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ELECTROKINETIC TRANSPORT AND MANIPULATION OF PARTICLES IN CURVED MICROCHANNELS

Junjie Zhu
Clemson University, junjiez@clemson.edu

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ELECTROKINETIC TRANSPORT AND MANIPULATION OF PARTICLES IN CURVED MICROCHANNELS

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Presented to
The Graduate School of
Clemson University

In Partial Fulfillment
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Junjie Zhu
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Accepted by:
Dr. Xiangchun Xuan, Committee Chair
Dr. Chenning Tong
Dr. Richard Miller
Dr. Rui Qiao
ABSTRACT

The investigation of electrokinetic particle transport in confined microchannels has practical significances in a variety of applications ranging from traditional gel electrophoresis to electrokinetic microfluidics-based lab-on-a-chip devices. To date, however, studies on particle electrokinetics have been limited to primarily theoretical or numerical analyses in straight microchannels of simple geometries. Very little work has been done on electrokinetic particle motions in real microchannels which usually consist of one or multiple turns. This thesis is dedicated to the fundamental and applied studies of electrokinetic transport and manipulation of particles in various curved microchannels using a combined experimental, theoretical, and numerical method.

First, a fundamental study of particle electrokinetics in a microchannel U-turn, a typical unit in LOC devices, was investigated. A 2-D numerical model based on finite element method was developed to understand and predict the particle motion within the U-turn. It is demonstrated that particles are deflected to the outer wall of the turn by curvature-induced dielectrophoresis (termed cDEP) due to the locally intrinsic electric field gradients. Moreover, this lateral displacement increases with the rise of either the applied electric field or the particle size.

Next, we utilize the cDEP in microchannel turns to implement a continuous electrokinetic focusing of particles in serpentine microchannels. Particles are demonstrated to gradually migrate to the centerline due to the periodically switched dielectrophoretic force they experience in a serpentine microchannel. This electrokinetic focusing favors large electric fields and large particles, and also increases when the number of serpentine periods increases. Such focusing also takes place in a spiral microchannel, where, however, particles are eventually focused to a stream flowing near the outer sidewall of the channel.
Then, we explore the applications of cDEP to continuous electrokinetic separation of particles in curved microchannels. We develop two different approaches based on what we have acquired from the studies of particle electrokinetics in serpentine and spiral microchannels. The first approach employs a sheath flow to focus particles to one sidewall of a serpentine microchannel, where particles are then deflected to different flow paths by cDEP and thus sorted at the exit of serpentine section. We use this method to separate particles and cells by size at low DC electric fields. The second approach eliminates the sheath flow focusing of particles by the use of particle deflection and focusing in a double-spiral microchannel. Specifically, particles are focused by cDEP to one single stream near the outer wall of the first spiral, which is then displaced by cDEP and divided into two or more sub-streams in the second spiral, enabling the continuous sorting. We use this approach to implement the separation of particles by size and by charge, respectively. Moreover, we also demonstrate a continuous ternary separation of particle by size and charge simultaneously.
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NOMENCLATURE

$\alpha$  \quad \text{ratio of the applied RMS AC field to the DC field}

c  \quad \text{numerical modeling correction factor}

$\varepsilon$  \quad \text{dielectric constant of the liquid}

$\varepsilon_f$  \quad \text{permittivity of the suspending fluid}

$\phi$  \quad \text{electric potential}

$E$  \quad \text{applied electric field}

$E_{AC}$  \quad \text{RMS value of the AC field}

$E_{DC}$  \quad \text{DC component of the applied electric field}

$f_{CM}$  \quad \text{Clausius–Mossotti (CM) factor}

$f_{p-w}$  \quad \text{coefficient for wall induced particle velocity}

$F_{DEP}$  \quad \text{dielectrophoretic force}

$\lambda$  \quad \text{numerical modeling correction factor}

$\mu$  \quad \text{fluid viscosity}

$\mu_f$  \quad \text{fluid dynamic viscosity}

$\mu_{DEP}$  \quad \text{dielectrophoretic mobility}

$\mu_{EK}$  \quad \text{electrokinetic mobility}

$u_{eo}$  \quad \text{electroosmotic velocity}

$u_{ep}$  \quad \text{electrophoretic velocity}

$U_c$  \quad \text{cell velocity}

$U_{EK}$  \quad \text{electrokinetic velocity}
\( U_{DEP} \)  dielectrophoretic velocity
\( U_p \)  particle velocity
\( \mathcal{R} \)  radius of channel curvature
\( \sigma_p \)  electric conductivity of the particle
\( \sigma_f \)  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

Over the last three decades, considerable advancement has been made in the field of miniaturization of all kinds of systems, such as mechanical, fluidic, electromechanical, or thermal down to microns [1]. In the beginning of the 1980s, a distinct new field, micro-electromechanical systems (MEMS), which typically refers to the mechanical devices driven by electricity, was born due to those achievements. First emerging as the natural generalization of the existing MEMS and further evolving into a brand new discipline in 1990s, microfluidics has been broadly employed in the development of inkjet print heads, DNA chips, Laboratory-on-a-Chip (LOC) technology, micro-propulsion, and micro-thermal technologies [2]. Microfluidics-based lab-on-a-chip devices manipulate fluids and samples in channels with dimensions of tens of micrometers, which has promised to offer numerous advantages over conventional analysis techniques, for instance, small quantities of samples and reagents; implementing separations and detections with high resolution and sensitivity; low cost; short time for analysis; small footprints for the analytical devices [3].

Generally, particle manipulation includes pumping, focusing, trapping, concentration, separation, and sorting, etc. As an overview, pumping is the most fundamental manipulation in microfluidic devices, and refers to moving fluids around on the chip from the original position they were introduced to the place they will be collected. Focusing refers to aligning scattered particles to a single file within a microchannel. Trapping and concentration refer to immobilizing
one or many particles to stagnation points for the ease of detection. Separation and sorting refer to transforming a mixture of particles into two or more distinct sub-streams.

Various force fields have been demonstrated to implement particle manipulation in microfluidic devices, which include hydrodynamic, magnetic, electric, acoustic, optical, and gravity etc. Among these methods, electrokinetic flow, which transports particles by application of electric fields, has received much attention for its simplicity and effectiveness in performing these operations. To date, however, studies of particle electrokinetics have been limited to primarily theoretical or numerical analyses in straight microchannels of simple geometries [5]. Very little work has been done on electrokinetic particle motions in real microchannels that may have one or multiple turns for reducing the devices size. Therefore, this thesis is dedicated to the study of electrokinetic transport and manipulation of particles in curved microchannels. The goals are to obtain a fundamental knowledge of electrokinetic particle transport in microchannel turns, and to explore the applications of microchannel turns as passive control elements of particle manipulation in microfluidic systems.

1.2 Background on Electrokinetic Phenomena

The first observation of electrokinetic phenomena dates back to the studies conducted by Reuss in 1809, however, the first theoretical developments of electrokinetic transport are attributed to Helmholtz and Smoluchowski [6], [7]. The follow-up development of these early works has been summarized by Dukhin and Derjaguin [8]. Since then, considerable attention has been conferred on electrokinetic phenomena.

Fluid handling devices, particularly pumps which operate in the macroscopic regime are incapable of handling the requirements of the microscopic transport realm since one of the
biggest challenges is the enormous pressure gradients are necessary to drive a fluid through a capillary of microscopic radius [9]. As a result, enormous effects have been made towards the exploration of alternative approaches of fluid transport in microchannels. Aside from pressure-driven, electric-field-driven flow was another most frequently adopted techniques applied to micro-scale transport. As liquid mediums are used frequently in handling particles, cells and other biological samples, colloidal systems and colloidal phenomena become a prerequisite study prior to manipulating them. Therefore, almost surely, electrokinetic transport in colloidal system became the most suitable substitute for providing the motive force to the fluids in microscopic domains without requiring large pressure gradients. The general classification of electrokinetic phenomena is the following: 1. Electro-osmosis which refers to the movement of an ionized fluid under the effect of an electric field. 2. Electrophoresis which refers to the movement of charged particles by an electric field in a resting fluid. 3. Streaming Potential which refers to the inverse of electro-osmosis, i.e. an electric field is induced by the circulation of an ionized fluid. 4. Sedimentation Potential which refers to the inverse of electrophoresis, i.e. an electric field is created by the movement of charged particles in a fluid. 5. Dielectrophoresis which refers to the movement of neutral particles by the application of an electric field [1]. This thesis will focus on electrokinetic phenomena using an applied electric field to induce motion and hence accompanying background on electrokinetic transport, electro-osmosis, electrophoresis, and dielectrophoresis will be discussed in depth as follows [10].

1.2.1 Electric Double Layer

Every fundamental phenomenon of the electrokinetic transport of particles in microchannels originates from the electric double layer (EDL). The EDL is formed in order to neutralize the charged surface and, in turn, causes an electrokinetic potential between the surface
and any point in the mass of the suspending liquid. This voltage difference is usually on the order of mill volts and is referred to as the surface potential or zeta potential $\zeta$. The magnitude of the surface potential is related to the surface charge and the thickness of the double layer.

When a microchannel surface subjected to a polar or aqueous medium, it is usually negatively charged. The formation of such interfacial charge could be explained by several important mechanisms: 1. Ionization of surface groups. 2. Differential dissolution of ions from surfaces of sparingly soluble crystals. 3. Isomorphic substitution. 4. Charged crystal surfaces. 5. Specific ion adsorption [9]. As a result, the free ions in the solution will be preferentially redistributed induced by the presence of charged surfaces. Specifically, counter-ions will be attracted to and co-ions be repelled from a charged surface simultaneously where the two layers on the interface were well known as EDL. The layer on the liquid side can be further divided into stern layer in which ions are immobilized and diffuse layer where ions are free to move. Figure 1 illustrates the formation of an EDL within the presence of a positively charged surface and the corresponding electric potential distribution.
Figure 1: Non-uniform electric double layer (EDL) ion distribution and corresponding potential for an electrolyte in the presence of a surface with surface charge $\zeta$, reprinted from [9].

The dimension of the potential distribution, in the transverse direction away from the shear plane, is characterized by the inverse Debye-screening length, $\kappa$, defined as:

$$\kappa = \sqrt{\frac{2z_v^2e^2n_\infty}{ek_bT}}$$

(1-1)

Where $n_\infty$ is the bulk ionic concentration, $k_b$ is Boltzmann’s constant, $T$ is the fluid temperature, $z_v$ is ionic valence, $e$ is the elementary charge, and $\varepsilon$ is the dielectric constant in the medium. Typically, the dimension of the EDL is within the range of $\frac{3\zeta_0\varepsilon}{\kappa}$ and on the order of several nanometers.
1.2.2 Electrokinetic Transport

Electrokinetic pumping techniques are widely used to move liquids and particles at micro-scales because they are implemented through surface forces, which scale well when length scales are reduced. It also has the advantage of being easily integrable into microfluidic systems when compared to external systems such as syringe pumps. Electrokinetics is a general term associated with the relative motion between two charged phases. Electrokinetic phenomena occur when one attempts to shear off the mobile part of the electric double layer. Then, as the charged surface (plus attached material) tends to move in the appropriate direction, the ions in the mobile part of the electric double layer undergo a net migration in the opposite direction, carrying solvent along with them, thereby causing the movement of the solvent. As we pointed out at the beginning of chapter 1, four types of electrokinetic phenomena (electro-osmosis, streaming potential, electrophoresis, and sedimentation potential) are more commonly encountered in electrokinetic phenomena due to relative motion between charged phases and electrolytes. These four types of electrokinetic phenomena are all based on the formation of electric double layer and two of them will be described as follows [9].

1.2.3 Electro-osmosis

When an ion-containing fluid (for example, water) is placed in a microchannel that has fixed charges on its surface (such as silicon dioxide or surface-oxidized PDMS), EDL will be formed according to aforementioned mechanism. Electro-osmosis is the bulk fluid motion induced by the migration of the excess counter ions within the EDL when external electric field applied along the microchannel. More specifically, in the presence of an applied electric field, the ions are subjected to an electrical force which acts tangential to the electric field lines and
serves to move them towards corresponding electrodes. Due to viscous effects, the ions drag the 
non-charged liquid molecules as they move through the channel and hence forming a plug like 
flow profile when there is no overlapping of EDL. (See Figure 2) The Electro-osmotic velocity 
profile of a fluid can be easily derived from the incompressible, steady state, fully developed, 
Navier-Stokes equation, with the addition of an electrical body force term [4]:

\[
0 = -\frac{\partial p}{\partial z} + \mu \left( \frac{\partial^2 u}{\partial y^2} \right) + E_z \rho_e 
\]

(1-2)

Where, \( z \) is the dimension along the length of the channel, \( y \) is the transverse dimension, \( E_z \) is 
the applied electric field(V/m), \( \mu \) is the fluid viscosity, \( dp/dz \) the pressure gradient, \( u \) is the fluid 
velocity and \( \rho_e \) is the net charge density within the channel which can be expressed through the 
Poisson equation:

\[
\rho_e(y) = -\varepsilon \frac{d^2 \psi}{dy^2}(y) 
\]

(1-3)

Plugging Equation (1-3) into Equation (1-2) and solving for the electroosmotic flow velocity \( u_{eo} \), 
in the absence of an applied pressure gradient with boundary conditions set such that: \( \psi(h) = \psi(-h) = \zeta \) and \( u(h) = u(-h) = 0 \) yields [4]:

\[
u_{eo} = -\frac{\varepsilon \zeta}{\mu} \left( 1 - \frac{\psi}{\zeta} \right) (E_z) 
\]

(1-4)
1.2.4 Electrophoresis

Likewise, when a particle with non-zero net charges suspended in a bulk medium, the particle will move relative to the suspending medium due to electrostatic force as an electric field applied. Such particle motion is so called electrophoresis. (See Figure 3) Because the same operating mechanism that drives electroosmotic flow is also responsible for electrophoresis, we can derive the electrophoretic velocity of a particle, with electro-static surface charge, $\zeta_p$, suspended in a bulk fluid within the presence of a thin double layer and an applied electric field $E$ to be:

$$u_{ep} = \frac{\varepsilon \zeta_p}{\mu} E$$  \hspace{1cm} (1-5)

Here, the difference between Equations (1-4) and (1-5) is that the zeta potential of the wall, $\zeta_{wall}$, is replaced by the zeta potential of the particle, $\zeta_p$. In the case of a uniform applied electric field the particles will follow the electric field lines.
Figure 3: Electrophoresis of a charged particle in an external electric field, reprinted from [9].

Usually, the electrophoretic and electroosmotic motions in microchannels are opposite to each other in flow 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.

### 1.2.5 Dielectrophoresis

Dielectrophoresis (DEP) is another important motion originated from the electric field gradient which usually occurs during electrokinetic transport of particles and resulting in translational particle motion across fluid streamlines. The dielectrophoretic force induced on a spherical particle in a DC electric field is given by [11]:

$$ F_{DEP} = (1/2) \pi \varepsilon_f d^3 f_{CM} (\mathbf{E} \cdot \mathbf{V}E) $$

(1-6)

where $\varepsilon_f$ is the permittivity of the suspending fluid, $d$ the particle diameter, $f_{CM}$ the so-called Clausius–Mossotti (CM) factor, $\mathbf{E}$ the electric field.
As seen in equation (1-6), the dielectrophoretic force scales with the third power of the diameter of particles. Therefore, it is a very useful technique in manipulating particles based on their size differences when other parameters are specified. This advantageous property of DEP can be fully used for particle manipulation and will be further studied in the following chapters, where the dielectrophoretic force induced by curved microchannels during electrokinetic flow is used to either focus or separate particles.

1.3 Literature review on particle electrophoresis in microchannels

Distinct from the classical particle electrophoresis in an unbounded and stationary liquid [63],[64], the presence of solid walls in microchannels causes at least three effects on the electrophoretic motion: (a) generating an electroosmotic flow of the suspending liquid due to the walls’ non-zero charge [65]; (b) enhancing the viscous retardation of particles due to the walls’ non-slip velocity [66]; (c) altering the electric field (and thus the flow field) distribution around particles due to the walls’ non-conducting condition [67]. Moreover, the last effect may induce particle dielectrophoresis as a result of polarization in non-uniform electric fields, which occurs in two circumstances [11]: one is when the particle moves near a wall such that the electric field around the particle is significantly distorted [68],[69], and the other is when the microchannel has variable cross-sections such that the applied electric field is intrinsically non-uniform [44],[56].

In straight microchannels with uniform cross-sections, liquid electro-osmosis and particle electrophoresis remain unvaried along the flow direction. Particle dielectrophoresis is generally negligible. In the limit of thin electric double layers (as compared to the particle size, of course, even thinner as compared to the channel dimension), many theoretical and numerical studies
have been conducted to determine the electrophoretic velocity of spherical or cylindrical particles moving close to a planar wall [70]-[72], or in a slit or cylindrical pore [73]-[82]. The predicted decrease in particle velocity due to the wall effects has been verified experimentally [45], [83]. These retardation effects become more significant at larger double layer thicknesses [84]-[86], which agree qualitatively with a recent measurement [87]. When particles move in close proximity to a channel wall, however, the predicted wall effect is to enhance the particle electrophoretic motion [70], [72], [74], [88], [89]. This enhancement has been verified by Xuan et al. [87] in an experiment on particle electrophoresis in cylindrical capillaries.

