Particle manipulation using moving dielectrophoresis

Kua, Chin Hock

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Particle Manipulation using Moving Dielectrophoresis

by

Kua Chin Hock

B.Eng.(Hons) in CAD/CAM Engineering

Universiti Malaya, 1999

S.M. in Innovation in Manufacturing Systems and Technology

Nanyang Technological University, 2001

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Signature of author:
IMST Programme
Jan 26, 2007

Certified by:
Prof. Lam Yee Cheong Prof. Kamal Youcef-Toumi
SMA Fellow, NTU SMA Fellow, MIT
Thesis Advisor Thesis Advisor

Certified by:
Assoc. Prof. Charles Yang Chun Dr. Isabel Rodriguez
MAE, NTU IMRE
Thesis Advisor Thesis Advisor

Accepted by:
Prof. Yue Chee Yoon Prof. David Hardt
Programme Co-Chair Programme Co-Chair
IMST Programme IMST Programme

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by

Kua Chin Hock

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ABSTRACT

After rigorous development over the years, dielectrophoresis has been established as an effective method to manipulate micron and sub-micron sized particles. In particular, it is a promising technology for lab-on-a-chip or micro total analysis system (μ TAS) to separate cells for biomedical applications. This technology is based on the knowledge that a particle suspended in a fluid medium experiences a net electrical force, due to a polarization effect, when non-uniform electrical fields are applied across the fluid. By varying the applied electric field frequencies, the magnitude and the direction of the dielectrophoretic forces on the particle can be varied and controlled. When the applied electric field only varies in magnitude over time, the dielectrophoretic force is 1-dimensional. This is commonly referred as conventional dielectrophoresis. When the applied electric field has a varying magnitude and phase, the dielectrophoretic force is 2-dimensional. This is commonly referred as traveling wave dielectrophoresis. While particle separations have been demonstrated with devices based on these two techniques, the separated particles were confined in space. To overcome this issue, fluid flow is generally used to carry the particles. In this investigation, moving dielectrophoresis (mDEP) is introduced for the manipulation and transportation of particles. The moving dielectrophoresis is generated by a series of electrodes which can be individually energized to induce an electric field that moves from one electrode to another. Beside the electric field frequency, the switching speed of the electrode is a second time parameter introduced in moving dielectrophoresis. A major difference of this technique from the traveling wave dielectrophoresis is that the moving speed of the energized electrodes is independent of the electric field frequency. By sequentially energizing the electrodes, a particle can be controlled to move in the same direction. By controlling the electric field frequencies and the energizing of the electrodes, other manipulation techniques like separation, isolation, fractionation and trapping can be achieved. A mathematical model is also presented to provide a theoretical basis for the use of the moving dielectrophoresis.

Keywords: dielectrophoresis, particle manipulation.

Thesis Advisors:

1. Prof. Lam Yee Cheong, SMA Fellow, NTU
2. Prof. Kamal Youcef-Toumi, SMA Fellow, MIT
3. Assoc. Prof. Charles Yang Chun, NTU
4. Dr. Isabel Rodriguez, IMRE

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List of Symbols

<u>Symbol</u>	<u>Units</u>	<u>Description</u>	<u>Symbol</u>	<u>Units</u>	<u>Description</u>
a	m	Particle radius	ϵ	F m ⁻¹	Permittivity
f	Hz	Frequency	$\tilde{\epsilon}$	F m ⁻¹	Complex permittivity
g	m s ⁻²	Gravitational body force	ϵ_0	F m ⁻¹	Free space permittivity
h	m	Microchannel height	Re[...]	-	Real part of ...
$\tilde{K}$	-	Clausius-Mossoti factor	Im[...]	-	Imaginary part of ...
$\tilde{K}^{(n)}$	-	Clausius-Mossoti factor of order n	*	-	Complex conjugate
K_x	-	Wall correction factor (drag) in x	ϵ'	F m ⁻¹	Real part of permittivity
K_y	-	Wall correction factor (drag) in y	ϵ''	F m ⁻¹	Imaginary part of permittivity
l	m	Microchannel length	ϕ	V	Electrical potential
m	kg	Mass	$\tilde{\phi}$	V	Complex potential phasor
$\mathbf{p}$	C m	Dipole moment $\mathbf{\tilde{p}}$	ϕ_R	V	Real part of potential phasor
$\mathbf{p}^{(n)}$	C m	Multipolar moment of order n	ϕ_I	V	Imaginary part of potential phasor
$\mathbf{r}$	m	Position vector	η	kg m ⁻¹ s ⁻¹	Viscosity
$\mathbf{u}$	m s ⁻¹	Fluid velocity	κ_m	-	Relative permittivity
u_x	m s ⁻¹	Fluid velocity in x-direction	ρ	C	Free unpaired charges
$\mathbf{v}$	m s ⁻¹	Particle velocity	ρ_m	kg m ⁻³	Medium density
A	m ²	Area	ρ_p	kg m ⁻³	Particle density
$\mathbf{E}$	V m ⁻¹	Electric field	σ	S m ⁻¹	Conductivity
E_x	V m ⁻¹	Electric field in x-direction	$\tilde{\sigma}$	S m ⁻¹	Complex conductivity
$\tilde{\mathbf{E}}$	V m ⁻¹	Electric field phasor	σ_q	C m ⁻²	Surface charge density
$\mathbf{F}$	N	Force	τ	s	Relaxation time
F_x	N	Force in x-direction	ζ	V	Zeta potential
$\mathbf{F}_{\text{buoy}}$	N	Buoyancy force	v	m ³	Volume
$\mathbf{F}_{\text{DEP}}$	N	Dielectrophoretic force	ω	rad s ⁻¹	Angular frequency
$\mathbf{F}_{\text{drag}}$	N	Viscous drag force	Φ	V	Electrostatic potential
$\mathbf{F}_{\text{grav}}$	N	Gravitational force	$\Delta...$	-	Change in ...
i	-	Imaginary unit $\sqrt{-1}$	$\langle...\rangle$	-	Time average operator
Q	C	Charge	$ \dots $	-	Magnitude operator
T	K	Tempature	$\nabla...$	-	Del operator
t	s	Time	$\cdot$	-	Dot product
V_0	V	Amplitude of voltage	$\times$	-	Vector cross product
			$\emptyset$	m	Diameter

Chapter 1

Introduction

1.1 Introduction

There is currently a high level of interest in developing means to manipulate biological particles, where expensive and time-consuming conventional analytical techniques to separate and identify cells, proteins, viruses and DNA, are replaced by low cost microfluidic devices. Such devices are commonly referred as lab-on-a-chip or micro-total analysis systems (μ TAS). To manipulate these bio-particles, whose diameters range from 10 nm to 100 μ m, researchers have exploited the use of electrostatic force as it becomes dominant at micrometer scales [Jones 2003]. The methods include electrophoresis [Li 2006], electroosmosis [Green *et al.* 2000, Mpholo *et al.* 2003, Debesset *et al.* 2004], electrofusion, electroporation, and dielectrophoresis [Pohl and Hawk 1966]. Among these methods, electrophoresis has been used to separate and detect molecules such as proteins and DNA [Zheng *et al.* 2004]. Devices based on this technique are commercially available. Dielectrophoresis, the center of discussion in this investigation, is traditionally recognized as a cell manipulation technique.

The term dielectrophoresis was first used by [Pohl 1951], which he described as the translational motion of neutral matter caused by polarization effects in a non-uniform electric field [Pohl 1978]. Originally, this term strictly referred to the phenomena of induced dipole on particles due to a non-uniform field. However, the term has now been broadened to include other

electrokinetic phenomena arising from non-uniform electric fields, in particular, traveling-wave dielectrophoresis [Masuda *et al.* 1988] and electrorotation [Wang *et al.* 1992].

In the early stage, dielectrophoresis was performed using pin-wire electrodes [Pohl 1951]. Manipulation was limited to large particles like cells. Using this electrode, [Pohl and Hawk 1966] demonstrated the separation of viable and non-viable yeast cells. [Pohl 1978] had subsequently extended the experiments to separate other biological cells, including canine thrombocytes, red blood cells, chloroplasts, mitochondria, and bacteria.

In recent years, microelectrodes with dimension as small as $0.5\mu\text{m}$ have been fabricated using photolithography technique [Morgan and Green 1997]. Electrodes are now small enough to generate high electrical field gradients to manipulate sub-micrometer particles. Dielectrophoresis can now be used to separate viruses [Morgan and Green 1997, Green *et al.* 1997, Hughes and Morgan 1998, Hughes *et al.* 1998, Morgan *et al.* 1999, Hughes *et al.* 2001, Hughes *et al.* 2002], proteins [Washizu *et al.* 1994], and DNA [Asbury and van 1998, Bakewell *et al.* 2000, Asbury *et al.* 2002, Ying *et al.* 2004].

1.2 Research Motivation

Using dielectrophoresis, particles suspended in a fluidic medium are electrically polarized when they are subjected to a DC or AC electric field. If the field is non-uniform, the particles experience a net electrical force where the magnitude and direction of the force is determined by the electrical properties (i.e. conductivity and permittivity) of the particles and the suspending medium. The magnitude and direction of this force are also determined by the electric field frequencies. Since different particles exhibit different electrical polarization, they thus experience different forces in the non-uniform electric field, allowing different particles to be separated.

This knowledge has been applied in particle separation, where two sub-populations having their own unique frequency-dependent dielectric properties experience different magnitude and direction of the dielectrophoretic force. This technique has been demonstrated

in the spatial separation of blood cells [Gascoyne *et al.* 1992], and separation of sheep erythrocytes cells from bacteria *M. luteus* [Wang *et al.* 1993]. The spatial separation of sub-micrometer particles on a castellated electrode array has also been demonstrated [Morgan *et al.* 1999]. The spatial separation of a heterogeneous population of sub-micrometer particles of identical size can also be accomplished using electrode arrays [Green and Morgan 1997]. However, in these methods, the particles are localized at the electrodes after separation. Additional efforts are required to collect the particles.

In the traveling-wave dielectrophoresis, an applied electric field having varying magnitude and phase is used. In the traveling wave dielectrophoresis, the real part of the Clausius-Mossotti factor determines the levitation of the particles from the electrode plane, and the imaginary part of the Clausius-Mossotti factor controls the translational movement of the particles along the electrode plane. Particle separation is achieved by applying a frequency where the first sub-population is levitated and translated, while the second sub-population is immobilized on the electrodes. This technique has been demonstrated for the separation of viable and non-viable yeast cells [Cui *et al.* 2001, Talary *et al.* 1996]. Particle separation is still possible even if both sub-populations are levitated and travel in the same direction, due to the fact that particles with different sizes travel at different velocities. This technique was demonstrated by Morgan and co-workers [Morgan *et al.* 1997] for the separation of erythrocytes and leukocytes cells. The major drawback of traveling wave dielectrophoresis is that the particle separation is now determined by the imaginary part of the Clausius-Mossotti factor, instead of the real part. In addition, a particle can only be transported when the real and imaginary parts of the Clausius-Mossotti factor are in the right combination, i.e. the real part has to be negative before it can be moved along the microchannel with the force induced by the imaginary part. This limitation restricts the workable-range of the applied electrical frequency.

In this work, moving dielectrophoresis (mDEP) is proposed and implemented as an alternative new method to manipulate cells. The moving dielectrophoresis is generated by sequentially energizing single electrode or an array of electrodes to form an electric field that

moves particles continuously along the microchannel. Particle separation is controlled by the applied electrical frequency, and particle transportation is controlled by the inter-electrode activation time. Therefore, unlike traveling wave dielectrophoresis which requires the imaginary part of the Clausius-Mossotti factor, this technique utilizes the conventional real part of the Clausius-Mossotti factor. In addition, unlike conventional dielectrophoresis technique where the particles are localized after separation, this technique transports the particles during separation.

1.3 Organization of Dissertation

The subsequent chapters describe the concept, methodology, results and conclusions to demonstrate the moving dielectrophoresis. In particular, Chapter 2 reviews the development of dielectrophoresis and describes various particle manipulation methods.

Chapter 3 describes the concept of moving dielectrophoresis. The chapter first illustrates the generation of a moving electric field, and later demonstrates with schematic diagrams on how a particle can be transported and separated using a moving electric field.

Chapter 4 presents the mathematical model for the equation of motion of the moving dielectrophoresis. It shows the effect of dielectrophoretic force, fluid drag, buoyancy force and gravitational force on a particle. A coupled partial differential equation is derived from the balance of those forces using Newton's second law. This analytical model is then compared with the experimental results.

Chapter 5 presents the sensitivity studies of several system parameters on the strength of the dielectrophoretic force, where the results provide indication on the effectiveness of the separation method. These parameters include applied voltage, electrode width, inter-electrode gap, microchannel height and number of energized electrodes. In addition, this chapter also presents the dielectrophoretic force strength of different types of electrode configurations.

Chapter 6 documents the device fabrication and the experimental setup to generate a moving electric field, and the sample preparation and experimental technique to use this

moving electric field to transport and separate cells. In this investigation, viable and non-viable yeast cells were used to demonstrate the concept of moving dielectrophoresis.

Chapter 7 shows the experimental results to transport and separate particle using moving dielectrophoresis, where viable and non-viable yeast cells were used as model particle. This chapter also documents the trajectory of the particle under a positive dielectrophoresis or a negative dielectrophoresis.

Finally, Chapter 8 reviews the contribution of this investigation.

Besides those chapters, additional research findings are documented in the appendices. Appendix A documents the analytical solutions for the electrical potential, electric field and dielectrophoretic force using the Wiener-Hopf technique. Appendix B discusses the wall and particle size effects on the dielectrophoretic force. Appendix C is the archives of the Matlab scripts for the equation of motion. Lastly, Appendix D discusses the electrohydrodynamic effects on the dielectrophoresis.

Chapter 2

Review of Particle Manipulations

2.1 Introduction

Cell separation technologies are important in biological applications as a mean to purify target cells from the sample, where the target cells can be subsequently collected for downstream testing. In the conventional approach, cells are separated based on density, size, affinity to antibodies, or fluorescent emission sorting by flow cytometry [Freshney 2005].

In the past two decades, there have been enormous efforts to miniaturize analytical equipment. Some of these conventional cell separation technologies have been extended to the microfluidic environment, together with numbers of new technologies that leverage on the physical and chemical phenomena specific to the micro-scale. These microfluidic techniques include among others electrophoresis [Ling *et al.* 2005], optical tweezers [Jordan *et al.* 2005], magnetophoresis [Pamme and Manz 2004], acoustic [Petersson *et al.* 2005], hydrodynamic [Lutz *et al.* 2006], bifurcation [Huang *et al.* 2004] and dielectrophoresis methods. A comprehensive overview of various techniques for particle manipulation can be found in the review papers, e.g. [Voldman 2006, Lim *et al.* 2005, Vilkner *et al.* 2004, Vykoukal and Gascoyne 2002, Kang and Park, Dittrich *et al.* 2006].

One major advantage of dielectrophoresis is that the technique can fulfill a broad range of particle manipulations in the microfluidic chips, including particle separation, particle fractionation, particle transportation, particle trapping, and particle sorting. This chapter begins with an overview of the physics of dielectrophoresis, and continues with revisions on various particle manipulations technique employing dielectrophoresis.

2.2 Principles of Dielectrophoresis

2.2.1 Particle Polarization

When an external electric field $\mathbf{E}$ is applied across a particle suspended in a fluid medium, both the particle and the suspending medium are polarized. The result is net unpaired surface charges σ_s cumulated at the interface between the particle and the fluid medium. These surface charges generate a second electric field and distort the original electric field. A typical resulting electric field is shown in figure 2.1. The amount of charges at the interface depends on the field strength and the electrical properties of the particle and the suspending medium. The key electrical properties involved are conductivity and permittivity, where conductivity is a measure of the ease with which charges can move through a material, while permittivity is a measure of the energy storage or charge accumulation in a system [Morgan and Green 2003].

Figure 2.1 Interfacial polarization and dielectrophoresis.

2.2.2 Dielectrophoretic Force

The surface charges interact with the electric field to produce Coulomb forces. Since the electric field distribution is not uniform in figure 2.1, the electric field density is higher on the right than on the left, resulting in a net force $\mathbf{F}$ in the direction as shown in figure 2.1.

Few methods have been developed to find the total electrical force on the particle, including effective moment method [Jones 1995] and Maxwell Stress Tensor method [Wang *et al.* 1997]. The effective moment method is more commonly used since it provides a simple analytical solution while maintaining a good physical insight of the behavior of the system. The basis of the effective moment method is the hypothesis that a force and torque upon a particle can be expressed in terms of the effective moments identified from the solution for the induced electrostatic field due to the particle [Jones 1995]. In contrast, the Maxwell Stress Tensor method requires a rigorous surface integration of stress tensor over the particle. The analytical solution has been limited to the case of a homogeneous spherical particle [Wang *et al.* 1997]. However, the Maxwell Stress Tensor method is preferred for numerical calculation.

Assuming that the observation point is far enough from the particle, the surface charges on the particle in figure 2.1 can be approximated as a dipole, which is oriented with the direction of the electric field. With this approximation, the total electrical force on the particle is found as [Jones 1995]

$$\mathbf{F} = (\mathbf{p} \cdot \nabla) \mathbf{E} \quad (2.1)$$

where $\mathbf{p}$ is the effective dipole moment specific to the particle-fluid system, and ∇ is the del operator. This electrical force is termed as dielectrophoretic force.

When the dipole approximation is not accurate, higher order multipoles have to be considered. The general solution was solved by Jones and Washizu [Washizu and Jones 1994, Jones and Washizu 1996, Washizu and Jones 1996, Washizu 2004].

2.2.3 Frequency Response

For an isotropic homogenous spherical particle with radius a , the time-averaged dielectrophoretic force in equation (2.1) can be generalized as [Wang *et al.* 1994],

$$\langle \mathbf{F}(t) \rangle = 2\pi\epsilon_m a^3 \left\{ \text{Re}[\tilde{K}] \nabla E_{rms}^2 + \text{Im}[\tilde{K}] (E_{x0}^2 \nabla \phi_x + E_{y0}^2 \nabla \phi_y + E_{z0}^2 \nabla \phi_z) \right\} \quad (2.2)$$

where E_{rms} is the root-mean-square of a sinusoidal electric field having magnitude (E_{x0} , E_{y0} , E_{z0}) and phases (ϕ_x , ϕ_y , ϕ_z), with $\text{Re}[\]$ and $\text{Im}[\]$ denote real part and imaginary parts respectively, and K is the Clausius-Mossotti factor defined as

$$\tilde{K} = \frac{\tilde{\epsilon}_p - \tilde{\epsilon}_m}{\tilde{\epsilon}_p + 2\tilde{\epsilon}_m} \quad (2.3)$$

and

$$\tilde{\epsilon}_p = \epsilon_p - i \frac{\sigma_p}{\omega} \quad \text{and} \quad \tilde{\epsilon}_m = \epsilon_m - i \frac{\sigma_m}{\omega} \quad (2.4)$$

$\tilde{\epsilon}_p$ and $\tilde{\epsilon}_m$ are the complex permittivity of the particle and the suspending medium, respectively. The Clausius-Mossotti factor is a frequency dependent variable. A normalized plot for the Clausius-Mossotti factor is shown in figure 2.2. When $\tilde{K} > 0$, the particle is said to be experiencing a positive dielectrophoresis, where the particle moves towards the high electric field gradient regions. Likewise, when $\tilde{K} < 0$, the particle experiences a negative dielectrophoresis and moves away from the high field gradient regions [Jones 1995]. By changing the electric field frequency, the Clausius-Mossotti factor can experience a transition from a positive value to a negative value, which causes the dielectrophoretic force on the particle to change its direction accordingly. The Clausius-Mossotti factor is a unique property of the particle under the specified suspending medium, and it is this property that is being utilized in dielectrophoresis for particle manipulation.

