PHYSICAL CELL SEPARATION USING MICROFLUIDIC FUNNEL RATCHETS

by

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Abstract

The separation of biological cells using non-chemical methods is important to many areas of medicine and biology. Filtration through microstructured constrictions is one such method where cells can be separated by a combination of size and deformability. This technique, however, is limited by unpredictable variations of the filter hydrodynamic resistance as cells accumulate in the microstructure. Applying a reverse flow to unclog the filter will undo the separation and reduce filter selectivity because of the reversibility of low-Reynolds number flow. This work introduces a microfluidic structural ratchet mechanism to separate cells using oscillatory flow through a 2-dimensional array of funnel-shaped structures. Devices are fabricated using multi-layer soft lithography of polydimethylsiloxane (PDMS) and flow is controlled using pressure sources and on-chip membrane valves. An iterative procedure of design and testing is used to produce a final device which is characterized by the sorting and separation of L1210 mouse lymphoma cells (MLCs), peripheral blood mononuclear cells (PBMCs) from healthy donors, as well as polystyrene microparticles. The ability of this mechanism to sort and separate cells/particles based on size and deformability is investigated and confirmed. Additionally, the spatial distribution of cells after sorting is demonstrated to be repeatable and the separation process is shown to be irreversible. This mechanism can be applied generally to separate cells that differ by size and/or deformability.
Preface

A version of the following sections is currently under review for publication: 1.4-1.6, 2.1, 2.3, 2.4, Chapter 3:5.1, 5.2, 5.3, 5.5, 5.6, and 6.3. I designed the device, and carried out the fabrication with the assistance of Lily So and Bill Lin. Testing of the device was performed jointly by myself and Bill Lin, and I wrote the manuscript.

Section 2.2 describes a numerical model used to predict the pressure asymmetry required in deforming a cell through a tapered pore. An initial version of this model for short channels was originally developed by Dr. Hongshen Ma, and early modeling was conducted by Quan Guo. I adapted this model for use in high channels where the maximum deformed radius of the cell is less than the channel height.

Chapter 4: describes three separate device generations. Generation 1 devices were designed and fabricated on silicon masters by Dr. Hongshen Ma. Isaac Tang and I assisted with the silicon master fabrication. PDMS molding was carried out by Isaac Tang, Keir Maguire, and Jenny Reimer. Experiments were done by Isaac Tang. I designed both the Generation 2 and Generation 3 devices. I was assisted in fabrication of the silicon masters for Generation 2 by Dr. Hongshen Ma. Much of the PDMS molding for Generation 2 was done by Remy Barois. I conducted the testing for these devices. I was assisted in fabrication of the silicon masters for Generation 3 by Dr. Hongshen Ma and Lily So. The PDMS molding was done by myself and Bill Lin, with assistance from Julia Koch. The testing of Generation 3 devices was jointly conducted by Bill Lin and myself.
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List of Abbreviations

BSA = bovine serum albumin
CTC = circulating tumor cell
DEP = dielectrophoresis
DLS = deterministic lateral displacement
MLC = mouse lymphoma cell
PBMC = peripheral blood mononuclear cell
PBS = phosphate buffered saline
PDMS = polydimethylsiloxane
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Dedication

To the glory of God who is Love,
Jesus who is Life
and Holy Spirit who is Freedom
Chapter 1: Introduction

Many areas in medicine and biology require individual populations of cells to be separated from heterogeneous samples. Examples include the isolation of fetal cells from maternal blood, blood cell fractionation, and the separation of circulating tumor cells (CTCs) from the peripheral blood of cancer patients. Chemical separation methods based on affinity capture of cell surface molecules are effective at isolating cells with known chemical markers, but can alter the properties of the cells in the process. These methods are undesirable when the separated components need to be preserved in their original state, or when a unique biochemical marker is not known.

Physical cell separation is a category of separation techniques which use the physical properties of the cell such as size, deformability, morphology, conductivity, and density to distinguish target cells from background cells. These separation mechanisms include those that are based on filtration and those based on flow. Filtration-based separation mechanisms typically use both size and deformability as sorting parameters. Microfluidic filtration consists of an applied pressure which drives a particle-laden fluid into microscale constrictions. These constrictions are sized such that certain particles can deform through while others are blocked. Specific filtration designs include membrane, weir-style, crossflow, and micropillar array filters. Filtration is of particular interest due to its ability to sort by deformability, which could potentially be a better parameter than size to distinguish cells based on phenotype or disease status.

Flow-based separation mechanisms include field flow fractionation and hydrodynamic separation. Field flow fractionation relies on an external field perpendicular to the direction of flow to act more strongly on one of the target cell phenotypes and is dependent on properties such as size, dielectric constant, or density. Specific realizations of this principle include dielectrophoresis, magnetophoresis, and acoustophoresis. Hydrodynamic separation methods are typically purely size-based, although a recent study suggests the potential to sort by deformability as well as size. Hydrodynamic separation methods include deterministic lateral displacement, hydrodynamic chromatography, and inertial microfluidics.

Section 1.1 presents the performance metrics associated with cell separation. Section 1.2 reviews filtration mechanisms. Section 1.3 reviews hydrodynamic sorting methods. These sections are followed by a short discussion of the limitations of prior techniques. Section 1.5
presents an asymmetrical pore design which we previously developed to allow cells passage through in only one direction. Finally, Section 1.6 presents our design concept wherein a filter is constructed using these pores to irreversibly sort cells.

1.1 Definition of Performance Metrics

1.1.1 Performance Metrics for Cell Separation

A separation mechanism can be thought of as a system with inputs and outputs. For binary separations, a single mixed sample enters the device at the input, and after the separation has taken place, the output consists of a purified sample of target cells. A number of parameters can be used to evaluate the effectiveness of various separation mechanisms. One of the most commonly reported metric is separation efficiency, which is defined as follows:

\[
\text{Efficiency} = \frac{\text{Target cells at the output}}{\text{Target cells at the input}} \times 100\% \quad (1.1)
\]

Efficiency is typically associated with a particular cell phenotype, since most studies focus on extracting a single cell population of interest from a mixture.

A second commonly reported parameter is the purity which is defined as:

\[
\text{Purity} = \frac{\text{Target cells in the output}}{\text{Total cells in the output}} \times 100\% \quad (1.2)
\]

In separations where the concentration of target cells is very low in relation to the total concentration of cells in the sample, sometimes an enrichment factor is reported instead of purity. The enrichment factor measures the factor by which the purity of target cells increases due to the separation process. This can be calculated as follows:

\[
\text{Enrichment} = \frac{\text{Target cell concentration at the output}}{\text{Target cell concentration at the input}} \quad (1.3)
\]

It is important to note that these parameters depend not just on the separation mechanism, but also on the cell types in the sample. Thus, separation mechanisms can only be directly compared when the same input cells are used in the separation.

1.1.2 Performance Metric for Cell Sorting

While separation parameters work well for binary separations, a performance metric is needed to evaluate mechanisms which sort the cells by size or deformability into separate fractions. We
define a metric for the selectivity of a sorting process based on metrics developed for liquid chromatography. Liquid chromatography is a classical technique for separating molecular components of a mixture using the differences in the time required to pass analytes through a fixed length column, which known as retention time. The concentration of analytes at the outlet of the column is monitored optically and typically results in time-varying plots similar to Figure 1.1. Since each molecular species has a characteristic retention time corresponding to the peak and width of the distribution, the resolution of a chromatographic process to separate two components is defined as the difference in retention times divided by average peak widths as follows 11:

$$R_s = \frac{2(t_{R,i} - t_{R,j})}{W_i + W_j}$$  (1.4)

where \(t_R\) is the retention time of cell phenotypes \(i\) and \(j\) and \(W\) is the width of each peak as shown in Figure 1.1.

![Figure 1.1 Example of a chromatography output showing signal vs. retention time for cell phenotypes i and j.](image)

Using equation 1.4 as a model, we define a parameter to measure the selectivity of a separation process for two cell phenotypes \(i\) and \(j\). For a purely size-based sorting process, the output fractionation should correspond to the size distribution of the cells. The average cell sizes of each population, \(\mu_i\) and \(\mu_j\), correspond to the retention times in equation 1.4. The width of the size distribution can be defined as two standard deviations, such that \(W_i\) and \(W_j\) correspond to \(2\sigma_i\) and \(2\sigma_j\). Substituting these new terms into equation 1.4 yields a new equation for selectivity:

$$S = \frac{(\mu_i - \mu_j)}{(\sigma_i + \sigma_j)}$$  (1.5)
Greater values of S correspond to more widely segregated size-distributions and therefore easier populations to separate. Figure 1.2 illustrates the size distributions of two populations for the cases where S has the values of 0.75, 1.5, and 5. The selectivity of a sorting mechanism can be characterized by the minimum value of S at which the system can achieve a desired efficiency or purity of separation.

Figure 1.2 Example of two cell size distributions $i$ and $j$ where $S = 0.75$ (A), $S = 1.5$ (B), and $S = 5$ (C).

1.2 Filtration Review

1.2.1 Membrane Filtration

Early cell filtration was demonstrated by Seal in 1965. With the advent of technology allowing small cylindrical holes on the size scale of individual cells to be created in a thin polymer
membrane, he used such a membrane as a sieve for cancer cells in blood. As a cell mixture encounters the filter, small cells are able to flow through while large cells become trapped in the pores. This type of design, shown in Figure 1.3, has been the subject of a number of studies recently for the capture of circulating tumor cells from blood samples. Variations include using multiple membranes with a different size of pore on each to increase the capture efficiency or using a second membrane just below the first to support captured cells and keep them from being harmed during the filtration process. Efficiencies from membrane filtration have been reported at 70-90% for filtering out cultured human cancer cells spiked into whole blood at low concentrations. In this method, once a larger cell blocks a pore, it becomes trapped there for the duration of the process. Post-filtration analysis is typically conducted on the membrane itself. Even though the concentration of target cells is extremely low when filtering out circulating tumor cells from blood, at high throughputs most of the pores will eventually become clogged by cancer cells or large leukocytes and render the filter inoperative.

**Figure 1.3 A membrane filter.**

### 1.2.2 Weir-Style Filtration

A second type of filtration involves a weir-style barrier as shown in Figure 1.4 where the depth of the channel changes to exclude larger less deformable cells. A notable recent example was fabricated by Huang et al. and involves using polydimethylsiloxane (PDMS) microvalves as a tunable filter. A pressure applied to a control channel deflects a PDMS membrane into the flow channel, with the degree of applied pressure corresponding to the amount of deformation. This design was tested using microparticles as well as chondrocyte cells. Although this design is versatile and adjustable, the selectivity is limited by the poor control over the void space in the
filtering area. As the membrane deflects, it does not block off the channel evenly but deflects more in the middle than at the edges, allowing larger particles to pass through near the channel sidewalls. It also suffered from clogging as large cells accumulated at the weir area and blocked the channel. The solution used in this case was to have periodic back-flushing to push clogged material away from the weir, however this strategy only temporarily removes the clogging particles or cells which return in greater concentrations as the filtering continues. Ji et al. tested each of the four types of filters for the application of separating leukocytes from red blood cells and found that the weir-style design yielded a better efficiency than their membrane filter, but a lower efficiency than their crossflow and micropillar array filters.

