

A Microfluidic Device for Single Cell Isolation

A Major Qualifying Project Report

WORCESTER POLYTECHNIC INSTITUTE

By:

Meghan Hemond

Kelsey Krupp

Emily Tierney

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Approved:

Professor Sakthikumar Ambady

Professor Dirk Albrecht

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Abstract

There exists a need for inexpensive and efficient methods to isolate single cells, especially single tumor cells for single cell analysis to improve treatment methods. We developed a microfluidic device that traps single beads ranging from 38 to 45 μm , similar to mammalian cells. Our results suggest our device could trap single beads in 60 μm microwells, indicating this device could allow isolation of similarly-sized cells. Our device could be used for pharmacological testing for personalized medicine and other applications.

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Authorship

All members of the team contributed equally to the writing and editing of this report.

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Chapter 1: Introduction

Despite all of the major technological advances over the last century, basic laboratory and cell culture techniques have remained nearly the same. Scientists are comfortable with the techniques they are using, they are well understood and they have been standardized to make results easier to produce, interpret, and share with scientists around the world. Despite the advantages of using these techniques, the tremendous opportunities to improve upon them should not be ignored.

Current cell analysis techniques have two issues that must be addressed in order for more accurate cell analysis to be performed: cells are cultured in heterogeneous populations and data is recorded on bulk properties of these cell populations. Both bulk analysis and heterogeneous population samples add a layer of complexity to cell culture. Bulk analysis only presents the average behavior of the cells and nuanced behaviors may be misrepresented or masked. While a population may appear homogeneous, rare cell types may exist within the population that display many interesting and unique properties and their behaviors may be masked (Tibbett and Anseth, 2009). If these cells cannot be studied individually, we are unable to understand these behaviors, which may hold the key to understanding the human body at its simplest level. For example, in tumor biopsies, there are many different types of cells present. By studying the cell population as a whole, the average behavior of the cells is studied rather than the behavior of the individual cells. Specific cells like cancer stem cells and certain aggressive cancer cells may have very different behavior from a typical cell in the population, but their behavior is being shadowed by the other cells. Therefore, single-cell analysis is a technique to overcome the inaccuracy of the current methods (Carlo, 2012).

The most common single-cell separation and analysis method is flow cytometry. Flow cytometry is currently the gold standard because it is incredibly high throughput, some 10,000 cells per second can be analyzed, but flow cytometry was not designed to perform multiple assays on the same cell (Carlo, 2012). Flow cytometry is able to collect data from a single cell at a single time point, but after the assay is complete cells are discarded as waste. This makes it difficult to identify which cells are behaving abnormally to study them further and determine the cause of their behavior. Flow cytometry is also a very expensive method of single cell isolation, which limits its use to labs that can afford or have access to the equipment.

In order to create a low-cost device for single cell analysis, the team was tasked with creating a microfluidic device to isolate single cells. Since single cells should be trapped within micron-sized devices using low flow rates that prevent cell damage and allow the cells to be cultured after isolation. Microfluidics can be used as a high throughput method, which is ideal for single cell analysis applications. If rare cell types are of interest, there is likely only a few in the cell population, so the more cells that are isolated, the higher the chance of seeing the individual cells of interest.

Our ranked objectives are that the device must be: compatible with common cell culture techniques, compatible with common microscopes, accurate, precise, inexpensive, and high throughput.

This device requires single cells to be trapped in order to study each cell individually. Media must also be delivered to the cells as they are studied in the device. To fabricate these devices, a Computer Aided Design program called DraftSight and standard

photolithography techniques are used to transfer the designs to a silicon wafer from which PDMS (polydimethylsiloxane) devices can be fabricated.

To perform proof of concept testing, Cospheric© polyethylene fluorescent beads were used in a suspension of mineral oil in the device. The beads were approximately the same size as PANC1 cells in suspension, we believe the behavior of the bead suspension would mimic the behavior of cells within the device.

In the remainder of the report, we will provide background into the different methods of single-cell isolation and how they compare to each other. We will also provide some background into microfluidics. We will discuss our objectives, constraints, and functions, and then explain the approach of our project. We will then provide alternative designs and the reasons behind our design choices. Later, we will explain the experiments that we ran and discuss the results. Finally, we will draw conclusions from our experiments and discuss the overall functionality of our device.

Chapter 2: Literature Review

2.1 History of Cell Culture

Tissue culture was first introduced in the early 1900's, and has been widely used ever since. Initially, it allowed scientists to maintain cells and tissues *in vitro* so they could perform experiments and study them over time, which was imperative to understanding basic biology and living systems. Today, cell culture is a universally accepted practice that influences many different industries and has enabled us to do things that wouldn't have been imaginable 100 years ago.

Traditionally cells are both cultured and analyzed as an entire population, and the results are the average behavior of all the cells within that population. This requires the assumption that the average response is representative of a typical cell in the population, which is not necessarily accurate. For example, an average of 50% protein expression in a cell population can represent either a 100% response in half the cells or a 50% response in all cells and therefore averages can be misleading due to the difficulty of differentiating between the two scenarios (Yin and Marshall, 2012). There is mounting evidence regarding the cellular differences that are found in isogenic and clonal populations, which were previously assumed to be identical throughout.

The population of cells present in tumors shows vast heterogeneity, which makes them a particularly important application of single cell analysis. Cancerous cells exhibit rapid changes in their genetic make-up due to either genetic drift, the rate of replication and age of that cell, or the processes occurring and proteins that cell is expressing (Yin and Marshall, 2012). Individual cells exhibit unique behavior in regards to protein expression and metabolic activity. Therefore, the oversimplification of bulk analysis is problematic

because it neglects this cell to cell variability. In order to understand the heterogeneity and inner workings of a cell population, each cell has to be analyzed individually. This enables researchers to study the factors that influence individual cell behavior and understand what causes the fundamental differences between cells. These differences dictate cell to cell interactions and it is important to see how the behavior of one cell can influence those around it and how that affects the overall health and function of the entire population (Yin and Marshall, 2012).

2.2 Growing field

Single cell analysis is a field that has developed rapidly in the last decade, but it still needs significant improvement and development before it can reach its full potential. At this point people are taking many different approaches in order to determine what works best, but there are still many unmet needs. The importance of single-cell analysis has caught the attention of the US National Institutes of Health (NIH). The agency launched a program to fund advances in single-cell research, with a budget of \$90 million over five years. The NIH recognizes the current shortcomings and challenges that come along with single cell analysis, but they also recognize the importance of this research and the potential that it has to improve our understanding of cell responses which will aid in better detection and treatment of diseases. This program funds research in a wide array of disciplines and applications and is geared toward changing the field of single cell analysis from a small highly specialized group of researchers, and making it more widely used and accessible by promoting commercialization (Single Cell Analysis Program, 2015).

2.3 Applications

In order to further improve single cell analysis, simple and reproducible techniques should be developed. There also exists a need to develop a method for culturing and expanding single cells for an extended period of time. If rare cells are captured and expanded, cell lines of rare cell types can be created. Rather than performing experiments using existing cell lines, which may be easy to grow but do not demonstrate the behavior found in rare cells, these cell lines could be used in order to create a more realistic cell culture where specific conditions need to be met in order for the experiment to be successful. Another application is cell-cell interactions. By isolating individual cells, certain cell types can be forced to interact in order to see their behavior and the way the cells interact with each other. Further experimentation can be done by co-culturing the individual cells together in hopes of advancing tissue engineering. By studying how the cells interact with each other, it can help to determine what cells types and environmental conditions are necessary for organ growth. Another limitation is that some methods depend on the size of the cell, but whether the cells are in in the process of dividing or already divided, the cell is always changing its size, which makes it difficult to use some methods (Carlo, 2012).

A promising application that stems from single cell analysis is personalized medicine. Personalized medicine tailors drug regimens to a specific patient based on how their cells respond to certain therapies. While personalized medicine is not the creation of novel drugs for individuals, it is the classification of individuals to sub populations who will most benefit from preexisting and defined therapies, especially cancer therapeutics. Ideally, a patient's tumor sample would be isolated into single cells then exposed to different drugs

so doctors can determine which treatment is most effective for that patient and the specific type of cancer cells present. Cancers vary widely because of their rapidly changing genome. Cancer cells, even within the same tumor biopsy sample display distinct molecular differences which lead to the development of different assays to provide prognoses (Bates, 2010). Tailoring treatments to patients who are most likely to respond to them has incredible potential for improving cancer prognosis and patient outcomes.

2.4 Current single cell analysis devices

2.4.1 Gold standard

Currently, a majority of single cell analysis is done using flow cytometry. With flow cytometry, hundreds of thousands of cells can be analyzed per minute. The cells can be sorted by their size, granularity, and fluorescence properties (Carlo, 2012). The method of flow cytometry does not require a lot of time and is easy to perform.

Though many researchers, scientists, medical workers, etc. use flow cytometry, for the goals of our project, a combination of other methods seem to be more beneficial. Flow cytometry requires expensive devices and the cells are discarded after each test, so the cells cannot be studied over a more relevant period of time or beyond a single experiment.

2.4.2 Single Cell Isolation Methods

The different methods of isolating single cells span a diverse spectrum. There are a number of mechanisms utilized and the processes vary greatly in complexity. A general overview of the methods that have been used successfully in the past will highlight the main advantages and limitations of each method to exemplify how they are selected for specific applications. Here, we present a sampling of common single cell isolation techniques. A more detailed list can be found in Appendix A.

One of the earliest and simplest methods of single cell isolation is serial dilution. This process entails repeatedly diluting a cell suspension until only single cells remain. Serial dilution is not a complicated process and requires only basic lab equipment such as micropipettes, microtiter well plates, and a microscope. Since the process results in cells contained within microwells, the cells are accessible and are compatible with many assays and standard cell culture techniques (Ishii, 2010). Despite the simplicity of this method, many of its limitations derive from its lack of automation. This process is done manually and is therefore very labor intensive and time consuming. The technique has very low throughput as a result, which reduces the probability of finding target cells especially if they are rare cell types (Ishii, 2010).

Another method that is relatively straightforward and fits the capabilities of most labs is micropatterning. Micropatterning can utilize a variety of techniques, material combinations, and surface treatments depending on the application. Micropatterning uses different surface modification mechanisms to create cytophilic and cytophobic regions that guide cell attachment. By designing cytophilic regions only large enough to permit a single cell to adhere, single cell isolation is achieved. An advantage of this method is that the cell containing regions can be made into any size or shape to adjust to specific cell types and the pattern can be scaled up to achieve desired throughput. Another attractive feature of this methodology is that the cells are accessible and therefore easily maintained. Media and other supplemental nutrients can be flowed over the immobilized cells for convenient exchange, but one must consider the shear stress caused by media perfusion and ensure it will not result in cell lysis. Different techniques of micropatterning vary in complexity. For example, this process can be simplified so that it doesn't require a microfabrication process

and can be done with standard laboratory materials as described by Lin *et al*, 2009 in a Microscale Oil-Covered Cell Array (MOCCA). In this process, a glass slide is treated to make it hydrophobic. It is then plasma treated while covered with a micropatterned aluminum screen that created small hydrophilic circles where cell containing drops will form on the slide. A cell suspension is poured over the treated glass slide, followed by mineral oil that forms and seals droplets as it moves across the slide. This process is done manually, without the requirement of controlled flow rates that require significant background knowledge and planning to achieve. This makes the process extremely practical, and it appeals to more users (Lin *et al*, 2009). Micropatterning can also incorporate photolithography to create more intricate patterns. For example, McDevitt *et al* (2001) used laminin coatings on polystyrene tissue culture plates to encourage cardiomyocytes to assemble single file and form multinucleated myofibrils. A linear pattern was designed and soft lithography was used to create a PDMS stamp of the device. Laminin was applied to the stamp and the design was then transferred to a polystyrene tissue culture plate. Individual cardiomyocytes were able to adhere to only the laminin patterned areas that were one cell wide. The researchers then saw cell fusion and coordinated contractile activity (McDevitt *et al*, 2001). Here, micropatterning was used to manipulate individual cells to form complex arrangements typically found exclusively *in vivo* to create a more accurate platform for studying cardiac activity at the cellular level. Using photolithography and soft lithography significantly elevates the complexity of the process and creates a need for more expensive and complicated equipment. A limitation of these methods is that they are only compatible with adherent cells that are capable of binding to the surface. Once cells are in the array it is not possible to remove particular cells of interest or to manipulate single cells since all

cells are exposed to the same factors. Lacking the ability to move or target specific cells restricts the possibility of further processing or expansion.

