Irvine. We spoke about his projects and which project he thought would fit me best. The project that initially assigned to me was the study of brain aneurysms, and finding the least invasive form of treatment.

However, a few months into me joining Dr. Tang's lab, in late October of 2013, I was diagnosed with breast cancer. The cancer was fortunately in its early stages, but not early enough that I could only have the surgery and radiation. I was diagnosed with a very aggressive form of breast cancer and I needed immediate attention. I had to have two surgeries, the first surgery was a lumpectomy and lymph node removal, the second surgery was required when my surgeon got the lab results back and was notified they did not get a clean margin. A short while after I had to go into the hospital to have a port placed into my arm, I was told my veins are not strong enough for them to inject the chemotherapy drugs directly into them. A week after the placement of the port I was scheduled to start my chemotherapy, but when I visited my oncologist she informed me that I have a slight temperature and she is not going to risk it. I had to wait another week for them to clear me to start my treatment. I was scheduled to go through six very painful chemotherapy sessions followed by 20 radiation sessions.

During my treatments at the hospital my friends and family were by my side each and every step of the way, my mother never left my side not even for a moment. My entire treatment took roughly about 8 months, during which Dr. Tang kept in contact with me and allowed me the

time off I needed to go through this trying time of my life. I can honestly say this experience has been by far the worst experience of my life and I could have not done it without my family and friends but mostly my mother. My mother made sure to keep me hydrated and fed, and she never allowed me to feel defeated or upset about my situation. She was there with me every second of every day, she sat with me through 7 hours of chemo each time, and she took care of me. I can honestly say without her I would have never made it.

When I came back from my leave, still bald from chemo and feeling weak, Dr. Tang asked me if I wanted to change my research topic to cancer cell detection since it was now personal for me. I initially said yes thinking it is a good idea, but what I didn't realize was how much damage this disease had done to me, both physically and mentally. Dr. Tang was kind enough not to expect me to come back full force since he knew I was not at 100% still. Once I started my research I realized I had a horrible case of PTSD, the first year back I struggled every day, every day I would break down and cry in fear of the cancer coming back and taking my life. To this day, I still have nightmares about this unfortunate event. There are days that I struggle to keep calm, every doctor visit is a couple of days of anxiety. At my last check up at the hospital in October of this year the radiologist told me she had seen a mass and was not sure what it was, after more painful testing and me having a few anxiety attacks they told me it was nothing.

At the end of 2014 I decided I did not want to pursue a PhD in electrical engineering and that I wanted to change my major to biomedical engineering. I spoke to Dr. Tang about this matter and he was more than understanding. Furthermore, he helped me reapply to UCI for a master's degree in biomedical engineering, he wrote all the required letters to the chair of the department and he never once gave me an indication that he wasn't pleased with me or this

process. Dr. Tang was and always is kind to me, he has always been understanding of my needs and he helped me on this journey through grad school every step of the way.

Therefore, I would like to take this opportunity to thank everyone from the bottom of my heart and to show my gratitude to my family, my friends and my professor who stood by me and never gave up on me. I will always remember my years here at UCI, the good ones and the tough ones, and I will always keep this beautiful campus and its vibrant people very close to my heart.

ABSTRACT OF THE THESIS

Study of Microfluidic Devices for Dielectrophoresis Separation of Cells in Liquid

By

Maryam Shakib

Master of Science in Biomedical Engineering

University of California, Irvine, 2017

Professor William Tang, Chair

In the path to finding a cure for cancer and saving a life it is essential to detect the disease in its early stages and to be able to eradicate it. In most cases of cancer, the disease is not detected until it is in later stages and has metastasized to vital organs. However, with current advances in medicine the doctors are able to extend the life expectancy of the patient through the use of chemotherapy drugs, radiation therapy and radical surgeries. Currently the focus of the researchers has been put on developing immunotherapy drugs, which are targeted therapies. Chemotherapy has been a somewhat effective therapy, but the side effects of chemotherapy are often too much to bear, loss of hair, loss of appetite, severe nausea, and lack of energy all contribute to a lower quality of life.

In order for the scientist to be able to design targeted therapies with much less side effect and higher rate of success in curing or managing the disease they need be able to have access to the cancer cells in the early stages of the disease. They need to be able to study the cell and its genomic makeup.

The objective of this research is to find a more effective and accurate way of screening for cancer cells, isolating and collecting them. Through the design of microfluidic devices, one can hope to be able to detect the circulating tumor cells in the blood, trap them and separate them from the other contents of blood for study.

The ultimate objective of this project is to design and fabricate a device capable of using Dielectophoresis to separate cancer cells from blood cells accurately. The design of the Microfluidic devices is done using the software SolidWorks, the simulation is ran using the software COMSOL, and the fabrication is done in the lab using photolithography. However due to the time constraints and not having the required trainings or certificates for handling live cells and blood specimens this project was not tested with cancer cells.

Chapter 1: Introduction

1.1 Cancer Diagnosis

In most diseases, often the most effective source of information is the blood. Physicians accomplish this task by collecting the blood and analyzing the molecules and cells contained within the blood. Depending on the concentration, absence or presence of leukocytes, platelets, and erythrocytes the physician can diagnose the disease [1].

In cancer research, early detection is the key to a more successful treatment and higher survival rate.

According to the National Cancer Institute, the following cancer types are the most common types of cancer diagnosed in the United States of America each year. In order for these cancers to be considered common, the annual incidence for the year of 2017 had to be 40,000 cases or more [2].

Bladder, Breast both Female and Male, Colon and Rectal (Combined), Endometrial, Renal Cell and Renal Pelvis, Leukemia, Liver and Intrahepatic Bile Duct, Lung, Melanoma, Non-Hodgkin Lymphoma, Pancreatic, Prostate, and Thyroid are the most commonly accruing cancers [2].

Each type of cancer depending on its location and symptoms has a different process of detection. In the case of a breast cancer, the cancer can be detected by annual mammograms or self-examination. In the case of pancreatic cancer however, the detection usually does not happen in the early stages. This is due to the location of pancreas deep inside the abdomen behind the stomach. As a result, diagnosis of the disease in the early stages through physical exams is impossible. The most effective way

is to look for the specific tumor markers (CA 19-9) in the blood [3], but unless it is suspected that the disease exists doctors often do not regularly test for these markers. Therefore, it is imperative to diagnose the cancer in its early stages, prior to spreading to other vital organs, for the effectiveness of the treatment, and increase in patient's life span and quality.

1.2 Molecular and Cell biology

Cells are the building blocks of life, and the biological world is composed of two types of cells, prokaryotic and eukaryotic cells. The difference between the prokaryotic and eukaryotic cells is the lack of a nucleus and other organelles in the prokaryotic cell [4].

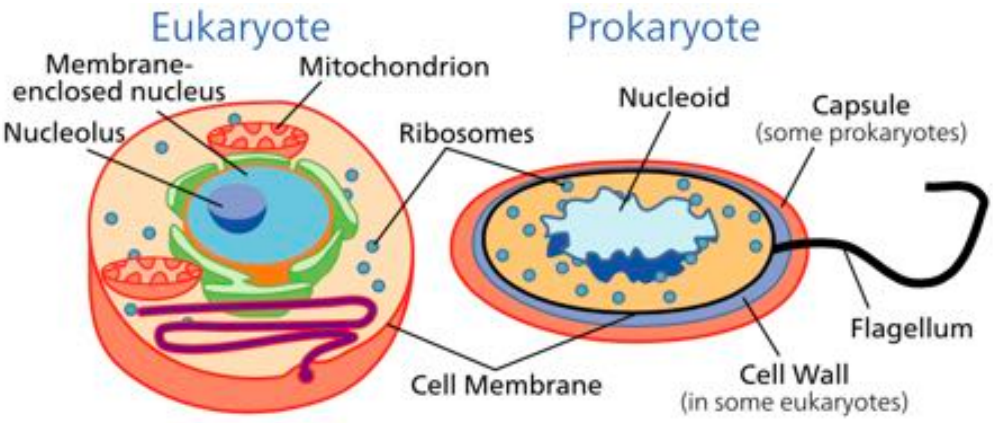

Figure 1. Cell Structures of Eukaryotes and Prokaryotes

Since the prokaryote cells are either bacteria or archaea, they are not the subject of study for cancer, the study of cancer involves the Eukaryotic cells.

The ideal situation for mitosis and cell division is for the DNA to replicate itself perfectly and produce an identical chromosol replica each time[5]. During this replication cells go through checkpoints to ensure there is no damage to the replicated DNA, if a damage is

detected, the cell will then enter apoptosis to prevent the cell from mutating and spreading without control. When one of these check points fail to recognize a damaged cell, the cell mutates, replicates and becomes cancerous[5].

Cell organelles and the nucleolus are separated from the outside world by a phospholipid bilayer, but for cells to be able to live and thrive they need to be able to have an intake if biological materials and exert byproducts of their biological activities. Cells also need to communicate with other cells in order to be able to function as a system, this action is done through cell signaling[6]. The biological molecules either will enter and exit the cell through diffusion or through the use of the ion channels. Our interest rests with the ions that travel through these channels, the concentrations of these ions will set the cell surface potential. This potential can then be utilized in separation from blood cells using an electric field.