In straight microchannels with variable cross sections, the applied electric field becomes non-uniform, causing variations in both liquid electro-osmosis and particle electrophoresis. Meanwhile, particle dielectrophoresis is no longer negligible unless the particle is small (e.g., point particles [90], [91]) and the imposed electric field is low. In a recent experiment on the electrophoretic motion of micro-particles in a converging–diverging microchannel, Xuan et al. [92] observed that the ratio of particle velocity in the throat to that in the straight part is significantly lower than their cross-sectional area ratio. Moreover, this ratio is a strong function of both the applied electric field and the particle size. All these phenomena, as confirmed numerically by Qian’s group [93], [94], are the consequences of particle dielectrophoresis induced in the channel throat region. More recently, particles were observed to migrate across streamlines by dielectrophoresis in microchannel constrictions formed by non-conducting posts, hurdles, or oil droplets [23], [101]-[103]. The result is a narrower or focused particle stream downstream of the constriction. If strong DC or DC-biased AC electric fields are applied, particles may even be trapped at the entrance of the constriction [23], [44], [56], [102], [104].
1.4 Overview of thesis

This thesis consisting of seven chapters will use a combined experimental, numerical, and theoretical method to achieve our objectives. Chapter 2 will be a fundamental study of particle electrophoresis in a single microchannel turn, i.e. a U turn. Following that will be the exploration of microchannel turns as passive control elements in microfluidic systems. Specifically, application studies of particle focusing in serpentine and spiral microchannels will be performed in the chapter 3 and 4, respectively. Chapter 5 investigates the combination of electrokinetic sheath flow and dielectrophoresis to perform continuous separation of particles by size in a serpentine microchannel. Based on the demonstrated particle focusing in a symmetric spiral channel in chapter 4, chapter 6 further studies continuous separation of particles by size in an asymmetric double spiral microchannel. Chapter 7 expands the use of spiral channels to separating particles by charge. In chapter 8, a brief overview of the key contribution of the thesis is given, and several future projects stemming from these findings are proposed.
CHAPTER 2: Fundamental Study of Particle

Electrophoresis in a U-turn

2.1 Background

In curved microchannels the applied electric field becomes non-uniform due to the variation of path length in the channel width direction, i.e., the higher electric field occurs close to the inner wall of a curved channel due to the shorter path length for electric current. Therefore, both liquid electro-osmosis and particle electrophoresis vary with positions within a curved microchannel, leading to increased band broadening of point-like solute particles via hydrodynamic dispersion [105]. For particles with finite sizes, dielectrophoresis may take effects if the applied electric field is not too small. Davison and Sharp [106] numerically examined the electrophoretic motion of a cylindrical particle through a 90° turn. While the full hydrodynamic interactions between the particle and fluid were considered, the dielectrophoretic force induced within the turn was ignored. Dielectrophoresis was also neglected in an earlier numerical study of particle electrophoresis in a T-shaped microchannel, where the applied electric field is non-uniform at the T-junction [107]. Such treatment may cause errors to the computed particle velocity as discussed in Xuan and Li’s recent experiment [35].

U-turn, the basic unit of serpentine microchannels, is commonly used to switch the transport direction of fluids and particles in LOC devices. The objective of this chapter is to perform an experimental and numerical study of particle electrophoresis in a U-turn. Subjected to external electric field, polystyrene micro-beads are found to migrate across streamlines and flow laterally towards the outer wall caused by negative dielectrophoretic force they experience. This
transverse deflection arises from the dielectrophoretic particle motion induced by the non-uniform electric fields intrinsic to curved channel turn.

2.2 Experiment

2.2.1 Microchannel Fabrication

The straight channel with a U-turn right in center was fabricated with PDMS using the standard soft lithography method [27], and the detailed fabrication process can be referred to in Appendix A. The microchannel for the experiments consists of three straight sections connected by a U-turn and an L turn respectively with two wells on two ends which were serving as reservoirs as indicated in Figure 4.

Figure 4: Picture of a PDMS-based microchannel with a U-turn (filled with green food dye for clarity) used in the experiment. The inset is a schematic view of the channel with actual dimensions. The width of the channel is 50 μm, and the radii of the inner and outer corners are 10 and 60 μm respectively.
The U-turn of the microchannel is used to produce the negative dielectrophoretic deflection of particles along the channel centerline as explained in the theory section. The total length of the microchannel is 10 mm, and the width and depth are 50 µm and 25 µm, respectively, throughout the entire channel.

2.2.2 Particle Solution Preparation

Green fluorescing polymer microspheres of 1 µm (Bangs laboratories, USA, Solid content: 1%), 5 and 10 µm in diameter polystyrene beads (Sigma-Aldrich, St. Louis, MO, USA, Solid content: 2.5%) were re-suspended in 1 mM phosphate buffer respectively with a concentration of about $10^6$ particles per milliliter. Tween 20 (0.5% v/v, Fisher Scientific) was added to the particle solution to suppress particle adhesions to channel walls as well as particle aggregations. The calculated electric conductivities of these three types of particles are 40, 8 and 4 µS/cm, respectively, if the surface conductance is assumed to be 1 nS. As the electric conductivity of the buffer solutions (200 µS/cm for 1mM phosphate buffer) is much higher than that of the particles, the CM factor, $f_{CM}$, was found close to −0.5 for all types of particles.

2.3 Theory

First we study the variation of particle speed and trajectory along channel centerline during the electrophoretic motion through a single U-turn in a microchannel of uniform width and height (see Figure 5). This fundamental study developed experimental and theoretical bases for the subsequent studies of particle electrophoresis in curved microchannels we will present later in chapter 3 and 4. To be consistent with typical electrokinetic microfluidic devices, the applied electric field was kept no more than 100 kV/m, at which the particle speed, $U_p$, and the fluid
speed, \( U_f \), will be generally smaller than 10 mm/s [12][13]. Therefore, the inertial effects of particles and fluid can be neglected because the channel Reynolds number, \( R_c \), and the particle Reynolds number, \( R_p \), are both much less than 1. Therefore, the bulk motion of fluid is laminar flow throughout the entire microchannel. Given that the Debye-screening length and corresponding thickness of EDL are on nanoscale order, the flow velocity profile can be considered as plug-like and hence slip boundary condition on channel sidewall. In addition, limiting the applied electric field will also help to reduce the Joule heating effects unless a high solution concentration is to be used in a large channel [14]. This extreme circumstance will be avoided throughout the research.

In order to clarify the channel turn-induced impact on particle electrophoresis, we need to identify the forces on the particle that may be newly created within a channel turn. Figure 5 shows the electric field lines (arrows indicate the direction) and the contour of electric field intensity (the darker the higher) in a microchannel U-turn in the absence of particles. It is apparent that the electric field becomes non-uniform and attains the maximum and minimum values at the inner and outer walls of the turn, respectively. Thus, particles will encounter a dielectrophoretic force, \( \mathbf{F}_{\text{DEP}} \), when they move through the turn. In DC electric fields, \( \mathbf{F}_{\text{DEP}} \) on an isolated spherical particle is approximated to the leading order as [15]

\[
\mathbf{F}_{\text{DEP}} = \left(1/2\right) \pi \varepsilon_f d^3 f_{CM} \mathbf{E} \cdot \nabla \mathbf{E} \tag{2-1}
\]

\[
f_{\text{CM}} = \left(\sigma_p - \sigma_f\right) / \left(\sigma_p + 2\sigma_f\right) \tag{2-2}
\]

where \( f_{\text{CM}} \) is the Clausius-Mossotti (CM) factor, \( \sigma_p \) is the electrical conductivity of particles, and \( \sigma_f \) is the fluid conductivity. When \( \sigma_p < \sigma_f, f_{\text{CM}} < 0 \) leading to a negative dielectrophoresis, i.e., particles are directed towards the lower electric field region. For the polystyrene beads to be used
in this research, \( \sigma_p = \sigma_b + 4K_s/d \) is the sum of bulk conductivity, \( \sigma_b \approx 0 \) (non-conducting), and surface conductivity with the surface conductance, \( K_s \approx 1 \) ns [16], hence, \( \sigma_p \leq 40 \mu\text{S/cm} \) for particles with diameter \( d \geq 1 \mu\text{m} \). In this research, we will adjust the conductivity of the physiological solution to ensure \( \sigma_p \ll \sigma_f \) and thus negative particle dielectrophoresis (\( f_{CM} \approx -1/2 \)). This is designed to accord with the dielectrophoresis of cells as they behave like poor conductors in DC electric fields [17], [18]. Therefore, the results obtained from this section will be directly useful to cell electrophoresis in curved microchannels, which is envisioned to have much broader applications.

**Figure 5:** Illustration of the dielectrophoretic forces, \( \mathbf{F}_{DEP} \), experienced by a particle during its electrokinetic motion through a U-turn. The background shows the contour of electric field intensity (the darker the higher). The arrowed curves show the electric field lines.

By balancing \( \mathbf{F}_{DEP} \) with the Stokes drag force, one can get the particle dielectrophoretic velocity, \( \mathbf{U}_{DEP} \), which will be superimposed to \( \mathbf{U}_{EK} \). Namely, the real particle velocity of
electrokinetic motion within a microchannel turn will be \( \mathbf{U}_p = \mathbf{U}_{DEP} + \mathbf{U}_{EK} \). In streamline coordinates [19], \( \mathbf{U}_p \) may be conveniently broken down into

\[
\mathbf{U}_p = (\mathbf{U}_{DEP,s} + \mathbf{U}_{EK}) \hat{s} + \mathbf{U}_{DEP,n} \hat{n}
\]  

where \( \hat{s} \) and \( \hat{n} \) are the unit vectors along and normal to the streamlines (similar to the electric field lines shown in Figure 5 [20], [21]), respectively. The velocity terms in Eq. (2-3) are given by

\[
\mathbf{U}_{DEP,s} = \mu_{DEP} \mathbf{E} \nabla / \partial s
\]  

(2-4)

\[
\mathbf{U}_{EK} = \mu_{EK} \mathbf{E}
\]  

(2-5)

\[
\mathbf{U}_{DEP,n} = \mu_{DEP} \mathbf{E}^2 / \mathcal{R}
\]  

(2-6)

\[
\mu_{DEP} = \varepsilon_f \rho^2 f_{CM} / 6 \mu_f
\]  

(2-7)

where \( \mathbf{E} = |\mathbf{E}| \), \( \mathcal{R} \) is the radius of curvature of the streamline which should follow closely the radius of curvature of the channel turn when fluid inertia is negligible, and \( \mu_{DEP} = \mathbf{F}_{DEP} / (\mathbf{E} \cdot \nabla \mathbf{E}) \) is the dielectrophoretic mobility. Eq. (2-3) indicates two effects of a microchannel turn on particle electrophoresis due to the induced non-uniform electric field (see Figure 5): (1) particles are deflected across streamlines from the inner wall to the outer wall of a channel turn due to the cross-stream dielectrophoretic motion, \( \mathbf{U}_{DEP,n} \), leading to variations in particle trajectory and speed (as \( \mathbf{U}_{EK} \) gets larger when a particle is further away from the inner wall of a turn); (2) other than the increase in \( \mathbf{U}_{EK} \) due to deflection, particle speed in Eq. (2-3) is also perturbed by the stream-wise dielectrophoretic motion, \( \mathbf{U}_{DEP,s} \), though at a smaller magnitude (because the channel width remains constant within the turn). More importantly, as \( \mathbf{U}_{DEP,n} \) is a second-order function of electric field while the stream-wise particle speed, \( \mathbf{U}_{DEP,s} + \mathbf{U}_{EK} \), is only roughly a
linear function, a larger electric field will draw a greater deflection of particles within a microchannel turn. This favoring of large electric fields makes the particle manipulation in curved microchannels very appealing as the performance and the particle throughput can be enhanced simultaneously.

### 2.4 Numerical Modeling

In order to quantitatively understand and predict the observed particle deflection behavior, we developed a two-dimensional numerical model to simulate the electric field-mediated particle transport through the U-turn. This model is based on that developed by [22]. For simplicity, the perturbations of particles on the flow and electric fields (via moving boundaries in both fields due to the finite particle size) were both neglected in our model, so were the particle-particle interactions which could significantly saved numerical time. Instead, a correction factor $c$ was introduced to account for the effects of particle size (and others if any) on the dielectrophoretic velocity. As such, the particle velocity in Eq. (2-3) is revised as

$$\mathbf{U}_p = \mu_{\text{EK}} \mathbf{E} + c \mu_{\text{DEP}} (\mathbf{E} \cdot \nabla \mathbf{E}).$$

(2-8)

This velocity was used in the particle tracing function of plot parameters menu of postprocessing in COMSOL (Burlington, MA) to compute the particle trajectories. Particles were assumed massless and started at the centerline of channel inlet. As particle dielectrophoresis happens only in the channel width direction, a 2D model was employed to predict the particle focusing without considering the effects of top and bottom channel walls on particle motion. This treatment has been proved reasonable in recent studies [22], [23].
In our numerical model, the electrokinetic mobility, $\mu_{EK}$, was obtained by measuring the average particle velocity in the straight section of the microchannel, which is $2.5 \times 10^{-8}$ m$^2$/s for both 5 μm and 10 μm particles and $3.2 \times 10^{-8}$ m$^2$/s for 1 μm particles. The dielectrophoretic mobility, $\mu_{DEP}$, was calculated from Eq. (2-4) with the typical dynamic viscosity, $\mu = 0.9 \times 10^{-3}$ kg/(m·s) and permittivity $\varepsilon_r = 6.9 \times 10^{-10}$ C/(v·m) for pure water at 25 °C. The measured electric conductivity of the solution, i.e., $\sigma_f$ is about 200 μS/cm. Therefore, the CM factor, i.e., $f_{CM}$ was determined as −0.37, −0.46 and −0.48 for 1, 5 and 10 μm particles, respectively. Under the assumption of a uniform electric conductivity of the suspending fluid, the electric field $\mathbf{E} = -\nabla \phi$ was computed by solving the Laplace equation $\nabla^2 \phi = 0$. The boundary conditions include the voltage drop between the two channel ends and the insulating condition on the channel walls. The correction factor $c$ was determined by fitting the predicted particle trajectories to the observed particle streak line in the U turn.
2.5 Results and discussion

2.5.1 Electric Field Effects

Figure 6: Comparison of 5 µm particle trajectories passing through a U turn with a 50 µm uniform width at 10, 20 and 30 kV/m DC electric fields: (a) experimentally observed particle streak-line images and (b) numerically predicted particle trajectories in a U turn. Particles deflection increases with the rise of electric field due to the electric field dependence of dielectrophoretic forces $F_{DEP}$. The arrows show the flow direction.

Figure 6 shows the particle trajectories of 5 µm particles starting from the centerline of the entrance region migrating through a U turn at 10, 20 and 30 kV/m DC electric field respectively. The applied electric potential drops across the entire channel (1 cm straight length) are 100, 200 and 300 V for left, middle and right images, corresponding to a nominal electric field of 10, 20 and 30 kV/m respectively. Under different electric fields, the original position of particles located at the centerline of the channel width was selected for convenience since the
centerline can be easily used as a reference line. As shown in Figure 6, particles shared the same starting position prior to entering the U turn. However, they all deviated from the centerline after migrating through the U turn at dissimilar extents as predicted. It is apparently that the deflection of the particles increased as the rise of electric field as we illustrated before because the negative DEP force they experienced is proportional to electric field intensity. The correction factor $c$ was set to be 0.6 for 5 μm polystyrene beads and close agreements between the experimentally and numerically obtained trajectories of a single particle were found for all the three cases.
2.5.2 Particle Size Effects

Figure 7: Comparison of 1, 5 and 10 μm particle trajectories passing through a U turn at 20 kV/m DC electric field: (a) experimentally observed particle streak-line images and (b) numerically predicted particle trajectories in a U turn. Particles deflection increases with the rise of particle size due to the size-dependence of dielectrophoretic forces \( F_{DEP} \). The arrows show the flow direction.

As shown in Figure 7, the lateral displacement of particles increases as the rise of the particle size due to the second order relationship between the negative DEP force and particle size as illustrated in the theory part. Apparently, the DEP velocity induced by DEP force exerted on 1 μm particle can be neglected compared to electrokinetic velocity and they basically follow the streamline as if no deflection occurs when passing a U turn. However, when comes to 5 μm particles, the DEP force they experienced and hence the DEP velocity are 25 times larger such that an observable deviation of particle trajectory from the centerline is found downstream.
Likewise, the negative DEP force exerted on 10 μm particles was strong enough to deflect them close to the side wall under the same electric field as former two types of particles. Based on the model, we also simulated the trajectories of 1, 5 and 10 μm particles at a 20 kV/m DC electric field when they pass through a U turn which agree well with the experimental results and will be employed for further study of the particle motion in different structure of curved microchannels.

