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Single cell Enrichment with High Throughput Microfluidic Devices

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Single cell Enrichment with High Throughput Microfluidic Devices

THESIS

submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Pedram Pakjesm Pourfard

Thesis Committee:
Assistant Professor Jered Haun, Chair
Professor Abraham Lee
Assistant Professor Elliot Hui

2017
DEDICATION

To

my parents and friends

in recognition of their worth, love, and help
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ABSTRACT OF THE THESIS

Single cell Enrichment With High Throughput Microfluidic Devices

By

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Master of Science in Biomedical Engineering

University of California, Irvine, 2017

Professor Jered Haun, Chair

Microfluidics is a rapidly growing field of biomedical engineering with numerous applications such as diagnostic testing, therapeutics, and research preparation. Cell enrichment for automated diagnostic is often assayed through measurement of biochemical and biophysical markers. Although biochemical markers have been widely used, intrinsic biophysical markers, such as, Shear migration, Lift force, Dean force, and many other label-free techniques, are advantageous since they don’t require costly labeling or sample preparation. However, current passive techniques for enrichment had limited adoption in clinical and cell biology research applications. They generally require low flow rate and low cell volume fraction for high efficiency. The Control increment filtration, T-shaped microfluidic device, and spiral-shaped microfluidic devices will be studied for single-cell separation from aggregates. Control increment filtration works like the tangential filter; however, cells are separated based off of
same amount of flow rate passing through large space gaps. Main microchannel of T-Shaped is connected to two perpendicular side channels. Based off Shear-modulated inertial migration, this device will enable selective enrichment of cells. The spiral shaped microfluidic device depends on different Dean and lift forces acting on cells to separate them based off different sizes. The spiral geometry of the microchannel will enable dominant inertial forces and the Dean Rotation force to cause larger cells to migrate to the inner side of the microchannel. Because manipulation of microchannel dimensions correlates to the degree of cell separation, versatility in design exists. Cell mixture samples will contain cells of different sizes and therefore design strategies could be utilized to maximize the effectiveness of single-cell separation.
CHAPTER 1: INTRODUCTION

Microfluidic devices are microscale devices that only use a small amount of liquid for sample processing. Therefore, these devices are the best for point of care purposes. Furthermore, microfluidic devices have been used in various fields, such as biotechnology, chemical engineering, physics, etc. In these microfluidic devices, new properties have been observed in fluid dynamics so that it can be used for various applications. In a macroscale level, viscosity and surface tension forces are usually neglected, whereas this is not true in microscale level devices. Moreover, the small size of microfluidic devices allows researchers to have better manipulation and control over their experiments. Additionally, the most important aspect of microfluidic devices is their minimal need of sample for experiments, which increases their application in health industry.

1.1 Background

The first known microfluidic device was used for gas chromatography in Stanford University in 1980s [1]. Their only goal was using microscale devices to do chromatography. In 1990, Manz et. Al. published a paper and drew the attention of researchers on various possible applications of microdevices and caused fast progress in the microfluidics field in industries and universities [2], [3]. Initially, microfluidic devices were being used for pumping and mixing at microscale levels, but these devices have much more variety today. These initial devices were mainly constructed by a glass-silicon method that was substituted
with a more efficient and inexpensive method that has a better compatibility with biology systems today [4].

Advent of Lab on Chip was another huge progress in microfluidic devices. Lab on Chip is a small laboratory that consists of a pump, microchannels, micromixer, and any other necessary equipment for doing specific experiments [5, 6]. There are two main concepts behind Lab on Chip. The first one is ease of access in any place in the world because of its small size, which doesn’t need any fully equipped laboratory or specialized personnel. The second concept is lowering the amount of sample it needs for processing an experiment. If the size of microchannels are a thousand times smaller than a laboratory tube, the laboratory will process the same experiment with one million times more of the sample. For example, if a macroblood test is happening with one liter blood, only one microliter of blood is needed for the same experiment in microfluidics experiment. In ther words, microfluidics can be used only one time, which will lower possibilities of contamination [7].

One of the important features of microfluidic devices is separation and cell sorting for automated sample processing that is being used in many health care fields, such as cancer and stem cell research. Separation of desired cells from blood is one of the most prevalent and effective ways for diagnosis and treatment. In many cases, selecting and sorting rare target cells are crucial in early diagnosis of malaria, cancer cells, circulating tumor cell, and HIV. Furthermore, purified or concentrated samples can be prepared for investigating on biochemistry and biophysical properties of them [8].
1.2 Importance of Thesis

Cell separation and sorting are critical in many biological related experiments and therapeutic methods. In conventional methods, biochemical labels were being used to determine and separate cells, but recently, there has been more interest in methods that use intrinsic biomarkers of cells. Although the conventional methods can sort cells efficiently in a short amount of time, the huge equipment, biohazardous aerosols and complex process of cell sorting cause researchers to be more inclined to miniature devices than before. Moreover, parallelization and lab-on-a-chip capability of these devices make them more suitable and attractive for cellular isolation and experimental processing.

Cell sorting is the first step for diagnostics. Improvement of miniaturize chips on one side and understanding of intrinsic properties of cells on the other side helps researchers to separate and enrich cells to desirable purity. Microfluidic devices are used for sorting hematopoietic stem cells for autologous patient treatments [9]. Instead of increasing need of isolating rarer cells from blood and performing experiments on them, it is crucial to separate different cells of the blood compartment and then analyze them. Among them are circulating tumor cells (CTCs), hematopoietic stem cells (HSCs), and circulating fetal cells (CFCs) from blood [10-13]. Cell free plasma is being used for early cancer detection [14]. However, there is no universal agreement about a marker which can be used to sort CTCs, and therefore, makes non-labeling methods a perfect choice for stratifying them. Other blood components are being used for diagnosing as well. For example, white blood cells (WBCs), or leukocytes, are required for few hematological tests and DNA sequencing [15]. Reticulocytes and mononucleated red blood cells are markers for abnormal red blood cell turnover [16].
Microfluidic devices could be used for therapeutics after diagnostics. Platelet transfusion after purifying during surgery is one example [17]. The other areas which researchers are interested in are enrichment of malaria-infected cells and removing CTCs and bacteria before transfusing again to patient to prevent cancer and infection. Moreover, label free devices are being used for cell biology. Understanding the cell behavior needs more of single or enrichment cells than heterogeneous mix of cells. Therefore, current methods for isolating CTCs, circulating stem cells, and white blood cells consist of staining and using biochemical markers. There are few drawbacks, however. First, there aren’t markers available for every cells. Furthermore, these markers change the phenotype of the cells. Adding markers could change the rate of differentiation and their expansion. Finally, these markers add more intricacy and cost to the whole process [18]. On the other hand, label free microdevices would be a great substitution for being used to purify cells.

1.3 Classification and Definitions

Different biomarkers are being used mainly to purify and enrich cell samples for further processing. Some of these biomarkers are size, electrical properties and hydrodynamics properties. Usually, a more generic term of microfluidics is being used for all of them, but it is possible to divide it into smaller subsidiaries. These devices could be classified in two broad categories [19].
1.3.1 Active Microfluidics

First category is microfluidics devices, which use nonfluidic body forces, such as acoustic, electric, magnetic, and optical to move cells around. These devices are active systems. Although active systems have higher efficiency levels than passive ones, all of them need expensive equipment, which occupy large spaces. Producing active devices is more challenging and requires more expensive techniques. In addition, larger equipment and sizes limit their point of care feature and make them less transportable and also, running an experiment consumes more energy compared to passive microfluidics. Moreover, as it was mentioned, some of these active devices can harm cells. Therefore, it makes them less favorable than passive microfluidics devices.

1.3.2 Passive Microfluidics

Passive systems use inertial forces, filters, sediment and gravity to sort and enrich cells. In passive microfluidics, researchers use channel geometry and fluid properties to design devices. Viscosity forces, surface forces, and capillary forces are comparable to other fluid forces in microscale and it can be used in designing. As it was mentioned, although active forces are more effective and faster, passive devices are cheaper, less challenging for mass production, more transportable, and need less equipment. Therefore, increasing the efficiency and improving passive devices to be more reliable in addition to their reasonable price can make them the best choice for industry purposes.
CHAPTER 2: LITERATURE REVIEW

In the following chapter, common microfluidic devices are explained and various methods for sorting and enrichment are being investigated. As it was mentioned before, there are two general categories of microfluidics: active and passive. In this chapter, after introducing flow cytometry, FACS and MACS, different passive microfluidic devices will be investigated.

2.1 Flowcytometry

One of the most important cell sorting strategy is flow cytometry. Flow cytometry is used to count and analyze the size, shape and properties of individual cells within a heterogeneous population of cells. This device sucks the sample and mixes the sample into a stream of saline solution in the cytometer, and leads to a cell suspension through a narrowing channel, causing the cells to form a single line before the cells pass through a laser at the interrogation point. Sample rate of these devices is close to 50’000 Cell/s [8]. In addition, in flow cytometry, it is common to use markers and detect emitted light from excited fluorescent molecules. This method was introduced in 1969 by Herzenberg et al. [20]. This commercial cell sorter exploits a technique called fluorescence-activated cell sorting (FACS). Similarly, magnetic-activated cell sorting devices (MACS), which generally operate by separating cells with magnetic labels from unlabeled cells via a permanent magnet, are widely used due to their rapid processing [21].
2.2 Cell Separation by Microfluidics Devices

In the following, different methods that separate cells are briefly reviewed. In general, all of cell sorting mechanisms can be divided into three broad categories, (i) fluorescent label-based, (ii) bead-based, and (iii) label-free cell sorting. The device used for this thesis project belongs to the third category.