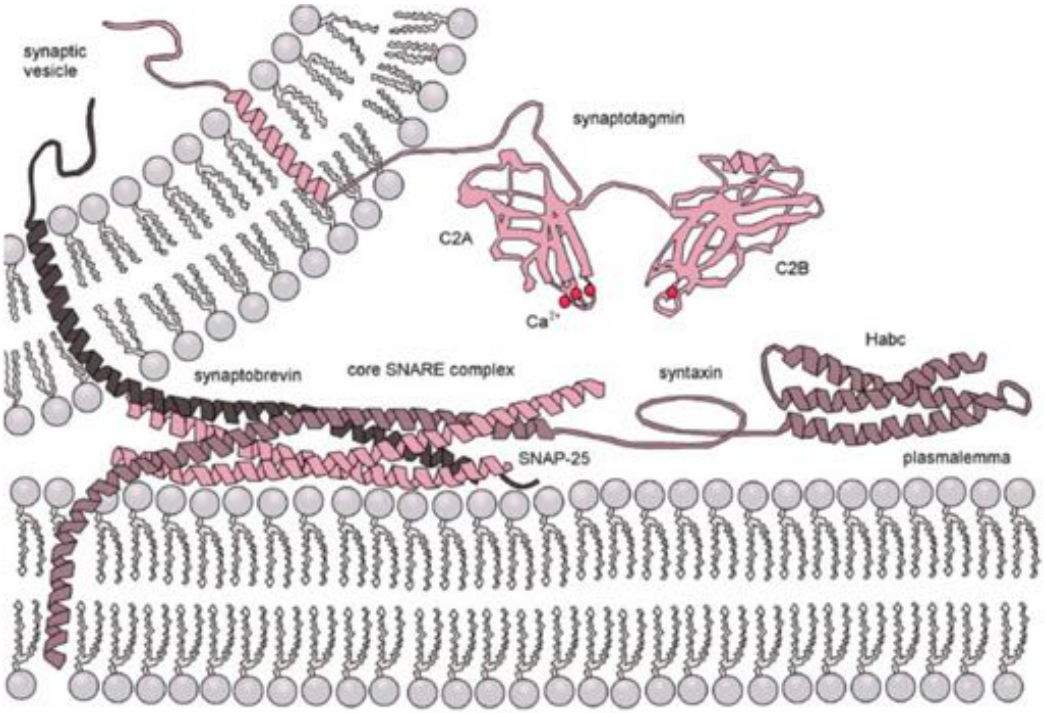

Figure 2. Phospholipid bilayer of a cell

1.3 Importance of early detection

"Cancers comprise a major part of the global chronic disease burden and increasingly contribute to the mortality in low and middle income countries"[7].

It is projected that by the year 2030 the resulting death from cancer will rise to 11.5 million people annually [7]. It is imperative to detect this disease in its early stages in order to have a higher chance of survival. Cancer cells have the ability to disseminate to distant organs, colonize and disable the orangs. Metastatic cancer is responsible for roughly 90% of cancer related deaths [8]. Early detection will allow for the physician to localize the disease and put in place an effective treatment plan, be it surgery, chemotherapy, radiation therapy, or any combination of the three. Tumor removal is the best course of action in cancers that have not metastasized. Metastatic cells can often remain dormant and not be effected by the chemotherapy drugs, for the chemotherapy drugs target cells that are going through cell division at higher rates. These cells can at a later stage become reactivated and claim the life of the patient. Hence the desire for an early detection, and methods that make early detection more efficient and successful.

1.3.1 Methods of detection

For centuries humans have been curious about blood and all its properties; in the year of 1668 a Dutch biologist and microscopist by the name of Jan Swammerdam observed and described the red blood cells [9]. After his break through scientist became more and more curious, from this curiosity came the science of hematology. For years scientists have tried different avenues in order to find the most optimum path to cancer detection, study, and treatment.

The most important tool in battling a disease is understanding the disease. In the case of cancer that falls on being able to locate the cancer cells in the early stages and isolating them from the surrounding cells.

The first and most commonly used technique for detecting cancer cells is through the collection of the patients' blood sample. After the blood is collected from the patient it needs to undergo testing, this testing is done through staining and studying the blood sample under a microscope. The stained blood is smeared as an either a thin film or a thick film on a glass slide [10], this glass slide is then looked at under a microscope to see if there are any cancer cells present. In order for the scientist to be able to detect the unwanted cells, the blood will go through different staining procedures. This technique however is not useful in separating the cancer cells from the blood sample.

Circulating Tumor Cells (CTCs) have been the main focus of researchers for years, CTCs allow for in-depth study of key behavior of metastatic cancer cells. Isolating the CTCs has proven to be challenging for the scientists, these cells are often rare and mixed with approximately 10 million leukocytes and 5 billion erythrocytes in 1 ml of blood [11]. One method currently in use for cell separation is flow cytometry, another is Dielectrophoresis.

1.4 Summary

It can be seen that the detection of cancer cells in the early stages of development is crucial for the fight against cancer. Scientist are continuously looking for more effective ways of cancer detection, treatment, and cure. The objective is to get a better understanding of the disease and find ways of treatment that does not reduce the quality of life for the patient. The current Chemotherapy drugs used in the market can have

severe side effects that cause the patient to have a lower quality of life. These treatments take a toll on the patients both physically and mentally.

2.1 Flow Cytometry Overview

The first generation of a particle sorting apparatus was invented by Mack J. Fulwyler in 1968 (Fig.3). The objective of this invention was to be able to separate cells based on their size, radioactivity, or luminescence [12].

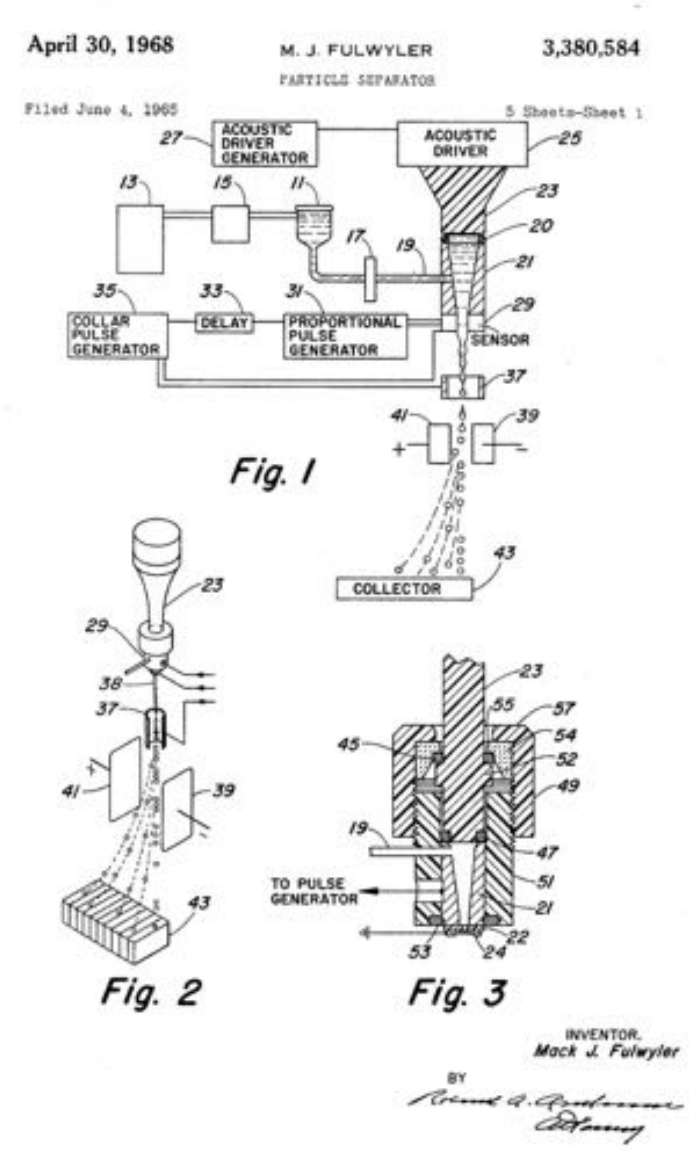

Figure 3. A Schematic Diagram of a Simplified System[12]

In flow cytometry cells that are labelled with fluorescent molecules are suspended in a liquid and passed through an array of lasers. A sensitive photomultiplier is then is used to measure the fluorescent intensity of each particle [13]. Thousands of cells pass one by one through one or more laser beams per second in a flow cytometer. The flow cytometer then measures the scattered lights at several angles and fluorescence emissions [14]. Flow cytometer is comprised of three parts, the fluidic system, the excitation source along with the detectors, and the electronic system for digitalizing the signal for analysis[14]. Flow cytometers can be used for multitude of applications, cell sorting, cell counting, multi-parametric DNA analysis and so on.

2.2 Dielectrophoresis overview

Dielectrophoresis (DEP) was named and first understood by Herbert Pohl in the 1950's. Pohl defined the dielectrophoresis effect as "the motion of suspensoid particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field" [15]. The term DEP is used when describing the motion of a particle exposed to an electric field gradient [16].

The idea is when a particle is placed in a non-uniform electric field it will become polarized and experience an induced electric dipole moment.

The magnitude of this induced electric dipole moment can be calculated by [16],

$$m_{eff} = 4\pi\varepsilon_m r^3 pE \quad (1)$$

where m_{eff} is the effective induced dipole moment, ε_m is the absolute dielectric permittivity of the fluid in which the particle is suspended, r is the radius of the spherical

particle, p is the effective polarizability per unit volume of the particle, and E is the applied electric field [16].

The importance of a non-uniform electric field is displayed when the particle is subjected to this field; the polarized cell when placed in a non-uniform filed. The effect of this field gradient will have a net electrostrictive force acting on the polarized cell. Depending on the polarity of this induced dipole moment, the particle can either move towards the regions of large spatial variation of the electric field (positive DEP) or away (negative DEP) [16].

For the purposes of particle separation this non-uniform electric field will need to be produced by an alternating current. The resulting field will then produce a force as stated in equation (2) [16],

$$F_{\mathrm{DEP}} = \left(m_{eff}.\nabla\right)E, \tag{2}$$

combining equations (1) and (2) the following equation can be derived:

$$F_{\mathrm{DEP}} = 4\pi\varepsilon_m r^3 p(E.\nabla)E \tag{3}$$

As it was discussed in section 1.1, cells have distinct electrical properties, these properties are defined by the ion channels and ion concentrations within the cells. These properties will cause the cells to polarize when subjected to an electric field, however this does not mean that the cells are electrically charged.