2.6 Summary

We have demonstrated particle electrokinetic transport through a U-turn would be perturbed by negative dielectrophoretic force induced by electric field gradient which are seldom considered in previous literatures. A 2-D numerical model was also developed to simulate the particle motion within a U-turn. Based on experimental and numerical results, it provided us potential use of similar structure for fundamental particle manipulation including focusing, separation etc. Therefore, our next chapter will be continuous particle transport and focusing in a serpentine channel in which each period can be considered as a combination of two U-turns. In addition, the final structure and dimensions of the serpentine channel were optimized by comparing the focusing performance at the exit region of microchannels based on the numerical model.
CHAPTER 3: Particle Focusing in Serpentine

Microchannels

3.1 Background on Particle Focusing

Focusing microparticles (both biological and synthetic) into a tight stream is usually a necessary step prior to separating and sorting them [24]-[27]. Particle focusing may be realized by using hydrodynamic [29]-[32] or electrokinetic [33]-[36] sheath flows to pinch the suspending medium and thus focus particles. This approach, however, requires a large amount of sheath fluid and a precise control of the flow rate of sheath fluid and particulate stream. Particle focusing may also be achieved by applying an external force, e.g., optical [37], acoustic [38], electrophoretic [39], or AC dielectrophoretic [40]-[43], to manipulate particles directly to their equilibrium positions. This approach, however, requires an additional pressure pumping of the particulate stream, not to mention the extra set-ups for generating the external forces. Moreover, the particle throughput is limited as the time for forces to act on particles decreases with increasing flow rate.

The concurrent pumping of the particle stream and focusing of particles have been realized by DC electrokinetic flow via the induced dielectrophoretic particle motion. However, an array or pairs of insulative microstructures such as oil menisci are necessary parts within the microchannel in order to create the non-uniform electric field [44]-[46]. Moreover, the generally strong electric field, shear stress and Joule heating in the constrictions formed by the microstructures may pose significant problems to cell viability [17]. Another approach to focusing particles is based on particle hydrophoresis that is generated by the lateral pressure
gradient induced in a channel comprising an array of obstacles on the top and bottom walls [47],[48]. This approach is, however, sensitive to the structure of the obstacles and requires a non-trivial fabrication. Recently, inertia has also been exploited to implement a continuous focusing of particles in curved microchannels [49]-[52]. The equilibrium position of the focused particle stream is, however, dependent on the Reynolds number. Moreover, particles become defocused when the Reynolds number is above one threshold value.

In this chapter, we introduce a novel particle focusing technique in DC electrokinetic flow through a planar serpentine microchannel with constant width and depth. This focusing stems from the cross-stream dielectrophoretic motion of particles induced within the channel turns. Neither an extra pressure-pumping nor embedded microstructures are required in this DC dielectrophoretic focusing of particles.

3.2 Experiment

![Figure 8: Picture of the serpentine microchannel used in the experiment with dimensions indicated in the inset.](image)
Figure 8 displays a picture of the serpentine microchannel used in our experiment. It was fabricated in polydimethylsiloxane (PDMS) using the soft lithography technique [27]. The detailed fabrication process can be referred to in Appendix A. The channel consists of a 1 cm long curved part in the middle and two 0.5 cm long straight parts at each end. It has a uniform depth of about 20 µm as calculated from the speed of photoresist spinning. The inset in Figure 8 shows the close-up view of the curved part. The channel is 50 µm wide. The width and height of every U-turn are both 200 µm. Polystyrene particles of 5 µm and 10 µm in diameter (Sigma-Aldrich, St. Louis, MO) were re-suspended in 1 mM KCl to ensure $\sigma_p << \sigma_f$ and thus $f_{CM} \approx -1/2$, yielding the maximum possible negative dielectrophoresis [15]. The electric field was supplied by a DC power supply (Glassman High Voltage Inc., High Bridge, NJ). Particle transport through the serpentine channel was visualized through an inverted microscope (Nikon TE2000-U) equipped with a CCD camera (Nikon DS Qi1MC).
3.3 Theory

Figure 9: Mechanism of the DC dielectrophoretic focusing of particles in a serpentine microchannel (only one s-shaped period is exhibited). Both the induced dielectrophoretic force in each turn and the particle velocity components in streamline coordinates are illustrated. The background shows the contour of electric field intensity (the darker the higher). The arrowed curves represent the electric field lines ($\mathbf{E}$).

Figure 9 shows the electric field lines (with arrows indicating the direction) and the contour of electric field intensity (the darker the higher) in one s-shaped period of a serpentine microchannel, i.e., a left U-turn immediately followed by a right U-turn. The electric field becomes non-uniform and attains the maximum and minimum values at the inner and outer walls of each of the four 90° turns. Hence, particles experience a dielectrophoretic force, $\mathbf{F}_{DEP}$ (bold...
symbol denotes a vector hereafter) when they move electrokinetically through a channel turn. In DC electric fields, $F_{DEP}$ on an isolated spherical particle is given by [15]

$$F_{DEP} = (1/2) \pi \varepsilon_f d^3 f_{CM} (E \cdot \nabla E)$$

(3-1)

$$f_{CM} = \left( \sigma_p - \sigma_f \right) / \left( \sigma_p + 2\sigma_f \right)$$

(3-2)

where $\varepsilon_f$ is the fluid permittivity, $d$ the particle diameter, $f_{CM}$ the so-called Clausius-Mossotti (CM) factor, $E$ the electric field vector, $\sigma_p$ the electric conductivity of particles, and $\sigma_f$ the electric conductivity of the suspending fluid. Note that Eq. 1 is valid only for weak electrolyte solutions [53] and small particles (more accurately, the size of the particle is much smaller than the characteristic length scale of the electric field) [15].

As polystyrene particles and live biological cells appear insulating in DC and low-frequency AC electric fields ($< 100$ kHz) [16], [17], we generally have $\sigma_p < \sigma_f$ and so $f_{CM} < 0$ leading to negative dielectrophoresis. In other words, $F_{DEP}$ is directed towards the lower electric field region as indicated in Figure 9. Therefore, particles will migrate across streamlines from the inner corner to the outer corner in each of the turns. As a matter of fact, the streamlines are equivalent to the electric field lines illustrated in Figure 9 due to the similarity between flow and electric fields in pure electrokinetic flows [20], [21]. Since the inner and outer corners switch between the left and right U-turns, particles will experience $F_{DEP}$ of alternate directions within the turns. Moreover, as $F_{DEP}$ is always stronger in the inner corner than in the outer corner, particles tend to be deflected towards the channel center region in each of the serpentine period (see Figure 9). The overall consequence will thus be a focused particle stream along the channel centerline.
By balancing $F_{DEP}$ and the electrophoretic force with the Stokes drag force, one can obtain the net particle velocity, $U_p$, as

$$U_p = U_{EK} + U_{DEP} = \mu_{EK}E + \mu_{DEP}(E \cdot \nabla E)$$

(3-3)

$$\mu_{DEP} = \varepsilon_f d^2 f_{CM} / 6 \mu_f$$

(3-4)

which is composed of the electrokinetic motion (a combination of fluid electroosmosis and particle electrophoresis), $U_{EK}$, and the dielectrophoretic motion, $U_{DEP}$. In the last equation, $\mu_{EK}$ is the electrokinetic mobility, $\mu_{DEP}$ the dielectrophoretic mobility which is negative as $f_{CM} < 0$, and $\mu_f$ the fluid viscosity. Note that the particle and fluid inertial motions have been neglected in Eq. 2 as the Reynolds and Dean numbers are both small ($\ll 1$) under the experimental conditions [49]. As the particle focusing is attributed to the cross-stream dielectrophoretic motion, we may conveniently express the particle velocity, $U_p$, in terms of streamline coordinates (see Figure 9),

$$U_p = (U_{DEP,s} + U_{EK}) \hat{s} + U_{DEP,n} \hat{n} = \left( \mu_{DEP} E \frac{\partial E}{\partial s} + \mu_{EK} E \right) \hat{s} + \mu_{DEP} \frac{E^2}{\Re} \hat{n},$$

(3-5)

where $U_{DEP,s}$ is the dielectrophoretic particle velocity in the streamline direction with the unit vector $\hat{s}$, $U_{EK}$ the stream-wise electrokinetic velocity, $U_{DEP,n}$ the dielectrophoretic particle velocity normal to the streamline direction with the unit vector $\hat{n}$, $E$ the electric field intensity, and $\Re$ the radius of curvature of the streamline which should follow closely the turn radius when fluid inertia is negligible.

The efficiency of the DC dielectrophoretic focusing of particles in a serpentine microchannel is determined by the ratio of the distance a particle moves perpendicular to the
streamline to the distance travelled along the streamline. This ratio, the larger the better focusing, is equivalent to the ratio of particle velocity perpendicular and parallel to the streamline, i.e.,

\[ \frac{U_{\text{DEP},n}}{U_{\text{DEP},s} + U_{\text{EK}}} \]

as seen in Eq. (3-5), which may be approximated as

\[
\frac{U_{\text{DEP},n}}{U_{\text{EK}}} = \left( \frac{\mu_{\text{DEP}}}{\mu_{\text{EK}}} \right) \frac{E}{9R}
\]

(3-6)

where \( U_{\text{DEP},s} \) has been assumed to have a much smaller magnitude than \( U_{\text{EK}} \). This assumption is generally valid in microchannels with a constant width unless \( U_{\text{EK}} \) is trivial due to the counter-balanced particle electrophoresis and fluid electroosmosis. Hence, a larger electric field and/or a smaller turn radius should provide a better focusing. However, it is important to note that a microchannel turn with a very small radius, i.e., a sharp turn, may induce a nonlinear electrokinetic flow due to the electric field leakage [54], which is believed to affect the particle focusing performance. In addition, as \( \mu_{\text{DEP}} \) is proportional to the particle diameter squared (see Eq. 2) while \( \mu_{\text{EK}} \) is only a weak function of particle size [55], the dielectrophoretic focusing should work more efficiently for larger particles.

### 3.4 Numerical Modeling

In order to understand and predict the observed particle focusing behavior, we developed a numerical model to simulate the electric field-mediated particle transport through the serpentine microchannel. This model is based on that developed by [22]. For simplicity, the perturbations of particles on the flow and electric fields (via moving boundaries in both fields due to the finite particle size) were both neglected in our model, so were the particle-particle interactions. Instead,
a correction factor $c$ was introduced to account for the effects of particle size (and others if any) on the dielectrophoretic velocity. As such, the particle velocity in Eq. (3-3) is revised as

$$U_p = \mu_{EK}E + c\mu_{DEP}(E \cdot \nabla E)$$  \hspace{1cm} (3-7)$$

This velocity was used in a particle tracing function in COMSOL (Burlington, MA) to compute the particle trajectory. Particles were assumed massless and uniformly distributed at the channel inlet. As particle dielectrophoresis happens only in the channel width direction, a 2D model was employed to predict the particle focusing without considering the effects of top and bottom channel walls on particle motion. This treatment has been proved reasonable in chapter 2.

In our numerical model, the electrokinetic mobility, $\mu_{EK}$, was obtained by measuring the average particle velocity in the straight section of the microchannel, which is 5.5×10^{-8} m^2/(V·s) for both 5 μm and 10 μm particles with an error of 10%. The dielectrophoretic mobility, $\mu_{DEP}$, was calculated from Eq. 2 with the typical dynamic viscosity, $\mu = 0.9 \times 10^{-3}$ kg/(m·s) and permittivity $\varepsilon_f = 6.9 \times 10^{-10}$ C/(v·m) for pure water at 25 °C. The measured electric conductivity of the solution, i.e., $\sigma_f$ in Eq. 1, is about 160 μS/cm. Therefore, the CM factor, i.e., $f_{CM}$ as defined in Eq. 1, was determined as −0.46 and −0.48 for 5 μm and 10 μm particles, respectively. Under the assumption of a uniform electric conductivity of the suspending fluid, the electric field $E = -\nabla \phi$ was computed by solving the Laplace equation $\nabla^2 \phi = 0$. The boundary conditions include the voltage drop between the channel ends and the insulating condition on the channel walls. The correction factor $c$ was determined by fitting the predicted particle trajectories to the width of the observed particle stream at the exit of the serpentine section.
3.5 Results and discussion

3.5.1 Electric Field Effects

Figure 10: Demonstration of the DC dielectrophoretic focusing of 5 μm particles in a serpentine microchannel at different electric fields: (a) experimentally observed particle streak images, and (b) numerically predicted particle trajectories in the entrance (top) and exit (middle and bottom with electric fields indicated) regions of the serpentine section of the microchannel. The block arrows indicate the flow direction.

Figure 10(a) illustrates the experimentally obtained streak images of 5 μm particles in the entrance (top) and exit (middle and bottom) regions of the serpentine section of the microchannel. The applied electric potential drops across the entire channel (2 cm straight length) are 200 V and 400 V for the middle and bottom images, corresponding to a nominal electric field of 10 kV/m and 20 kV/m (the true values are 7.7 kV/m and 15.4 kV/m in the straight section), respectively. At both electric fields, particles are almost uniformly distributed prior to entering the serpentine section (top), indicating a zero-focusing effect in the straight section of the channel. In the exit region of the serpentine section, however, particles are moving in a focused stream along the channel centerline as expected. Moreover, the width of the focused particle
stream decreases with the rise of electric field as noted above. It is estimated that over 1200 particles can be dielectrophoretically focused within one minute in this serpentine microchannel at the electric field of 20 kV/m.

3.5.2 Length Effects

Figure 10b shows the numerically predicted trajectories of 5 μm particles in the serpentine microchannel at the same conditions as those in the experiments. The correction factor $c$ was set to 0.5. Close agreements between the experimentally and numerically obtained widths of the focused particle stream were found for both electric fields. Using this model, we have also extracted the width of 5 μm particle stream with respect to the number of serpentine periods (refer to Figure 9) that particles have passed through. The applied electric field was 20 kV/m. As illustrated in Figure 11, the initially uniformly distributed particles (with an assumed width of 50 μm) get focused as they migrate electrokinetically through the serpentine channel. Moreover, the focusing performance decreases along the channel (reflected by the decreasing slope of the illustrated curve in Figure 11) because the overall cross-stream dielectrophoretic motion in one serpentine period becomes smaller when particles move closer to the centerline.
Figure 11: Illustration of the 5 μm particle stream width with respect to the number of serpentine periods (refer to Figure 9) that particles have passed through. The applied electric field is 20 kV/m.

3.5.3 Particle Size Effects

Figure 12(a) compares the streak images of 5 μm (top) and 10 μm (bottom) particles in the exit region of the serpentine section of the microchannel at a nominal electric field of 10 kV/m. The measured width of the focused 10 μm particle stream is 13 μm, which is as expected smaller than that for 5 μm particles (the measured width is about 23 μm). The numerically predicted trajectories of 10 μm particles in the serpentine microchannel at the experimental condition are displayed in Figure 12(b) (bottom). The correction factor $c$ was set to 0.3 for the best fit to the measured width of the focused particle stream. While $c$ has been found insensitive to the applied
electric field for both 5 µm and 10 µm particles, it is still uncertain whether \( c \) varies with the particle concentration and the channel structure etc. We are currently examining this issue.

![Comparison of (a) experimentally observed streak images and (b) numerically predicted trajectories of 5 µm (top) and 10 µm (bottom) particles in the exit region of the serpentine section of the microchannel at a nominal electric field of 10 kV/m. The block arrow indicates the flow direction.](image)

Figure 12: Comparison of (a) experimentally observed streak images and (b) numerically predicted trajectories of 5 µm (top) and 10 µm (bottom) particles in the exit region of the serpentine section of the microchannel at a nominal electric field of 10 kV/m. The block arrow indicates the flow direction.

Besides the ratio of particle velocity perpendicular and parallel to the streamline as given in Eq. (3-5), the ultimate width of the focused particle stream in a serpentine microchannel is also dependent on the channel width and length (the total channel length in the serpentine section). In order to be focused to the channel centerline, all particles should have sufficient times to traverse at least half of the channel width before travelling out of the serpentine section. Therefore, reducing the width and increasing the length of the serpentine section should both improve the DC dielectrophoretic focusing of particles. More accurately, the latter condition should be replaced by the number of serpentine periods because particle dielectrophoresis only takes effects within the channel turns and the straight channels between them contribute little to focusing. It is also beneficial to use an AC electric field to generate the cross-stream dielectrophoretic particle motion while the pumping of the particle stream may be realized by a DC electric field or pressure gradient. We note that DC-biased AC electric fields have been
recently employed to improve the dielectrophoretic separation of particles in structured microchannels [56], [57].