Ji et al. tested each of the four types of filters for the application of separating leukocytes from red blood cells and found that the weir-style design yielded a better efficiency than their membrane filter, but a lower efficiency than their crossflow and micropillar array filters.

![Figure 1.4 A weir-style filter.](image)

### 1.2.3 Crossflow Filtration

Crossflow filtration refers to the situation where smaller more deformable cells are removed by a flow perpendicular to the main flow through pores in the side of the channel as shown in Figure 1.5. VanDelinder et al. demonstrated the utility of this technique to separate leukocytes from red blood cells with 98% efficiency and an average purity of 71%.

Kuo et al. constructed a variation on this filter type by using the fluid momentum instead of an applied crossflow to direct cells through the channel walls. They designed a microchannel consisting of many S-shaped bends with pores on the edges of the bends. As the fluid travels around the bend, a portion of the
velocity carries some of the fluid through the pores allowing smaller more deformable cells to transit through. They tested this design as a method for separating circulating tumour cells from blood using a cultured human cancer cell line. The efficiency of this method was inversely correlated with flow rate, with 90% separation efficiency measured at 25 µL/min, but dropping to 50% at 100 µL/min as more target cells were lost through the pores at this higher flow rate.

1.2.4 Micropillar Array Filtration
The final filtration method uses an array of micropillars across the channel as shown in Figure 1.6 to retain larger less deformable cells while allowing smaller more deformable cells to pass through. Some recent examples include a filter fabricated by Lay et al. which used raindrop-shaped micropillars to filter bacteria. This design included two rows of pillars in the array, with a small bypass region where spaces between the pillars were larger. As the filter became clogged during filtration at a constant flow rate, the bypass allowed fluid to continue flowing and relieved the pressure buildup from obstruction of the pores. Although this bypass keeps the hydrodynamic resistance of the channel from increasing past a certain point, it also reduces the efficiency as target cells are lost through the bypass.

Another example fabricated by Mohamed et al. uses an array of rectangular pillars to filter cancer cells from blood. Their device utilizes multiple stages of pore sizes, each containing multiple rows of pillars. Using various cultured human cancer cells spiked into whole blood, they demonstrated that these cancer cells could be retained within the device and removed
via backflow after filtration. They note that different cell lines have differing size and deformability ranges, therefore they are retained at different stages within their device.

Another variation of micropillar array filtration was fabricated by Tan et al. also for the purpose of detecting circulating tumor cells from blood. Their design uses micropillars to create small perforated wells that retain cancer cells while allowing blood cells to flow either through the perforations or around the well after a cancer cell has been captured \(^{24}\). By leaving plenty of room between these wells but arranging them in a staggered format, they avoid the problem of increased hydrodynamic resistance that accompanies clogging. This design is optimal for situations where target cells are rare, and they demonstrate an efficiency of greater than 80% for cultured human cancer cells spiked into whole blood. However, in situations where there are larger concentrations of target cells the efficiency of the separation will drop once all the wells become filled.

### 1.3 Hydrodynamic Sorting Review

Hydrodynamic sorting refers to a number of techniques which rely upon the flow properties of particles or cells inside of a microchannel to sort by size. Typically, these methods involve introducing cells into a single inlet at a constant flow rate, and then relying on the internal structure of the device to transport particles of different sizes into separate outlets. This goal is accomplished through moving particles of certain sizes across streamlines as they travel through the device, either through arrays of obstacles, inertial forces, or compressing and expanding streamlines. These methods can be high-throughput and the separation process is simple to perform. However the selectivity and efficiency of the separation depends very strongly upon the geometrical parameters of the device in relation to the size and shape of the cells or particles undergoing the separation.

#### 1.3.1 Deterministic Lateral Displacement (DLD)

Deterministic lateral displacement, also known as the "bump array" method was first demonstrated as a method to sort particles by Huang et al. \(^{25}\) and relies on an array of microfluidic posts with each successive row slightly offset from the previous. The post sizes and positions are selected such that larger particles interacting with a post will be bumped into a
different streamline and migrate diagonally through the array, whereas smaller particles do not experience this lateral migration as they flow through the channel. Huang et al. were able to achieve a resolution of 10 nm while sorting particles between 0.8 and 1 µm in size with nearly 100% purity and efficiency. Davis et al. developed a version of this system for the separation of whole blood and demonstrated the extraction of purified blood plasma in one case, and the enrichment of white blood cells (WBCs) in another. In the second case, this mechanism was able to isolate 99% of WBCs but at a purity of less than 10%. Additionally, Loutherback et al. built upon this concept to fabricate a ratchet mechanism using triangular posts such that particles of certain sizes exhibit irreversible behavior, taking different paths through the array when the flow is reversed. DLD devices have recently been used by Holm et al. for separating worm-shaped trypanosome parasites from blood cells with a purity of 99%.

1.3.2 Hydrodynamic Chromatography

Another hydrodynamic cell sorting method is known as pinched flow fractionation, first demonstrated by Yamada et al. This method involves a narrowing of the channel where the particles are pushed against the sidewall. Such a device include one inlet that introduces a particle mixture while a second contains only buffer and has a higher flow rate. These inlets join together and narrow into one channel, where the buffer stream pushes the particles against the wall of the channel. When the pinched channel widens, the particles can be directed into different outlets. In Yamada’s study, the separation efficiency was found to be 99% and 92% for a mixture of 15 and 30 µm particles.

A related method, simply known as hydrodynamic filtration, relies on carefully controlled flow rates in perpendicular channels branching off from the main channel to siphon off fluid or small particles. Yamada et al. demonstrated that by using different flow rates in these side channels they could concentrate a dilute particle mixture, siphon off plasma from whole blood, or enrich a sample of leukocytes by removing excess erythrocytes. In this method, they demonstrated that over 80% of the plasma could be siphoned off. They also demonstrated an enrichment of leukocytes by a factor of 29 using repeated filtrations. However, this enrichment still results in less than 10% purity.
1.3.3 Inertial Microfluidics

Inertial microfluidics relies upon inertial forces acting upon the cells to bring them to a size-specific equilibrium position within the channel. The combination of a wall effect lift force and a shear gradient lift force causes particles to settle into an equilibrium flow position which can be controlled by the channel geometry. In such a system, Di Carlo et al. demonstrated that separation can be performed by balancing the inertial force with another force such as a Dean drag force introduced by arcing channel segments \(^{31}\). The ratio of these two forces changes based on particle size, allowing particles to be sorted and extracted on the basis of equilibrium position within the channel. The mathematical relationship of these forces in regard to designing inertial focusing and separation systems has been discussed in-depth previously \(^{32}\) and these systems have been used in the separation of bacteria from blood \(^{33}\), blood components from one another \(^{31}\), and neural cells \(^{34}\). Efficiencies ranged from 80% to over 99% for these various applications. The input mixtures must be diluted significantly to avoid particle interactions with one another, however the operational flow rates are on the order of milliliters per minute. Additionally, a recent study by Hur et al. has demonstrated the ability to sort by deformability as well as size \(^{10}\). This technique was demonstrated for the enrichment of cultured cancer cells from blood, with an efficiency of 95-98%. The purity of the samples in these results can be calculated by multiplying the enrichment factor with the initial purity of the sample which yields only 4.48% and 4.28% for the experiments conducted.

1.4 Limitations and Challenges of Filtration

Filtration is important because it can sort by deformability and in most cases it allows for the extraction of unaltered cells following separation. However, as was mentioned in the previous section, existing filtration methods are prone to clogging which limits the selectivity of the mechanism. As cells become lodged in the microscale constrictions during the sorting process, the overall hydrodynamic resistance (\(R_H\)) of the filter changes and diminishes the effect of the applied pressure gradient in an unpredictable manner\(^{35}\), as shown in Figure 1.7 for a micropillar array filter. The selectivity of traditional filtration approaches is further limited by cytoskeleton remodeling, whereby a persistent force wedging cells into the filter induces changes in the internal structure of the cell \(^{36}\), enabling some trapped cells to gain passage over time. Furthermore, prolonged contact between the cells and the filter surface increases the potential for
non-specific adsorption, which exacerbates clogging and prevents the recovery of the cells after separation \(35\).

![Figure 1.7 Clogging in a filter mechanism. As cells block the pores of the microstructure, the hydrodynamic resistance of the filter changes unpredictably.](image)

### 1.5 Ratchet Mechanism

The cells that accumulate in a filter microstructure could seemingly be removed by periodically reversing the direction of flow to dislodge cells from the filter, and re-open the pores to reset the hydrodynamic resistance of the filter to its initial value. However, the effectiveness of such a remedy is limited by the reversibility of inertia-less, low-Reynolds number flow, which will undo the initial separation performed by the filter microstructure. Flow reversibility in low-Reynolds number systems can be countered using a ratchet mechanism, such as those that have been developed using thermal gradients \(37\), electromagnetic fields \(38\), physical geometry \(39-41\), and various combinations thereof \(42, 43\). However, these mechanisms have only presented the possibility of separating particles based on size but not deformability.

Previously, we proved the existence of a structural microfluidic ratchet created using funnel-shaped micrometer-scale constrictions and demonstrated unidirectional transport of single cells when excited using an unbiased oscillatory flow of the bulk liquid \(44\). The funnel microstructures are sized such that the entrance side of the funnel is larger than the diameter of the cells, while the exit side is smaller than the diameter of the cells. The pressure required to deform single cells through such a constriction along the direction of the taper is less than the
pressure required against the direction of the taper. When a cell is deformed into a constriction, the pressure required to advance the position of the cell increases until a point of instability, known as Haines' jump, where the cell is rapidly pulled through the constriction\textsuperscript{45}. The threshold pressure for Haines' jump depends on the radius of the leading cell surface and the radius of the following cell surface, which are in turn determined by the geometrical constraint provided by the funnel taper.

1.6 Design Concept and Research Goals

In this work, we leverage this ratchet effect to create a physical cell separation device where cell samples are transported through a matrix of funnel constrictions using an oscillatory flow. The small and deformable cells transit through the constrictions in forward flow, while the large and rigid cells are blocked (Figure 1.8A). When the flow direction is reversed to unclog the constrictions, small and deformable cells are unable to pass back through (Figure 1.8B). Using multiple linear arrays of these constrictions enables the sorting of cells based on their size and deformability.

The goal of this research is to develop and characterize this mechanism for the sorting of cells by size and deformability. Specifically as follows:

- Optimize device features, geometry and dimensions through an iterative process of design and testing
- Develop supporting microfluidic circuitry to generate the flow conditions necessary for reliable cell sorting and separation
• Demonstrate cell sorting using a single cell population based on size and deformability
• Demonstrate cell separation using two model cell populations of differing sizes and deformabilities
• Utilize model cells and particles of similar sizes to study the relative effects of size and deformability in the device
• Characterize the effects of varying different experimental parameters
• Confirm the irreversibility of the ratchet mechanism
Chapter 2: Device Design

This chapter describes the design of the cell separation device. Section 2.1 briefly describes the funnel geometry used in the device. Section 0 describes the numerical model used to estimate the expected pressure asymmetry of a cell deforming through a tapered pore and examines the effect of varying the funnel shape. Section 3.2 outlines how these funnels are arranged into an array and describes the operation of the sorting process. Lastly, Section 2.4 describes the design features used to precisely control the flow inside the device.