Microdroplets are an additional method of single cell isolation. Droplets can be generated using multiple techniques, and usually result in a single cell that is encapsulated in an aqueous solution surrounded by a carrier oil. Microdroplet formation allows for high throughput; some have been shown to generate droplets at rates exceeding 10,000,000 per second. However, not all of these droplets contain single cells and the percentage of successful single isolation may be lower than is desirable (Lindstrom and H. Andersson-Svahn, 2010). The volume of microdroplets usually ranges from several nanoliters to microliters (Mazutis *et al*, 2013), and allows the droplet to function as a microreactor for the encapsulated cell. The small volume allows the cell's secretions to quickly change the concentration within the droplet to detectable levels, and this information can in turn be used to analyze or sort cells (Mazutis *et al*, 2013). The individual droplets don't allow cross-contamination between drops and do not allow cells to influence each other as long as coalescence is prevented. In order to lower the risk of coalescence, drops need to be stabilized, usually with the use of a biocompatible fluorinated surfactant. These surfactants can be very expensive, around \$1,000/mL. One of the most appealing characteristics of cell containing microdroplets is that they can be sorted and manipulated in many ways while still maintaining integrity and isolation. Droplets are compatible with cell culture and are shown to survive for several days without being removed from their original droplets (Claussell-Tormos *et al*, 2008). All of these characteristics make this method versatile and give it great potential in current and future applications.

2.4.3 Limitations of Current Single Cell Analysis Techniques

Limitations of some previous techniques are that they are not high throughput.

Only a few cells can be tested at a time, so a large number of tests have to be performed one after another. This is much less efficient than running parallel experiments where all cells can be tested at once under the same conditions. Another limitation is that cells are not able to be analyzed over a long period of time. Often, cells are discarded, mixed, and not cultured in media, so they will not survive long enough to be analyzed further. Also, since many experiments are done on a larger-scale, on 96 or 384 well plates, the environment is much larger than the size of the cell, so it's hard to control the environment of the cell. The experiments and assays that can be performed are also limited because of the single-cell analysis method. Another disadvantage to some techniques is that they do not prevent contamination between different cell types. For accurate single cell analysis techniques, this is essential. During the process of some separation techniques, the cells can be exposed to residues or chemicals and this adds an uncontrolled variable that could influence cell behavior, which is another disadvantage (Carlo, 2012).

2.5 Microfluidics

2.5.1 Why use microfluidics

The goal of our project is to create a device that is inexpensive, reproducible and marketable to scientists and researchers. The device should also be able to view the cells over a biologically significant time period. A method that has a high throughput is a necessity for our device. The device needs to trap cells in a way that allows further experimentation to be performed. Microfluidics is a technique that has been proven to successfully isolate single cells and expand them over a relevant time period, beyond the scope of most single cell analysis techniques

Microfluidics is the study of fluids in the scale of nanometers to a few hundred microns. Microfluidic applications were previously restricted to silicon based devices but have since expanded to life science applications since the development of soft lithography techniques that have allowed for polymer based applications. Microfluidics are appealing to those performing research in cell biology because of their small size, customization, and diagnostic potential (Streets and Huang, 2013).

Microfluidics is an emerging field that has been shown to effectively isolate single cells and culture cells in three dimensional constructs over a period of time. In these systems it is easier to control the cells. The suspension flows into the device and the geometry and channels arrange the cells to be cultured. These microfluidic devices typically incorporate a network of small channels that range from about 10 microns to 200 microns in width. The microfluidic systems can have very specific designs for certain studies, making them more customizable than a simple petri dish. For example, there can be gradients, valves, channels, wells, or pillars incorporated into the device. Microfluidics is beneficial for single cell analysis because features like wells or pillars can be used to capture the single cells and allow them to stay isolated from each other.

Microfluidics has the ability to control fluids at a very small scale and can create systems with laminar flow rather than turbulent flow. Using different types of flow driven by either hydrostatic pressure or syringe pumps gives precise control of flow rates in the devices, and allows cells to be processed without being damaged by rapid or uncontrolled flow rates (Mehling and Tay, 2014). Like common cell culture techniques, microfluidics can allow cells to be maintained over a long period of time, but the system is more automated

because there is limited need for pipetting fluids, which reduces user variability and gives more predictable and reproducible results. Microfluidics also offers precise control over the microenvironment of the cells due to the small volumes of reagents used, and the microenvironment can be adjusted to more closely mimic the *in vivo* environment (Folch, 2013). Since the volumes of reagents required are on the nanoliter scale, devices are cost efficient to run and produce very little waste.

Another advantage to microfluidics is the high throughput. Microfluidic devices allow for parallel experiments, so many cells can be tested at once to yield a large quantity of results (Folch, 2013). For example, droplet generation is an efficient method of isolating single cells in a microfluidic device while retaining high throughput. This technique preserves the viability of cells and allows for cells to be manipulated within the droplet rather than having to be removed from the system and manipulated manually with a micropipette (Mazutis *et al*, 2013).

We have provided the necessary background for understanding all aspects of our project. Single cell analysis by way of single cell isolation and single cell culture has been explained in depth. Next, we will talk about the objectives and constraints of our projects and the specific functions of our designs.

Chapter 3: Project Strategy

3.1 Initial Client Statement

After speaking with our advisor, the initial client statement read:

“The aim of the project would be to develop a system that would isolate and expand single cells from tissue biopsies. The system created must be able to trap and expand the cells in micron sized hydrogels of varying stiffness representing different tissues. Ideally, the system should allow placement and/or arrangement of cell laden microgels to produce precise geometries that can facilitate organ engineering, tissue engineering and the study and analysis of cell-cell interactions.”

Our client expressed a desire for a microfluidic device because of the range of applications it's compatible with. Microfluidics is an emerging field and is best suited to the resources available here at WPI. There are opportunities to introduce a novel concept to the field of single cell analysis using microfluidic devices that are cost efficient. After further research, we determined it was necessary to expand the scope of our project.

3.2 Objectives

Using our initial client statement, we established a list of objectives that would need to be met in order to successfully complete the project to the satisfaction of our client. These objectives can be seen in the objective tree below in Figure 1, and are further explained after.

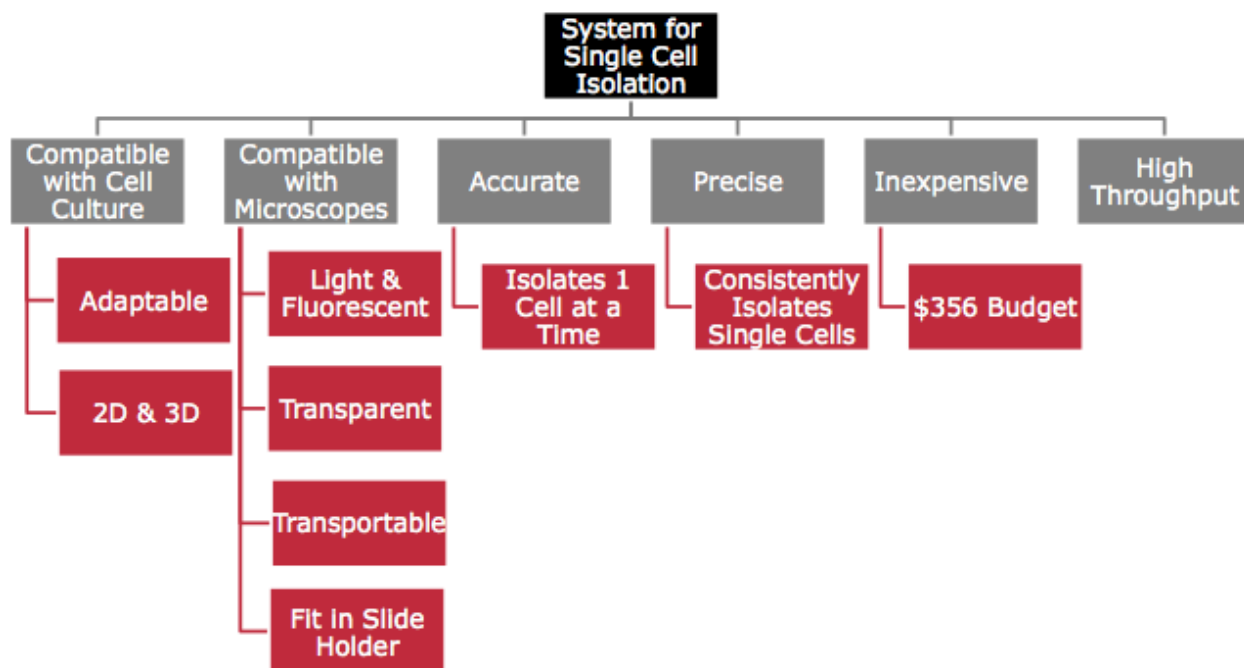


FIGURE 1: OBJECTIVES TREE

Compatible with common cell culture techniques: Since cells are to be flowing through our device, we thought that compatibility with cell culture techniques was most important. Without the correct cell culture technique, the cells will not survive; therefore our device will not be useful.

Compatible with common microscopes: Once single cells have been isolated, they need to be analyzed. In order for our system to be useful in a wide range of labs, it has to be compatible with common laboratory equipment. By bonding PDMS to a glass slide it gives the system transparency, and the cells inside can be analyzed using a common light microscope.

Accurate: The main function of our system is isolating single cells, so it is important that it can do so accurately. If cells clump or if more than one is isolated in the same bubble or

pocket, the user can't analyze them as single cells, and extra steps would need to be taken to further separate them.

Precise: Ideally we want our device to be able to do a large number of tests and analyze a large number of cells at once, so the device should be able to isolate a large number of single cells.

Inexpensive: This is most applicable to completing our project while staying within our \$365 budget. The resulting device is going to be extremely inexpensive compared to other single cell separating technologies that are currently on the market, which will be one of its key features.

High Throughput: This objective isn't essential in order for our device to function, however it would be a desirable feature. It would make the device compatible with applications that require a large number of cells to be processed and screened and it would make it more marketable. However, 100 is the median number of isolated single cells used per experiment (Single Cell Technologies Trends, 2014), so this is a realistic number to aim for.

Primary Objectives

- compatible with common cell culture techniques
- compatible with common microscopes
- accurate
- precise
- inexpensive

- high throughput.

Secondary Objectives

- compatible with 2D and 3D cell culture
- compatible with a variety of cell culture techniques
- compatible with light/fluorescent microscopes
- should be transparent
- should be transportable
- should fit in a typical slide holder
- should capture a single cell
- should be able to capture a large number of single cells at once
- should not exceed \$356

We then ranked our objectives using a pairwise comparison, shown below in Table 1:

TABLE 1: PAIRWISE COMPARISON CHART

Pairwise Comparison Chart								
	Compatible with Cell Culture	Inexpensive	High Throughput	Precise	Accurate	Compatible with Microscopes	Variety of Cell Types	Score
Compatible with Cell Culture		1	1	0	0	0.5	1	3.5
Inexpensive	0		0	1	1	0	1	3
High Throughput	0	1		0	0	0	1	2
Precise	1	0	1		0.5	1	1	3.5
Accurate	1	0	1	0.5		1	1	3.5
Compatible with Microscopes	0.5	1	1	0	0		1	3.5
Variety of Cell Types	0	0	0	0	0	0		0

After identifying each of our objectives, our team utilized a pairwise comparison chart to establish a ranking of our objectives according to their importance and relevance to our project. In this chart each of the objectives are compared to the others one by one. In each comparison, the more important objective receives a 1 and the less important objective receives a 0. If the two objectives are of equal importance they each receive a score of 0.5. These scores are then totaled horizontally, and their final score determines their ranking with the highest scoring being most important and the lowest scoring being least important.

3.3 Revised Client Statement

Our final client statement reads as follows:

“The aim of this project is to develop an efficient system to isolate single cells from tissue biopsies. Ideally, the device should allow multiple applications such as (a) sorting of single cells from cell lines or tissue biopsies for clonal expansion and analysis (personalized medicine), (b) high throughput- ability to screen multiple pharmacological agents on hundreds of clonally expanded cells.”