3.6 Summary

We have demonstrated a novel particle focusing technique based on the cross-stream dielectrophoretic particle motion in the turns of a serpentine microchannel. The favoring of large electric fields makes this focusing technique very appealing as the efficiency and the particle throughput may be enhanced simultaneously. Moreover, the footprint of a serpentine microchannel can be readily made very small to save space for other integrated functionalities on microfluidic chips. This DC dielectrophoretic particle focusing technique is superior to the existing AC dielectrophoretic focusing technique in that both the in-channel micro-electrodes and the external pumping force are eliminated. Hence, the fabrication and operational costs are reduced. We envision direct near-term applications of this novel focusing technique in continuous bioparticle separation and flow cytometry for a wide range of technological solutions in biology, medicine and industry.
CHAPTER 4: Particle Focusing in Spiral Microchannels

4.1 Introduction

Aside from studying the particle motion in serpentine microchannels in chapter 3, we herein perform an experimental and numerical study of particle electrophoresis in spiral microchannels. This type of curved channels has been often used to reduce the device size of, for example, micro-reactors and micro-mixers [110], [111]. Different from a serpentine channel whose turns change directions alternately (i.e., left and right), a spiral channel maintains the direction of its turns. As a result, particles will be focused to the side wall instead of channel centerline due to its intrinsic pattern. More specifically, because of the length difference between the channel boundaries along the flow direction, the electric field will attain maximum and minimum at inner and outer wall respectively. Therefore, in spiral microchannels particles will be continuously experience negative DEP force which maintains a fixed angle to the flow direction and thus finally deflected to the outer wall. It is noticed that these two microchannels have both been recently demonstrated to focus and separate particles via inertia effects [49], [51], [52], [112]-[114].

4.2 Experiment

Figure 13 displays a picture of the spiral microchannel (filled with green food dye for clarity) used in the experiment. It was fabricated in Polydimethylsiloxane (PDMS) using the standard soft lithography technique. The detailed fabrication process can be referred to in Appendix A. The microchannel consists of two spirals that are symmetric with respect to the
channel center (i.e., the junction of the two spirals) while in opposite directions: the counterclockwise one is indicated as the first spiral through which particles entered from the inlet reservoir in experiments; the clockwise one is indicated as the second spiral through which particles exited to the outlet reservoir in experiments. Each spiral has four equally separated loops, and measures in total 2.5 cm long including the straight part from/to the reservoir. The diameter of the most inner semi-circle is 100 µm as indicated in the inset of Figure 13. The channel is everywhere 50 µm wide and 25 µm deep. The radial distance (or the shortest distance) between adjacent loops is 150 µm (or the center-to-center distance between loops is 200 µm).

![Figure 13: Picture of the double-spiral microchannel used in the experiment with dimensions indicated in the inset. The block arrows indicate the particle flow directions in experiments, of which the inflow from the inlet reservoir takes place in the first spiral and the outflow to the outlet reservoir happens in the second spiral. Polystyrene particles of 5 µm and 10 µm in diameter (Sigma-Aldrich, USA) were re-suspended in 1mM phosphate buffer at a concentration of at least $10^7$ particles per milliliter. A 0.5% Tween 20 (Sigma-Aldrich, USA) was added to the particle solution to suppress particle aggregation or adhesions to the channel wall. The calculated electric conductivities of the two particles are 8 µS/cm and 4 µS/cm, respectively, if the surface conductance is assumed to be 1 nS.](image-url)
As the measured electric conductivity of the buffer solution is 207 μS/cm, the CM factor, i.e., $f_{CM}$ in Eq. (2), was determined as –0.47 and –0.49 for 5 μm and 10 μm particles, respectively. Electric field was supplied by a DC power supply (Glassman High Voltage Inc., High Bridge, NJ). Electrophoretic motion of particles through the spiral microchannel was visualized through an inverted microscope imaging system (TE2000-U with DS Qi1MC CCD camera, Nikon Instruments, TX), and recorded in the form of both images and live videos (about 19 frames per second).

4.3 Theory

We first analyze the possible variations in speed and trajectory for particle electrophoresis through a microchannel turn of uniform width and depth, see Figure 14. To be consistent with typical electrokinetic microfluidics where the fluid speed is on the order of mm/s [17], [59], [62], the inertial motions of fluid and particles are safely neglected, as the channel and particle Reynolds numbers are both very small [113], [114]. Figure 14 illustrates the electric field lines (E, with short arrows indicating the directions) and the contour of electric field intensity (the darker the higher) in the turn. Due to the variation in path length for electric current, electric field attains the maximum and minimum values near the inner and outer corners, respectively. Therefore, particles are subject to a transverse dielectrophoretic motion, $\mathbf{U}_{DEP}$ (bold symbols denote a vector hereafter) when they move electrokinetically, $\mathbf{U}_{EK}$, through the turn. In DC electric fields, particle dielectrophoresis is characterized as [15]

$$
\mathbf{U}_{DEP} = \mu_{DEP} \nabla E^2
$$

(4-1)

$$
\mu_{DEP} = \varepsilon_f d^2 f_{CM} / 12 \mu_f
$$

(4-2)
\[ f_{CM} = \frac{(\sigma_p - \sigma_f)}{(\sigma_p + 2\sigma_f)} \]  \hspace{1cm} (4-3)

where \( \mu_{DEP} \) is the particle dielectrophoretic mobility, \( \varepsilon \) the fluid permittivity, \( d \) the particle diameter, \( f_{CM} \) the Clausius-Mossotti (CM) factor, \( \mu_f \) the fluid viscosity, \( \sigma_p \) the particle conductivity, and \( \sigma_f \) the fluid conductivity. Depending on the relative magnitude between \( \sigma_p \) and \( \sigma_f \), the CM factor may be negative or positive, yielding a negative or positive dielectrophoresis [11], [15]. Accordingly, \( U_{DEP} \) may point towards the outer (if negative dielectrophoresis) or inner (if positive dielectrophoresis) corner of the turn.

Figure 14: Velocity analysis of particle electrophoresis in a microchannel turn of uniform width and depth. Also illustrated are the electric field lines (\( E \), short arrows indicate the directions) and the contour of electric field intensity (the darker the higher).
Since live biological cells and polymer microparticles often behave like poor conductors in DC and low-frequency AC (< 100 kHz) fields [16], [17], $\sigma_p$ is smaller than $\sigma_f$ so that $f_{CM} < 0$. Hence, these particles should be deflected across streamlines by negative dielectrophoresis and migrate towards the outer corner of the turn, as indicated in Figure 14. It is important to note that the electric field lines illustrated in Figure 14 are identical with the streamlines due to the similarity between the flow and electric fields in pure electrokinetic flows [20]. Hence, we may conveniently express the particle velocity, $U_p$, in terms of streamline coordinates,

$$U_p = U_{EK} + U_{DEP} = \mu_{EK} E + \mu_{DEP} \nabla E^2$$

$$(U_{EK} + U_{DEP,3}) \hat{s} + U_{DEP,n}\hat{n} = \left(\mu_{EK} E + \mu_{DEP} \frac{\partial E}{\partial s}\right) \hat{s} + \mu_{DEP} \frac{E^2}{\mathcal{R}} \hat{n}$$

where $\mu_{EK}$ is the particle electrokinetic mobility (a combination of liquid electroosmosis and particle electrophoresis [65]), $U_{EK}$ the stream-wise electrokinetic velocity, $U_{DEP,3}$ the particle dielectrophoretic 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 $\hat{n}$, $E$ the electric field intensity, and $\mathcal{R}$ the radius of curvature of the streamline which should follow closely the radius of channel curvature in low-Reynolds number flows.

Eq. (4-4) indicates that a microchannel turn causes two effects on particle electrophoresis via the curvature-induced electric field gradients (see Figure 14): (a) the cross-stream dielectrophoretic motion, $U_{DEP,n}$, shifts particles across streamlines towards the outer (if negative dielectrophoresis) or inner (if positive dielectrophoresis) corner, leading to variations in particle trajectory and speed; (b) the stream-wise dielectrophoretic motion, $U_{DEP,3}$, also perturbs the particle electrokinetic velocity though to a much smaller extent if the width and depth of the turn
remain unvaried. It is the ratio of particle velocity normal and parallel to the streamline that
determines the particle deflection obtained through a channel turn,

\[
\frac{U_{DEP,n}}{U_{EK} + U_{DEP,s}} = \left( \frac{\mu_{DEP}}{\mu_{EK} + \mu_{DEP} \frac{\partial E}{\partial s}} \right) \frac{E}{9R}
\]  

(4-5)

Therefore, a larger electric field and/or a smaller turn radius should provide a more apparent
demonstration of the above-mentioned curvature effects on particle electrophoresis. Moreover, as
\(\mu_{DEP}\) is proportional to the particle diameter squared [see Eq. (4-2)] while \(\mu_{EK}\) is only a weak
function of particle size [55], the velocity ratio in Eq. (4) should also increase with the rise of
particle size.

### 4.4 Numerical Modeling

We developed a numerical model to understand and predict the observed particle
electrophoretic motions in the spiral microchannel. Briefly, the perturbations of particles on the
flow and electric fields were neglected in the model, so were the particle-wall and particle-
particle interactions. A correction factor, \(c\), was introduced to account for the effects of particle
size (and others if any) on the dielectrophoretic velocity. This is because Eq. (4-1) is valid only
when the particle size is much smaller than the characteristic length scale of the electric field.
Hence, the particle velocity in Eq. (4-4) is rewritten as

\[
U_p = \mu_{EK} E + c\mu_{DEP} \nabla E^2.
\]  

(4-6)

The new velocity was then used in a particle tracing function in COMSOL (Burlington, MA) to
compute the particle trajectory.
For simulation, the electrokinetic mobility, $\mu_{EK}$, was determined by measuring the particle velocity in a straight uniform microchannel of the same width and depth as the spiral channel. We obtained an almost equal value of $\mu_{EK} = 3.2(\pm0.3)\times10^{-8} \text{ m}^2/\text{(V}\cdot\text{s})$ for the 5 $\mu$m and 10 $\mu$m particles used in experiments. The dielectrophoretic mobility, $\mu_{DEP}$, was calculated from Eq. (4-2) with the typical viscosity, $\mu = 0.9\times10^{-3} \text{ kg/(m}\cdot\text{s})$ and permittivity $\varepsilon_f = 6.9\times10^{-10} \text{ C/(v}\cdot\text{m)}$ for pure water at 25 °C. The values for the CM factor, $f_{CM}$, involved in $\mu_{DEP}$ were assigned as $-0.47$ and $-0.49$ for the 5 $\mu$m and 10 $\mu$m particles as noted above. The electric field $E = -\nabla \phi$ was computed by solving the 2D Laplace equation $\nabla^2 \phi = 0$ using the electrostatics module in COMSOL. The boundary conditions include the voltage drop between the channel ends and the insulating condition on the channel walls. The correction factor, $c$ in Eq. (4-6), has been recently found to be dependent on particle size and channel geometry but insensitive to electric field [22],[23], [108]. Since only one channel was used in the experiment, $c$ was determined by fitting the predicted particle trajectory to the experimental data at the electric field of 200 V/cm. The obtained $c$ value for each particle size was then used for all other electric fields if applicable.

4.5 Results and discussion

4.5.1 Electric Field Effects

Figure 15 illustrates and compares the experimentally observed electrophoretic motions (top row: snap-shot images; middle row: superimposed images) and the numerically predicted trajectories (bottom row) of 5 $\mu$m particles in the asymmetric double spiral microchannel. The applied DC electric field was 200 V/cm on average, corresponding to a 1000-V-voltage drop across the 5-cm-long channel. The correction factor, $c$ in Eq. (4-6), was set to 0.6 in the modeling.
by examining the width of the particle stream in the superimposed image. At the inlet of the first spiral (see the images in the left column), particles were uniformly distributed by nature and covered the whole channel width except very close to the walls due to particle-wall interactions [68], [69], [115]. Once they moved into the curved part of the first spiral, particles started experiencing negative dielectrophoresis as explained above and were thus pushed towards the outer wall. The result was seen to be a squeezed particle stream near the outer wall of the first spiral at the channel center (see the images in the middle column). When they entered into the second spiral, particles were still subject to negative dielectrophoresis but in the opposite direction due to the switching of inner and outer walls between the two spirals. Eventually, particles moved out of the double-spiral microchannel in a focused stream near the outer wall of the second spiral (see the images in the right column). These observations were confirmed by the numerical modeling.
Figure 15: Illustration and comparison of experimentally observed electrophoretic motions (top row: snap-shot images; middle row: superimposed images) and numerically predicted trajectories (bottom row) of 5 µm particles in the spiral microchannel (left column: inlet; middle column: center; right column: outlet). The applied DC electric field was 200 V/cm on average across the channel length. The block arrows indicate the flow directions.

Figure 16 compares the experimentally observed electrophoretic motions (left column: snap-shot images; middle column: superimposed images) and the numerically predicted trajectories (right column) of 5 µm particles at the outlet of the spiral microchannel at different electric fields (from 100 V/cm to 400 V/cm). The correction factor, $c$, remained at 0.6 for all fields in the modeling. With the increase of electric field, particles were focused to a tighter stream, which was also observed to move nearer to the outer wall of the channel (movies are available in Supporting Information). Especially at the electric field of 400 V/cm, we noticed that particles were nearly moving in a single file (see the images in the bottom row). This function is
expected to find applications in microfluidic flow cytometry [27] and particle separation [26] etc. The experimental observations are consistent with the theoretical analysis as the particle velocity ratio in Eq. (4-5) does indicate that the curvature-induced dielectrophoretic focusing effects should increase with the rise of electric field. Moreover, these observations are in reasonable agreement with the numerical simulations despite the fact that the same correction factor was used in the modeling.

Figure 16: Comparison of experimentally observed electrophoretic motions (left column: snap-shot images; middle column: superimposed images) and numerically predicted trajectories (right column) of 5 μm particles at the outlet of the spiral microchannel at different electric fields (as indicated). The block arrows indicate the flow directions.
4.5.2 Particle Size Effects

As alluded to earlier, the particle velocity ratio in Eq. (4-5) also becomes greater for larger particles. This analysis is supported by Figure 17 which shows the experimentally observed electrophoretic motions (top row: snap-shot images; middle row: superimposed images) and the numerically predicted trajectories (bottom row) of 10 μm particles in the spiral microchannel at the electric field of 200 V/cm. The correction factor, c, was set to 0.3 by fitting the width of the focused particle stream in the images at the channel outlet to that predicted in the modeling (see the right column). As compared to the electrophoretic behaviors of 5 μm particles in Figure 16, we notice that 10 μm particles clearly exhibit a larger deflection and a better focusing due to a stronger curvature-induced dielectrophoresis in the two spirals. These enhanced effects are also apparent in the predicted particle trajectories at the channel center, which, however, seem to be underestimated in the modeling if one compares the position of the focused particle stream in the second spiral of the channel (see the middle column). We attempted to increase the correction factor in the modeling, but still couldn’t get a satisfactory agreement with the experimental data. This discrepancy may be partially attributed to the neglect of particle-wall interactions [23],[68],[69], [116] in our model.
4.6 Summary

We have studied particle electrophoresis in a spiral microchannel using a combined experimental and numerical method. Due to the variation in path length for electric current,
electric field gradients are formed by channel curvatures. As such, particle dielectrophoresis is induced in curved microchannels, which was found to deflect particles across streamlines. The result is a focused particle stream flowing near the outer wall of the spiral channel. Moreover, the width and position of the particle stream at the channel outlet were found to depend on the electric field magnitude and the particle size as predicted from the theoretical analysis. In addition, a numerical model was developed, which simulates closely the observed particle electrophoretic behaviors in the spiral channel in most cases. It is anticipated that the curvature-induced dielectrophoretic focusing effect will find applications in continuous bioparticle separation and flow cytometry for a wide range of technological solutions in biology, medicine and industry.

In short, we have demonstrated that curved microchannels with different patterns such as serpentine and spirals are capable of focusing particles either to the centerline or the side wall. Focusing, however, is not our unique objective because it is usually a prerequisite step prior to detection or sorting. The following chapters will be our work on continuous particle focusing and separation in curved microchannel based on our preexist designs.
CHAPTER 5: Particle Separation by Size in Serpentine Microchannels

5.1 Background on Particle Separation

Particle separation, no matter synthetic or biological, is substantial to chemistry, biomedicine, food industry, and fundamental and clinical pharmacology. Micro/nanofluidic devices gain more and more favor due to their rapid real time analytics with small amounts of sample required and low cost [117]. The development of those devices facilitated the emergence of numerous separation techniques which are typically categorized as batch-wise and continuous-flow approaches. Separation of samples in batch processes includes filtration [118], centrifugation [119], chromatography [120], electrophoresis [121], and field-flow fractionation [122]. Continuous-flow separation operates continuously in the time axis and requires either an external (i.e., active) or internal (i.e., passive) force field to act on the focused particulate stream [26], [122], [123], [124], [125]. In the active mode, one or more coupled force fields are used to implement particle separation in these devices, which includes hydrodynamic [127], magnetic [128], electrical [129], acoustic [130], optical [131], gravity forces [132]. Accordingly, topology-induced internal forces were taken advantage of by the passive methods to direct particles into diverse sub-streams during the transport which involve hydrodynamic filtration [133], hydrophoresis [134], and inertia forces [135], [136], [137].