2.1 Funnel Design

The funnel structures are comprised of planar tapers with straight or parabolic shaped sidewalls. These funnels have a rectangular cross-section where the depth of the channel is 25 µm to accommodate typical mammalian cells in suspension, and the width of the funnel varies according to Equation 2.12 in the following section with a parabolic coefficient of 2000 m$^{-1}$. The parabolic geometry increases the repeatability and robustness of the fabrication procedure for small funnels. The pore size of the funnel is varied depending on the funnel's location within the sorting area as will be discussed in Section 2.3.

2.2 Model of Cell Deformation in Funnels

![Figure 2.1](image)

Figure 2.1 Three dimensional view of a cell deforming through a funnel constriction showing the pressure inside and outside the cell.

The problem of a cell deforming through a microscale constriction can be modeled by considering the cell to be a liquid-filled sac surrounded by a membrane with a cortical tension, $T_C$ that is assumed to be isotropic and constant$^5$. As a cell deforms through a constriction with
a rectangular cross-section as shown in Figure 2.1, the internal pressure of the cell is denoted $P_{\text{int}}$ while pressures $P_1$ and $P_2$ are the pressures acting on the leading and trailing membranes of the cell respectively. We can examine the balance of forces acting on these membranes separately as illustrated in Figure 2.2. The membrane has two radii of curvature, one which is constrained by the geometry of the funnel, and another which is not. The constrained radius is denoted $R_a$ for the leading edge of the membrane and $R_b$ for the trailing edge. The unconstrained radius is denoted $R_{c1}$ and $R_{c2}$ for the leading and trailing edges respectively. Figure 2.3 illustrates these radii from the oncoming view.

In the case of the leading edge as shown in Figure 2.2A, the normal components of the membrane tension forces, $F_{Tc1}$ and $F_{Ta}$, are equal to the force from the difference in pressure on either side of the membrane as follows:

$$ (P_1 - P_{\text{int}})ac_1 = F_{Tc1} \cos \theta_1 + F_{Ta} \cos \varphi_1 $$

(2.1)

![Figure 2.2 Schematic of forces acting on a curved membrane. (A) Leading edge membrane. (B) Trailing edge membrane.](image)

Next, the membrane tension forces are defined as the cortical tension multiplied by the length of the membrane edges over which they act, such that:

$$ F_{Tc1} = T_c (2a) \quad (2.2) $$

$$ F_{Ta} = T_c (2c) \quad (2.3) $$

Substituting these tension force terms into Equation 2.1 yields:

$$ (P_1 - P_{\text{int}})ac_1 = 2T_c (a \cos \theta_1 + c_1 \cos \varphi_1) \quad (2.4) $$

Furthermore, by geometry:
\[ \cos \theta_1 = \frac{c_1}{2R_{c1}} \]  
\[ (2.5) \]

\[ \cos \phi_1 = \frac{a}{2R_a} \]  
\[ (2.6) \]

Substituting Equations 2.5 and 2.6 into equation 2.4, and dividing through by a factor of \( ac_1 \) gives:

\[ P_1 - P_{\text{int}} = T_c \left( \frac{1}{R_{c1}} + \frac{1}{R_a} \right) \]  
\[ (2.7) \]

Following the same procedure for the trailing edge membrane yields:

\[ P_2 - P_{\text{int}} = T_c \left( \frac{1}{R_{c2}} + \frac{1}{R_b} \right) \]  
\[ (2.8) \]

To find an expression for the applied pressure on the entire cell, we can subtract Equation 2.7 from Equation 2.8:

\[ P_2 - P_1 = T_c \left( \frac{1}{R_a} + \frac{1}{R_{c1}} - \frac{1}{R_b} - \frac{1}{R_{c2}} \right) \]  
\[ (2.9) \]

The difference between \( R_{c1} \) and \( R_{c2} \) is considered small enough to be negligible, therefore these two radii are approximated as being equal. This reduces Equation 2.9 to the following final form:

\[ \Delta P = T_c \left( \frac{1}{R_a} - \frac{1}{R_b} \right) \]  
\[ (2.10) \]
As the cell deforms through the funnel, the pressure given in Equation 2.9 will increase until it reaches a maximum. At this point, the cell undergoes the Haines’ jump instability and the entire cell is rapidly pulled through the constriction.

We considered both straight tapers and parabolic tapers of the following forms:

\[ y = \pm (mx + W_0) \]  
\[ y = \pm (kx^2 + W_0) \]

where \( m \) and \( k \) are coefficients defining the shape of the taper and \( W_0 \) is the half-width of the funnel pore as shown in Figure 2.4A. The parameter \( m \) corresponds to a certain angle \( \theta \) measured from the vertical according to the equation:

\[ m = \tan(\theta) \]

Figure 2.4 illustrates a cell deforming through a parabolic constriction in the forward (A) and reverse (B) directions. The reference axis is placed as shown in Figure 2.4A with the y-axis placed horizontally and the x-axis vertically at the centre of the funnel pore. The z-axis (not drawn) is perpendicular to the page. The z-axis height of the channel is larger than the radius of the cell even under extreme deformation.

The maximum pressure to deform a cell through a funnel can be calculated from Equation 2.9 based on the geometrical parameters of the funnel constriction and the size of the cell by assuming volume conservation. This maximum pressure is found numerically using Matlab, as is outlined in the following sections.
2.2.1 Deformation Along a Parabolic Funnel Taper

To model the deformation of a single cell in a parabolic funnel along the direction of taper, the cell can be divided into 3 portions as shown in Figure 2.5A. To determine the volume of each portion, we need to calculate \( R_c \). This value is approximated by considering the compression of a cell between two parallel plates. The compression causes the cell to become a spheroid with the two uncompressed radii having an equal value. If we consider the mean value of \( W \), denoted \( W_{\text{mean}} \), along portion 2 as the distance between these two plates, \( R_c \) can be calculated using volume conservation as follows:

\[
R_c = \sqrt[3]{\frac{3V_0}{4\pi W_{\text{mean}}}}
\]  \hspace{1cm} (2.14)

where \( V_0 = \frac{1}{2}\pi R_0^3 \)

\[
V_0 = \frac{1}{2}\pi R_0^3
\]  \hspace{1cm} (2.15)

Although \( R_c \) is in reality a function of \( x \), these variations are considered small enough that we can apply our approximate mean value to each portion.
Figure 2.5  Modeling the cell transiting through a parabolic pore along the direction of taper.  (A) Total cell volume is divided into three regions.  (B) Elliptical cross-section in region 2.

The radii $R_a$ and $R_b$ are determined by the width of the funnel and volume conservation.  The total volume of the cell is a sum of the volumes of each portion as follows:

$$V_0 = V_1 + V_2 + V_3$$  \hspace{1cm} (2.16)

The volumes $V_1$ and $V_3$ are half-spheroids:

$$V_1 = \frac{2}{3} \pi R_a^2 R_c$$  \hspace{1cm} (2.17)

$$V_3 = \frac{2}{3} \pi R_b^2 R_c$$  \hspace{1cm} (2.18)

The volume $V_2$ is approximated as a sum of elliptical slices (Figure 2.5B) each with a thickness of $\Delta x$ and a radius on the $y$-axis of $W(x)$, which is the width of the funnel at that point.

$$V_2 = \sum_{i=a}^{b} \pi W(i) R_c \cdot \Delta x$$  \hspace{1cm} (2.19)

Remembering that:

$$W(x) = kx^2 + W_0$$  \hspace{1cm} (2.20)

These equations allow us to solve for $R_a$ and $R_b$, and thus calculate $\Delta P$ for each position of the cell inside the funnel.  In this case we consider a 50 µm long funnel, and find the maximum deformation pressure.

2.2.2  Deformation Along a Linear Funnel Taper

To model the deformation of a single cell in a linear funnel along the direction of taper, a small modification must be made when the model includes larger values of $\theta$.  The previous model assumes that region 3 is a half-spheroid, which closely approximates the geometry when $\theta$
is small. However a better approximation is to consider region 3 to be a partial spheroid truncated at the point where the tangent to the spheroid is equal to the plane of the sidewall. This modification is most significant at small compressions and wide sidewall angles when very little of the cell is in contact with the funnel walls. At the truncation point the half-width of the funnel is represented by:

\[ W_{\text{trunc}} = R_b \cos \theta \]  

(2.21)

and the height of the truncated portion by:

\[ h = R_b - \sqrt{R_b^2 - W_{\text{trunc}}^2} \]  

(2.22)

Using this information gives a modified equation for the volume of the third region as follows:

\[ V_3 = \frac{4}{3} \pi R_b^2 R_c - \frac{\pi R_c}{3R_b} h^2 (3R_b - h) \]  

(2.23)

Figure 2.6 Modeling the cell transiting through a linear funnel along the direction of taper. Total cell volume is divided into 3 regions.

2.2.3 Deformation Against the Funnel Taper

To model the deformation of a single cell against the direction of taper, the maximum pressure occurs when the cell forms a half-spheroid in the funnel opening. In this case, the cell can be divided up into two separate shapes (Figure 2.7A). The first is a half-spheroid of radius \( R_a \) that is equal to \( W_0 \). The second is a spheroid of radius \( R_b \). The intersection of volumes 1 and 2 forms a third volume, \( V_3 \), which is an ellipsoidal cap (Figure 2.7B).
Figure 2.7 Modeling the cell transiting through the pore against the direction of taper. (A) Total cell volume is divided into two main regions with the intersection between them representing a third region. (B) Modeling region 3 which is an ellipsoidal cap with a base radius of $W_0$ and a height of $h$.

By volume conservation, we can equate these volumes as follows:

$$V_0 = V_1 + V_2 - V_3$$

(2.24)

In order to calculate these volumes, we again need to approximate the radius of the cell in the $z$-direction, $R_c$. For small amounts of cell compression where $R_0$ is similar to $W_0$, this value can be approximated as before as if the cell is being compressed between two parallel plates of a distance apart equal to the pore size.

$$R_c = \frac{3V_0}{4\pi W_0}$$

(2.25)

This approximation is useful for small compressions where the cell is compressed close to the centre, but becomes less accurate at larger compression amounts where the cell is significantly larger than the pore size.

Subsequently, the volumes $V_1$ and $V_2$ can be calculated:

$$V_1 = \frac{3}{2}\pi R_c^2 R_c k$$

(2.26)

$$V_2 = \frac{4}{3}\pi R_c^2 R_c k$$

(2.27)

The intersection of these two areas is given by the following equation:

$$V_3 = \frac{\pi R_c}{3R_b} h^2 (3R_b - h)$$

(2.28)
The value $h$ is the height of the ellipsoidal cap (Figure 2.7B) and can be represented in terms of $R_b$ and $W_0$ from geometry:

$$h = R_b - \sqrt{R_b^2 - W_0^2} \quad (2.29)$$

Using the above equations to solve for $R_a$ and $R_b$, we can use Equation 2.10 to solve for the maximum pressure needed to push the cell through the funnel against the direction of taper.