3.4 Project Approach

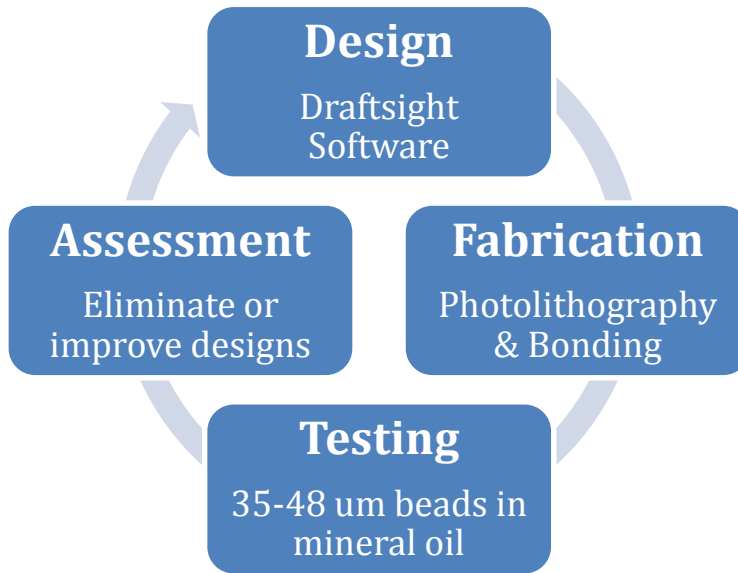


FIGURE 2: DESIGN PROCESS

After establishing each of our objectives and ranking them in order of importance, we used them to assess different design ideas. A thorough review of current literature on microfluidics gave our team an idea of what is currently being done in terms of single cell separation with microfluidics and what is possible and realistic. We were able to use some of our original ideas in combination with concepts demonstrated in contemporary literature to develop a set of preliminary designs.

Figure 2 demonstrates a brief outline of our design process. Each cycle begins with a basic drawing in DraftSight. In order to determine the effectiveness of each design the devices need to be fabricated and tested. After each design is tested, it is either eliminated or improved upon, and then the next iteration begins.

Chapter 4: Alternative Designs

4.1 Needs Analysis

Our device needs to isolate single cells and allow for further analysis of individual cells. The single cell isolation would create a homogenous cell population so that the behavior of the cells is specific to that individual cell and the testing can take into account the different cell types present in a tumor biopsy. We would like the single cells to be able to be removed from the device for further testing, but we realize this may not be possible due to the time constraints of our project. Our device must create a system to efficiently isolate the single cells. It is preferable that the method of separation is a semi-automated microfluidic device, based on the client statement.

Preferred method of separation

Separation mechanism must not cause cell lysis

Cells must be 10 microns apart to be considered isolated

4.2 Functions (Specifications)

The function of our device is to capture single cells in wells. To accomplish this successfully, the cells must remain isolated and not contact any other captured cells. The device must capture single cells so that when treatments are tested using our device, the researcher can see the behavior of every individual cell and not just a representative behavior of the entire population of cells. A specification of our device is that minimally 50% of the wells have to be filled with a single cell. Some may have more than one cell or no cells. This specification is necessary to create a high throughput system. The single cells are the ones that will be studied, so this specification ensures that there will be a higher number of single cells to investigate.

4.3 Conceptual Designs

4.3.1 Alternative Design 1

The first device we tried was based on the publication *Microscale Oil-Covered Cell Array* (Lin *et al*, 2009). We created a simple system where a cell suspension would be poured over a grid of microwells. The suspension would be manually spread over the top surface of the PDMS to ensure cells have spread over the entire surface and reached all of the wells. The goal was to have cells fall into wells that were just slightly bigger than the size of the cells, so that no more than one cell could fit in each well. After cells are allowed to settle into wells and the excess suspension is removed from the top surface, a second microfluidic device would be flipped over and placed on top of the grid of wells. The top device would have wells much bigger than the cells and would have channels connecting the wells that allow media to flow through them. The diameter of the bottom wells ranged from 15 to 100 microns whereas the wells on the top device were 800 microns in diameter. The top device would be the method of cell culture media perfusion. The larger well size on the top would also allow for the cells to expand (Figure 3).

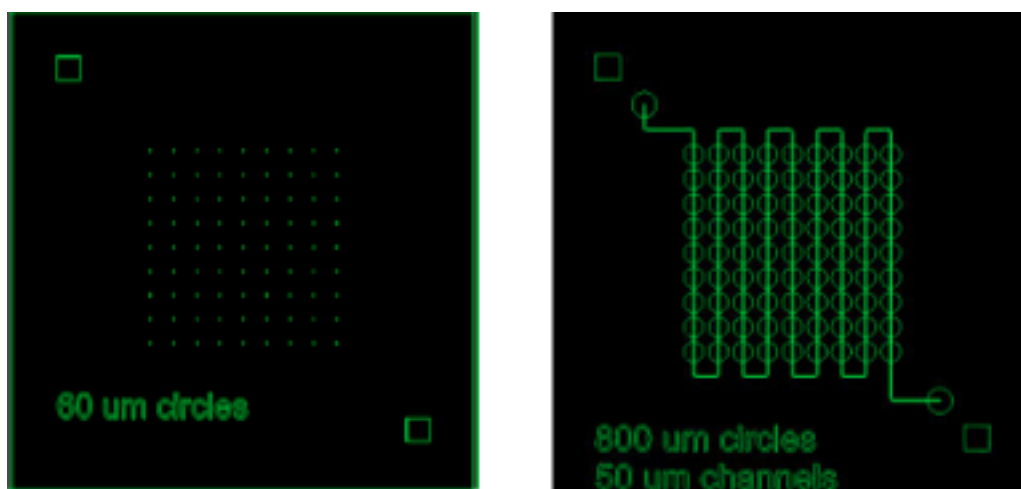


FIGURE 3: ALTERNATIVE DESIGN 1

After creating this device and conducting preliminary testing, we discovered the difficulty associated with aligning the larger top device above the smaller wells. We included square markers in the upper corners of each device to assist with alignment but the devices must be placed under a microscope to properly visualize these place-markers. This restriction limits the reproducibility and ease of use of this device, because misalignment of the two devices is likely to cause malfunction. We were able to capture single cells in over 50% of the wells in the bottom device, but another reason we did not choose this device is because it would not be sufficient for a mixed cell population. When the device is designed, a specific well size is chosen and all the wells are the same size. If there were varying cell sizes, only some of the wells would be trapping single cells because some may be trapping 2 smaller cells or they would be unable to trap the larger cells. Another problem with this device is that cells would not be able to be easily removed from the device. A biopsy punch could be used to punch out a cell of interest, but once the cells started growing, the cells would become mixed populations if they were to flow out of the microfluidic device. Though we liked the simplicity of this device, we decided to pursue a different device that would be easier to use and would be useful for a wider variety of applications.

4.3.2 Alternative Design 2

We created a device that uses a droplet generator as the mechanism of single cell isolation. This design idea came from a recent publication (Chabert, 2008). In this device, a cell suspension would flow through the center channel where it is met by two streams of mineral oil, one on each side, in a flow focusing channel. This forces the formation of droplets, ideally capturing one cell in each droplet. The droplets are then sorted using

hydrodynamic flow so that that large, cell containing droplets drift to one side of a barrier, while empty droplets move to the other. This leads empty droplets to flow out one outlet to be discarded, while cell containing droplets flow to the other outlet where they can be removed or put in a second device where further testing and manipulation can occur. This device relies on delicate flowrates that dictate how efficiently the system works, and these would have to be adjusted and finely tuned for each cell type, which takes away from the device's adaptability. Another drawback of this device is that it required fluorinated oils and surfactants to keep droplets from merging together, and these far exceeded the budget of this project. We decided this device would not be feasible for our team to use (Figure 4).

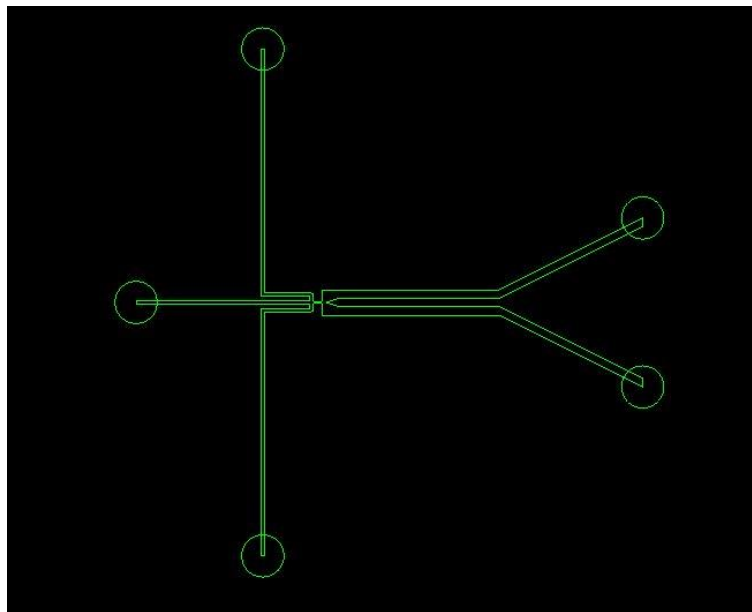


FIGURE 4: ALTERNATIVE DESIGN 2

4.3.3 Alternative Design 3

Next, we designed a device based on a serpentine channel with one well on each side of the horizontal portions of the main channel. The wells have a tiny channel connecting them, which creates a valve system. In this device, a cell suspension flows from the inlet to the outlet, ideally capturing a single cell in each well. When cells pass the wells the downward flow through the tiny channel would pull them downwards and trap them in the well. Once a cell is trapped it blocks the tiny channel and cuts off the downward flow, preventing other cells from drifting into the well with it. This device would allow for media perfusion so cells can be cultured within the device, or they could be easily removed by reversing the flow from the outlet to the inlet, which would push cells out of the wells. When testing the device, we discovered that the channels connecting the wells were too wide, so our suspension flowed in a vertical line from the inlet to the outlet rather than back and forth through the channels, and nothing was trapped in the wells. Time constraints prevented us from redesigning this device with more appropriately sized wells and channels, but we do believe this device has potential as a simple method of single cell isolation (Figure 5).

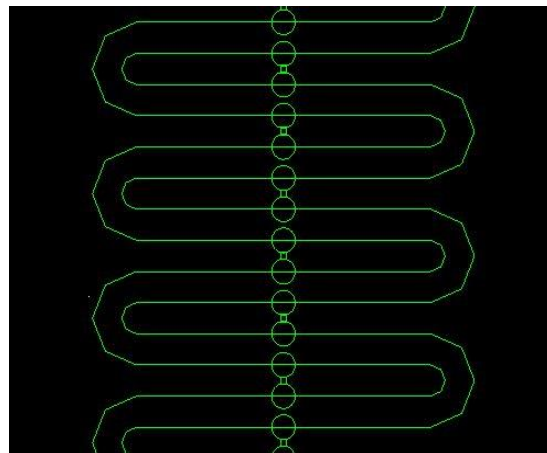


FIGURE 5: ALTERNATIVE DESIGN 3

4.3.4 Alternative Design 4

The next device that was tested was a simple serpentine device. From the inlet to the outlet, the device had one channel shaped as a serpentine that was aligned vertically. On the horizontal segments of the channel, there were 3 wells that took the shape of a semicircle and were approximately 60 microns in diameter. In this device, cells would start in the inlet and flow through the single channel towards the outlet. The goal was to have cells fall in the wells as they flowed through the device. Since the wells ideally, would only be large enough to hold one cell, single cells would be isolated. When watching the flow through the microscope as the device was being tested, there were beads flowing over the tops of the wells without being trapped. We determined that the flow rate was too high. Once we lowered the flow rate, the beads were still not getting trapped, there was nothing pulling the beads from the main channel into the wells. With further testing and modifications to the size and shape of the wells, this device could have potential. We were unable to establish a method of removing any captured cells or beads, and we eliminated this design because of its initial inability to capture any single beads (Figure 6).

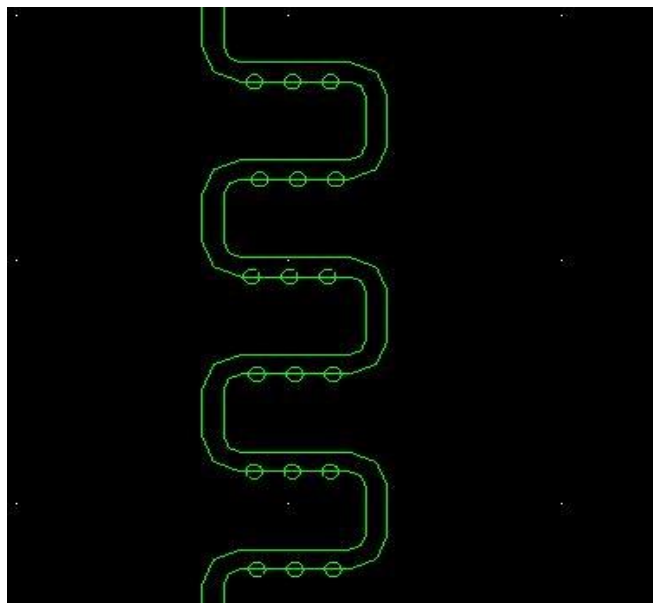


FIGURE 6: ALTERNATIVE DESIGN 4

4.3.5 Alternative Design 5

This brought us to our last design (Figure 7).