When the applied electric field only varies in magnitude, only the real part of the Clausius-Mossotti factor is induced. This is represented as $\text{Re}[\tilde{K}]$ in figure 2.2. When the applied electric field varies in magnitude and phase, both the real part and the imaginary part of the Clausius-Mossotti factor are induced. The imaginary part is represented as $\text{Im}[\tilde{K}]$ in

figure 2.2. It is clear that the $\text{Im}[\tilde{K}]$ cannot be independently induced without inducing $\text{Re}[f_{CM}]$. However, $\text{Re}[\tilde{K}]$ can be induced without inducing $\text{Im}[\tilde{K}]$.

Figure 2.2 Normalized Clausius-Mossotti factor vs. frequency.

2.3 Dielectrophoretic Methods for Particle Separation

2.3.1 Conventional Dielectrophoresis

One of the most important applications of dielectrophoresis is particle separation. It relies on the fact that a specific sub-population of particles has unique frequency-dependent dielectric properties that are different from other sub-populations. The relative magnitude and direction of the dielectrophoretic force exerted on a given population of particles depends on the conductivity and permittivity of the suspending medium, together with the frequency and magnitude of the applied field. Therefore, differences in the dielectric properties of particles manifest themselves as variations in the dielectrophoretic force magnitude or direction thereby producing separation.

The transition of particles from negative dielectrophoresis into positive dielectrophoresis on castellated electrodes was demonstrated by Pethig and co-workers [Pethig *et al.* 1992]. Spatial separation of blood cells [Gascoyne *et al.* 1992] and separation of blood cells from bacteria was also performed on such electrode array [Wang *et al.* 1992]. The spatial

separation of sub-micrometer particles on a castellated electrode array has also been demonstrated [Morgan *et al.* 1999]. The spatial separation of a heterogeneous population of sub-micrometer particles of identical size can also be accomplished using electrode arrays [Green and Morgan 1997].

It has been demonstrated that small biological particles such as viruses, DNA and macromolecules can be separated using dielectrophoresis, e.g., the spatial separation of two different viruses, Tobacco Mosaic Virus and Herpes Simplex Virus, using a polynomial electrode was advised by [Morgan *et al.* 1999]. Herpes Simplex Virus was trapped under negative dielectrophoretic forces at the field minimum at the center of the electrode array, while simultaneously Tobacco Mosaic Virus experiences positive dielectrophoresis and is collected at the high-field regions at the electrode edges, resulting in the physical separation of the two virus types.

Physical separation of a mixture of particles into two populations can be achieved by subjecting the electrode array to a flow of liquid of sufficient pressure to remove particles trapped at field minima leaving the other particles trapped at the electrode tips. The remaining particles can then be removed by switching off the field and flushing fresh liquid across the electrodes [Huang *et al.* 2002]. This physical separation technique is based on the knowledge that the particles trapped at field gradient maxima by positive dielectrophoresis are held by a stronger force than those experiencing negative dielectrophoresis [Pethig *et al.* 1992].

These are the basic separation techniques using dielectrophoresis. It only relies on the real part of the Clausius-Mossotti factor, since the applied electric field only changes in magnitude but not phase. The major disadvantage is that the particles are trapped at the electrodes after separation, and flushing needs to be performed to collect the separated particles. A more advantageous technique is to separate and transport the particles at the same time. This is achieved using traveling-wave dielectrophoresis (twDEP) or dielectrophoretic – field flow fractionation (DEP-FFF) techniques.

2.3.2 Traveling Wave Dielectrophoresis

In the traveling-wave dielectrophoresis (twDEP), the applied electric field has varying magnitude and phase, which induces both the real and imaginary part of the Clausius-Mossotti factor on the particles [Wang *et al.* 1994]. For a interdigitated electrode [Cui and Morgan 2000, Cui *et al.* 2001], the real part of the Clausius-Mossotti factor determines the levitation of the particles from the electrode plane, whereas the imaginary part of the Clausius-Mossotti factor controls the translational movement of the particles along the electrode plane. Particle separation is achieved by applying a frequency where the first sub-population is levitated and translated, while the second sub-population is immobilized on the electrodes. This technique has been demonstrated to separate viable and non-viable yeast cells [Cui *et al.* 2001, Talary *et al.* 1996].

Particle separation is still possible even if both sub-populations are levitated and travel in the same direction, due to the fact that particles with different sizes travel at different velocities. This technique was demonstrated by Morgan and co-workers [Morgan *et al.* 1997] to separate erythrocytes and leukocytes cells.

2.3.3 Dielectrophoretic - Field Flow Fractionation

Dielectrophoretic forces can be combined with hydrodynamic forces in a separation method known as field flow fractionation (FFF), which is a general chromatographic separation technique in chemistry and biology [Giddings 1984]. In DEP-FFF, particles are separated according to a combination of their effective polarizability and density [Huang *et al.* 1997, Markx *et al.* 1997]. Particles are repelled from the electrodes under a dielectrophoretic levitation force, which acts on a suspension of particles. This force is combined with fluid flow with a parabolic velocity profile, where particles levitated at different height are transported in different speeds. In contrast to other DEP separation methods, where particles remain on the same plane and are either eluted or remain trapped, DEP-FFF exploits the velocity gradient in the flow profile to achieve highly selective separation. Recent examples of applications

include the separation of latex particles [Markx *et al.* 1997, Wang *et al.* 1998] and blood cells [Yang *et al.* 1999, Wang *et al.* 2000].

However, due to randomness, the particles travel at a Gaussian-shaped distribution, and as a result, subpopulations often overlap. Thus, the separated subpopulation often contains residue of other subpopulations [Yang *et al.* 2000]. This is an area which needs to be improved.

2.3.4 Insulator-based Dielectrophoresis

An initially uniform electric field in a parallel channel can be turned into a non-uniform electric field by positioning insulating pillars along the channel. Cells and DNA can be separated into two regions of high electric field gradient and low electric field gradient, as demonstrated by [Chou *et al.* 2002, Lapizco-Encinas *et al.* 2004]. Latex can be separated into two different flow paths by coupling this technique with fluid flow [Kang *et al.* 2005]. The advantage of this technique is that metallic electrodes are not needed to generate the non-uniform electric fields; thus, avoiding the problem associated with metallic electrodes, like electrolysis and AC electroosmosis.

2.4 Particle Transportation

Dielectrophoresis can also be used to transport particles, like conventional pump and electrohydrodynamic methods. This is achieved through the use of interdigitated electrodes generating traveling-wave dielectrophoresis. Particles are moved in a traveling electric field which is energized with a four-phase signal [Fuhr *et al.* 1991, Hagedorn *et al.* 1992]. Pumping of liquid along the device in order to produce horizontal motion is not required.

However, the traveling-wave dielectrophoresis can only allow one-dimensional transportation of the particle. An improved design is a grid electrode system, which allows two-dimensional movement of a particle [Suehiro and Pethig 1998]. It is constructed of two glass plates, where there are vertical electrode strips on the top glass, and horizontal electrodes

strips on the bottom glass. A high field region is developed at the intersection of the two strip electrodes to which AC signals are applied. The particles are attracted or repelled from the intersection depending on whether the particles are experiencing positive or negative dielectrophoresis.

2.5 Particle Trapping

Another important application of dielectrophoresis is the non-contact trapping of single particles. This method uses a polynomial electrode system which generates a potential energy well at the center of the electrodes. Particles are trapped at the center of the electrodes under negative dielectrophoresis. The trapping of single sub-micrometer particles in quadrupole microelectrode structures has been demonstrated experimentally by [Hughes and Morgan 1998]. Such trapping is of particular interest, since it allows single particles to be isolated without resorting to invasive physical or chemical methods.

Similar trapping has been shown by other researchers [Hughes and Morgan 1998, Müller *et al.* 1996, Green *et al.* 2000]. [Müller *et al.* 1996] were designing a quadrupole electrode array that was expected to trap 650 nm latex beads. However, to their surprise, they were able to trap particle as small as 14 nm. This prompted them to question the minimum particle size that can be stably trapped and the role of electrohydrodynamics. It was later proved by other researchers that the minimum radius is proportional to 1/3 of the trap width and the gradient of the electrical field [Hughes and Morgan 1998]. It was later demonstrated that electrohydrodynamic phenomena are responsible for this discrepancy, where electrothermal flows dominate at high frequencies, and AC electroosmotic flows dominate at low frequencies [Green *et al.* 2000].

However, such quadrupole microelectrode structures are not a closed trap. It has an open top and gravity is responsible for the downward force holding the particle on the surface. Particles with near neutral buoyancy are less likely to be held in the trap by gravity. A closed

trap was made using two polynomial electrodes placed one above the other, to produce an octopole [Schnelle *et al.* 2000].

An improved design of a particle trapping system was demonstrated by Voldman, who created extruded-quadrupole electrode array that consists of a set of four metallic gold posts [Voldman *et al.* 2002, Voldman *et al.* 2003]. Their measurements show that it can confine particles over 100 times more strongly than a planar counterpart, yet it allows the flow-chamber height and trap geometry to scale independently.

The particle trapping technique has been extended to trap massive number of particles. Such technique involves building arrays of microelectrode traps [Manaresi *et al.* 2003, Gray *et al.* 2004, 2004, Rosenthal and Voldman 2005, Chiou *et al.* 2005]. Recently, particle trapping using positive dielectrophoresis has been achieved using ring-dot electrodes [Taff and Voldman 2005]. The design can trap single cell in a ring-dot electrode.

2.6 Particle Sorting

A major development in particle handling occurred through the integration of several types of electrokinetic particle trapping and manipulation devices into one microchip [Fiedler *et al.* 1998, Müller *et al.* 1999]. Such device [Müller *et al.* 1999] consists of two layers of electrode structures separated by a 40 μm thick polymer spacer forming a flow channel. The electrode elements are formed by funnel, aligner, cage and switch; which are designed to focus, trap and separate eukaryotic cells or latex particles with a diameter of 10–30 μm . Each set of electrodes can be independently addressed with suitable AC fields and frequencies. Particles are suspended in an electrolyte of high conductivity such that the system operates under negative dielectrophoresis. In the experiment, efficient handling of particles could be achieved with flow rates up to 3500 mm/s, with electrodes operated at 5~11 V and 5~15 MHz.

The field cage is the most critical design in this type of integrated system. [Müller *et al.* 1999] showed a dependency of critical voltage required to hold a latex particle in the cage subject to a laminar flow field. A decrease of the amplitude resulted in displacement of the

particle from the field minimum (along the x -axis) up to a point where the particle left the cage due to the applied flow. This poses a critical problem to such design, where the dimensions of the cage must be optimized for the size of the particles which are to be handled by the system, and greatly limits the type of particles that can be processed.

The core to the particle sorting is the dielectrophoretic gate, which acts to deflect particles into one of two microfluidic channels. More recent examples are [Dürr *et al.* 2003, Kentsch *et al.* 2003, Holmes *et al.* 2005].

Particle sorting can also be achieved by combining massive number of individual particle traps, as proposed by [Voldman *et al.* 2002]. The device consists of an array of extruded-quadrupole electrodes. The system can simultaneously load, interrogate, and sort an ensemble of single cells. Other improved designs include [Taff and Voldman 2005, Gadish and Voldman 2006].

2.7 Other Dielectrophoretic Methods

In the electrosmeear method [Das *et al.* 2005], electrical signals were sent to two electrode arrays to generate a spatially varying dielectrophoretic strength along the channel. This setup allowed cells to be attracted to the electrodes at different regions of the channel based on their dielectrophoretic affinity. This method was anticipated to be useful for microscopic examination.

There are also numerous attempts to couple the dielectrophoretic technique with other techniques like cell-targeted antibody [Yang *et al.* 2006], DEP marker [Hu *et al.* 2005], ultrasonic standing wave [Wiklund *et al.* 2006], AC electroosmosis [Zhou *et al.* 2005], and electrohydrodynamic flow [Tuval *et al.* 2005] methods to improve cell selectivity.

2.8 Programmable Control Device

Arrays of individually excitable electrodes can be built to form electric field cages to transport particles. This approach involves incorporating programmable switches to control the excitation pattern and sequence. Such devices can be used to massively trap single cells in a microfluidic chamber. Some of the examples include square electrode array on a CMOS chip [Manaresi *et al.* 2003], optoelectronic tweezers [Chiou *et al.* 2005] and array of elongated electrodes [Vulto *et al.* 2006, Medoro *et al.* 2004, Shaikh and Ugaz 2006]. Such devices function by generating pockets of electrical potential cages to allow single cells to be trapped and positioned from one electrode to another electrode. The virtual programmable devices allow cells to be manipulated in many fashions limited only by the imagination of the users. For example, such devices have been demonstrated to be able to perform cell trapping [Manaresi *et al.* 2003, Chiou *et al.* 2005], positioning [Manaresi *et al.* 2003], transportation [Chiou *et al.* 2005], separation [Manaresi *et al.* 2003, Chiou *et al.* 2005], and isolation. In addition, cells can be aligned along different parts of the microchannel after separation [Medoro *et al.* 2004]. In addition, unlike other methods which require fluid flow, such devices can manipulate cells in a static liquid.

Programmable electrode arrays have also been used to transport and manipulate liquid droplets, which is expected to be useful in performing chemical and biological assays [Vykoukal *et al.* 2001, Gascoyne *et al.* 2003, Gascoyne *et al.* 2004, Gascoyne and Vykoukal 2004, Schwartz *et al.* 2004].

2.9 Summary

Dielectrophoresis is one of the most efficient techniques in microfluidics to achieve particle separation. The technique is based on electrical response of particle-medium system. Many techniques have been introduced to manipulate particle using dielectrophoresis. The conventional dielectrophoresis (cDEP) and traveling-wave dielectrophoresis (twDEP) are two

basic methods that describe the manipulation using only the magnitude gradient of the electric field, or the magnitude gradient and phase difference of the electric field. Dielectrophoresis can be used to perform particle separation, particle transportation, particle trapping, and particle sorting. Dielectrophoretic methods have been coupled with other methods to achieve more efficient or selective separation and to achieve higher throughput.

Chapter 3

Moving Dielectrophoresis

3.1 Introduction

Dielectrophoresis has been established as a cell or particle separation technology in microfluidics. However, the conventional dielectrophoresis only provides localized cell separation, whereas the traveling wave dielectrophoresis revokes both real and imaginary part of the polarization factor in order to achieve simultaneous particle separation and transportation. This chapter introduces a new particle separation and transportation concept termed moving dielectrophoresis. The underlying mechanism is discussed in detail in the coming sections.

3.2 Generation of Moving Dielectrophoresis (mDEP)

Consider an electrode structure where independently excitable electrodes are arranged at the top plane and a common ground electrode is arranged at the bottom plane, where the top electrodes can be activated sequentially as shown in figure 3.1. By switching the top electrodes on and off in this manner, an electric field that move from left to right is generated. In general, the moving speed of the electric field is determined by the activation period of the top electrodes. This electric field motion is defined as moving electric field. The corresponding

particle motion due to the polarization by the electric field is termed moving dielectrophoresis (mDEP).

Figure 3.1 Generation of moving electric field.

There are two independently controllable time parameters in the mDEP, the electric field frequency from the AC power supply and the inter-electrode activation time. In the conventional dielectrophoresis, the electric field frequency is the only controllable time

parameter¹. Thus, conventional dielectrophoresis can only achieve localized particle manipulation. Using moving dielectrophoresis developed in this work, the particle can be controlled to move within a moving electric field through sequential serial electrode activation/deactivation, providing a second time parameter, in addition to the particle manipulation using the electric field frequency. The second time parameter provides an additional control on the particle motion.

The activation time scale and the activation pattern determine whether the real part or the imaginary part of the Clausius-Mossotti factor is induced. For instance, when the inter-electrode activation time is significantly longer than the system charge relaxation time, only the real part of the Clausius-Mossotti factor is important. On the other hand, when the inter-electrode activation time is in the same order of magnitude as the electric field frequency and the electrodes are activated like in the traveling wave mode, both the real and imaginary parts are important. In this investigation, the focus is only on the former case.

3.3 Particle Transportation using mDEP

The moving electric field technique can be used to transport particles spatially across a microchannel. As illustrated in figure 3.2, a particle experiencing a positive dielectrophoresis moves toward the high electric field gradient region. Thus, the particle is attracted to the electrode edge when the center electrode is energized. After the particles reach the center electrode, the adjacent electrode is energized and the center electrode is de-energized. At this instance, the particle experiences an attractive force from the adjacent electrode and moves toward this adjacent electrode. By sequentially energizing and de-energizing the electrodes sequentially, the particle travels behind the electric field, with a speed capped by the activation time. This forward motion is possible as long as the dielectrophoretic force is larger than the fluid drag or hydrodynamic force, gravitational force, and the Brownian motion. In addition,

¹ Although strictly speaking, the switching time (on/off) to release the trapped cells on the electrodes can be considered as another time-dependent parameter in the conventional dielectrophoresis.

the inter-electrode activation time has to be long enough so that the particle has sufficient time to travel to the next electrodes.

Similarly, a particle experiencing a negative dielectrophoresis can be transported using the same technique, as shown in figure 3.3. Under negative dielectrophoresis, the particle moves away from the high electric field gradient region. Thus, the particle is repulsed from the center electrode when the center electrode is energized. After the particle reaches the adjacent electrode, the adjacent electrode is energized and the center electrode is de-energized. The particles are repulsed further to the subsequent electrode. By sequentially energizing and de-energizing the subsequent electrodes, particles move in front of the electric field.

However, when the electric field frequency is same as the particle-medium relaxation frequency, a particle experiences a zero dielectrophoretic force, i.e. the particle remains stationary when the moving electric field passes through the particle. Thus, particles are not transported in this condition.

Figure 3.2 A particle experiencing a positive dielectrophoresis is transported under a moving electric field.

Figure 3.3 A particle experiencing a negative dielectrophoresis is transported under a moving electric field.

3.4 Particle Separation using mDEP

The moving electric field can be further extended to perform particle separation using various schemes. In particular, particles having different electrical properties can be separated based on their dielectrophoretic affinity. It is also possible to separate particles based on the force balance against the fluid drag and friction.

3.4.1 Separation based on Dielectrophoretic Affinity

As discussed in Chapter 1, particles having different electrical properties have different frequency responses. Such frequency responses determine the magnitude and direction of the dielectrophoretic forces exerted on the particles. These responses can be controlled by changing the applied electrical frequency, the voltage amplitude, and the medium conductivities. Separation of two particle groups is achieved when those parameters are controlled such that different particle groups travel in a different direction.

This knowledge can be further utilized in conjunction with the moving electric field to achieve particle separation. For instance, for a given electrical frequency and medium conductivity, consider the case where the first particle experiences a positive dielectrophoresis and the second particle experiences a negative dielectrophoresis. As shown in figure 3.4, when

the center electrode is energized, the particle under the positive dielectrophoresis is attracted to the electrode, while the particle under the negative dielectrophoresis is repulsed from the electrode. After the first particle arrives at the center electrode and the second particle moves to the adjacent electrode, the subsequent electrode is energized. The first particle is still trapped at the center electrode due to positive dielectrophoresis, while the second electrode is repulsed further from the adjacent electrode. Thus, the first particle and the second particle are separated at least one electrode-distance. This distance can be further increased by sequentially switching on more than one (several) adjacent electrodes.

