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Electrokinetic Manipulation of Particles and Cells in Microfluidic Devices

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ELECTROKINETIC MANIPULATION OF PARTICLES AND CELLS IN MICROFLUIDIC DEVICES

A Thesis
Presented to
The Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Mechanical Engineering

by
Christopher S. Church
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Accepted by:
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ABSTRACT

With the recent advancement in micro-fabrication technology, lab-on-a-chip devices have been developed in order to perform biological analysis through cell manipulation. Microchannels used in these lab-on-a-chip devices have been demonstrated to accurately perform many different cell manipulation techniques such as focusing, separation, trapping, and lysis. Although there are many methods available for these techniques, electrokinetics has been rapidly gaining popularity due to the simplicity of application and removal of the need for in channel micro-structures. This thesis studies the use of electrokinetic flow and accompanying phenomena in various structured microchannels to perform focusing, separation, trapping, and lysis of cells. Three related projects were conducted in series.

First, a parametric study of the focusing of yeast cells using negative dielectrophoresis in a serpentine microchannel was studied. Focusing cells into a single stream is usually a necessary step prior to counting and separating them in microfluidic devices such as flow cytometers and cell sorters. This work demonstrated a sheathless electrokinetic focusing of yeast cells in a planar serpentine microchannel using DC-biased AC electric fields. The concurrent pumping and focusing of yeast cells arose from the DC electrokinetic transport and the turn-induced AC/DC dielectrophoretic motion, respectively. The effects of electric field (including AC to DC field ratio, and AC field frequency) and concentration (including buffer concentration and cell concentration) on the cell focusing performance were studied experimentally and numerically. A
continuous electrokinetic filtration of *E. coli* cells from yeast cells was also demonstrated via their differential electrokinetic focusing in the serpentine microchannel.

Next, negative and positive dielectrophoretic focusing were also studied in their application to particle separation in a serpentine microchannel. This work first demonstrated negative and positive dielectrophoretic focusing of by changing only the electric conductivity of the suspending fluid. Due to the channel turn-induced dielectrophoretic force, particles were focused to either the centerline or the sidewalls of the channel when their electric conductivity was lower (i.e., negative DEP) or higher (i.e., positive DEP) than that of the fluid. These distinctive dielectrophoretic focusing phenomena in the serpentine microchannel were then combined to implement a continuous separation between particles of different sizes and electric conductivities. Such separation eliminates the fabrication of in-channel microelectrodes or micro-insulators that are typically required in DEP-based separation techniques.

Lastly, red blood cells were used to study cell lysis and trapping in a microchannel constriction. Cell Lysis is an important step in the analysis of intracellular contents. Electrical lysis of red blood cells was demonstrated in a hurdle microchannel using a low continuous DC-biased AC electric field amplified by channel geometry. Trapping of cells was also demonstrated using this DC-biased AC electric field, and the transition between trapping and lysis of red blood cells in this microchannel was demonstrated by simply adjusting the applied DC voltage. Further, these phenomena were used in conjunction to demonstrate the separation of Leukemia cells from red blood cells.
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NOMENCLATURE

\(\alpha\) ratio of the applied RMS AC field to the DC field

\(\varepsilon_m\) permittivity of the suspending fluid

\(\phi\) electric potential

\(E\) applied electric field

\(E_{AC}\) RMS value of the AC field

\(E_{DC}\) DC component of the applied electric field

\(f_{CM}\) Clausius–Mossotti (CM) factor

\(f_{p-w}\) coefficient for wall induced particle velocity

\(F_{DEP}\) dielectrophoretic force

\(\lambda\) numerical modeling correction factor

\(\mu\) fluid viscosity

\(\mu_m\) fluid dynamic viscosity

\(\mu_{DEP}\) dielectrophoretic mobility

\(\mu_{EK}\) electrokinetic mobility

\(u_{eo}\) electroosmotic velocity

\(u_{ep}\) electrophoretic velocity

\(U_c\) cell velocity

\(U_{EK}\) electrokinetic velocity

\(U_{DEP}\) dielectrophoretic velocity
$U_p$  particle velocity

$R$  radius of channel curvature

$\sigma_p$  electric conductivity of the particle

$\sigma_m$  electric conductivity of the fluid

$t$  time period from the initiation

$x_p$  instantaneous position of a particle

$x_0$  initial location of the particle

$\gamma$  particle-wall separation distance

$\psi$  electric double layer potential

$\zeta$  surface potential

$\zeta_p$  static surface charge of the particle
CHAPTER 1: Introduction

1.1 Aims and Motivation

In recent years, breakthroughs in micro-fabrication technology have greatly increased the speed of new technological development and sharply reduced costs. With the advent of these new micro-fabrication techniques, “Lab-on-a-chip” microfluidic devices have been introduced to harness the tremendous potential of applying these new technologies to the mechanical and biomedical engineering fields. Lab-on-a-chip (LOC), a rapidly expanding area of research, refers to reducing a chemical or biological laboratory to the size of a credit card by incorporating a network of microchannels, electrodes, and sensors built onto an integrated micro-fluidic chip. These microchannels, which typically range in width and height from about 20 to 200 µm are able to perform the same functions as previous room-size methods including but not limited to disease diagnosis via cell analysis and DNA electrophoretic separation. Further benefits of these microfluidic devices include the reduction of materials necessary for the experiments, which leads in turn to a large reduction in the cost of carrying out the experiments [1].

As liquid mediums are used constantly in handling cells and other biological samples, LOC lends itself well to biomedical analysis due to its small scale, fluid based transport, and ability to accurately manipulate cells and particles at the micro-scale level. Currently, LOC devices have made significant contributions to the biomedical field through biochips for disease detection and cell manipulation as well as mapping the human genome [2].
Microchannels on LOC devices can be used to perform a number of cell manipulations including focusing, separating, trapping, and lysis, which can each be achieved using a number of techniques. As an overview, focusing refers to taking a scattered sample of cells introduced to the inlet of a microchannel and manipulating them to a single streamline by the exit of the microchannel. This is a necessary step prior to detection or sorting. Separation refers to separating cells or particles to different regions of the microchannel based on inherent properties such as size or conductivity. Trapping refers to locally increasing the concentration of a cell sample by restricting movement past certain areas of the microchannel, and lysis refers to permanently disrupting the cell membrane such that the contents of the cell are released and may be analyzed.

Electrokinetic flow, which transports cells by application of an electric field, has received much attention for its simplicity and effectiveness in performing these operations. Particles or cells suspended in a fluid medium being transported electrokinetically inside a microchannel will experience a dielectrophoretic force proportional to their radius when introduced to a non-uniform electric field. The non-uniform electric field necessary to induce dielectrophoresis can be generated using either in channel electrodes or the inherent microchannel geometry. The result of this dielectrophoretic force is cross streamline migration of cells as they are transported through the microchannel. Chapters 2, 3, and 4 of this thesis study the use of electrokinetic transport and the dielectrophoresis resulting from the microchannel geometry to perform focusing, separation, trapping, and lysis of cells and particles in structured microchannels. Accompanying background on these particle and cell handling
techniques will be discussed in depth in the respective chapters. Presented below is the background of electrokinetic transport phenomena involved in this thesis.

1.2 Background of Electrokinetic Phenomena

1.2.1 The Electric Double Layer

A fundamental phenomenon that allows for the electrokinetic transport of cells and particles in microchannels is the electric double layer. When a solid object (e.g., a channel, cells or particles) is placed in contact with an aqueous solution, it often becomes charged such that a preferential distribution of ions takes place adjacent to its surfaces. This occurs as the electrostatic charges on the surface attract the counterions in the fluid medium inside the channel resulting in a higher concentration of counterions near the solid surface and a lower concentration of counterions far away from the solid surface. On the other hand, the coion concentration near the surface is lower than the coion concentration far away from the surface. This imbalance in counterions and coions near the solid surface produces a net charge close to the surface. The resulting region consisting of the charged surface and the layer of liquid balancing the charge is referred to as the electric double layer [1]. Fig. 1 shows a schematic of this electric double layer for an arbitrary microchannel surface.
**Figure 1:** Diagram of the ion distribution and the resulting electric double layer formed near a solid surface, reprinted from [3].

### 1.2.2 Electroosmosis

Upon the application of a tangential electric field, the excess counterions within electric double layer move. While the counterions move, they also drag the surrounding liquid molecules with them due to viscous effects causing a bulk liquid motion, which is referred to as electroosmosis [1]. The resulting electroosmotic velocity is one of the main motions in microchannels contributing to the transport of cells and particles and typically is the dominant driving force. The electrosmotic velocity in a microchannel for an incompressible fluid assuming steady state and fully developed flow can be expressed as follows [1]:

$$u_{eo} = -\frac{\varepsilon_m \zeta}{\mu_m} \left(1 - \frac{\psi}{\zeta}\right) (E)$$  \hspace{1cm} (1)

where $u_{eo}$ is the electroosmotic velocity, $\varepsilon_m$ is the dielectric constant of the medium, $\mu_m$ is the viscosity of the medium, $\zeta$ is the surface potential, $\psi$ is the electric double layer potential, and $E$ is the applied electric field.

### 1.2.3 Electrophoresis
As the applied electric field induces electroosmosis, another force is introduced to particles suspended in the bulk liquid. The surface of the particle carries an electrostatic charge, which can cause the particle to move relative to the suspending liquid. This motion is called electrophoresis. The resulting electrophoretic velocity for a particle can be expressed as follows:

\[
\mathbf{u}_{ep} = \frac{\varepsilon_m \zeta_p}{\mu_m} (E) \tag{2}
\]

where \(\mathbf{u}_{ep}\) is the electrophoretic velocity, and \(\zeta_p\) is the static surface charge of the particle.

Frequently, the electrophoretic and electroosmotic motions in microchannels are opposing in direction. As is typically the case, the electroosmotic motion dominates and the particles are dragged through the channel by the bulk flow when the electric field is applied.