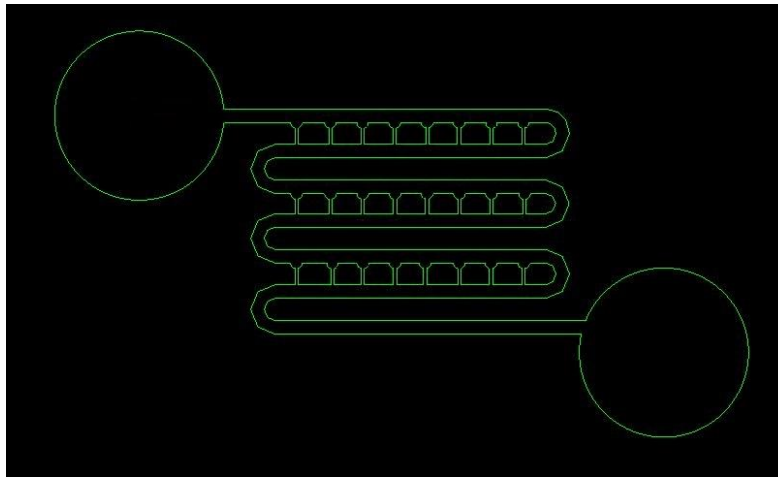


FIGURE 7: ALTERNATIVE DESIGN 5

Similar to the previous design, it would have wells along the horizontal part of the channel. Connecting the well and the next horizontal portion of the channel flowing underneath one row of wells would be small rectangles that are smaller than the size of the beads. The wells were 60 microns in diameter and the small channels were 20 microns wide, designing the pocket in which cells would ideally be captured. These small rectangles provided the suction that the beads needed to be isolated in the wells. This design was simple and did not require a precise flow rate, so hydrostatic pressure could be used to drive fluid flow through the device. A microscope image of this device can be seen in Figure 8.

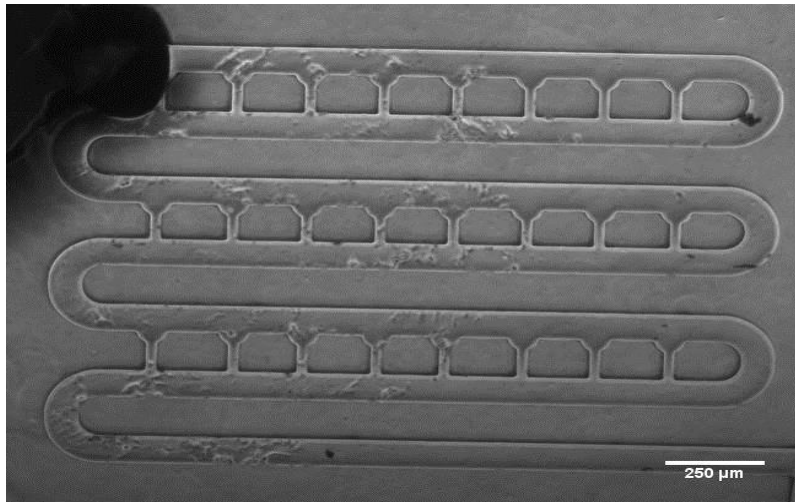


FIGURE 8: ALTERNATIVE DESIGN 5, MICROSCOPE VIEW

4.4 Feasibility Study/ Experiments

Based on the complexity of using microfluidic devices, we determined that removing the cells from the device would not be feasible for our team. The process of manufacturing and testing devices included many steps and took a long time, therefore creating a device solely for single cell isolation would be the only process feasible in our timeframe. The next step in improving our device would be creating a method to remove the cells from the wells and place them in another microfluidic device where they could be cultured for a longer period of time in order for long-term studies to be performed.

4.5 Modeling

4.5.1 Design Calculations

We trypsinized PANC1 cells to determine what size would be required for the wells to be able to isolate just one cell. The trypsinized cells were approximately 40 microns, so we decided to design the wells to have a diameter of 60 microns. This size would allow enough room for a single cell to settle in the well and prevent another from entering. The

small vertical rectangular channel underneath the well had a width of 20 microns, so this prevents the cell from flowing through.

4.5.2 Decisions

Because of our budget, we decided that using the expensive surfactants needed for the droplet generator or the design iterations that required a new photomask for the other designs were not possible for our team. This left the air pocket design with the wells as the primary option for our team.

4.5.3 Optimization

We decided to create our device out of PDMS because it would allow us to make more devices for a lower cost. We also produced designs with a variety of well sizes on the same photomask to reduce cost and increase the chances of success with one of these devices.

4.6 Preliminary Data

TABLE 2: PRELIMINARY DATA (GRID OF WELLS DEVICE)

Device	Desired Cells/Well	Cell Density	Empty Wells	Single Cell Wells	Capture Efficiency
40um	1	9900000	47	34	49.38271605
			41	40	
			35	46	
60 um	1	4400000	25	56	67.48971193
			26	55	
			28	53	

This table shows the preliminary data collected from the grid of wells device (Alternative Design 1). After pouring the cell suspension over the device and letting the cells fall into the wells, the wells were inspected to determine whether there was a cell present in the well. Table 2 shows, out of 81 wells, how many wells were empty or filled. Though this device had success capturing cells, it could not be determined if there was one

cell or more than one cell in each well. As described above, there were other characteristics of this device that resulted in discontinuing experiments with the grid of microwells.

Chapter 5: Design Verification

This chapter verifies that we met our ranked objectives, which were: compatibility with common cell culture techniques, compatible with common microscopes, accurate, precise, inexpensive, and high throughput. Testing our devices with fluorescent beads verified compatibility with microscopes, accuracy, precision, and the ability to be high throughput. Testing of our devices with fibroblast cells verified the compatibility with common cell culture technique.

5.1 Device Fabrication

Devices were created using “DraftSight software. A photomask was created at CAD/Art Services Inc. and returned back to us. Using standard photolithographic processes, the designs were transferred from the photomask to a silicon wafer, some steps are shown in Figures 9 and 10.



FIGURE 9: SILICON WAFER SPINCOATED WITH PHOTORESIST



FIGURE 11: SILICON WAFER EXPOSED TO UV LIGHT

The photomask contained 3 wafers of designs, each wafer containing 12 devices. PDMS was poured over the silicon wafer and baked. The devices were then cut from the PDMS mold and plasma bonded to a glass slide, shown in Figure 11.



FIGURE 10: PDMS DEVICE ON GLASS SLIDE

5.2 Device Setup

In order to start testing the device, a syringe setup needed to be attached to the device. The device setup is shown in Figure 12. One vertical syringe was to hold the suspension and the horizontal one was to hold fluid in order to flush or prime the device. The tubing from the syringe was then inserted into the inlet hole in the microfluidic device.

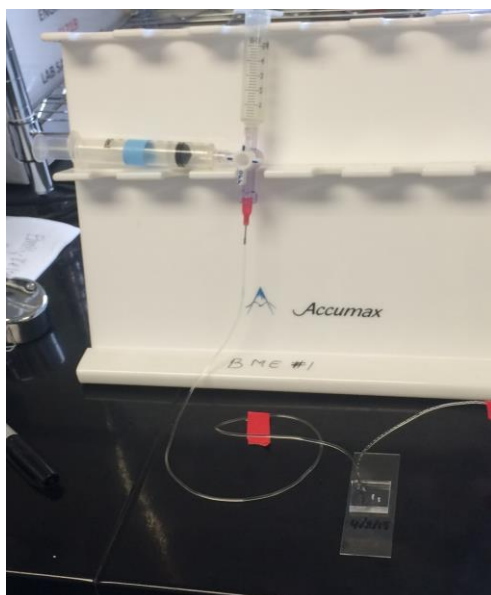


FIGURE 12: DEVICE SET UP

5.3 Proof of Concept Testing

In order to perform proof of concept testing, we used polyethylene fluorescent beads ordered from the company, Cospheric© (Product ID: UVPMS-BR-1.20) with diameters ranging between 38 and 45 microns. This size was chosen because they are similar in size to trypsinized PANC1 cells. To prevent clumping of the beads in the devices, a solution of Tween surfactant was created (Appendix D). The fluorescent beads were added to the Tween and water solution and spun. Depending on the desired density, 0.25g or 0.5g of the beads and Tween were added to mineral oil and placed in the vertical syringe. The device was flushed with oil to clear dust or PDMS particles and the syringes were primed to remove bubbles. Hydrostatic pressure was created in

the device to initiate flow, and flow from the top syringe was turned on, causing beads to flow into the device. Beads flowing through the main channel, and were too small to flow through the small vertical rectangular channels, causing some to get trapped in the wells.

5.4 Cell Testing

Fibroblast cells were used to test in the devices to determine if they were compatible with cell culture techniques and to determine if the devices were able to capture single cells. Since cells were able to go in suspension in the water, water and media were used to flush the device and prime the syringes. Since a surfactant wasn't being added to the solution, Pluronic-127 was pipetted into the device and let sit to coat the sides of the device in order to reduce the clumping of cells. A suspension of cells at a density of 20,000 cells/ 1 mL was added to the top syringe. Again, hydrostatic pressure was created to initiate flow, and cells were allowed to flow through the device.

Chapter 6: Discussion

6.1 Proof of Concept Testing

The results of this device were unique because when using beads, the small channels underneath the wells created suction because of the oil flowing in the main channel above as well as below the small channels. The suction was able to pull the beads into the wells, causing them to get trapped and remain in the wells as others flowed past them in the main channel. The design of this device was based on a previous publication *On-site formation of emulsions by controlled air plugs* (Huang, 2014) where they used a similar device to create air bubbles within their device. We modified the design and operating protocol to allow us to isolate single beads or cells and then contain them individually within droplets.

By varying the density of the beads in the suspension, the number of beads getting trapped in the wells would change. Using 0.5g of beads in the suspension was creating a density of beads that was too high, and multiple beads were getting trapped in one well, usually up to three beads per well (Figure 13).

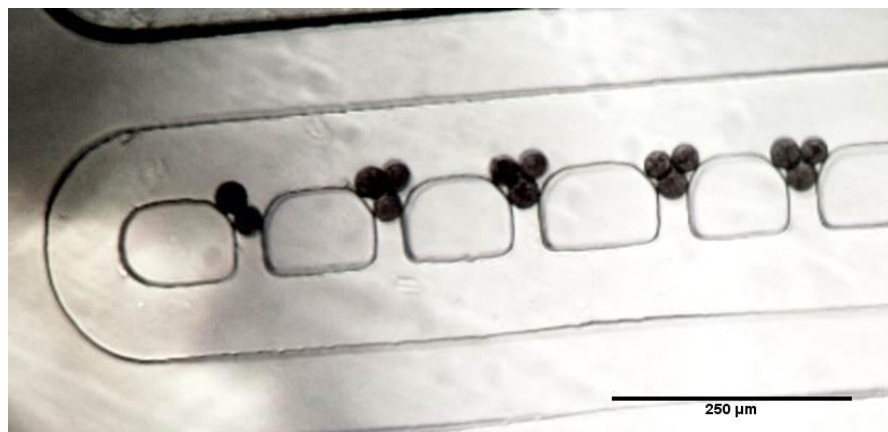


FIGURE 13: BEAD CAPTURE AT 0.5G DENSITY

When the amount of beads was reduced to 0.25g, single beads were trapped in the 60 micron diameter wells (Figure 14).

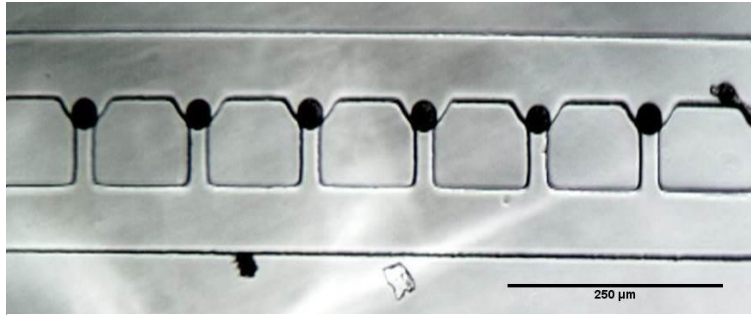


FIGURE 15: BEAD CAPTURE AT 0.25G DENSITY

Using one of the bigger devices resulted in beads getting trapped in multiple rows of the device, shown in Figure 15.