Figure 3.4 Illustration of particle separation using moving electric field. The particle experiencing a positive dielectrophoresis is mobilized at the local position, while the particle experiencing a negative dielectrophoresis is transported along with the moving electric field.

In addition, separation of a mixture of heterogeneous particles having different dielectric properties may be achieved by applying an electric field frequency which is the same as the relaxation frequency of the target particles. As the moving electric field sweeps across

the electrodes, the target particles experience a zero dielectrophoretic force while all other particles experience a net dielectrophoretic force. The target particles remain stationary at the original position, and all other particles are being transported away. Thus, the target particles are being separated from the mixture. However, any slight deviation from the ideal situation, the zero dielectrophoretic force will become either positive or negative. Thus, in general, one cannot rely on the relaxation frequency of the particles for their immobilization.

3.4.2 Separation based on Particle Speed

Particle separation can be achieved by controlling the inter-electrode activation time. Since it takes a finite time for a particle to travel to the next electrode, the particle can only travel a finite distance. It will be left behind if the traveling speed of the particle is smaller than the moving speed of the electric field. Thus, particles that travel faster than the moving electric field are transported from the original location, leaving behind the particles that travel slower. This traveling speed depends on the combination of dielectrophoretic force, hydrodynamic drag force and friction. In principle, electric field frequency and voltage can also be used to achieve the same effect, as long as the net force ($=$ dielectrophoretic force $+$ hydrodynamic drag $+$ friction) on the different sub-population of particles is not the same. Here, the drag force refers to the resistant force from the fluid acting on the solid particle; whereas the friction refers to the pulling force when the particle is in a physical contact with the microchannel walls.

3.5 Summary

A moving electric field can be generated when a single or an array of independently excitable electrodes are energized sequentially. The corresponding particle polarization effects are termed moving dielectrophoresis. There are two independently controllable time parameters in this system, which are, the electric field frequency from the AC power supply and the inter-

electrode activation time. The former controls the particle separation, whereas the latter determines the particle transportation.

Chapter 4

Equation of Motion under Single-Energized Electrode

4.1 Introduction

It is important to understand the motion of the particle under the moving electric field described in the previous chapter. The response of the particle determines whether the particle can be successfully transported from one energized electrode to another energized electrode. Such response can be modeled by considering only a single energized electrode, which is essentially the basic module of the moving electric field. The trajectory of the particle under an energized electrode can then be traced by balancing the forces acting on the particle.

There are many methods to calculate various forces present in a system. A rigorous approach is to use surface/volume integral methods to sum all the stress or pressure components on the particle. This method has been used for calculating the hydrodynamic force, as demonstrated by [Glowinski *et al.* 1999, Singh *et al.* 2000]. The integral methods are potentially able to accommodate the wall and particle size effects in the microfluidic channel, but are more computational intensive when used to solve equation of motion. On contrast, the point method assumes all forces can be represented as equivalent forces acting on a point,

which is typically the center of mass. The point method is convenient for calculating the equation of motion, but typically ignores wall and particle size effects.

This section describes the mathematical modeling for a particle under a single energized electrode. The equation of motion is found by balancing the dielectrophoretic force, fluid drag, buoyancy and gravitational force by taking the point force method.

4.2 Forces Acting on a Particle

In general, there are numerous forces acting on a particle suspended in a fluid medium and driven by a dielectrophoretic force. These forces include, but not limited to, dielectrophoretic force, electrohydrodynamic force, buoyancy, gravitational force, Brownian motion, Coulombic force, wall friction, and forces arise from the electrical double layer effect. However, the following assumptions can be made pertaining to this research, which allow some of these forces to be ignored in the modeling:

- (a) The particle size of interest is in the range of 1~20 μm . Thus, the Brownian motion can be ignored.
- (b) The applied electrical potential is between 5 V to 20 V with frequency between 100 kHz to 10 MHz. At this potential, and considering the particle size at micron range, the dielectrophoretic force is dominant compared to the surface force exerted by the fluid due to the electrothermal effect. The AC electroosmotic force is observable only at frequency below 10 kHz. Thus, these two forces, which are collectively referred to as electrohydrodynamic force, can be ignored.
- (c) This study only focuses on the macroscopic response of the particle, and the particle is assumed to be at a distance significantly larger than the Debye length. Thus, the electrical double layer effect can be ignored.
- (d) The particle is assumed to be electrically neutral. This behavior is generally true for cells, although DNA and protein are generally charged. Thus, the Coulombic force can be ignored, when applying the subsequent model for cells.

- (e) When the particle slide on the microchannel surfaces, the particle experiences a frictional force. It is assumed that the particle does not touch the surface of the microchannel wall. This assumption is further implemented as one of the simulation constraint to stop the program when the particle actually touches the wall.

Following these assumptions, the modeling of the particle motion in the microchannel is simplified to consider four major forces only, namely dielectrophoretic force $\mathbf{F}_{\text{DEP}}$, fluid drag $\mathbf{F}_{\text{drag}}$, buoyancy force $\mathbf{F}_{\text{buoy}}$ and gravitational force $\mathbf{F}_{\text{grav}}$, as shown in figure 4.1.

For the structure shown in figure 4.1, the geometry of the electrodes depends on the x - and y -coordinates only, and the scale in the z -axis is significantly larger than the x - and y -axis. Thus, the potential and the electric field in the structure can also be described as a function of x - and y -coordinates only, without the z -coordinate dependence. Furthermore, although these forces are exerted either on the surface or the volume of the particle, these forces can be described as equivalent point forces acting on the centre of the particle. Having this further assumption, the analysis of the equation of motion for a particle can be fully simplified to x - and y -axis dependent only.

Figure 4.1 Illustration of the component of forces acting on a particle in a microchannel under the dielectrophoretic force.

From Newton's laws of motion, the rate of change in the momentum of the particle is directly proportional to the sum of the forces acting on the particle, which can be represented as a differential equation,

$$\mathbf{F}_{\text{DEP}} + \mathbf{F}_{\text{drag}} + \mathbf{F}_{\text{buoy}} + \mathbf{F}_{\text{grav}} = m \frac{d^2 \mathbf{r}}{dt^2} \quad (4.1)$$

Equation 4.1 can be expanded into its individual components in the xy -space as

$$F_{\text{DEP}_X} \hat{x} + F_{\text{DEP}_Y} \hat{y} - F_{\text{drag}_X} \hat{x} - F_{\text{drag}_Y} \hat{y} + F_{\text{buoy}} \hat{y} - F_{\text{grav}} \hat{y} = m \frac{d^2 x}{dt^2} \hat{x} + m \frac{d^2 y}{dt^2} \hat{y} \quad (4.2)$$

Alternatively, the components of forces along the x -direction and y -direction are, respectively,

$$F_{\text{DEP}_X} - F_{\text{drag}_X} = m \frac{d^2 x}{dt^2} \quad (4.3)$$

$$F_{\text{DEP}_Y} - F_{\text{drag}_Y} + F_{\text{buoy}} - F_{\text{grav}} = m \frac{d^2 y}{dt^2} \quad (4.4)$$

The value of these forces can be obtained analytically or numerically. The equation 4.3 and 4.4 are then solved using differential equation solver. The following sub-sections discuss the method to obtain the value for each component of these forces. The solution for these differential equations is discussed in the next section.

The structure shown in figure 4.1 represents the basic module for the moving electric field structure presented in figure 3.1. The behavior of the system can be obtained by analyzing this basic module. The full behavior of the moving electric field system can be found by repeating this basic module, where the initial conditions and boundary conditions from the previous module are transferred to the next module.

4.2.1 Dielectrophoretic Force

The calculation of the dielectrophoretic force involves solving the Laplace's equation for a boundary value problem to find the electrical potential and the electric field. The calculation can be performed analytically or numerically. In general, the analytical approach is mathematically involved, and the solution is only available for certain electrode configurations.

Green's theorem [Wang *et al.* 1996] and Fourier series [Morgan *et al.* 2001] have been used to obtain analytical solutions for simple electrode arrays and interdigitated electrode arrays. On the other hand, the numerical approach yields a solution for almost any electrode configurations. Finite element method [Green *et al.* 2002] and the weighted least-square finite difference scheme [Chen *et al.* 2005] have been used to obtain numerical solutions for two-phase electrode arrays and interdigitated electrode arrays.

For this study, the numerical approach was used; although an analytical solution is presented in Appendix A by approximating certain boundary conditions. The governing equations for the dielectrophoretic force are derived from the electromagnetic field, where the unknown is the electrical potential Φ in the xy -space, given the boundary conditions at the bounding edges. In a microfluidic device, the electric field wavelength is typically several orders of magnitude larger than the particle or electrode size. Thus, the electroquasistatic approximation can be used. This assumption allows the time-dependent problem in figure 4.1 to be analyzed as a static problem, where the boundary condition $\Phi = V_0 \cos \omega t$ is analyzed as $\Phi = V_0$. In general, as long as the potential difference between the top and the bottom electrodes is V_0 , the potentials in these two electrodes can take any values for the static analysis.

Applying electroquasistatic assumptions to the Maxwell equations, and assuming no free charges in space, the Gauss' law reduces to [Haus and Melcher 1989]

$$\nabla \cdot \nabla \Phi = 0 \text{ or } \nabla^2 \Phi = 0 \quad (4.5)$$

Equation 4.5 is the governing equation to obtain the electrical potential Φ in the xy -space.

For the geometry as shown in the figure 4.1, the top and the bottom plate are glass material. The structure is symmetric at the left edge. Physically, the right edge goes to infinity, but the distance of the right edge from the symmetric line can be approximated as a finite distance l , as long as l is at least one characteristic length longer than d and h following the St. Venant's principle. The microchannel is filled with fluid medium. The electrical potential is applied on the top and bottom electrodes. Since the electrode thickness is typically less than

100 nm, which is significantly smaller than the microchannel height of at least 20 μm , it is reasonable to model the electrodes as having a zero thickness. These physical conditions translate into boundary conditions are shown in figure 4.2. The detail reasoning in reaching these boundary conditions can be referred at [Green *et al.* 2002].

Figure 4.2 The geometries and the boundary conditions for the analysis of the electrical potential, the electric field and the spatial strength of the dielectrophoretic force.

Correspondingly, the electric field $\mathbf{E}$ is found by taking the gradient of the potential

$$\mathbf{E} = -\nabla\Phi \quad (4.6)$$

Separately, from the equivalent dipole method [Jones 1995], the time-averaged dielectrophoretic force acting on a spherical particle with radius a and suspended in a medium with permittivity ϵ_m is given by [Morgan and Green 2003]

$$\mathbf{F}_{\text{DEP}} = \pi\epsilon_m a^3 \text{Re}[\tilde{K}] \nabla |\mathbf{E}|^2 \quad (4.7)$$

It can be seen that the term $\pi\epsilon_m a^3 \text{Re}[\tilde{K}]$ on the right hand side of equation 4.7 is a constant for any specific particle and medium condition, and only the term $\nabla |\mathbf{E}|^2$ determines the spatial strength of the dielectrophoretic force for any electrode configuration. Realizing this concept, it is possible to use electric field $\mathbf{E}$ obtained in equation 4.6 to calculate a separate gradient of electric field square term $\nabla |\mathbf{E}|^2$ on a same simulation software, and later combine with the remaining factors $\pi\epsilon_m a^3 \text{Re}[\tilde{K}]$ to find the full dielectrophoretic force strength on another software package. (Note that the exact mathematical representation of a time-averaged

dielectrophoretic force will require an angle bracket on both side of the vector term, such as $\langle \mathbf{F}_{\text{DEP}} \rangle$. However, since dielectrophoretic force analysis almost certainly involves time-average integration, it is generally convenient to write it in shorthand without the angle bracket. The exception to this shorthand form is in the derivation of the dielectrophoretic force, where clear differentiation is required to distinguish different steps in the derivations.)

It is important to note that the dielectrophoretic force calculated from equation 4.7 has ignored the wall and particle size effect. Such effect is significant when the particle size is in the same order of magnitude as the microchannel height. This effect is further discussed in Appendix B.

Using the parameter values as shown in table 4.1, the boundary value problem as illustrated in figure 4.2 is solved using finite element simulation software (FlexPDE 5.0 Professional version). Figure 4.3 shows the simulation results for the electrical potential, the magnitude of the electric field, and the magnitude of the dielectrophoretic force factor. From figure 4.3(c), it can be seen that the dielectrophoretic force decays exponentially away from the top electrode. The force is reduced by an order of magnitude for approximately every 20 μm .

Table 4.1 Generic values for the electrical field simulation. These values are used through out the rest of the simulation results, unless otherwise specified.

Parameters	Value	Unit
Microchannel length, l	100	μm
Microchannel height, h	25	μm
Electrode width, d	10	μm
*Amplitude of voltage, V_0	5	V

*Note: The physical function generator usually specified the peak-to-peak voltage (V_{pp}) or root-mean-square voltage (V_{rms}). For 10 V_{pp} , the amplitude of the voltage V_0 is 5.

The time-averaged dielectrophoretic forces shown in equation 4.7 can be expanded to their individual x - and y -components which are, respectively,

$$F_{\text{DEP}_x} = \pi \epsilon_m a^3 \text{Re}[\tilde{K}] \frac{\partial}{\partial x} |\mathbf{E}|^2 \quad (4.8)$$

$$F_{DEP_Y} = \pi \epsilon_m a^3 \text{Re}[\tilde{K}] \frac{\partial}{\partial y} |\mathbf{E}|^2 \quad (4.9)$$

Figure 4.4 and figure 4.5 show the simulation results for the gradient of the electric field square as depicted in the equation 4.8 and 4.9, respectively. Note that the x - and y -components are in the same order of magnitude. The numerical results of $\partial|\mathbf{E}|^2/\partial x$ and $\partial|\mathbf{E}|^2/\partial y$ are the basis in calculating the dielectrophoretic force in the final equation of motion program.

Figure 4.3 The simulation result for (a) Potential, V; (b) Magnitude of electric field, V/m; (c) Magnitude of gradient of electric field square, V^2/m^3 .

Figure 4.4 Magnitude of gradient of electric field square of x -component along $y = 0 \mu m$, $10 \mu m$ and $20 \mu m$.

Figure 4.5 Magnitude of gradient of electric field square of y -component along $y = 0 \mu m$, $10 \mu m$ and $20 \mu m$.

4.2.2 Fluid Drag

A particle moving in a fluid medium experiences a dampening force which acts in the direction against the motion. This dampening force is called fluid drag. For a spherical particle moving slowly in an unbounded viscous fluid, the fluid drag $\mathbf{F}_D$ acting on the particle is proportional to the drag coefficient $A = 6\pi\eta a$ and the particle velocity $d\mathbf{r}/dt$ [Deen 1998]

$$\mathbf{F}_D = -6\pi\eta a \frac{d\mathbf{r}}{dt} \quad (4.10)$$

where a is the particle radius and η is the viscosity of the fluid medium. The negative sign in the right hand side of equation 4.10 indicates that the drag acts in the opposite direction to the particle velocity.

For a particle moving in a microchannel bounded by two parallel plates, as the case in figure 4.1, a wall correction factor has to be added to the drag relation. The fluid drag in the x - and y -component are now, respectively, [Ganatos 1978, Ganatos *et al.* 1980, Ganatos *et al.* 1980]

$$F_{drag_X} = 6\pi\eta a K_x \frac{dx}{dt} \quad (4.11)$$

$$F_{drag_Y} = 6\pi\eta a K_y \frac{dy}{dt} \quad (4.12)$$

where K_x and K_y are the wall correction factors in the x - and y -direction, respectively, and dx/dt and dy/dt are the instantaneous particle velocity in the x - and y -direction, respectively. The wall correction factors depends on the ratio of the microchannel height to the particle radius (i.e. h/a) and the ratio of the particle y -position to the microchannel height (i.e. b/h). The values for certain h/a ratio are plotted in figure 4.6. The wall correction factors in the y -direction is few times larger than the wall correction factors in the x -direction at distance near to the wall, which implies that a particle faces larger resistance in the y -directional motion compared to the x -directional motion when the particle tries to move near to the wall. Thus, a particle tends to stay in a constant elevation when it moves along the microchannel.

Other methods have been demonstrated to simulate the particle motion in a confined space. [Glowinski *et al.* 1999, Singh *et al.* 2000] demonstrated the sedimentation of sphere using a distributed Lagrange multiplier/fictitious domain method. [Feng and Michaelides 2002] has performed simulation for the hydrodynamic force acting on a sphere that settles in orthogonal cylindrical or prismatic enclosures. Such methods take into account of the finite size of the particle directly in simulating the particle motion in the fluid, which is a possible extension to the approach used in this work.

Figure 4.6 The wall correction factor of fluid drag for a spherical particle in a parallel plate channel in the x-direction, K_x and in the y-direction, K_y , respectively. (a = sphere radius, h = microchannel height, b = distance between centre of the sphere to the bottom of the microchannel) The values are adapted from [Ganatos 1978].

4.2.3 Buoyancy and Gravitational Forces

Since a particle fully submerged in a fluid displaces a fluid volume equivalent to the particle's body volume, there is a buoyancy force acting on the particle. Following Archimedes's principle, this buoyancy force is equal to the weight of the displaced fluid

$$F_{buoy} = \rho_m V_p g \quad (4.13)$$

where ρ_m is the density of the fluid, V_p is the volume of the particle, and g is the acceleration due to gravity.

The gravitational force also acts on the particle, which is given by

$$F_{grav} = mg = \rho_p V_p g \quad (4.14)$$

where $m = \rho_p V_p$ is the mass of the particle, and ρ_p is the density of the particle. For a spherical particle, the volume of the particle is given by

$$V_p = \frac{4}{3} \pi a^3 \quad (4.15)$$

Since these two forces are always related to each other, they are sometimes being lumped together and presented as just a buoyancy force or a gravity force, as done by [Green *et al.* 2000, Castellanos *et al.* 2003, Morgan and Green 2003].

4.3 Equation of Motion

Using the x -component of the dielectrophoretic force from equation 4.8 and the fluid drag from equation 4.11, the x -directional force balance on the particle in equation 4.3 becomes

$$\pi\epsilon_m a^3 \operatorname{Re}[\tilde{K}] \frac{\partial}{\partial x} |\mathbf{E}|^2 - 6\pi\eta a K_x \frac{dx}{dt} = m \frac{d^2 x}{dt^2} \quad (4.16)$$

Using the y -component of the dielectrophoretic force from equation 4.9, the fluid drag from equation 4.12, the buoyancy force from equation 4.13 and the gravitational force from equation 4.14, the y -directional force balance on the particle in equation 4.4 becomes

$$\pi\epsilon_m a^3 \operatorname{Re}[\tilde{K}] \frac{\partial}{\partial y} |\mathbf{E}|^2 - 6\pi\eta a K_y \frac{dy}{dt} + \rho_m V_p g - \rho_p V_p g = m \frac{d^2 y}{dt^2} \quad (4.17)$$

Equation 4.16 and 4.17 can be further simplified by substituting the particle mass m as $\rho_p V_p$ and the particle volume V_p as $\frac{4}{3}\pi a^3$. The equation of motion in the x - and y -directions becomes

$$\frac{3\epsilon_m \operatorname{Re}[\tilde{K}]}{4\rho_p} \frac{\partial}{\partial x} |\mathbf{E}|^2 - \frac{9\eta K_x}{2\rho_p a^2} \frac{dx}{dt} = \frac{d^2 x}{dt^2} \quad (4.18)$$

$$\frac{3\epsilon_m \operatorname{Re}[\tilde{K}]}{4\rho_p} \frac{\partial}{\partial y} |\mathbf{E}|^2 - \frac{9\eta K_y}{2\rho_p a^2} \frac{dy}{dt} + \frac{(\rho_m - \rho_p)g}{\rho_p} = \frac{d^2 y}{dt^2} \quad (4.19)$$

By solving these two second order differential equations, the particle position, velocity and acceleration in the xy -space can be obtained. Note that these two differential equations are non-linear and coupled. The dielectrophoretic force and fluid drag components depend on both x - and y -position.