As one of the force field manipulating particles based on their intrinsic properties, dielectrophoresis (DEP) is frequently utilized to separate or sort particles which can generally be categorized as electrode-based [138],[139], insulator-based [140],[141],[142] and contactless
approaches [143], [144]. Generally, as indispensible components in a typical electrode-based
dielectrophoresis (eDEP) device, either interdigitated micro-electrode or electrode array are
aligned along the entire microchannel and high-frequency AC electric voltages are required to
generate electric field gradients for particle DEP. [145] - [153] However, the complicated
fabrication and surface fouling are the two main disadvantages of eDEP devices, not to mention
the extra pumping components are also required. As an optional substitute for eDEP devices,
Insulator-based dielectrophoresis (iDEP) devices avoids the above drawbacks using in-channel
micro-obstacles such as blocks, hurdles, posts and ridges etc. to reshape the local electric field
distribution to obtain non-uniform electric field [22], [39], [56], [57], [102], [103], [154]-[157].
However, those in-channel constrictions may cause at least two side effects on the particles
(especially to bio-particles). One is the Joule heating effect due to locally amplified electric field
induced by those obstacles and the other is particle clogging or adhesion to the channel wall [17].

Among the diverse particle focusing methods, sheath flow focusing may be the most
common one that has been adopted in microfluidic devices. This type of focusing has been
realized by using either pressure-driven or electric field-driven particle-free sheath flows to pinch
the particle suspension flow and thus focus particles into a single file. In general, one or more
sheath fluids should be used in order to obtain a 2D or 3D particle focusing.

In this chapter we introduce a particle separation technique in DC electrokinetic flow through
a planar serpentine microchannel. The technique combines the electrokinetic sheath focusing and
particles separation via dielectrophoresis induced by the non-uniform electric field inherent to
channel curvature that has been recently demonstrated in [108], [159], [160]. DC electric fields
provides pumping, focusing, and separation of particles simultaneously in the serpentine
microchannel without any additional pumping components or in-channel microstructures
(microelectrodes or micro-insulators). It is noticed that similar serpentine microchannels have been recently used to filtrate and separate microparticles by size in pressure-driven flow via inertia [49], [112].

5.2 Experiment

5.2.1 Microchannel fabrication

Figure 18 reveals a close-up shot of the serpentine microchannel (filled with dark green food dye for clarity) used in the separation experiments. The inset in the bottom right corner indicates the channel dimensions of the center region. Such channel was fabricated with PDMS using the standard soft lithography method. The detailed fabrication process can be referred to in Appendix A. The channel consists of 4 straight sections which were extended to 4 reservoirs respectively at each end and they were connected by a serpentine section in the center. All of the branch channels are 3.5 mm long and 15 μm in depth with a 50 μm uniform width. In addition, the channel is symmetric to the Y axis as shown with 2 reservoirs on each end along the X direction hence either end can be served as a pair of inlet or outlet reservoirs. Inlet reservoir 1, 2 and out reservoir 1, 2 were labeled within each reservoir for the convenience of further illustration. The serpentine section of the microchannel is comprised of 33 periods and has a uniform width and depth of 50 μm and 15 μm respectively. Each end of the serpentine section was bifurcated to a pair of straight branches at a right angle (see Figure 18).
Figure 18: Picture of microchannel used in experiment to separate particles by size (Each reservoir was filled with green food dyed for clarity). Channel dimensions of either entrance or exit region are labeled in the inset at the bottom right corner. The block arrow indicates the flow direction.

5.2.2 Particle Solution Preparation

Green fluorescing polymer microspheres of 1 (Bangs laboratories, USA, Solid content: 1%) and 5 µm in diameter (Duke Scientific, USA, Solid content: 1%) were re-suspended in 1 mM phosphate buffer at 1:50 volume ratio with a concentration of about $10^7$ particles per milliliter. Tween 20 (0.5% v/v, Fisher Scientific) was added to the particle solution to suppress particle adhesions to channel walls as well as particle aggregations. The calculated electric conductivities of these two sizes of particles are 40 and 8 µS/cm, respectively, if the surface conductance is assumed to be 1 nS. As the electric conductivity of the buffer solutions (200 µS/cm for 1 mM
phosphate buffer) is much higher than that of the particles, the CM factor, $f_{CM}$, was found close to $-0.5$ for both types of particles.

5.3 Theory

Figure 19: Illustration of continuous focusing and separation of particles with different sizes in the serpentine microchannels. The electric field lines with dark or light color (E, short arrows indicate the directions) and the contour of electric field intensity (the darker the higher) are also illustrated.

The electric field distribution (denoted by the background color, the darker the higher) and the streamlines (represented by short arrows) at the entrance region of the serpentine microchannel were sketched in Figure 19, where the light and dark streamlines denote sheath flow and particle mixture respectively. It also indicates the mechanism of continuous particle focusing via sheath flow and separation by negative dielectrophoresis in a serpentine
microchannel. More specifically, sheath flow which has a relatively large flow rate was precisely controlled by the voltage difference applied in the inlet reservoirs and move towards the junction region. Due to relatively large flow rate of the sheath flow from the top branch, the streamlines from the bottom branch will be compressed to a narrow band against the sidewall before entering the serpentine part. Correspondingly, the particle mixture within the flow will electrokinetically follow the streamlines and also be squeezed to a tight stream close to the sidewall, achieving the first function, i.e. focusing of particle mixture. As particle mixture migrating to the serpentine part, the electric field attains maximum and minimum at each inner and outer corner respectively due to the variation of path length which can be revealed from the color intensity of the background. As a result, particles will experience corresponding dielectrophotic force $\mathbf{F}_{\text{DEP}}$ (a bold symbol denotes a vector henceforth) generated by the non-uniform electric fields induced by channel turns. For an insulating spherical particle of radius $a$ subjected to an electric field $\mathbf{E}$, the time average of $\mathbf{F}_{\text{DEP}}$ force is given by [15]

$$
\mathbf{F}_{\text{DEP}} = \left(1/2\right)\pi\varepsilon_m d^3 f_{\text{CM}} (\mathbf{E} \cdot \nabla \mathbf{E})
$$

(5-1)

$$
f_{\text{CM}} = \left(\sigma_p - \sigma_m\right) / \left(\sigma_p + 2\sigma_m\right)
$$

(5-2)

where $\varepsilon_m$ is the permittivity of the suspending medium, $d$ the particle diameter, $f_{\text{CM}}$ the so-called Clausius–Mossotti (CM) factor, $\mathbf{E}$ the root-mean-square (RMS) value of the applied electric field, $\sigma_p$ the electric conductivity of particles, and $\sigma_m$ is the electric conductivity of the medium.

Generally the electric conductivity of polystyrene beads is smaller than that of biological solutions. In addition, biological cells usually behave like poor conductor when subjected to DC electric fields since the cell membrane blocks the DC current [15]. In both cases, the electric conductivity of particles is smaller than that of medium, leading to $f_{\text{CM}} < 0$ and hence negative
dielectrophoresis. Therefore, the $F_{\text{DEP}}$ force particles experienced will direct from the higher electric field region to the lower one of each turn as indicated in Figure 19. Under certain electric fields, larger particles will be gradually deflected to the center region of the channel during each period as they move electrokinetically through the serpentine section since the turns in the serpentine channel alternatively switch directions. Meanwhile, smaller particles migrate laterally much less than larger ones due to the fact that dielectrophoretic force scale with the second power of particle size. The accumulated effect of the above results is a focused sub-stream of larger particles along the channel centerline paralleled with an unfocused sub-stream of smaller particles at the exit region of the serpentine section. Therefore, continuous separation of particles by size was achieved at downstream region of the serpentine section which served as the second function, i.e. separation of particle mixture.

The particle velocity, $U_p$, is a combination of electrokinetic motion and dielectrophoretic motion caused by DC fields as shown in Eq. (5-3),

$$U_p = U_{\text{EK}} + U_{\text{DEP}} = \mu_{\text{EK}} E + \mu_{\text{DEP}} (E \cdot \nabla E)$$

(5-3)

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

(5-4)

where $\mu_{\text{EK}}$ denotes the electrokinetic mobility, $\mu_{\text{DEP}}$ the dielectrophoretic mobility, $E$ the applied DC electric field, and $\mu_m$ is the dynamic viscosity of the suspending medium. As the mechanism for particle deflection in the serpentine microchannel is the cross streamline migration of particles due to dielectrophoresis, the particle velocity can be conveniently expressed in streamline coordinates as illustrated in Figure 19,
\[ \mathbf{U}_p = \left( \mathbf{U}_{EK} + \mathbf{U}_{DEP,s} \right) \hat{s} + \mathbf{U}_{DEP,n} \hat{n} = \left( \mu_{EK} E + \mu_{DEP} \frac{\partial E}{\partial s} \right) \hat{s} + \mu_{DEP} \frac{E^2}{\mathfrak{R}} \hat{n} \] (5-5)

where \( \mathbf{U}_{EK} \) is stream-wise electrokinetic velocity, \( \mathbf{U}_{DEP,s} \) the dielectrophoretic particle velocity in the streamline direction with the unit vector \( \hat{s} \), \( \mathbf{U}_{DEP,n} \) the dielectrophoretic particle velocity normal to the streamline direction with the unit vector \( \hat{n} \), \( E \) the electric field intensity (i.e., \( E = |E| \)), and \( \mathfrak{R} \) is the radius of curvature of the streamline. Notice that the electric field lines shown in Figure 19 resemble the streamlines in the serpentine channel due to the similarity between flow and electric fields in pure electrokinetic flows [20], [21].

Accordingly, the deflection is a function of particle mobility ratio, which in DC electric fields is given by [162]

\[
\frac{\mu_{DEP}}{\mu_{EK}} = \frac{d^2 f_{CM}}{6 \left( \zeta_p - \zeta_w \right)}
\]

where \( d \) is the particles diameter, \( f_{CM} \) the so-called Clausius–Mossotti (CM) factor [15], [161], \( \zeta_p \) the zeta potential (a measure of surface charge) of particles, \( \zeta_w \) the zeta potential of the channel wall (assume it is uniform). Therefore, the dependence of deflection on the intrinsic properties of particles such as diameter enables the continuous separation of particles by size in serpentine microchannels, which will be elaborated in results and discussions section.

### 5.4 Numerical Modeling

We developed a numerical model to understand and predict the observed particle electrophoretic motions in the serpentine microchannel. Briefly, the perturbations of particles on the flow and electric fields were neglected in the model, so were the particle-wall and particle-
particle interactions. A correction factor, $c$, was introduced to account for the effects of particle size (and others if any) on the dielectrophoretic velocity. This is because Eq. (5-1) is valid only when the particle size is much smaller than the characteristic length scale of the electric field. Hence, the particle velocity in Eq. (5-3) is rewritten as

$$U_p = \mu_{EK} E + c \mu_{DEP} \nabla E^2.$$  

(5-7)

The new velocity was then used in a particle tracing function in COMSOL (Burlington, MA) to compute the particle trajectory.

For simulation, the electrokinetic mobility, $\mu_{EK}$, was determined by measuring the particle velocity in a straight uniform microchannel of the same width and depth as the spiral channel. We obtained an almost equal value of $\mu_{EK} = 3.5 \times 10^{-8}$ m$^2$/s for the 1 $\mu$m and 5 $\mu$m particles used in experiments. The dielectrophoretic mobility, $\mu_{DEP}$, was calculated from Eq. (5-4) with the typical viscosity, $\mu = 0.9 \times 10^{-3}$ kg/(m·s) and permittivity $\varepsilon_f = 6.9 \times 10^{-10}$ C/(V·m) for pure water at 25 $^\circ$C. The values for the CM factor, $f_{CM}$, involved in $\mu_{DEP}$ were assigned as $-0.5$ for both types of particles as noted above. The electric field $E = -\nabla \phi$ was computed by solving the 2D Laplace equation $\nabla^2 \phi = 0$ using the electrostatics module in COMSOL. The boundary conditions include the voltage drop between the channel ends and the insulating condition on the channel walls. Since only one channel was used in the experiment, $c$ was determined by fitting the predicted particle trajectory to the experimental data at the electric field of 100 V/cm. The obtained $c$ value for each particle size was then used for all other electric fields if applicable.
5.5 Results and discussion

5.5.1 Separation of Polystyrene Beads by Size

Figure 20: Continuous focusing and separation of 1&5 μm particles in serpentine microchannel: (a) Snapshot, (b) Superimposed images and (c) numerically predicted trajectories of particle mixture migrating from one branch to the entrance region (top) squeezed by the electrokinetic sheath flow from the other branch against the channel side wall and separated into two sub-streams at the exit region (bottom).

As illustrated above, the magnitude of the lateral displacement of particles is proportional to the DEP force acting on the particles, and hence the particle size. Therefore the trajectories of the polystyrene beads of different sizes can be diverted into bifurcate sub-streams after they pass the
serpentine section. Figure 20 demonstrated the case of continuous separation of 1 and 5 μm fluorescent polystyrene beads in the serpentine channel as shown. The DC voltages applied in the inlet reservoir 1 and 2 are 270 V and 300 V respectively to attain a focused particle stream at the entrance region (see the first row of Figure 20). The average electric fields in the top and bottom straight branches are 11.8 and 3.5 kV/m respectively which were estimated in a 2D modeling in COMSOL (Burlington, MA, USA). The electrokinetic mobility \( \mu_{EK} \) for both particles approximate \( 3.5 \times 10^{-8} \) (m\(^2\)/V·s), indicating their similar electrokinetic velocity. The negative DEP force ratio between two types of particles is predicted to be 125:1 since they are 5 times difference in size. Moreover, the lateral displacement ratio of two should be 25:1 as predicted.

As seen from the snapshot image in Figure 20(a) and the superimposed image in Figure 20(b), particle mixture move electrokinetically from the inlet reservoir 1 entering the junction of two straight branches. Meanwhile the sheath fluids from the other branch also move towards the junction, resulting in a squeezed particulate stream against the channel side wall. More specifically, particle stream almost cover the entire channel width of the straight branch when they are further away from the junction. When close to or at the bifurcation region, however, sheath flow will pinch the particle stream into a tight file due to its larger flow rate than that of particle mixture. As a result, particle stream was squeezed to a single file with the width that large particles can only pass through the bifurcation region one by one as Figure 20(b) indicated. As illustrated before, 5 μm particles were gradually deflected to the centerline of the channel due to larger negative DEP force they experienced while 1 μm particles migrated less than one half of the channel width due to their smaller mobility ratio, \( \mu_{DEP}/\mu_{EK} \). If both outlet reservoirs are grounded, however, the squeezed stream of large particles will occupy the centerline and thus
split into two sub-streams at the exit which compromise the separation. Therefore, the separation of particles at the exit bifurcation region must be achieved by providing a small voltage difference in two outlet reservoirs which yields slightly difference in the flow rate of two outlet branches. Eventually, at the exit region 5 \( \mu \)m particles were directed to the top branch and 1 \( \mu \)m ones flowed to the bottom branch by adjusting the voltage applied in two outlet reservoirs as predicted in the second row of Figure 20(c). It is noticed that the shape of both types of fluorescent particles at the entrance region seemed slightly different from the ones at the exit region. They are two reasons: the main one is that particle stream were slowed down at the entrance region induced by the pressure from the sheath flow while recover to the normal speed when migrating to the exit region; the other is that exposure time were quadrupled at exit region compare to the entrance for the convenience of making superimposed images. Compared with the simulated trajectories of 1\( \mu \)m particles, the width of corresponding sub-stream was underestimated as indicated. It could be reasonably explained by particle diffusion induced by the concentration gradient at the entrance region where particles were highly concentrated in bottom layer. Such diffusion which usually prefer small particles gave rise to the dispersion of 1 \( \mu \)m beads to center region where particle concentration was lower and thus resulted in a larger stream width than simulated.

5.5.2 Electric Field Effect

In addition, we investigated the effect of overall electric field on the focusing and separation of 1 and 5 \( \mu \)m polystyrene beads in the serpentine microchannel. Figure 21 compares the simulated particle trajectories in the entrance and exit region of the serpentine part when the applied DC voltages in the inlet reservoir 2 are 100 V (a), 300 V (b), and 500 V (c), respectively.
Correspondingly, the voltages in the inlet reservoir 1 and outlet reservoirs are also labeled beside each straight section since the applied voltages in the rest reservoirs to the inlet reservoir 2 maintain at constant ratio.