Different markers are being used to sort cells without labels. These markers can sort according to inherent properties of cells such as, size, shape, electrical polarizability, electrical impedance, density, deformability, magnetic susceptibility, and hydrodynamics. Furthermore, these markers can be divided in two larger groups of active or passive. Active microfluidic devices rely on outside forces (e.g. magnetic, acoustic, dielectrophoretic) to convey particles to the desired channel or output. This group usually not only requires to have large devices to create outside forces, but also works with low throughput and complex sample preparation. On the other hand “Passive” microfluidics approaches usually either use hydrodynamic flow by designing specific kinds of channels to divert one type of particles to a different channel, (e.g. few of these kind of microfluidic devices are deterministic lateral displacement (DLD) [22], microfluidic crossflow filtration [23], plasma skimming [24], pinched flow fraction [25] and hydrodynamic filtration [26]) or using the natural inclination of particles to distinguish them, such as velocity profile (e.g. the tubular pinch effect [27]) and Dean flow fractionation [28]).

Development of new microfluidic devices to separate cells needs a mechanism to implement a sort of force or fractionation. According to low Reynolds number, the ratio of inertial to viscous forces in laminar flow in microfluidics, indicate that viscous forces are
more dominant than inertial forces. This forms into an idea that an external force is needed to manipulate particles in the channel. However, nowadays many observations have been noticed of which streamlines could be implemented and questioned the idea of using external forces. In sum, the scale of microfluidic systems provides an interface to manipulate cells with kinetic, equilibrium, and elution separation [8].

2.2.1 Microscale Filters (Size, Deformability)

The first and most common biomarker is size. There are so many filters with different pore sizes which separate different particles according to their size. Clogging and other reasons cause poor efficiency for these filtration systems. Microscale filters have better efficiency for doing this task and four types of them has been introduced: weir, pillar, cross-flow, and membrane. In the following picture, three of these types can be seen.

![Figure 1] Three Types of Filter for Separation [8]. a) Weir-type b) Pillar-type c) Cross-flow

Weir type filtration has a planar surface at the top section and blocks most of the flow except the small slit at the top. This microscale filter is being used to separate cells from plasma. When blood is passing through, plasma is deviated to a new direction, but cells which are small enough to pass through the little space can pass through the top section. The amount of produced plasma is limited to several nanoliters, but new design of continuous weir-type
filters improve it to larger volumes [29]. Pillar filtration is almost the same as dead end filtration because cells are trapped in the direction of flow and cause clogging and folding. In this design, the space between pillars specify the cutoff diameter of particle and is gradually decreased in each step, which makes them capable for sorting different cells with different sizes. Mohamed et al. isolated fetal nucleated red blood cells (fNRBCs) from maternal cells in cord blood with this design [30]. Cross-flow design uses the same concept as the other two designs, but in this type, pillars are used in another direction and larger particles pass through the main channel and smaller particles are dragged out in the side channel and thus, filtration is being done. The key fact about this method is the gap between pillars should be small enough to not let larger particles pass them. This method has been used for separating plasma from whole blood [31]. The last type is membrane filters, which are comprised of well-defined pores and the gap size helps define the size of cutoff. These types of filtration still have issues with clogging, but by increasing the surface area this would be of less concern. The efficiency of 90% is being observed with massive array of oval pores [32]. To summarize, all four types of filters have been investigated and been compared with each other. Ji et al. [33] illustrates that cross flow filter has higher efficiency for separating white blood cells from red blood cells, but in general, microscale filters were unsuccessful in separating cells based on size.

Filters based on size-based separation have a few drawbacks. First and most important, in biology, the particles are not the same size. Therefore, while trying to separate a target cell, there is a wide range of cells with different diameters that have to be considered. Clogging is always a problem with these devices, although, the cross flow mitigates this
problem among those four types of filter devices. Furthermore, some cells are deformable, such as CTCs, and may be damaged when they are passing through the filters, especially when the flow rates are increased [34]. The ability to mix these microdevices with on chip protein analysis for point of care diagnostics allows for an interesting way for separation.

2.2.2 Hydrodynamic Filtration (Size, Shape)

Hydrodynamic filtration or pinched flow fraction both use the same concept for sorting. In this technique, by assuming laminar flow, center of mass of particle will follow streamlines. Two inlets are designed, one of them carry all of the cells and the other one is being used to pinch the cell to the wall [33]. The aligned cells are near the wall and then cells will encounter a sudden expansion. This expansion will increase the distance of the particle with different sizes. Center of mass for smaller size particles will be closer to the wall and this will result in a different path for cells.

2.2.3 Pinched Flow Fraction

Pinched flow fraction is one of the passive methods that separate particles based on size. In laminar flow, particles follow stream line in stokes regime [35]. As it could be seen in Figure 2, two channels, in which one of them that contains the fluid and the other one that contains cells or particles with different sizes will be joined together. In the narrower section, cells or particle are pinched to the wall by controlling the flow rate of channel that only contains the fluid. In laminar flow, cells will follow a streamline that passed through their mass center.
Therefore, larger particles will follow a different streamline than the one for smaller cells. Finally, cells will be separated based on their size in the expansion region of the device.

\[ y_0 = \left( w_p - \frac{D}{2} \right) \frac{w_0}{w_p} \]  

(1)

In this method, laminar flow should be used, which will dictate low flow rate over design. On the other hand, the cells volume fraction doesn’t affect the efficiency of the design. The overall efficiency of the design relates to flow rate of two channels and the expansion region design section. If the expansion width of microchannel is more than specific, the overall efficiency will decrease. Yamada et. al. [37] proposes an experimental equation based on different width lengths:

*Figure 2) Schematic of Pinched Flow Fraction [36].*
In equation 1, \( w_0 \) is expansion channel length, \( w_p \) is joined channel width, \( D \) is particle size, and \( y_0 \) is mass center of particle in exit channel. Therefore, based on \( y_0 \), different channel cut of size will be designed to collect appropriate cells. The channel width is not the only factor, but also hydraulic diameter, cell size population, and smoothness of channels are as important. Thus, particles smaller than surface roughness cannot be separated based on this method [37].

As it is illustrated in Figure 3, exit channels are designed radially and with the same channel width. Thus, all of them will collect the same volume fraction. Larger particles will stay in streamline and will exit from the main center channel, whereas smaller particles will be separated in a different exit channel.
Figure 3) Cells Pathway in Two Different Flow Rates of Inlet 2. In the left picture, higher flow rate cause cells to be pushed toward top section. Right picture has lower flow rate and have different cell pathway [26].

In Asymmetric Pinch Flow Fraction, exit channels are in a semicircle fashion, as it is shown in Figure 4, for higher efficiency. Contrary to the previous design, exit channels have different widths and lengths. Therefore, different volume fraction can be extracted by different exit channels [26]. The higher collecting channel are designed in front of the main channel and by flow rate control, no cells will be collected from this channel. In this way, higher efficiency is obtainable and all of the channels will be used.
It is possible to increase the efficiency of pinched flow fraction by designing curved microchannels and rotating the whole substrate [38]. In the new design, the microfluidic device has curved microchannels with a small radius and the whole substrate will rotate the microfluidic device. When cells enter the curved microchannel section, they will experience inertial radial force perpendicular to their pathway based on their sizes and densities. Therefore, this new design will separate particles not only based on their size, but also based on their density. Particles with same sizes, but higher densities will be trapped in the initial channels. In general, sedimentation speed, $U_s$, of particles can be calculated based on equation 2.
\[
U_s = \frac{\rho_p}{18 \mu r_c} D_p^2 U^2
\]  

(2)

In the above equation \(\mu\) is viscosity, \(\rho_p\) is particle density, \(r_c\) is curved channel radius, \(D_p\) is particle diameter, and \(U\) is the average velocity. Sedimentation velocity is proportional to square of velocity and will use the pinched flow fraction method, as the microchannel velocity decreases because inertial forces will decrease, respectively. On the other hand, if the same design is being rotated with few hundred rpm, total sedimentation velocity will be the sum of centrifugal force and previous sedimentation velocity. Therefore, the total sedimentation velocity can be calculated based on equation 3.

\[
U_s^t = U_s + U_c = \frac{\rho_p}{18 \mu r_c} D_p^2 U^2 + \left( \frac{\rho_p}{18 \mu} - \frac{\rho_f}{18 \mu} \right) D_p^2 r_d \omega^2
\]  

(3)

In the equation 3, \(U_c\) is centrifugal velocity due to rotation, \(r_d\) is particle distance from center of rotation, and \(\omega\) is rotational velocity in the microfluidic device. Morijiri et. al. [38] used this method with different rotations per minute and the result shows that rotation of device doesn’t have much influence on efficiency and it could only be used as a pump.

**Figure 5** Two Different Radius of Curved Microchannel Pinched Flow Fraction
2.2.4 Deterministic Lateral Displacement

The newer version of separation in microfluid devices is deterministic lateral displacement. This microfluidic device is comprised of many posts and these posts are being used to change the pathway of different cells based on their size. In this design, different cells and particles will be positioned differently based on their size, with respect to the posts. Smaller particles will be closer to the post and each row of posts will have an offset from the previous row. Therefore, the posts are not in the same line and they will be directing cells differently with respect to size.