2.3 Microfluidic Overview

In the year 1959 Richard Feynman gave a thought-provoking speech in which he stated, "There is Plenty of Room at the Bottom" [17]. This quote has become a driving force for many scientists, and since humans have made ground breaking advancements in the

world of technology and engineering. In 1947 two physicists by the names of John Bardeen and Walter Brattain let by William Shockley invented the transistor commonly known as the Bipolar Junction Transistor. A few years later in 1956, Shockley, Bardeen and Brattain won the Nobel prize in the field of physics for inventing a revolutionary solid-state amplifier[18].

In the following years researchers and scientists made advances in fabrication and production of smaller and more efficient transistors. In the late 1970's silicon technology was used for machining mechanical microdevices [17]. In late 1980's the development of microflow sensors, micropumps, and microvalves dominated the early stages of microfluidic devices [17]. With the advancement in silicon wafer etching, and moving to submicron feature sizes, the field of microfluidic has been able to take advantage of these processes in design and production of microchannels used in microfluidic devices.

One of the most important part of study of microchannels is the behavior of the liquid in the channel. From the study of fluid dynamics it is know that fluids in channels can behave in different ways, they can either have a turbulent flow or a laminar flow [19]. Turbulent flow and laminar flow are characterized by their Reynolds number (which is unit less), this number can be calculated using the below equation,

$$Re = \frac{\rho v D}{\mu} \tag{4}$$

Where ρ is the density of the fluid, v is the mean velocity of the fluid, D is the hydraulic diameter of the circular channel, and μ is the dynamic viscosity of the fluid. When the Reynolds number is less than 2300, the flow is laminar and when it is larger than 2300 it is turbulent [19]. In the case of microchannels this fluid flow is of the laminar flow.

2.4 Summary

In this chapter a brief over view of flow cytometry, dielectrophoresis, and microfluidics was given. One can conclude, in the past century scientist have made strides in the advancement of both electronic and biomechanical fields. For the remainder of this thesis the focus will be on the design, fabrication and assembly of microfluidic devices utilizing dielectrophoresis for particle separation.

Chapter 3: First Generation Microfluidic Design

This research project is a continuation of a previously designed microfluidic device by a fellow researcher at UCI. Having helped the fellow student in his experiment and having learned from him, it was decided to continue in his footsteps and evolve his design.

3.1 Channel Design

The initial design of the channels for the first generation of the microfluidic device for this project was a simple three input, three output channels. The first step of the design was to determine how the channels and electrodes should be located in respect to each other for maximum effect. For simplicity, it was decided to have a design where there are three inputs, one main channel, and three outputs. The idea was that a constant stream of fluid in the center of the channel can be stablished, containing particles that were later going to be separated downstream in the channel. The objective of the design was to use the center input for this fluid and the top and bottom inputs for the guide buffer. The guide buffer would keep the fluid roughly at the center of the channel prior to exposure to the electric field. After the exposure of the mixture to the electric field the particles, depending on being negative or positive DEP, would move towards one or the other electrode in the channel and ultimately be removed from the top or the bottom collection wells at the output.

The diameter of the input channels where chosen to be 1mm, the main channel width was chosen to be 100μm with a depth of 50μm, the collection well diameters were chosen to be 3mm. The preliminary image of the channels are displayed in fig (4).

Figure 4. 2D Channel Structure

The next step in designing the microfluidic device was to take into account the potential existence of lager particles (>20μ) in the fluid. In order to ensure these particles were not going to enter the system and cause blockage or damage to the device, it was decided to have three rows of filters at the input. These filters were made up of three rows of pillars, each row having a different size pillars and a different distance between the pillars. The space between any two pillars ranged from 60μm to 30μm, with 60μm being the row closest to the input and 30μm being the row closest to the main channel. This design was put in place to ensure only particles of 20μ and smaller can enter the main channel, (Fig. 5). In figure (5) it can also be seen the two guiding pillars toward the end of the main channel. The objective of these guide pillars was to direct the separated particles in the flowing fluid into the different output wells, i.e. the stream containing the separated particles will either flow at the top wall of the channel or the bottom wall of the channel, and these guide pillar will ensure they continue to do so after the they are no longer under the influence of the electric field.

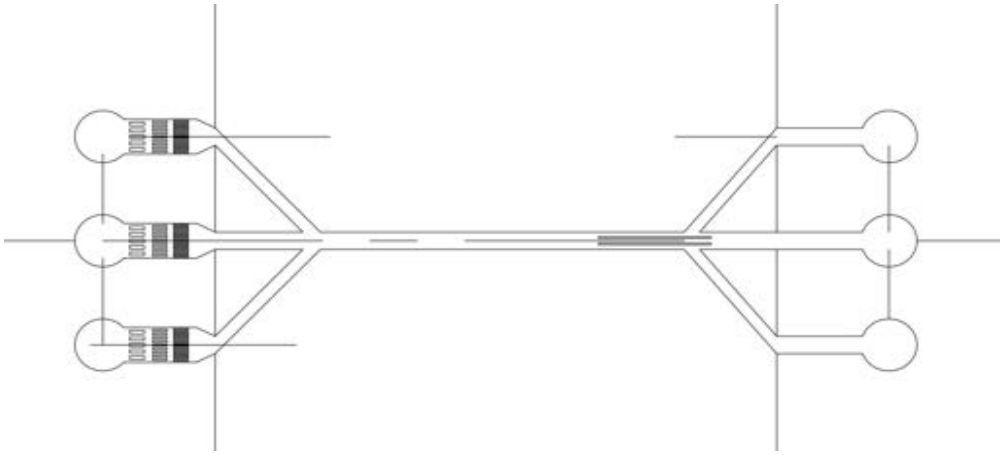

Figure 5. Channels with Input Filters

3.2 Electrode Design

For the initial design, it was decided for the device to have two electrodes, one on each side of the main channel. The width of the channels was chosen to be equal to each other, the intensity of the electric field across the channels however was controlled by the micro openings in the side walls of the channels. These openings on the top and bottom wall of the channel allowed for the electric field to travel across the channel; the frequency of these channels and the distance between each channel allowed for an ununiformed field across the channels. As it can be seen in figure (6) the frequency of these openings is much more on top than on the bottom wall of the channel. The width of the channels also differed in size to allow for a much stronger field on one side and a weaker field on the opposite side. This ununiformed electric field would then be used to separate particles based on their size and the surface potential, attract them either to locations where the field is the strongest or to locations where the field is the weakest. In the case of this project negative DEP was used.

The electrode material used for this design was solder wire. The reason for this choice was the low melting point and ease of use.

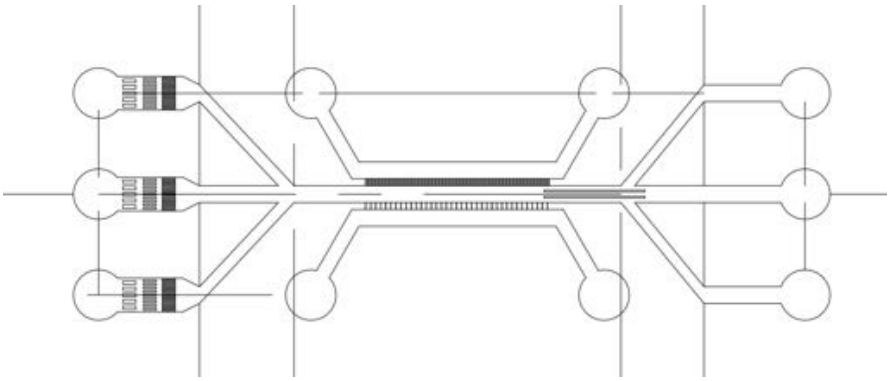

Figure 6. Completed Channel and Electrode Design

3.3 Fabrication and assembly of Device

3.3.1. Mask production

After the design of the device was completed using SolidWorks®, the saved file was then transferred to AutoCAD® format for printing of the mask. The AutoCAD® file was then sent to CAD/Art Services, Inc. for printing (fig 7). As it can be seen in figure 7, the CAD file was designed for a 4-inch silicon wafer that could accommodate the placement of three complete channels with the electrodes. The design also included a clear ring of width 1-inch around the 4-inch wafer, this clear ring was placed on the mask for use in the alignment of the mask with the wafer in the fabrication process. The mask used was a quartz film (transparent) which was then covered with chrome in the areas which needed to be exposed to the ultra violet light and eventually dissolve away. Negative photolithography was used for the processing of this wafer.