### 2.2.4 Pressure Asymmetry

The pressure asymmetry is defined as the maximum reverse pressure over the maximum forward pressure.

$$Asymmetry = \frac{\max(\Delta P_{reverse})}{\max(\Delta P_{forward})} \quad (2.30)$$

The individual pressures are dependent on the cortical tension of the cell, however the asymmetry is independent of tension. Thus, the asymmetry will depend solely on the shape of the funnel and the size of the cell, not the deformability of the cell.

We modeled funnel shapes corresponding to a funnel inlet half-width ranging from 3.5 to 25 µm. The lower bound was chosen as the size which would allow a 7 µm diameter cell to enter a pore resulting in 50% compression without experiencing any compression at the inlet. The upper bound results from constraints on the distance of the funnels from one another, assuming a funnel length of 50 µm. The pitch of the funnel array in early designs was 50 µm, therefore half of this value is used as the maximum inlet half-width.

Matlab code to calculate the asymmetry is shown in Appendix A for linear and parabolic funnel shapes. Input parameters include $R_0$ and $W_0$. To determine actual pressures needed, $T_C$ can be entered. Figure 2.8 shows the results of this model for a 10 µm diameter cell transiting through a 8 µm pore.
### 2.2.5 Funnel Design Parameters

Expectedly, more gradual tapers, as defined by smaller $k$ and $\theta$ values, result in greater asymmetries as shown in Figure 2.8. However, these more gradual shapes also increase the length for which the cell is in contact with the funnel walls, leading to increased risk of clogging or cell damage. Furthermore, parabolic tapers yield greater directional asymmetry at smaller cell compression levels, allowing ratcheting to be enabled at smaller pressures. To balance these considerations, we chose a parabolic funnel shape with $k=2000 \text{ m}^{-1}$.

### 2.3 Cell Sorting Funnel Array

The funnel microstructures are arranged in a 2D array of funnel constrictions consisting of 12 rows with 128 funnels in each row (Figure 2.9B). The funnel pore size is constant in each row, but decreases in increments of 1 µm from 15 to 4 µm in one design and from 12 to 2 µm in another. The funnel constrictions are aligned vertically to one another in order to maintain a linear streamline from one row to the next during the sorting process. Membrane microvalves\textsuperscript{46}, V1 to V6 in Figure 2.9C, are used to create precisely controlled flows through the funnel array in the horizontal direction for infusion and extraction, as well as in the vertical direction for separation.
The separation process, shown in Figure 2.9B, involves initially infusing the cell sample below the first row of funnels using a horizontal flow. The cell infusion process is enabled by opening valves V1 and V2 while sealing V3 through V6. The sample is then sorted using the microfluidic ratchet mechanism by applying an oscillatory flow in the vertical direction, which is generated using a fluidic H-bridge created by valves V3 through V6 to switch on/off the forward and reverse pressure. Specifically, during the forward flow phase, V3 and V4 are opened, while V5 and V6 are closed. During the reverse flow phase, the states of these valves are inverted. Finally, the separated cells are extracted using a horizontal flow from a buffer inlet with valves configured as the infusion process. If necessary, this process can be repeated to increase the throughput of the separation process.

Figure 2.9  Device design schematic. (A) Funnels and cell retention constrictions showing dimensions of features. (B) Schematic of the cell separation area within the device illustrating the inflow/outflow phase (1) and separation phase (2). (C) Overview of device layout showing inlets, outlets, and valves (V1 to V6).

2.4 Precision Flow Control

The flow profile in the funnel array is controlled using an elongated microchannel in series with the tree microchannel network. This microchannel provides a dominant hydrodynamic resistance that dictates the fluid flow resulting from the pressure applied between the oscillation pressure inlet and the oscillation outlet in Figure 2.9C. The geometry of this microchannel (50 µm wide X
25 µm tall X 32,800 µm long) is designed to generate a target flow rate on the order of 100 µm/s in the funnel array region based on an oscillatory pressure amplitude of 3.5 kPa according to the following formula:

\[ R_H = \frac{\Delta P}{Q} \]  

(2.31)

\( R_H \) is the hydrodynamic resistance, \( \Delta P \) the pressure drop, and \( Q \) the volumetric flow rate. For a rectangular channel in laminar flow, the hydrodynamic resistance can be calculated using:

\[ R_H \approx \frac{12 \mu L}{wh^3} \]  

(2.32)

where \( \mu \) is the viscosity of the fluid; \( L, w, \) and \( h \) are the length, width, and depth of the channel; and \( w > h \).
Chapter 3: Fabrication and Experimental Procedures

The microfluidic device is fabricated using standard techniques of photolithography and multilayer soft lithography. The inverse of the desired pattern is first formed on a silicon wafer and used as a master for replication in polydimethylsiloxane (PDMS). The PDMS device is then bonded to a glass slide and controlled using pressure sources. This chapter describes the exact details of this process as well as the experimental procedures for preparing and analyzing samples.

3.1 Fabrication of Silicon Masters

Patterns of the required microstructures are drawn using Solidworks DWG-Editor to generate commercially-produced optical photomasks. Inverse versions of the required microstructures are fabricated on a silicon wafer using two photolithographic steps. First, the silicon wafer is coated with a layer of SU-8 negative photoresist (Microchem) and spun at a speed of 1200 rpm for 50 s. This wafer is baked on a 95°C hotplate for 5 minutes and then exposed to UV light through an optical photomask (Advance Reproductions, Andover, MA). The wafer is then baked at 65, 95, then 65°C for 1,4, and 1 minutes respectively. The patterned wafer is developed using SU-8 developer (Microchem) and washed with isopropanol. To harden the patterned microstructures, the wafer is baked at 200°C for one hour. This final bake temperature is reached by a slow ramp at a rate of 10 degrees every 12 minutes. After baking, the wafer is allowed to equilibrate to room temperature on top of the hotplate. SPR220-7.0 photoresist (Microchem) is added to the cooled wafer by spin coating at 600 rpm for 50 s. The edge bead is removed from the wafer manually and then softbaked for 1, 5, and 1 minutes at 65, 95, and 65°C respectively. A second photomask (CAD/Art Services, Brandon, OR) is aligned to the previous set of patterns and subsequently exposed and developed in MF 319 developer (Microchem). Finally, the wafer is baked on a hotplate at 95°C for 5 minutes to allow the SPR220-7.0 photoresist layer to reflow and take on a parabolic profile. Great care is taken throughout the process to prevent exposing the wafer to thermal shocks which can cause the micro-scale SU-8 features to warp and bend. The fluid control layer is fabricated on a second silicon wafer using a single layer of SPR 220-7.0 using the same procedure as described above.
3.2 Fabrication of PDMS Devices

The PDMS masters are fabricated using standard multi-layer soft lithography techniques. The flow layer is formed using a 5:1 base to hardener ratio of RTV 615 silicone (Momentive Performance Materials), while the flow layer is formed using a 20:1 ratio. The flow and control layers are bonded together by diffusion. The bonded flow and control layer is subsequently attached to a glass slide following 45 seconds activation in air plasma (Harrick Plasma). Inlets and outlets are punched manually using a 0.5 mm punch (Technical Innovations).

3.3 Sample Preparation

Experiments were conducted using peripheral blood mononuclear cells (PBMCs), L1210 mouse lymphoma cells (MLCs), and microparticles. Peripheral blood mononuclear cells were prepared from whole blood obtained from healthy volunteers. Whole blood was drawn into 6 ml sodium heparin containing tubes. PBMCs were separated out using Histopaque 1077 (Sigma-Aldrich) according to the manufacturer’s instructions, and then re-suspended at a concentration of 1 x 10^7 cells/ml in AIM 5 media (GIBCO-Invitrogen). MLCs were cultured in suspension using RPMI 1640 (Gibco, Invitrogen) with 10% fetal bovine serum and 1% penicillin/streptomycin. These cells were incubated at 37 °C with 100% humidity and 5% CO2. Prior to experimentation, the cells were concentrated via centrifugation and re-suspended in a solution of phosphate buffered saline (PBS) containing 0.4% bovine serum albumin at a concentration of 1 x 10^7 cells/ml. Cells were used for experimentation between 3 to 4 days after passaging. In experiments where cells were stained, the L3224 LIVE/DEAD Viability/Cytotoxicity kit (Invitrogen) was used according to the manufacturer's directions. Microparticles of sizes 6.37 ± 0.48 µm and 10.14 ± 1.04 µm (Bangs Laboratories) were used for particle experiments. 50 µL of each were suspended in 1 mL deionized water and 0.5% of Tween 20 (Invitrogen).

3.4 Experimental Setup and Preparation

Precise control of fluid flow in the device was created using a commercial pressure controller (Fluiqent, Paris, France). Microvalves integrated in the PDMS microstructure were actuated using a custom-designed pressure controller described previously (24). Cell distributions were determined by visual inspection using an inverted microscope (Nikon Ti-U) and CCD camera (Nikon DS-2MBW).
Prior to each experiment, a solution of 1% BSA in PBS was used to fill the device prior to operation and incubated for 10 minutes to prevent nonspecific adsorption of cells onto the PDMS surface. The device was then flushed with PBS. To load cells into the separation area, a pressure of 2 kPa was applied to both the cell and buffer inlets simultaneously. Equal oscillation pressures were applied to both the upper and lower oscillation inlets and ranged from 7 to 51 kPa. All pressures remained constant at the inlets throughout the course of each experiment.
Chapter 4: Previous Generation Devices

The design presented in Chapter 3 is the result of an iterative process of design and testing. The original device, denoted as Generation 1, was designed by Dr. Hongshen Ma. I assisted in the fabrication of this device, and was primarily responsible for the design, fabrication, and testing of the Generation 2 and Generation 3 devices. The device presented in Chapter 3 is the Generation 3 device. Table 1 summarizes the main design differences between these devices, which are discussed in more detail for the remainder of this chapter accompanied by the reasons for adopting these changes.

<table>
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<tr>
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<th>Generation 1</th>
<th>Generation 2</th>
<th>Generation 3</th>
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<tbody>
<tr>
<td><strong>Oscillation</strong></td>
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<td>Narrowest oscillation</td>
<td>Narrowest oscillation</td>
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<td>Oscillation channels separated from sorting area by rectangular filter blocks</td>
<td>Oscillation channels separated from sorting area by semi-circular filter blocks</td>
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<td>Oscillation flow switching off-chip</td>
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<td>10° and 0° linear, and parabolic funnels</td>
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<td>• Short oscillation inlet channel provides dominant $R_H$</td>
<td>• Tall serpentine oscillation inlet channel provides dominant $R_H$</td>
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4.1 Generation 1 to Generation 2

4.1.1 Oscillation

Figure 4.1 Details of sorting area and oscillation channels in the Generation 1 device.