![Deterministic Lateral Displacement Schematic](image)

*Figure 6* Deterministic Lateral Displacement Schematic. A) displacement of second row compare the first one B) Three different flow pathway that start from the same position C) gradual cell migration of cell based on position of the center mass [39].

The main reason for lateral migration of cells is due to their size and shape [39]. Therefore, different sized cells will migrate differently in the microchannels. In this design, smaller particles will stay closer to the flow direction and as the cell size increases, lateral migration will increase. The amount of lateral migration can be calculated based on the shift of the post in each row and distance between the posts [23]. This device has been used to separate...
leucocytes, red blood cells, and thrombocytes [40]. There are advantages and disadvantages for this device. The main feature of deterministic lateral displacement is their high resolution. This device has 10 nanometer resolution which is much higher than other methods but there are a few drawbacks:

1) Cells can be separated only in laminar flow. Thus, flow rate of these devices should be low.

2) Complicated fabrication because of many posts.

3) Clogging issue in high cell volume fraction in these devices.

Industrial deterministic lateral displacement device (Artemis Health) has shown its ability for separation of nucleated red blood cells. Although this method will lower the blood concentration, it has a high efficiency.

2.2.5 Gravity and Sedimentation

As it was mentioned, in pinched flow fraction method, sedimentations depend on density. Therefore, in the sedimentation and gravity method, density has an important role in separation. The general idea is the same, that cells enter channels in the direction of gravity with normal distribution and two other channels on each side are being used to direct cells to the middle and line them up. The overall schematic of design can be seen in Figure 7. After the preparation step that lines the cells up, a 90-degree angle is being used to change the direction of flow, with respect to the gravitational direction. Therefore, gravity is perpendicular to fluid flow after the angle. In the separation region, the channel width is
increased and sedimentation velocity depends on particle diameter and difference of density of particle and fluid. The sedimentation velocity can be calculated by:

$$U_{sed} = 2gr^2\Delta \rho / 9\mu$$

(4)

Sedimentation velocity is usually slow and to increase separation efficiency, hydraulic force is usually added by changing the geometry of the microchannels. Huh et. al. [41] used asymmetrical channel expansion to increase efficiency. Although in their experiment, 1,3, and 20 micrometer polystyrenes were separated with 1 ml/h flow rate, actual biological cells were never used to validate this experiment.

Figure 7) Gravity Microfluidic Device For Separation [41]

### 2.2.6 Biomimetic

There is always a tendency to use biomimetic devices for separation. Separation of cells is naturally happening in the body. For instance, waste product is always separated
from blood in veins. By investigating this phenomena, researchers have used this for separation of blood based components. Three different methods have been used.

1) Plasma skimming
2) Leukocyte margination
3) Zweifach-Fung effect

Although a complete mechanism of them have not been completely understood, different microfluidic devices are being designed by mimicking this actual phenomena. In microcapillary, red blood cells have the tendency to stay at the center, but leukocytes usually migrate toward walls with plasma rich regions. Also, bifurcation law is being observed when a microcapillary splits. Red blood cells predominantly choose the larger of the daughter capillaries. As it was mentioned, a number of techniques have been used to take advantages of these effects. Shevkoplyas et. al. [42] separate leukocytes by bifurcation law. In their experiment, 70 microliter blood was injected and they enriched leukocytes from 4300 to 42300 cells per microliter and increased the ratio of leukocytes to red blood cells from 1:1 to 1:32. As it is mentioned, red blood cells predominantly choose the larger splitting section. The result of experiment can be seen in Figure 8. Moreover, they also showed that the channel height didn't affect separation. Thus, by increasing the channel height, higher flow rate is achievable.
Faivre et al. [43] used the same method to separate plasma from a diluted blood sample. They demonstrated the cell-free layer could be enhanced by a sudden channel expansion. Furthermore, they applied a trifurcating outlet following this sudden expansion to separate plasma in a 16% hematocrit blood suspension at 200 μL/h, isolating 24% of the initial plasma, and they increased flow rate by increasing the channel height. Designing parameters in this device are viscosity, size, and shape of microchannel, and flow rates.

These devices have different applications based on their flow rates. The high flow rate plasma skimming devices, tested by Faivre et al., could be used in large volume samples in blood donor preparation, which has high volumes. On the other hand, leukocytes
margination and bifurcation devices can be utilized on lab-on-chip devices, handling much smaller volumes.

2.2.7 Inertial Devices

These devices have higher Reynolds number compared to all the previously mentioned devices and they usually run at a Reynolds number of 1-100. The particle Reynolds number is defined based on their environment. Reynold number of particles can be defined as \( R_{ep} = Re \times \left( \frac{\text{particle dimension}}{\text{channel dimension}} \right)^2 \).

For a high Reynold number, particles will not follow a streamline completely and inertial forces will deviate cells from streamlines. For instance, in a circular tube and with normal distribution at entry, particle will be focused at 0.6R, where R is the tube radius [27]. Equilibrium position in downstream is occurring because two forces are at balance with each other. The two forces are: 1) lift force due to shear gradient 2) wall effect force on particle. Geometry of microchannels affect number and position of equilibrium positions. Therefore, different shapes of channels have been used to change the equilibrium position [44, 45]. The second group of inertial microfluidics, using curved channels. In these channels, lower momentum flow is being replaced by a higher momentum flow. In curved channels, higher momentum flow is the one close to walls and low momentum flow is the one close to the center. Therefore, particles migrate with fluid circulation and will be deviated from their original pathway. Different sized particles have a different tendency to follow circulation. Smaller particles will be trapped in circulation, while larger particles cannot follow the path.
line and will stay close the wall, as shown in Figure 9. Different parameters have been shown in Figure 9 in curved channels.

Equilibrium positions can be found in this device. In order to separate with high efficiency, one should consider two things. 1) desired and undesired particles should be gathered at equilibrium position and 2) the equilibrium position for these two should be different. Although inertial lift force depends on particle size, the equilibrium position for various particle sizes in long channel is same. Therefore, in order to have different equilibrium positions, some other types of forces, which are almost the same magnitude of the lift force,

---

*Figure 9* Migration of Particles in Inertial Focusing Devices. A) secondary flow is being made at cross-section of channel due to higher flow rate and curved channel B) cell migration through curve C) effect of dean number on migration of particles D) Particle migration velocity at different mean flow velocity [45].
should be used. Dean drag is one of these forces. In curved microchannels, the secondary flow will cause migration of cells based on their size. By application of the Dean force, it is possible to separate cells based on their sizes again. Due to higher flow rates, usually a pump is being used. Cells at the entrance have normal distribution and larger particles, based on the criteria that the device had to be designed for, will be captured at equilibrium position, while smaller particles will circulate in secondary flow. Thus, enriched target cells can be separated from the designed outlet channel [46].

This device was used to enrich thrombocytes 100 times [47]. Besides, 10, 15, and 20 micrometer beads were separated by this device. These devices show their capability by 80 percent separation efficiency and high throughput close to few milliliter per minutes. It should be mentioned that these devices only work at low cell volume fractions. If there is a high concentration sample, the sample should be diluted before injected into the device because cells can affect each other. Furthermore, it is being predicted that fluids have higher viscosities due to the presence of cells in solution and longer devices are needed for complete separation. However, increasing velocity can solve the higher viscosity problem. These devices are using high flow rates and are capable to be coupled with other instruments that makes them more popular and attractive.
CHAPTER 3: FORMULATION OF MOVEMENT OF PARTICLES IN FLUID

Investigating and designing microfluidic devices has two parts. The first one is the effect of different design and producing various forcing and the second part is how these forces will act and move particles in microchannels. Therefore, in this chapter, different forces are introduced and their effect on cell movement will be analyzed and mathematical equations will be provided for each type of the forces.

Discrete phase approach is the general method for numerically solving particle movement in fluid that considers particles as second phase in fluid phase. In this approach, the path of each particle will be investigated separately and its equation will be solved in Lagrangian point of view. Forces on fluid particles were first investigated by Stokes and it was developed by Basset, Boussinesq, and Ossen. Therefore, the governing equation on particles in fluid dynamics is named after these three scientists and it is called the Basset-Boussinesq-Ossen (BBO) equation. The law of newton for a particle in fluid is:

\[
\frac{m_p du_p}{dt} = F_D + m_p g + F
\]  

The \(F_D\) is drag force, \(m_p g\) is gravity force, and \(F\) is other forces that will be elaborated below. Drag force is the effect fluid on particle so it never can be neglected. The effect of other forces depend on particle density, particle size and should be considered separately. Equation 5 can be rewritten by dividing the whole equation by \(m_p\) and is considered to be a linear form of the drag force equation and becomes:

\[
\frac{du_p}{dt} = \frac{C_D 18 \mu Re}{\rho_p d_p^2} \frac{(u_f - u_p)}{24} + g + f
\]  

(6)
Where $C_D$ is the drag coefficient and it depends on the particle's Reynolds number, which is:

\[
Re_p = \frac{d_p |u_f - u_p|}{\gamma}
\]  

(7)

The actual drag coefficient is measured in the experiment by Morsi and Alexander and it could be seen in Figure 10.

![Figure 10: Unitless drag coefficient based on fluid Reynolds number](image)

As it is illustrated in figure 10, drag coefficient is equal to $24/Re$ for Stoke's flow. In order to have a better prediction on particle movement in fluids, other type of forces should be taken into consideration. Overall, some of the forces acting upon the particles are:

1) Virtual mass force
2) Basset force
3) Buoyancy force
4) Pressure gradient force
5) Saffman force
6) Brownian force

7) Body force

In the following, the description and magnitude of these forces will be elaborated.