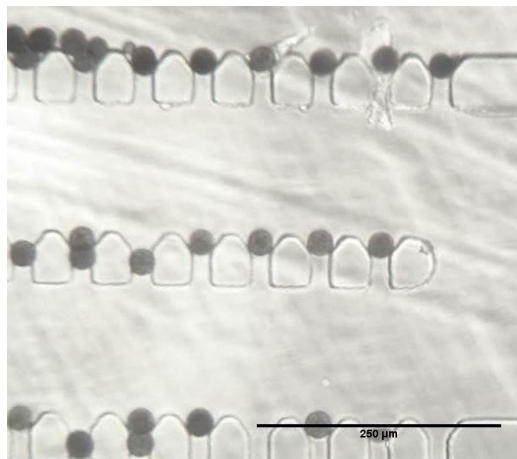


FIGURE 14: MULTIPLE ROWS OF SINGLE BEAD CAPTURE

Working with an oil suspension for the beads made it very difficult to get flow from hydrostatic pressure in the device. Oil had to be used because the beads would not stay in suspension when they were in water. The beads wanted to stick to the sides of the syringe, so they would not flow into the device. Therefore, we decided to use mineral oil, but because of the change in viscosity between the mineral oil and water or media, the flow rate drastically changed and it was more difficult to achieve natural flow without forcing fluid into the device. This often caused the 3-way valve to get clogged with oil and beads and would prevent anything from flowing into the device.

Another challenge that occurred when flowing fluid through the device was dust or PDMS particles clogging the channels. Because we were not in a clean room and not under sterile

conditions, dust or remnant PDMS particles were often appearing in the device after it was flushed initially with oil. Since the channels were only 80 microns wide, this meant that single particles of dust or PDMS would completely clog the channels and not allow beads to get trapped in the wells (Figure 16).

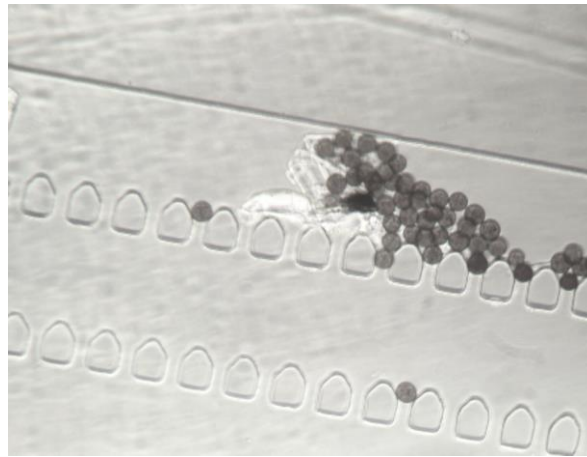


FIGURE 16: DUST CLOG

We also faced challenges in the proper fabrication of our device. We needed to incorporate small features into our device to capture beads and cells but plasma bonding such small features posed a problem. In Figure 17, we show a bead that was able to flow under small features that had not been plasma bonded to the glass slide. For future work, the aspect ratio (height:width) could be adjusted to increase the stability of these features. We developed our silicon wafers with a height of 80 microns. A shorter height may increase stability and the likelihood of features bonding appropriately to the glass slide.

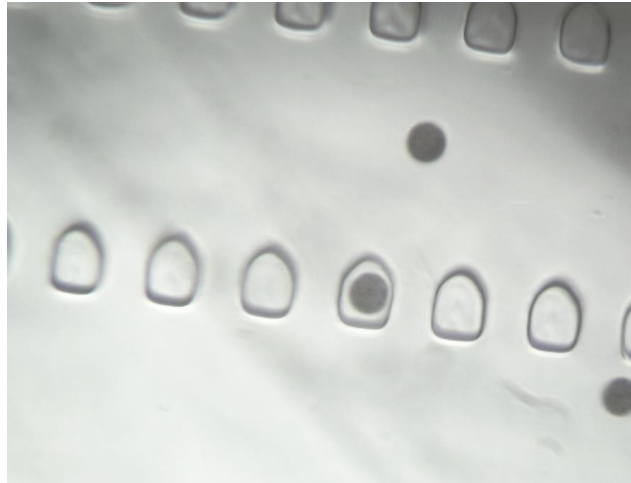


FIGURE 17: POORLY BONDED FEATURE WITH BEAD

Optimization of suspension density and minimization of dust are factors that could greatly improve the throughput of our device. Once our device yields a higher throughput, we would be able to determine how well the device meets the objectives of accuracy and precision. We were unable to obtain numerical data and further testing is required to determine the accuracy and precision of this device. To obtain this data we would want to flow bead suspensions through the device until wells were filled. We would then calculate the percentage of single beads isolated in wells compared to empty wells or wells containing multiple beads. We would run these trials in triplicate and then repeat these same tests with a suspension of PANC1 cells.

6.2 Cell Testing

After getting results with the fluorescent beads, we tested the device with cells. To start, we used human primary fibroblast cells. When flowing water through the device to start, there were air bubbles that were formed in the small rectangular channels underneath the wells (Figure 18). The air bubbles were a positive result of this device because these could later be utilized to help removed the cells from the device. These were formed because the channels were too small to allow any water to enter. After flowing the water, media was flowed through to coat the device before flowing cells. When the fibroblasts entered the devices, they were too small to get trapped in the wells. The cells would flow into the wells and the small channels and none were getting isolated. Because of time constraints, we were not able to change the cell type or the size of the device. For future testing of these devices, the first method of testing would be using a larger cell type like the PANC1 cells.



FIGURE 18: AIR BUBBLES PRESENT IN CELL TESTING

Our device was able to meet some of the objectives we established for this project. The device is able to be used with common microscopes. We have been able to use our devices successfully with both a fluorescent and a light microscope. Our device is also compatible with common cell culture techniques. It has the ability to be used in the hood and it is able to be sterilized by autoclaving which is a common sterilization technique that is available in most labs. Microfluidic devices made of PDMS are frequently used for cell culture applications and while we

did not specifically test for cell viability, we can assume they will be biocompatible. It is also relatively inexpensive. The cost to start producing these devices would be expensive because of the costs of a clean room and the photomasks, but after those are acquired, the cost is cheap. All that is needed to produce the devices would be silicon wafers and the materials to make PDMS. These devices, while they have demonstrated potential, have not been high throughput up until this point. Optimization of suspension density and minimization of dust are factors that could greatly improve the throughput of our device. Once our device yields a higher throughput, we would be able to determine how well the device meets the objectives of accuracy and precision. We were unable to obtain numerical data and further testing is required to determine the accuracy and precision of this device. To obtain this data we would want to flow bead suspensions through the device until wells were filled. We would then calculate the percentage of single beads isolated in wells compared to empty wells or wells containing multiple beads. We would run these trials in triplicate and then repeat these same tests with a suspension of PANC1 cells.

While we are able to make very preliminary assessments about the success of our device, more testing is required and more data must be gathered before any conclusive statements can be made. Our trials were not reproducible and adjustments to the device protocol must be made.

6.3 Design Considerations

6.3.1 Economics and Society

Our device provides a low cost method for single cell isolation, leading to the possibility of analyzing gene expression or clonal expansion for varied applications such as development of pure populations of cells, drug and molecule testing. The device size can also be increased to lead to higher throughput and increased cost-effectiveness.

6.3.2 Environmental Impact

The devices and associated set up are single-use only and would therefore create some plastic waste. However, only the research community would be using these devices and the impact should remain relatively small. The protocol could be optimized to reduce waste and this would also increase the sustainability of the device.

6.3.3 Political Ramifications

This project has very minimal projected political ramifications. This device would be used for research purposes and would therefore have limited impact on cultures of other countries even though it may affect the culture of scientific research by producing a shift in the paradigm of cell analysis and traditional culture techniques.

6.3.4 Ethics

Our project follows good ethical practices because it does not require any animal or human testing. The only testing done in our devices uses previously established cell lines. When eventually using human tumor samples, privacy considerations should be upheld to protect patient confidentiality.

6.3.5 Health and Safety

As long as the device is used for the purposes described in the report, we do not see any health and safety concerns for users.

6.3.6 Manufacturability

The most expensive part of manufacturing the device is the cost of a clean room. Assuming a company already had access to a clean room, the only costs would be printing photomasks and transferring the designs onto the silicon wafer. The photomask is approximately \$120 including shipping and the silicon wafers are approximately \$7 per wafer. The photomask makes 3 wafers, so each wafer costs about \$47. Each wafer will make 12 devices. A company could make the silicon wafers for \$47 dollars and send them to labs who would only have to pour the PDMS, which would

be inexpensive for them. For us to pour the PDMS as well, each device costs around \$4.91. But a company could sell the silicon wafers instead of manufacturing the PDMS molds, so each device would come out to around \$3.91, 1 silicon wafer being \$47. The device is very reproducible. Once the design is made in DraftSight, the steps following are very standard procedures. The photomask is made from the computer image and the design is transferred to the silicon wafer. PDMS is then poured over the wafer. If the protocols are followed correctly, the device will be very reproducible.

Chapter 7: Final Design and Validation

7.1 Device Fabrication

1. Devices were created using the “DraftSight” software. Features were dimensioned and the polarity was indicated to determine which features were raised and which were channels.
2. 1 silicon wafer was produced with 12 devices, including some duplicates.
3. On the photomask, the features that would stay as solid PDMS were clear and the channels, wells or inlet/outlet holes were black.
4. The Designs were sent to CAD/Art Services Inc. in order for a photomask to be produced with our devices.
5. Using the standard photolithography process described in Appendix B, the designs on the photomask were transferred to a silicon wafer.
6. PDMS was then poured over the wafer and baked at 65 degrees C overnight after the wafer was fluorinated; the full soft lithography process is described in Appendix C.
7. The devices were cut out from the PDMS slab, inlet and outlet holes were punched, and the device was plasma bonded to a glass slide. The protocol for plasma bonding is also described in Appendix C.
8. Devices were then ready to be tested.

7.2 Device Setup

1. A three-way valve was connected to two syringes and a luer valve. The luer valve was then attached to plastic tubing. The syringe setup is shown in Figure 19.

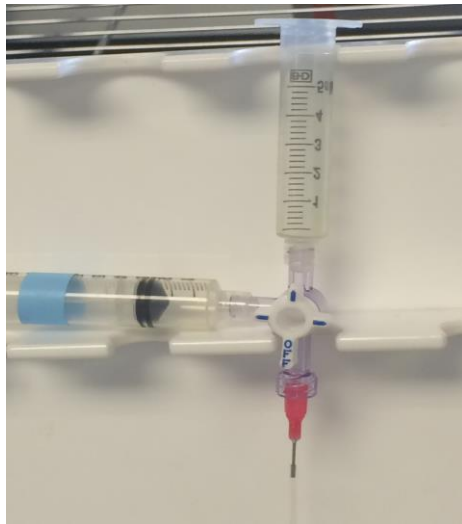
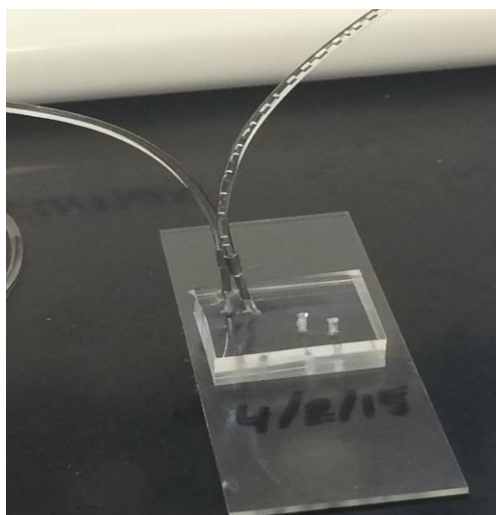


FIGURE 19: SYRINGE SET UP

2. One syringe held about 5 mL of the suspension to flow into the device. The second syringe had about 1 mL of a solution used to flush the device to minimize dust before the solution would flow into the device.
3. The plastic tubing connected to the luer valve was inserted using a metal pin into the inlet of the device, shown in Figure 20. A second set of tubing and pin was inserted into the outlet and ran into a small petri dish to collect the fluid.