In the original design, the hydrodynamic resistance was provided by the 64 oscillation channels directly above and below the sorting area of the device as shown in Figure 4.1. These channels were 3 µm wide and 2250 µm long in order to provide sufficient hydrodynamic resistance to
achieve a desired flow rate of 100 µm/s at a pressure of 3.5 kPa as described in Section 2.4. The other function of the 3 µm channel width was to prevent cells from entering, ensuring that no cells were lost into the oscillation channels. However, this configuration had two main difficulties. The first difficulty was in the manufacturing stage. During photolithography, the cross-flow channels are essentially thin and long walls of SU-8 photoresist upon the silicon substrate. During processing, the mismatch between the thermal expansion rate of the SU-8 and the silicon substrate lead to buckling of the SU-8 causing it to become wavy (Figure 4.2A), often breaking and delaminating from the surface (Figure 4.2B). We also discovered that for the >10 µm channel heights we require for working with cancer cells, the thin channels were prone to falling over due to their high aspect ratio. To bypass these difficulties we incorporated the hydrodynamic resistance into the oscillation inlet in the Generation 2 device and narrowed the oscillation channels only at the entrance to the separation area.

Figure 4.2 Micrograph of fabrication defects in oscillation channels of Generation 1 device. (A) Buckling during processing due to different thermal expansion rates of SU-8 and silicon substrate. (B) Oscillation channels delaminated from surface.

The second difficulty with this configuration was that despite the small size of these channels, cells that drifted too close to the opening would be pulled inside the channel, usually
destroying the cell in the process. This effect occurs because a cell blocking the opening of one of these channels experiences the full magnitude of the pressure drop associated with that channel, which is a significantly larger force than the viscous forces we are using to move the cells. Thus, any cell that blocks the entirety of an opening of an oscillation channel would experience a large force and be pulled into the channel. To counteract this difficulty we separated out the cell-blocking functionality by adding an additional filter structure composed of small rectangular blocks to keep the cells from entering the oscillation channels. The gaps between these filter blocks (denoted as cell retention constrictions in Figure 2.9A) are 2 µm.

Lastly, oscillation switching in the Generation 1 device is accomplished by means of a fluidic H-bridge using on-chip valves exactly as described in Section 3 above for the Generation 3 device. We removed the oscillation switching control to off-chip in an effort to conserve space and reduce complexity of the design. Oscillation control in Generation 2 was accomplished by means of our custom-built pressure board.

### 4.1.2 Sorting Area

Each of the funnels in the Generation 1 device is linearly tapered with an angle of 15° from vertical, as shown in Figure 4.3A. After performing some preliminary modeling as presented in Section 0, we implemented three different funnel shapes: 10° linear, parabolic according to the equation $y = 2000x + W_0$, and straight (Figure 4.3B). Theoretical calculations predict that the parabolic design will have a greater asymmetry and therefore a higher efficiency for cell-sorting. However, the theoretical model is greatly simplified and does not take into account factors such as the potential for the cells to become wedged inside the funnel area or damaged. Therefore, both linear and parabolic were implemented in separate devices on the Generation 2 wafer to determine which is the optimal design for cell sorting applications. Straight constrictions were included to characterize the baseline efficiency of separation against which to measure the separation achieved in both the parabolic and 10° funnels.
More multi-level array devices with decreasing pore sizes were added on the Generation 2 wafer, specifically, two of each funnel shape. These were considered to be more important for demonstrating ratcheting behavior and sorting capabilities across a range of cell sizes, and more useful for different types of experiments.

The channels between subsequent funnel rows were decreased from 100 to 50 µm in width to shorten the time needed for a cell to travel between funnel rows and to conserve space on the wafer. However the inlet channel at the bottom of the sorting area was left at 100 µm to allow a larger number of cells to be brought in for sorting at any given concentration.

### 4.1.3 Channels and Materials

One significant drawback of the Generation 1 device was the inability to completely seal the valves and isolate the sorting area during oscillation. These devices were formed wholly from
SU-8 photoresist which has a rectangular cross-section. As shown in Figure 4.4A, when the valve is closed a small amount of void space remains which allows fluid to pass through. The hydrodynamic resistance of this void space is less than that of the oscillation channels, causing fluid to preferentially flow horizontally out of the sorting area when an oscillation pressure is applied. In the Generation 2 device, channels requiring valves were fabricated using SPR photoresist. After patterning and developing, this photoresist can be heated to cause it to become liquid again for a short time and take on a curved profile. After molding, these curved channels allow the valves to seal fully as shown in Figure 4.4B.

Another impediment to device operation in Generation 1 was the depth of the channel. We observed that with 10 µm deep devices, cancer cells had a high probability of becoming stuck anywhere in the microstructure due to constantly being compressed by the PDMS walls of the device. However, the rigorous fabrication criteria required to keep the oscillation channels intact excluded the possibility of making higher structures. In Generation 2, the thickness of the SU-8 layer was increased to 15 µm.

Finally, the Generation 1 device included a widening in each of the inlet and outlet channels supported by rectangular posts, to be used for counting cells as they traveled through. However it was found that these areas had a tendency to accumulate debris and cells, causing the entire device to become clogged and cease functioning. These cell counting areas were removed in the Generation 2 device.

Figure 4.4 Membrane microvalves in open (top) and closed (bottom) configurations. (A) Rectangular channel profile (leaky valve). (B) Curved channel profile (sealed valve).
4.2 Generation 2 to Generation 3

4.2.1 Oscillation

The narrowest portions of the oscillation channels in the Generation 2 devices still exhibited a tendency for fabrication defects when the photolithography process was not tightly controlled. Specifically, the channels would bend or break causing the device to become unusable. Since extreme narrowing of the channel mouths is not necessary after the addition of filter blocks, these channels were widened to 10 µm. Additionally, we observed that the filter blocks were not always successful in creating a barrier to the transport of cells. During the course of experiments, some cells still traveled below the lower filter blocks and became trapped there. To aid in the recovery of these cells, the filter blocks in the Generation 3 device were given a semi-circular profile to further utilize the ratchet mechanism such that cells which became trapped underneath would more easily deform back into the sorting area.

Oscillation switching in the Generation 2 device was controlled off-chip by our custom-built pressure controller, however the large amount of fluidic circuitry between the pressure controller and the sorting area caused considerable delays in the application of the oscillatory pressure to the cells. This resulted in unequal pressure application between the forward and reverse modes as visualized in Figure 4.5. We returned to on-chip oscillation control for the Generation 3 device in order to improve the precision of the forward and reverse pressures as well as to allow for higher frequency oscillation.

![Figure 4.5 Schematic of ideal, on-chip, and off-chip pressure waveforms.](image)
4.2.2 Sorting Area
During fabrication of the Generation 2 silicon masters we observed that linear tapers were unpredictable to fabricate due to the tendency of sharp corners to become rounded during the photolithography process. This process-dependent error widened the pore size in an unpredictable manner, reducing the repeatability of fabrication. Straight pores also exhibited fabrication difficulties as the film stress in the SU-8 layer created warping and often breaking of the thin pore connections. Parabolic structures were more robust than the straight pores and more precise than the linear tapers during fabrication. In testing, we also found that the cells were undamaged by deformation through every pore shape in both the forward and reverse directions over a range of oscillation pressures. Thus, the parabolic shape was implemented across all the devices in Generation 3 of the design due to its higher asymmetry and greater ease of fabrication.

In order to process a greater amount of cells, the size of the funnels was reduced to 50 µm in height with a 25 µm pitch (Figure 4.3C) which doubled the amount of funnels in each row and reduced the amount of space taken up by the device on the chip. The size of the funnel opening is still wide enough such that MLCs (~11µm diameter) will be undeformed when initially entering the funnel.

4.2.3 Channels and Dimensions
In the 15µm tall Generation 2 device we still observed the effects of friction in the compression of larger cells flowing through the channels. These cells had a much lower velocity than uncompressed free-flowing cells and had a higher probability of adhering to the channel wall and becoming stuck. In the Generation 3 device we increased the channel depth to 25 µm such that the cells would not be constrained by the depth even while deforming through a funnel constriction.

A consequence of increasing the channel depth is a reduction in the hydrodynamic resistance of the oscillation inlet channel. To compensate, we elongated these channels in a serpentine manner to increase the hydrodynamic resistance to its previous value.
Chapter 5: Results and Discussion

This chapter reports the results of experiments using the Generation 3 device to sort and separate cells. The first section discusses cell sorting and deformability effects. Section 5.2 discusses cell separation followed by an analysis of various experimental parameters in Section 5.3. We compare the experimental results with our model in Section 5.4. Cell viability during the sorting process is discussed in Section 5.5, and lastly an investigation of the ratcheting effect is presented in Section 5.6.

5.1 Cell Sorting

To evaluate our separation mechanism, L1210 mouse lymphoma cells (MLCs) and human peripheral blood mononuclear cells (PBMCs) were used as mechanical models of two cell types with distinct mechanical properties. From measurements taken on our microscope, MLCs have diameters ranging from 8 to 14.5 µm with a mean of 11.1 µm and a standard deviation, $\sigma_{\text{size}} = 1.2$ µm. PBMCs have diameters ranging from 6 to 8.5 µm with a mean of 7.2 µm and $\sigma_{\text{size}} = 0.6$ µm. Cell deformability, as defined by cortical tension, was measured to be $231\pm15$ pN/µm and $68.4\pm13.1$ pN/µm respectively for these cell types. Therefore, the deformability of L1210 MLCs is expected to be approximately 3.5 times that of PBMCs. For the purpose of device validation, PBMCs serve as a model of a smaller and more deformable cell, while MLCs serve as a model of a larger and more rigid cell.

Initially, each cell sample containing 75-150 cells was separately infused into a device and sorted using an oscillatory pressure of 7 kPa. The timing of the oscillatory flow consisted of 3 seconds of forward pressure followed by 1 second of reverse pressure. The forward biased oscillation flow enables all cells to travel to a funnel constriction sufficiently small for ratcheting to occur. After 60 seconds of oscillation, the cells segregated in each row were counted manually using an optical microscope.

Both MLCs and PBMCs were observed to form consistent and narrow distributions in the matrix of funnel constrictions. The location of these distributions can be characterized using the funnel sizes behind which a sub-population of the sorted cells were distributed. Specifically, the sorted MLC distribution had a mean funnel size of 9.7 µm and standard deviation, $\sigma_{\text{sorted}} = 1.3$ µm. This result is shown superimposed on the size distribution discretized in 1 µm increments (Figure 5.1A). Similarly, the sorted PBMC distribution had a mean funnel size of 5.2 µm with
\( \sigma_{\text{sorted}} = 1.2 \, \mu m \) (Figure 5.1B). See Table 2 for a summary of the means and standard deviations from various experiments. For MLCs, \( \sigma_{\text{sorted}} \approx \sigma_{\text{size}} \), whereas for PBMCs, \( \sigma_{\text{sorted}} \approx 2 \cdot \sigma_{\text{size}} \). The broadening of the sorted PBMC distribution likely results from the slight increase in force experienced by the PBMCs at higher funnel rows. The force applied to the cells is determined by the velocity of the flow through the pore, which increases as the pore size narrows as will be discussed below in Section 5.4.