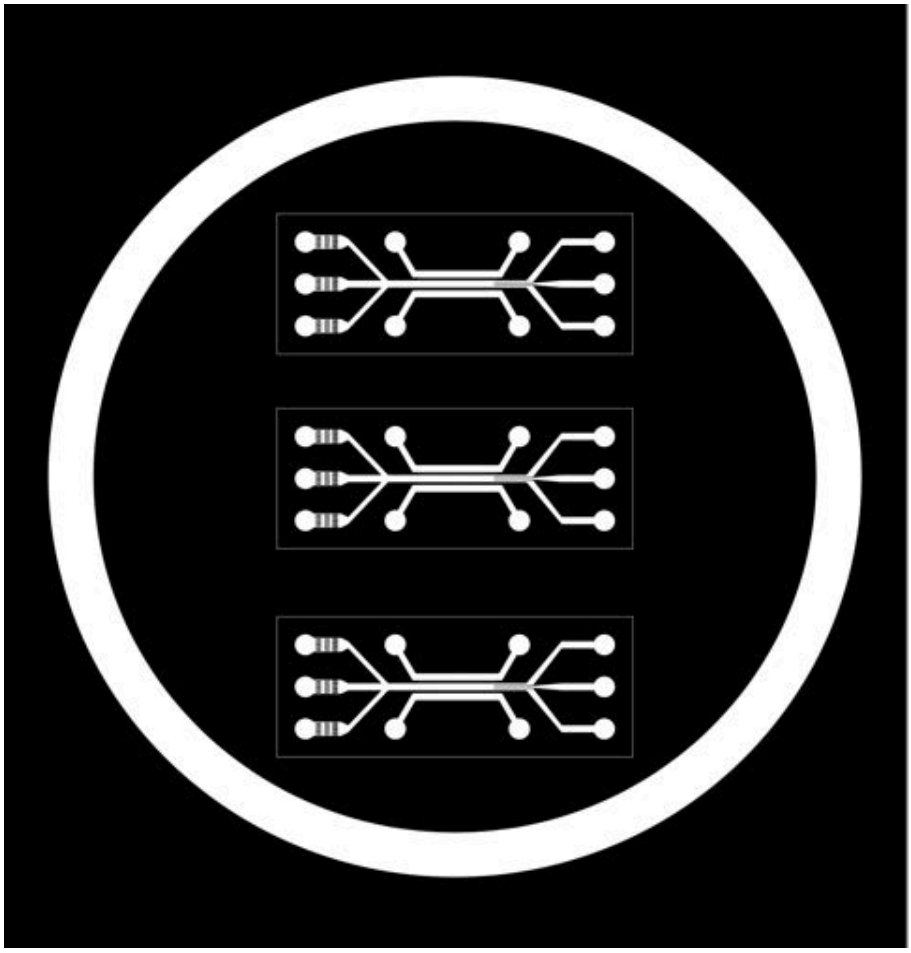

Figure 7. Mask CAD Design

After the mask was processed, produced, and returned by the CAD/Art Services, Inc. it was sent to the UCI's cleanroom lab for cleaning, fabrication and development. Figure 8 is the picture of the actual mask in the cleanroom.

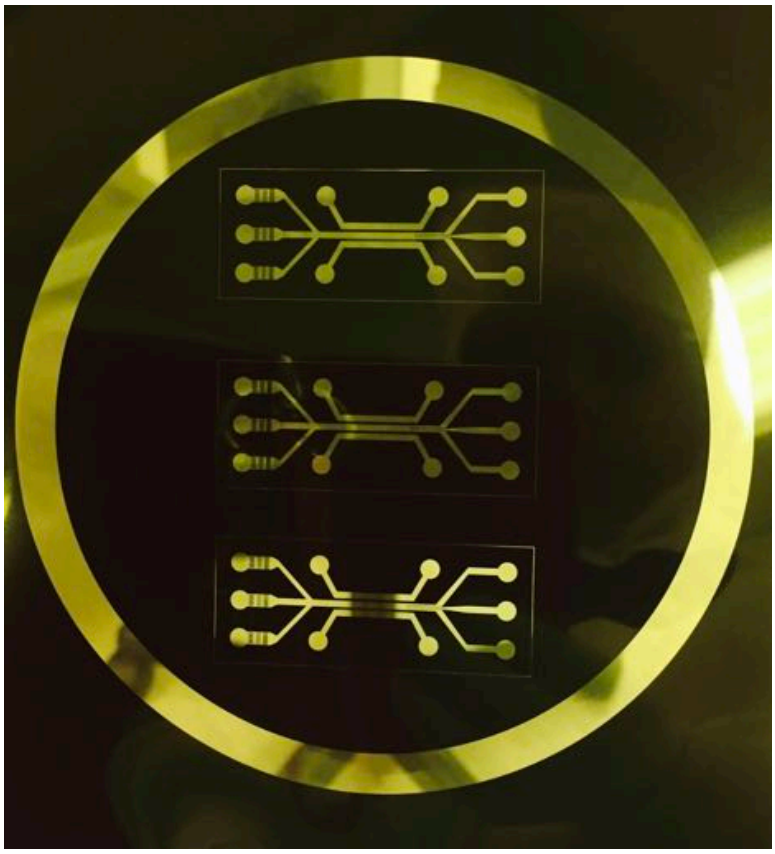

Figure 8. Printed Mask on a Quartz Film

3.3.2. Photolithography and mold production

Photolithography is vastly used in the world of integrated circuits, it is used to etch Micro and even Nano sized transistors onto positively or negatively doped silicon wafers. Photolithography is the process of exposing a photosensitive polymer to a light source through a mask with patterns [20].

The steps to negative photolithography are as follows: Cleaning of the Wafer, dehydration bake, deposition of SU8 (negative photoresist), soft baking, exposure to UV light, post exposure bake, wet developing, and post develop baking.

The photolithography process was done in UCI cleanroom located in Calit2 building by an authorized UCI personnel.

Prior to photoresist deposition, the wafer needs to be cleaned of any contaminates or particles that could cause a defect in the production of the mold.

A hydrofluoric acid dip is used to clean the wafer from any potential contaminates or particles. After the HF dip the wafer is then put in an oven for a dehydration bake to remove any water or water vapor that might have been deposited on the wafer. The presence of water or water vapor can hinder the adhesion of the SU8 to the substrate. Figure 9 is the image of the wafer after an HF dip and dehydration bake.

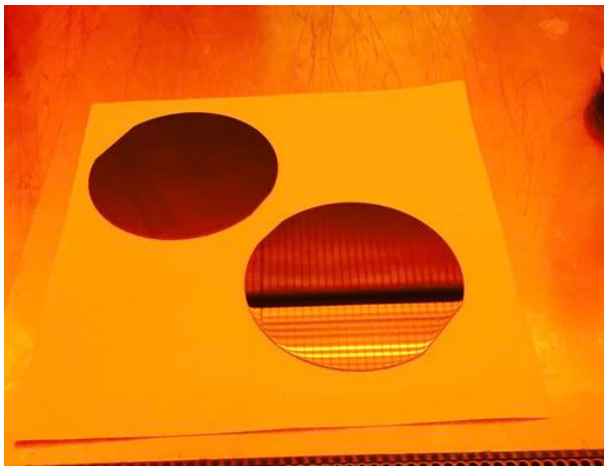

Figure 9. Cleaned and Dehydration Baked Wafer

Figures 10, and 11 demonstrate the wafer being placed on the vacuum chuck resist spinner and SU8 depositing on the wafer. Since a channel depth of 50µm was desired the wafer was spun at 2000rmp for one minute [21].

After the spin cycle was completed the wafer with the SU8 was transferred to an oven for a soft bake at approximately 80°C. The soft bake will secure adhesion of the resist layer to the wafer [20].

Figure 10. Wafer Placement on the Vacuum Chuck Spinner

Figure 11. SU8 Pour

Figure 12 is the image of the wafer after the soft bake was completed.

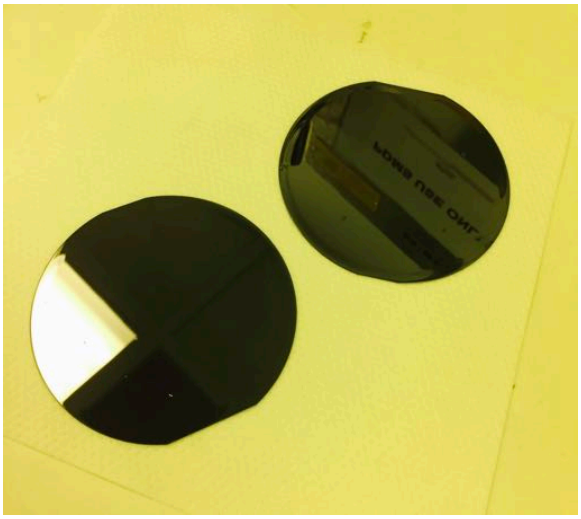

Figure 12. Soft Baked Wafer with SU8

After the completion of the soft bake, the wafer was transferred to a UV chamber (Fig. 13) for exposure. The mask is then placed on the wafer and aligned by using the clear ring printed on the mask. After the mask is placed on the wafer the wafer is exposed to a UV light (Fig. 14), the exposure of SU8 to UV light will cause the SU8 molecules to be photochemically rearranged and form an insoluble product [22].