### 1.2.4 Dielectrophoresis

While electrophoresis and electroosmosis are the two driving motions that occur during electrokinetic transport, dielectrophoresis is a third motion that arises when the local electric field is spatially non-uniform. Dielectrophoretic motion induces cross streamline migration of cells and particles as they are transported electrokinetically through the channel. The time-average dielectrophoretic force on a spherical particle in a DC electric field and low frequency (<100kHz) AC electric fields is written as [4]

\[
\mathbf{F}_{DEP} = \left( \frac{1}{2} \right) \pi \varepsilon_0 \omega d^3 f_{CM} (E \cdot \mathbf{V}E) \tag{3}
\]
where $d$ the particle diameter, $f_{CM}$ the so-called Clausius–Mossotti (CM) factor that has been assumed approximately the same in DC and low-frequency AC fields [5,6], and $E$ the root-mean-square (RMS) electric field.

As can be seen in Equation 3, the dielectrophoretic force is proportional to the diameter of the cell or particle. As such, it is a very useful technique in separating particles and cells based on their size. This phenomenon is further studied in Chapter 2 of this thesis, where the dielectrophoretic force induced by the channel geometry during electrokinetic flow is used to focus yeast cells.

1.3 Structure of Thesis Work

The following work presented in Chapters 2-4 demonstrates the use of these electrokinetic phenomena to perform focusing, separation, trapping, and lysis of cells and particles in structures microchannels. First, Chapter 2 presents a parametric study of the focusing of yeast cells in a serpentine microchannel. Next, Chapter 3 demonstrates negative and positive dielectrophoretic focusing and their application to particle separation in the serpentine microchannel. Finally, Chapter 4 studies the lysis and trapping of red blood cells in a microchannel constriction. Accompanying background on the particle and cell manipulation techniques is discussed in the respective chapters. In these chapters, the experimental method and numeric modeling have been repeated in order to facilitate the readers understanding of each project without having to refer back to previous chapters too frequently.
CHAPTER 2: Electrokinetic Focusing and Filtration of Cells in a Serpentine Microchannel

2.1 Background on Cell Focusing

Focusing particles or cells into a single stream is usually a necessary step prior to counting and separating them in microfluidic devices such as flow cytometers and cell sorters [7-11]. Previously, particle focusing in microchannels has been achieved by pinching the suspending medium with hydrodynamic [11-14] or electrokinetic [15-18] sheath flows. This sheath flow focusing method requires the precise control of the flow rate of both the sheath flows and the particulate stream. Particle focusing has also been achieved through the use of external force fields such as acoustic [19], optical [20], magnetic [21], electrophoretic [22], and AC dielectrophoretic forces [23-26]. Although these approaches directly manipulate particles to the desired positions, both external pressure-pumping of the particle stream and extra setups for generating the external forces are typically required. Recently, hydrophoresis has been used to focus particles in a microchannel using obstacles on the top and bottom channel walls [27,28]. This approach is dependent on fabrication as the focusing effectiveness is sensitive to the structure of the obstacles. Particle focusing has also been obtained using inertia in curved microchannels [29-31], where the equilibrium position of the focused particle stream is sensitive to the Reynolds number [32].

In addition, the concurrent pumping and focusing of particles have been demonstrated using dielectrophoresis induced in DC electrokinetic flow. In creating the
non-uniform electric field in this focusing technique, however, an array or pairs of microstructures such as insulating posts and oil menisci are required [5,33,34]. Furthermore, high electric fields in the constriction areas formed by the microstructures can cause significant Joule heating and shear stress, both of which may have strong adverse effects on cell viability, especially severe to mammalian cells [6]. In order to overcome these issues, a sheathless DC electrokinetic focusing of particles in a planar serpentine microchannel was recently introduced by Xuan’s group [35]. Due to the turn-induced negative dielectrophoretic motion, particles migrate across streamlines and flow in a focused stream along the channel centerline. While this method eliminates the in-channel microelectrodes and micro-insulators and hence the accompanying adverse effects, relative large electric fields or long channels are still required in order to focus small particles. This issue can be addressed using DC-biased AC electric fields [36,37]. As both DC and AC fields contribute to dielectrophoresis while only the former generates the net particle motion, focusing can be implemented at lower field magnitudes.

The work in this chapter presents a systematic study of electrokinetic focusing of yeast cells in a serpentine microchannel under DC-biased AC electric fields. The effects of electric field (including magnitude, AC to DC field ratio, and AC field frequency) and concentration (including cell and buffer concentrations) on the cell focusing performance are examined. Further, this focusing technique is used to demonstrate a continuous filtration of *Escherichia coli* (*E. coli*) cells from yeast cells. In addition, numerical modeling is performed to predict and verify the effectiveness of the electrokinetic focusing and filtration of cells in a serpentine microchannel.
2.2 Experiment

2.2.1. Microchannel Fabrication

The serpentine microchannel was fabricated with PDMS using the standard soft lithography method [38], and the in depth fabrication process can be referred to in Appendix A. The microchannel for the experiments is a straight channel connecting two wells (serving as reservoirs) with a serpentine section in the center. Figure 2 shows a picture of the fabricated channel whose dimensions are as indicated.

![Figure 2: Picture and dimensions of the serpentine microchannel used in the experiments.](image)

The serpentine section of the channel is comprised of 33 periods and used to produce the dielectrophoretic focusing of cells along the channel centerline as explained in the Theory section. The total length of the microchannel is 30 mm, and the width and depth are 50 µm and 25 µm, respectively, throughout the length of the channel.

2.2.2 Cell Preparation

ATCC4098 yeast cells (*Saccharomyces cerevisiae*) were cultured at 37°C in the Sabouraud Dextrose broth (Becton and Dickinson Company, USA) medium. After about
24 hours, the cells were harvested and washed three times with phosphate buffered saline (PBS). The cells were then collected at an approximate concentration of \(90 \times 10^7\) cells per mL in \(1\times\) PBS, and the cell concentration was diluted down to about \(4.5 \times 10^7\) cells per mL prior to use. The average diameter of the yeast cells measured about 5 \(\mu\)m.

In preparing \(E.\ coli\) cells, a single colony of \(E.\ coli\) ORN208 was inoculated into a Tryptic Soy Broth (TSB) and incubated at 37°C overnight. After being cultured, the \(E.\ coli\) was centrifuged at 3000g for 3 minutes before being re-suspended in \(1\times\) PBS. After repeating this process three times, the cells were collected at an approximate concentration of \(8.9 \times 10^9\) cells per mL in \(1\times\) PBS. As their diameter (about 1 \(\mu\)m) is much smaller than the yeast cells, \(E.\ coli\) cells were not diluted significantly prior to the experiments for visibility purposes in the recorded images. Tween 20 (Fisher Scientific) was added to the cell suspensions at 0.5% v/v in order to suppress the cell adhesions to channel walls and the cell aggregates as well.

### 2.2.3 Experimental Technique

The electrokinetic focusing and filtration of cells in the serpentine microchannel was achieved through negative dielectrophoresis by application of an electric field. A function generator (33220A, Agilent Technologies, Santa Clara, CA) combined with a high-voltage amplifier (609E-6, Trek, Medina, NY) was used to supply both the DC and DC-biased-AC fields required in the experiments. The behavior of cells in the microchannel was visualized using an inverted microscope (Nikon Eclipse TE2000U, Nikon Instruments, Lewisville, TX), and videos were recorded using a CCD camera (Nikon DS-Qi1Mc) at a rate of 19 frames per second. The captured videos and images
were then processed using the Nikon imaging software (NIS-Elements AR 2.30). Pressure-driven cell motions were eliminated by carefully balancing the liquid heights in the two reservoirs prior to each measurement.

2.3 Theory

2.3.1 Operating Mechanism

Figure 3 shows the distribution of electric field intensity (the darker the higher) and the electric field lines (with arrows showing the direction) in one period of the serpentine microchannel. Due to the variation of path length for electric current, the electric field at each of the four 90° turns becomes non-uniform and attains the local maximum and minimum values at the inner and outer corners, respectively. As a result of the electric field gradients at these corners, cells experience a dielectrophoretic force $F_{DEP}$ (a bold symbol denotes a vector henceforth) as they move electrokinetically through the channel turns. The time average of $F_{DEP}$ on an isolated spherical particle is modeled as previously discussed in the introduction by Equation 3, where

$$f_{CM} = \left( \sigma_c - \sigma_m \right) \left( \sigma_c + 2\sigma_m \right)$$

In Equation 4, $\sigma_c$ the electric conductivity of cells, and $\sigma_m$ is the electric conductivity of the medium.

Since biological cells appear to be poorly conducting in DC and low-frequency AC electric fields [38], their conductivity is generally smaller than the medium conductivity, leading to $f_{CM} < 0$ and hence negative dielectrophoresis. Therefore, $F_{DEP}$ is directed towards the lower electric field region at the outer corner of each turn as indicated in Figure 3.
Figure 3: Mechanism for electrokinetic cell focusing in a serpentine microchannel. The diagram shows the dielectrophoretic force experienced by the cell at each turn in one serpentine period as well as the velocity components in streamline (similar to the electric field lines as demonstrated) coordinates. The background shows the electric field contour (the darker the higher).

Since the turns in the serpentine channel alternate direction, and since the electric field gradient is stronger at each inner radius than outer radius, the cells are gradually deflected towards the center region of the channel during each period as they move electrokinetically through the channel. The compounding effect results in a focused stream of cells along the channel centerline as the cells exit the serpentine section of the microchannel.