**FIGURE 20: TUBING AND METAL PINS
INSERTED INTO DEVICE**

7.3 Proof of Concept Testing

1. The detailed protocol to make the Tween20 surfactant is described in Appendix D.
2. About 90 uL of the Tween20 was added to 100 mL of boiling DI water and mixed for about 30 seconds. This created a 0.1% Tween solution.
3. About 2.0 mL of the Tween and water solution was added to 5g of the fluorescent beads.
4. The solution with the beads was spun for 5-10 minutes and the clumped beads from the top of the conical tube were removed.
5. Then 0.25g or 0.5g beads (Depending on the desired density of the suspension) and Tween were added to 10 mL mineral oil.
6. The conical tube was inverted to mix the beads into the oil.
7. Approximately 5 mL of the solution was added to the top vertical syringe.
8. Approximately 1 mL of mineral oil from the left horizontal syringe was pushed through the device to clean out any dust particles.
9. The syringes were primed to remove bubbles.
10. The syringe setup was placed about 12 inches above the device to create hydrostatic pressure. For this device, the flow rate did not need to be precise, so the height during each trial could vary. The flow from the top syringe was turned on, allowing the beads to flow into the device at the inlet hole.
11. Beads flowed through the main channels but could not pass through the small horizontal channels. This would cause some of the beads to get trapped in the wells.
12. As beads exited the device, they flowed out of the outlet and into a small petri dish as waste.

7.4 Cell Testing

1. Water was flowed through the device to reduce particulates in the device, this created air bubbles in the small channels underneath the wells.

2. Approximately 20 μ L of Pluronic-127 was pipetted into the inlet in order to coat the device so cells would remain in solution and not get stuck in channels and would be less likely to clump.
3. Media was flowed through the device to coat the surfaces that cells would be in contact with and to flush the Pluronic-127.
4. About 1 mL of media was pulled into the left horizontal syringe.
5. A suspension of cells at a density of 20,000 cells/1 mL was added to 10 mL of media.
6. 5 mL of the cell suspension was poured into the top vertical syringe.
7. The media from the left syringe was manually pushed through the device to clear any of the remaining Pluronic-127 and dust.
8. The syringes were primed and again, hydrostatic flow was created with a height change of 12 inches between the syringe setup and the device.
9. Flow from the top device was turned on, allowing cells to flow into the device.
10. Again cells would flow through the main channel and out the outlet into a petri dish. In our test, the cells were too small for our devices and they were able to travel through the small horizontal channels.

We successfully created a microfluidic device that demonstrated potential to isolate single beads from a solution. These same principles can be applied to a cell suspension and the device could be used to isolate single cells from a tumor biopsy sample.

Chapter 8: Conclusions and Recommendations

Our device isolated single polyethylene beads. If more design iterations were to be performed, we believe there is demonstrated potential to isolate single cells from a cell suspension as well. Large-scale pharmaceutical testing could be done on these cells for applications in personalized medicine. The cells would remain in their own wells to ensure that their behavior was from that one specific cell, making it easier to understand how the patient's individual cells react to the specific drugs.

Though our device isolated single polyethylene beads from suspension, the next step in development should allow for a retrieval method of these single beads, or eventually cells. Our device provides minimal space for the cells to grow and expand, so the cells would not be viable in this device for a significant period of time. If the isolated cells are retrieved from our device and transferred to a microfluidic cell culture platform, more effective analysis could be conducted.

This device or a subsequent device could also be manufactured out of a hydrogel such as gelatin. Cell culture would then occur in a three dimensional environment, more closely mimicking the way they would grow *in vivo*. There are existing protocols for creating devices out of a hydrogel. After making the PDMS mold, a hydrogel is cast over it to create an entirely hydrogel device. Variable hydrogel stiffness could be obtained to match the tissue of origin of the cells being studied.

Also it would be beneficial to conduct work in a clean room. Particulate contamination, via dust particles, frequently clogged channels within the microfluidic device. Clogging prevented flow through the device and caused device failure. Decreasing the likelihood of dust entering the system would allow devices to function more successfully and over longer periods of time.

Finally, well size could also be altered to tailor the device to more specific application or to isolate more specific cells. A variety of well sizes could also be used to isolate from a heterogeneous population as opposed to the homogenous population we used throughout the course of this project.

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Appendices

Appendix A: Single Cell Isolation Methods

Method	Advantages	Limitations	Process
Serial Dilution (Ishii, 2010)	Compatible with standard microtiter plates, easy to culture	Manual process, labor intensive, time consuming, low throughput, low chance of finding rare cells	Cell suspensions are repeatedly diluted until only single cells remain
Microscale Oil-Covered Cell Array (MOCCA)	Simple, no microfabrication required, inexpensive, array formation only takes 2 minutes, number of droplets can be changed, only requires common laboratory supplies.	Plasma treatment extends beyond the micropatterned filter and causes larger droplets to be formed. Variability in droplet size. Most of the process is done manually	Glass slide is treated to make it hydrophobic, the plasma treated with an aluminum screen to make small hydrophilic circles where drops will form. Cell suspension is poured over glass, followed by mineral oil that forms and seals droplets
Flow Cytometry	'gold standard', High throughput (up to 10,000 cells/s), cells can be sampled at multiple time points, semi-quantitative data Compatible with FACS fluorescence activated cell sorting, single cells can be encapsulated in droplets and cultured	It wasn't designed to work with single cells, one cell can't be followed or identified over time, expensive	Flow cytometry (FCM) is an approach to quantitatively analyze multiple characteristics of millions of single cells and other particulate matter from a heterogeneous population (Brehm-Stecher and Johnson 2004)
Microscopy (automated microscopy, high throughput microscopy...ect)	Time dependent data can be collected and a single cell can be followed, qualitative data regarding cell division and expansion	Low throughput, multiple cell parameters can't be analyzed, a lot of time is spent collecting data, cumbersome process, not ideal for single cells, difficult to get single cells in wells	Cells are fixed or placed in a multi-well plate, and a microscope takes hundreds of pictures of them, then they go through automated image analysis to find useful information
Microwells	The number of wells, and their shape, size, depth and dimensions can be customized according to cell types and applications. Different materials and fabrication methods can be used. Capable of holding cells for a longer period of time. Compatible with microscopy	Throughput is limited to the number of wells. It is difficult to remove cells of interest from the array, it has to be done manually	Cells are mechanically separated. An array of wells is created, each well being small enough that only a single cell can fit within each.
Micropatterning	Large arrays can be made to increase throughput, many	Only compatible with adherent cells that will	A surface is treated to make cytophilic and

	different combinations of surface treatments have been used, cells can be replenished easily by flowing media or nutrients over the array	bind to the surface, can't easily remove cells of interest, substances used to attract and bind cells can affect their behavior, flowing media over cells can cause shear stresses.	cytophobic regions that guide cell attachment and arranges single cells in an array
Mechanical Traps	Compatible with microscopy, high throughput, time efficient, cells can be organized into arrays of traps Compatible with cell culture, can be transferred out of traps	Not designed for long term analysis (<24hrs), flow has to be considered to prevent cell damage,	Cell suspension flows over traps that physically separate single cells
Magnetic Traps	Specific cell types of cells of interest can be selectively sorted out, cells can be sorted according to a variety of factors	Sort term analysis, magnetic components could have an effect on cells	Uses immunomagnetic labeling or binds a magnetic marker to cells so they can be sorted and trapped when they interact with a magnetic field at designated time points
Hydrodynamic Traps	High throughput, cells can be placed in an array, compatible with non-adherent cells	Short term analysis, potential harm or cell damage must be considered,	Most common method of cell trapping in microfluidics, uses small channels or holes next to the main channel that allow enough flow through them to trap single cells as they pass by.
Optical Traps	Very high precision and control of cellular arrangement, can be used to selectively move cells of interest, has been improved to handle higher throughput Cells can be moved within enclosed chambers bc no physical contact is needed, compatible w cell culture	Extremely expensive, laser energy can cause increased heat and photodamage that can harm cells. careful, complex planning and good understanding is required to prevent photodamage	Optical tweezers (focused laser beams): cells are trapped at the focus point of the laser beam, where they can then be repositioned in any direction
Dielectrophoretic Traps	High throughput (10,000 cells/s), allows heat removal that prevent cell damage, sensitive enough to detect a rare event and sort cells according to it	Controlling more cells increases complexity of the design	Cells are moved by forces generated in a non-uniform electric field that direct them If target cells can be labeled and bound to a polystyrene bead they can be sorted from a population

Acoustic Traps	Offers dynamic control over cell environment	Only capable of short term analysis, need thermal control to prevent cell damage	Ultrasonic waves create pressure gradients that isolate cells
Droplets	Low risk of cross-contamination between droplets, the small volume of droplets allows concentrations to reach detectible levels quickly, droplets can be sorted and manipulated, cells can be incubated within their droplets, drops can be merged or split, high throughput ($>10^7$)	Risk of drops coalescing, stabilizing droplets to prevent this requires the use of expensive surfactants, channel dimensions and microfluidic design must be extremely accurate,	Droplets are formed to encapsulate single cells typically using an aqueous cell suspension surrounded by a carrier oil

Appendix B: Photolithography Process

Preliminary Setup. Determine photolithography parameters

Before beginning any photolithography process, the entire procedure must be planned. The primary determinants to spin speeds and duration of baking and development steps are the photoresist material and the desired resist thickness. Refer to the photoresist spec sheets for more information. For example, for a 80 μ m thick process using SU8 2035, we find the following information from the data below:

1. Spin speed: 1600 rpm
2. Soft-bake times: 3 min @ 65 $^{\circ}$ C; 9 min @ 95 $^{\circ}$ C
3. Exposure energy: 215 mJ/cm²
4. Relative dose: 1x
5. Post-exposure bake: 2 min @ 65 $^{\circ}$ C; 7 min @ 95 $^{\circ}$ C
6. Development time: 7 min

The bake times directly relate to the experimental plan, but the UV exposure time must be calculated from the exposure energy, relative dose, the illumination intensity, and an empirical correction factor. The illumination intensity of the UV-KUB should be stable at 23.4 mW/cm², and the correction factor is 1.5 due to the narrow spectrum of UV exposure at 365 nm. For example, from the data above, the UV exposure time should be: $215 \text{ mJ/cm}^2 \times 1 \text{ (multiplier)} \times 1.5 \text{ (correction factor)} / 23.4 \text{ mW/cm}^2 = 13.8 \text{ s}$

Procedure 1. Dehydration Bake

The dehydration bake removes residual water molecules from the wafer surface by heating up the wafer on a hot plate or convection oven. Removing residual moisture increases the adhesion of the photoresist on the substrate.

1. Turn on the blower and light on the cleanhood. Let it run for a few minutes before working inside.
2. Power on the PMC Dataplate hot plate in the clean hood. Ensure the hotplate surface is clean.
3. Set the desired temperature to 120 $^{\circ}$ C. Press the following buttons in order: [SET], "Plate Temp" [1], [1], [2], [0], [ENT]. The display cycles between the set temperature and current

temperature about once per second.

4. Place a clean new wafer onto the hotplate surface. The whole wafer should completely fit on the hotplate surface so that heat can conduct evenly to the wafer.

5. Once the plate reaches the desired temperature, heat for 5 min. To set a timer, press the following buttons in order: [SET], "Timer (h:m)" [4], [5], [ENT]. Or: [SET], "Timer (m:s)" [5], [5], [0], [0], [ENT].

6. Carefully remove from the hotplate with wafer tweezers and allow to cool to room temperature. The wafer is now ready for the next procedure.

Procedure 2. Spin-coating

Spin-coating is a step to apply photoresist onto the wafer. This section will outline the steps of spin coating SU-8, a common type of negative photoresist that is used in the MicroFabrication Laboratory. The procedure is similar for AZ1512, a positive photoresist, except it is deposited via syringe rather than pouring due to its lower viscosity. This step uses the Laurell spin-coater in the fume hood.

Preparation stage:

1. Turn on the spin coater using the left power strip switch under the fume hood. If the display does not light up, turn on the unit power switch at the back of the unit.

2. Turn on the two 7" Dataplate hotplates (Figure 5) using the right power strip switch under the fume hood and set the left one to 65 °C and the right one to 95 °C as in Proc 1,

Step 2 above. (Note, the "5" button sticks on one hotplate so use 96 °C if necessary).

If foil is absent, damaged, or dirty, replace with new foil.

3. Press [Select Process] and choose the appropriate spin program according to your desired parameters. If none exist yet, you must enter a new spin program. Refer to the User Manual or Appendix 1 for programming. If you make any changes or additions, note your changes in

the MFL logbook.