Matlab (version 7 R14, The MathWorks Inc.) has been used to solve these differential equations. Since Matlab only accepts first order differential equations, it is necessary to rewrite equation 4.18 and 4.19 into systems of first order equations.

Defining,

$$y(1) = x \quad (4.20)$$

$$y(2) = \frac{dx}{dt} \quad (4.21)$$

$$y(3) = y \quad (4.22)$$

$$y(4) = \frac{dy}{dt} \quad (4.23)$$

A system of differential equations equivalent to differential equations in equation 4.18 and 4.19 is now,

$$y(1)' = y(2) \quad (4.24)$$

$$y(2)' = A - B \cdot y(2) \quad (4.25)$$

$$y(3)' = y(4) \quad (4.26)$$

$$y(4)' = C - D \cdot y(4) \quad (4.27)$$

where $A = \frac{3\epsilon_m \text{Re}[\tilde{K}]}{4\rho_p} \frac{\partial}{\partial x} |\mathbf{E}|^2$, $B = \frac{9\eta K_x}{2\rho_p a^2}$, $C = \frac{3\epsilon_m \text{Re}[\tilde{K}]}{4\rho_p} \frac{\partial}{\partial y} |\mathbf{E}|^2 + \frac{(\rho_m - \rho_p)g}{\rho_p}$ and

$$D = \frac{9\eta K_y}{2\rho_p a^2} .$$

As shown in the next section, the solutions to this system of equations is *stiff* – a term used in differential equations to refer to solutions where the time scale vary greatly across the whole time frame [Shampine *et al.* 2003]. It is impractical to solve such system using the classical methods like Euler or fourth-order Runge-Kutta. Instead, a stiff ODE solver is required. Equation 4.24, 4.25, 4.26 and 4.27 are solved in Matlab using a trapezoidal rule solver ‘ode23t’. This solver is designed for moderately stiff ordinary differential equation. The Matlab codes used for this program are documented in Appendix C.

4.4 Simulation Results

Three sample cases have been run to understand the general behavior of the system, using the typical values shown in table 4.2. The particle is assumed to have a Clausius-Mossotti factor of -0.4, thus, the particle experiences a negative dielectrophoresis and tends to move away from the electrodes. The particle is assumed to have a radius of $2.5\ \mu\text{m}$ and a density of $1040\ \text{kg m}^{-3}$, typical for latex sphere. The fluid is assumed to be a water-based solution, with medium relative permittivity of 78, viscosity of $0.0089\ \text{kg m}^{-1}\ \text{s}^{-1}$, and density of $1000\ \text{kg m}^{-3}$.

The simulation is performed using start time of 0 s, and end time of 5 s, with an initial velocity of $0\ \text{ms}^{-1}$. Three initial positions have been considered at different elevation of $y = 3.5\ \mu\text{m}$, $12.5\ \mu\text{m}$, and $21.5\ \mu\text{m}$, respectively, with x fixed at $10\ \mu\text{m}$. The simulation results of the particle trajectories in the xy -space are shown in figure 4.7. The particle position and velocity over time in the x -direction and y -direction are shown in figure 4.8(a), (b), (c) and (d), respectively.

Table 4.2 Typical values for the parameters used in the simulation.

Parameters	Value	Unit
Free space permittivity, ϵ_0	8.854×10^{-12}	Farads m^{-1}
Medium relative permittivity, ϵ_m	78	
Particle radius, a	2.5	μm
Clausius-Mossotti factor	-0.4	
Fluid viscosity, η	0.0089	$\text{kg m}^{-1}\ \text{s}^{-1}$
Medium density, ρ_m	1000	kg m^{-3}
Particle density, ρ_p	1040	kg m^{-3}
Gravitational constant, g	9.81	m s^{-2}

The simulation results indicate that particles moves relatively fast in the initial time steps and move significantly slower in the later time steps. Such behavior is typically seen in the solution of a stiff differential equation. The x -directional motion is more important than the y -directional motion, since particles are separated from each other based on their horizontal traveling distance. When a particle is near to the top electrode, the electric field gradients are large, thus, the dielectrophoretic force acting on the particle is also large. Therefore, the particle can move further than a position away from the top electrode. When a particle is near to the channel wall, the fluid drag in the y -direction is large.

Figure 4.7 Particle trajectories for particles at different initial positions. The markers ‘ Δ ’, ‘ $\square$ ’ and ‘ $\circ$ ’ represent the simulation results at initial positions of (10, 3), (10, 12.5), and (10, 22), respectively.

Figure 4.8 Simulation results against time plots for three different initial positions at $y_0 = 3.5 \mu\text{m}$, $12.5 \mu\text{m}$, and $21.5 \mu\text{m}$, respectively. (a) Particle x-position against time; (b) Particle y-position against time; (c) Particle x-velocity against time; (d) Particle y-velocity against time.

Thus, particle tends to stay in its own course. From figure 4.8(a), a particle with same electrical and mechanical properties can travel a different distance in the microchannel based on its initial y -position.

4.5 Comparison with Experimental Observations

The mathematical model presented in the previous section is a theoretical prediction of particle movement based on a number of assumptions and simplifications on the actual physical conditions. This section covers the experimental setup and the corresponding results to access the accuracy of the theoretical model in predicting the trajectory of particles. A short discussion follows.

4.5.1 Experimental Methods and Setup

The experimental setup and devices used for the work in this section is described in Chapter 6, with some modifications and customizations on certain apparatus. Borosilicate glass microspheres (Duke Scientific Corp.) with diameter of $5\text{ }\mu\text{m}$ were suspended in a solution with 0.1% sodium dodecyl sulfate [or SDS] (Sigma). The SDS surfactant was added to the solution to promote higher ionic charge and prevents the microspheres from sticking onto the electrodes or microchannel walls. The conductivity of the solution was measured to be $245\text{ }\mu\text{S/cm}$. The suspension was injected into the microchannel. The microchannel height was measured to be (average of data from four corners = $22.85 + 23.75 + 24.10 + 25.80 = 24\text{ }\mu\text{m}$). A pre-processing was performed on the suspension where the glass microspheres were first transported to a pre-determined location. This pre-processing was achieved using the moving dielectrophoresis concept as described in the previous chapter. This step ensured that the microspheres were in the same initial x -position. After that, a single electrode was energized. The applied electrical voltage, the electrical frequency and the top electrode width were 10 Vpp , 500 kHz , and $10\text{ }\mu\text{m}$, respectively. The microspheres were repelled from the electrode

edge due to the negative dielectrophoresis. The motion of the microspheres was recorded using a camcorder (Handycam DCR-DVD803E PAL, Sony), through the microscope (Eclipse TE2000-S, Nikon).

The DVD video was downloaded to a computer, and converted from mpeg2 to avi format. The microspheres trajectories were traced using the *Manual Tracking* plug-in of the image processing software *ImageJ*. The diameter of the microspheres was measured in the image with three repetitions.

4.5.2 Experimental Results and Discussions

The trajectories of three different glass microspheres were plotted in figure 4.9. Five repetitions were performed for each sphere. The initial x -position of the microspheres was around $10\ \mu\text{m}$, measured from the center of the energized electrode. The results show that the glass microspheres exhibit a high initial velocity and a small velocity at longer time.

Figure 4.9 Experimental results showing the trajectories (x -position over time) of three borosilicate spheres in a sodium dodecyl sulfate (SDS) 0.1% solution with measured medium conductivity of $245\ \text{mS/cm}$. The diameter of the borosilicate spheres are shown next to the respective data. Five repetitions were performed for each sphere.

Table 4.3 shows the comparison between the experimental observations with the analytical predictions. Since the video was recorded on the xz -plane, the y -position of the

microspheres in the microchannel was not known. For this reason, three initial y -positions were used for the theoretical model to facilitate a more comprehensive comparison. The error was at the range of 35% to 53%, which was calculated as $(\text{experiment} - \text{analytical})/\text{experiment}$.

Table 4.3 Comparison between experimental observations and theoretical predictions.

Time (s)	Experiment (μm)	Theoretical - at different initial position of y (μm)					
		@ 3.5 μm	% error	@ 12.5 μm	% error	@ 21.5 μm	% error
1.0	43.39	20.3745	53.0	25.5207	41.2	27.9839	35.5
2.0	53.07	25.4296	52.1	30.8156	41.9	32.4087	38.9
3.0	58.22	28.6612	50.8	34.0748	41.5	34.8774	40.1
4.0	62.24	31.1527	49.9	36.3314	41.6	36.9020	40.7
5.0	65.39	33.0390	49.5	38.0840	41.8	38.6120	41.0

The result shows a qualitative match between the experimental observations with the theoretical predictions. The trajectories of the microspheres in the experiment results exhibit a consistent trend as predicted in the theoretical model. There is a rapid increase in velocity at the initial time, and a much slower increase in velocity at a longer time. However, the quantitative comparison shows that experiment values are approximately double the theoretical values.

As studied in the next chapter, the sensitivity analyses show that this discrepancy can not be accounted for by the variation in the parameters in the existing model. The most likely scenario is that the electrohydrodynamic effect was not taken into account in the model. Such electrohydrodynamic effect is due to the interaction of the applied electric field with the fluid medium, where the fluid medium is polarized, resulting in induced fluid flow. A more detail discussion on the electrohydrodynamic effect is documented at Appendix D. It is predicted that the SDS surfactant promotes stronger ionization in the fluid medium, thus induced a stronger fluid flow compared to a solution without this surfactant. As a result, the motion of the glass microspheres is determined by the net force from dielectrophoresis and the induced fluid flow. If the induced fluid flow is in the same direction as the dielectrophoretic force, the glass microspheres would travel farther than acted by the dielectrophoretic force alone. Therefore, the theoretical model presented here is useful for conditions where electrohydrodynamic effect is not dominant. A further comparison of this theoretical model

with experiments involving yeast cells show a closer match, where the fluid medium is mannitol solution. The discussion of this result for yeast cells is deferred until Chapter 7, where it is included together with the discussion on the moving dielectrophoresis technique. This electrohydrodynamic effect is not incorporated in the model because the induced fluid flow exerts a surface force on the particle, and thus requires a summation of forces around the surface of the particle to calculate the particle trajectory. Our current model assumes that all the forces can be simplified to point force acting on the center of mass of the particle.

4.6 Summary

There are at least four types of forces acting on a particle, namely dielectrophoretic force, fluid drag, gravitational force, and buoyancy force. A mathematical model has been built. The trajectory of a particle in the microchannel can be predicted by solving the equation of motion arising as the balance of these forces. The numerical solutions and experimental results show a qualitative match, although they differ by as much as forty percent empirically. This discrepancy is expected to be due to the fluid flow or electrode polarization effects which are not included in the model.

Chapter 5

Parameters Affecting Moving DEP

5.1 Introduction

It is crucial to understand the factors affecting separation efficiency in mDEP. This chapter documents the study of the parameters affecting the equation of motion described in the previous chapter. These parameters include initial positions, particle polarization factor, particle size, applied voltage, electrode width, inter-electrode gap, microchannel height, number of energized electrodes, and types of electrode configurations. Since the underlying differential equations are not linear, it is not trivial to predict the particle trajectories based on a simple scaling analysis. Instead, the analysis has to be performed for each condition, by resolving the differential equation. Experimental results are presented along the theoretical predictions to validate the predictions. These experimental results were obtained using the experimental setup as discussed in the previous chapter. Due to electrohydrodynamic effects, the experimental observations of the traveled distance are always larger than the theoretical predictions. Nevertheless, the qualitative comparison shows a good agreement.

5.2 Effect of Initial Positions

The dielectrophoretic forces exerted on a particle is a function of the position of the particle. For the electrode configuration shown in figure 4.1, the dielectrophoretic forces are strongest near to the top electrode. At a distance $x > 20 \mu\text{m}$, the x -component of the dielectrophoretic force is almost independent of y -position. The x -component of the dielectrophoretic force is also significantly larger than the y -component of the force.

Along the positive x -direction, the dielectrophoretic forces decay in the magnitude as illustrated in figure 4.3(c). In general, a particle further from the electrodes experiences a smaller dielectrophoretic force than a particle near the electrodes. Consequently, a particle experiences a smaller momentum when the particle is far from the electrodes. As a result, the particle initial position has a diminishing effect on the trajectory of the particle, as illustrated in figure 5.1. The particles at $x_0 = 10 \mu\text{m}$, $20 \mu\text{m}$, and $30 \mu\text{m}$, travel to x -positions of $38.3 \mu\text{m}$, $39.4 \mu\text{m}$, and $41.1 \mu\text{m}$, respectively, after 5 s. The experimental observation supports this argument, as shown in the same graph. The experimental results for three particles with diameters of $4.32 \mu\text{m}$, $4.90 \mu\text{m}$, and $5.56 \mu\text{m}$, respectively, travel to a final position of $65 \mu\text{m} \sim 74 \mu\text{m}$ after 5 s. Comparing this result with the particles with initial x -position of $10 \mu\text{m}$, having final position of $60 \mu\text{m} \sim 71 \mu\text{m}$, as shown in figure 4.9, the difference is approximately $5 \mu\text{m}$.

However, this conclusion is only true when the particle is at least one characteristic length away from the top electrode. The final distance differs more when particles are near to the top electrode. The particle trajectory is sensitive to the initial position in the y -direction, as shown in figure 4.7 and figure 4.8.

Figure 5.1 Particle trajectories in x -direction for different initial x -positions. The data clouds represent the experimental results, where the measured particle diameters are written next to those data. The analytical solutions are plotted in solid curves, where the markers ‘ Δ ’, ‘ $\square$ ’ and ‘ $\circ$ ’ represent initial conditions of $x_0 = 10 \mu\text{m}$, $20 \mu\text{m}$, and $30 \mu\text{m}$, respectively. Other simulation parameters use the typical values as in table 4.2.

5.3 Effect of Polarization Factor

The polarization factor of a particle depends on the applied electrical frequency, and the conductivity and permittivity of the fluid and particle. The polarization factor is typically characterized by the Clausius-Mossotti factor as depicted by equation 2.3. As shown in figure 5.2 and figure 5.3, the particle trajectories have a non-linear dependence on the polarization factor.

The polarization factor has an upper limit of 1.0 and a lower limit of -0.5.

In general, the difference in the final traveled distance reduces with the increase in the magnitude of the polarization factor. For a particle experiencing a negative dielectrophoretic force, the final x -position for a particle with polarizability of -0.5 is $39.99 \mu\text{m}$ compare to $38.30 \mu\text{m}$ for a particle with polarizability of -0.4. The difference is $1.69 \mu\text{m}$. Comparing this to the case where particles with polarizabilities of -0.2 and -0.1, which migrate $32.52 \mu\text{m}$ and $27.27 \mu\text{m}$, respectively, the difference is $5.25 \mu\text{m}$. Similar results can be seen on a particle experiencing a positive dielectrophoretic force, as shown in figure 5.3. Particles having high polarizabilities have a closer response time compared to particles having low polarizabilities.

The results shown in figure 5.2 and figure 5.3 also reveal an important particle manipulation strategy. For a particle experiencing negative dielectrophoresis, the particle is transported to the next electrode by a dielectrophoretic force which pushes it across the inter-electrode gap. Since the dielectrophoretic force is large near the energized electrode, the particle experiencing negative dielectrophoresis always experience a large pushing force at the initial time. As such, the particle experiencing negative dielectrophoresis can be transported to the next electrode even if the magnitude of the polarization factor is small. In contrast, a particle experiencing positive dielectrophoresis is pulled to the energized electrode by the dielectrophoretic force. Since the dielectrophoretic force is weak far from the energized electrode, the particle experiencing positive dielectrophoresis has to have a large polarization factor in order to gain enough momentum to move the particle.

Figure 5.2 The particle trajectories for different Clausius-Mossotti factors ranging from -0.01 to -0.5. The graph shows the typical behavior of particle experiencing a negative dielectrophoresis.

Figure 5.3 The particle trajectories for different Clausius-Mossotti factors ranging from 0.01 to 1.0. The graph shows the typical behavior of particle experiencing a positive dielectrophoresis. The particle initial condition is assumed to be 30 μm from the origin.

5.4 Effect of Particle Size

Figure 5.4 shows the particle trajectories in the xy -space for different particle sizes, a , including 2.5 μm , 5 μm , 6.25 μm , 8.33 μm , and 10 μm . The corresponding x -position vs. time is plotted in figure 5.5. These particle sizes are chosen based on the h/a ratio of 10, 5, 4, 3, and 2.5, respectively, where the microchannel height h is assumed to be 25 μm . By making such selection, the drag forces for these particle sizes can be obtained by interpolating within the data points of the same h/a ratio tabulated in the previous chapter.

In general, the particle trajectories depend on the particle size. A larger particle travels a longer distance within the same time, although such relationship is not linear. Note that particle with diameter 10 μm has a significantly smaller travel distance because it touches the microchannel floor after ~ 0.2 s. This constraint is imposed to avoid calculating trajectory for particle position smaller than its radius.

Figure 5.4 Analytical prediction of the particle trajectories for different particle sizes a of 2.5 μm , 5 μm , 6.25 μm , 8.33 μm , and 10 μm . The initial position of the particle is assumed to be (10, 12.5). Other simulation parameters use the typical values as in table 4.2.

It is interesting to study the dependent of the forces on the particle size. The dielectrophoretic force is dependent on cube of the particle radius, whereas the fluid drag is linearly dependent on the particle radius (but subjected to a correction factor that depends on the microchannel height to particle size ratio). The buoyancy and gravitational forces are significantly smaller than the dielectrophoretic force such that they can be ignored. Although the comparison suggests a square dependence of particle radius for the balance of forces, the traveled distance does not increase by a similar proportion when the particle size doubled. This result is due to the non-linear effect from the fast decaying dielectrophoretic force away from the energized electrode, as shown by the non-linear differential equation 4.18 and 4.19.

Figure 5.5 The corresponding particle trajectories in x -direction plotted over time.

5.5 Effect of Applied Voltage

From the scaling laws, the dielectrophoretic force scales with the square of the applied voltage.[Castellanos *et al.* 2003, Jones 2003]

$$\mathbf{F}_{\text{DEP}} \sim V^2$$

The dielectrophoretic force increases four times when the applied voltage is doubled. This effect is significant at short range, and fade at longer distance away from the electrodes. The analytical solutions illustrate this effect, as shown in figure 5.6. The particle only travels an extra 8 μm after 5 s, although the applied voltage is increased from 6 V_{pp} to 10 V_{pp} . The experimental results confirm this prediction. Such phenomenon is due to the effect from the fluid drag, where the drag force is linearly proportionate to the particle velocity.