The cell velocity, $U_c$, is a combination of electrokinetic motion caused by the DC field and dielectrophoretic motion caused by both the AC and DC fields as shown in Equation 5,

$$U_c = U_{EK} + U_{DEP} = \mu_{EK} F_{DC} + \mu_{DEP} (E \cdot V E)$$

(5)

$$\mu_{DEP} = \varepsilon_m d^2 f_{CM} / 6 \mu_m$$

(6)
where $\mu_{EK}$ denotes the electrokinetic mobility, $\mu_{DEP}$ the dielectrophoretic mobility, $E_{DC}$ the DC component of the applied electric field, $E = E_{DC} + E_{AC}$ with $E_{AC}$ being the RMS value of the AC field, and $\mu_m$ is the dynamic viscosity of the suspending medium. As the mechanism for cell focusing in the serpentine microchannel is the cross streamline migration of cells due to dielectrophoresis, the cell velocity can be conveniently expressed in streamline coordinates as illustrated in Figure 3,

$$U_c = (U_{EK} + U_{DEP,s})\hat{s} + U_{DEP,n}\hat{n} = \left(\mu_{EK}E_{DC} + \mu_{DEP}\frac{\partial E}{\partial s}\right)\hat{s} + \mu_{DEP}\frac{E^2}{\Re}\hat{n}$$  \hspace{1cm} (7)

where $U_{EK}$ is streamwise electrokinetic velocity, $U_{DEP,s}$ the dielectrophoretic particle velocity in the streamline direction with the unit vector $\hat{s}$, $U_{DEP,n}$ the dielectrophoretic particle velocity normal to the streamline direction with the unit vector $\hat{n}$, and $\Re$ is the radius of curvature of the streamline. It is important to note that the electric field lines shown in Figure 3 resemble the streamlines in the serpentine channel due to the similarity between flow and electric fields in pure electrokinetic flows [40,41].

The effectiveness of cell focusing in the serpentine microchannel is determined by the ratio of the distance a cell moves perpendicular to the streamline to the distance the cell moves along the streamline. This ratio can be expressed as the ratio of the cell velocity components perpendicular and parallel to the streamline, which, as referred to Equation 7, is given by

$$\frac{U_{DEP,n}}{U_{EK} + U_{DEP,s}} \approx \frac{U_{DEP,n}}{U_{EK}} = \frac{\mu_{DEP}}{\mu_{EK}} \frac{E^2}{E_{DC}\Re} = \frac{\mu_{DEP}}{\mu_{EK}} \frac{E}{\Re}(1 + \alpha)$$  \hspace{1cm} (8)

$$\alpha = \frac{E_{AC}}{E_{DC}}$$  \hspace{1cm} (9)
where $U_{DEP,s}$ has been assumed to have a much smaller magnitude than $U_{EK}$ in the current channel geometry, and $\alpha$ is the ratio of RMS AC field to DC field, i.e., $E = E_{DC} + E_{AC} = E_{DC} (1 + \alpha)$. Equation 8 indicates that a larger $E$ or a larger $\alpha$ should provide a better focusing. Moreover, as $\mu_{DEP}$ is proportional to the square of the cell diameter (see Equation 6) whereas $\mu_{EK}$ is only a weak function of cell size [42], bigger cells should be focused more effectively than smaller ones. This enables the electrokinetic filtration of cells by size in a serpentine microchannel.

2.3.2 Numerical Modeling

In order to predict and understand the effects of working parameters on cell focusing, a numerical model was developed in order to simulate the electrokinetic transport of cells through the serpentine microchannel. This model is based on the one developed by Kang et al. [43,44], and has recently been used by the authors to simulate the particle focusing in various structured microchannels [35,37,45]. In this model a correction factor, $\lambda$, was introduced to account for the perturbation of cell size and cell-cell interactions etc. on the dielectrophoretic velocity. Thus, Equation 7 can be revised to show the simulated cell velocity as

$$U_c = \mu_{EK}E_{DC} + \lambda \mu_{DEP} (E \cdot \nabla E) = \mu_{EK}E_{DC} + \lambda (1 + \alpha)^2 \mu_{DEP} (E_{DC} \cdot \nabla E_{DC}).$$

(10)

This velocity was used as an input to the particle tracing function in COMSOL (Burlington, MA) for computing the cell trajectory. Note that the correction factor, $\lambda$, decreases with the increase in cell size [35,37,43-45], which indicates a smaller
difference in the real dielectrophoretic response of cells with different sizes than that predicted by Equation 3.

In order to determine the cell velocity in Equation 10, the electric field distribution, $E_{DC}$, was solved from the Laplace equation in COMSOL. The electrokinetic mobility, $\mu_{EK}$, was obtained by measuring the average cell velocity in the straight section of the serpentine microchannel. The dielectrophoretic mobility, $\mu_{DEP}$, was determined from Equation 6 where the dynamic viscosity, $\mu_m = 0.9 \times 10^{-3}$ kg/(m·s), and permittivity, $\varepsilon_m = 6.9 \times 10^{-10}$ C/(v·m), of pure water at 25 °C were used as they closely approximate the respective properties of the PBS solution. As the electric conductivity of live cells at DC and low frequency AC electric fields is far smaller than that of the PBS buffer, the CM factor, $f_{CM}$ in Equation 4 was found to be approximately $-1/2$. The correction factor $\lambda$ was determined by matching the predicted cell trajectory to the visible thickness of the cell stream at the exit of the serpentine channel under a 50 V/cm DC field. This obtained value was then used for all other fields and buffer concentrations.

2.4 Results and Discussion

This section presents the parametric study of the effects of electric field and concentration on the electrokinetic focusing of yeast cells in the serpentine microchannel. The electric field effects examined include electric field magnitude, AC to DC field ratio, and AC field frequency, and the concentration effects examined include buffer concentration and cell concentration. In each experiment, all parameters were fixed except for the parameter being tested to ensure only the tested parameter was affecting the cell focusing. The standard parameters used in the experiments include the electric
field magnitude, $E = 100$ V/cm, the AC to DC field ratio, $\alpha = 2$, the AC field frequency 1 kHz, the buffer solution $1\times$ PBS, and the standard cell concentration as prepared in the Cell Preparation section of this chapter. The electrokinetic mobility of yeast cells in $1\times$ PBS was measured to be $\mu_{E,K} = 3(\pm 0.6) \, (\mu m/s)/(V/cm)$ where the 20% variation is due to the variance in cells and the experimental error. The electrokinetic focusing technique as demonstrated here was also used to demonstrate a continuous filtration of $E. \text{coli}$ cells from yeast cells. The results from these experiments were all compared with the simulated results from numerical modeling.

### 2.4.1 Electric Field Effects

With all other parameters as given above being fixed, the electric field magnitude was varied from 50 V/cm to 100 V/cm in order to study its effect on cell focusing. The snapshot (left column) and superimposed (middle column) images of the focused yeast cells at the exit of the serpentine section of the channel are shown in Figure 4.

![Figure 4](image)

**Figure 4:** Experimentally obtained images (left and middle columns) and numerically predicted trajectories (right column) of yeast cells at the entrance (a) and exit (b,c) of the serpentine section of the microchannel under an electric field of 50 V/cm (b), and 100 V/cm (c). Other parameters are referred to the text.
The cell images at the entrance (Figure 4(a)) are also included to show how focusing improves as cells progress through the serpentine section. It is apparent from comparing Figure 4(c) with Figure 3(b) that increasing the field magnitude improves the focusing as the width of the cell stream at 100 V/cm is narrower than at 50 V/cm. Moreover, the cell throughput is also enhanced at a larger field. This result is consistent with Equation 8, and also agrees with the simulated cell trajectories as demonstrated in Figure 4 (right column). The correction factor was set to $\lambda = 0.18$ for both field magnitudes, which is much smaller than that previously obtained for 5-μm-diameter polystyrene beads ($\lambda \approx 0.5$) [35,37,43-45]. This may be attributed to the distinctly different internal structure of yeast cells from that of polymer beads. The intrinsic variance in cell size and shape etc. may also be part of the reason. This issue will be addressed in the future by studying the electrokinetic motion of single cells in a microchannel turn.

In examining the effect of the AC to DC electric ratio on cell focusing, $\alpha$ was varied from (a) $\alpha = 0$ (i.e., pure DC) to (b) $\alpha = 1$ (i.e., 1DC:1AC) and (c) $\alpha = 2$ (i.e., 1DC:2AC), see Figure 5 for the comparison of snapshot (left column) and superimposed (middle column) images at the exit of the serpentine section.
Figure 5: Experimentally obtained images (left and middle columns) and numerically predicted trajectories (right column) of yeast cells at the exit of the serpentine section of the microchannel for: (a) $\alpha = 0$ (i.e., pure DC), (b) $\alpha = 1$ (i.e., 1DC:1AC), and (c) $\alpha = 0$ (i.e., 1DC:2AC) at a total field magnitude of 100 V/cm.

As expected from Equation 8 cells obtain a better focusing when $\alpha$ is increased. It is, however, important to note that increasing $\alpha$ decreases the DC field component and hence reduces the cell throughput. The simulation results in Figure 5 (right column) show the same trend as in the experimental images. The correction factor was still set to $\lambda = 0.18$ as it is independent of electric field [35,37,43-45]. In all cases, the simulated cell trajectories agree well with the experimental results, which justifies the use of the correction factor in the modeling.

The effect of AC field frequency on cell focusing was also examined, where three different frequencies, 100 Hz, 1 kHz, and 10 kHz, have been tested. No higher frequency was applied due to the limitation of the amplifier. At these frequencies, there was no significant change in the width of the focused cell stream at the exit of the serpentine section. This was expected because the frequency effect is represented by the CM factor in Equation 3, which only has a substantial impact on dielectrophoresis once the frequency exceeds 100 kHz [5, 6].

2.4.2 Concentration Effects
The effects of buffer and cell concentrations on yeast cell focusing in the serpentine microchannel were both examined. Figure 6 compares the superimposed cell images (left column) from the exit of the serpentine section at three different buffer solutions: (a) 0.01× PBS, (b) 0.1× PBS, and (c) 1× PBS.

![Figure 6](image)

**Figure 6**: Experimentally obtained images (left column) and numerically predicted trajectories (right column) of yeast cells at the exit of the serpentine section of the microchannel for buffer concentrations of 0.01× PBS (a), 0.1× PBS (b), and 1× PBS (c).