![Figure 5.1](image_url)  

**Figure 5.1** Results of size measurements and sorting experiments. (A) Distribution of MLCs in the microfluidic funnel matrix after sorting and distribution of sizes measured external to the device. (B) Sorted distribution and size distribution of PBMCs.
5.1.1 Effect of Deformability

To further investigate size versus deformability effects in the sorting process, we compared the distribution of MLCs in the device with that of similarly sized polystyrene microparticles sorted under identical conditions. Microparticles (Bangs Laboratories, Fishers, IN) with diameters of 6.37±0.48 µm and 10.14±1.04 µm were mixed together to mimic the size range of MLCs. Since polystyrene microparticles are effectively incompressible relative to the PDMS structure, they can be considered to be sorted based on size alone. After sorting (oscillation pressure = 14 kPa), the diameters of both MLCs and microparticles were measured (measurement error = ±0.8µm) as a function of their trapping funnel size (Figure 5.2). In both cases, a strong correlation between cell/particle size and funnel size was observed. For similarly sized cells and particles, the mean trapping funnel size differed by approximately 2 µm, which suggests that the cells are compressed by this amount on average during the sorting process. These results validate the potential to use our mechanism to sort cells and particles based on size and/or deformability, therefore expanding the current repertoire of separation methods.

Table 2: Mean and standard deviation of various cell distributions within the device

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cell type</th>
<th>Oscillation pressure (kPa)</th>
<th>Mean (µm)</th>
<th>Standard deviation (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML alone</td>
<td>ML</td>
<td>7</td>
<td>9.748</td>
<td>1.34</td>
</tr>
<tr>
<td>PBMC alone</td>
<td>PBMC</td>
<td>7</td>
<td>5.18</td>
<td>1.23</td>
</tr>
<tr>
<td>Mixed</td>
<td>ML</td>
<td>7</td>
<td>9.45</td>
<td>1.05</td>
</tr>
<tr>
<td>Mixed</td>
<td>PBMC</td>
<td>7</td>
<td>5.51</td>
<td>1.08</td>
</tr>
<tr>
<td>Repeatability - Trial 1</td>
<td>ML</td>
<td>14</td>
<td>8.31</td>
<td>1.19</td>
</tr>
<tr>
<td>Repeatability - Trial 2</td>
<td>ML</td>
<td>14</td>
<td>8.84</td>
<td>1.15</td>
</tr>
<tr>
<td>Repeatability - Trial 3</td>
<td>ML</td>
<td>14</td>
<td>8.63</td>
<td>1.09</td>
</tr>
<tr>
<td>Repeatability - Trial 4</td>
<td>ML</td>
<td>14</td>
<td>8.58</td>
<td>1.22</td>
</tr>
<tr>
<td>Repeatability - Trial 5</td>
<td>ML</td>
<td>14</td>
<td>8.81</td>
<td>1.22</td>
</tr>
<tr>
<td>Pressure 1</td>
<td>ML</td>
<td>7</td>
<td>9.84</td>
<td>1.29</td>
</tr>
<tr>
<td>Pressure 2</td>
<td>ML</td>
<td>14</td>
<td>8.8</td>
<td>1.31</td>
</tr>
<tr>
<td>Experiment</td>
<td>Cell type</td>
<td>Oscillation pressure (kPa)</td>
<td>Mean (µm)</td>
<td>Standard deviation (µm)</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>---------------------------</td>
<td>-----------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Pressure 3</td>
<td>ML</td>
<td>28</td>
<td>8.64</td>
<td>1.33</td>
</tr>
<tr>
<td>Pressure 4</td>
<td>ML</td>
<td>41</td>
<td>7.45</td>
<td>1.41</td>
</tr>
<tr>
<td>Pressure 5</td>
<td>ML</td>
<td>55</td>
<td>7.15</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Figure 5.2  Cell diameter versus trapping funnel size of sorted microparticles and MLCs

5.2  Cell Separation

To evaluate the ability of our device to separate cells from a mixture, we combined PBMCs with fluorescently-stained MLCs and sorted the sample under identical experimental conditions as
described above. Example bright-field and fluorescence images are shown in Figure 5.3 and the resulting distribution is shown in Figure 5.4A. The distributions of MLCs and PBMCs overlapped in the 7 and 8 µm rows. Therefore, using the 8 µm pore as a cutoff yields a separation efficiency of 98% with a purity of 99% for the MLCs and correspondingly a 97% efficiency with 95% purity for the PBMCs in this sample (Figure 5.4B).

![Figure 5.3](image.png)

**Figure 5.3**  (A) Bright-field micrograph of a mixture of MLCs and PBMCs after separation. PBMCs labeled using red circles in photo. (B) Corresponding fluorescent micrograph showing the distribution of stained MLCs.

The selectivity of this separation depends both on the size and deformability of the input cells. Equation 1.5 in Section 1.1 defines the selectivity for size alone. To consider both size and deformability, we must use the mean and standard deviation of the steady state positions of the cells within the device. This yields:

\[
S_{\text{sorted}} = \frac{\mu_{\text{sorted (MLC)}} - \mu_{\text{sorted (PBMC)}}}{\sigma_{\text{sorted (MLC)}} + \sigma_{\text{sorted (PBMC)}}}
\]  

(5.1)

For the separation of MLCs from PBMCs, we achieve a selectivity of 1.8 using Equation 5.1. Based on the size distributions of these cells, the selectivity, according to Equation 1.5, would be 2.2. Therefore, sorting within our device does not significantly degrade the natural separation occurring between these two types of cells.
5.3 Device Parameters

The mean and width of the cell distribution after sorting are primarily governed by three experimental parameters: the amplitude of the pressure oscillation, the timing of each oscillation cycle, and the total oscillation time. For the majority of our experiments, the standard operating conditions involved an oscillation pressure of 14 kPa, where each oscillation cycle consists of 3 s of forward pressure followed by 1 s of reverse pressure, applied for a total of 60 seconds.

5.3.1 Repeatability

The repeatability of this mechanism was evaluated over several sorting trials using MLCs under standard operating conditions. As shown in Figure 5.5, the resulting distributions were
consistent and repeatable. Specifically, the means and standard deviations of the distributions are within 6% and 12% of one another (see Table 2).

![Graph showing repeatability of results](image)

**Figure 5.5**  Repeatability of results. Multiple trials show consistent and repeatable distributions of MLCs within the device at an oscillation pressure of 14 kPa.

### 5.3.2 Oscillation Pressure Magnitude

To study the effect of oscillation pressure, MLCs were sorted using pressures of 7, 14, 28, 41, and 55 kPa while all other parameters were kept constant. Table 3 shows the correspondence between the applied oscillation pressure and the fluid velocity in the wide channels of the sorting area as well as in an 8 µm funnel constriction (calculations are shown in detail in Section 5.6). In the wide channel, the cells are unconstrained by the microstructure and will travel at approximately the same velocity as the fluid. In the funnel constrictions, the cells will deform into a constriction according to the pressure generated by the fluid flowing around the cell.
Table 3: Pressure, flow rate, and fluid velocity during oscillation

<table>
<thead>
<tr>
<th>Oscillation pressure (kPa)</th>
<th>Bulk flow rate ( (m^3/s) )</th>
<th>Fluid velocity in channels ( (m/s) )</th>
<th>Fluid velocity in 8 ( \mu m ) funnel ( (m/s) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.39E-11</td>
<td>1.74E-04</td>
<td>5.43E-04</td>
</tr>
<tr>
<td>14</td>
<td>2.78E-11</td>
<td>3.48E-04</td>
<td>1.09E-03</td>
</tr>
<tr>
<td>28</td>
<td>5.56E-11</td>
<td>6.95E-04</td>
<td>2.17E-03</td>
</tr>
<tr>
<td>41</td>
<td>8.14E-11</td>
<td>1.02E-03</td>
<td>3.18E-03</td>
</tr>
<tr>
<td>55</td>
<td>1.09E-10</td>
<td>1.36E-03</td>
<td>4.26E-03</td>
</tr>
</tbody>
</table>

Expectedly, our results show an inverse relationship between the oscillation pressure and the mean of the resulting distribution (Figure 5.6). In addition, \( \sigma_{\text{sorted}} \) increased at higher oscillation pressures (Table 2 in Section 5.1). This is believed to be due to the effect mentioned in Section 5.1 where cells in smaller pores are subject to slightly greater forces.
5.3.3 Oscillation Pressure Timing

To evaluate the effect of oscillatory pressure timing, we sorted MLCs using forward pressure durations of 2, 3, 6, and 8 seconds, while keeping the reverse pressure duration at 1 second. The results, displayed in Figure 5.7, show a thresholding effect where a minimum of 3 seconds is necessary for the sample cells to assume a characteristic distribution, while further increases in the period of the forward pressure do not lead to an altered steady-state distribution.
5.3.4 Number of Oscillation Cycles

To determine the total number of cycles necessary to reach a steady-state distribution, we tracked the time-evolution of MLC and PBMC distributions after 0, 2, 4, 6, and 20 oscillations with oscillation pressure of 7 kPa (Figure 5.8). A steady-state distribution is reached after approximately 6 oscillations for MLCs. For PBMCs, more oscillation cycles were necessary to reach a steady state distribution because of the greater distance that needed to be traversed by these cells in the device.
Figure 5.8  Evolution of MLC distribution after 0, 2, 4, 6, and 20 oscillations.

5.4 Comparing Model with Experiment

In order to validate the model used in section 0, we must determine the pressure experienced by a cell as it enters a constriction in the device. First, the flow rate inside the device from an applied oscillation pressure can be calculated using a rearrangement of Equation 3.23 from Section 2.4 as follows:

\[ Q_{\text{total}} = \frac{\Delta P}{R_H} \]  \hspace{1cm} (5.2)

The value of \( R_H \) is calculated as shown in Equation 3.24 using the channel parameters given in Section 2.4. The total flow is used to solve for the average flow velocity through each funnel pore using the channel height, \( h \), and full pore width \( 2W_0 \), for a funnel row consisting of 128 identical funnels.

\[ v_{\text{funnel}} = \frac{Q_{\text{total}}}{128h(2W_0)} \]  \hspace{1cm} (5.3)

The force experienced by a cell inside such a constriction can be estimated using the Stokes drag force \( F_d \):

\[ F_d = 6\pi \cdot \mu \cdot R \cdot v_{\text{funnel}} \]  \hspace{1cm} (5.4)

where \( \mu \) is the viscosity of the fluid and \( R \) is the radius of a sphere in Stokes flow. This calculation is approximate because the flow can only bypass the cell on two sides when it is being deformed inside a constriction, the velocity of the flow increases as the constriction narrows, and also because the cell is not a perfect sphere in this situation. The radius of the
undeformed cell $R_0$ is used in this approximation. This force is converted into a pressure by dividing by the average cross-sectional area of the cell as follows:

$$P_{cell} = \frac{F_d}{\pi \frac{1}{2}(R_u + R_w)R_c}$$

This pressure increases slightly as cells travel up through the device due to the increase in fluid velocity as the pore size decreases.