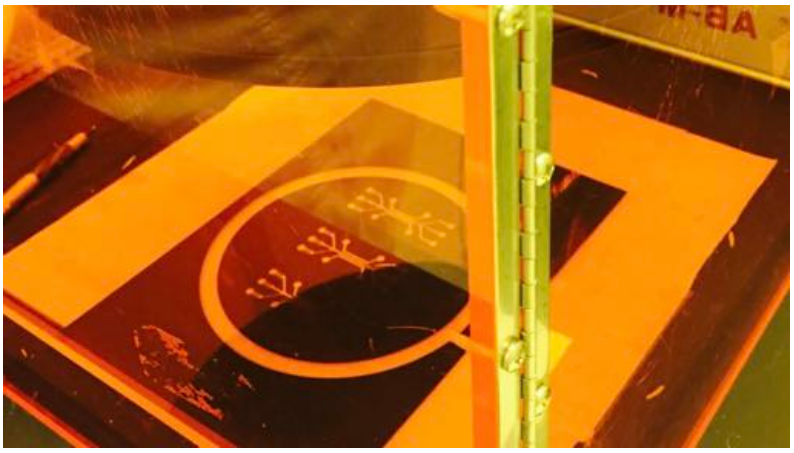

Figure 13. UV Chamber

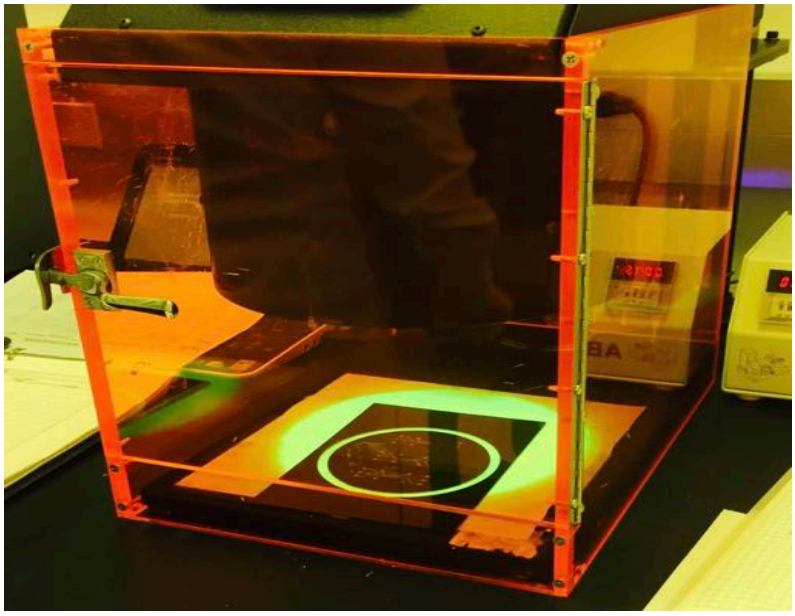

Figure 14. UV Exposure

After the exposure is completed the wafer is transferred to the oven for a postexposure bake. Upon completion of the postexposure bake the wafer is then moved to a container which contains 1-Methoxy-2-propyl acetate [23], the acetate will then dissolve the areas of the wafer that have not been exposed to the UV light (Fig. 15). This will leave the wafer with a 50μm high channel mold (Fig. 16), this mold will have to then go through another bake. This bake was done gradually over a period of two and a half hours on a hotplate. The starting temperature was set to 60°C, this temperature was risen by increments of 20°C every 20 minutes until reached 120°C. The wafer was left at 120°C for a period of 20 minutes, after this time had lapsed the temperature was brought down, at the same rate it was risen, back to 60°C. When the bake was done the wafer was allowed to cool down to room temperature before placing it into a vacuum chamber for Silane depositing. When the wafer was cooled down to room temperature it was placed in the vacuum chamber with 5μL of Silane enclosed in chemical hood. The wafer was

placed in the chamber for a period of 24 hours, at which time the Silane was completely deposited on the wafer, this step is necessary to prevent PDMS from attaching to the wafer.

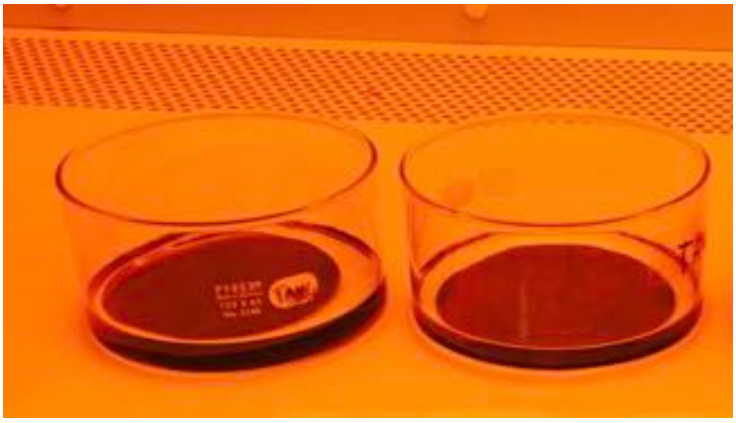

Figure 15. Wet Developing

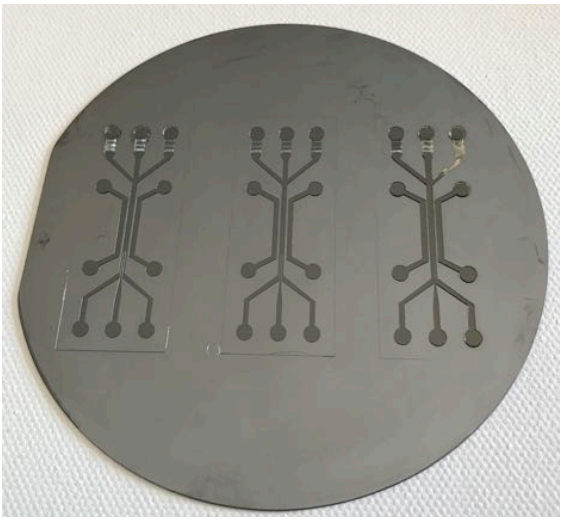

Figure 16. Micro Channel Mold-First Generation

3.3.3 PDMS molding

The fabricated mold was then placed into a petri dish for a Polydimethylsiloxane (PDMS) pour. PDMS was mixed at a ratio of 10:1 (silicone elastomer base : curing agent) and poured over the mold. The mold containing the PDMS was then placed into a vacuum

chamber for a period of two hours for any all air to be completely removed from the mixture. The presence of air in the mixture produces problems downstream for testing, the air bubbles could disrupt the flow of liquids in the channel or even render the device useless.

After degassing the PDMS the dish was placed in an oven at a temperature of 65°C for a period of two hours to completely cure the PDMS. After the curing of the PDMS the dish was removed from the oven and the area containing the channels was cut using a surgical blade. The cut PDMS contained the channels for the inputs and outputs, the main channel, and the electrode channels. The channels were then plasma bonded to a slide glass. The purpose for this was to be able to see through the glass on the microscope, and also PDMS could be plasma bonded to glass very efficiently.

3.3.4 Electrode Instalment

The next step for the device was the placement of the electrodes. The electrode was chosen to be a low melting point solder wires, the approach was to place the device on a hotplate at a temperature of 50°C and very slowly feed the wire into the electrode channel. Once the electrode would come in contact with the slide glass on the bottom of the PDMS sitting on the hot platen it would melt, and by feeding more wire into the electrode channel it would fill the channel completely.

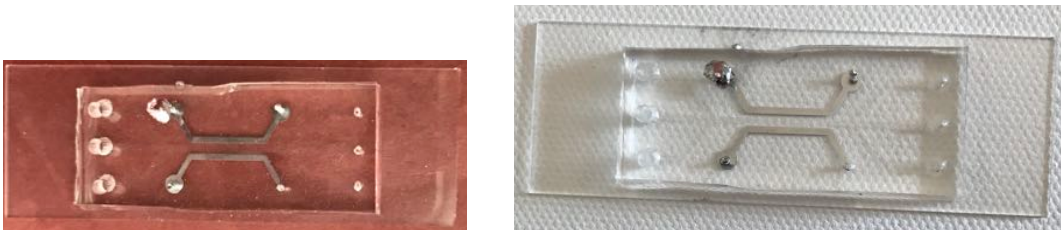

Figure 17. Completed Device

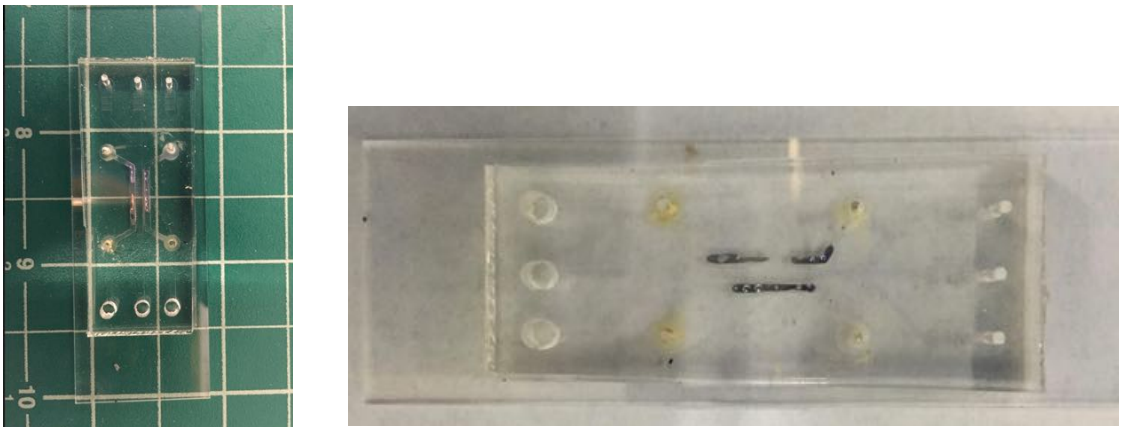

Figure 18. Incomplete Electrode Placement

However, placing the electrodes in this manner proved to be more difficult than anticipated. When placed under a microscope it could be seen that the metal had not uniformly filled the channel and as a result it was not possible to get the desired effect. Figures 17, and 18 display the results of the completed devices, with figure 17 having a complete electrode placement, and figure 18 showing an incomplete electrode placement.

3.4 Testing and Results

For the testing of the device two different size microspheres were purchased from Polysciences, Inc. 6.0µm Polychromatic Red Microspheres, and 20.0µm Yellow-Green Microspheres.

DEP buffer containing the 6μm beads was injected into the center input to the channel via a syringe pump at a rate of 0.8μL/min along with the DEP buffer in the top and bottom inputs to the channel at 1.2μL/min.