Figure 21: Effect of overall electric field level on the focusing and separation of 1 and 5 μm polystyrene beads. The DC voltage applied at the inlet reservoir 1 varied from 100V (a), 300 V (b) to 500V (c) and maintained the ratio of voltage applied to inlet reservoir 2 to inlet reservoir 1 and outlet reservoir 2 at 30:27:1 while the outlet reservoir 2 was grounded.

The focused particle stream at the entrance region maintained the same width when varying the overall applied voltage as indicated. This is because of the linear relationship between the electric field and electrokinetic flow rate without pressure driven flow, [161] when the voltage ratio between sheath flow and particle stream was fixed. The focusing effect of 5 μm beads was enhanced as the rise of overall average electric field due to the DEP force scaling with the
second power of particle size. This trend is obviously revealed by the simulated particle trajectories after particle passing through the first serpentine period. The case (b) has been expatiated both experimentally and numerically above, where the electric field difference between the reservoir 1 and 2 is sufficient to focus both types of particles into a tight stream near the side wall at entrance region for subsequent separation in the exit region. In case (a), the estimated average electric field is 5 kV/m which is not sufficient enough to focus 5 µm beads into a tight stream and hence still mix with 1 µm beads at the exit region (See Figure 21 (a)). In case (c), both particle sub-streams are conspicuously over focused prior to separation which indicating the unnecessary large electric field for particle sorting.

5.5.3 Length Effects

Moreover, we also study the length effect of the serpentine part on particle focusing and separation. When applied average electric field was maintained at 15 kV/m, the two types of particles in the first five periods maintain mixing with each other and thus no observable separation occurred. When particles migrating to the 8th period, the separation distance between two sub-streams become 1 µm as simulated in COMSOL. This separation distance gradually increased from 8 µm to 16 µm as they move from the 16th period to the 24th one. However, after the 24th period, the separation distance has almost no change due to the fact that the final width of focused sub-stream was limited by particle diameter along channel centerline. Therefore, theoretically the length of the serpentine part can be shrunk to at least 3/4 of the original design based on the given average electric field and particle size.
5.5.4 Separation Resolution

Figure 22: Continuous focusing and separation of 3 μm beads and yeast cells in serpentine microchannel: Snapshot (left column), Superimposed images (middle column) and numerically (right column) predicted trajectories of particle mixture migrating from one branch to the (a) entrance region squeezed by the electrokinetic sheath flow from the other branch against the channel side wall and separated into two sub-streams at the (b) exit region.

Moreover, we also explored the separation resolution of the serpentine channel. 3 μm and 5 μm beads can be easily separated under the average electric field of 10 kV/m as predicted. Even 2 μm and 3 μm beads can be separated with 1-μm separation distance by adjusting the voltage applied in the outlet reservoir 2 to 20 V when the average electric field was fixed at 15 kV/m. The overall separation efficiency could be guaranteed by increasing the average electric field as previously demonstrated in case (c). Therefore, theoretically any two types of particles can be separated in the serpentine channel as long as they differ in the mobility ratio under certain average electric field. The separation of 3 μm beads and yeast cells whose size range from 4 to 8 μm in diameter demonstrates flexible application of our serpentine microchannels.
Figure 22 shows the snapshot and superimposed images obtained from experiment at (a) the entrance and (b) the exit of the serpentine section. To minimize the average electric field for separation, the applied voltage in the inlet reservoir 1 and 2 are 200 and 170 V respectively and corresponding nominal electric field is 10 kV/m. Likewise, the voltages in the outlet reservoir 1 and 2 are, respectively, 5 and 0 V to guarantee the flow rate difference is sufficient to separate two sub-streams. Both the beads and yeast cells were focused to a tight stream at the entrance prior to entering the serpentine section (See Figure 22(a)). After passing through the serpentine section, however, yeast cells were aligned along the channel centerline while 3 μm beads were slight scattered to a narrow band at the exit region (See Figure 22(b)). This also can be easily differentiated by comparing the darkness of superimposed images between the two sub-streams at the exit region, in which polystyrene beads appeared darker than the yeast cells. Notably the predicted particle trajectories closely agree with the experimental results, as shown in Figure 22 (right column), where the green trajectories represent beads and the red trajectories represent yeast cells.
Figure 23: Percentages of the 3 µm polystyrene beads and yeast cells in outlet reservoir 1 (a) and outlet reservoir 2 (b) of the serpentine microchannel after the size-based separation using sheath flow combined with DEP.

The throughput of the cell separation is estimated to be 1 µl/h, however, the channel depth does not affect the separation performance in the serpentine microchannel as the underlying deflection effects remain unvaried in the vertical direction. This advantage, of which is the so-called insulator-based DEP [18], [154], can be exploited to enhance the particle throughput without mitigating the separation performance. The efficiency of such size-based separation was also tested by counting the number of 3 µm beads and yeast cells in two outlet reservoirs. Figure 23 shows the percentages of beads and cells in outlet reservoirs 1 and 2, respectively, where a total of over 1700 beads were sorted. It was found that about 98% of the
particles collected in outlet reservoir 1 are 3 \( \mu \)m beads while over 94\% of the particles in outlet reservoir 2 are yeast cells.

5.6 Summary

The continuous focusing and separation of particles in a planar serpentine microchannel has been demonstrated both numerically and experimentally using dc electric fields. This technique combines the sheath flow which account for focusing and negative dielectrophoretic responsible for separation of the particles through a serpentine channel. Continuous focusing and separation of polystyrene beads with dissimilar sizes in the serpentine channel were demonstrated. Moreover, the overall average electric field effect and serpentine length effect on the performance of particle separation have been systematically investigated. Furthermore, based on our model, we predicted separation of particles with small size difference and further validate our conclusion by the separation experiment of polystyrene beads and yeast cells. It is found that as long as particles are different in mobility ratio, focusing and separation can occur continuously within the channel using such technique. Such channel could be used for separation of cells in lab-on-a-chip devices or even singling out monosized particles from sample mixture having a wide range of diverse sizes for further analysis along with numerous other applications.
CHAPTER 6: Particle Separation by Size in Spiral Microchannels

6.1 Introduction

Although the modified version of serpentine channel was demonstrated to be able to separate particles by size, there are at least two drawbacks of such technique: first, sheath flow is indispensable to the precise control of particle focusing at entrance region; second, the introduction of extra sheath solution will dilutes and disperses the original sample. Therefore, in this chapter we introduce a particle separation technique in DC electrokinetic flow through a planar spiral microchannel. The technique utilizes the dielectrophoretic focusing of particles induced by the non-uniform electric field inherent to curved channels that has been recently demonstrated in [108], [109]. The electrokinetic pumping, focusing, and separation of particles take place continuously in the spiral microchannel without additional pressure pumping and fabricated in-channel microstructures (microelectrodes or micro-insulators). It is noticed that curved microchannels have been recently used to filtrate and separate micro-particles by size in pressure-driven flow via inertia [49], [51], [52], [112]-[114].

Recall that we have verified the viability of particle focusing technique in symmetric double spiral microchannels in chapter 4. Moreover, focusing is always a necessary step prior to separation and if separation is required in our new design, the symmetry of the double spiral structure must be broken. Thereafter, we modified previous symmetric double spiral channel by diminishing the width of the second spiral in which DEP force drop down to an appropriate
magnitude such that particles with dissimilar sizes can be deflected into different sub-streams other than focusing to a tight stream.

### 6.2 Experiment

#### 6.2.1 Microchannel fabrication

![Figure 24: Picture of the double-spiral microchannel used to separate particles. The inset indicates the channel dimensions. The block arrows indicate the particle flow directions in the experiment.](image)

Figure 24 displays a picture of the spiral microchannel (filled with dark food dye for clarity, the inset indicates the channel dimensions) used in the experiment. It was fabricated in PDMS using the standard soft lithography technique [27]. The detailed fabrication process can be referred to in Appendix A. The microchannel consists of two spirals that have dissimilar widths and switch directions at the channel center (i.e. the junction of the two spirals). The first spiral is
counterclockwise and 50 µm wide, which starts with a short straight channel from the inlet reservoir as labeled in Figure 24. It is followed by a 100-µm-wide clockwise spiral, which is then split into two straight channels at the bifurcation prior to ending at the outlet reservoirs 1 and 2. Each spiral has four equally separated loops and measures in total 2.5 cm long including the straight part from/to the reservoir. The channel is everywhere 25 µm deep. The diameter of the innermost semi-circle is 100 µm. The radial distance (or the shortest distance) between the neighboring two loops is 125 µm (or the center-to-center distance between loops is 200 µm), as indicated in the inset of Figure 24.

6.2.2 Particle Solution Preparation

Polystyrene particles of 3, 5, and 10 µm in diameter (Sigma-Aldrich, USA) were re-suspended in either 1 or 10mM phosphate buffer at a concentration of about 10^7 particles per milliliter. Tween 20 (0.5% v/v, Fisher Scientific) was added to the particle solution to suppress particle adhesions to channel walls as well as particle aggregations. The calculated electric conductivities of these three sizes of particles are 13, 8, and 4 µS/cm, respectively, if the surface conductance is assumed to be 1 nS. As the electric conductivity of the buffer solutions (200 and 1900 µS/cm for 1 and 10 mM phosphate buffer respectively) is much higher than that of the particles, the CM factor, f_{CM}, was found close to −0.5 for all the three particles. Electric voltages were supplied by a DC power supply (Glassman High Voltage, High Bridge, NJ, USA) in conjunction with a custom-made voltage controller. Particle motion through the spiral microchannel was visualized through an inverted microscope imaging system (TE2000-U equipped with a DS Qi1MC CCD camera, Nikon Instruments, TX, USA).
6.3 Theory

Figure 25: Velocity analysis of electrokinetic particle transport in a microchannel consisting of two arcs. The electric field lines (E, short arrows indicate the directions) and the contour of electric field intensity (the darker the higher) are also illustrated.

Figure 25 shows the electric field lines (E, short arrows indicate the directions) and the contour of electric field intensity (the darker the higher) in a microchannel consisting of two arcs. Due to the variation in path length for electric current, electric field attains the maximum and minimum values near the inner and outer channel walls, respectively. Therefore, particles are subjected to a transverse dielectrophoretic motion, $U_{DEP}$ (a bold symbol denotes a vector hereafter), when they move electrokinetically, $U_{EK}$, through the arc channel. In DC electric fields, these two motions in the cross-stream and stream-wise directions, respectively, are given by
\[ \mathbf{U}_{\text{DEP}} = \mu_{\text{DEP}}(\mathbf{E} \cdot \nabla \mathbf{E}) = \left( \mu_{\text{DEP}} \frac{E^2}{\mathcal{R}} \right) \hat{n} \] (6-1)

\[ \mu_{\text{DEP}} = \varepsilon_f d^2 f_{\text{CM}} / \mu_f \] (6-2)

\[ \mathbf{U}_{\text{EK}} = \mu_{\text{EK}} \mathbf{E} = (\mu_{\text{EK}} E) \hat{s} \] (6-3)

\[ \mu_{\text{EK}} = f_g \varepsilon_f \left( \zeta_p - \zeta_w / \mu_f \right) \] (6-4)

where \( \mu_{\text{DEP}} \) is the particle dielectrophoretic mobility, \( \mathbf{E} \) the electric field strength with a magnitude of \( E \), \( \mathcal{R} \) the radius of curvature of a streamline that is equivalent to the electric field line illustrated in Figure 25 due to the similarity between velocity and electric fields in pure electrokinetic flow [20], [21], \( \hat{n} \) the unit vector normal to a streamline, \( \varepsilon_f \) the fluid permittivity, \( d \) the particle diameter, \( f_{\text{CM}} \) the Clausius–Mossotti (CM) factor, \( \mu_f \) the fluid viscosity, \( \mu_{\text{EK}} \) the particle electrokinetic mobility, \( \hat{s} \) the unit vector along a streamline, \( f_g \) the factor accounting for the wall effects on particle electrokinetic motion [55], and \( \zeta_p \) and \( \zeta_w \) are the zeta potentials of the particle and channel wall, respectively.

As biological cells and polymer beads often behave like poor conductors in DC and low-frequency AC (<100 kHz) electric fields [16], [17], their electric conductivity, \( \sigma_p \), is smaller than that of the suspending fluid, \( \sigma_f \). As such, the CM factor, \( f_{\text{CM}} = (\sigma_p - \sigma_f) / (\sigma_p + 2\sigma_f) \) is smaller than zero, leading to negative particle DEP. In other words, particles should be deflected across streamlines and toward the outer wall of each arc microchannel where the local electric field is the lowest, as illustrated in Figure 25. It is the velocity ratio

\[ \frac{|\mathbf{U}_{\text{DEP}}|}{|\mathbf{U}_{\text{EK}}|} = -\frac{\mu_{\text{DEP}} E}{\mu_{\text{EK}} \mathcal{R}} \] (6-5)
that dictates the particle deflection in the arc channel. Note that \( \mu_{\text{DEP}} < 0 \) for negative particle DEP. Apparently, the velocity ratio, \(|U_{\text{DEP}}|/|U_{\text{EK}}|\), increases with the rise of the applied electric field and the intrinsic particle mobility ratio (Eq. 6-6), if the channel structure, or more specifically, \( R \), is fixed.

\[
\frac{\mu_{\text{DEP}}}{\mu_{\text{EK}}} = \frac{d^2}{6f_e (\zeta_p - \zeta_w)} \frac{\sigma_p - \sigma_i}{\sigma_p + 2\sigma_i}
\]

(6-6)

As \( \mu_{\text{DEP}}/\mu_{\text{EK}} \) is a function of particle size \( (d) \), charge \( (\zeta_p) \), and conductivity \( (\sigma_p) \), it is natural to suppose that particles may be deflected by DEP to different flow paths in a spiral microchannel and thus be separated by one or more of their intrinsic properties. The necessary condition for this separation is that all the particles should be flowing in a single focused stream prior to the differential deflection. Therefore, a double-spiral microchannel is employed to implement the dielectrophoretic focusing of particles in the first spiral and subsequently the dielectrophoretic separation in the second spiral. Moreover, the second spiral is designed wider than the first one to break the symmetry of the dielectrophoretic effects. Otherwise, particles will be simply focused by DEP to a stream flowing near the outer wall of the first spiral, which is then dielectrophoretically deflected (while remaining focused) to the outer wall of the second spiral without discrimination.

The continuous separation of particles in an asymmetric double-spiral microchannel depends on not only the difference in the velocity ratio, \(|U_{\text{DEP}}|/|U_{\text{EK}}|\), between the two (or multiple) types of particles to be separated, but also the magnitude of these ratios themselves. The former determines the relative displacement between the two types of particles in the second spiral and thus the sensitivity of the separation. The magnitude of \(|U_{\text{DEP}}|/|U_{\text{EK}}|\) determines the extent of particle focusing and deflection in the first and second spirals, respectively, and thus
the effectiveness of particle separation. Principally, any two particles of dissimilar $|\text{UDEP}|/|\text{UEK}|$ (essentially $\mu_{\text{DEP}}/\mu_{\text{EK}}$ due to the difference in particle size, surface charge, or conductivity as noted above) can be separated by DEP in the asymmetric spiral microchannel. Such separation can be enhanced by using a larger electric field and/or a longer spiral because the dielectrophoretic focusing and deflection are both accumulative effects. Moreover, this continuous separation should work more effectively for bigger particles or particles with a smaller electrokinetic mobility due to a greater $\mu_{\text{DEP}}/\mu_{\text{EK}}$ (Eq. 6-6).
6.4 Results and discussion

6.4.1 Separation of Polystyrene Beads by Size

Figure 26: Demonstration of the continuous dielectrophoretic separation of 5 and 10 mm particles in the spiral microchannel: (A) snap-shot (left) and superimposed (right) images of the particles at the channel inlet; (B) superimposed image of the focused 5- and 10-μm particle stream at the channel center; (C) superimposed image of the separated 5 and 10 μm particles at the channel bifurcation. Particles were re-suspended in 1mM phosphate buffer. The DC voltages imposed on the reservoirs are indicated in the bottom-left image. The block arrows indicate the flow directions. UDEP in (B) is the dielectrophoretic particle velocity induced in the curved channel (refer to Figure 24).
Figure 26 shows the continuous dielectrophoretic separation of 5 and 10 µm particles in the fabricated double-spiral microchannel. Particles were mixed and re-suspended in 1mM phosphate buffer. The imposed DC voltage at the inlet reservoir was 600 V, while those at the outlet reservoirs 1 and 2 were 0 and 15 V, respectively. The average electric fields in the two spirals were numerically computed (COMSOL, Burlington, MA, USA) as 186 and 93 V/cm, respectively. The highest electric field, 265 V/cm, occurred near the innermost semi-circle of the 50-µm wide spiral, where, however, particles hardly approached due to the induced negative dielectrophoretic motion directing toward the lower field region (see U_{DEP} in Figure 26B).