The cell concentration was maintained at the standard concentration as used in all previous tests. It was observed that increasing the buffer concentration increases the effectiveness of the cell focusing. This is because cells move slower when the buffer concentration is increased. The measured electrokinetic mobility of yeast cells are $\mu_{EK} = 7(\pm 1.4)$ and $5(\pm 1)$ ($\mu$m/s)/(V/cm) in the 0.01× and 0.1× PBS, respectively, in contrast to $\mu_{EK} = 3(\pm 0.6)$ ($\mu$m/s)/(V/cm) in the 1× PBS. The decrease in cell mobility with increasing buffer concentration is mainly attributed to the reduced electroosmotic flow [46,56]. At lower velocities, there is more time for the dielectrophoretic force to affect the cells at each turn as they progress through the channel, which leads to an improved focusing in the serpentine channel. This trend can also be clearly seen in the simulation.
results in Figure 6 (right column), where the measured cell electrokinetic mobilities have been used and the correction factor was still fixed at $\lambda = 0.18$.

In testing the effect of cell concentration on focusing, the original yeast cell sample was not diluted. The cell images thus obtained from the exit of the serpentine section (Figure 7(a)) is compared to those at the standard cell concentration (Figure 7(b)).

![Figure 7](image)

**Figure 7:** Experimentally obtained images (left and middle columns) and numerically predicted trajectories (right column) of yeast cells at the exit of the serpentine section of the microchannel for high cell concentration (a) and standard cell concentration (b).

Apparently increasing the cell concentration can decrease the effectiveness of cell focusing. This is due in part to the cell-cell interactions which affect cell dielectrophoresis and the fact that there is not always room for the cells to form a thin stream at the exit regardless of the effectiveness of the focusing. The latter is clearly seen in comparing the snapshot images (left column in Figure 7) for the two cell concentrations. The reduced yeast cell focusing at the high cell concentration appears to be well predicted by decreasing the correction factor from $\lambda = 0.18$ to 0.12 in the simulation as demonstrated in Figure 7 (right column).

### 2.4.3 Electrokinetic Filtration of *E. coli* Cells From Yeast Cells

The electrokinetic cell focusing method was also used to demonstrate an electrokinetic filtration of cells by size in the serpentine microchannel. For this purpose,
*E. coli* cells were mixed with yeast cells in 1× PBS. The standard parameters as presented earlier were employed. Figure 8 shows the snapshot and superimposed images recorded from (a) the entrance and (b) the exit of the serpentine.

**Figure 8:** Experimentally obtained images (left and middle columns) and numerically predicted trajectories (right column) of yeast and *E. coli* cells at the entrance (a) and exit (b) of the serpentine section of the microchannel.

Both the yeast and *E. coli* cells were unfocused at the entrance to the serpentine section, see Figure 8(a). This can be easily seen by observing the widths of the cell streams in the superimposed (center top) image, where yeast cells appeared dark while *E. coli* cells appeared grey. At the exit of the serpentine section, however, yeast cells were aligned along the channel centerline while *E. coli* cells still scattered, see the center image in Figure 8(b). This differential electrokinetic focusing lies in the size difference between the two types of cells. Although it is not a complete separation, the unfocused *E. coli* cells can be filtered from the focused yeast cells if a three-branch outlet is designed to follow the serpentine section. Notably the predicted cell trajectories closely agree with the experimental results as shown in Figure 8(c), where the green trajectories represent *E. coli* cells and the red trajectories represent yeast cells. The correction factors for yeast
and *E. coli* cells were set to $\lambda = 0.18$ and 1, respectively. The measured electrokinetic mobility of *E. coli* is approximately $5 \, (\mu m/s)/(V/cm)$.

### 2.4.4 Joule Heating and Cell Viability Test

As 1× PBS is highly conductive and was used in the majority of the cell focusing experiments, Joule heating may have caused a temperature rise in the solution affecting cell viability [6]. In order to ensure that Joule heating was not an issue in these tests, the electric current in 1× PBS was monitored when the highest electric field, 100 V/cm, was applied. The current was found to remain at 30 $\mu$A for 5 minutes with no noticeable increase, indicating negligible Joule heating in all the tests [48].

Other adverse effects on cell viability may be caused by the electric field-induced trans-membrane voltage, especially from the DC field [6]. For this reason, a cell viability test was performed by staining a sample of the yeast cells from both the inlet and outlet reservoirs with methylene blue. As viable cells with intact cellular membrane exclude methylene blue and remain translucent while non-viable cells are stained blue, the impact of electric field exposure on cell viability can be determined by comparing the percentage of viable cells in the inlet and outlet reservoirs. It was confirmed that more than 95% of the yeast cells were still alive after being exposed to the most abrasive electric field used in the experiments, that is, the 100 V/cm DC electric field.

### 2.5 Summary

A sheathless cell-friendly electrokinetic focusing technique has been demonstrated in a planar serpentine microchannel using DC-biased AC electric fields. This technique uses the DC electrokinetic motion to pump the cell suspension while
taking advantage of the induced cross-stream AC/DC dielectrophoretic motion within the turns to focus cells along the channel centerline. The fabricated in-channel microstructures (either electrodes or obstacles) and/or the external pressure-driven pumping that are typically required in dielectrophoresis-based particle focusing approaches are thus eliminated. This greatly simplifies the device fabrication as well as the device operation. Using a combined experimental and numerical method, the effects of five parameters, including electric field magnitude, AC to DC field ratio, AC field frequency, buffer concentration, and cell concentration, on the focusing performance of yeast cells in a serpentine microchannel has been examined. It is found that the effectiveness of cell focusing is enhanced with increasing field magnitude, AC to DC field ratio, and buffer concentration, or decreasing cell concentration. The electrokinetic cell focusing in serpentine microchannels has also been demonstrated to continuously filter *E. coli* cells from yeast cells. This serpentine cell focusing microchannel can be envisioned as a front-end device for cell detection and sorting in lab-on-a-chip devices for numerous other applications.
CHAPTER 3: Continuous Particle Separation in a Serpentine Microchannel via Negative and Positive Dielectrophoretic Focusing

3.1 Background on Particle and Cell Separation

Dielectrophoresis (DEP) is a powerful tool that has been widely used to focus and separate cells and particles in microfluidic devices [9,49,50]. So far DEP has been implemented using both electrode-[51,52] and insulator-based [53,54] approaches to generate a non-uniform local electric field. In the former, pairs of electrodes are placed inside a microchannel, and AC voltages are applied locally through those electrodes to achieve dielectrophoretic particle deflection for either focusing [23-25,55] or separation [26,56-65]. This approach suffers from several problems such as the need of hydrodynamic pumping of the sample, the increased complexity in microchannel fabrication, and the surface fouling of electrodes due to electrochemical reactions etc. [6,66]. These problems are not encountered in the insulator-based approach, where both AC and DC fields can be applied through the electrodes positioned outside a microchannel, and the non-uniform electric field is generated by in-channel insulators.

Two types of in-channel insulators have been demonstrated to produce the dielectrophoretic force for particle and cell manipulations. The first type is the insulating obstacles (e.g., hurdles, posts, and ridges) that are fabricated inside a microchannel to partially block the electric current. As a consequence, the electric field is locally amplified around the insulating obstacles, which on one hand can induce DEP for particle focusing [34,37] or separation [36,44,67-73], while on the other hand may cause adverse
effects on both the sample and the device due to Joule heating and particle clogging etc. [6]. These drawbacks are overcome in the second type of insulator-based approach where the insulating walls of a curved microchannel are directly used to control the particle motion [74]. Due to the variation in path length for electric current, the electric field becomes inherently non-uniform in a curved channel [75]. Thus induced dielectrophoretic force can generate a cross-stream particle deflection, which has been demonstrated to focus particles and cells in curved microchannels [35,45,76].

In our previous work, particles and cells experienced only negative DEP and were thus focused to either the centerline of a serpentine microchannel [35,76] or the outer sidewall of a spiral microchannel [45]. The work in this chapter extends previous work to demonstrate both the negative and positive dielectrophoretic focusing of particles in a serpentine microchannel. Moreover, these two focusing phenomena are combined to demonstrate a continuous separation between particles of different sizes and conductivities. Additionally numerical modeling is developed to simulate the observed particle focusing and separation behaviors.

3.2 Experiment

The serpentine microchannel was fabricated with polydimethylsiloxane (PDMS) using the standard soft lithography method as described in detail in Appendix A and also in [76]. The channel studied in this chapter has the same geometry as that of Chapter 2. For more detail on the channel measurements, the reader is referred back to Figure 2.

The serpentine section serves to focus and separate particles as explained below, and is comprised of 33 serpentine periods. As previously mentioned, the channel has a
uniform width and depth, which are 50 µm and 25 µm, respectively. Polystyrene particles of 2.2 µm (fluorescent, G0220, Duke Scientific Corp.) and 5 µm (Fluka 79633, Sigma-Aldrich) in diameter were re-suspended in either deionized water or 1 mM phosphate buffer at a concentration of at least $10^7$ particles per milliliter. Tween 20 (0.5% v/v, Sigma-Aldrich) was added to the suspensions to suppress particle adhesions to the channel wall as well as particle aggregations. The DC-biased AC electric field (with a fixed 1 kHz frequency) across the serpentine microchannel was supplied by a function generator (33220A, Agilent Technologies) in conjunction with a high-voltage amplifier (609E-6, Trek, Inc.). Particle motions were observed using an inverted microscope (Eclipse TE2000U, Nikon Instruments). Videos and images were recorded using a CCD camera (Nikon DS-Qi1Mc), and processed using the Nikon imaging software (NIS-ELEMENTS AR 2.30).

### 3.3 Theory

#### 3.3.1. Operating Mechanism

Figure 9 displays the contour of electric field intensity (the darker the higher) and the electric field lines (i.e., streamlines due to the similarity between flow and electric fields in pure electrokinetic flows [40] in one period of the serpentine microchannel in the absence of particles. Due to the variation of electrical path, the electric field distribution at each turn becomes non-uniform generating a local maximum and minimum at the inner and outer corners, respectively. Therefore, particles experience a cross-stream dielectrophoretic force as they move electrokinetically through the channel at a velocity
of \( U_{EK} \). Equations 3 and 4 can be referred to for the dielectrophoretic force on a particle in a suspending medium.