3.1 Virtual Mass Force

Force required for accelerating fluid phase due to accelerating motion of particle is virtual mass force and is equal to:

\[ f_v = \frac{1}{2} \frac{\rho_f}{\rho_p} \frac{d}{dt} (u_f - u_p) \]  
\( (8) \)

If density ratio of particle over fluid is high, it is possible to neglect this force.

3.2 Basset Force

In accelerating motion of particles, steady state boundary layer is different from actual boundary layer. Therefore, force due to delay in development of boundary layer on particles is called basset force. As it is clear, this force is time dependent and in low Reynolds number, is equal to:

\[ f_{ba} = 9 \sqrt{\frac{\rho_f \mu}{\pi \rho_f d}} \int_0^1 \frac{d}{d\xi} (u_f - u_p) \sqrt{t - \xi} \]  
\( (9) \)

If density ratio of particle over fluid is high, it is possible to neglect this force.
3.3 Buoyancy Force

In a situation of fluid statics, the net upward buoyancy force is equal to the magnitude of the weight of fluid displaced by the body. This force directly depends on density difference of fluid and particle. Overall, it can be shown that buoyancy force is equal to:

\[ f_{bo} = -\frac{\rho_f}{\rho_p} g \]  \hspace{1cm} (10)

If density ratio of particle over fluid is high, it is possible to neglect this force compared to gravity force.

3.4 Pressure Gradient Force

The change of fluid profile will induce a force on particles that can be shown as a pressure gradient force:

\[ f_{gf} = -\frac{\rho_f}{\rho_p} u_p \cdot \nabla u_f \]  \hspace{1cm} (11)

Pressure gradient force can be neglected if density ratio of particle over fluid is high.

3.5 Saffman Force

This is a lift force due to shear force in direction of velocity gradient acting on a particle, which can lift the particle in fluid. The force can be calculated by:

\[ f_{s,i} = 2K \frac{v^{5.0}}{d_p} \frac{\rho_f}{\rho_p} \frac{d_{ij}}{(d_{ik}d_{kl})^{0.25}} (u_f - u_p)_j \]  \hspace{1cm} (12)
Where $K=2.594$ and $d$ is fluid deformation tensor. Saffman force is higher when closer to walls because of higher shear forces in that area. Therefore, this force should be considered in small microchannels.

### 3.6 Brownian Force

This force is the erratic random movement of microscopic particles in a fluid, as a result of continuous bombardment from molecules of the surrounding medium. Brownian motion would be the dominant force for particles less than micrometer. This force will be smaller quickly, as particle diameter increases.

### 3.7 Body Force

A body force is a force that acts throughout the volume of a body. Gravity is the most important body force. In passive microfluidic devices, usually no other body force is present but many active microfluidics have dominant body forces. Electromagnetic forces and electrical fields are considered as body force, but none of them are being considered for this thesis project.
CHAPTER 4) DESIGNING PROCEDURE AND SET UP

4.1 Designing Procedure

Three types of experiments will be elaborated during this section. For single cell separation, different designs had been developed to separate cells based on their sizes. Although many devices had been proposed and analyzed for separation and some of them were explained literature review, they usually have few general drawbacks. First, many of them are active microfluidics, which means they need expensive and large devices. Thus, it makes it hard to use them as point of care devices. Second, many of proposed methods in passive devices have low flow rates. Accuracy of these devices drop fast as the flow rate increases. Therefore, high throughput methods are developing to compensate for drawbacks. Three different methods have been designed and their efficiency has been evaluated. These three types of devices use special forces to separate cells, as some of them was introduced in Chapter 3.

4.1.1 Control Incremental Filtration

The first device is control incremental filtration [42]. The overall design picture can be seen in Figure 11. Two side channels are separated by row of posts with specific gap sizes. The gap size is larger than an actual single cell size diameter. Therefore, less blockage is being observed in these devices compared to filtration devices. The main reason for selective enrichment of particles is side wall expansion. Side channel width increases gradually through the device. Gap distance will be the same and flow rate passing between each gap is
the same based on side wall expansion profile. Therefore, smaller particle than certain cut
off size would be separated and enter to side channel.

![Figure 11](image)

Figure 11) Schematic Illustration of a Controlled Incremental Filtration (CIF) Array. (A) Overall design of a CIF-based device comprising a central flow channel which retains the particles being concentrated, and two side channels which carry the filtrate. The width of the side channels, $w_s(i)$, gradually increases with increasing gap row number, $i$, while the width of the central channel, $w_c$, remains constant throughout the length of the device. (B) A close-up view of the CIF array showing the overall geometry of the obstacle placement in the device. (C) A 3D rendering of the CIF array. Arrows indicate the direction of flow [42].

The central channel has constant width $w_c$, however, side channel width differs with gap $i$ number. $w_s(i)$ is being calculated based on $f_{gap}$ and previous amount of side channel width $w_s(i - 1)$. This recursive approach will be able to automate the design and make it easier to
design in a CAD software and make it ready for fabrication. The first few side channel width will be defined based on designing criteria and it is close to zero. By assuming constant pressure across the width of the device, the recursive equation can be developed. The key aspect of designing is $f_{gap}$, that is the amount of liquid that is being removed from the central channel at each gap. The threshold value for $f_{gap}$ was found experimentally by testing on beads of various diameters.

As seen in Figure 12, increasing of central channel width needs a lower $f_{gap}$ for certain particle sizes. Based on these calculations, few $f_{gap}$ with different central channel widths were selected. The second step is determining the side channel width at different gap numbers. In order to calculate the gap width at each gap, a MATLAB code was generated that
will use the recursive equations to solve the width of side channel. A simple equation for volumetric flow rate in central channel, and the side channel at gap row $i$ is:

$$Q_s(i) = Q_s(i - 1) + f_{gap}Q_c(i - 1)$$ \hfill (13)

By knowing that total volumetric flow is the same before and after the each gap, and using resistance of side channel $R_s$ and central channel $R_c$, then equation 13 becomes:

$$R_s(i) = \frac{(1 - 2f_{gap})R_cR_s(i - 1)}{R_c + f_{gap}R_s(i - 1)}$$ \hfill (14)

The fluidic resistance of each channel segment between the rows of gaps (of which there are typically several thousands) can be estimated using the approximation of each of the individual segments as rectangular channels and according to literature, is equal to

$$R(w, d, \mu, L) = \frac{12\mu L}{d^3} \left[ 1 - \frac{192w}{d} \sum_{n=1,3,5,...}^{\infty} \frac{\tanh\left(\frac{n\pi d}{2w}\right)}{(n\pi)^5} \right]^{-1}$$ \hfill (15)

Where $L$ is the length, $w$ is the width and $d$ is the depth of the channel, and $\mu$ is the viscosity of the fluid. By using last two equations and considering desired value for the first side channel width close or equal to zero, the width of each row can be calculated. The code Flowchart can be seen in Figure 13.
Before designing the new devices for single cell enrichment, the same algorithm was being used and the result was compared with the results of the original developers. For doing so, data values were extracted by Digitizer and were compared to MATLAB code that it was developed. Figure 14 shows $w_s(i)$ and $Q_s(i_T)/Q_c(i_T)$ for 6 different $f_{gap}$. The result shows that the code is reliable so it can be used to redesign with desired $f_{gap}$, and central channel width based on Figure 12.
The minor difference can be from different viscosity values, different starting value for \( w_s(1) \), and different error cut off magnitudes. The \( f_{\text{gap}} \) value is approximate and multiple design is needed to identify the right \( f_{\text{gap}} \) that is needed for single cell enrichment. Therefore, multiple devices were designed to find the best parameters experimentally. Ten devices were designed based on the MATLAB code. Table 1 illustrates parameters for these 10 devices.
Table 1) Design Parameter For CIF Design

<table>
<thead>
<tr>
<th></th>
<th>( w_c )</th>
<th>( w_s )</th>
<th>( d )</th>
<th>gap distance</th>
<th>( f_{gap} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>150</td>
<td>100</td>
<td>20</td>
<td>0.00125</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>150</td>
<td>100</td>
<td>20</td>
<td>0.00075</td>
</tr>
<tr>
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<td>15</td>
<td>100</td>
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<td>0.0005</td>
</tr>
<tr>
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<td>100</td>
<td>400</td>
<td>100</td>
<td>20</td>
<td>0.00125</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>150</td>
<td>100</td>
<td>20</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>150</td>
<td>100</td>
<td>20</td>
<td>0.0015</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0.001</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0.0015</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0.002</td>
</tr>
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<td>100</td>
<td>100</td>
<td>20</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Based on this information, automated code in MATLAB will generate the final script for AutoCAD in text file and could be opened in AutoCAD automatically. The MATLAB code can be found in Appendix A. The schematic for one of these devices can be seen in Figure 15 and based on these designs, lithography mask was ordered. Silicon wafers have a 100 \( \mu m \) diameter. Therefore, the total length of the device was divided to smaller pieces and these segments were added to each other with half circles. The effect of these half circles was investigated and it will be explained later. Using the design above, a photo mask of 30 \( \mu m \) resolution was prepared by Finleline Inc.
Figure 15) AutoCAD Design For One of the Devices and Lithography Photo Mask.
4.1.2 Continues Particle Separation in Spiral Microchannel