Table 4 below shows the calculated pressure applied to a MLC with 11 $\mu$m diameter in various pores using a number of applied pressures, along with the forward and reverse threshold pressures calculated by our deformation model in Section 2.2. From these values, we can predict where a 11 $\mu$m diameter cell should end up within the device following sorting. At the 10 $\mu$m pore, the cell experiences a pressure of 0.495 Pa, which is greater than the threshold pressure of 0.38 Pa that our model predicts is necessary for this cell to transit through this pore, therefore the cell should pass through. At the next row where each pore is 9 $\mu$m in size, the cell experiences a force of 0.576 Pa which is less than $\Delta P_{fwd} = 0.89$ Pa. Therefore we expect this cell to be prevented from transiting through this pore and will end in this row at steady state. Indeed, this is exactly what we find in Figure 5.1A. The peak of the distribution represented by this average 11 $\mu$m cell passes through the 10 $\mu$m pore size row and transits up to the 9 $\mu$m row.

These calculations are approximately consistent with the experimental results shown in Figure 5.6. At higher pressures, however, the cells are retained by funnel sizes that are larger than predicted by the model. For instance, the model predicts that the peak will shift to the 4 $\mu$m pore row at an applied oscillation pressure of 55 kPa, but the peak is found in the 6 $\mu$m pore row instead. There are two potential factors that could account for this discrepancy. First, the model does not take into account the effects of friction. At greater deformations, the cell membrane is in contact with the funnel microstructure with greater force and greater area, which leads to greater friction forces opposing the passage of the cell. Secondly, the liquid drop model of cell deformation may not be accurate at high compressions when the pore size becomes more similar to the size of the nucleus.
Table 4: Predicted forward and reverse threshold pressures as well as calculated pressure applied to a 11 µm diameter MLC with $T_c = 250$ pN/µm. Green indicates the situations where the cell is predicted to pass through the pore, whereas red indicates situations where the cell is predicted to be retained.

<table>
<thead>
<tr>
<th>Pore size (µm)</th>
<th>∆P&lt;sub&gt;fwd&lt;/sub&gt; (Pa)</th>
<th>∆P&lt;sub&gt;rev&lt;/sub&gt; (Pa)</th>
<th>7 kPa applied</th>
<th>14 kPa applied</th>
<th>28 kPa applied</th>
<th>41 kPa applied</th>
<th>55 kPa applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.38</td>
<td>0.82</td>
<td>0.495</td>
<td>0.990</td>
<td>1.980</td>
<td>2.899</td>
<td>3.889</td>
</tr>
<tr>
<td>9</td>
<td>0.89</td>
<td>3.45</td>
<td>0.576</td>
<td>1.153</td>
<td>2.305</td>
<td>3.376</td>
<td>4.528</td>
</tr>
<tr>
<td>8</td>
<td>1.81</td>
<td>8.19</td>
<td>0.687</td>
<td>1.374</td>
<td>2.749</td>
<td>4.025</td>
<td>5.399</td>
</tr>
<tr>
<td>7</td>
<td>3.35</td>
<td>15.39</td>
<td>0.836</td>
<td>1.673</td>
<td>3.345</td>
<td>4.899</td>
<td>6.571</td>
</tr>
<tr>
<td>6</td>
<td>5.92</td>
<td>25.74</td>
<td>1.055</td>
<td>2.110</td>
<td>4.220</td>
<td>6.179</td>
<td>8.288</td>
</tr>
<tr>
<td>5</td>
<td>10.41</td>
<td>40.67</td>
<td>1.369</td>
<td>2.738</td>
<td>5.476</td>
<td>8.018</td>
<td>10.756</td>
</tr>
<tr>
<td>4</td>
<td>18.97</td>
<td>63.36</td>
<td>1.875</td>
<td>3.750</td>
<td>7.501</td>
<td>10.984</td>
<td>14.734</td>
</tr>
</tbody>
</table>

5.5 Cell Viability

To investigate changes in cell viability as a result of the sorting process, we stained a sample of MLCs using a live/dead assay. The cells were incubated in a solution containing the stain for 10 minutes prior to experimentation. When a sample entered the sorting area, the cells were counted under fluorescence to enumerate the live and dead cells. The oscillation media contained additional dye, and the sample was oscillated for 5 minutes. After oscillation the device was allowed to sit for 10 minutes to allow the dye to penetrate the membranes of any cells which may have been damaged by the sorting process. Finally, the live and dead cells were again enumerated under fluorescence. When the total cell count decreased after sorting, the difference was attributed to rupturing of the cell membrane causing a cell to be destroyed. We observed that membrane rupture only occurred to cells which were already dead prior to sorting, and occurred in less than 1% of the cells in the sample. Since the possibility of compromising cell viability increases when higher pressures are applied to the cells, we evaluated cell viability over several oscillation pressures between 7 and 41 kPa. More than 95% of the sample cells were viable before and after sorting in each case.
5.6 Ratcheting versus Filtration

A fundamental question associated with our cell separation mechanism is whether separation is achieved by irreversible ratcheting or by reversible filtration. To characterize the ability of our mechanism to irreversibly separate cells from the bulk liquid we evaluated the fraction of irreversibly separated cells in the following way: A sample of MLCs was first sorted using 60 s of oscillatory pressure using standard experimental parameters. Immediately following, the oscillation pressure was applied in reverse (3 seconds backward and 1 second forward) for another 60 s as shown in Figure 5.9.

![Figure 5.9](image)

The cells that were irreversibly separated remained trapped within their steady state positions whereas the cells that were only reversibly separated all returned to their starting positions. Using an oscillation pressure of 10 kPa, over 50% of the cells were irreversibly separated. This fraction increases monotonically with increasing pressure until 100% of the cells were irreversibly separated using 41 kPa (Figure 5.10). We previously showed that ratchet transport through microscale funnel constrictions can be enabled when the applied pressure exceeds a certain threshold, dependent on cell radius and pore size. At small compression values, the asymmetry is low, giving rise to a gap between the reverse threshold pressure for the previous funnel and the forward threshold pressure of the next row that a cell encounters during oscillation. For example, in Section 5.4, Table 4 shows that for an 11 µm diameter cell, the reverse threshold pressure for a 10 µm pore is 0.82 Pa, while the forward threshold pressure for the 9 µm pore is 0.89 Pa. For applied pressures in this range, the cell has insufficient pressure applied to transit through the 9 µm funnel row, but enough pressure to return back through the 10 µm row and therefore will not ratchet. Since the cell sample presents a range of cell sizes and
deformabilities, increasing the applied pressure advances each to a higher compression level, thereby increasing the asymmetry and ensuring that most of the cells will ratchet and become irreversibly separated. While both reversible and irreversible separations are achievable using this device, operating in the irreversible regime increases the robustness of the separation for integration within automated cell processing systems.

![Graph showing the percentage of cells separated by ratcheting as a function of oscillation pressure.](image)

**Figure 5.10** Percentage of cells separated by ratcheting as a function of oscillation pressure.
Chapter 6: Conclusion

6.1 Summary of Results

We have developed a cell sorting and separation device using an iterative process of design, modeling, fabrication, and testing. The resultant device irreversibly sorts cells by both size and deformability using microfluidic funnel constrictions arranged in a 2-dimensional array. These constrictions form structural ratchets which prevent cells from returning back through the constriction under the force of an oscillatory pressure. We confirmed the ability of this device to sort cells by size using cells of a single phenotype, and also the ability to sort by deformability by comparing cells with rigid microparticles in a similar size range. Using MLCs and PBMCs as model cells for a mixed separation experiment yields a separation efficiency of 98%. The distributions of cells within the device were found to be consistent and repeatable from trial to trial. We observed that higher oscillation pressures caused cells to travel farther into the device and increased the contribution of deformability to the sorting results. We also observed a threshold time needed for cells to deform through a funnel and reach a characteristic distribution. This distribution does not change after the system reaches steady state even if oscillation continues. The cells were remained viable and were not harmed by the process of deforming through the constrictions.

6.2 Limitations

Operation of this device can be limited by clogging in several different forms. First, if the concentration of cells is too high (>10^7 cells/ml) the cells will begin to clog the inlet and reduce flow into the sorting area. Additionally, at such concentrations a large fraction of funnels become blocked by 2 or more cells during oscillation, preventing smaller cells from passing through to higher funnel rows and reducing the selectivity of the filter. Concentrations of this magnitude can also be created locally due to sedimentation effects. All inlets in the device are accessed using small needles connected to vertical holes through the top PDMS layer as shown in Figure 6.1. When the flow is stopped for extended periods of time, such as during manual counting, sedimentation due to gravity causes the local concentration of cells at the device inlet to increase.
Another form of clogging occurs when the cells stick to one another and form aggregates. Aggregates are far more likely to form at high input concentrations due to the increased interaction of cells with one another. When an aggregate forms it is treated by the device as one very large cell and usually does not pass through even the largest funnel row. Aggregates are undesirable because they cause smaller cells to be sorted into larger pore size rows and reduce the selectivity and efficiency of the filter.

6.3 Comparison with Filtration Based Cell Separation

The oscillatory flow and irreversible cell transport created in our mechanism improves the selectivity and scalability of traditional microfluidic filtration methods. Selectivity is enhanced by the oscillatory flow which prevents clogging, and thereby avoids altering the hydrodynamic resistance of the separation microstructure. Scalability of the separation process is enhanced because the irreversible transport of cells aids the extraction of separated cells, thereby enabling the microstructure to be refreshed for repeated separations. Compared to current microfluidic filtration methods, the separation efficiency of our device is 98%, whereas other cell filtration mechanisms typically report efficiencies of 80-90% for cells in similar size ranges. Furthermore, those studies used cultured cells from human cancer cell lines as target cells to separate from blood, which are larger than the MLCs used here.
Figure 6.2 shows the average cell sizes we measured for a human cancer cell line (UC-13) cultured in the lab (17 µm diameter) as compared to MLCs (11 µm diameter) and PBMCs (7 µm diameter). We demonstrated a higher efficiency using two populations of cells that are more similar in size, indicating greater selectivity. An additional advantage of our device is its ability to sort cells into multiple spatially separated regions much like hydrodynamic sorting, whereas previous filtration mechanisms have largely been binary sorters \textsuperscript{14, 16, 18, 19, 22-24}. In our device, multiple cell populations can be sorted simultaneously and the outlets from each sorting region can be arbitrarily grouped together to optimize for efficiency or purity, enabling this mechanism to be adapted for different cell types.

![Figure 6.2 Visual representation of average cell sizes](image)

Currently, we have demonstrated a throughput of approximately 9000 cells/hour using a device with a sorting area of 3,200 µm X 1,340 µm. Although the throughput is low compared to other filtration devices, it scales linearly with the size of the sorting area, with the ultimate limit being the total patternable area on a silicon wafer.

### 6.4 Future Work

The work represented in this thesis has been a proof-of-concept device to demonstrate the ability to sort cells by size and deformability in an array of tapered funnel constrictions. The future work will be focused on optimization and developing the mechanism for specific applications. Research into process and device optimization has already been initiated by Bill Lin and Emily Park. The goals for future work on this mechanism are as follows:
Process optimization

- Automate the entire operation of the device: repetitions of sample loading, oscillation, and clearing of the separation area until the desired amount of sample has been processed.
- Automate the cell counting process using a camera and image processing software. Enable this counting to be done in real-time as an experiment is running.
- Demonstrate successful collection of samples from the outlets following sorting. Develop a standard method of collecting cells from the outlets.