When the particle is less conductive than the suspending fluid, \( f_{CM} \) will be negative producing negative DEP [4,77]. So the particle will be pushed by \( \mathbf{F}_{DEP} \) towards the region where the electric field is lower, i.e., the outer corners of the turns in the serpentine microchannel. On the contrary, when it is more conductive than the fluid, the particle will experience positive DEP and thus be pulled by \( \mathbf{F}_{DEP} \) to the inner corners of the turns where the local electric field is higher. See Figure 9 for the directions of \( \mathbf{F}_{DEP} \) on a particle in these two circumstances. As the electric field gradient is also larger at the inner corner of a turn, the particle should experience a stronger \( \mathbf{F}_{DEP} \) if it is closer to the inner corner. For this reason, particles undergoing negative DEP will be deflected gradually towards the channel centerline by the alternating turns as they progress through the serpentine microchannel. This phenomenon is schematically illustrated in Figure 9 (a), where the magnitude of \( \mathbf{F}_{DEP} \) is denoted by its vector length. Such sheathless electrokinetic focusing in a serpentine microchannel has been recently demonstrated by the authors with both polymer particles and biological cells [35,76].
Figure 9: Mechanisms of negative (a) and positive (b) dielectrophoretic focusing of particles (shown as shaded circles) in a serpentine microchannel. The background shows the electric field contour (the darker the higher) and the electric field lines (or equivalently the streamlines, $U_{EK}$) in the absence of particles. The approximate magnitude of the dielectrophoretic force, $F_{DEP}$, on a particle at a location is denoted by its vector length.

In a similar manner, particles with positive DEP particles will be gradually pulled in to the inner corners of the turns where the electric field strength and gradient are both stronger, and then follow the channel wall. This eventually causes a splitting with the particles lining both sidewalls of a serpentine microchannel, which is schematically illustrated in Figure 9 (b). Recognizing the distinctive focusing behaviors for particles experiencing negative and positive DEP in Figure 9, one can anticipate a continuous separation between them in a serpentine microchannel. This separation mechanism was utilized to separate particles with different sizes and electric conductivities in the present work. Specifically, as suggested by Equation 4, a suspending fluid with an intermediate
conductivity between those of the two particles was chosen to attain simultaneously the negative and positive dielectrophoretic focusing.

3.3.2. Numerical Modeling

In order to predict and verify the effects of negative and positive DEP on particles, a numerical model similar to that discussed in Chapter 2 was developed to simulate the electrokinetic particle transport through the serpentine microchannel. Again, the perturbations of particles on the flow and electric fields were neglected. Instead, a correction factor, $c$, was introduced to account for the effects of particle size on $F_{DEP}$ or the resulting dielectrophoretic velocity. This model has been validated through comparisons with the experimental observations of electrokinetic particle and cell motions in various microchannels [35,37,44,45, 73,76]. In this model the particle velocity, $U_p$, is expressed as [43] with $\mu_{DEP}$ previously given by Equation 6

$$U_p = \mu_{EK}E_{DC} + \lambda \mu_{DEP} (E \cdot \nabla E) + f_{p-w} (E \cdot E) \hat{n}$$  \hspace{1cm} (11)

$$f_{p-w} = 0.176 \exp \left[-5.734 \left(\frac{\gamma}{d}\right)\right] \epsilon_m d / 3\pi \mu_m$$  \hspace{1cm} (12)

where the three terms in Equation 11 represent the particle velocities induced by electrokinetic flow, DEP, and particle-wall interactions, respectively, and $f_{p-w}$ is the coefficient characterizing the wall-induced particle velocity in terms of the particle-wall separation distance $\gamma$ [43]. Note that the inertial and centrifugal motions have been neglected in Equation 11 because the Reynolds and Dean numbers are both very small under the experimental conditions. The instantaneous position of a particle, $x_p$, is then obtained by integrating the particle velocity $U_p$, i.e.,
\[ x_p = x_0 + \int_0^t U_p(t') dt' \]  

(14)

where \( x_0 \) represents the initial location of the particle, and \( t \) is the time period from the initiation.

The numerical modeling was performed in COMSOL® (Burlington, MA) with the MATLAB® interface. A 2D model of the serpentine microchannel was first developed in COMSOL®, where the effects of the top and bottom channel walls on particle motions were ignored [35,37,44,45,73,76]. Then, the electric field distribution that was needed to compute the particle velocity, \( U_p \), from Equation 11 was solved from Laplace equation in COMSOL®. Next, the finite-element-model (FEM) structure was exported into MATLAB® to determine the trajectory of a particle whose initial position was specified at the channel entrance. A custom-written program in MATLAB® was used to determine the particle position \( x_p \), where the key function is to calculate the particle-wall separation distance \( \gamma \) and thus the coefficient \( f_{p-w} \) from Equation 12.

Other parameters required in the modeling were obtained as follows: the electrokinetic mobility, \( \mu_{EK} \), was attained by measuring the average particle velocity in the straight section of the serpentine microchannel; the dielectrophoretic mobility, \( \mu_{DEP} \), was determined from Equation 6 using the dynamic viscosity, \( \mu_m = 1.0 \times 10^{-3} \text{ kg/(m·s)} \), and permittivity, \( \varepsilon_m = 6.9 \times 10^{-10} \text{ C/(V·m)} \), of pure water at 20 °C; the CM factor, \( f_{CM} \), depends on the electric conductivities of the fluid and the particle where the latter was unable to measure and will be discussed in the next section; the correction factor, \( \lambda \), was determined by matching the predicted particle trajectory to the observed particle motion.
3.4. Results and Discussion

3.4.1. Negative and Positive Dielectrophoretic Particle Focusing

Negative dielectrophoretic focusing in the serpentine microchannel was studied using 2.2 µm fluorescent particles suspended in 1 mM phosphate buffer. Figure 10 shows the superimposed images (left column) at the entrance (a) and exit (b) of the channel serpentine section. The applied voltage at the inlet reservoir was 550 V AC (RMS value, 1 kHz frequency) with a 50 V DC bias while the outlet reservoir was grounded. The average electric field through the channel was about 200 V/cm. In the entrance image (Figure 10 (a)) the particles appear uniformly distributed when they enter into the serpentine section as they cover the majority of the channel width. They, however, begin to be focused in the first few serpentine periods due to the induced negative dielectrophoretic motion. At the exit of the serpentine section (Figure 10(b)), the particles are observed to be focused to a single stream at the channel centerline with a measured width of 9 µm. This correlates well with the expected position of the particles under the influence of negative DEP, as the particles are alternately deflected away from the inner corner of each turn towards the channel centerline [35,76].

Figure 10: Superimposed images (left column) and predicted trajectories (right column) showing the negative dielectrophoretic focusing of 2.2 µm particles at the entrance (a) and exit (b) of the serpentine section of the microchannel.
The numerically predicted particle trajectories at the experimental condition are also illustrated in Figure 10 (right column) for a clear comparison. As the particle conductivity is much lower than that of 1 mM phosphate buffer (measured as 210 \( \mu \)S/cm) [78], the CM factor, \( f_{CM} \), was close to –0.5 in the modeling. The particle electrokinetic mobility, \( \mu_{EK} \), was measured to be 3.0 \( (\mu \text{m/s})/(\text{V/cm}) \), and the correction factor, \( \lambda \), for particle DEP was set to 0.7, which was found to give a close match to the experimental results.

Positive dielectrophoretic focusing in the serpentine microchannel was tested using the same 2.2 \( \mu \)m particles as in the negative DEP experiment. In order to ensure positive DEP, deionized water was used as the suspending fluid due to its extremely low electric conductivity. Figure 11 (left column) shows the superimposed images at the entrance (a) and exit (b) of the channel serpentine section. The same electric field as used in the negative DEP experiment was applied. At the entrance region (Figure 11 (a)), the particles once again appear scattered and uniformly distributed covering the majority of the channel width. However, from the exit image in Figure 11 (b), the particles can be observed lining the sidewalls of the channel with a measured width of about 9 \( \mu \)m on each side. This is also consistent with the theory as the particles should be attracted by positive DEP to the high electric field region at the inner corner of the channel turns.
Figure 11: Superimposed images (left column) and predicted trajectories (right column) showing the positive dielectrophoretic focusing of 2.2 μm particles at the entrance (a) and exit (b) of the serpentine section of the microchannel.

The numerically predicted particle trajectories for positive dielectrophoretic focusing in the serpentine microchannel are illustrated in the right column of Figure 11. For the modeling, the average electrokinetic mobility, $\mu_{EK}$, of 2.2 μm particles in deionized water was measured to be $3.5 \, (\mu m/s)/(V/cm)$. The CM factor, $f_{CM}$, was assumed to be $+0.2$ while the correction factor, $\lambda$, was set to $0.4$. The CM factor was obtained by assuming that: (1) the electric conductivity of 2.2 μm particles is about $9 \, \mu S/cm$, equivalent to a $0.5$ nS surface conductance [78], and (2) the electric conductivity of the deionized water is around $5 \, \mu S/cm$ which could not be measured accurately due to the limitation of the conductivity meter (accumet AP85, Fisher Scientific, unable to be calibrated below $12 \, \mu S/cm$). The correction factor used here is significantly smaller than that used in modeling the negative dielectrophoretic focusing of the same particle (which is $0.7$). The reason behind this discrepancy is currently unknown. With these parameters, the modeling results seem to predict the experimental observations reasonably well.

3.4.2 Particle Separation via Negative and Positive Dielectrophoretic Focusing
The negative and positive dielectrophoretic focusing phenomena demonstrated above were combined to achieve particle separation in the serpentine microchannel. For this experiment, 2.2 µm and 5 µm particles were mixed and suspended in deionized water. The calculated electric conductivity of 5 µm particles was about 4 µS/cm if the surface conductance was still assumed to be 0.5 nS [78]. Thus, the CM factor for 5 µm particles in deionized water (with the electric conductivity being still assumed as 5 µS/cm) was approximately −0.07, yielding negative DEP. Therefore, 5 µm and 2.2 µm particles should be focused to the centerline and the sidewalls of the serpentine microchannel, respectively, and can thus be continuously separated without any other external force. Figure 12 (left column) shows the superimposed images of particle separation at the entrance [(a) for 2.2 µm particles and (b) for 5 µm particles] and exit [(c) for 2.2 µm particles and (d) for 5 µm particles] of the channel serpentine section. The applied voltage was 500 V AC (RMS value, 1 kHz frequency) with a 100 V DC bias. Note that the superimposed images for the two types of particles are from the same video though they are displayed individually.