Although increasing the applied voltage increases the dielectrophoretic force exerted on a particle, it also has an adverse effect, increasing the electrohydrodynamic flow. Such body force exerted on the particle increases faster than the dielectrophoretic force when the voltage is increased². Therefore, there is an optimum value for applied voltage such that the

² AC electroosmosis is proportionate to square of voltage, whereas electrothermal effect is proportionate to power four of voltage. More detail comparison can be found from the scaling law notes by Castellanos A., Ramos A., González A.,

dielectrophoretic force dominates against electrohydrodynamic force. This optimum value also depends on medium conductivity and particle size. From the experimental observation, an applied voltage of $10 V_{pp}$ is sufficient to drive the particle using dielectrophoresis for a particle size of $5\sim 10 \mu\text{m}$, and medium conductivity of $1\sim 1000 \mu\text{S/cm}$.

Figure 5.6 Particle trajectories in x -direction when subjected to $6 V_{pp}$, $8 V_{pp}$, and $10 V_{pp}$ applied voltage using the typical values in table 4.2. The initial y -positions are assumed to be $12.5 \mu\text{m}$.

5.6 Effect of Electrode Width

The effect of the electrode was studied for three different widths, namely $10 \mu\text{m}$, $15 \mu\text{m}$ and $20 \mu\text{m}$. Figure 5.7a and figure 5.7b show the magnitude of gradient of electric field square plots for electrode widths of $15 \mu\text{m}$ and $20 \mu\text{m}$, respectively. The plot for an electrode width of $10 \mu\text{m}$ has been shown in the previous chapter in figure 4.3c. These simulation results indicate that the dielectrophoretic forces are similar for different electrode widths at regions $x < -d/2$ or $x > d/2$. Since these two regions represent the space where the particle has to travel over to achieve continuous motion in the moving dielectrophoresis, it is sufficient to consider these two regions only. At the region $-d/2 < x < d/2$, although the dielectrophoretic forces

distribution varies for different electrode widths, it does not play a significant role in particle transportation or particle separation. Particles are always moved away from the edge of the energized electrode, or attracted to the edge of the energized electrode.

Figure 5.7 The simulation results of magnitude of gradient of electric field square, $\nabla|E|^2$, unit V^2/m^3 , for top electrode widths of (a) 15 μm . and (b) 20 μm , respectively. Other parameters of the model are kept constant as in table 4.1.

Figure 5.8 shows the effect of electrode width on the particle trajectories. The electrode width of 10 mm has been used as base condition to facilitate the comparison in figure 5.8a and figure 5.8b. The theoretical results and experimental observations on the particle trajectories are consistent with the analysis on the dielectrophoretic force for different electrode widths. The theoretical results for electrode widths of 15 μm and 20 μm are identical with the results for electrode width of 10 μm , where the particles travel a same total distance when measured from the electrode edge. Therefore, the electrode width has minimal effect on the particle trajectories.

Figure 5.8 The effect of electrode width on the particle trajectories in x -direction. The theoretical results are plotted as continuous lines with hollow markers, whereas the experimental results are plotted as solid markers. (a) Comparison between electrode width of $10\ \mu\text{m}$ with electrode width of $15\ \mu\text{m}$. (b) Comparison between electrode width of $10\ \mu\text{m}$ with electrode width of $20\ \mu\text{m}$. The trajectories merely shifted $5\ \mu\text{m}$ or $10\ \mu\text{m}$ in the positive x -direction in both conditions, respectively, compared to the base case of electrode width of $10\ \mu\text{m}$.

5.7 Effect of Inter-electrode Gap

Referring to figure 4.3c, the dielectrophoretic force distribution decays exponentially from the edge of the electrode towards the positive x -direction. At a distance $10\ \mu\text{m}$ from the edge of the electrode, the dielectrophoretic force decreases one-order of magnitude compared to the value at the electrode edge. At a distance of $20\ \mu\text{m}$, the force decreases almost two-order of magnitude. Since the inter-electrode gap represents the distance a particle has to travel over before it is polarized and moved by the subsequent energized electrodes, it is advantageous to keep the inter-electrode gap as small as possible. A small gap ensures that the particle always experience a dielectrophoretic force that is large enough to propel it to the next electrodes.

5.8 Effect of Microchannel Height

Figure 5.9 shows the $\nabla|\mathbf{E}|^2$ plot for a microchannel height of 50 μm . Comparing this plot to figure 4.3c which has a microchannel height of 25 μm , the dielectrophoretic force is smaller in general. Therefore, it is advantageous to design a dielectrophoretic system with a small microchannel height.

Figure 5.9 The simulation result of magnitude of gradient of electric field square, $\nabla|\mathbf{E}|^2$, unit V^2/m^3 , for a microchannel with a height of 50 μm . The other parameters of the model are kept constant as in table 4.1.

Figure 5.10 shows the particle trajectories for a microchannel height of 50 μm . The simulation results show that the particle transportation at this condition is highly dependent on the particle y-elevation. A particle at initial y-position of 5 μm travels a distance less than 5 μm in 5 s, whereas a particle at initial y-position of 45 μm travels ~ 25 μm for the same period. Figure 5.11 gives a xy -space view of the particle trajectories at different initial conditions. Referring back to the $\nabla|\mathbf{E}|^2$ plot, it can be seen that a particle at initial y-position of 5 μm experiences at least an order of magnitude smaller dielectrophoretic force compared to a particle at initial y-position of 45 μm . The experimental results also show that a glass

microsphere in a microchannel height of $50\text{ }\mu\text{m}$ travels almost $10\text{ }\mu\text{m}$ less than a glass microsphere in a microchannel height of $25\text{ }\mu\text{m}$.

Figure 5.10 Particle trajectories in x -direction under a microchannel with height of $50\text{ }\mu\text{m}$. The data clouds represent the experimental results, where the measured particle diameters are written next to those data. The analytical solutions using the typical values in table 4.2, except that the microchannel height is changed to $50\text{ }\mu\text{m}$, are plotted as curves. The markers ' Δ ', ' $\square$ ' and ' $\circ$ ' represent initial conditions of $y_0 = 45\text{ }\mu\text{m}$, $25\text{ }\mu\text{m}$, and $5\text{ }\mu\text{m}$, respectively.

Figure 5.11 Particle trajectories at different initial positions for a microchannel height of $50\text{ }\mu\text{m}$. The markers ' Δ ', ' $\square$ ' and ' $\circ$ ' represent the simulation results at initial positions of $(10, 5)$, $(10, 25)$, and $(10, 45)$, respectively.

5.9 Effect of Number of Electrodes

The effect of energizing two and three electrodes is shown in figure 5.12. The effect of energizing more electrodes is similar to the effect of increasing the electrode width. The dielectrophoretic force components along the x -direction are similar for the region beyond the last electrode edge. Therefore, the dielectrophoretic force distribution at $x > 15 \mu\text{m}$ when energizing two electrodes, see figure 5.12a, is same as the dielectrophoretic force distribution at $x > 25 \mu\text{m}$ when energizing three electrodes, see figure 5.12b.

Figure 5.12 The effect of energizing two-pair or three-pair of electrodes. The value plotted are the magnitude of $\nabla|\mathbf{E}|^2$, with unit of V^2/m^3 . (a) Two-pair of electrodes; (b) Three-pair of electrodes.

The theoretical results for particle trajectories when energizing one, two and three electrodes are shown in figure 5.13. Each particle is assumed to have an initial x -position of $5 \mu\text{m}$ away from the last electrode edge. The simulated results show that the curves merely shifted $\sim 10 \mu\text{m}$ or $\sim 20 \mu\text{m}$ in the x -direction when two or three electrodes are energized,

respectively. The experimental results also support this result where the final travel distance is approximately 10 μm apart. Each experimental result was repeated five times for a same glass microsphere. Thus, the plot represents the spread of five repetitions.

Figure 5.13 Particle trajectories in x -direction when 1, 2 or 3 electrodes are switched on, respectively, using the typical values in table 4.2. The initial y -positions are assumed to be 12.5 μm .

5.10 Different Electrode Configurations

A moving electric field can be generated by many other electrode configurations. For instance, the moving electric field can be generated by a series of two pair of electrodes arranged in top-down configuration, as illustrated in figure 5.14. Another configuration is to arrange the pair of electrodes on a left-right condition, as shown in figure 5.15. Simulations were performed using the geometry and boundary conditions as denoted in figure 5.14a and figure 5.15a.

The dielectrophoretic force space factor, $\nabla|\mathbf{E}|^2$, for these two configurations decay faster than the existing configuration in figure 4.3 when moving away from the origin. At a

distance of $x = 30 \mu\text{m}$, the dielectrophoretic force space factor decays to $\sim 10^{14} \text{ V}^2/\text{m}^3$ for these two configurations. The existing configuration only decays to $\sim 10^{15} \text{ V}^2/\text{m}^3$. The dielectrophoretic force space factor is shown in figure 5.14b for top-bottom electrode configuration and figure 5.15b for left-right electrode configuration. The particle trajectories plot in figure 5.16 further support this theoretical prediction. For a particle at initial position of $(10 \mu\text{m}, 12.5 \mu\text{m})$, the particle can be transported $10 \mu\text{m}$ more in the existing configuration than the other two configurations.

Figure 5.14 The top-bottom electrode configuration that can generate moving electric field. (a) Geometry and boundary conditions. (b) Magnitude of gradient of electric field square, $\nabla|\mathbf{E}|^2$.

Figure 5.15 The left-right electrode configuration that can generate moving electric field. (a) Geometry and boundary conditions. (b) Magnitude of gradient of electric field square, $|\nabla E|^2$.

Figure 5.16 Analytical prediction of the particle trajectories for different electrode configurations, i.e. single electrode on top with planar electrode at bottom, left-right electrode configuration, and top-bottom electrode configuration.

5.11 Summary

There are a number of parameters involved in the model that govern the particle manipulation using a moving electric field. The sensitivity of the particle manipulation due to the variation in these parameters can be predicted numerically using the equation of motion model developed in the previous chapter. The simulation results provide an idea of conditions or parameter range where the particle manipulation is sensitive to. A quick study on a group of selected parameters indicates that the distance a particle travels in the x -directional is sensitive to the initial y -position when the particle is near to the origin. It is also sensitive to the particle polarization factor, but has a diminishing effect at higher polarization magnitude. Other factors like particle size, applied voltage, inter-electrode gap, and microchannel height are important also. On contrast, the particle travel distance is not sensitive to electrode width and the number of electrode energized. A comparison with two other configurations that can generate moving dielectrophoresis suggests that the existing configuration is better in transport particle to a longer distance. The experimental results agree qualitatively with the theoretical predictions.

Chapter 6

Device Fabrication and System

Setup

6.1 Introduction

The concept, model and sensitivity analysis have been discussed in the previous chapters. The generation of a moving electric field requires an experimental setup that can generate an electric field across an array of electrodes sequentially. The core of the setup is the microelectrodes chip where the actual particle manipulation occurs. An array of individually excitable microelectrodes has to be built, where those electrodes are linked to a programmable system. Some of the programmable systems that have been demonstrated include square electrode array on a CMOS chip [Manaresi *et al.* 2003], optoelectronic tweezers [Chiou *et al.* 2005], array of elongated electrodes [Vulto *et al.* 2006, Medoro *et al.* 2004, Shaikh and Ugaz 2006] and liquid droplet manipulator [Vykhoulal *et al.* 2001, Gascoyne *et al.* 2003, Gascoyne *et al.* 2004, Gascoyne and Vykhoulal 2004, Schwartz *et al.* 2004]. This chapter discusses the fabrication, setup of the experiment devices, and the preparation of the biological samples.

6.2 Microchip Fabrication

The microfluidic chip consists of 3 layers, i.e. a top plate, a spacer, and a bottom plate, as shown in figure 6.1. The top plate is a 500 μm thick glass pre-coated with a layer of indium tin oxide (ITO) on one of the planar surfaces. The ITO is a conductive material which serves as the common ground for the microfluidic chip. Since the ITO material is transparent, it allows the observation of particle activities during experiment. Two $\varnothing 1$ mm holes were drilled on the ITO glass to act as fluid inlet and outlet. The drilling was achieved by first gluing the ITO glass to a support plate using mounting adhesive (Crystalbond 509 Clear, Aremco). The holes were drilled using $\varnothing 1$ mm diamond drill bit (Diamond Solid Drills 4ED10, UKAM) mounted on a precision vertical mill machine (Model 5410, Sherline). After drilling, the epoxy was dissolved using acetone. Finally, the ITO glass was cleaned using acetone, isopropanol (IPA) and deionized water, in the stated order.

Figure 6.1 The assembly of the microfluidic chip consists of three layers, i.e. a top plate, a spacer and a bottom plate. All dimensions are in mm.

The spacer is a layer of 25 μm thick acrylic polymer (ARclear™ 8154, Adhesives Research, USA) which has adhesive on both surfaces. A slot of approximately 16 mm wide $\times$ 2 mm width was cut on the spacer using laser cutting machine (M-300, Universal Laser Systems, USA) to form the fluidic channel.

The bottom plate is a 500 μm thick glass wafer (Pyrex 7740, Corning, USA). Electrodes were deposited onto the glass wafer using a photolithographic lift-off process. The glass wafer was first cleaned by soaking in a piranha solution for approximately 15 min. It was then washed with deionized Millipore water, blown dry, and kept in an oven for 10 min at 110 °C. A layer of chromium was sputtered onto the glass wafer to act as an anti-reflection layer with a sputtering power of 100 W for 20 s. A layer of photoresist (AZ7220, Clariant) was spin coated onto the glass wafer with a spread speed of 500 rpm for 5 s and spin speed of 5000 rpm for 40 s. The glass wafer was then softbaked for 60 s at a temperature of 95 °C. The photoresist was exposed under UV light for 32 s, as shown in figure 6.2(a), and developed using AZ developer solution (50 % water + 50 % developer) for 30 s. The chromium and gold layers were sputtered onto the glass wafer using a sputtering power of 100 W, and for 30 s and 90 s, respectively, as shown in figure 6.2(b). Lift-off process was performed by soaking the glass wafer in acetone and placing it in an ultrasonic bath for approximately 30 min until the electrodes were fully developed. After the lift-off process, the glass wafer was cleaned with acetone, IPA and DI water, and blown dry. The chromium and gold layers were measured to have an average thickness of 330.0 Å (33.00 nm) using surface profiler (P-10, KLA Tencor). The chromium layer which is not covered with the gold layer was removed using chromium etchant (50% chromium etchant + 50% DI water). The final electrode is as illustrated in figure 6.2(c).

Figure 6.2 The microelectrode fabrication processes using the photolithography lift-off technique. (a) The photoresist is exposed to the UV light; (b) The deposition of the chromium and gold, after the exposed photoresist is cleaned; (c) The final electrode.

The microfluidic chip was designed to have a total of 60 independent electrodes with constant width and spacing. Three configurations of microfluidic chip were built, each having a 10 μm / 10 μm, 15 μm / 15 μm, and 20 μm / 20 μm width and spacing, respectively. For the 20 μm configuration, the width and spacing were measured to be 20.42 μm width and 19.45 μm spacing using the imaging software (analySIS, Soft Imaging System), CCD camera (DXC-950P, Sony) and microscope (BX60, Olympus).

The microfluidic chip was assembled by pasting the spacer between the bottom plate and the top plate. The slot on the spacer formed the fluidic channel, which allowed the passage of cells suspension. The channel height was measured to be approximately 24 μm, using the auto-focus capability of a microscope (Eclipse TE2000-S, Nikon) with a motorized stage. The ITO layer on the top plate was electrically connected to the ground line on the bottom plate using conductive epoxy (CW2400 Circuitworks, Chemtronics).

6.3 Device Setup

The microfluidic chip was physically attached to a custom-built printed circuit board (PCB) (Circuit Image, Singapore). The PCB has 62 independent via on the top surface and 31 via on the bottom surface. The microfluidic chip was electrically connected to the PCB using wire bonding technique (4534AD, Kulicke & Soffa). Each bonding pad on the microfluidic chip was connected to a corresponding via on the PCB. The ball bond was formed on the via, while the wedge bond was formed on the bonding pad. Both end of the PCB was connected to 31-way edge connectors (IBM PC AT, RS component), respectively. Thus, this setup provided 2 sets of 30 independently-controllable inputs, and 1 common ground. The assembly view of the microfluidic chip is shown in figure 6.3.

One of the edge connectors was connected to a series of 30 relays (HE3621A0510, Breed Electronics) through two 16-way color coded IDC ribbon cables. The relays were soldered in parallel on a PCB. The power lines of the relays were electrically connected to a function generator (33250A, Agilent) and a wideband amplifier (WA301, TTI). The control lines of the relays were electrically connected to a digital I/O card (PCI-6509, National Instruments) installed on the PCI slot of a personal computer. The switching of the relays was controlled by the LabView software (Labview version 8, National Instruments). The electrical connection for the relays is illustrated in figure 6.4.

Using this setup, each electrode on the microfluidic chip can be independently energized through the switching of relays controlled by the personal computer. For instance, an I/O icon can be established on the LabView software to link to certain physical electrical line on digital I/O card. When this icon is activated, the electrical line receives a digital ‘high’ (5V) signal. This digital ‘high’ signal in turn closes the circuit on the corresponding relays. This allows the current from the function generator to past through, which forms a close circuit on the corresponding electrode on the microfluidic chip.

A LabView program has been written to generate a sequential control signal with a user-specified activation period. By default, all the relays were open circuit. The program

sequentially triggered the relays into the close circuit condition. The result was an electric field that moved from one electrode to another on the microfluidic chip. This setup can achieve an activation period as fast as 10 ms.

The full experimental setup is illustrated in figure 6.5. The corresponding information flow among the equipment is shown in figure 6.6. An enlarged view of the microfluidic chip is shown in figure 6.7.

Figure 6.3 The top view and the cross-sectional view of the microfluidic chip, assembled to PCB. The schematics are not to scale.

Figure 6.4 Illustration of the electrical connections for the relays system. The control signal is connected to the National Instruments digital I/O card, while the power signal is connected to a function generator.

Figure 6.5 The picture of the physical experimental setup. Legend: (1) Control algorithm on the LabView platform, (2) Digital I/O card installed on a PC, (3) Voltage amplifier, (4) Function generator, (5) Connection block for the digital I/O card, (6) Multiplexer, (7) Camera system, (8) Microscope, (9) Microfluidic chip assembly.

Figure 6.6 The schematic of the building blocks and the information flow in the experimental setup.

Figure 6.7 The enlarged view of the microfluidic chip assembly. Legend: (9a) Ribbon cables, (9b) Connectors, (9c) Custom printed circuit board, (9d) Microfluidic chip.

6.4 Sample Preparation

This section describes the preparation of the viable yeast and non-viable yeast sample for the experiments. The initial yeast sample was suspended in cell culture media. This culture media was replaced with mannitol solution to obtain a desired medium conductivity.

6.4.1 Suspending Medium Preparation

The suspending medium was prepared by adding mannitol (M-9546 D-Mannitol, Sigma) into deionized water to obtain a final concentration of 250 mM mannitol. The conductivity of the suspending medium was adjusted by adding sodium chloride (Lot #9224, GCE Laboratory Chemical). The conductivity was measured using a conductivity meter (ION check65, Radiometer Analytical). Three bottles of suspending medium were prepared, with conductivity 58 $\mu\text{S}/\text{cm}$ (at 24 °C), 305 $\mu\text{S}/\text{cm}$ (at 20.6 °C) and 1002 $\mu\text{S}/\text{cm}$ (at 24 °C), respectively. The solution was kept in the freezer at approximately 4 °C for future usage.