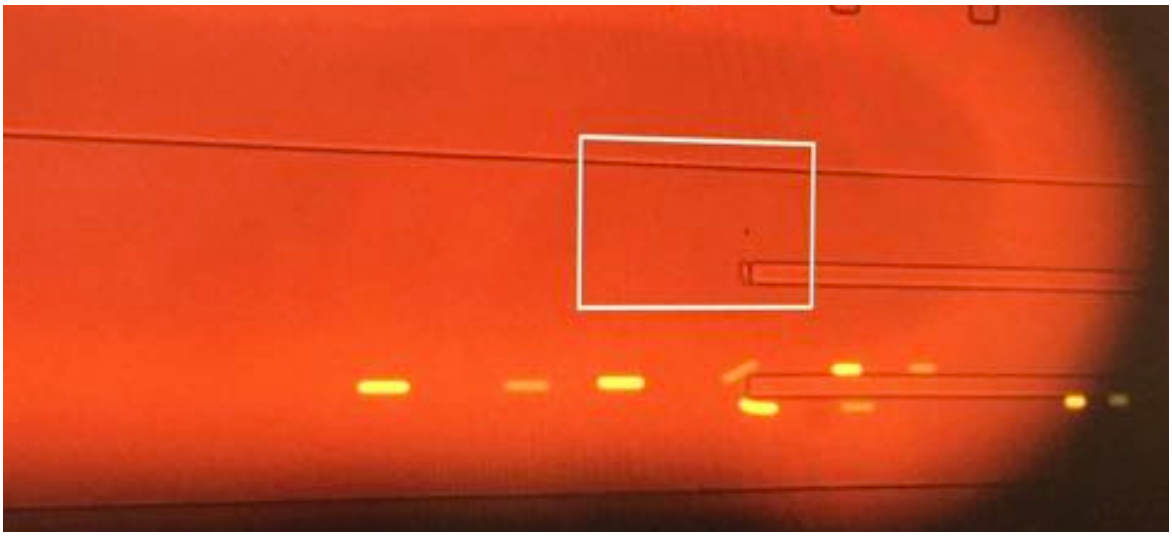

Figure 19. DEP Buffer Containing 6μm Beads

It was observed that after a few minutes the beads which entered the channel would flow into the openings on the top and bottom wall of the channel. This was a cause for concern, since those openings were to allow the electric field to travel across the main channel. This crowding and getting stuck in the openings cause the device to not function as intended. The result of this initial testing resulted in the abandoning of this design and moving to a different design.

Chapter 4: Second Generation Design

4.1 Channel design

Since the previous channel design proved to be ineffective it was decided to change the design of the channel. The next design would move away from placing the electrodes on the same plane as the channel on the PDMS. It was decided that the channel would have a similar design to the previous design with some changes. The major change was to remove the electrode channels from the main channel design and place it on a different plane. Therefore, the main channel would not have any openings to the electrode channels. It was decided to utilize the movement of the beads into the openings and place side channels for the transport of the beads on the main channel, the idea was derived from freeway exits. The electrodes then would be printed on a glass slide and placed directly over the main and side channels. This would allow for the microspheres to be pulled into the side channels by the non-uniform electric filed.

The overall design of the inputs and outputs are essentially the same. Much like the previous design, it was chosen to have three inputs with the filters and three outputs. However, in this design the outputs were not all connected to the main channel. Each channel, the main and side channels, each had their own specific output well. This would make it more efficient for collection of the particles. The two side channels were connected to the main channel through side branches. The main channel was designed to have five side branches on each side. The side channels would then join together along the way and end in an outlet well. At the beginning the sizes for these side channels and the main channel was chosen without taking into account the pressure and the flow rate

of the liquid. As it soon became evident through COMSOL simulation the side channels had little to no flow in them due to the fact that the main channel remained the same size after each branch (Fig. 20).

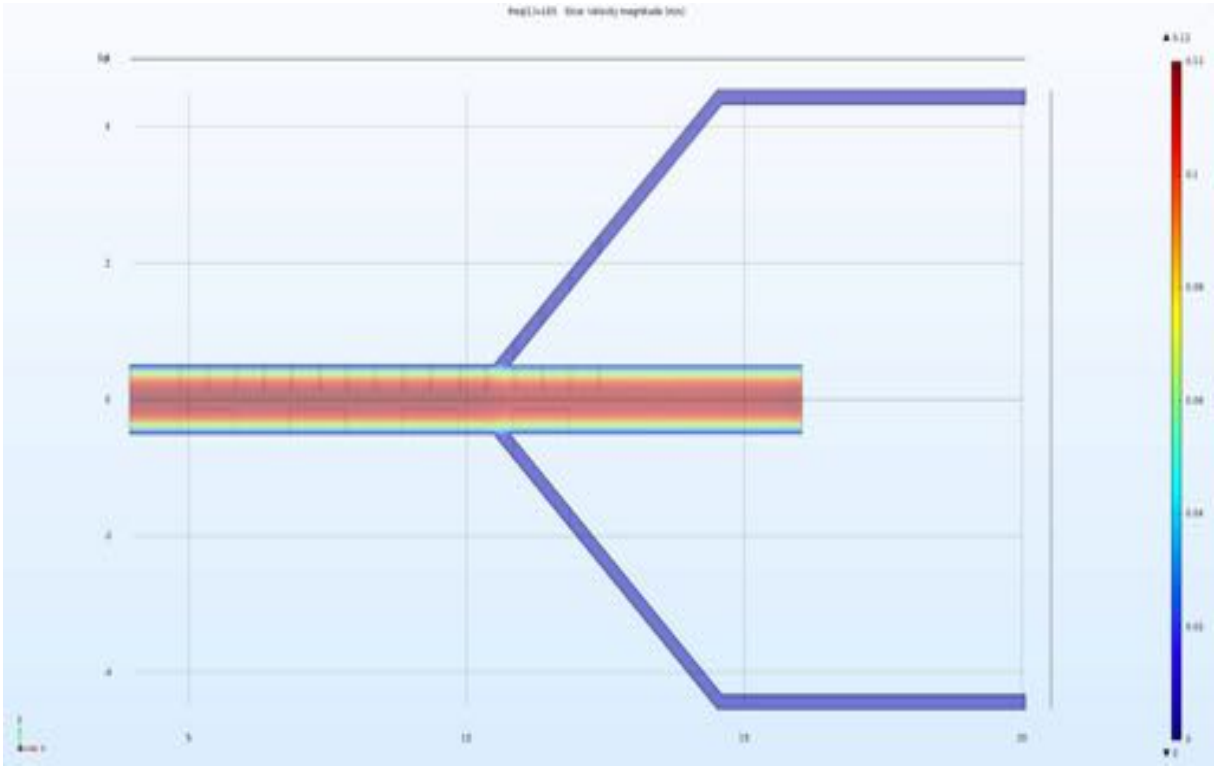

Figure 20. Fluid Flow in the Channels

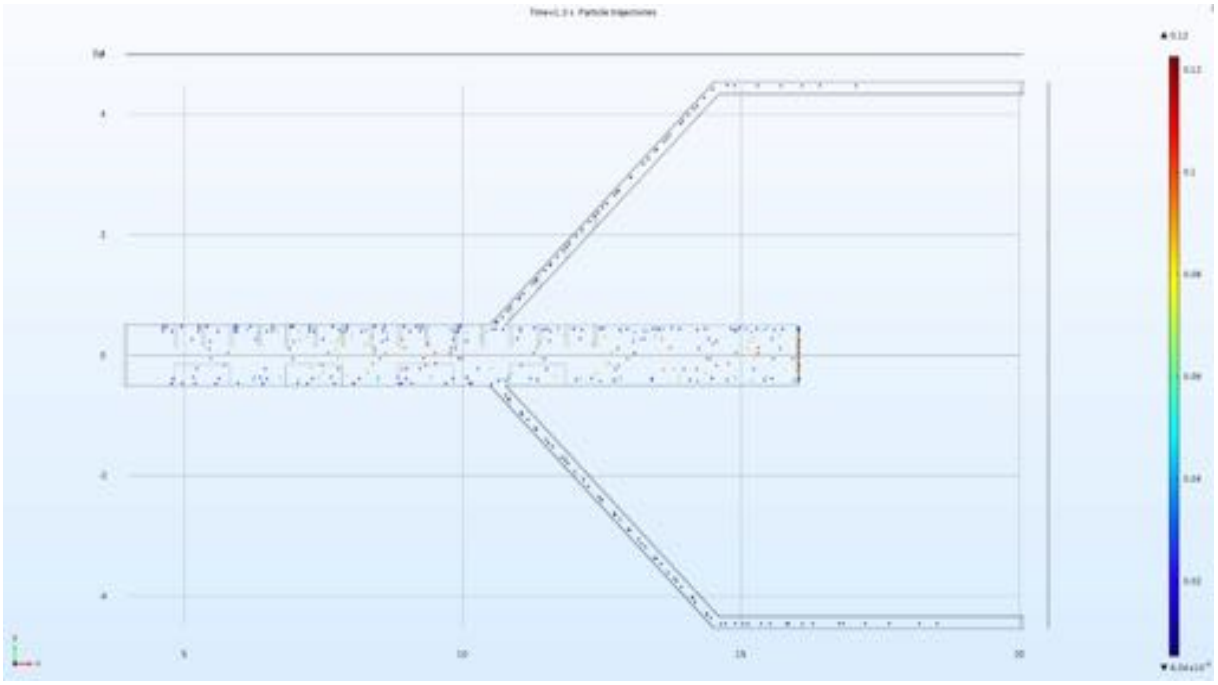

Figure 21. Particle Flow in the Channels

As it is seen in figure 20, and 21 the side channels have little to no flow whilst all the liquid is flowing through the main channel. Since this design did not provide the desired flow or pressure to carry the separated particles in the side channels it was slightly modified. The new design would require the main channel to have a smaller width after each side branch. The idea for this new design was taken from the blood vessel branching[24].

For the 2D model of this design the following equation was applied:

$$D_2 = D_0 - 2\times D_1$$

Where D_0 is the initial width of the main channel and D_2 is the width of the channel after the branch width of $2\times D_1$ has been removed. The objective for this was to compensate for the fluid loss in the side channels and to be able to have a better flow and pressure in the side channels. As it can be seen in figure 22, as the main channel loses width after each

side branch connection, the side channels gain width after each side branch connection, but the side branches remain the same width throughout the design.