In the entrance region of the serpentine section, both the fluorescent 2.2 µm particles (Figure 12 (a)) and the non-fluorescent 5 µm particles (Figure 12 (b)) are unfocused and (nearly) uniformly distributed. At the exit region, the two different dielectrophoretic focusing behaviors can be seen clearly. As expected 2.2 µm particles are observed in Figure 12 (c) to line the channel sidewalls due to positive DEP while 5 µm particles are focused by negative DEP to the channel centerline in Figure 12 (d).
However, the positive dielectrophoretic focusing of 2.2 μm particles (with a measured stream width of about 15 μm on either sidewall) appears to be less effective than the negative dielectrophoretic focusing of 5 μm particles (with a measured stream width of about 8μm). This is mainly attributed to the strong dependence of dielectrophoretic force on particle size, see Equation 3. In addition, the focusing of 2.2 μm particles is weaker than that in Figure 11 because the particles spend less time in the channel to experience the dielectrophoretic force in a larger DC field. At this point, if a three-fork branch could be included slightly downstream of the serpentine section, the 2.2 μm fluorescent particles would be filtered outward through the side branches while the 5 μm particles continued forward along the center branch.

**Figure 12:** Superimposed images (left column) and predicted trajectories (right column) showing the separation of 2.2 μm fluorescent particles and 5 μm non-fluorescent particles in the serpentine microchannel: (a) 2.2 μm particles at the entrance, (b) 5 μm particles at the entrance, (c) 2.2 μm particles at the exit, and (d) 5 μm particles at the exit of the serpentine section.

The numerically predicted trajectories for 2.2 μm and 5 μm particles in the separation experiment are displayed in the right column of Figure 12. In the modeling the
electrokinetic mobility, $\mu_{EK}$, of 2.2 $\mu$m particles in deionized water was as given earlier while that of 5 $\mu$m particles was measured to be $5.5 \ (\mu\text{m}/\text{s})/(\text{V/cm})$. The CM factor of 2.2 $\mu$m particles was still assumed to be $+0.2$ with the same correction factor as in the positive dielectrophoretic focusing experiment, i.e., $\lambda = 0.4$. For 5 $\mu$m particles, the CM factor was $-0.07$ as discussed above, and the correction factor was set to 0.5 which is consistent with previous studies [35,37,45,76]. With these parameters, the modeling results agree with the experimental observations reasonably well.

### 3.5 Summary

Negative and positive dielectrophoretic focusing of particles have been both demonstrated in a serpentine microchannel by changing the electric conductivity of the suspending fluid. Particles were observed to either move along the channel centerline or line the channel sidewalls when their electric conductivity was lower (yielding negative DEP) or higher (yielding positive DEP) than that of the fluid. In both cases focusing takes place due to the cross-stream migration of particles caused by the dielectrophoretic force induced by the channel turns. As particles are focused to different regions of the serpentine microchannel with negative and positive DEP, the two phenomena have also been combined to demonstrate a continuous separation between particles of different sizes. In this separation the more conductive smaller particles experienced positive DEP and were focused to the channel sidewalls, while the less conductive larger particles experienced negative DEP and were thus focused to the channel centerline. A numerical model based on Lagrangian tracking method has also been developed, which can predict
reasonably the observed particle focusing and separation behaviors in the serpentine microchannel.
CHAPTER 4: Integrated Electrical Concentration and Lysis of Cells in a Microfluidic Chip

4.1 Background on Cell Lysis and Trapping

Cell Lysis is an important step prior to the analysis of intracellular contents. As lysis allows for the examination of intracellular contents, recognition of abnormalities in genes or proteins can allow for early disease diagnosis. Recently, lysis has been demonstrated using four techniques: chemical lysis, mechanical lysis, thermal lysis, and electrical lysis. During chemical lysis, lytic acids such as sodium dodecyl sulfate or hydroxide are used to dissolve the membrane or react with the membrane lipids [83,85,93]. In some applications, water may also be used to lyse cells by osmosis [87]. Whereas chemical lysis has many applications, the lytic acids used to disrupt the cell membrane may also denature proteins and interfere with intracellular contents, reducing the effectiveness of the technique. Thermal lysis has been demonstrated to lyse cells using high temperatures [92]. However, this process can also cause the denature of proteins and therefore is limited in practicality. Mechanical lysis is another technique that has been used to lyse cells in microchannels. This has previously been performed by microscale sonification and nano-barb filtration [80,81,90].

Electrical lysis has rapidly gained popularity due to its simplicity and practicality in application in microchannel devices. This process is based on electroporation caused by the application of an electric field, and has been performed using two different methods: an electric pulse, and continuous DC electric fields. The electric pulse lyses
cells by applying a high electric field in short bursts, which causes the cell membrane to be irreversibly disrupted. Fabrication for this technique can be complex as a high density of microscale electrodes or structures must be used. Another challenge with the high electric field application is the formation of bubbles in the microchannel due to joule heating [86]. Electrical lysis has also been demonstrated using low dc electric fields [91]. This technique uses the microchannel geometry to create a locally high electric field which causes the cells to lyse as they pass through the designated section of the microchannel. As the two determining factors for cell lysis are the electric field magnitude and the exposure time to the electric field, pressure driven flow must often be used in this technique to slow down the cells and ensure that they spend adequate time in the high electric field region to be lysed [91]. The need for a pressure driven flow to adjust the cell speed can be eliminated using a DC-biased AC electric field. Using this method, the total electric field necessary for cell lysis can be achieved with a lower DC component, which results in a lower cell velocity. As only the DC component of the electric field contributes to the electrokinetic transport, the exposure time to the locally high electric field is increased without the need for a counteractive pressure driven flow.

Trapping of cells is also an important operation that can have practical applications in microfluidic cytometers. Trapping can be used, for example, to increase the concentration of cells prior to lysis to provide for easier sampling of intracellular contents after lysis. Previously, trapping has been demonstrated using negative dielectrophoresis induced by a DC-biased AC electric field [35]. This method is particularly useful as it can be implemented in much smaller electric field magnitudes.
than in pure DC cases, which stems from the independent control of DC electrokinetic motion and AC/DC dielectrophoretic motion. Further, cell trapping behavior can be altered by simply adjusting the AC to DC field ratio.

The work in this chapter demonstrates the electrokinetic trapping and lysis of red blood cells in a hurdle microchannel using an applied DC-biased AC electric field. Lysis is caused by a locally amplified electric field due to channel geometry, and trapping is achieved by dielectrophoresis. Further, the transition between trapping and lysis is demonstrated by simply adjusting the DC component of the electric field. Finally, trapping and lysis in this channel are used in conjunction to demonstrate a separation between Leukemia cells and red blood cells. Simulation is used to predict trapping and lysis conditions and simulation results are shown to be in agreement with experimental results.

4.2 Experiment

4.2.1 Microchannel Fabrication

The microfluidic chip was fabricated using the standard soft lithography technique as described in detail in Appendix A and used in [38]. The microfluidic chip fabricated for the experiment consists of a straight microchannel connecting two wells with a constriction in the middle. Figure 13 shows a picture of this chip where the inset is the zoom-in view of the constriction with dimensions indicated.
Figure 13: Picture and dimensions of the hurdle channel used in these experiments.

Specifically, the channel length is 1 cm between the two wells with a width of 400 µm. The width and length of the constriction are 40 and 200 µm, respectively. The radii of corners in the constriction are all 20 µm, and the depth is uniform at 15µm.

4.2.2 Cell Preparation

In preparing the blood cells for the experiments, a sample of sheep blood was centrifuged, re-suspended in RPMI 1640, and stored in a refrigerator until use. Prior to use, the cells were washed 3 times and suspended in 1xPBS (Phosphate Buffered Saline, Fisher Scientific). The original concentration of the sample was then diluted 400 times before the sample was introduced to the microchannel.

K652 Leukemia cells (chronic myelogenous leukemia) were also centrifuged and suspended in RPMI 1640. These cells were prepared at a concentration of 8.75x10⁵ cells/mL and were stored at room temperature. Prior to use, the cells were washed 3 times and suspended in 1xPBS. No further dilution was necessary before the cells were introduced to the microchannel.
4.2.3 Experimental Technique

The electrical concentration and lysing of red blood cells in the microchannel constriction was achieved by application of DC-biased AC electric fields. A function generator (33220A, Agilent Technologies, Santa Clara, CA) combined with a high-voltage amplifier (609E-6, Trek, Medina, NY) was used to supply the electric fields. The behavior of cells in the microchannel was visualized using an inverted microscope (Nikon Eclipse TE2000U, Nikon Instruments, Lewisville, TX), through which videos and images were recorded using a CCD camera (Nikon DS-Qi1Mc). The captured digital videos and images were processed using the Nikon imaging software (NIS-Elements AR 2.30). Pressure-driven cell motions were eliminated by balancing the liquid heights in the two reservoirs prior to every experiment.

4.3 Theory

4.3.1 Operating Mechanism

The electric field distribution around the constriction region in the fabricated microchannel is shown in Figure 14, where the lines indicate the electric field direction and the contour indicates the field intensity (the darker the higher). Due to the reduction in cross-sectional area, the root-mean-square (RMS) electric field becomes strongly non-uniform in the constriction region. Thus, cells experience a dielectrophoretic force when they move electrokinetically towards the constriction. Using the dipole moment approximation, the time average of the induced dielectrophoretic motion, \( U_{DEP} \), of an isolated spherical cell is given by [3]

\[
U_{DEP} = \mu_{DEP} (\mathbf{E} \cdot \mathbf{V_E})
\]  

(14)
with \( \mu_{DEP} \) as previously denoted in Equation 6 in Chapter 2:

Since they [6, 39, 89] appear to be poorly conducting in DC and low-frequency AC fields leading to \( f_{CM} < 0 \) and \( \mu_{DEP} < 0 \), biological cells undergo negative dielectrophoresis. Therefore, \( U_{DEP} \) points away from the constriction and opposes the streamwise electrokinetic motion, \( U_{EK} \), of the incoming cells; see Figure 14.