Edit Program 10

Step:001/002 Vac ↓ req Step:002/002 Vac ↓ req

Time:00:10.0 Cpm:00 Time:00:30.0 Cpm:00

Rpm : +00500 Loop:000 Rpm : +01600 Loop:000

Acel: 0100 Goto:001 Acel: 0300 Goto:001

Valv: Valv:

Sens: Sens:

The first step is a slow ramp to 500 rpm at 100 rpm/s and is designed to slowly spread the resist across the wafer. The second step spins faster to determine the final resist film thickness. Only the spin speed (in rpm) needs to be changed for different resist thicknesses; all other parameters should remain unchanged.

4. Remove the spin-coater lid and verify the presence of a foil liner. If the foil is not present, line the bowl with foil to catch photoresist that is removed from the wafer during spinning. Ensure that the bowl periphery is covered above the height of the chuck and wafer, and also completely covering the bottom to the chuck. Rotate the chuck and ensure that the foil does not touch the chuck or impede rotation.

5. Select [Run Mode].

6. Turn on the N2 supply by opening the main tank valve. Ensure an output pressure of 60-70 psi. If the display reads "Need CDA," open the round valve attached to the pressure regulator.

Open the vacuum valve by aligning the black handle with the tubing

7. Make sure that the wafer is clean and dry. Visible dust on the wafer can be removed by gently blowing the wafer using the nitrogen gun, which is located on the right side of the fume hood.

8. Position the 4" wafer alignment tool against the chuck, and using wafer tweezers or your gloved hand, touching only the edge, place the wafer on the chuck aligning to the marks on the alignment tool.
9. Before removing the alignment tool, press the [Vacuum] button. A hiss should be audible, and the display should change from "Need vacuum" to "Ready". The wafer should now be held down on the chuck.
10. Test your alignment by beginning the spin program. Press [START] and observe the edge of the wafer as it turns. It should wobble less than 5 mm. If not, press [STOP], then [Vacuum] to release the vacuum, realign, and return to step 8. Reset the spin program if necessary by pressing [Edit Mode] then [Run Mode] and ensuring the display reads "Ready".

Coating Stage:

1. Ensure the wafer is centered and the spin-coater is programmed and ready to spin.
2. For SU8 2035 photoresists and similar high-viscosity materials, pour the resist directly from a 50 mL conical tube. It will flow very slowly. Pour approximately 8-10 mL of resist onto the wafer in one continuous motion, with the tube far enough to avoid contact with the wafer but close enough to prevent thin filaments of resist from forming: about 1 cm. Once the resist blob covers about 5cm diameter, quickly move the tube toward the edge while tilting the tube upwards and twisting to prevent drips on the outside of the tube.
3. Press the [START] button of the spinner to start spin coating. The spin coating process takes about 1 minute, depending on the program. [OPTIONAL:] Near the end of the second spin step, use a piece of Al foil, rolled into a rod to collect resist streams that fly off of the wafer. Do not touch the edge, but bring the rod close. This will clean up the resist at the edge and somewhat

reduce the edge bead, or thicker later at the edge due that forms due to surface tension.

4. The spinner will stop automatically when spin coating is completed.

5. Verify that the photoresist has been uniformly coated. If striations and streaks are

observed, the spin coating was not successful. Some causes may include:

- dust particles on the surface (clean it better),

- bubbles in the photoresist (heat the resist tube to 40-50 °C in a water bath to remove them; see resist datasheet for more information)

- insufficient resist volume applied

6. Press [Vacuum] to release the chuck vacuum.

7. When the last wafer has been coated, close the vacuum and CDA valves at the N2 tank.

Procedure 3. Prebake (Soft Bake)

The prebake (Soft Bake) procedure is required to densify the photoresist following spin coating and evaporate the solvent. In order to reduce thermal stresses due to the substantial difference in coefficient of thermal expansion between Si and resist, the temperature should be raised and lowered gradually in a 2-step process, first at 65 °C, then at 95 °C, then back to 65 °C.

This step uses the two 7" Dataplate hotplates in the fume hood.

1. Use the "removal tool" to transfer the wafer from the spinner chuck to the 65 °C hotplate.

Set the timer for the desired time at this temperature, and cover the wafer with a foil tent.

2. Transfer the wafer from the 65 °C hotplate to the 95°C hotplate. Set the timer for the desired

time at this temperature and cover with a foil tent. Use wafer tweezers to lift up the edge, but

don't grab the wafer edge, since the resist is still very soft. Instead, slide the "removal tool" underneath and lift.

3. Return the wafer to the 65 °C hotplate for 3 minutes, covered, then transfer it to the clean hood to cool to room temperature. Be sure to place your hand underneath as you move the wafer from the fume hood to clean hood: if you drop it, it'll shatter.

Procedure 4. UV exposure

The UV exposure procedure exposes the photoresist layer to collimated 365 nm UV light via an

LED source through a photomask. A negative resist becomes cross-linked and insoluble in developer when exposed, whereas a positive photoresist becomes soluble in developer when exposed. This procedure assumes that a transparency photomask will be used in direct contact with the resist layer. This step uses the UV-KUB exposure system in the clean hood.

Preparation stage:

1. Turn on the UV-KUB via the power switch at the back left, just above the power cord. Press the silver power button on the front panel, lower right. The touchscreen should light up and display "UV-KUB"
2. Touch the screen to reach the main menu. Touch [Settings] and [Drawer] to unlock the drawer. Wave your hand near the door sensor at the lower left to open the drawer. If there is a wafer or mask present, remove them. Place the 4"x 5" glass slide on the tray and wave near the door sensor to close it.
3. Return to the [Settings] menu (touch the [X] in the upper right of the screen). Touch [Illumination] to calibrate the UV intensity. It should display about 23.4 mW/cm² through the glass plate. If not, adjust your exposure time calculations in "Preliminary Setup".
4. Return to the main menu and select [Full Surface] then [New cycle] then [Continuous]

5. Program the desired exposure duration and intensity. Enter the time using the touchscreen numbers, then a unit ([h], [m], [s] for hours, minutes, seconds), then [v] to confirm. Note that decimal values are not permitted, so round to the nearest second. Next enter the intensity in %, usually 100%, and [v] to confirm.
6. Test the exposure by touching [Insolate]. The drawer will open. Wave it closed. The display should read "Loading in Progress". Touch the screen to start the exposure. Verify that the countdown timer begins at the proper duration.
7. The exposure will end automatically and alert with a loud beep (silence by touching the screen). The drawer will open automatically. Remove the glass slide if present.

Mask alignment stage:

1. Transfer the room temperature, resist-coated wafer to the UVKUB tray, centering it in the circular pattern.
2. Observe the position of any defects in the resist layer. You will try to rotate your photomask such that these defects are removed during development; i.e. they are covered with black mask regions if a negative resist, or are covered with clear mask region if a positive resist.
3. Cut out the photomask circle using scissors, taking care not to kink the transparency film. Ensure it is free of dust, and gently wipe with a lint-free cleanroom wipe or blow with the N2 gun if necessary.
4. Place the photomask over the resist-coated wafer and orient it such that any defects will be removed during development
5. Place the 4" x 5" glass slide over the wafer and mask to keep it flat and in direct contact. First tilt the 5" side to the back corner supports, then gently move it toward you so it rests on the bottom tray surface.

Finally, gently lower the glass plate onto the wafer, ensuring it is fully covering the mask and wafer, and that it did not move the mask while lowering.

Exposure stage:

1. When you are satisfied with the mask orientation and glass plate placement, wave the door closed. Touch the screen.

2. When it asks: "What do you want to do?", touch [Continue] on the screen.

The last used program will begin automatically after 1-2s. Verify the correct exposure. If anything is awry, immediately press the large red button to abort and retry.

3. The exposure will end automatically and alert with a loud beep (silence by touching the screen). The drawer opens automatically.

4. Gently lift the glass slide with wafer tweezers and set aside. Gently lift the photomask with wafer tweezers and set aside.

5. Observe the resist surface. At this point, no pattern should be easily visible. If it is, the exposure time was too long.

6. Wave the drawer closed when done exposing, then touch the screen and select [Cancel].

Procedure 5. Post-Exposure Bake (PEB)

The post-exposure bake completes the process of crosslinking a negative resist or solubilizing a positive resist. As in the prebake, a two-step heating and cooling is required to minimize resist layer thermal stresses. This step uses the two 7" Dataplate hotplates in the fume hood.

1. Transfer the wafer from the UV-KUB to the 65 °C hotplate in the fume hood. Be sure to place your hand underneath as you move the wafer so it doesn't drop. Set the timer for the desired time at this PEB temperature.
2. Observe the resist surface. With ideal exposure, the mask pattern will become slightly visible in 5-30 s. Cover with a foil tent.
3. Transfer the wafer from the 65 °C hotplate to the 95 °C hotplate and cover. Set the timer for the desired time at this temperature.
4. Return the wafer to the 65 °C hotplate for 3 minutes, then transfer it to a cleanroom wipe on the work surface to cool to room temperature. At this point, the mask pattern should be clearly visible. If not, exposure and/or baking times were too short.

Procedure 6. Development

The development step dissolves away the unexposed negative photoresist (or exposed positive photoresist). It is performed by immersing the wafer in developer liquid and agitating until the resist is dissolved and only the insoluble pattern remains. This procedure uses a glass dish and developer chemical in the fume hood. Developers are located in the flammable cabinet below the fume hood, left side.

1. Ensure the glass dish is clean. Clean and dry with a cleanroom wipe if necessary. Pour developer in the dish to about 0.5-1 cm depth.
2. Immerse the wafer in developer and gently slosh/agitate, taking care not to splash developer out of the dish. Start a timer on the hotplate with the desired development time.
3. Observe the wafer periodically. Bare Si regions will become visible after ~30s - 1 min. The resist at the edge is thicker than in the center, and therefore tends to be the last part to dissolve away.

4. When all resist appears dissolved, remove it from the developer bath with wafer tweezers and run under a gentle stream of water in the hood sink. Grasp the wafer in your hands at the edges to ensure it doesn't fall and break! Note the time of development in your lab notebook.

5. After both front and back sides are rinsed in H₂O, dry both sides with the N₂ gun. Bring the nozzle close to the wafer and sweep side to side, especially in areas with small resist features.

6. Inspect the wafer as described in Procedure 7 below, and then perform a final cleaning development by holding the wafer with tweezers horizontally over the dish and squirting a small amount of fresh developer on the wafer. Gently slosh side-to-side for about 15s. Rinse with H₂O and dry with a N₂ gun.

Procedure 7. Inspection

Inspection is a step to verify general process quality and the development process. This section

will outline the main feature distortions that are encountered in photolithography process. The

Zeiss Stemi-2000 stereo microscope is equipped with a fiber-optic light ring and is used to visualize the wafer in reflectance mode.

After initial development and rinsing, the wafer will appear dirty. This is OK! It is due to the resist that has dissolved in the developer and will be cleaned to a shiny surface after brief wash with fresh developer. Also, sharp corners and large resist fields will likely display surface cracks. This is also OK! It is due to the thermal stresses during bakes, which were minimized by gradual heating and cooling but not fully eliminated. These cracks will be eliminated with the Post-bake, Procedure 8.

1. Development time. Pay attention to the smallest features in the resist pattern. Lines should be sharp, with no evidence of resist material in regions where it should be removed. If not, development is incomplete.

Return the wafer to the developer bath and repeat for ~30s, then rinse, dry, and re-inspect. Instead, if the

resist layer that should remain looks especially cloudy or rough, the wafer may be over-developed.

Additionally, overdevelopment may narrow a resist feature or widen a resist "hole", and underdevelopment may do the opposite.

2. Bake times and temperatures. The extent to which a feature deviates from its ideal size is a function of the exposure time, prebake temperature, prebake time, development temperature and development time. Any of these parameters could be the cause for overdevelopment or underdevelopment and it is therefore important that one understand some important troubleshooting techniques. The key idea to troubleshoot the distorted feature is to observe the effect of changing a parameter while holding the other parameters at constant. The following example illustrates this idea.

It can be observed that by changing the exposure time while holding the other parameters at constant, there is a time window where the feature size is optimal, i.e. between 15s and 25s in this example. If changing of this parameter does not produce the desired feature size, the problems are most likely to be caused by other parameters or combinations of several parameters. Repeated troubleshooting with other parameters should be carried out.

Procedure 8. Post-bake

The Postbake procedure is required to stabilize and harden the developed photoresist prior to

processing steps that the resist will mask. Typical post-bake temperature is 150 °C for 30 min for SU8 (or 90-120°C for 5 min for other thin resists). This procedure uses any of the Dataplate hotplates.