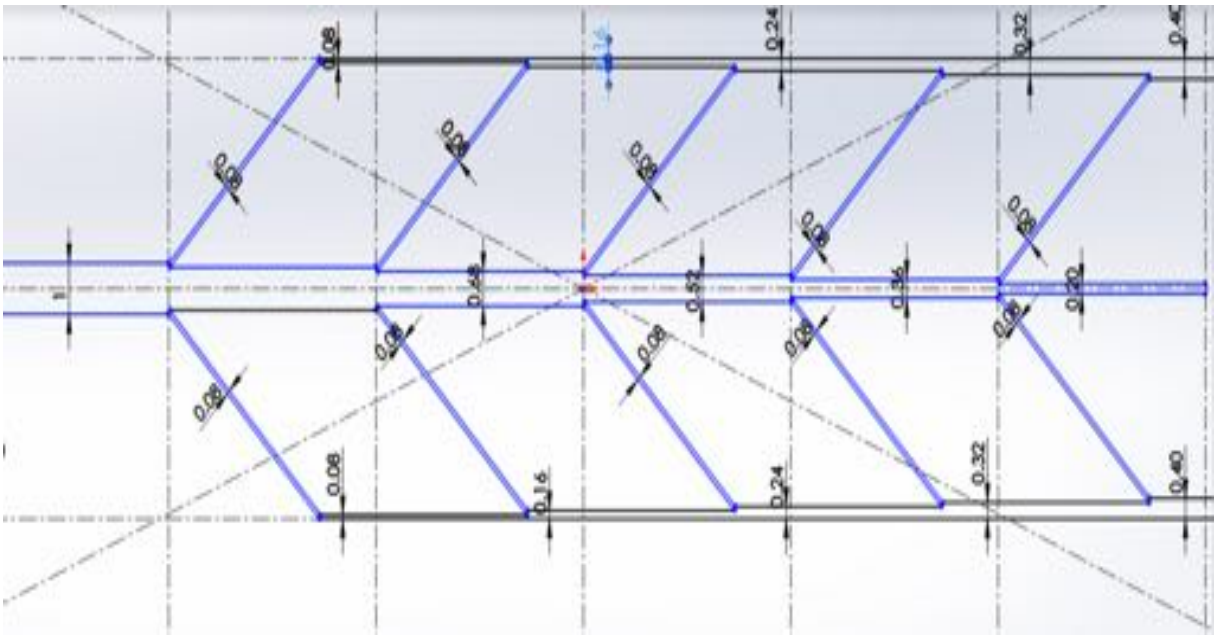

Figure 22. Channel and Branch Sizes

4.2 Electrode design

The next step of the device design is the electrodes. Initially it was planned to utilize the electrode design from the initial design, however it was not possible to integrate the electrode design the same way as the first design due the presence of branches.

There was no design where the electrodes could be placed on the same plane as the channels, therefore it was decided to have the electrodes printed on glass slides and then plasma bond the glass to the PDMS containing the channels.

The electrode design was a simple one, the finger design was used with the bottom fingers large than the top fingers in width, and the bottom fingers being less in frequency than the top fingers, as it is demonstrated in figure 23. The two circular pads on the ends of the electrodes were placed there as contact pads. This design was chosen for the

purpose of having a non-uniform electric filed across the channel. The electrodes were then chosen to be printed on a slid glass using gold.

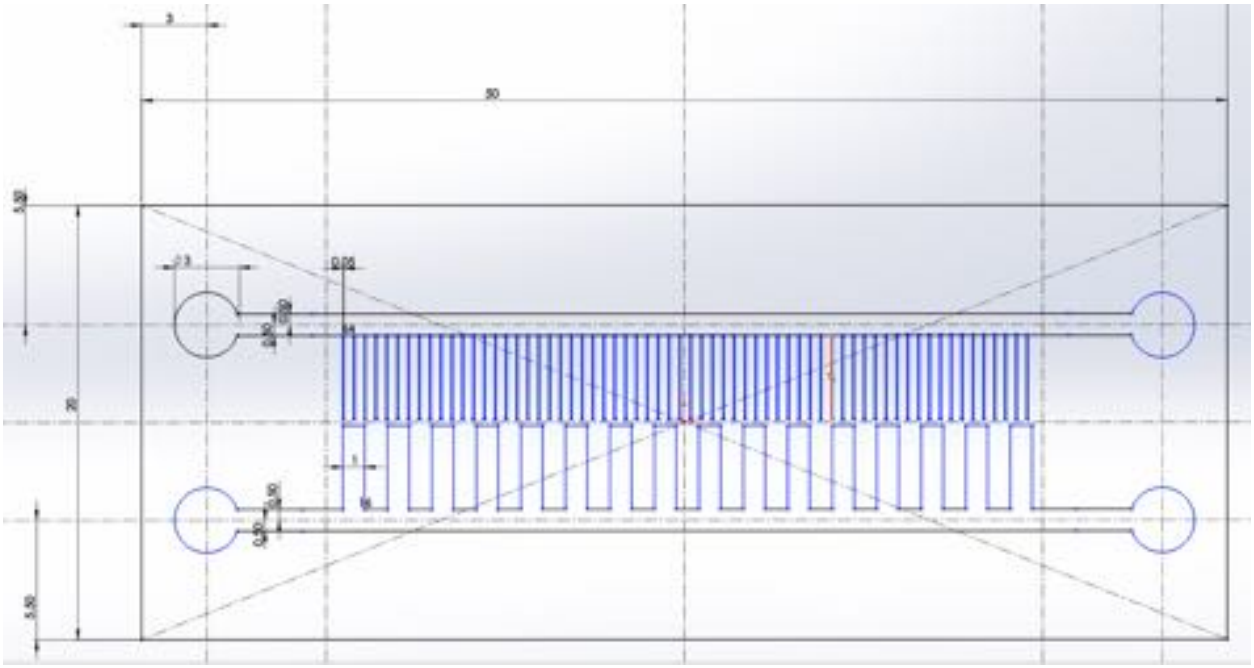

Figure 23. Electrode Design

4.3 Fabrication and assembly

4.3.1 Mask design

Since the electrodes were separate from the channel design, the mask had to be divided into two sections, the top section containing the channel design and the bottom section containing the electrode design. The mask was then sent CAD/Art Services, Inc. for printing. As it can be seen in figure 24 three different sizes of branches were chosen for this mask, 80µm, 100µm, and 200µm. The purpose of this was to test the effects of different branch sizes on the fluid flow in the main and side channels.

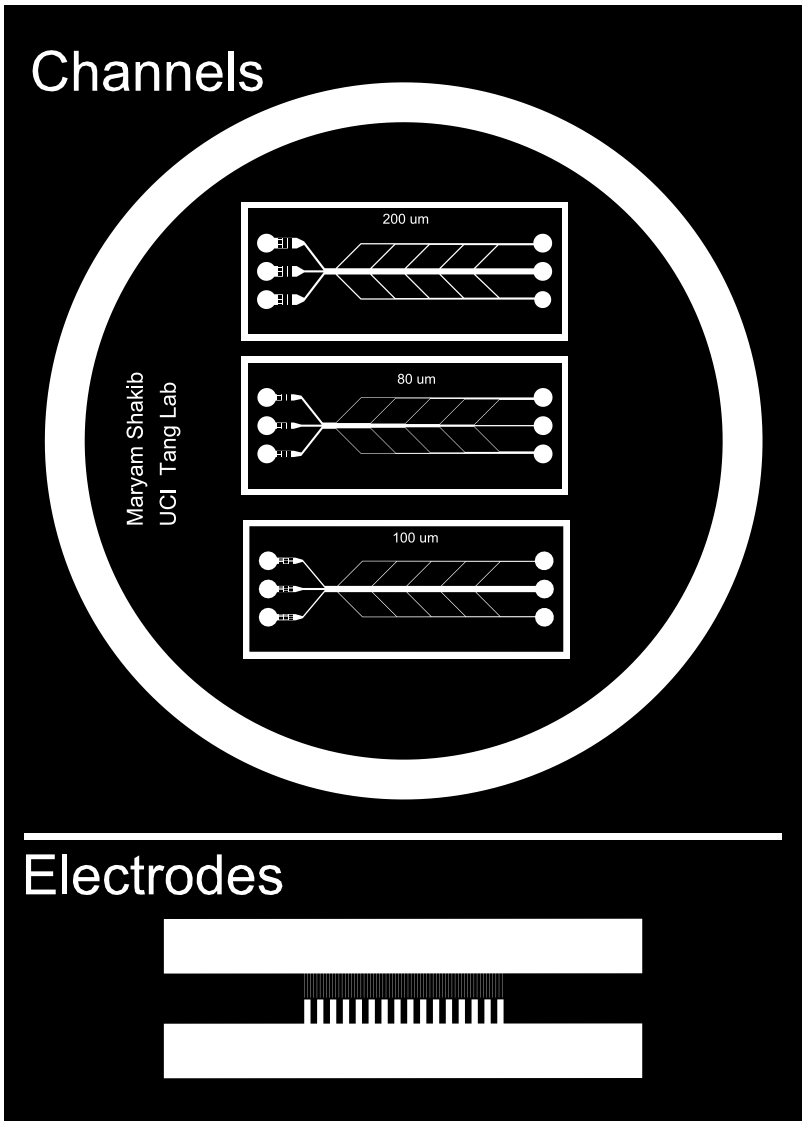

Figure 24. Mask CAD Design

4.3.2 Mold and Electrode Fabrication

The fabrication process for the production of the new design was essentially the same as the old design explained in chapter 3. Same steps were taken for the production of the wafer, however the gold depositing of the electrodes on glass slides was a different process. Much like the wafer fabrication photolithography was used for the electrode fabrication on glass.

The glass slides were cleaned prior to electrode depositing to ensure no particles were on the glass that could cause a defect.

The next step was to deposit a layer of chromium on the glass followed by a layer of gold. Then to pattern the gold electrodes using photolithography[25]. The actual electrode fabrication was done in the UCI cleanroom in the Calit2 building.

Figure 25. Micro Channel Mold-Second Generation

4.3.3 PDMS Molding and Device Assembly

The fabricated mold was placed into a petri dish for PDMS pour. PDMS was mixed at a ratio of 10:1 and poured over the mold. The mold containing the PDMS was then placed into a vacuum chamber (fig. 26) for a period of two hours for any all air to be completely removed from the mixture. The presence of any gas in the mixture produces problems downstream for testing, the gas bubbles can disrupt the flow of liquids in the channel or even render the device useless.