At the channel inlet (Figure 26A), 5 and 10 µm particles were distributed uniformly and covered nearly the entire channel width. Once they moved into the curved part of the 50-µm-wide first spiral, both particles started experiencing negative DEP as explained above and were thus pushed toward the outer channel wall. The result was seen to be a squeezed particle stream near the outer wall of the first spiral at the channel center (Figure 26B), where the 10 µm particles obtained a better focusing due to their larger mobility ratio, μ_{DEP}/μ_{EK} in Eq. (6-6), than 5 µm ones. As the measured electrokinetic mobility for both the sizes of particles in 1mM phosphate buffer is about μ_{EK} = 3.3×10^{-4} (cm²/V s), the mobility ratio can be calculated as μ_{DEP}/μ_{EK} = -43.6 and -174.2 µm²/V for 5 and 10 µm particles, respectively. When they entered into the 100-µm-wide second spiral, 5 and 10 µm particles were still subjected to negative DEP but in the opposite direction due to the switching of inner and outer walls between the two spirals (see the direction change of particle dielectrophoretic velocity, U_{DEP}, in Figure 26B). Therefore, both particles were now deflected toward the outer channel wall of the second spiral while at dissimilar rates due to the difference in μ_{DEP}/μ_{EK}. As a consequence, the single stream of 5 and 10 µm particles in the 50-µm-wide first spiral was seen being gradually divided into two sub-
streams in the 100-μm-wide second spiral. These two sub-streams were eventually separated at the channel bifurcation, as illustrated in Figure 26C. Specifically, 10 μm particles were collected into outlet reservoir 1, while 5 μm particles were collected in outlet reservoir 2.

The applied 600 V DC voltage at the inlet reservoir is possibly the minimum value in our experiment for a 100% separation of the 5 and 10 μm particles. To achieve this, a 15 V DC bias voltage is also necessary at outlet reservoir 2 to sort 10 μm particles into outlet reservoir 1. If the bias voltage is less than 15 V, part of the 10 μm particles was observed to flow into reservoir 2. As analyzed in Section 2, the electric field threshold for particle separation depends on the properties of the particle-fluid-channel system. It can be reduced when the channel length is increased or the channel width is decreased. Larger electric fields will be needed to separate particles with smaller sizes or smaller size differences. The same spiral microchannel as that in Figure 26 was also used to separate 3 and 5 μm particles in 1mM phosphate buffer. It was observed that the application of a 1500 V DC voltage at the inlet reservoir was still not sufficient to yield a 100% separation of these two sizes of particles. At this voltage level, the average electric field in the 50-μm wide first spiral reached over 450 V/cm, which can cause adverse effects to bio-particles especially mammalian cells [27, 54]. One easy way to reduce the electric field is to slow down the particle electrokinetic motion, i.e. reduce $\mu_{EK}$ in Eq. (6), as noted in Section 2. This can be performed simply by increasing the buffer concentration, which has been long known to cause a decrease in the surface charge of both the channel walls and the particles (i.e. $\zeta_p$ and $\zeta_w$).

6.4.2 Separation Resolution
Figure 27: Snapshot at the entrance (A) and Superimposed images at the center (B) and bifurcation (C) of the spiral microchannel for the continuous dielectrophoretic separation of 3 and 5 μm particles. Inset at the bottom left indicates the zoom in view of the entrance region for clarity. Particles were re-suspended in 10mM phosphate buffer.

Figure 27 shows the images at the center (B) and bifurcation (C) of the spiral microchannel for the continuous dielectrophoretic separation of 3 and 5 μm particles. Particles were re-suspended in 10mM phosphate buffer with a measured electric conductivity of around 1900 μS/cm. The measured electrokinetic mobility was about $\mu_{EK}=0.76\times10^{-4}$ (cm$^2$/V s) for both the sizes of particles, which is significantly lower than that of 5 and 10 μm particles in 1mM phosphate buffer as given above. With this new electrokinetic mobility in 10mM buffer, the mobility ratio were determined as $\mu_{DEP}/\mu_{EK} =-64.7$ and $-179.7$ μm$^2$/V for 3 and 5 μm particles,
respectively. The imposed DC voltages at the inlet reservoir and outlet reservoir 2 (see the labels in Figure 24) were 700 and 4 V, respectively. Outlet reservoir 1 was still grounded. It is straightforward to find from Eqs. (5) and (6) that the velocity ratios $|U_{DEP}|/|U_{EK}|$ of 3 and 5 μm particles in the 10mM buffer are 70 and 20% larger than those of 5 and 10 μm particles in the 1mM buffer. Therefore, the former two particles should be more effectively focused and deflected by DEP, which is consistent with the observations in between Figure 27A (with the 10mM buffer) and Figure 26B (with the 1mM buffer). As such, 3 and 5 μm particles could be effectively separated at a relatively low electric field (Figure 27C), although their size difference is smaller than that between 5 and 10 μm particles.

6.5 Summary

In this chapter, a novel curvature-induced particle separation technique has been demonstrated in spiral microchannels. This technique exploits the electrokinetic and dielectrophoretic motions induced in an asymmetric double-spiral microchannel to implement a continuous pumping, focusing, and separation of particles. The exclusion of in-channel microelectrodes and micro-insulators not only simplifies the device fabrication and operation, but also eliminates the regions with large electric fields, shear stresses, and Joule heating. Therefore, the technique can potentially be used to separate biological cells including vulnerable mammalian cells. Moreover, the channel depth does not affect the particle separation performance in the spiral microchannel as the underlying dielectrophoretic focusing and deflection effects remain unvaried over the entire depth. This feature, an advantage of the so-called insulator-based DEP [18], [154], can be used to increase the particle throughput while not alleviating the separation performance. The compact spiral microchannel is envisioned to be
integrated with other functional components into lab-on-a-chip devices toward numerous other applications.
CHAPTER 7: Particle Separation by Charge in Spiral Microchannels

7.1 Introduction

In chapter 5 and 6, we have developed approaches to separating particles in serpentine and spiral microchannels respectively by the use of curvature-induced dielectrophoresis [108], [109], which is essentially a new form of iDEP and herein termed cDEP. This method exploits the intrinsic electric field gradients formed within microchannel turns to manipulate particles by DEP [163]. As such, the adverse effects caused by the micro-obstacles in iDEP devices are mitigated. We found that in an appropriate suspending medium large particles experience negative cDEP in a serpentine microchannel and migrate toward the channel center while small particles undergo positive cDEP and line the channel sidewalls. These distinctive focusing phenomena were combined to implement a continuous separation of particles by size [160]. In another study we utilized the negative cDEP in a serpentine microchannel to push small particles to the channel center and meanwhile bounce large particles between the two sidewalls for a continuous sorting [164]. Additionally we demonstrated a continuous binary separation of particles by size in a double-spiral microchannel, where particles are focused by negative cDEP to a stream flowing near the outer wall of the first spiral and then deflected by negative cDEP to size-dependent flow paths in the second spiral [165]. However, these methods are basically capable of separating particles by size only.

As reviewed above, the majority of the particle separations demonstrated so far in microfluidic systems have been limited to be size based and binary [26], [98], [123], [124]-[126].
In this chapter we demonstrate that cDEP can also be exploited to separate particles by surface charge in a double-spiral microchannel. Moreover, we use this cDEP method to implement a continuous ternary separation of particles by charge and size at the same time.

7.2 Experiment

7.2.1 Microchannel fabrication

The microchannel was fabricated with polydimethylsiloxane (PDMS) using the standard soft lithography technique. The detailed fabrication process can be referred to in Appendix A. As shown in Figure 28, the channel is composed of two spirals that have three loops in each and are asymmetric with respect to the channel center (i.e., the junction of the two spirals). The first spiral is uniformly 50 μm wide and connected to the inlet reservoir (see the labels in Figure 28) through a short straight segment of equal width. It circulates counter-clockwise till the channel center and measures about 13 mm long. The second spiral starts at the channel center and extends clockwise with a gradually increasing width from 50 μm to 100 μm. It is followed by a 1.5 mm-long straight segment, which trifurcates into three equal branches of 100 μm width prior to ending at the outlet reservoirs 1, 2 and 3 (see the labels in Figure 28). The diameter of the innermost semi-circle is 100 μm for both spirals. The radial distance between neighboring loops increases from the inner 150 μm to the outer 350 μm in order to avoid the electrical leakage through the PDMS wall [166]. The channel is 25 μm deep everywhere and measures 39 mm long in total with an overall footprint of less than 2 cm × 2 cm including all the reservoirs.
7.2.2 Particle solution preparation

In demonstrating the particle separation by charge, plain non-coated polystyrene beads of 10(±0.2) μm in diameter (Sigma-Aldrich, USA) and fluorescent carboxyl-coated polystyrene beads of 10.14(±1.04) μm in diameter (Bangs laboratories, USA) were mixed and re-suspended in 1 mM phosphate buffer at a concentration of about 10^7 particles per milliliter for each type. In
the experiment of particle separation by charge and size, plain non-coated polystyrene beads of 5(±0.1) μm in diameter (Sigma-Aldrich, USA) were added to the above particle mixture, which was then re-suspended in 0.1 mM phosphate buffer. Tween 20 (0.5% v/v, Fisher Scientific) was added to both types of particle solutions to suppress particle adhesions to channel walls as well as particle aggregations. Moreover, particle solution was stirred before being introduced to the inlet reservoir.

7.2.3 Particle Manipulation and Visualization

Electric voltages were supplied by a DC power supply (Glassman High Voltage, High Bridge, NJ, USA) in conjunction with a custom-made voltage controller. Pressure-driven flow was eliminated by carefully balancing the liquid heights in the reservoirs prior to every experiment. Particle motion was monitored using an inverted microscope (Nikon Eclipse TE2000U, Nikon Instruments, Lewisville, TX), through which videos and images at the inlet, center and outlet regions of the spiral microchannel 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). The trajectories of plain and fluorescent beads were obtained by superimposing sequential images in the Nikon imaging software with reference to a dark and bright background, respectively. They were then combined in Matlab® to achieve the graphical demonstration of particle separation in terms of discrete particle trajectories.
7.3 Theory

7.3.1 cDEP for Particle Deflection

Figure 29 shows the streamlines (short arrows indicate the directions) and contour (represented by the background color, the darker the higher) of electric field, \( E \), in the center region of the spiral microchannel. Only the first half-loop is included for each spiral, and the width change in the second spiral is exaggerated for a better demonstration. In both spirals electric field reaches the maximum and minimum values at the inner and outer walls, respectively, due to the variation in path length for electric current. Therefore, particles experience a transverse dielectrophoretic motion, \( U_{DEP} \), when they follow the electric field lines (see Figure 29) to move electrokinetically through the curving channel with velocity, \( U_{EK} \). Using the effective dipole moment method \[15\], one can obtain \[165\]

\[
U_{DEP} = \mu_{DEP} (E \cdot \nabla E) = \left( \frac{\mu_{DEP} E^2}{\gamma R} \right) \hat{n} \tag{7-1}
\]
Figure 29: Illustration of cDEP for particle (represented by spheres) focusing and separation in an asymmetric double-spiral microchannel. Also illustrated are the electric field lines and contour (background, the darker the higher field magnitude).

where $\mu_{DEP}$ is the dielectrophoretic mobility of particles, $E$ the electric field with a magnitude of $E$, $R$ the radius of the local curvature of an electric field line (equivalent to a streamline in pure electrokinetic flows [20], see Figure 29), and $\hat{n}$ the unit normal vector of the streamline. Hence, the particle deflection across the channel width due to cDEP can be approximately characterized as

$$\text{deflection} \approx U_{DEP} \frac{R \theta}{U_{EK}} = \frac{\mu_{DEP}}{\mu_{EK}} E \theta \quad (7-2)$$

where $\mu_{EK}$ is the electrokinetic mobility of particles, and $\theta$ is the rotating angle of the spiral channel in one direction. It is straightforward that increasing the electric field and/or employing
multiple loops (each loop has a rotating angle of $2\pi$) for the spiral enhance the particle deflection. More importantly, this deflection is a function of particle mobility ratio, which in DC electric fields is given by [162]

$$\frac{\mu_{\text{DEP}}}{\mu_{\text{EK}}} = \frac{d^2 f_{\text{CM}}}{6\left(\zeta_p - \zeta_w\right)}$$

$$f_{\text{CM}} = \frac{\sigma_p - \sigma_f}{\sigma_p + 2\sigma_f}$$

where $d$ is the particles diameter, $f_{\text{CM}}$ the so-called Clausius–Mossotti (CM) factor [15], $\zeta_p$ the zeta potential (a measure of surface charge) of particles, $\zeta_w$ the average zeta potential of the channel wall, $\sigma_p$ the electric conductivity of particles, and $\sigma_f$ the electric conductivity of the suspending fluid. The dependence of deflection on the intrinsic properties of particles including diameter, charge and conductivity enables the continuous separation of particles by one or a combination of these properties in spiral microchannels, which will be explained below.

### 7.3.2 cDEP for Particle Focusing and Separation

Traditionally, a negative $f_{\text{CM}}$ indicates negative DEP which shifts particles to the region of lower electric field [11]. Since polystyrene beads [16] and biological cells [167] as well often appear poorly conducting in DC electric fields, they experience negative DEP in typical buffer solutions due to $\sigma_p < \sigma_f$ and migrate toward the outer channel wall in either spiral as illustrated in Figure 29. Therefore, we can use cDEP to focus all particles to a stream flowing near the outer wall of the first spiral if the electric field, $E$, and/or the number of loops in the first spiral, reflected by $\theta$ in equation (7-2), are sufficiently large. The minimum value of the product $E\theta$ for
such cDEP focusing is determined by the particle with the smaller (or the smallest if more than two types of particles are present in the mixture) mobility ratio, \( \mu_{DEP}/\mu_{EK} \), small.

When the focused particle stream flows into the second spiral with electric field magnitude/gradients being lower than in the first spiral (see Figure 29), those particles with mobility ratios larger than \( \mu_{DEP}/\mu_{EK} \), small can still be displaced by cDEP to near the outer channel wall as they actually attain an over-focusing in the first spiral. In contrast, the smaller (or the smallest) particles with \( \mu_{DEP}/\mu_{EK} \), small are deflected at a lower rate and thus by a smaller distance though still toward the outer wall of the second spiral. As a result, particles with dissimilar mobility ratios, \( \mu_{DEP}/\mu_{EK} \), can be continuously separated by cDEP in the second spiral and eventually sorted into the three outlet reservoirs 1, 2 and 3 after passing the channel trifurcation (see Figure 28). According to equation (7-3), this method can apply to the separation of particles by size, charge, and/or conductivity, among which a binary separation of polystyrene beads by size has been demonstrated by the authors in chapter 6 [165].

### 7.4 Numerical Modeling

The deflection of particles towards the outer wall of a microchannel turn brings out a dielectric interaction force between the particle and wall, \( F_{p-w} \), due to the non-uniform distribution of the applied electric field around the particle. This repulsive force has a similar origin to \( F_{DEP} \) in Eq. (5-1). However, the latter is attributed to the intrinsic non-uniformity of the applied electric field within a microchannel turn while \( F_{p-w} \) is induced when a finite-sized non-conducting particle approaches a non-conducting channel wall and thus perturbs the local electric field distribution. \( F_{p-w} \) is also distinguished from the classic electrostatic repulsion which is a result of the electric double layer interactions [168], [169]. Moreover, it becomes non-trivial at a
much longer range (~ 1 μm between the particle and wall surfaces) than the electrostatic repulsion and the van der Waals attraction (both on the order of nm) do. So the latter two forces may be safely neglected. \( F_{p-w} \) prevents particles from approaching channel walls too closely, and is obtained by integrating the Maxwell stress tensor over the particle surface, which for a non-conducting particle is given as [11], [67]

\[
F_{p-w} = \oint_S \left( \frac{1}{2} \varepsilon_0 \left( E_p \cdot E_p \right) \right) \hat{n} dS
\]  

(7-5)

where \( \hat{n} \) is the unit vector normal to the streamline while passing through the particle center, and \( E_p \) indicates the electric field distribution in the fluid with consideration of the particle. As \( E_p \) is dependent on the particle size and the location with respect to the wall, \( F_{p-w} \) varies with the dynamic position of the particle during its electrophoresis. Therefore, the calculation of \( F_{p-w} \) would require a full account of the electro-hydrodynamic interactions among the particle, electric field and fluid.

In order to quantitatively understand the particle electrophoresis in spiral microchannels, we revised the numerical model developed in previous chapter. We modified particle-wall interaction force by introducing another correction factor \( C_{p-w} \) instead of considering the perturbations of particles on the flow and electric fields in the model. Hence, the particle velocity in Eq. (5-3) is rewritten as

\[
U_p = \mu_{ex} E + c \mu_{DEP} \nabla E^2 + c_{p-w} f_{p-w} (E \cdot E) \hat{n}
\]

(7-6)

where the last term represents the virtual particle velocity arising from the particle-wall interaction force, \( F_{p-w} \) in Eq. (7-5), and \( f_{p-w} \) is a factor that characterizes this velocity in terms of the electric field \( E \) without considering the presence of particles. For the case that the particle
radius is much smaller than the radius of curvature of the channel, \( f_{p-w} \) has been obtained in previous work as [23],

\[
f_{p-w} = 0.705 \exp\left[-2.687 \left(\frac{\delta}{a}\right)\right] \frac{\varepsilon_f a}{6\pi \mu_f} \quad (7-7)
\]

where \( \delta \) is the separation distance between the particle and the wall. With this treatment, the complicacy and difficulty in solving for \( E_p \) in Eq. (7-5) through a full consideration of particle, fluid and electric field interactions are avoided.