1. Place the developed wafer on a hotplate at no more than 65 °C.
2. Set the ramp rate to 6 °C/min or 360 °C/hr: [SET], "Ramp °C /hr" [6], [3], [6], [0], [ENT].

Set temperature to 150°C. Set the timer for 45 minutes. Set the hotplate to automatically turn off then the timer ends, by pressing "Auto Off" [8]. Cover with a foil tent.

3. The hotplate will slowly ramp up to 150°C over about 15 mins, maintain temperature for

~30mins, then turn off and slowly return to room temperature. This will take around 1 hr total.

4. After the wafer has returned to room temperature, inspect the wafer again and verify that surface cracks have disappeared. Document selected microscope fields with a camera.

Appendix C-Soft Lithography SOP

PROCEDURE 1: Fluorination of the Micropatterned Substrate

This procedure facilitates mold release by covalent treatment of Si or glass surfaces with a fluorosilane chemical by vapor deposition. The treatment renders the Si or glass hydrophobic, and maintains the Micropatterned SU8 features as long as possible without delamination by reducing the forces applied during PDMS de-molding.

1. Set up the vacuum dessicator inside a fume hood. Line the bottom surface with foil if damaged, missing, or dirty. Prepare a support ring (cardboard or other material) and line up Si wafers (or glass slides) along the inner part of the ring, with the side to be treated facing inwards.
2. Make aluminum foil boat big enough to hold 40 uL (about 1 drop) and place in the center of the platform.
CAUTION (Tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (TFOCS; Gelest, SIT8174.0; or United Chemical Technology, 6H-9283) is corrosive and toxic! Avoid direct contact and always handle it in the fume hood.
3. Pipette 40ul of the TFOCS chemical directly from stock bottle and place into the aluminum foil boat you just made. Remove the pipette tip by hand and gently place into the vacuum chamber (Do not eject it!)
4. Close the chamber and vacuum for 1 hour.
5. After 1 hr, remove the treated Si wafers (or glass). If any hazy film appears, remove with 15 - 30s contact with isopropanol, rinse with water, and dry in an air stream.
6. Fluorinated pieces are ready to use right away. Verify hydrophobicity by observing contact angle of water drops on the treated surface. Water drops should roll off the surface, leaving it dry.
7. After a few hours, the chemical liquid will have evaporated. Discard foil boat and pipette tip in hood waste bag.

PROCEDURE 2: Preparing the PDMS Mixture

This procedure prepares a PDMS mixture for casting. We use Sylgard 184, which comes as a kit with Part A (monomer) and Part B (cross linker). A typical ratio is 10:1 (w/w). For simplicity, we typically weigh out the components into a single weigh boat on a balance.

1. Set up a paper tower on the balance, ensuring it does not hang over the edges, and a large weigh boat. Remove any visible dust.
2. Determine your desired PDMS volume. Each wafer requires about 50-60g PDMS. Ideally, you should make about 80-120 g PDMS per weigh boat, up to three boats at a time.
3. Tare the weigh boat (set weight to 0.0g). Pour Part A) into the weigh boat until the desired weight (e.g., 91.2g). Then divide this value by 10 (for 10:1 ratio), tare again to 0.0g, and pour Part B to the desired weight (e.g. 9.1g). Within -0.2/+0.5g is ok.
4. Using a transfer pipette, slowly and gently fold (as in baking) the low-viscosity Part B into the high-viscosity Part A. Once Part B is no longer visible on the surface, increase your folding speed. Ensure that all edges have been mixed. Mixing should take at least 1 min, ideally >2. (Technique is more important than time here). *There should be lots of bubbles!*
5. Place the weigh boat into the vacuum chamber. If more than one is prepared, invert a second weight boat on top, rotated such that the PDMS in the lower boat is visible, and place the second PDMS boat on top. Repeat one more time for three total, as needed.
6. Apply a vacuum and observe bubble enlargement. Release the vacuum after 1 min as necessary if bubbles appear as though they may overflow. This pops many of them, and reduces the likelihood of spillage.
7. Degas for 1 hr. At this point, all bubbles should be gone and PDMS is ready to pour in Procedure 3. Be careful when releasing vacuum! Air rushing in could knock over the PDMS boats.

PROCEDURE 3: Casting and Curing PDMS

During this procedure, mixed PDMS is poured over the Si/SU8 mold master in a dish or foil vessel, bubbles and/or dust particles are removed, and the PDMS is cured by baking at 65C for >3hrs.

1. Prepare casting vessels by bending a foil sheet over the bottom of a 500 mL Erlenmeyer flask. Flatten the edges until they are about 10 - 15mm high. Ensure the bottom surface is flat.
2. Set up the masters to be cast on the bench top covered with absorbent mats and a paper towel. If dust is visible, blow with the air gun. Weigh the master and vessel, recording the weight.
3. Once the PDMS mixture has been degassed for 1hr, and surface bubbles are gone, bring them to the masters.
4. Pour PDMS mixture across the wafer, from one side to the other, in a continuous movement. This reduces the number of bubbles formed. At this stage only the wafer needs to be covered.
5. Weigh the PDMS+master+vessel and subtract the master and vessel weight. About 60g PDMS is the target. If more is needed, bring the vessel back to the absorbent pad and pour more. Repeat until the desired PDMS weight is achieved.
6. Cover to prevent dust and observe after a few minutes any bubbles or dust remaining.
7. Surface bubbles can be removed by mouth blowing (from about 10 cm away). Deeper bubbles can be left until they rise to the surface. Bubbles adherent to the Si or SU8 surface can be dislodged by tilting the vessel back and forth (causing shear forces). Be careful not to spill any PDMS! It's messy, sticky, and hard to clean off
8. Large dust particles can be moved or aspirated with a disposable transfer pipette.
9. Once you are satisfied with the casting, place it onto a level shelf in the 65C oven, and bake for at least 3hrs. Leaving overnight is also OK.

PROCEDURE 4: Preparing a PDMS device

This procedure completes a PDMS device, including punching inlet and outlet holes for microfluidic devices.

1. Demold the cured PDMS from the Si master. Peel off the foil and carefully remove the Si wafer. If PDMS coated the underside of the wafer, you may need to cut it out with a scalpel or razor blade. Store the Si master in a safe place, ideally a wafer holder.
2. Set up the rubber cutting pad. Use a straight razor blade to identify the indentation line that separates adjacent devices, if present. Then, align the razor vertically and apply pressure to complete the cut. If

necessary, move the razor to the next position and cut with downward pressure. *Do not slide the razor through the PDMS! It will deform as you cut.*

3. Once your device has been trimmed, determine the size of any inlet and outlet holes.
4. Apply Scotch Magic tape to the micropatterned side. If desired, mark the center of each hole for easier viewing.
5. Flip over the device, tape and channel side on the rubber cutting pad.
6. Using a dermal punch of desired diameter, punch downward and in a straight line until contact with the rubber cutting pad.
7. Lift up the device, *leaving the punch inserted*, and a cored PDMS piece should protrude from the channel/tape side. Remove it before gently removing the punch.
8. Repeat steps 6-7 until all holes are punched.
9. Clean the punched holes by squirting water through each hole with a wash bottle. Repeat with ethanol and water again. Then dry in an air stream. This process removes any PDMS particles that may have been left behind during punching.

PROCEDURE 5: Plasma Bonding

This procedure covalently binds PDMS to glass, Si, or PDMS by oxygen plasma treatment of clean surfaces.

After plasma activation, surfaces are brought into contact, forming an instant and irreversible bond. Oxygen plasma is also useful for cleaning substrates and vaporizing organic materials. (This is a relatively slow process, and it will remove organic thin films, not clean off dust.)

Materials and equipment needed: glass tray, test slide and scrap PDMS piece, tape, plasma cleaner, vacuum pump

Plasma bonder/cleaner setup:

(Set-up required only if plasma system has not been used recently)

1. Turn on the vacuum pump and open the "specialty vacuum" valve on the fume hood (labeled "SV"). A hissing noise should be heard in the chamber.

2. Close both valves on the round metal door. Align it to the glass vacuum chamber, and after a few seconds ensure that it is firmly held onto the chamber. *Support it and do not let it drop!*
3. Start a timer. After about 15s, turn on the power and set power level to [High]. A purple glow should be visible through the vent holes after a few seconds.
4. Once a purple-glowing plasma is visible, *slowly* open the needle valve *a very small amount* to let in room air and oxygen. The plasma should brighten and become more orange. If it dims too much, close the needle valve slightly and observe the bright plasma return after a few seconds.
5. Allow the chamber to clean for 1-2 minutes.
6. When plasma treatment is completed, turn off the unit power and the vacuum pump power. Slowly open the exhaust valve until the vacuum has been released. *HOLD ONTO THE DOOR*, or it will fall!

PDMS Bonding:

1. (Optional) Prepare a test bonding sample, such as a scrap of clean PDMS and a clean glass fragment (or two PDMS scraps). Remove dust with tape. Then follow Steps 2-10, and if successful, repeat Steps 2-10 with the desired parts to be bonded.
2. Seal the PDMS on the tray slide with treatment side facing up. Next to it, place the glass fragment (or the second PDMS piece).
3. Insert the tray into the chamber. Ensure the door valves are closed, turn on the vacuum pump, and align the door until it is held in place.
4. After ~5s, turn on unit power and wait for purple plasma as described in steps 3-4 above. Start a time when it appears and adjust needle valve to generate brighter plasma.
5. Treat PDMS surfaces for 60s.
6. Turn off unit power and the vacuum pump power. Slowly open the exhaust valve until the vacuum has been released. As before, *HOLD ONTO THE DOOR*, or it will fall!
7. Carefully remove the plasma-activated PDMS and glass.
8. Gently invert the glass onto the activated PDMS surface. Bonding is covalent and instantaneous, so there is no opportunity to realign! Make sure you align before any contact, and be as gentle as possible.

9. Once the PDMS is sealed, apply light pressure to remove any air bubbles that may have been trapped inside.

10. Wait about 15 - 30s, and test an edge for bonding by *very gently* peeling up at the corner. A successfully bonded PDMS piece will not peel away from the substrate, and will break internally before debonding!

Appendix D- Tween 20 Surfactant

Preparing Tween Solutions

- Fill the container for making the solution with desired amount of de-ionized water and place it on the heating surface, such as the hotplate.
- Heat until the water reaches a rolling boil and leave water boiling for ~5 minutes.
- While the water is heating, using a precise scale and a pipette measure 0.10grams of Tween per each 100ml of water. (Example: 1500ml de-ionized water, $0.10\text{g} \times 15 = 1.5\text{g}$ of Tween)
- Slowly add pre-measured Tween to hot water and mix with immersion mixer for about 30 seconds. (Wait until water cools and any bubbles have settled before using solution)
- When finished, the solution should look clear and uniform.

Using Tween Solutions to Suspend Particles

The specifics of this section will pertain to creating Cospheric's Density Marker Beads (DMB) products which are defined to have 20% solids in 2.5ml. The process is easily modified to other situations.

- Using a 2.5ml vial, add 0.5g of the desired microspheres. (Any container and hydrophobic particles may be used).
- Using a pipette or syringe, dispense 2.0ml of the 0.1% Tween solution on top of the spheres.

- Weigh the microsphere/Tween solution and add any additional Tween solution to each vial to ensure vial weights are equal to better balance the centrifuge while it is operation.
- Secure the cap on the vial.
- Centrifuge for 5 – 10 minutes to get the spheres wetted and into solution.

http://www.cospheric.com/tween_solutions_density_marker_beads.htm

Appendix E: BME Educational Objectives

3. An ability to design a system, component, or process to meet desired needs *within realistic constraints such as economic, environmental, social, political, ethical, health and safety, manufacturability, and sustainability* (ABET 3c) *while incorporating appropriate engineering standards* (ABET Criterion 5) (*need to assess each of these separately, but since 'or' and "such as" not all need to be met separately*).

- i) multiple realistic constraints (*economic, environmental, social, political, ethical, health and safety, manufacturability*) – **page(s) 54-55**
- ii) appropriate engineering standards - **page(s) 23-28**

4. An ability to function on multidisciplinary teams (3d). **page(s) 8**

6. An understanding of professional and ethical responsibilities (3f)

- i) Professional – **page(s) 23-27**
- ii) Ethical – **page(s) 54**

7. An ability to communicate effectively (3g). **page 24**

8. The broad education necessary to understand the impact of engineering solutions in a global, *economic, environmental*, and societal context (3h). (both economic AND environmental need to be addressed)

- i) Economic – **page(s) 54**
- ii) Environmental – **page(s) 54**

10. A knowledge of contemporary issues (3j). **page(s) 13-20**