Another passive device is spiral microchannel and separate single cells by using dean flow and differential migration. The design takes advantage of different amount of lift force and Dean force acting on particle with various sizes. Lift force is always present in microchannels and tend to migrate cells towards the wall, but on the other hand, Dean forces occur because of the spiral channel geometry. As a result, larger particles occupy single equilibrium position at the inner half of the microchannel wall due to Dean force and smaller particles migrate from inner half of the channel to outer half of the channel. At the end, two distinct stream of particles would be collected from two outputs. Fluid flowing through a curvilinear channel experiences centrifugal acceleration leading to two counter-rotating vortices known as Dean vortices [48]. The magnitude of these secondary flows is quantified by dimensionless Dean number (De) as:

\[
De = \frac{\rho U_f D_h}{\mu} \sqrt{\frac{D_h}{2R}}
\]

where \( \rho \) is the density of fluid \((kg.m^{-3})\), \( U_f \) is the average fluid velocity \((m.s^{-1})\), \( \mu \) is the fluid viscosity \((kg.m^{-1}.s^{-1})\), \( R \) is the radius of curvature \((m)\), and \( Re \) is the flow Reynolds number. Therefore, particles flowing in curvilinear microchannels experience different Dean drag force based on the position of the particle in vortices and is illustrated in Figure 16.
Figure 16) (a) Schematic of the Proposed Spiral Microparticle Separator. The design consists of two inlets and two outlets with the sample being introduced through the inner inlet. Neutrally buoyant particles experience lift forces ($F_L$) and Dean drag ($F_D$), which results in differential particle migration within the microchannel. (b) Microchannel cross-sections illustrating the principle of inertial migration for particles with $\alpha_p/D_h \sim 0.1$. The randomly dispersed particles align in the four equilibrium positions within the microchannel where the lift forces balance each other. Additional forces due to the Dean vortices reduce the four equilibrium positions to just one near the inner microchannel wall [48].

Thus, particles move either toward inner or outer wall. In addition to Dean drag, particles also experience Stoke’s drag forces that moves particles in stream of fluid and Lift forces that are a combination of the shear-induced inertial lift force and the wall-induced inertial lift force. In Poiseuille flow, the parabolic nature of the velocity profile result in the shear-induced inertial lift force and the wall-induced force and migrate the cell away from the center channel. On the other hand, when particles get close to the wall, asymmetric forces
induce around the particles that push cell back to the center. This force is called wall-induced force. Chun et al. [49] showed the preferential focusing of particles are dominant for particles with \( \frac{a_p}{D_h} \) ratio \( \sim 0.1 \). As a result, four equilibrium position will be made because of lift force and these four-equilibrium position could be reduced to one by using Dean drag force. The key design parameter in this microfluidic device is curvature radius and amount of lift and dean forces acting on particles. For a high Dean number, complete recirculation will be observed and it should be avoided. However, Dean force will be much lower in low flow rate and continuous separation was observed in lower Re number. In order to calculate design parameters, Dean drag force and lift force should be calculated. Ookawara et al. [50, 51] formulated the expression for the average Dean velocity and by using stocks drag, Dean drag force can be calculated as:

\[
F_D = 3\pi\mu U_{Dean} a_p = 5.4 \times 10^{-4} \pi \mu De^{1.63} a_p \quad (N)
\] (17)

Asmolov [52] derived an expression describing the magnitude of the lift forces as a function of the particle position across the channel cross-section.

\[
F_L = \rho G^2 C_L a_p^4 \quad (N)
\] (18)

where \( G \) is the shear rate of the fluid \( (s^{-1}) \) and \( C_L \) is the lift coefficient, which is a function of the particle position across the channel cross-section but in low flow rate it can be assumed as 0.5. Although both of these two forces are size dependent, Dean drag force is related to first order particle diameter whereas lift force will changed rapidly\( (\sim a_p^4) \). By varying ratio of these two forces at specific Dean number, continuous migration will eventually result in complete separation of particles with certain sizes. The force calculation for various cell sizes was performed and it is presented in Table 1. To achieve complete separation between cells,
length of the device should be determined. Using Asmolov's lift force and Stoke's drag, an expression for cells lateral migration velocity can be derived. Therefore, the channel length necessary for the particle to completely migrate from inner to outer wall ($L_M$) is then given by:

$$U_L = \frac{\rho U_f^2 C_L a_p^3}{3\pi \mu D_h^2} \to L_{Lift} = \frac{U_f}{U_L} \times L_M$$

(19)

Similarly, the distance for complete migration due Dean force can be calculated similarly by using $U_{Dean}$ instead of $U_L$ in the previous equation. Finally, total length required for cell separation was calculated based on these two equations and it is presented in Table 3.

**Table 2) Parameters Used For Calculation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$</td>
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</tr>
<tr>
<td>$\rho$</td>
<td>1000</td>
</tr>
<tr>
<td>$w$</td>
<td>0.0001</td>
</tr>
<tr>
<td>$h$</td>
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</tr>
<tr>
<td>$R$</td>
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<tr>
<td>$D_h$</td>
<td>6.6E-05</td>
</tr>
<tr>
<td>Re</td>
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</tr>
<tr>
<td>$U(m/s)$</td>
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</tr>
<tr>
<td>$Q(ml/min)$</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

**Table 3) Force Calculation and Necessary Length**

<table>
<thead>
<tr>
<th>D (um)</th>
<th>$a_p/D_h$</th>
<th>$F_{Dean}/N$</th>
<th>$F_{Inertial}/N$</th>
<th>$U_{Dean}/m/s$</th>
<th>$U_{Inertial}$</th>
<th>$L_{Inertial}$</th>
<th>$L_{Dean}$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4.05E-14</td>
<td>4.60E-06</td>
<td>1.07E-06</td>
<td>1.12E+00</td>
<td>2.61E-01</td>
</tr>
<tr>
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<td>2.60E-13</td>
<td>2.05E-14</td>
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<td>2.61E-01</td>
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<td>2.32E-04</td>
<td>5.17E-03</td>
<td>2.61E-01</td>
</tr>
</tbody>
</table>
In conclusion, one general device was designed based on both force calculation and length required for particle to completely migrate from one side to the other side of the channel. The amplitude of the force is highly related to particle size and curvature radius. Therefore, changing these two factors can result in comparable forces. As it is shown in Table 3, smaller particles have higher Dean forces than inertial forces so smaller particle will rotate in cross-section while larger cell will stay in one position. On the other hand, length required for complete migration for smaller cell size has been shown in the same Table. Design parameter based on these calculation can be found in Table 4.

Table 4) Parameters For Spiral Design

<table>
<thead>
<tr>
<th>Width(μm)</th>
<th>Height(μm)</th>
<th>$D_0$(μm)</th>
<th>Number of turn</th>
<th>Position on spiral</th>
<th>Outlet channel 1,2,3 width(μm)</th>
<th>Outlet channel 4 width(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>180</td>
<td>7000</td>
<td>3</td>
<td>$D_0 \times \frac{\text{Angle}(i)}{2\pi}$</td>
<td>60</td>
<td>70</td>
</tr>
</tbody>
</table>

The final schematic design for spiral channel can also be seen in Figure 17. To design, spiral position was calculated in MATLAB and it was written in text file and was imported in AutoCAD. Finally, four outlets were designed. The first three channel have 60 μm and the last one has 70 μm width.
4.1.3 Shear-modulated Inertial Migration

Cell filtration can be done with much simpler design. Bhagat et. al [53] used the idea that inertial lift will migrate particles toward microchannel walls in high aspect ratio rectangular geometry. High aspect ratio will increase the lift force generated and complete filtration can be done in short distance in low flow rates (Re<50). Segre and Silberberg [54] were the first to show inertial migration of cell in a circular tube. They illustrate nonuniform cells at the entrance will be in equilibrium position at 0.6R. In square channels, there would be eight equilibrium position and by increasing the Reynolds number (Re>500) this equilibrium position will be reduced to 4 positions at channel corners. Although all particles will get to equilibrium positions, different particles migrate differently based on few parameters. Asmolov derived an expression for the net lift force acting on particles:

\[ F_L = \rho G^2 C_L a_p^4 \]  

(20)

Where \( \rho \) is the fluid density, \( G \) is the fluid shear rate \( G = 2U_f/D_h \), \( U_f \) is the average flow velocity, and \( C_L \) is the lift coefficient that is a function of the particle position across the
channel cross section and it varies with Re number but in low flow rate it can be assume is constant and equal to 0.5. In rectangular microchannel, hydraulic diameter is almost equal to the narrowest width and using stokes law \( F_L = 3\pi\mu a_p U_L \) lift equation can be rewritten as:

\[
U_L = \frac{F_L}{3\pi\mu a_p} = \frac{4\rho U_f^2 C_L a_p^4}{3\pi \mu w^2} \quad (21)
\]

Finally, the microchannel length \( L \) necessary for a complete migration is equal to:

\[
L = \frac{U_{max} L_p}{U_L} = \frac{2 U_f L_p}{U_L} = \frac{3\pi \mu w^2}{2\rho U_f^2 C_L a_p^4} \quad (22)
\]

Where \( L_p \) is equal to width of the channel. Equation 22 was used to design passive microfluidic for cell separation. Therefore, new design parameters were developed based on Equation 22. Table 5 illustrates parameters that have been used and microchannel length values based on those parameters.