6.4.2 Yeast Preparation

Yeast cells, *Saccharomyces cerevisiae* (strain ATTC 18824), were grown at 30 °C in a culture medium of pH 6.5. The dead yeast cells were obtained by heating the live yeast cells contained in a 6 ml test tube, which was submerged in a beaker of ~500 ml of water at a temperature of 75 °C for 15 min. The water was boiled using a magnetic stirrer hotplate (MR3001K, Heidolph).

0.25 ml of live yeast sample and 0.25 ml of dead yeast sample were drawn using pipette and mixed with 0.1 ml of 0.4% trypan blue stain (Invitrogen). The solutions were mixed thoroughly using a shaker, and allowed to stand for 5 min at room temperature. The trypan blue was used to check the viability of the yeast cells. A viable yeast cell would not be stained by the trypan dye, whereas a non-viable yeast cell would be stained by the trypan dye.

The conductivity of the live yeast sample, dead yeast sample and the mixed sample was changed by replacing the original culture medium with the suspending medium discussed in the previous section. The process involved centrifuging the samples, removing the culture medium and replacing with the suspending medium using pipette. The process was repeated three times, and the samples were mixed thoroughly before each centrifugation.

6.4.3 Sample Introduction

A small amount (0.05~0.1 ml) of particle suspension was drawn into the syringe from the test tube. The suspension was later carefully dropped into the inlet of the microfluidic chip. A suction cap (Silicone Flat Pad stock no. 227-3856, RS component) assembled on another syringe was used to draw the suspension into the channel by sucking from the outlet. Care was taken to ensure no gas bubble was formed inside the electrode area in the channel. The channel was cleaned by deionized water before testing on a different sample.

6.5 Summary

The microelectrodes were fabricated using the photolithographic lift-off processes on glass wafers. The microelectrodes glass chip was assembled to ITO glass using acrylic polymer as spacer. The assembled chip was connected to a function generator, relays, and a computer. A custom-written algorithm was used to generate sequential signal to energized electrodes. Viable yeast cells and non-viable yeast cells suspended in a different medium of conductivities were prepared. Non-viable yeast cells were stained blue for differentiation.

Chapter 7

Cell Transportation and Separation using mDEP

7.1 Introduction

In this chapter, the method presented in Chapter 3 is validated. Yeast cells were used as model particle. The experimental results are organized into particle transportation under a positive dielectrophoresis and negative dielectrophoresis, respectively, and particle fractionation.

7.2 Transportation of Yeast Cells

Yeast cells can be transported using moving electric field when the dielectrophoretic forces are the dominant force against the fluid drag, gravitational force, friction and Brownian motion. The experiments to demonstrate the transportation of yeast cells were conducted at several conditions. Two sets of conditions are discussed for each case of transportation under a positive dielectrophoresis and negative dielectrophoresis, respectively. For cells experiencing positive dielectrophoresis, the cells move by trailing the electric field. In a negative dielectrophoresis condition, the cells move by leading the electric field.

7.2.1 Cells Transportation under Positive Dielectrophoresis

The cell transportations under a positive dielectrophoresis were demonstrated for two operating conditions, namely at medium conductivity of 305 $\mu\text{S}/\text{cm}$ and applied electrical frequency of 2 MHz, and at medium conductivity of 21.5 $\mu\text{S}/\text{cm}$ and applied electrical frequency of 100 kHz. Using the two-shell sphere model [Huang *et al.* 1992, Talary *et al.* 1996], the polarization factor for viable yeast cells was calculated to be 0.52 and 0.14, respectively.

Figure 7.1 shows transportation of viable yeast cells at an applied electrical frequency of 2 MHz and in a medium conductivity of 305 $\mu\text{S}/\text{cm}$. The inter-electrode activation time was 2 s. The applied sinusoidal voltage was measured to be 9.3 Vpp. A total of six images were extracted from a video clip, using a two second interval. Under the moving electric field, the viable yeast cells were attracted to the electrode edge and trailing the moving electric field. Since the inter-electrode activation time was 2 s, the viable yeast cells were observed to move from one electrode to another electrode at a two second interval.

Figure 7.2 shows the DEP transportation of viable yeast cells at an applied electrical frequency of 100 kHz and in a medium conductivity of 21.5 $\mu\text{S}/\text{cm}$. The images were extracted at a time frame of 0.04 s for a total of 0.44 s. The detail motion of viable yeast cells can be properly studied at this finer time frame. The inter-electrode activation time was 2 s. The applied voltage was 10 Vpp. The viable yeast cells were aligned at the top-most electrode in figure 7.2 at $t = 0$ s. These series of frames show the motion of a group of yeast cells moving to the next electrode when the subsequent electrode was energized. The results indicate that viable yeast cells were capable of moving a distance of 10 μm in less than 0.5 s.

Figure 7.1 Transportation of viable yeast cells experiencing positive dielectrophoresis. The medium conductivity was $305 \mu\text{S}/\text{cm}$. The electrical potential and frequency were $9.3 \text{ V}_{\text{pp}}$ and 2 MHz , respectively. A 2 s inter-electrode activation time was used. The dark-field horizontal stripes were electrodes. The word "ON" represents the electrode that was energized at the corresponding time instance. Viable yeast cells can be seen to align with the energized electrode during motion.

Figure 7.2 Enlarged images showing the detailed motion of the viable yeast cells at 0.04 s time interval. The medium conductivity was $21.5 \mu\text{S}/\text{cm}$. The electrical potential and frequency were 10 V_{pp} and 100 kHz , respectively. A 2 s inter-electrode activation time was used. Viable yeast cells experienced positive dielectrophoresis under these operating conditions. The cells appeared smeared in the photos since the microscope field was focused on the electrode. The dark-field horizontal stripes were electrodes.

Figure 7.3 shows the cells trajectories from the sampling of three viable yeast cells at medium conductivity of $298 \mu\text{S/cm}$. The trajectories were recorded using image processing software (ImageJ) system as described in Section 4.5.1. The trajectories of these cells were traced at each 0.04 s time frame as recorded from the video. The plot shows that the viable yeast cells traveled $20 \mu\text{m}$ successively at a 2 s interval. This mode of transportation using positive dielectrophoresis produces a cell motion that has a sudden transition when moving from one electrode to another electrode. This mode of transportation is also sensitive to cells-to-electrode distance. A cell at a distance too far from the energized electrode may not be attracted to the electrode, and thus, not transported.

Figure 7.3 Trajectories of three viable yeast cells. The medium conductivity was $298 \mu\text{S/cm}$, the applied frequency was 2 MHz , the electrical voltage was 9.3 Vpp , and the inter-electrode activation time was 2 s . The numbers in the legend represent the measured yeast cell diameters. All the trajectories are overlapping.

7.2.2 Cells Transportation under Negative Dielectrophoresis

The cell transportations under a negative dielectrophoresis were also demonstrated at two different operating conditions, namely at medium conductivity of $305 \mu\text{S/cm}$ and applied electrical frequency of 2 MHz , and at medium conductivity of $966 \mu\text{S/cm}$ and applied electrical frequency of 100 kHz . Using the two-shell sphere model [Huang *et al.* 1992, Talary *et al.* 1996], the polarization factor for viable yeast cells was calculated to be -0.31 and -0.48 , respectively.

Figure 7.4 shows transportation of non-viable yeast cells at an applied electrical frequency of 2 MHz and in a medium conductivity of 305 $\mu\text{S}/\text{cm}$. The inter-electrode activation time was 2 s. The applied sinusoidal voltage was measured to be 9.3 Vpp. Again, a total of six images were extracted from the video, using a two second interval. Under the moving electric field, the non-viable yeast cells were repelled from the electrode edge and leading the moving electric field. Unlike the cell transportation under a positive dielectrophoresis which shows a clear alignment of cells along energized electrode at each two second interval, the non-viable yeast cells experiencing negative dielectrophoresis moved from one electrode to another electrode in a more subtle manner. The non-viable yeast cells always traveled in front of the energized electrodes dispersedly.

Figure 7.4 Transportation of non-viable yeast cells experiencing negative dielectrophoresis. The medium conductivity was 305 $\mu\text{S}/\text{cm}$. The electrical potential and frequency were 9.3 Vpp and 2 MHz, respectively. A 2 s inter-electrode activation time was used. The dark-field horizontal stripes were electrodes. The word “ON” represents the electrode that was energized at the corresponding time instance. Non-viable yeast cells can be seen to move in front of the energized electrode.

Figure 7.5 shows transportation of non-viable yeast cells at an applied electrical frequency of 100 kHz and in a medium conductivity of 966 $\mu\text{S}/\text{cm}$. The images were extracted at a time frame of 0.04 s to capture the detailed motion of this mode of transportation. The inter-electrode activation time was 2 s. The applied voltage was 10Vpp. Under the moving electric field, the non-viable yeast cells were repulsed away from the electrode edge and moved in front of the moving electric field. These frames show that non-viable yeast cells were capable of moving a distance of 10 μm in less than 0.2 s, at this operating condition.

Figure 7.5 Enlarged images showing the detailed motion of the non-viable yeast cells at 0.04 s time interval. The medium conductivity was 966 $\mu\text{S}/\text{cm}$. The electrical potential and frequency were 10 Vpp and 100 kHz, respectively. A 2 s inter-electrode activation time was used. Non-viable yeast cells experienced negative dielectrophoresis under these operating conditions. The cells appeared smeared in the photos since the microscope field was focused on the electrode. The dark-field horizontal stripes were electrodes.

Figure 7.6 shows the cells trajectories from the sampling of three non-viable yeast cells at medium conductivity 305 $\mu\text{S}/\text{cm}$. The trajectories were recorded using an image processing software (ImageJ) system as described in Section 4.5.1. The trajectories of these cells were traced at each 0.04 s time frame as recorded from the video. The plot shows that the non-viable yeast cells traveled rather linearly at each two second interval. There is no abrupt

motion in the transportation under the positive dielectrophoresis. Cells are always being pushed forward when the electrode was energized. Thus, this mode of transportation is not sensitive to the cell-to-electrode distance. Therefore, negative dielectrophoresis is a more efficient mode of transporting cells compared to positive dielectrophoresis.

Figure 7.6 Trajectories of three non-viable yeast cells. The medium conductivity was $305 \mu\text{S}/\text{cm}$. The electrical potential and frequency were $9.3 \text{ V}_{\text{pp}}$ and 2 MHz , respectively. The inter-electrode activation time was 2 s . The numbers in the legend represent the measured yeast cell diameters. Trajectories for $\varnothing 4.95 \mu\text{m}$ yeast cell and $\varnothing 4.13 \mu\text{m}$ yeast cell are overlapping.

7.3 Fractionation of Viable and Non-viable Yeasts

Fractionation of viable and non-viable yeast cells were demonstrated at an operating condition where viable yeast cells experience a positive dielectrophoresis and non-viable yeast cells experience a negative dielectrophoresis. This method was based on the knowledge that viable and non-viable yeast cells had different electrical properties, thus, exhibit different frequency response. Therefore, viable yeast cells and non-viable yeast cells can be separated based on dielectrophoretic affinity under a moving electric field, using the concept illustrated in Chapter 3, figure 3.4.

As shown in figure 7.7, three electrodes were energized simultaneously. At a two second interval, the last electrode was de-energized and leading electrode was energized to

create a moving electric field that traveled from the top to the bottom of the picture. The medium conductivity was 305 $\mu\text{S}/\text{cm}$. The applied voltage and frequency were 9.3 V_{pp} and 2 MHz, respectively. The inter-electrode activation time was 2 s. Under the positive dielectrophoretic force, the viable yeast cells were attracted to the energized electrodes. At the same time, the non-viable yeast cells were repelled from the energized electrodes due to the negative dielectrophoretic force. As the electric field traveled from top to the bottom plane, the viable yeast cells moved behind the electric field, whereas the non-viable yeast cells moved in front of the electric field. Since there were three electrodes energized, the viable yeast cells and non-viable yeast cells were spatially separated at three electrode distances. During the motion, there were viable yeast cells which moved in the opposite direction to join the rest of the viable yeast cells on the last electrode. Such transient motion was due to viable yeast cells under positive dielectrophoresis, and would be attracted to the electrode when the electric field approaching them.

Figure 7.8 shows the viable and non-viable yeast cells separation with a 50× optical zoom. It can be seen in figure 7.8(h) that there are two bands of cells, the cells on the first band (top of the picture) is yellow in color, and the cells on the second band (bottom of the picture) is dark blue in color. The cells in yellow were viable yeast cells, whereas the cells in dark blue were non-viable yeast cells. This coloring effect was due to the application of trypan blue stain to the specimen where the viable yeast cells were capable of removing the stain, whereas the non-viable yeast cells would absorb the stain and gained a dark blue color. The existence of two color bands serve to prove that there are indeed two groups of cells having different frequency response. The arrow points on figure 7.8(d), (e) and (f) show the motion of viable yeast cells moving towards the electric field when the electric field was approaching.

Figure 7.7 Fractionation of a mixture of viable and non-viable yeast cells at operating conditions of $305 \mu\text{S}/\text{cm}$, $9.3 \text{ V}_{\text{pp}}$, 2 s activation time, and 2 MHz . The non-viable yeast cells experiencing negative dielectrophoresis moved in front of the energized electrodes, whereas the viable yeast cells experiencing positive dielectrophoresis moved behind the energized electrodes. The viable and non-viable yeast cells were separated by three-electrode distance. The images were recorded at $20\times$ optical magnification. The dark-field horizontal stripes are electrodes.

Figure 7.8 Fractionation of a mixture of viable and non-viable yeast cells at the same operating conditions as above, at 50x optical magnification. The viable yeast cells were in yellow color, whereas the non-viable yeast cells were in dark blue. The dark-field horizontal stripes are electrodes. Viable yeast cells were trailing the electric fields, whereas non-viable yeast cells were leading the electric fields. These two bands of cells can be clearly seen in (h). Picture (d), (e), (f) show the intermediate process, where several viable yeast cells (white arrow) were moving up, when the electric fields were traveling down.

7.4 Cell Trajectories under Single-Energized Electrode

The moving dielectrophoresis method is achieved by sequentially energizing an array of single electrodes. Thus, the behavior of the cells under moving dielectrophoresis can be understood by studying the cells motion under a single electric pulse. The trajectory of the cells under such pulse reviews the net force acting on the cells and is useful in determining the cells separation strategy.

7.4.1 Methods

Table 7.1 shows the experimental conditions to study the cell trajectories under a single electric pulse. The viable yeast cells and non-viable yeast cells samples were prepared at three different medium conductivities of 21.5 $\mu\text{S/cm}$, 305 $\mu\text{S/cm}$ and 966 $\mu\text{S/cm}$, respectively. The microelectrodes width and inter-electrode gap were both 10 μm , respectively. The microchannel height was 25 μm .

Table 7.1 Experimental conditions to study the cells trajectory.

	Yeast Type	Medium Conductivity	Electrical Frequency	Electrical Voltage
Condition A	Viable	21.5 $\mu\text{S/cm}$	100 kHz	10 V _{pp}
Condition B	Dead	21.5 $\mu\text{S/cm}$	100 kHz	10 V _{pp}
Condition C	Viable	305 $\mu\text{S/cm}$	2 MHz	9.3 V _{pp}
Condition D	Dead	305 $\mu\text{S/cm}$	2 MHz	9.3 V _{pp}
Condition E	Viable	966 $\mu\text{S/cm}$	100 kHz	10 V _{pp}
Condition F	Dead	966 $\mu\text{S/cm}$	100 kHz	10 V _{pp}

An electrode was energized for 5 s. The duration of the time was chosen such that it was long enough for the cells motion to be fully stabilized, while short enough to prevent electrolysis. The motion of the cells was recorded through CCTV camera, using a commercial tape recorder. The analog video in the tape was converted to digital video using Movie Studio EzCoder 3.0 system. The final digital video had twenty-five frames of images per second. The cells trajectories recorded in the digital video were recorded using image processing software

(ImageJ). The “Manual Tracking” plug-in in the ImageJ software was used for the cells tracking. Five samples were taken for each condition from the pool of digital video.

7.4.2 Results

Figure 7.9 shows the trajectories of viable and non-viable yeast cells at different operating conditions under a single electric pulse. The values in the legend represent the measured cell diameters. The 21.5 $\mu\text{S}/\text{cm}$ live yeast and 305 $\mu\text{S}/\text{cm}$ live yeast were both experiencing positive dielectrophoresis. Those curves have negative slopes. All other cases were under negative dielectrophoresis. The slopes were positive in those cases. The origin was taken at the centre of the energized electrode. In the cases where the slope was negative, the cells moved toward the centre of the energized electrode. This behavior represented positive dielectrophoresis. In the cases where the slope was positive, the cells moved away from the centre of the energized electrode. This behavior represented negative dielectrophoresis.

Several characteristics can be observed from the trajectory results. In the case of positive dielectrophoresis, there was a slow velocity at the beginning, following by a fast velocity when it was near to the electrode. The cells under positive dielectrophoresis always moved to the centre of the electrodes.

In the case of negative dielectrophoresis, there was a fast initial speed, following by a slow speed when the cells were further away from the centre of the energized electrode.

Figure 7.9 Position of yeast plotted against time for different medium conductivities and cell viabilities. The numeric numbers on the legend represent the measured cell diameters. Different frequencies were used.

7.5 Summary

The experimental results indicate that particle transportation and particle separation can be achieved using the moving electric field. Both the viable yeast cells and non-viable yeast cells were transported using the method. Non-viable cells experiencing negative dielectrophoresis traveled in front of the moving electric field whereas viable yeast cells experiencing positive dielectrophoresis stayed behind the moving electric field. In general, cells can be transported irrespective whether they experienced positive dielectrophoresis or negative dielectrophoresis. However, the particle transportation under the negative dielectrophoresis was more efficient than the particle transportation under the positive dielectrophoresis. In addition, a mixture of viable and non-viable cells was fractionated based on their dielectrophoretic affinity.

Chapter 8

Conclusions

8.1 Research Contributions

This dissertation presented the concept of particle manipulation using a moving electric field. The dissertation had shown the necessary equipments to generate the moving electric field. This concept was demonstrated by using viable and non-viable yeast cells. The experimental results indicated that cells can be transported under both positive dielectrophoresis and negative dielectrophoresis. In addition, the test results also demonstrated that cells can be spatially separated and transported across the channel based on their dielectrophoretic affinity.

A mathematical model had been built to support the use of this particle manipulation technique. The model took into account of the dielectrophoretic force, fluid drag, gravitational force and buoyancy force. The model managed to capture the basic properties of particle manipulation using moving dielectrophoresis. The model is valid as long as the particle size is at least few microns and the electrical frequency is much larger than 100 kHz, such that the dielectrophoretic force dominates other forces.

The sensitivity analyses using the developed mathematical model highlighted the parameters that are important to the operation. Such simulation results provided an idea on the range or condition where the particle manipulation was sensitive to those parameters. The

parameters which were important include the initial y-position, particle polarization factor, particle size, applied voltage, inter-electrode gap, and microchannel height.

This technique offers several advantages. First, fluid flow is not required to achieve particle separation. It had been particularly difficult to integrate the macro-world to the micro-devices to pump the fluid into the microdevices. Such devices were generally large. Second, this technique can avoid electrolysis, since each electrode was switched on for several seconds during operation. In the conventional technique, the electrodes had to be switched on continuously for operation, which can induce electrolysis after only several seconds, depending on the voltage. Finally, the separation method depended on the real part of the Clausius-Mossotti factor only, unlike the traveling wave dielectrophoresis which induced both real and imaginary part of Clausius-Mossotti factor. This advantage allowed the particle separation to be based on the conventional dielectrophoretic affinity, reducing the number of relevant parameters.