Figure 14: Picture of the electric field distribution around the constriction region in the hurdle microchannel. The electric field lines are shown with arrows indicating the direction of the electric field, and the electric field contour is shown where the darker colors represent regions where the electric field magnitude is higher. The two cell motions, \( U_{EK} \), and \( U_{DEP} \), are included on the figure and represent the electrokinetic motion and dielectrophoretic motion respectively with arrows indicating the direction of each motion.

The real cell velocity, \( U_c \) can be expressed as shown previously in Equation 5.

As demonstrated previously by the authors [35,37], it is convenient to rewrite the cell velocity in streamline coordinates, which can be referred to in Equation 7.
As cells experience negative dielectrophoresis, i.e., $\mu_{\text{DEP}} < 0$, $U_{\text{DEP},3}$ is against $U_{\text{EK}}$ and thus slows down the cells when they approach the constriction; see Figure 14. When the magnitude of $U_{\text{DEP},3}$ is everywhere smaller than $U_{\text{EK}}$ or $U_{\text{DEP,3}}/U_{\text{EK}} > -1$, the electrokinetic motion moves cells through the constriction where they are subjected to a locally amplified electric field. Meanwhile, since $U_{\text{DEP,3},i}$ points towards the channel center plane, cells are displaced across streamlines and focused to a stream near the center of the constriction. If the local electric field within the constriction is high enough, cells can be lysed during the passage. However, when the magnitude of $U_{\text{DEP,3}}$ becomes greater than $U_{\text{EK}}$ or $U_{\text{DEP,3}}/U_{\text{EK}} \leq -1$ in an area, cells are unable to enter into the constriction and get trapped in its front. It follows from Equation 7 that the relative magnitude between $U_{\text{DEP,3}}$ and $U_{\text{EK}}$ is expressed as

$$\frac{U_{\text{DEP,3}}}{U_{\text{EK}}} = \frac{\mu_{\text{DEP}} E}{\mu_{\text{EK}} E_{\text{DC}}} = \frac{\mu_{\text{DEP}}}{\mu_{\text{EK}}} \frac{(1 + \alpha)^2}{\alpha} \frac{\partial E_{\text{AC}}}{\partial s},$$

(15)

Therefore, the transition between cell lysing and concentration can be realized by simply adjusting $E_{\text{DC}}$ or equivalently $\alpha$ if $E_{\text{AC}}$ is maintained. Note that the pre-factor $(1 + \alpha)^2/\alpha$ in Equation 15 decreases monotonically with $\alpha$ in the range of $0 < \alpha < 1$. That is to say lowering $\alpha$ will allow the dielectrophoretic motion to dominate causing cells to be concentrated while increasing $\alpha$ allows the electrokinetic motion to dominate causing cells to be lysed.

4.3.2 Numerical Modeling
A numerical model was developed similar to that presented in Chapter 2 in order to predict the electrical concentration and lysing of cells in the microfluidic chip. Again, the effects of cells on the electric and flow fields are neglected, and a correction factor, $\lambda$, is introduced to account for the influence of cell size and cell-cell interactions etc. on the dielectrophoretic velocity. Thus the simulated cell velocity is expressed by Equation 10, which is used as an input to the particle tracing function in COMSOL (Burlington, MA) for computing the cell trajectory. This model has been validated in simulating the electrical manipulations of particles and cells in various microchannels [35,37,43,44,73].

The model, which was set up as per Chapter 2, used the electrokinetic mobility, $\mu_{EK}$, of red blood cells in 1× PBS which was determined to be approximately $1.5(\pm0.3)$ ($\mu$m/s)/(V/cm). This was determined by measuring their average velocity in the microchannel distant from the constriction. For the Leukemia cells, the electrokinetic mobility was determined to be approximately $1.2(\pm0.3)$ ($\mu$m/s)/(V/cm). The obtained variation in $\mu_{EK}$ is due to the inherent variance in cells and the experimental error as well. The dielectrophoretic mobility, $\mu_{DEP}$, was determined using Equation 6, where the dynamic viscosity and permittivity of pure water at 25°C were assumed. The correction factor, $\lambda$, was determined as 0.5 for the red blood cells and 0.3 for the Leukemia cells by matching the predicted cell trajectories to the experimentally observed cell streaks at a 50 V/cm pure DC field. This value is close to that obtained previously for 5-$\mu$m-diameter and 10-$\mu$m-diameter spherical polystyrene beads [35,37,43,44,73].

4.4 Results and Discussion
4.4.1 Electrical Trapping and Lysis of Red Blood Cells

In demonstrating the effectiveness of this dielectrophoretic technique, lysis and trapping were first individually examined in this hurdle channel by fixing the total electric field and adjusting the ratio of the AC to DC applied voltage. As the onset of electrical lysis was detected at an average of 100kV/m in the constriction, which is consistent with other sources [82], it followed that any field higher than this threshold value would induce lysis. As such, 16kV/m (160kV/m in the constriction) was chosen as it would be sufficiently high to guarantee complete lysis. With the electric field fixed, the applied voltage ratio of AC to DC was adjusted from pure 160V DC in increments down to 15V DC and 145Vrms AC. The following conditions were tested: 1) 0Vrms AC, 160V DC, 2) 80Vrms AC, 80V DC, 3) 120Vrms AC, 40V DC 4) 145Vrms AC, 15V DC.

In the first case of 160V DC, the electrokinetic motion driving the cells was significantly stronger than the dielectrophoretic opposing motion at the constriction. In addition, the corresponding electrokinetic velocity caused by the pure DC field was quite fast, so cells moved quickly through the constriction and were only exposed to the locally amplified electric field for a short duration. As the two determining factors for lysis are the electric field magnitude and the duration of exposure to the electric field, not many cells were lysed in this case. This is also why in many cases of pure DC cell lysis, opposing pressure driven flow is often introduced in order to slow down the cell velocity and allow adequate exposure time to the locally high field. The superimposed image from this test can be referred to in Figure 15(a), where the cell trajectories are still visible past the constriction indicating that little to no lysis has taken place. The right column in
Figure 15(a) shows the corresponding simulation results, which also show that cells should flow through the constriction under these conditions.

Figure 15: Superimposed images demonstrating the effects of the DC to AC ratio on cell lysis. The left column represents the experimental results, and the right column represents the simulated cell trajectories for: (a) 160V DC, No Lysis (b) 80V DC 80V AC, Partial Lysis (c) 40V DC 120V AC, complete lysis (d) 15V DC 145V AC, Trapping. In (d) the fewer cell trajectories correspond to the lower cell velocity caused by the low DC field.

In the second case, 80 Vrms AC and 80V DC were applied while maintaining the electric field constant at 160kV/m in the constriction. As the DC component of the electric field was reduced by half from the previous test, the corresponding cell velocity
resulting from electrokinetic motion was also reduced by half. At this ratio, the electrokinetic motion driving the cells was still stronger than the opposing dielectrophoretic motion, so cells moved through the constriction. However, as the cell velocity was lower than the pure 160V DC case, some cells were lysed as they passed through the constriction. The superimposed image for this test can be referred to in Figure 15(b). In this image, the cell trajectories are still visible past the constriction, however there are more gaps between the superimposed cell trajectories due to a higher percentage of cells lysing as they moved through the constriction. The right image in Figure 15(b) shows the corresponding simulation image for this case. In this case, the focusing of the cells after the constriction is slightly better predicted in the simulation case than in the experimental case, however the trend is still present. This could be due to the simulation predicting the ideal case while not taking into account non-uniformities and other irregularities present in actual cell samples.

The AC to DC ratio was then further increased to 120Vrms AC and 40V DC while still maintaining the total electric field at 160kV/m in the constriction. Similar to the first two cases, the electrokinetic motion was still stronger than the opposing dielectrophoretic motion and cells passed through the constriction. However, as the DC voltage had been further reduced, the corresponding cell velocity caused by the electrokinetic motion was also slower. In this case, all cells were lysed as they moved through the constriction as a result of the longer exposure time to the locally high electric field. This test demonstrated the usefulness of DC biased AC electric fields, as the cell velocity can be controlled while keeping the total electric field fixed without the need for
additional external forces. Figure 15 (c) shows the superimposed image from this test. In examining this figure, it is evident that the cell trajectories fade and disappear as they move through the constriction. This is consistent with complete lysis taking place as the cells are exposed to the high electric field in the constriction for an adequate length of time such that all cells are lysed by the time they exit the constriction. The right image in Figure (c) shows the simulation result for this test. As COMSOL cannot currently show if cells will be lysed under the applied conditions, the simulation was used to verify whether or not cells should pass through the constriction at this AC to DC ratio of applied voltages.

The AC to DC voltage ratio was further increased to 145Vrms AC and 15V DC. At this ratio, the electrokinetic motion was not strong enough to overcome the opposing dielectrophoretic motion, and the cells were trapped in front of the constriction. Whereas the previous three cases demonstrated the effect of AC to DC ratio on lysis, this test showed that further increasing this ratio would induce trapping. Figure 15 (d) shows the cell trajectories for this test. As is evident in the figure, there is no trace of the cells moving into the constriction as there was in Figure 15 (c). Rather, the cells are preventing from moving into the constriction and accumulate in front of it. It should be noted that in this test, the cell velocity was very low due to the low DC, which resulted in the fewer streamlines in Figure 15 (d) even over the course of a longer video. The right image of Figure 15 (d) shows the simulated cell trajectories for this test and is consistent with the experimental results as cells are predicted to become trapped in front of the constriction.
4.4.2 Trapping and Lysis Transition via DC Voltage Adjustment

After lysis and trapping had been individually studied by adjusting the AC to DC ratio, a DC biased AC electric field was used to show that transition between trapping and lysis could be achieved through small adjustment of the DC portion of the electric field. To this end, the AC voltage was fixed at 145Vrms AC. The results from this test can be referred to in Figure 16.