<table>
<thead>
<tr>
<th>Design parameter</th>
<th>Value</th>
<th>Particle Diameter (µm)</th>
<th>L(cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu ) (kg/(m.s))</td>
<td>0.0008</td>
<td>10</td>
<td>2.3844375</td>
</tr>
<tr>
<td>( \rho ) (kg/m³)</td>
<td>1000</td>
<td>15</td>
<td>0.7065</td>
</tr>
<tr>
<td>( Re )</td>
<td>100</td>
<td>20</td>
<td>0.298054688</td>
</tr>
<tr>
<td>( w(m) )</td>
<td>0.00015</td>
<td>25</td>
<td>0.152604</td>
</tr>
<tr>
<td>( h(m) )</td>
<td>0.00015</td>
<td>30</td>
<td>0.0883125</td>
</tr>
<tr>
<td>( D_h(m) )</td>
<td>0.00015</td>
<td>35</td>
<td>0.055613703</td>
</tr>
<tr>
<td>( U(m/s) )</td>
<td>0.533333333</td>
<td>40</td>
<td>0.037256836</td>
</tr>
<tr>
<td>( Q(ml/min) )</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 5) Parameter Design for Shear-modulated Inertial Migration on the Right and Calculation Values Used on the Left*
These calculations were performed to determine the length required to get to equilibrium position. Different cell lines have different diameter that will be discussed later but single cells have less than 15 $\mu m$. The only factor that is determinant is length of the device. The length of the device is chosen based on Table 5. Therefore, 0.7065cm is length needed to position 15 $\mu m$ cells at their equilibrium position and all cells less than 15 $\mu m$ would have uniform distribution across the width of the channel. In order to capture cells at the equilibrium, a junction was added 0.7065cm downstream of the main inlet to collect all equilibrium position cells. Theoretically, all cells larger than 15 $\mu m$ should migrate to side channels, while smaller cells stay at the center channel. Finally, two shorter and longer devices were designed and their specifics can be found in table 6.

*Table 6) Design Parameters for Shear-modulated Inertial Migration*

<table>
<thead>
<tr>
<th>Width ($\mu m$)</th>
<th>Longer device</th>
<th>Shorter device</th>
<th>Multi side channel device</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height ($\mu m$)</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Width of main channel ($\mu m$)</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Width of side channel ($\mu m$)</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Distance to first side channel ($\mu m$)</td>
<td>20000</td>
<td>10000</td>
<td>4000</td>
</tr>
<tr>
<td>Distance between two side channel ($\mu m$)</td>
<td>-</td>
<td>-</td>
<td>10000</td>
</tr>
<tr>
<td>Distance from side channel to outlets ($\mu m$)</td>
<td>4000</td>
<td>2000</td>
<td>10000, 2000</td>
</tr>
</tbody>
</table>

Two different lengths were considered to compare their performance in low flow rate. Also, a third design was added to increase the efficiency of the method. The performance of device
was evaluated both in low and high flow rates. Importantly, these devices show different characteristics in high flow rates. Three devices that is shown in Figure 18 were used and their result is presented in the next chapter.

![Figure 18](image)

**Figure 18** A) Schematic Design For Longer Device B) schematic design for shorter device C) schematic design for Multi side channel device

### 4.2 Setups

#### 4.2.1 Imaging and Data Analyzing

Nikon microscope was used to take pictures of the cell with a 10X objective lens. For the first part of research, purity of each channel was assessed by counting cells in 12-well plates. The entire population will be divided into four categories of single cells, Cluster (containing two and three cells), Small aggregate (4-6 cells), and Big aggregate (>6 cells). In each picture, the number of the event for each category will be counted and being compared with other devices. The need to make data analysis process more efficient and user-friendly drove us to the development of a custom MATLAB code. The actual code can be seen in appendix B. This code is fully automated and it can be used to measure cell number and divide them between the four defined categories.
The Code is written in MATLAB script and can process all pictures together. The overall process is making a black and white binary picture and measuring the area and categorized cell based on their size to four bins that it was defined earlier. Make a direct binary picture in ImageJ would result in inaccurate counting with a lot of noises. Therefore, the image is being processed before counting them. For processing, the edge of cells is being detected with ‘edge’ command and image will be dilated to fill any undetected edges. Then, full close detected edges will be filled and it is ready to become binary. Then ImageJ would be opened automatically to measure areas and the results will be obtained by MATLAB. Finally, result would be categorized in specified bins. The overall procedure can be found in Appendix C.

![Figure 19) Processed Binary Picture Comparing to Actual Picture](image)

To increase the accuracy of counting second method was used to determine singles versus cluster. The aspect ratio of single cells is usually close to one but doublet cells have dumbbells shape. Thus, they usually have higher aspect ratio and this was used because cells have wide range of cell diameters and finding cut off for single cells based on area is hard. In the following
one picture was assessed and compared with real image and error percentage has been calculated.

Table 7) Comparing MATLAB Image Processing and Actual Counting

<table>
<thead>
<tr>
<th></th>
<th>Single</th>
<th>cluster</th>
<th>Small Aggregate</th>
<th>Big Aggregate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATLAB</td>
<td>54</td>
<td>30</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Counting</td>
<td>49</td>
<td>34</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Error</td>
<td>10%</td>
<td>11%</td>
<td>14%</td>
<td>8.33%</td>
</tr>
</tbody>
</table>

As it was measured, error for counting is close to 10% that it is acceptable. Cell has wide range of diameters and definitive cut off is hard to determined. Besides, picture resolution would affect the result. Therefore, consistent picture was taken to ensure the data analyzing are perfect.

4.2.2 Cell Culture Procedure

MCF 7 and HCT 116 cell line were used for these experiments. HCT 116 was being used for CIF design and MCF 7 cell line had been used for the rest of the devices. The general cell culture procedure is almost the same for these two cell lines and the only difference is duration needed for trypsin to detach cells from the bottom of flask. The overall procedure can be found in Appendix D.

4.2.3 Experiment Set Up

In photolithography silicon wafer was provided and PDMS devices was produced based on the silicon wafer. The general procedure for photolithography and PDMS is presented in
Appendix E, and F, respectively. Then the PDMS devices were used for experiment that their general procedure could be found in the following.

1. Aspirate cell media and dilute cells to 1e5 cell/ml with PBS+
2. Using Orfio-cell counter MOXI Z to count the cells at the beginning as control
3. Divide cells 1.5ml portions for each run.
4. Use 5ml Syringe to load cells.
5. Use Harvard apparatus pump and set the flow rate.
6. Flushed with DI water at a 500 μl/min flow rate for 20 minutes.
7. Wash with PBS+ to flush all water.
8. Use three-way to avoid any bubble formation. (use one end for PBS+ and the other end for cell)
9. Switch the three-way valve and set the desire flow rate to run device with cell.
10. Wash with 1ml of PBS+ after each run to flush all cells.
11. Repeat last two steps for all flow condition.
12. Measure cell count with MOXI counter for measuring recovery
13. Wash device with DI water at a 500 μl/min flow rate for another 20 minutes.
14. Use 12 well-plate for microscope images.
15. Use 500ml water and 200ml cell to have enough cell for analyzing.
16. Use the MATLAB code to count cells and categorized them.
Figure 20) Experiment Setup
CHAPTER 5: RESULTS AND DISCUSSION

In this section, result of three separation devices will be presented and the best device will be chosen. Comparing devices is complicated and consistent parameter should be used to evaluate all of them. Therefore, two main parameters are defined and it will be used to evaluated devices together. The aim of these devices is single cells enrichment for further processing. The control population is homogenous mixture of cells that contain single cells, clusters and aggregates. Two aspects will be used to identify the efficiency of the device. These two aspects were recovery and purity, with the former being how much an outlet of a channel is receiving cells from the bulk fluid moving down the main channel. Purity, on the other hand, would examine how many single cells would the outlet of a channel receive as a fraction of all the cell aggregates there. Therefore, each device will be compared based on these two parameters. Purity of each channel is normalized by itself. For example, purity of single cells is equal to the number of single cells divided by total number of single, cluster, small and big aggregates. On the other hand, cell counter was used to identify recovery. Therefore, each outlet cell/ml number will be multiplied by the volume that it was extracted and it was divided by control so it is possible to have more than 100% recovery based on definition. Higher than 100% show dissociation large aggregates and it is normal.

5.1 Control Incremental Filtration

In Control Incremental Filtration, calculated side wall expansion, filter same amount of flow rate at each gap and gradually enrich smaller particle in side channel. Purity of these devices have been evaluated and it has been shown in Figure 21. Each experiment was done at least
three time. Purity of each sample is being evaluated by dividing each channel single cell count over whole cell count in that experiment. Then normalized data have been averaged and is presented. On the other hand, Recovery is being calculated by MOXI cell counter for each output. Then value measured in each experiment was divided by their control cell count. And the average of them was presented in Figure 21.
It was mentioned that ten devices were fabricated. The original design used low flow rate but in this thesis, all devices were evaluated at high throughput. These devices are planned to be coupled with another high flow rate device so all of them is evaluated in both high and low Reynolds number. The second microfluidic is using high flow rate and the aim of this thesis is designing high flow rate microfluidic device that has high recovery and purity. These microfluidics are using same principal as tangential filters. Schematic of this design is presented in the Figure 22.