This particle separation technique can be used for bio-diagnostics to separate and identify cells, bacteria, proteins or DNA.

Appendix A

Analytical Solutions for DEP Forces

A.1 Introduction

The boundary value problem shown in figure 4.2 was solved numerically using Matlab. The major disadvantage of the numerical method is that any subsequent attempt to compute the mathematical differential derivations from the calculated values generally yields poor results, if not impossible. For the electrostatic case, the potential Φ was calculated based on the boundary conditions using the finite element method. The electric field $\mathbf{E}$ is a derivation of potential Φ , where $\mathbf{E} = -\nabla\Phi$. Finally, the dielectrophoretic force is a derivation of electric field square, which is $\nabla|\mathbf{E}|^2$. An extremely fine mesh has been used in Chapter 4 to find the dielectrophoretic force; otherwise, a poor gradient was obtained. Although the analytical method does not have this constraint, the analytical solution is usually available for relatively simple conditions. Usually, simplifications have to be made in order to obtain an analytical solution. The solutions presented in the following paragraphs approximate the finite electrodes to infinite electrodes to derive the series solutions.

A.3 Potential

The Wiener-Hopf technique was used to obtain an analytical solution for the potential Φ . The technique utilizes the Fourier transform to change the initial xy -dependent problem to a y -dependent boundary value problem. The resulted boundary value problem was then solved, before inverting back to the xy -space. The crucial part of the Wiener-Hopf technique is that the solving process involves factorization of the functions such that one part is analytical in some lower half-plane while the other part is analytical in some upper half-plane.

The following Fourier transforms functions are defined for the potential Φ ,

$$U(k, y) = \int_{-\infty}^{\infty} \Phi(x, y) e^{ikx} dx \quad (\text{A.5})$$

$$U_+(k, y) = \int_0^{\infty} \Phi(x, y) e^{ikx} dx \quad (\text{A.6})$$

$$U_-(k, y) = \int_{-\infty}^0 \Phi(x, y) e^{ikx} dx \quad (\text{A.7})$$

so that $U(k, y) = U_+(k, y) + U_-(k, y)$. These transforms convert the xy -dependent problems into y -dependent problems.

The Fourier transforms are applied to the partial differential equation and the boundary conditions in equations A.1, A.2, A.3 and A.4. The Fourier transform is done by multiplying each term in the equations with e^{ikx} and integrating from $-\infty$ to ∞ along x . The governing equation now becomes an ordinary differential equation,

$$\frac{d^2 U}{dy^2} - k^2 U = 0, \quad 0 < y < h \quad (\text{A.8})$$

The Fourier transform of the boundary conditions are,

$$U(k, 0) = 0 \quad (\text{A.9})$$

$$U_{-}(k, h) = \frac{iV_0}{k} \quad (\text{A.10})$$

$$\frac{dU_{+}(k, h)}{dy} = 0 \quad (\text{A.11})$$

The equation A.8 has been widely studied. The solutions to equation A.8 can be a combination of *sine*, *cosine*, *sinh* or *cosh*. Since the boundary condition of $U(k, 0) = 0$ spans across the whole x -space, it seems appropriate to pick *sinh* function as the base solution. Therefore, the generic solution to the ordinary differential equation is

$$U(k, y) = A(k) \sinh(ky) \quad (\text{A.12})$$

Correspondingly, the first and second derivatives of equation A.12 are

$$U'(k, y) = kA(k) \cosh(ky) \quad (\text{A.13})$$

$$U''(k, y) = k^2 A(k) \sinh(ky) \quad (\text{A.14})$$

where $U' = \frac{dU}{dy}$ and $U'' = \frac{d^2U}{dy^2}$.

The solution in equation A.12 also has to satisfy the other two boundary conditions. At $y = h$, the value and the first derivative of equation A.12 have to conform to boundary conditions depicted by equation A.10 and A.11. The specific solution by substituting equation A.10 to equation A.12 is,

$$\begin{aligned} U(k, h) &= A(k) \sinh(kh) \\ U_{+}(k, h) + U_{-}(k, h) &= A(k) \sinh(kh) \\ U_{+}(k, h) + \frac{iV_0}{k} &= A(k) \sinh(kh) \end{aligned} \quad (\text{A.15})$$

Similarly, the specific solution by substituting equation A.11 to equation A.12 is,

$$\begin{aligned} U'(k, h) &= kA(k) \cosh(kh) \\ U'_{+}(k, h) + U'_{-}(k, h) &= kA(k) \cosh(kh) \\ U'_{-}(k, h) &= kA(k) \cosh(kh) \end{aligned} \quad (\text{A.16})$$

The unknowns k and $A(k)$ can be obtained by solving equation A.15 and A.16 simultaneously. By eliminating the $A(k)$ from the equations A.15 and A.16, the resulting equation can be factored as,

$$U_+(k, h) + \frac{iV_0}{k} = \frac{\sinh(kh)}{k \cosh(kh)} U'_-(k, h) \quad (\text{A.17})$$

The sinh and cosh functions can be represented as infinite product, which are, respectively,

$$\sinh(a) = a \prod_{p=1}^{\infty} \left(1 + \frac{a^2}{p^2 \pi^2} \right) \quad (\text{A.18})$$

$$\cosh(a) = \prod_{p=1}^{\infty} \left(1 + \frac{4a^2}{(2p-1)^2 \pi^2} \right) \quad (\text{A.19})$$

Therefore, the sinh and cosh terms in equation A.17 can be rewritten as,

$$\begin{aligned} K(kh) &= \frac{\sinh(kh)}{k \cosh(kh)} \\ &= \frac{kh \prod_{p=1}^{\infty} \left(1 + \frac{k^2 h^2}{p^2 \pi^2} \right)}{k \prod_{p=1}^{\infty} \left(1 + \frac{4k^2 h^2}{(2p-1)^2 \pi^2} \right)} \\ &= K_+(kh) \cdot hK_-(kh) \end{aligned} \quad (\text{A.20})$$

$$\text{where } K_+(kh) = K_-(-kh) = \prod_{p=1}^{\infty} \frac{1 - \frac{ikh}{p\pi}}{1 - \frac{2ikh}{(2p-1)\pi}}$$

The sinh and cosh terms in equation A.17 are replaced with equation A.20. After rearranging the terms, the resulting equation is,

$$\frac{U_+(k, h)}{K_+(kh)} + \frac{iV_0}{kK_+(kh)} - \frac{iV_0}{kK_+(0)} = hK_-(kh)U'_-(k, h) - \frac{iV_0}{kK_+(0)} \quad (\text{A.21})$$

Both sides of equation A.21 go to zero as $|k| \rightarrow \infty$. Invoking Liouville's theorem, both sides of equation A.21 equal to zero. Therefore, equation A.21 becomes two equations, i.e. the left

hand side of the equation equals zero, and the right hand side of the equation equals zero. Rearranging the results, the $U_+(k, h)$ and $U'_-(k, h)$ are, respectively,

$$U_+(k, h) = \frac{iV_0 K_+(kh)}{kK_+(0)} - \frac{iV_0}{k} \quad (\text{A.22})$$

$$\text{and, } U'_-(k, h) = \frac{iV_0}{khK_+(0)K_-(kh)} \quad (\text{A.23})$$

Substituting equation A.22 into equation A.15, the $A(k)$ term can be found as,

$$\begin{aligned} \frac{iV_0 K_+(kh)}{kK_+(0)} - \frac{iV_0}{k} + \frac{iV_0}{k} &= A(k) \sinh(kh) \\ A(k) &= \frac{iV_0 K_+(kh)}{kK_+(0) \sinh(kh)} \quad \text{for } x < 0 \end{aligned} \quad (\text{A.24})$$

Similarly, replacing equation A.23 into equation A.16, the $A(k)$ term can be found as,

$$\begin{aligned} \frac{iV_0}{khK_+(0)K_-(kh)} &= kA(k) \cosh(kh) \\ A(k) &= \frac{iV_0}{k^2 h K_+(0) K_-(kh) \cosh(kh)} \quad \text{for } x > 0 \end{aligned} \quad (\text{A.25})$$

The first solution is good for case $x < 0$, while the second solution is good for case $x > 0$.

Substituting the $A(k)$ depicted by equation A.24 and A.25 into the generic solution in equation A.12, the $U(k, y)$ is now,

$$U(k, y) = \frac{iV_0 K_+(kh) \sinh(ky)}{k \sinh(kh)} \quad \text{for } x < 0 \quad (\text{A.26})$$

$$\text{and, } U(k, y) = \frac{iV_0 \sinh(ky)}{k^2 h K_-(kh) \cosh(kh)} \quad \text{for } x > 0 \quad (\text{A.27})$$

where $K_+(0) = 1$.

The reverse Fourier transform for equation A.15 is defined as,

$$\Phi(x, y) = \frac{1}{2\pi} \int_{-\infty+\epsilon i}^{\infty+\epsilon i} U(k, y) e^{-ikx} dk \quad (\text{A.28})$$

Applying the reverse Fourier transform and substituting the $U(k, y)$ with results in equation A.26 and A.27, the potential $\Phi(x, y)$ becomes,

$$\Phi(x, y) = \frac{i}{2\pi} \int_{-\infty+\epsilon i}^{\infty+\epsilon i} \frac{V_0 K_+(kh) \sinh(ky)}{k \sinh(kh)} e^{-ikx} dk \quad \text{for } x < 0 \quad (\text{A.29})$$

$$\text{and, } \Phi(x, y) = \frac{i}{2\pi} \int_{-\infty+\epsilon i}^{\infty+\epsilon i} \frac{V_0 \sinh(ky)}{k^2 h K_-(kh) \cosh(kh)} e^{-ikx} dk \quad \text{for } x > 0 \quad (\text{A.30})$$

The remaining task is to find the integration along k . To find an analytical solution for integration in equation A.29 and A.30, the residue theorem is used, where,

$$\begin{aligned} \text{Res} \left[\frac{V_0 K_+(kh) \sinh(ky) e^{-ikx}}{k \sinh(kh)}; 0 \right] &= \frac{1}{1!} \lim_{k \rightarrow \infty} \frac{d}{dk} \left[\frac{(k-0)^2 V_0 K_+(kh) \sinh(ky) e^{-ikx}}{k \sinh(kh)} \right] \\ &= \lim_{k \rightarrow \infty} \frac{d}{dk} \left[\frac{k^2 V_0 \cdot \left(\frac{1-ikh/\pi}{1-2ikh/\pi} \right) (\dots) \cdot ky (1+(ky)^2/3!+(ky)^4/5!+\dots) \cdot (1-ikx-kx/2\dots)}{k \cdot kh (1+(kh)^2/3!+(kh)^4/5!+\dots)} \right] \\ &= \lim_{k \rightarrow \infty} \frac{d}{dk} \left[\frac{k V_0 y}{h} (1+\dots) \right] \\ &= \frac{V_0 y}{h} \end{aligned}$$

$$\begin{aligned} \text{and, } \text{Res} \left[\frac{V_0 K_+(kh) \sinh(ky) e^{-ikx}}{k \sinh(kh)}; k_n \right] &= \lim_{k \rightarrow k_n} \frac{(k-k_n) V_0 K_+(kh) \sinh(ky) e^{-ikx}}{k \sinh(kh)} \\ &= \lim_{k \rightarrow k_n} \frac{V_0 K_+(kh) \sinh(ky) e^{-ikx}}{\sinh(kh) + kh \cosh(kh)} \\ &= \lim_{k \rightarrow k_n} \frac{V_0 K_+(n\pi i) \sinh(n\pi y/h) e^{n\pi x/h}}{n\pi i \cosh(n\pi i)} \quad \left(k = \frac{n\pi i}{h} \right) \\ &= \frac{(-1)^n V_0}{n\pi} K_+(n\pi i) \sin(n\pi y/h) e^{n\pi x/h} \end{aligned}$$

$$\begin{aligned}
 \text{and, } \quad \text{Res} \left[\frac{V_0 \sinh(ky) e^{-ikx}}{k^2 h K_-(kh) \cosh(kh)}; k_n \right] &= \lim_{k \rightarrow k_n} \frac{(k - k_n) V_0 \sinh(ky) e^{-ikx}}{k^2 h K_-(kh) \cosh(kh)} \\
 &= \lim_{k \rightarrow k_n} \frac{V_0 \sinh(ky) e^{-ikx}}{2kh K_-(kh) \cosh(kh) + k^2 h K'_-(kh) \cosh(kh) + k^2 h^2 K_-(kh) \sinh(kh)} \\
 &= \lim_{k \rightarrow k_n} \frac{V_0 \sinh[-(2n-1)\pi i y / 2h] e^{-(2n-1)\pi x / 2h}}{-(2n-1)^2 \pi^2 K_-[-(2n-1)\pi i / 2] \sinh[-(2n-1)\pi i / 2]} \quad \left(k = -\frac{(2n-1)\pi i}{2h} \right) \\
 &= \frac{4V_0}{\pi^2} \frac{(-1)^{n+1}}{(2n-1)^2} \frac{\sin[(2n-1)\pi y / 2h]}{K_-[-(2n-1)\pi i / 2]} e^{-(2n-1)\pi x / 2h}
 \end{aligned}$$

These solutions are obtained by taking note that the poles at $\sinh(kh) = -i \sin(ikh) = 0$ is $k_n = \pm n\pi i / h$ and the poles at $\cosh(kh) = \cos(ikh) = 0$ is $k_n = \pm(2n-1)\pi i / h$. However, $k_n = n\pi i / h$ and $k_n = -(2n-1)\pi i / h$ would not yield analytical results, and, thus, dropped from the derivations. Hyperbolic identities $\sinh(a) = -i \sin(ia)$ and $\cosh(a) = \cos(ia)$ have been used to simplify the solutions.

Finally, by substituting the results from applying the residue theorem, the analytical solutions for potential $\Phi(x, y)$ are,

$$\Phi(x, y) = \frac{V_0 y}{h} + V_0 \sum_{n=1}^{\infty} \frac{(-1)^n}{n\pi} K_+(n\pi i) \sin(n\pi y / h) e^{n\pi x / h} \quad \text{for } x < 0 \quad (\text{A.31})$$

$$\text{and, } \quad \Phi(x, y) = \frac{4V_0}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{(2n-1)^2} \frac{\sin[(2n-1)\pi y / 2h]}{K_-[-(2n-1)\pi i / 2]} e^{-(2n-1)\pi x / 2h} \quad \text{for } x > 0 \quad (\text{A.32})$$

where the K_+ and K_- terms can be simplified to,

$$K_+(n\pi i) = \prod_{p=1}^{\infty} \frac{(p+n)(2p-1)}{p(2p-1+2n)} \quad (\text{A.33})$$

$$K_-[-(2n-1)\pi i / 2] = \prod_{p=1}^{\infty} \frac{(2p-1+2n)(2p-1)}{4p(p-1+n)} \quad (\text{A.34})$$

A.4 Electric Field

For the electroquasistatic condition, the electric field is the negative of the gradient of the potential,

$$\mathbf{E}(x, y) = -\nabla\Phi(x, y) \quad (\text{A.35})$$

where ∇ is the del operator. The electric field in a Cartesian coordinate is,

$$\mathbf{E}(x, y) = -\frac{\partial\Phi(x, y)}{\partial x} \hat{x} - \frac{\partial\Phi(x, y)}{\partial y} \hat{y} \quad (\text{A.36})$$

The electric fields for the potential in equation A.31 and A.32 are

$$\begin{aligned} \mathbf{E} = & -\hat{x} \frac{V_0}{h} \sum_{n=1}^{\infty} (-1)^n K_+(n\pi i) \sin(n\pi y/h) e^{n\pi x/h} \\ & - \hat{y} \frac{V_0}{h} \left\{ 1 + \sum_{n=1}^{\infty} (-1)^n K_+(n\pi i) \cos(n\pi y/h) e^{n\pi x/h} \right\} \end{aligned} \quad \text{for } x < 0 \quad (\text{A.37})$$

$$\begin{aligned} \text{and, } \mathbf{E} = & \hat{x} \frac{2V_0}{\pi h} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{(2n-1)} \frac{\sin[(2n-1)\pi y/2h]}{K_-[-(2n-1)\pi i/2]} e^{-(2n-1)\pi x/2h} \\ & - \hat{y} \frac{2V_0}{\pi h} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{(2n-1)} \frac{\cos[(2n-1)\pi y/2h]}{K_-[-(2n-1)\pi i/2]} e^{-(2n-1)\pi x/2h} \end{aligned} \quad \text{for } x > 0 \quad (\text{A.38})$$

A.5 DEP Force

The gradient of electric field square, $\nabla|\mathbf{E}|^2$, represents the component of the dielectrophoretic force that is particular to any specific electrode and microchannel geometry. The expanded form of the gradient of electric field square is

$$\nabla|\mathbf{E}|^2 = \hat{x} \frac{\partial}{\partial x} (E_x^2 + E_y^2) + \hat{y} \frac{\partial}{\partial y} (E_x^2 + E_y^2) \quad (\text{A.39})$$

The corresponding solutions for electric fields in equations A.37 and A.38 can be found by first finding the square of each electric field components E_x^2 and E_y^2 , and, later taking partial derivative of x and y . The E_x^2 and E_y^2 can be simplified by taking note that the multiplication of two identical single-summations becomes one double-summation. For the case $x < 0$, the square of the x - and y - component of the electric field are, respectively,

$$E_x^2 = \frac{V_0^2}{h^2} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} K_+(m\pi i) K_+(n\pi i) \sin\left(\frac{m\pi y}{h}\right) \sin\left(\frac{n\pi y}{h}\right) e^{\frac{(m+n)\pi x}{h}} \quad (\text{A.40})$$

$$\text{and, } E_y^2 = \frac{V_0^2}{h^2} \left\{ 1 + 2 \sum_{n=1}^{\infty} (-1)^n K_+(n\pi i) \cos\left(\frac{n\pi y}{h}\right) e^{\frac{n\pi x}{h}} + \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} K_+(m\pi i) K_+(n\pi i) \cos\left(\frac{m\pi y}{h}\right) \cos\left(\frac{n\pi y}{h}\right) e^{\frac{(m+n)\pi x}{h}} \right\} \quad (\text{A.41})$$

The x - and y -components of the gradient of the electric field square for the case $x < 0$ are, respectively,

$$\begin{aligned} \frac{\partial}{\partial x} (E_x^2 + E_y^2) &= \frac{2\pi V_0^2}{h^3} \sum_{n=1}^{\infty} (-1)^n n K_+(n\pi i) \cos\left(\frac{n\pi y}{h}\right) e^{\frac{n\pi x}{h}} \\ &+ \frac{\pi V_0^2}{h^3} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} (m+n) K_+(m\pi i) K_+(n\pi i) \cos\left[\frac{(n-m)\pi y}{h}\right] e^{\frac{(m+n)\pi x}{h}} \end{aligned} \quad (\text{A.42})$$

$$\begin{aligned} \text{and, } \frac{\partial}{\partial y} (E_x^2 + E_y^2) &= -\frac{2\pi V_0^2}{h^3} \sum_{n=1}^{\infty} (-1)^n n K_+(n\pi i) \sin\left(\frac{n\pi y}{h}\right) e^{\frac{n\pi x}{h}} \\ &- \frac{\pi V_0^2}{h^3} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} (n-m) K_+(m\pi i) K_+(n\pi i) \sin\left[\frac{(n-m)\pi y}{h}\right] e^{\frac{(m+n)\pi x}{h}} \end{aligned} \quad (\text{A.43})$$

These results are obtained by simplifying the derivations using the identities $\cos(a-b) = \cos a \cos b + \sin a \sin b$, and $\sin(a-b) = \sin a \cos b - \cos a \sin b$.