Figure 16: Sequence of snapshot images demonstrating the transition from trapping to lysis and back to trapping. The sequence starts at 15V DC 145Vrms AC, which results in
trapping (a). The DC is increased to 25V resulting in lysis through the constriction (b) and later (c). The DC is then reduced to 15V again resulting in trapping (d) and later (e). The time at which each snapshot was taken is displayed on each image in the sequence.

When the test began, the DC voltage was set at 15V DC consistent with the trapping condition previously determined in the voltage adjustment study. At this ratio of AC to DC, the electrokinetic motion could not overcome the opposing dielectrophoretic motion, and cells were trapped in front of the constriction as can be seen in Figure 16 (a). Once enough red blood cells had accumulated in front of the constriction, the DC voltage was increased to 25V DC. This increase in DC caused the electrokinetic motion to become dominant and cells passed through the constriction and were lysed. The start of this process can be seen in Figure 16 (b), where the accumulation in front of the constriction has started to become smaller as cells have began to move through the constriction and lyse. As lysis was allowed to continue, all of the previous accumulation caused by trapping passed through the constriction, which can be seen in Figure 16 (c). At this point, the DC voltage was once again reduced to 15V DC and the dielectrophoretic motion again became dominant resulting in trapping. This can be seen in Figure 16 (d) where trapping has caused red blood cells to begin accumulating in front of the constriction once more. After allowing this experiment to run longer under these conditions, the accumulation caused by trapping continued to grow as can be seen in Figure 16 (e). Thus, with a small 10V adjustment in DC while keeping the AC field fixed, the transition between trapping and lysis can be realized in this microchannel.

As the applied DC voltages responsible for the electrokinetic movement of the cells in this experiment are very low, special care must be taken to remove pressure
driven flow prior to each test as it will have a significant effect on the DC voltage adjustment required for trapping and lysis. This effect could be reduced by increasing the size of the reservoirs used in the microchannel. During the trapping phases, it was observed that the trapped cells could form chains in front of the constriction. In experimentation, chains were harder to force through the constriction than single cells when switching from trapping to lysis.

4.4.3 Continuous Separation of Leukemia Cells from Red Blood Cells

After demonstrating the transition between trapping and lysis by a small DC adjustment, both trapping and lysis were used in conjunction to demonstrate a separation of Leukemia cells from red blood cells. As the dielectrophoretic motion is a function of the square of the cell radius, it follows that larger cells experience a higher dielectrophoretic force at the same electric field magnitude. In this case, as the Leukemia cells are larger than the red blood cells, there should exist an AC to DC ratio, where the Leukemia cells can be trapped and the red blood cells can move through the constriction. Therefore, an experiment was performed in which the DC voltage was set to 30V DC and the AC voltage was set to 170Vrms AC. When a combined sample of Leukemia cells and red blood cells was introduced into the channel under these conditions, the red blood cells moved through the constriction and were lysed, while the Leukemia cells experienced a higher opposing dielectrophoretic force and were trapped in front of the constriction. Figure 17 shows the results from this experiment.
Figure 17: Snapshot image demonstrating the separation of Leukemia cells from red blood cells. The experimental results (a) show the larger Leukemia cells trapped in front of the constriction, while the smaller red blood cells pass through the constriction and are lysed. The simulation results (b) show the predicted cell trajectories of the red blood cells and a trapping zone for the Leukemia cells. The trapping zone represents the predicted region where the Leukemia cells should be trapped in front of the constriction.

Figure 17 (a) shows a snapshot of this process where the Leukemia can be seen trapped in front of the constriction and the blood cells can be seen passing through the constriction and lysing. This results in a continuous separation process by trapping the Leukemia and lysing the red blood cells once they move through the constriction. The numerical modeling results for the test are shown in Figure 17 (b), where the blue lines
are the cell trajectories on the Red Blood Cells, and the contour of the trapping zone is included for the Leukemia cells. By comparing Figure 17 (a) to Figure 17 (b), one can see that the path of the red blood cells matches that of the experiment while the Leukemia cells are trapped in the trapping zone predicted by the simulation. One current issue that makes this separation process difficult at times during experiments is the interaction between the blood cells and Leukemia cells. As Leukemia cells are trapped in front of the constriction, some red blood cells may attach to the Leukemia during the process. If enough of these red blood cells attach, it is possible for them to push the Leukemia cell through the constriction.

4.4.4 Joule Heating

As the working buffer in these experiments was 1xPBS, which is highly conductive, and as the constriction region amplifies the local electric field, the experiments were monitored for Joule heating. In doing so, the current was measured as soon as the electric field was applied and monitored every 10 seconds for 2 minutes to observe the rise in current over time. Under the maximum applied 16kV/m electric field (160 kV/m in the constriction), the current on application was about 250 μA and rose to approximately 260 μA after one minute, where the increase after this point became minimal.

4.5 Summary

The process of trapping and lysing red blood cells in a hurdle channel using negative dielectrophoresis has been studied, and the ability to switch between trapping and lysing by making a small adjustment to the applied DC voltage without significantly
changing the overall electric field was demonstrated. Further, both trapping and lysis were used in conjunction to demonstrate a separation between Leukemia cells and red blood cells in this microchannel. This process could be envisioned to be highly useful for future biomedical applications.
CHAPTER 5: Conclusions and Future Work

This thesis presents multiple studies using electrokinetic transport and the dielectrophoretic force induced by the microchannel geometries to manipulate cells and particles. The second chapter examines the use of negative dielectrophoresis to focus yeast cells in a serpentine microchannel and performed a parametric study in order to determine how different parameters contributed to the effectiveness of the negative DEP focusing. From this parametric study, it was determined that increasing the electric field magnitude, increasing the ratio of applied AC rms voltage to DC, increasing the buffer concentration, and avoiding high cell concentrations were all methods of improving focusing effectiveness. This work should be very useful for those looking to optimize focusing in microchannels using negative DEP for either optical detection or separation.

In addition, the research on this project showed how negative DEP could be used to perform a filtration of *E. coli* from yeast cells by taking advantage of the difference in size between the two types of cells. The next step in this area could be to examine the possibility of using positive dielectrophoresis in conjunction with negative dielectrophoresis to achieve a complete separation between cells in this channel. Additionally, a new channel geometry could be designed such that complete separation could be achieved using only negative dielectrophoresis.

The third chapter of this thesis examined both positive and negative dielectrophoresis on particles in a serpentine microchannel. The purpose of this work was to demonstrate how dielectrophoresis could be used to separate particles based on
their conductivity and size. Negative and positive DEP were used in conjunction to demonstrate a separation between different particles. Whereas most separation methods require both particle types to start from a single focused stream to which a deflective force is applied, this method was shown to work regardless of the starting position of the particles. Future work for this project would include applying positive and negative DEP separation to biological cells.

The fourth chapter in this thesis examined the trapping and lysing of red blood cells in a microchannel constriction using negative dielectrophoresis. This work first examined the lysing process by showing the effects of the duration of exposure to the electric field by way of adjusting the applied AC to DC field ratio. It also served to highlight one of the benefits of using a DC biased AC electric field as the velocity may be controlled without the need for an additional counter pressure driven flow. Further, this work demonstrated that a small voltage adjustment could be used to transition between trapping and lysis operations. Such a transition could be useful for biological analysis where the cell concentration must first be increased prior to examining the intracellular contents. Lastly, this project took advantage of the dependence of DEP on cell size in order to demonstrate a separation of red blood cells from Leukemia cells. In this separation, Leukemia cells were trapped infront of the microchannel constriction while red blood cells passed through the constriction and were lysed. Future work in this area could be geared towards dealing with some of the cell interaction problems that make this continuous separation challenging, as well as optimizing the channel geometry for more effective cell lysis.
Electrokinetic manipulation of cells and particles in microchannels has great potential with regards to biomedical applications. DC biased AC electric fields have been demonstrated to perform focusing, separation, trapping, and lysis operations without the need for additional external forces or built in microstructures. In addition, these electric fields have been demonstrated to work effectively both at low magnitudes such that the cells are not harmed in the process and at high magnitudes when cell lysis is needed. Future work could entail improving these methods by optimizing the channel geometries for each desired manipulation technique as well as applying the positive and negative DEP separation technique demonstrated in chapter 3 to biological cells.
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APPENDIX A: Microchannel Fabrication

In order to make the photomask, the channel geometry was drawn in AutoCAD® and printed onto a transparent thin film at a resolution of 10,000 dpi (CAD/Art Services, Bandon, OR). Photoresist was applied to a clean glass slide by spin-coating (WS-400E-NPP-Lite, Laurell Technologies, North Wales, PA) at a terminal speed of 2000 rpm, which yielded a nominal thickness of 25 µm. After spin-coating, the slide was baked on hotplates (HP30A, Torrey Pines Scientific, San Marcos, CA) using a two-step soft bake (65 °C for 3 minutes and 95°C for 7 minutes). The photoresist film was then exposed to near UV light (ABM Inc., San Jose, CA) through the negative photomask before being subjected to another two-step hard bake (65 °C for 1 minute and 95°C for 3 minutes). Following the hard bake, the photoresist was developed in SU-8 developer solution for 4 minutes, which left a positive replica of the microchannel on the glass slide. After briefly rinsing the slides with Isopropyle alchahol, the slides were subjected to one final hard bake at 150 °C for 5 minutes. The cured photoresist was then ready for use as the mold of the microchannel.

The channel mold was placed into a Petri dish and covered with liquid PDMS before being degassed for 30 minutes in an isotemp vacuum oven (13-262-280A, Fisher Scientific, Fair Lawn, NJ). Following the degassing, the liquid PDMS was cured in a gravity convection oven (13-246-506GA, Fisher Scientific, Fair Lawn, NJ) for 2 hours at 70°C. Once cured, the PDMS covering the microchannel was cut with a scalpel and peeled off of the mold. Next, two holes were punched into the PDMS cast to serve as
reservoirs. The channel side of the PDMS and a clean glass slide were then plasma treated (PDC-32G, Harrick Scientific, Ossining, NY) for one minute. Immediately after the plasma treating, the two treated surface were bonded irreversibly to make the microchannel. Once sealed, the working buffer was dispensed into the channel by capillary action to prime the channel and maintain the wall surface properties.