*Figure 21) Recovery and Purity for CIF Design*
Throughout of the device posts are presented and main flow has cells in it with high cell volume fraction. The high cell volume fraction is the second key factor for designing. Most of these methods only work in low cell volume fraction. Therefore, both high flow rate and high cell volume fraction is being used to assure that these devices can be coupled with any other devices for separation. Based on result that is presented in Figure 21, both device number 6 and 9 have high purity in all flow rates. First, purity is increased as the flow rate gets higher almost in all devices. Purity higher than 90 percent can be seen in those two devices and more interesting both have same $f_{gap}$ that means side channel width is increasing at the same rate but two devices have different central channel width. As it was mentioned, this device has the same principle as tangential flow filtration but the only difference is the amount of flow being filtered at each gap. On the other hand, all microfluidics have low recovery in side channel. Recovery increase as flow rate increase but maximum recovery is less than 20 percent. Although device number 6 have high purity, general low recovery of these devices let us to move on to other devices.
5.2 Continues Particle Separation in Spiral Microchannel

Curve microchannels are using Dean drag force and Lift force to enrich two different size cells. Dean Drag force has different direction depends on position in cross-section. Two vortices are being formed and smaller cells will be trapped in circulating vortices. On the other hand, larger cells are predominantly under lift force and will migrate to inner wall. Channel 1 is the closest to inner wall and channel 4 is closest to outer wall. Result for recovery and purity is being shown in Figure 23.
Based on figures, by comparing purity of four channels to control, all channels have almost equal distribution and no purification is observable. On the other hand, recovery of microfluid devices increases at higher flow rates. There are two possible explanations. First, in low flow rate, cell sediment on three-way valve and syringe itself depends on the orientation that is being used. Cells have less time to sediment in higher flow rate and flow itself don’t let cell to sediment. Second, cells are being dissociated in the device. Therefore, cell count was being used to check the second hypothesis. By measuring number of cells in 0.1, 0.5 and 1 ml/min, increase of single cell was observed but this increase was saturated as flow rate passes 0.5 ml/min and no more cell separation was observable. This microfluidic device had low performance due to designs problems that will be mentioned in next chapter.
5.3 Shear-modulated Inertial Migration

Shear-modulated inertial migration was designed to enrich cells based on their equilibrium position in channels. Particle moving in channel have ability to being at equilibrium position based on geometry shape of the channel. The equilibrium position was discussed earlier in previous chapter. In conclusion, in low flowrate there should be two equilibrium position in rectangular channel close to the wall. For separation, microfluidic device uses the amount of the time it takes for different cells diameter to get to equilibrium position. Larger cells migrate faster and will be in equilibrium position sooner than smaller cells. Therefore, two longer and shorter device was design to collect all single cells in main channel and larger cell in side channel. The same condition was used again which was high flow rates and high cells volume fraction. Interestingly, the separation wasn’t observed even in low flow rates for high volume fraction but enrichment was observed in the other channel. The Side channels population was more enriched by single cells than Main channel. Beside enrichment was higher at higher flow rate. In my point, inertia of cells and high cell volume fraction was the main reason for observing the opposite trends that it wasn’t expected. First, device with shorter length was used for purification. Purity of side channel is higher than main and control result and this difference increases at higher flow rates. Higher standard deviation can be seen in this device compare to longer and multi side channel device. The reason could be from the position of the outlet. The outlet in this design is closer to junction compare to other two designs so outlet would affect junction streamline more than the other two microfluidic devices. The result for purity and recovery is presented in Figure 24.
According to Figure 24, main channel purity is higher at low flow rates as it was expected in theory section but because of high cell volume fraction, it is not possible to have complete enrichment at low flow rate. At higher flow rate side channel purity increases and reach to maximum close to 70 percent. On the other hand, Purity of this design is much higher than 57.
spiral and CIF design. The dissociation is observable but this dissociation reach saturation after 0.5 ml/min. The second design with longer straight channel has better result. Purity is get close to 80 percent and is higher than main channel even in low flow rates. Besides, both main and side channel still have their high recovery and dissociation can also be seen.

Figure 25) Recovery and Purity for Longer T-junction Device

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Finally, based on these two channels third microfluidic device was developed. In multi side channel, almost same purity is being observed in low flow rate but in higher flow rates results improve gradually. Purity of the first side channel get close to 95% and even the second side channel have purity close to 90 percent. And by looking at recovery data, both first and second side have high recovery and their recovery don’t fall as the purity increase.
Therefore, it is possible to conclude the last design is capable for enrichment cells with high accuracy compare to other two mentioned design.
CHAPTER 6) SUMMARY AND FUTURE DIRECTIONS

Automate cell-based sample processing, labeling, and detection is ultimate goal of many microfluidic and Lab-On-Chip devices. Raw sample should be dissociated and single cells is needed for further analysis. Therefore, having platform for separating single cells from aggregate is usually the first step of sample preparation. Many approaches had been proposed for enrichment that each of them have their pros and cons. The usual method is adding chemical and enzymes to break the cell-cell bonds that their advantages and disadvantages was mentioned. Microfluidics are being used to enrich cells and separate the target cells by different cell markers. In this thesis, three different passive microfluidics is being used for single cell enrichment. Control incremental filtration was designed based on gradually filtration of fluid from the main channel to side channels by passing between post with large gap. High purity was observed and the best design was chosen based on the results. This method had low recovery and even with high purity of single cells at side channel, only 15 percent of control cells was recovered from side channel. COMSOL simulation was performed on these devices in to improve their purity. Particle trajectory simulation is illustrated in Figure 27.
Figure 27) Particle Trajectory At Different Time and Comparison Between Resistance Model and Simulation
Based on these simulations, the amount of flow rate passing from each post was measured and resistance model was compared with simulation result that it can be seen Figure 28. The overall flowrate is constant but close to turning point, upstream stream flow will affect the gaps close to half circles and will filter more than it was designed (Figure 28). Therefore, it is suggested to use complete straight version of this design for further improvement. But the inherent low recovery of this design made us to move on the new method.

![Flow rate passing from gaps at each post](image)

*Figure 28) Evaluating Flow Rate Passing from Each Post and Compare it by Straight Line.*

The spiral channel device didn’t have high purity but the recovery problem was solved. Besides, the spiral channel had design problem. Close to the outlet, multiple channel was branched from the same spot. The multiple branching from the same point will cause sharp edges that it is hard to be fabricate either in silicon wafer or PDMS. Thus, during PDMS fabricating, walls close to this multi branching point wouldn’t have their designed shape.
Besides, in original paper, it was suggested to have two inlet and direct incoming cells close to wall by the second guiding fluid sheath. Then smaller cells would migrate from inner wall to outer wall and being separated at the end. Therefore, data for spiral channel is not accurate and it should be tested again with a new design. In new design two inlet should be presented and for branching is better to extract channel one by one instead of branching form the same point. Finally, the last design is simplest design and it consist of only two crossed straight channels. T-junction device was designed to enrich single cells in the main channel but high cell fraction volume doesn’t let to separation happened properly. On the other hand, in high flow rate opposite effect was observed because of cells inertia. Larger cells with higher inertia would stay in the main channel and single cells would almost equally travel in either of two junctions. Therefore, single cell enrichment would be observed at the side channel. Finally, using same method multi side channel device was designed. High purity and recovery was obtained with this last design. Therefore, this device can be used to enrich cells with high purity and recovery. Potential of simple straight channel design was shown for cell separation based on size marker. As it was mentioned, this method at high flow rate is collecting aggregates at main channel but it doesn’t have any control on single cell migration. Many of single cells are in the main channel. Therefore, it is possible to recover the rest of them by using main channel output and rerun it through the microfluidic device. Finally, it is highly suggested to investigate further parameters that could improve both purity and recovery. Among those parameter, the angle of side channel seems to play an important role. Besides, main and side channel width can be adjusted for better purity. Based on comparison between shorter and longer T-junction devices, Results are different. Thus,
either the length of straight channel from inlet to side channel is important or distance of junction till outlet. Therefore, these design parameters can be changed in order to have higher efficiency.
REFERENCES


12. <31-bischoff.pdf>.


55. <ol201902y.pdf>.
APPENDIX A) MATLAB CODE FOR CIF AUTOCAD DESIGN

clear all
clc

%% initial values
L = 20; %Length of gap (mic m)
d = 100; %d=depth of channel (mic m)
wc = 100; %width of centeral channel (mic m)
mu = 10*10^-4; %viscosity of water (Pa.s or N.s/m^2)
wsmax = 100; % %viscosity, L=length, w=width, and d=depth of the channel
f_gap = 9.6*10^-5;
Q = 25 * 1.666667*10^7; %25 mic L/ min, 1.666667*10^-5 m^3/s,
v = 25 * 1.666667*10^-5 / (92.38*10^-6 * 150 * 10^-6 );

%% initializing
i=1;
ws(1)=0.01;
alpha=10^9/60;%coefficient for converting to micro litter /s
Rc=alpha*Rseries(50,d,wc,L,mu);
Rs(1)=alpha* Rseries(50,d,ws(1),L,mu);

%% calculating ws
errr = 1;
% for the first 50 ws useing smaller stepsize
ws(i) = 2;
while i < 50
    i = i+1
    ws(i)=ws(i-1);
    Rs(i) = (1-2*f_gap)*Rc*Rs(i-1)/(Rc+f_gap*Rs(i-1));
    while errr > 0.0001
        ws(i) = ws(i) + 0.5*10^-6;
        Rs_trial=alpha*Rseries(50,d,ws(i),L,mu);
        errr = abs(Rs(i)-Rs_trial);
    end
    ws(i)
    errr = 1;
end
while ws(i) < wsmax
    i = i+1
    ws(i)= ws(i-1);
    Rs(i) = (1-2*f_gap)*Rc*Rs(i-1)/(Rc+f_gap*Rs(i-1));
    while errr > 0.00001
        ws(i) = ws(i) + 0.5*10^-5;
        Rs_trial=alpha*Rseries(50,d,ws(i),L,mu);
        errr = abs(Rs(i)-Rs_trial);
    end
    ws(i)
    errr = 1;
end