Figure 26. Degassing of the PDMS

After degassing the dish containing the PDMS was placed in an oven at a temperature of 65° for two hours to ensure the PDMS is completely cured. After the curing of the PDMS the dish was removed from the oven and the area containing the channels was cut using a surgical blade. The cut PDMS contained only the inlets and the outlets, the main channel, the side channels, and the branches. The channels were then plasma bonded onto the glass containing the electrodes (fig. 27).

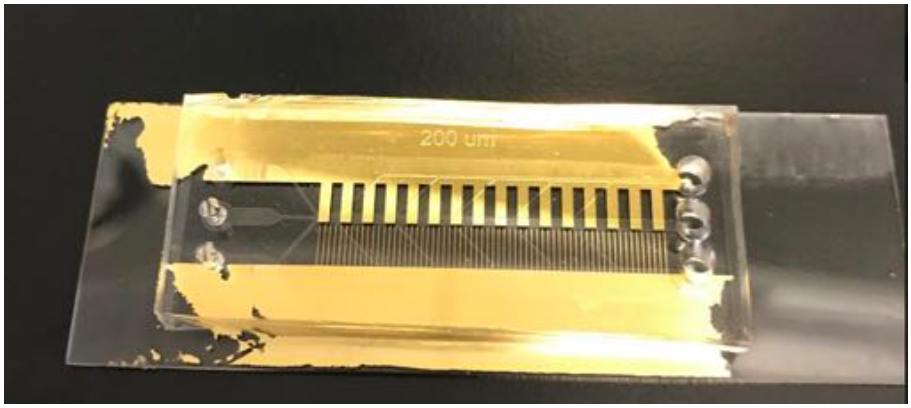

Figure 27. The Completed Device

However, as it can be observed in figure 27 the gold electrode placement on the glass did was not successful, and the contact pads were completely detached from the glass. This result caused the devices to become less than desirable, and ineffective. However, as demonstrated in figure 28 the plasma bonding of the channels to the glass with the electrodes proved to be very effective. As it can be seen the fluid flows in the main and the side channels and it is collected in the collection wells at the end.

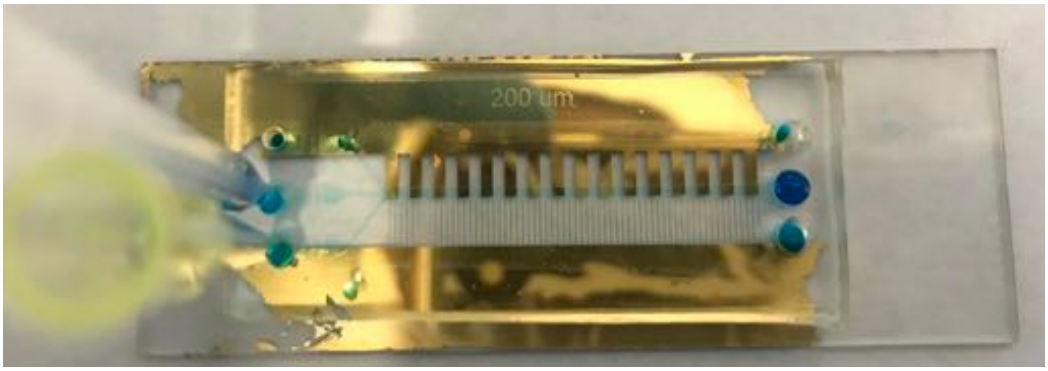

Figure 28. Liquid Flow in the Device

5.1 Simulation Results

After the SolidWorks designs were completed COMSOL Multiphysics® Modeling Software was used to run simulations for the theoretical study of the behavior of the channels.

Figure 28 is a COMSOL image taken to show the channel/electrode assembly for COMSOL simulation purposes. Figure 29 is the simulation of the liquid in the channels, as it can be observed the fluid has a higher velocity at the entrance of the main channel, and as it continues to flow down the channel, it loses velocity. At the same rate it can be seen that the side channels increase in velocity as the liquid moves towards the end of the channel.

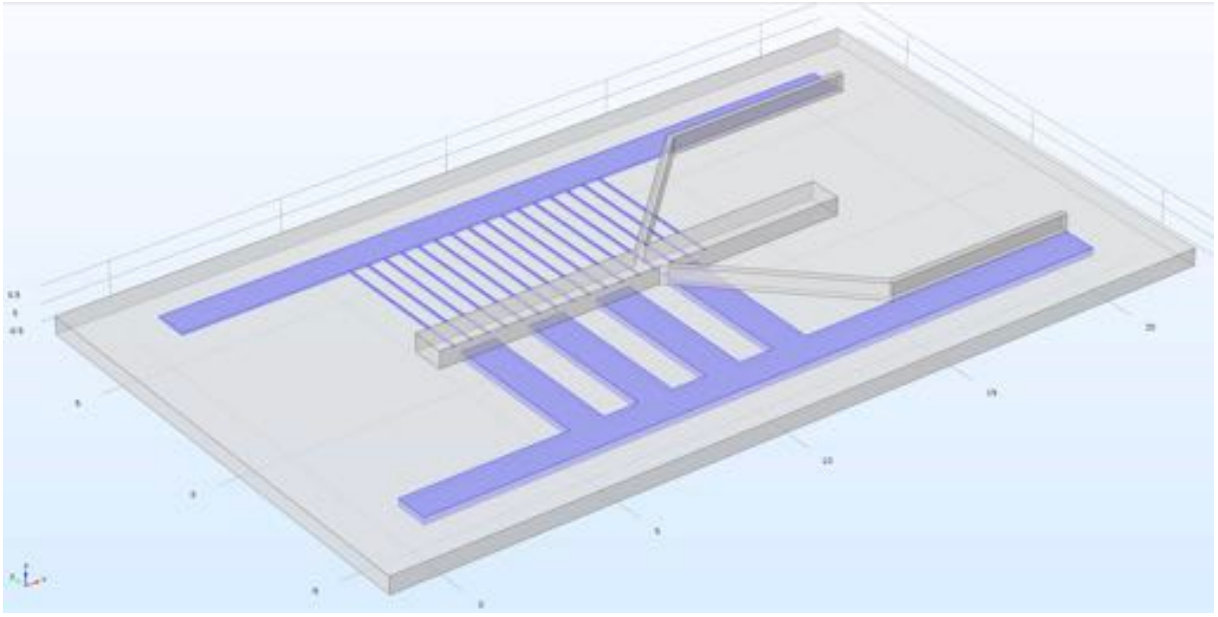

Figure 29. Channel and Electrode Assembly in COMSOL

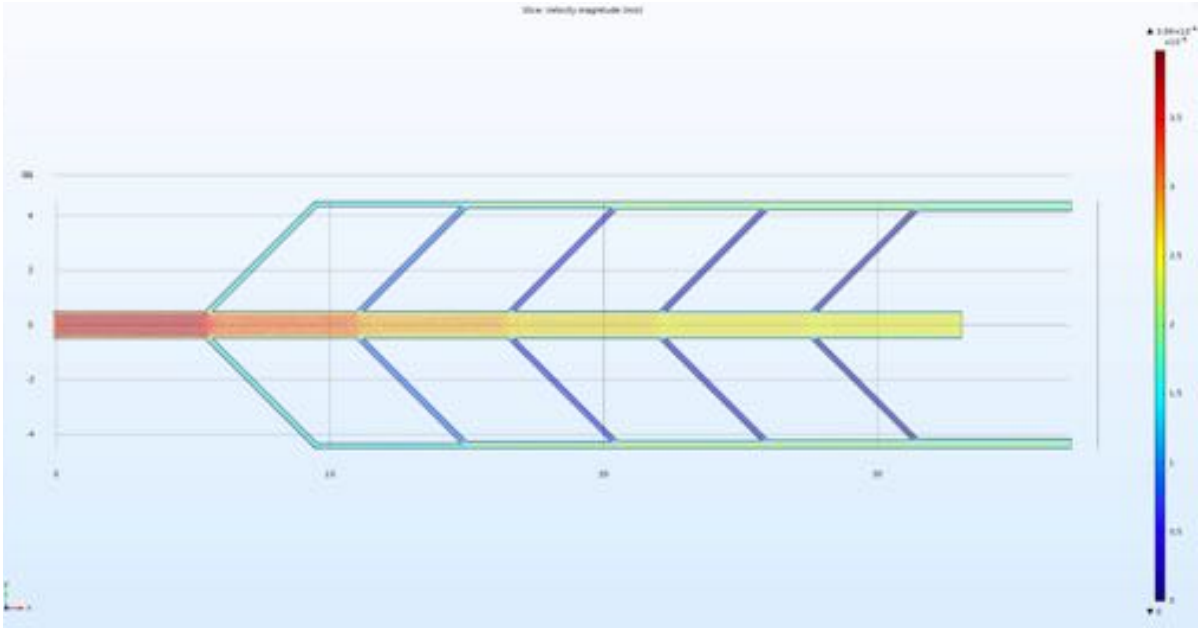

Figure 30. Fluid Flow in the Channels COMSOL

5.2 Summary

Over the course of this project, two different devices were designed, fabricated, and assembled. It was seen that the first-generation design placing the electrodes on the same plane as the channels introduced crowding into the main channel which in turn caused the blockage of the channel and resulting in a device failure.

The second-generation design remedied this failure by placing the electrodes on a separate plane and then bringing the channels and the electrodes in contact with each other. This would eliminate the crowding of the beads caused by the openings in the side walls of the main channel. Instead the It was shown through that this movement of the beads can be utilized in separating and collecting them in the side channels.

Through COMSOL simulations it was seen that the new design does meet the expected fluid flow in the side channels, and it is over all a more complete design than the first generation.

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