Device optimization

- Increase the throughput of the device by scaling up the sorting area and parallelizing multiple sorting areas to process clinically relevant sample volumes
- Tailor the device parameters (pore sizes, channel height, etc.) for specific applications

Application - CTCs

- Demonstrate separation of very low numbers of cultured human cancer cells spiked into larger volume of WBCs and verify that the efficiency and purity of the separation is preserved.
- Conduct tests using the peripheral blood of cancer patients. Compare the number of CTCs found in this device with the number detected by the Veridex Cellsearch® device.
References


Appendices

Appendix A

Matlab codes to calculate asymmetry of a cell deforming through linear and parabolic funnel constrictions

A.1 Comparison of Funnel Shapes

% Here we model the pressure required to deform cells across parabolic and linear funnels with a rectangular cross-section, with a height greater than the cell radius at the point of maximum deformation. The funnels are 50 um long and the funnel opening ranges from 3.5 to 25 um (plus W0).

% First the parameters:
clear;
icc = 2e-8;        %increment
L1 = 0:inc:30e-6;
L2 = zeros(1, length(L1));
Rb = zeros(1, length(L1));
dPforward = zeros(1, length(L1));
dPmaxforward = 0;

W0 = 3.5e-6;        %Half-width of minimum pore size
R0 = 5e-6;          % The radius of the cell in its relaxed state
tension = 415e-6;   % Cortical tension ~415e-6 N/m

x = 0:inc:50e-6;

k = 1400:50:10000;  % Coefficient on the parabolic funnel shape
theta = 4:0.1:26.6; % Angle of linear funnel

asymmetryk = zeros(1, length(k));
asymmetrym = zeros(1, length(theta));

%% Linear

for q=1:length(theta)
    m = tan (theta(q)*pi/180);
    V0 = 4*pi*(R0^3)/3;  %Volume of cell in relaxed state
    W = m*x + W0;        % Array of funnel half-widths
    Rb = W0;
    Rc = sqrt(3*V0/(4*pi*W0)); %Approximate vertical radius of cell
    Ra = W0;
    V1 = (2*pi/3)*Rc*(W0)^2; %Start by assuming no compression and increase
    V2 = 0;
    V3 = (2*pi/3)*Rc*(Rb)^2;
    vol = V0 - (V1 + V2 + V3);
    j = 1;                %Initialize counter variable
while (vol > 0) && (j < length(x))
    h = Rb - (Rb^2 - W(j)^2)^{(1/2)};
    V3 = (4*pi/3)*Rc*(Rb)^2 - pi*Rc*h^2*(3*Rb - h)/(3*Rb);
    wt = Rb*cos(theta(q)*pi/180);
    width=W(j);

    while (wt > width) && (vol > 0)
        j = j+1;
        V2 = V2 + inc*pi*Rc*W(j);
        vol = V0 - (V1 + V2 + V3);
        width = W(j);
    end
    Rb = Rb + 1e-9;
    vol = V0 - (V1 + V2 + V3);
end
L2 = x(j);
dPforward(q) = tension*(1/Ra - 1/Rb);

%% Deforming cells in the reverse direction
Ra2 = W0;
Rc = sqrt(3*V0/(4*pi*W0));

%Va = 2*pi*Ra2*Ra2*Rc/3; %Half-ellipsoid volume inside pore
%Rb2 = (R0^3 - Rc*W0^2/2)^{(1/3)}; %Uses circle assumption
Vtot = 0;
Rb2 = W0;

while Vtot<V0
    Va = 2*pi*Ra2*Ra2*Rc/3;
    h = Rb2 - (Rb2^2 - W0^2)^{(1/2)}; %Height of elliptical cap
    Vb = 4*pi*Rb2*Rb2*Rc/3; %Sphere with radius Rb
    Vint = pi*Rc*h^2*(3*Rb2 - h)/(3*Rb2); %Intersection of Va and Vb
    Vtot = Va + Vb - Vint; %Calculate combined volume
    Rb2 = Rb2 + 1e-9; %Increment Rb2 by 0.001um
end
dPreverse = tension*(1/Ra2 - 1/Rb2);

asymmetry(q) = dPreverse / dPforward(q);

%% Parabolic
for q=1:length(k)
    V0 = 4*pi*(R0^3)/3; %Volume of cell in relaxed state
    W = k(q)*x.^2+W0; % Array of funnel half-widths
    for i=1:length(L1)
        j = i;
if $W(i)<R_0$
   $W_{\text{mean}} = W(i); \quad \%\text{Mean pore width}$
   $R_c = \sqrt{3*V_0/(4*\pi*W_{\text{mean}})}; \quad \%\text{Approximate vertical radius of cell}$
   $\text{vol} = V_0 - \left( (2*\pi/3)*R_c*(W(i))^2 \right. + (\text{inc})*\pi*R_c*\text{sum}(W(i:j)) \left. \right) \ldots$
   $+ \left(2*\pi/3)*R_c*(W(j))^2; \right)$
while ((vol > 0)&&(j<length(L1)))
   $j = j + 1;$
   $W_{\text{mean}} = \text{sum}(W(i:j))/(j-i);$  
   $R_c = \sqrt{3*V_0/(4*\pi*W_{\text{mean}})};$  
   $\text{vol} = V_0 - \left( (2*\pi/3)*R_c*(W(i))^2 \right. + (\text{inc})*\pi*R_c*\text{sum}(W(i:j)) \left. \right) \ldots$
   $+ \left(2*\pi/3)*R_c*(W(j))^2; \right)$
end
$L_2(i) = x(j);$  
$R_a(i) = W(i);$  
$R_b(i) = W(j);$  
$dP_{\text{forward}}(i) = \text{tension}*(1/R_a(i) - 1/R_b(i));$
else
   $L_2(i) = x(j);$  
   $dP_{\text{forward}}(i) = 0;$
end
end
$dP_{\text{maxforward}} = \max(dP_{\text{forward}});$

%% Deforming cells in the reverse direction
$R_{a2} = W_0;$
$R_c = \sqrt{3*V_0/(4*\pi*W_0)};$

%$Va = 2*\pi*R_a2*R_a2*R_c/3;$  \quad \%\text{Half-ellipsoid volume inside pore}$
$R_{b2} = (R_0^3 - R_c*W_0^2/2)^{(1/3)};$  \quad \%\text{Uses circle assumption}$
$V_{\text{tot}} = 0;$
$R_{b2} = W_0;$

while $V_{\text{tot}}<V_0$
   $Va = 2*\pi*R_a2*R_a2*R_c/3;$
   $h = R_{b2} - (R_{b2}^2 - W_0^2)^{(1/2)}; \quad \%\text{Height of elliptical cap}$
   $V_b = 4*\pi*R_b2*R_b2*R_c/3; \quad \%\text{Sphere with radius } R_b$
   $V_{\text{int}} = \pi*R_c*h^2*(3*R_b2 - h)/(3*R_b2); \quad \%\text{Intersection of } Va \text{ and } V_b$
   $V_{\text{tot}} = Va + V_b - V_{\text{int}}; \quad \%\text{Calculate combined volume}$
   $R_{b2} = R_{b2} + 1e-9; \quad \%\text{Increment } R_{b2} \text{ by } 0.001\mu m$
end

$dP_{\text{reverse}} = \text{tension}.*(1./R_{a2} - 1./R_{b2});$

asymmetryk(q) = $dP_{\text{reverse}} / dP_{\text{maxforward}};$
end

%% Plotting
figure(1);
plot(L2, dPforward, 'o-r');
title('Forward Pressure Required for Transport(parabolic)');
xlabel('Position');
A.2 Calculation of Transit Pressures

% Here we model the pressure required to deform cells across a parabolic funnel with a rectangular cross-section, with a height greater than the cell radius at the point of maximum deformation.
% First the parameters:
clc;
clear;
L1 = 0:1e-7:50e-6;
L2 = zeros(1, length(L1));
Ra = zeros(1, length(L1));
Rb = zeros(1, length(L1));
dPforward = zeros(1, length(L1));
dPmaxforward = 0;

WO = 5e-6; %Half-width of minimum pore size
R0 = 5.5e-6; % The radius of the cell in its relaxed state
tension = 250e-6; % Cortical tension ~250e-6 N/m

x = 0:1e-7:100e-6;
k = 2000; % Coefficient on the parabolic funnel shape

V0 = 4*pi*(R0^3)/3; %Volume of cell in relaxed state
W = k*x.^2+W0; % Array of funnel half-widths
for i=1:length(L1)
j = i;

if W(i)<R0
    Wmean = W(i); %Mean pore width
    Rc = sqrt(3*V0/(4*pi*Wmean)); %Approximate vertical radius of cell

volatile V = V_0 - ((2\pi/3)Rc*(W(i))^2 + (1e-7\pi*Rc*sum(W[i:j]))) ...
+ (2\pi/3)Rc*(W(j))^2);
while ((vol > 0) && (j<length(L1)))
    j = j + 1;
    Wmean = sum(W(i:j))/(j-i); 
    Rc = sqrt(3*V_0/(4*\pi*Wmean));
    vol = V_0 - ((2\pi/3)Rc*(W(i))^2 + (1e-7\pi*Rc*sum(W[i:j]))
... 
+ (2\pi/3)Rc*(W(j))^2);
end
L2(i) = x(j);
Ra(i) = W(i);
Rb(i) = W(j);
dPForward(i) = tension*(1/Ra(i) - 1/Rb(i));
else
    L2(i) = x(j);
    dPForward(i) = 0;
end
end
dPmaxForward = max(dPForward)

%% Deforming cells in the reverse direction
Ra2 = W_0;
Rc = sqrt(3*V_0/(4*\pi*W_0))

%Va = 2\pi*Ra2*Ra2*Rc/3; %Half-ellipsoid volume inside pore
%Rb2 = (R_0^3 - Rc*W_0^2/2)^{(1/3)}; %Uses circle assumption
Vtot = 0;
Rb2 = W_0;
while Vtot<V_0
    Va = 2\pi*Ra2*Ra2*Rc/3; 
    h = Rb2 - (Rb2^2 - W_0^2)^{(1/2)}; %Height of elliptical cap
    Vb = 4\pi*Rb2*Rb2*Rc/3; %Sphere with radius Rb
    Vint = pi*Rc*h^2*(3*Rb2 - h)/(3*Rb2); %Intersection of Va and Vb
    Vtot = Va + Vb - Vint; %Calculate combined volume
    Rb2 = Rb2 + 1e-9; %Increment Rb2 by 0.001um
end
dPReverse = tension.*(1./Ra2 - 1./Rb2)

%% Plotting
asymmetry = dPReverse / dPmaxForward

k = 0.002;
figure(1);
plot(L2, dPForward, 'o-r');
title('Forward Pressure Required for Transport(parabolic)');
xlabel('Position');
ylabel('Forward Pressure (Pa)');

figure(2);
plot(k, asymmetry, 'o-b');
xlabel('funnel shape k');
ylabel('asymmetry');