%% plot
L_device = (L+20)*i*10^-4; %device length
QsOverQc = Rc ./ Rs;
figure (1)
hold on
plot(ws)
APPENDIX B) IMAGE PROCESSING CODE

clear all
clc

NumberOfPicture = 1;
CellCount=zeros(NumberOfPicture,4);
CellCount2=zeros(NumberOfPicture,4);
bin_counts_total=zeros(NumberOfPicture,29);

for n=1:NumberOfPicture
    I=imread(['a' num2str(n) '.jpg']);

    %read image
    %I = imread('mainchannel15.jpg');
    test = numel(size(I))>=3;  % if it is 3D will be 1
    if test == 1
        I = rgb2gray(I);
    end

    %detect cells
    %[junk, threshold] = edge(I, 'sobel', 0.1);
    %fudgeFactor = .4;
    BWs = edge(I,'sobel', threshold * fudgeFactor);
    I = imresize(I,900/1392);
    BWs = edge(I,'canny',0.2);

    %dilate the image
    se90 = strel('disk', 1, 0);
    se0 = strel('disk', 1, 0);

    %fill interior gaps
    BWsdil = imdilate(BWs, [se90 se0]);
    BWdfill = imfill(BWsdil, 'holes');

    seD = strel('diamond',1);
    BWfinal = imerode(BWdfill,seD);
    BWfinal=1-BWfinal;

    %display image
    %warning('off', 'Images:initSize:adjustingMag');
    %figure, imshow(BWfinal);
    % ImageJ
    % opening imageJ and adding java to our directory
    javaaddpath 'C:\Program Files\MATLAB\R2016a\java\mij.jar'
    javaaddpath 'C:\Program Files\MATLAB\R2016a\java\ij.jar'
    MIJ.start

    %Doing all of the procedures
    MIJ.createImage(BWfinal)
MIJ.run('Make Binary')
MIJ.run('Watershed')
MIJ.run('Set Measurements...', 'area redirect=None decimal=3')
MIJ.run('Analyze Particles...', 'size=19-Infinity display exclude');
A=MIJ.getResultsTable;
MIJ.run('Set Measurements...', 'area fit redirect=None decimal=3')
MIJ.run('Analyze Particles...', 'size=19-Infinity display exclude');
B=MIJ.getResultsTable;

%% Histogram
% creatin beans and histogram
data = A;

% specify groups if the dots are black
bin_edges = [20.5 35.5 40.5 50.5 60.5 70.5 80.5 90.5 100.5 110.5
120.5 130.5 140.5 150.5 160.5 170.5 180.5 190.5 200.5 210.5 220.5 230.5
240.5 250.5 260.5 270.5 280.5 290.5 300.5 inf];
num_bins = length(bin_edges) - 1;
bin_names = cell(num_bins, 1);
for i = 1:num_bins, bin_names[i] = ['group_' num2str(i)]; end
% count
[bin_counts,bin_edges] = histcounts(data,bin_edges);
for i=1:size(A,1)
  B(1,:) = [ ];
end
B=sortrows(B,1);
AspectRatio = zeros(size(B,1), 1);
AspectRatio = B(:,2)./B(:,3);
SingelCells = 0;
Clusters = 0;
SmallAggregates = 0;
for i=1:size(B,1)
  if B(i,1) > 80 && B(i,1) < 101
    if AspectRatio(i,1) <= 1.1 && AspectRatio(i,1) >= 0
      SingelCells = SingelCells + 1;
    elseif AspectRatio(i,1) >= 1.1 && AspectRatio(i,1) <= 10
      Clusters = Clusters + 1;
    end
  end
end
for i=2:6
  SingelCells = SingelCells + bin_counts(1,i);
end
for i=9:17
  Clusters = Clusters + bin_counts(1,i);
end
for i=18:27

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SmallAggregates = SmallAggregates + bin_counts(1,i);
end
BigAggregates = bin_counts(1,29) + bin_counts(1,28);
% BigAggregates+Clusters+SingelCells+SmallAggregates
% sum(bin_counts)
for i=1:29
    bin_counts_total(n,i)=bin_counts(1,i);
end
CellCount(n,1)=SingelCells;
CellCount(n,2)=Clusters;
CellCount(n,3)=SmallAggregates;
CellCount(n,4)=BigAggregates;
figure, imshowpair(BWfinal,I,'montage')
end
% MIJ.closeAllWindows
% MIJ.close
1. Convert raw image to binary image in MATLAB.

2. Compute event areas in ImageJ.
   1) Process -> binary -> make binary
   2) Process -> binary -> watershed
   3) Analyze -> set measurements -> check “area” only
   4) Analyze -> analyze particles
      Size: 21 – infinity
      Circularty: 0.00 – 1.00
      Show: nothing
      Check “display results” and “exclude on edges”
   5) Results -> options -> enter “85”, “-1”, and “.txt” and check “use file chooser to import sequences” and “copy row numbers”
   6) export result in MATLAB

3. Generate events/bin in MATLAB

4. Go back to binary image in ImageJ, analyze -> set measurements -> check “fit ellipse”, repeat step 2 and 3, and export data to MATLAB

5. Compute aspect ratio as major/minor

6. Look for events between 81 and 100, check their aspect ratios and if an event’s aspect ratio is equal to or smaller than 1.1, bin it as a single cell; else bin it as a cluster.

7. In MATLAB, based on event area and aspect ratio, bin events into the following groups:
   Single cells: 21 – 80 pixels;
Clusters: 91 – 200 pixels;

Small aggregates: 201 – 300 pixels;

Big aggregates: 301 - \infty.
APPENDIX D) CELL CULTURE PROCEDURE

1. Observe the desired cell line under microscope to make sure that it has reached 70% confluency.

2. Place Trypsin, cell media, and PBS in the water bath at 37°C for 15 minutes.

3. Aspirate the old media from the flask which contains the cells in a sterile culture hood.

4. Wash the cells with 5ml of PBS.

5. Add 3 ml of Trypsin to cells and place the flask in 37°C, 5% CO₂ incubator for few minutes until the cells detach from the bottom of flask. The time required depends on the cell line.

6. Once the cells are detached and floating, add 7ml of the cell media to the flask to deactivate the Trypsin. Pipette up and down for 5 minutes.

7. Transfer the 10ml of the cell solution to a 15ml conical tube. Centrifuge for 3 minutes at 1500 rpm.

8. Once complete, cell pellet could be observed on the bottom of the conical tube. Aspirate the media and trypsin solution without touching the cells.

9. After aspiration, add 5 ml of the cell media to the conical tube to break down the pellet. Pipette up and down until a homogenous mixture is obtained.

10. Take 1ml of the cell solution and add to a new flask with 14ml of fresh media.
11. Place the new flask in the incubator.
APPENDIX E) PHOTOLITHOGRAPHY PROCEDURE

1. Blow the wafer surface with air to remove particles and clean the surface.

2. Pour negative photoresist (SU-8) on the wafer and spin coat it at 2000 rpm for 40 seconds.

3. Soft bake the wafer at 65°C for 5 minutes.

4. Hard bake the wafer at 95°C for 25 minutes.

5. Place the photo mask on the wafer and cover it with another glass slide, UV expose it for 18 seconds.

6. Bake the wafer again to stabilize the features on the wafer at 65°C for 3 minutes followed by 95°C for 7 minutes.

7. Pour developer in a cup and place the wafer there, shake well for 4 minutes and then let the wafer stay in there for another 2 minutes.

8. Rinse the wafer with more developer and ethyl alcohol and dry it off with air gun.

9. The silicon wafer is then baked for another 2 hours at 120°C to remove any moisture and to ensure that SU-8 adheres to the wafer firmly. The wafer is salinized then to makes sure that the mold does not stick to the wafer and also prevents the feature from getting off the wafer when a mold is removed. Salinization procedure is as follows:
10. Place the wafer in desiccator.

11. Pour 10 μl of 2-Mehoxysilane in a small container and place it in the same desiccator.

12. Vacuum the desiccator overnight.

13. Remove the wafer and place it in room temperature for 1 hour.
APPENDIX F) PDMS PROCEDURE

1. Polydimethylsiloxane and curing agent is mixed at the ratio of 9:1 respectively.

2. The mixture was poured over the silicon wafer, then the wafer is placed in a vacuum desiccator to remove air from the uncured PDMS.

3. When all air bubbles have disappeared, the silicon wafer will be placed in the oven at 65°C for 12 for PDMS curing.

4. When the wafer is cooled down, the mold is being cut out and openings are punctured.

5. The device is cleaned with an air gun and tape to get rid of any unwanted particles.

6. The PDMS device is then plasma bonded for 70s to a glass slide.

7. Put the PDMS bonded to glass slide for another day for better binding in the oven at 65°C

8. Plastic tubing with the needle gauge 30 and the inner and outer diameter of 0.25mm and 0.75 is inserted.

9. The length of the tubing for all inlets are 40 mm. (Syringes and needles that used are from BD Sciences with the following specifications; 5 ml syringes with 1ml tip used along with needles of 0.25 mm outer diameter and a 0.06mm inner diameter.)

10. Finally, the device is flushed with DI water at a 500 μl/min flow rate for 20 minutes.