The instantaneous position of a particle, \( x_p \), is then obtained by integrating the particle velocity \( U_p \), i.e.

\[
x_p(t) = x_0 + \int_0^t U_p(t')dt' \quad (7-8)
\]

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 spiral microchannel was developed in COMSOL®, where the effects of the top and bottom channel walls on particle motions were ignored. Then, the electric field distribution that was needed to compute the particle velocity, \( U_p \), from equation (7-6) was solved from the 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 script 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 (7-7).
7.5 Results and discussion

7.5.1 Binary Separation of Polystyrene Beads by Charge

Figure 30: Continuous binary separation of plain non-coated (dark) and fluorescent carboxyl-coated (bright) 10 µm polystyrene beads in a spiral microchannel using cDEP: snapshot (a1) and superimposed (a2) images in the inlet region, superimposed (b1) and simulated (b2) image in the center region, snapshot (c1), superimposed (c2), and simulated (c3) images in the trifurcation region. The inlet reservoir was imposed a 400 V DC voltage while the three outlet reservoirs (see Figure 28), labeled as OR 1-3 in (c1), were all grounded. The block arrows indicate the flow directions.

Figure 30 shows the continuous binary separation of plain non-coated (dark) and fluorescent carboxyl-coated (bright) 10 µm polystyrene beads in the spiral microchannel using cDEP. The beads were re-suspended in 1 mM phosphate buffer with a measured electric conductivity 200 µS/cm. The inlet reservoir was imposed a 400 V DC voltage while the three outlet reservoirs were all grounded (see Figure 28). The average electric field in the 50 µm wide first spiral is about 160 V/cm, which was obtained from a full-scale modeling in COMSOL (Burlington, MA, USA). The electric field in the second spiral is lower than this value since its width increases from 50 µm to 100 µm. The electrokinetic mobility, $\mu_{EK}$, of beads was determined by tracking
the motion of individual beads in the straight part of the channel at a given electric field. We found $\mu_{EK} = 3.3 \times 10^{-8}$ and $1.6 \times 10^{-8}$ (m$^2$/V·s) for the non-coated and coated beads, respectively, indicating their dissimilar surface charges. As they are of nearly identical sizes and made of the same material, the two types of beads are expected to experience similar DEP or possess similar dielectrophoretic mobility, $\mu_{DEP}$. As such, the coated beads should have a mobility ratio, $\mu_{DEP}/\mu_{EK}$, roughly twice that of the non-coated beads.

At the inlet of the spiral microchannel the coated and non-coated beads entered into the straight segment uniformly, as seen from the snapshot image in Figure 30(a1) and the superimposed image in Figure 30(a2). They were then both deflected by negative cDEP in the first spiral and gradually focused into an overlapping stream near the outer wall as demonstrated in Figure 30(b). Immediately following that, the focused two types of beads in Figure 30(b) were observed to quickly migrate away from the inner wall of the second spiral (i.e., continuation of the outer wall of the first spiral) while at different rates. Apparently, the coated beads were displaced more than the non-coated ones due to their nearly doubled mobility ratio, $\mu_{DEP}/\mu_{EK}$, of the latter. As a consequence, the single focused particle stream in the first spiral continuously and autonomously split into two sub-streams based on charge at the end of the second spiral, see the snapshot image in Figure 30(c1), the superimposed image Figure 30(c2), and simulated image in Figure 30(c3). Eventually, the coated and non-coated beads were sorted in the channel trifurcation and collected into the outlet reservoirs 1 and 2, respectively.
Figure 31: Percentages of the coated and non-coated 10 μm polystyrene beads in outlet reservoir 1 (a) and outlet reservoir 2 (b) of the spiral microchannel after the charge-based separation using cDEP.

We tested the efficiency of such charge-based separation by counting the number of dark non-coated and bright coated beads in the three outlet reservoirs. Figure 31 shows the percentages of these two types of beads in outlet reservoirs 1 and 2, respectively, where a total of over 800 beads were sorted. No beads were noticed in outlet reservoir 3. It was found that over 95% of the beads collected in outlet reservoir 1 are coated beads while over 98% of the beads in outlet reservoir 2 are non-coated beads.
7.5.2 Effect of the Voltage at the Inlet Reservoir

Figure 32: Effects of the DC voltage at the inlet reservoir on the binary separation of plain non-coated (dark) and fluorescent carboxyl-coated (bright) 10 μm polystyrene beads in the spiral microchannel: (a) 200 V, (b) 400 V, and (c) 600 V. The three outlet reservoirs, labeled as OR 1-3 in (b), were grounded in all cases. The left and right columns show the snapshot and superimposed images, respectively. The block arrows indicate the flow directions in all three cases.
We also studied how the voltage at the inlet reservoir affects the cDEP separation of non-coated (dark) and carboxyl-coated (bright) 10 μm beads in the spiral microchannel.

Figure 32 shows the particle behaviors in the trifurcation region under the inlet voltages of 200 V (a), 400 V (b), and 600 V (c), respectively. The three outlet reservoirs were grounded in all cases. The 400 V case has been explained above, where the electric field in the first spiral is sufficient to focus both types of beads into a single stream near the outer channel wall for subsequent complete separation in the second spiral; see Figure 32(b). If, however, the inlet voltage is decreased to 200 V, the resulting 80 V/cm electric field in the first spiral is barely enough to deflect even the coated beads (with a larger mobility ratio $\mu_{DEP}/\mu_{EK}$) to the outer channel wall, leading to an incomplete separation. As shown in Figure 32(a) the coated beads moved to outlet reservoirs 1 and 2 while the non-coated beads were still collected into reservoir 2 though in a wider stream than the 400 V case. When the inlet voltage was increased from 400 V to 600 V, both types of beads were over-focused in the first spiral. As such, the lateral displacement between the two particle sub-streams in the second spiral was small so that all particles travelled to outlet reservoir 1 without separation, see Figure 32 (c).
7.5.3 Effect of the Voltages at the Outlet Reservoirs

Figure 33: Effects of the DC voltages at the outlet reservoirs, labeled as OR 1-3 in (a), on the binary separation of plain non-coated (dark) and fluorescent carboxyl-coated (bright) 10 μm polystyrene beads in the spiral microchannel. The outlet voltages for each case are marked on the superimposed images (right column) along with the block arrows indicating flow directions. The inlet voltage was fixed at 400 V in all three cases.
Further, we studied how the DC voltages at the outlet reservoirs may affect the binary separation of non-coated (dark) and carboxyl-coated (bright) 10 µm beads in the spiral microchannel. The inlet voltage was fixed at 400 V. Figure 33(a) shows the exact case that we have explained in Figure 30 and revisited in Figure 32(b), where the three outlets were all grounded so that the coated and non-coated beads were sorted into outlet reservoirs 1 and 2, respectively. However, when the voltages at these two reservoirs were tuned to 37 V and 4 V, we observed that the coated and non-coated beads were sorted into reservoirs 2 and 3, respectively, as demonstrated in Figure 33(b). This is because the new voltages at the outlet reservoirs changed the flow splitting at the trifurcation and redirected the majority of the flow toward outlet reservoirs 2 and 3. We also implemented the separation of coated and non-coated beads into outlet reservoirs 1 and 3, respectively, as seen in Figure 33(c). For this to happen, we floated outlet reservoir 2 and applied a 12 V to outlet reservoir 1 while still maintaining outlet reservoir 3 grounded. As such, there was actually no flow into reservoir 2 during the particle separation. This experiment demonstrates the flexibility of the cDEP separation in spiral microchannels.

7.5.4 Ternary Separation of Particles by Charge and Size

To test the versatility of the cDEP separation in spiral microchannels, we conducted another experiment to attempt a ternary separation of particles by charge and size. For this purpose, we fabricated a new spiral microchannel that is similar to the one in Figure 28 but with a doubled width everywhere. The gap distances between neighboring loops were also adjusted accordingly, yielding a total channel length of 46 mm. Plain non-coated 5 µm beads were added into the above-used binary particle mixture, which was then re-suspended in 0.1 mM phosphate buffer
(electric conductivity measures 26 μS/cm) for an improved separation. Using the method described earlier, we obtained the electrokinetic mobility, \( \mu_{EK} = 4.3 \times 10^{-8}, 4.5 \times 10^{-8}, \text{and} 2.2 \times 10^{-8} \text{ (m}^2/\text{V} \cdot \text{s)} \) for the non-coated 5 μm, non-coated 10 μm, and coated 10 μm beads, respectively. As the dielectrophoretic mobility, \( \mu_{DEP} \), is proportional to particle diameter squared (see equation (3)), it is expected that the mobility ratio, \( \mu_{DEP}/\mu_{EK} \), of 5 μm beads is about one quarter of that of the non-coated 10 μm beads. The mobility ratio of the coated 10 μm beads is the largest among the three due to their smallest electrokinetic mobility.

Figure 34 shows the experimental result. The DC voltage at the inlet reservoir is 1000 V, and those at the outlet reservoirs 1, 2 and 3 are 33 V, 20 V and 0 V, respectively. The computed electric field in the first spiral is about 330 V/cm on average, which as explained earlier, served to deflect and focus with cDEP all three types of beads to a single stream near the outer wall of the first spiral. Subsequently the discrepancy in the particle mobility ratio led to differential lateral displacements of the focused bead streams in the second spiral. As such, the coated 10 μm beads, non-coated 10 μm beads, and non-coated 5 μm beads were sorted into outlet reservoirs 1, 2 and 3, respectively, as illustrated in Figure 34 (a1 for snapshot, a2 for superimposed). We ran this separation experiment for over 10 minutes without interruption and adjustment. After that we took an image (top view) of each of the three outlet reservoirs, which, as seen in Figure 34(b1, b2 and b3 for outlet reservoirs 1, 2 and 3), indicates a high separation efficiency of the three types of beads by charge and size.
Figure 34: Continuous ternary separation of plain non-coated 5 µm (grey), plain non-coated 10 µm (dark), and fluorescent carboxyl-coated 10 µm (bright) polystyrene beads in a spiral microchannel using cDEP: snapshot (a1), superimposed (a2) and simulated (a3) images in the trifurcation region; top-view images of the three outlet reservoirs (b1, b2 and b3), labeled as OR 1-3 in (b1). The block arrows indicate the flow directions.

7.6 Summary

We have performed a continuous binary separation of particles by surface charge in a double-spiral microchannel using negative cDEP. The effects of the DC voltages applied to the inlet and outlet reservoirs on the particle separation have been examined systematically. As compared to the traditional elution-based capillary electrophoresis [97], [98], and field flow fractionation [122] for charge-based separation, this cDEP technique has the advantage of continuous-flow process
and is thus more suitable for integration with pre- and/or post-separation parts into lab-on-a-chip devices. We have also implemented a continuous ternary separation of particles by charge and size in a similar spiral microchannel. These experiments demonstrate that the developed cDEP technique may be used with potential to separate multiple particle targets by intrinsic properties (e.g., particle size, charge and conductivity).
CHAPTER 8: Conclusions and Future Work

Beginning with the fundamental study of particle electrophoresis in a U-turn microchannel, this thesis extensively investigated particle electrokinetic and dielectrophoretic motions in different types of curved microchannel both experimentally and numerically. The first part concentrates on the particle focusing which is usually an indispensable step prior to separation and demonstrated that both serpentine and spiral microchannels are capable of focusing particles to the channel centerline and side wall respectively. The second part of the thesis concerns the particle separation technique in different curved microchannels. More specifically, combining dielectrophoresis with electrokinetic sheath flow, the modified version of serpentine microchannel can be exploited for the separation of particles by size. Moreover, asymmetric double spiral microchannels have also been verified of being capable of continuously focusing and separation of particles by size difference in the first and second spiral respectively due to curvature-induced Dielectrophoresis (cDEP). Furthermore, we further demonstrated its capability of continuous focusing and separation of particles by charge difference which was rarely explored by other techniques. At last, we use this cDEP method to accomplish a continuous ternary separation of particles by charge and size simultaneously. Specifically the major contribution and conclusion of this thesis are:

1. In Chapter 2, a fundamental experimental study of particle motion within a U-turn was conducted which can be considered as a typical structure in curved microchannels. A 2-D numerical modeling based on finite element method has been developed to investigate particle deflection within the U-turn. It is found out that the lateral displacement of particles away from their original position increase as the rise of either
applied electric field or particle size. Therefore, such curvature-induced particle deflection can be used to implement other fundamental manipulation of particles such as focusing and separation.

2. In Chapter 3, we demonstrated a particle focusing technique based on the cross-stream dielectrophoretic particle motion in a serpentine microchannel in which each period consists of two U-turns. The focusing performance and particle throughput can both be enhanced by applying large electric fields which make the technique very promising. Moreover, the reduction in the footprint of the serpentine pattern also provides extra space for potential other integrated functionalities on microfluidic chips. Furthermore, compared to the existing AC dielectrophoretic focusing technique, this technique avoids both the in-channel micro-electrodes and extra pumping without compromising focusing performance.

3. In Chapter 4, instead of changing directions alternately, a spiral channel maintains the direction of its turns and hence curvature-induced particle dielectrophoresis also retain at constant angle to the electrokinetic flow. Correspondingly, particle will focus to a tight stream flowing near the outer wall of the spiral channel. Likewise, focusing performance can be enhanced by either increasing applied electric fields or replace the particles with large size in diameter. This size-dependent property provides potential particle separation technique in spiral microchannels which was demonstrated in chapter 6.

4. In Chapter 5, we developed a particle separation technique in modified serpentine microchannels based on particle focusing technique demonstrated in chapter 3. This
method takes the advantage of electrokinetic sheath flow for focusing and curvature-induced dielectrophoresis for separation of particles by size. Compared to other techniques, neither additional pumping components nor in-channel microstructures (microelectrodes or micro-insulators) are required to implement pumping, focusing, and separation of particles in the serpentine microchannel simultaneously by DC electric fields. Moreover, we also demonstrated the high separation resolution of our serpentine microchannel by singling out 3 μm beads from yeast cells whose sizes varied from 4 μm to 8 μm.

5. In Chapter 6, we introduced another particle separation technique in DC electrokinetic flow through a planar spiral microchannel based on the focusing technique we demonstrated in chapter 4. This technique takes the advantage of curvature-induced dielectrophoresis in an asymmetric double-spiral microchannel to implement a continuous transport, focusing, and separation of particles by size. It also eliminates the regions with large electric fields, shear stresses, and Joule heating by avoiding in-channel microelectrodes and micro-insulators. Moreover, the channel depth does not affect the particle separation performance in the spiral microchannel because the underlying dielectrophoretic focusing and deflection effects remain unvaried over the entire depth, which can be used to increase the particle throughput without alleviating the separation performance.

6. In Chapter 7, different from most of the particle separation techniques demonstrated so far in microfluidic systems which mainly focused on size based and binary aspects, we demonstrated that curvature-induced dielectrophoresis can also be used to separate particles by surface charge other than size in a double-spiral microchannel. Moreover,
we use this technique to implement a continuous ternary separation of particles by charge and size simultaneously. Furthermore, a modified numerical model considering the particle wall interaction was developed, which simulates closely the observed particle electrophoretic behaviors in the spiral channel in most cases. We anticipated that the curvature-induced dielectrophoretic effect will find promising applications in lab-on-a-chip devices as pre- and/or post-separation parts.

Future work could entail improving these focusing and separation methods by optimizing the channel geometries as well as applying dc-biased ac electric fields which has been demonstrated recently. There are many aspects of optimization of channel geometries for future work, and some of them are discussed below. First, under certain electric field, the length of the serpentine part or the loops of the spirals can be minimized based on the numerical modeling without compromising particle focusing performance. Second, the numerical modeling developed in chapter 7 can guide the optimal design of curved microchannels in order to implement the curvature-induced dielectrophoretic separation process. Moreover, dc-biased ac dielectrophoresis can be also applied into curved microchannels to decrease the required magnitude of average electric fields for diminishing the potential damage to biological cells.
References


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APPENDIX A

Microchannel fabrication

All of the microchannels were fabricated with PDMS using the standard soft lithography method [27]. 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 Isopropyl alcohol, 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.

**Experimental setup**

The electrophoretic motions of particles in different microchannels were achieved by application of an electric field provided by a DC power supply (Glassman High Voltage Inc., High Bridge, NJ) in conjunction with a custom voltage controller. The behavior of particles 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 motions were eliminated by carefully balancing the liquid heights in all the reservoirs prior to each measurement.