Similarly, the analytical solution can be derived for the case $x > 0$. The square of the x - and y - component of the electric field are, respectively,

$$E_x^2 = \frac{4V_0^2}{\pi^2 h^2} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n}}{(2m-1)(2n-1)} \frac{\sin\left[\frac{(2m-1)\pi y}{2h}\right] \sin\left[\frac{(2n-1)\pi y}{2h}\right]}{K_-[-\frac{1}{2}(2m-1)\pi i] K_-[-\frac{1}{2}(2n-1)\pi i]} e^{\frac{(m+n-1)\pi x}{h}} \quad (\text{A.44})$$

$$\text{and, } E_y^2 = \frac{4V_0^2}{\pi^2 h^2} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n}}{(2m-1)(2n-1)} \frac{\cos\left[\frac{(2m-1)\pi y}{2h}\right] \cos\left[\frac{(2n-1)\pi y}{2h}\right]}{K_-[-\frac{1}{2}(2m-1)\pi i] K_-[-\frac{1}{2}(2n-1)\pi i]} e^{\frac{(m+n-1)\pi x}{h}} \quad (\text{A.45})$$

The x - and y -components of the gradient of the electric field square for the case $x > 0$ are, respectively,

$$\frac{\partial}{\partial x}(E_x^2 + E_y^2) = -\frac{4V_0^2}{\pi h^3} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} (m+n-1)}{(2m-1)(2n-1)} \frac{\cos[(n-m)\pi y/h]}{K_{-m} K_{-n}} e^{-\frac{(m+n-1)\pi x}{h}} \quad (\text{A.46})$$

$$\text{and, } \frac{\partial}{\partial y}(E_x^2 + E_y^2) = -\frac{4V_0^2}{\pi h^3} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} (n-m)}{(2m-1)(2n-1)} \frac{\sin[(n-m)\pi y/h]}{K_{-m} K_{-n}} e^{-\frac{(m+n-1)\pi x}{h}} \quad (\text{A.47})$$

where $K_{-m} = K_-[-\frac{1}{2}(2m-1)\pi i]$ and $K_{-n} = K_-[-\frac{1}{2}(2n-1)\pi i]$.

A.6 Discussion

The analytical solutions for potential, electric field, and gradient of electric field square are plotted, as shown in Fig. A.2(a), Fig. A.3(a) and Fig. A.4(a). The parameters used in the solutions are $V_0 = 5$ and $h = 25 \mu\text{m}$. The Matlab was used to perform the summations. A total of 200 terms was used for the potential and electric field summations, while 100 terms was used for the gradient of electric field square summations.

The results are compared with the finite element simulation using FlexPDE, as shown in Fig. A.2(b), Fig. A.3(b) and Fig. A.4(b). The boundary conditions for the finite element simulation on left and right edge are changed to $\partial\Phi/\partial x = 0$, respectively, in place of the infinity conditions in Fig. A.1. To compensate for this edge effect from the change of the boundary conditions, the finite element simulation was ran for x -space equal to $\pm 100 \mu\text{m}$. However, only the $x = \pm 50 \text{ mm}$ space were plotted.

The analytical solutions and numerical solutions were matching. This conclusion was true for analytical solutions of potential and electric field. However, the analytical solutions for $\nabla|\mathbf{E}|^2$ are only valid for $x < 0$ and $x > 0$, the solutions do not converge at $x = 0$. A surface plot for $\nabla|\mathbf{E}|^2$ is shown in Fig. A.5, where the data points for $x = 0$ are not defined. Convergence tests at $x = 0^-$ and $x = 0^+$ are shown in Fig. A.6. The analytical solutions do not converge for both cases, and the results do not match for same number of terms too.

Figure A.2 The contour plots for potential, Φ . (a) Analytical result using Wiener-Hopf method, where $V_0 = 5$, $h = 25 \mu\text{m}$; (b) Numerical result using finite element method.

Figure A.3 The contour plots for the magnitude of electric field, $|\mathbf{E}|$. (a) Analytical result using Wiener-Hopf method, where $V_0 = 5$, $h = 25 \mu\text{m}$; (b) Numerical result using finite element method.

Figure A.4 The contour plots for the magnitude of gradient of electric field square, $|\nabla \mathbf{E}|^2$. (a) Analytical result using Wiener-Hopf method, where $V_0 = 5$, $h = 25 \mu\text{m}$. The data point at $x = 0$ have been omitted; (b) Numerical result using finite element method.

Figure A.5 The 3D surface plot for the magnitude of gradient of electric field square, $|\nabla \mathbf{E}|^2$ using the analytical Wiener-Hopf method. The data point at $x = 0$ have been omitted. The analytical solutions for $\nabla \mathbf{E}^2$ do not converge at $x = 0$.

Figure A.6 The convergence tests for analytical solutions of $|\nabla|E|^2|$ at $x = 0$. (a) Using Eq. A.42 and Eq. A.43 for case $x < 0$; (b) Using Eq. A.46 and Eq. A.47 for case $x > 0$.

Consider any of the double summation equations above. At $x = 0$, the exponential term of x equals to one. As the result, the double summations of the product of $K_{\pm(m)}$ and $K_{\pm(n)}$ diverge. On the other hand, at $x \neq 0$, the exponential term of x decreases and approach zero, as the m or n terms increases. In this case, the double summations of the product of $K_{\pm(m)}$ and $K_{\pm(n)}$ converge. This is the reason the $\nabla|E|^2$ equations do not converge at $x = 0$.

Appendix B

Wall and Particle Size Effects

B.1 Introduction

Dielectrophoresis has been demonstrated for the manipulation of bio-particles having characteristic length in micrometers. For instance, the separation of viable and non-viable yeast cells [Wang *et al.* 1993], the separation of mixtures of *Bacillus subtilis*, *Escherichia coli* and *Micrococcus luteus* bacteria [Markx *et al.* 1996], the separation of human leukemia cells [Becker *et al.* 1994] and the separation of human breast cancer cells from blood [Becker *et al.* 1995].

Effective dipole moment method [Jones 1995] has been commonly employed to predict the magnitude and direction of the dielectrophoretic forces in such devices. According to the effective dipole method, the dielectrophoretic force, $\mathbf{F}$, on a particle can be approximated as the dot product of the equivalent dipole induced on the particle, $\mathbf{p}$, with the gradient of the applied electric field, $\mathbf{E}$, [Jones 1995]

$$\mathbf{F} = (\mathbf{p} \cdot \nabla) \mathbf{E} \quad (\text{B.1})$$

where ∇ is the del operator. For a more rigorous approach, effective multipolar moment method [Jones and Washizu 1996] can be used to determine the dielectrophoretic force induced by the higher order electric field terms, where the multipolar dielectrophoretic force is,

$$\mathbf{F} = \frac{\mathbf{p}^{(n)} [\cdot]^n (\nabla)^n \mathbf{E}}{n!} \quad (\text{B.2})$$

where $\mathbf{p}^{(n)}$ is the effective multipolar moment of order n . It was found that it is generally sufficient to expand the multipolar terms till octopole, i.e. $n = 3$, in estimating the dielectrophoretic force. This formulation has been applied in predicting dielectrophoretic force in field cages [Schnelle *et al.* 1999, Schnelle *et al.* 2000, Voldman *et al.* 2001].

There are two major assumptions in using the effective moment method. Firstly, it is assumed that the characteristic length of interest is significantly larger than the particle size. Secondly, it is assumed that the equivalent dipole or multipoles induced on the particle under a non-uniform electric field is the same as the dipole or multipoles induced under a uniform electric field. In microfluidic devices where the microchannel thickness or width can be as small as 100 μm , and the nominal cell diameter in the range of 5~20 μm , these assumptions are often violated.

Parallel to the effective moment method, Maxwell stress tensor approach has been investigated for application in dielectrophoretic force. [Mognaschi and Savini 1983] has derived analytical solutions for a lossy sphere subject to the electric field of a point charge. Similar analytical solution is shown by [Akyuz *et al.* 1999] for a dielectric sphere under a point charge. A computer-aided approach was presented by [Goossens and Van 1988]. Other attempts to compare the Maxwell stress tensor with the effective dipole moment method include [Paul and Kaler 1990, Rosales *et al.* 2005, Benselama *et al.* 2004, Benselama *et al.* 2006]. However, [Wang *et al.* 1997] has later shown that the analytical solutions using Maxwell stress tensor [Mognaschi and Savini 1983] are equivalent to the multipolar moment solution [Jones and Washizu 1996]. It is important to note that these analytical solutions based on Maxwell stress tensor treat the sphere as an infinitesimal point in an unbounded space, as in the case of multipolar moment. [Wang *et al.* 1997] highlighted that the influence of the sphere finite-size on the applied field is not fully studied.

The present investigation shows that the particle size does in fact affect the applied field, and the dielectrophoretic force can even be generated under a uniform electric field. This work further extends the numerical approach to accommodate a three-dimensional model, as well as, the electric field in phasor form. The formulations can be used for time- or frequency-domain analysis of dielectrophoretic force for both conventional and traveling wave dielectrophoresis.

B.2 DEP force using Maxwell Stress Tensor method

The dielectrophoretic force is derived here using Maxwell stress tensor for both time-domain and frequency-domain analyses. Maxwell stress tensor has been derived in phasor form by [Wang *et al.* 1997]. Those expressions are expanded so that they can be represented as individual electrical field amplitude terms. For this reason, the first part of this section revisits the fundamental of the phasor notation. A clear understanding of these terms is crucial for the subsequent numerical modeling.

B.2.1 Phasor Electric Fields

A general sinusoidal electric field $\mathbf{E}(\mathbf{r}, t)$ having amplitude $\mathbf{E}_0(\mathbf{r})$, phase angle ϕ and angular frequency ω can be represented as,

$$\mathbf{E} = \mathbf{E}_0 \cos(\omega t + \phi) \quad (\text{B.3})$$

This electric field can be written in phasor form as [Jones and Washizu 1996, Wang *et al.* 1997],

$$\mathbf{E}(\mathbf{r}, t) = \text{Re}[\tilde{\mathbf{E}}_0(\mathbf{r})e^{i\omega t}] = \frac{1}{2}(\tilde{\mathbf{E}}(\mathbf{r}, t) + \tilde{\mathbf{E}}^*(\mathbf{r}, t)) \quad (\text{B.4})$$

where all the notations in bold represent vector, i is a complex unit, the asterisk $*$ represents complex conjugate, $\mathbf{r}$ is position vector, t is time, and $\text{Re}[\]$ represents real part of. Distinction is made between phasor electric field terms that depends on position only, i.e. $\tilde{\mathbf{E}}_0(\mathbf{r})$, and the

phasor electric field terms that depends on both position and time, i.e. $\tilde{\mathbf{E}}(\mathbf{r}, t)$, these terms are defined as,

$$\tilde{\mathbf{E}}_0(\mathbf{r}) = \mathbf{E}_{0R}(\mathbf{r}) + i\mathbf{E}_{0I}(\mathbf{r}) \quad (\text{B.5})$$

where $\mathbf{E}_{0R}(\mathbf{r}) = \mathbf{E}_0 \cos \phi$ and $\mathbf{E}_{0I}(\mathbf{r}) = \mathbf{E}_0 \sin \phi$;

$$\text{and, } \tilde{\mathbf{E}}(\mathbf{r}, t) = \mathbf{E}_R(\mathbf{r}, t) + i\mathbf{E}_I(\mathbf{r}, t) \quad (\text{B.6})$$

where $\mathbf{E}_R(\mathbf{r}, t) = \mathbf{E}_{0R} \cos \omega t - \mathbf{E}_{0I} \sin \omega t$ and $\mathbf{E}_I(\mathbf{r}, t) = \mathbf{E}_{0R} \sin \omega t - \mathbf{E}_{0I} \cos \omega t$.

The Gauss' law relates the electric field intensity to its source. For a linear isotropic medium, the Gauss' law in the differential form is [Haus and Melcher 1989],

$$\nabla \cdot \epsilon \mathbf{E} = \rho \quad (\text{B.7})$$

where $\epsilon = \epsilon_0 \epsilon_r$ is the medium permittivity obtained as the product of free space permittivity ϵ_0 and medium relative permittivity ϵ_r , and ρ is the free charge density.

The conservation of charge relates the current $\mathbf{J}$ to the rate of change of the free charge, given by,

$$\nabla \cdot \mathbf{J} = -\frac{\partial \rho}{\partial t} \quad (\text{B.8})$$

In a linear isotropic medium having conductivity σ , the current is related to the electric field by Ohm's law,

$$\mathbf{J} = \sigma \mathbf{E} \quad (\text{B.9})$$

Substituting equation (B.9) into equation (B.8), and combine with equation (B.7), the governing equation for the electric field in a lossy medium is obtained,

$$\nabla \cdot \sigma \mathbf{E} + \frac{\partial}{\partial t} (\nabla \cdot \epsilon \mathbf{E}) = 0 \quad (\text{B.10})$$

In a microfluidic device, the electric field wavelength is typically several orders of magnitude larger than the particle or electrode size. Thus, the electroquasistatic approximation can be

used. The Faraday's law is reduced to curl free electric field condition, and, the vector electric field can be defined as the gradient of a scalar potential [Haus and Melcher 1989],

$$\mathbf{E} = -\nabla\Phi \quad (\text{B.11})$$

Similar, the potential can be defined in phasor notation as,

$$\tilde{\mathbf{E}}_0 = -\nabla\tilde{\Phi}_0 \quad (\text{B.12})$$

where $\mathbf{E}_{0R} = -\nabla\Phi_{0R}$ and $\mathbf{E}_{0I} = -\nabla\Phi_{0I}$

In numerical modeling, the scalar potential Φ provides a direct approach in representing the physically applied voltage, as compared to the electric field.

B.2.2 Maxwell Stress Tensor in Phasor Electric Field

The sinusoidal electric field in equation (B.4) can be written in indicial form as,

$$E_j = \text{Re}[\tilde{E}_{j0} e^{i\omega t}] = \frac{1}{2}(\tilde{E}_j + \tilde{E}_j^*) \quad (\text{B.13})$$

Such index notation is convenient in providing information on the summation of the terms in an equation, especially for mathematical operation involving tensor. For this reason, the index notation would be used in the subsequent derivation involving Maxwell stress tensor. In this notation, the subscript j in equation (B.13) can be 1, 2, or 3, in which case it represents the electric field in x_1 , x_2 , or x_3 directions.

Similarly, the phasor electric field $\tilde{E}_j$ comprises of a real part E_{jR} and an imaginary part E_{jI} ,

$$\tilde{E}_j = E_{jR} + iE_{jI} \quad (\text{B.14})$$

where the lower case i represents $\sqrt{-1}$, and the subscripts “ R ” and “ I ” denote the real and the imaginary parts, respectively. Accordingly, the complex conjugate $\tilde{E}_j^*$ is defined as,

$$\tilde{E}_j^* = E_{jR} - iE_{jI} . \quad (\text{B.15})$$

The Maxwell stress tensor in its general form comprises of an electric field component and a magnetic field component. The subsequent derivations assume that the effects from the magnetic field can be ignored, which holds true when the electric field wavelength is several orders of magnitude larger than the dimensions of the dielectrophoretic electrodes [Wang *et al.* 1997]. However, it is important to note that there is application which utilizes magnetic field to separate magnetic particle, in what is termed magnetophoresis [Pamme and Manz 2004]. Such magnetophoresis applications would require Maxwell stress tensor with the magnetic component, which is not covered in this derivation. Assuming electric field effects only, the Maxwell stress tensor for a medium with permittivity ϵ can be written in index notation as [Grodzinsky],

$$T_{jk} = \epsilon \left(E_j E_k - \frac{1}{2} \delta_{jk} E_l E_l \right) \quad (\text{B.16})$$

where the subscripts j , k and l represent the summation index. In this summation convention, when an index appears twice in one term it is to be summed from 1 to 3. The Kronecker delta function δ_{jk} is given by,

$$\delta_{jk} = \begin{cases} 1, & j = k \\ 0, & j \neq k \end{cases} . \quad (\text{B.17})$$

Equation (B.16) can be expanded in phasor electric field form by replacing the electric field terms in equation (B.16) with the phasor terms in the right hand side of equation (B.13). The result is derived by [Wang *et al.* 1997] and shown to comprise of a time-averaged term $T_{jk}^{(\text{ave})}$ and an instantaneous term $T_{jk}^{(\text{ins})}$, respectively, which in indicial form are,

$$T_{jk}^{(\text{ave})} = \frac{1}{4} \epsilon \left(\tilde{E}_j \tilde{E}_k^* + \tilde{E}_j^* \tilde{E}_k - \delta_{jk} \tilde{E}_l \tilde{E}_l^* \right) \quad (\text{B.18})$$

$$T_{jk}^{(\text{ins})} = \frac{1}{4} \epsilon \left(\tilde{E}_j \tilde{E}_k + \tilde{E}_j^* \tilde{E}_k^* - \frac{1}{2} \delta_{jk} \left(\tilde{E}_l \tilde{E}_l + \tilde{E}_l^* \tilde{E}_l^* \right) \right) \quad (\text{B.19})$$

Using these results, we have further expanded equation (B.18) and (B.19) into its individual real and imaginary parts, by substituting the phasor electric field terms with equation (B.14)

and (B.15). The time-averaged term and instantaneous term for Maxwell stress tensor are now, respectively,

$$T_{jk}^{(\text{ave})} = \frac{1}{2} \epsilon \left[(E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR}) + (E_{jI} E_{kI} - \frac{1}{2} \delta_{jk} E_{II} E_{II}) \right] \quad (\text{B.20})$$

$$T_{jk}^{(\text{ins})} = \frac{1}{2} \epsilon \left[(E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR}) - (E_{jI} E_{kI} - \frac{1}{2} \delta_{jk} E_{II} E_{II}) \right] \quad (\text{B.21})$$

Note that both the resultant stress tensors comprise of real quantities only. All the imaginary quantities were cancelled during the mathematical expansion.

The total force on a volume is given by integrating the divergence of Maxwell stress tensors over the entire volume, which in indicial form is,

$$F_j^{(\text{vol})} = \int_V \frac{\partial}{\partial x_k} (T_{jk}^{(\text{ave})} + T_{jk}^{(\text{ins})}) dV \quad (\text{B.22})$$

Substituting equations (B.20) and (B.21) into equation (B.22), the time-averaged and instantaneous total force on a volume in individual electric field components are, respectively,

$$F_j^{(\text{v-ave})} = \frac{1}{2} \epsilon \int_V E_{jR} \frac{\partial E_{kR}}{\partial x_k} + E_{jI} \frac{\partial E_{kI}}{\partial x_k} dV \quad (\text{B.23})$$

$$F_j^{(\text{v-ins})} = \frac{1}{2} \epsilon \int_V E_{jR} \frac{\partial E_{kR}}{\partial x_k} - E_{jI} \frac{\partial E_{kI}}{\partial x_k} dV \quad (\text{B.24})$$

The total force on a volume can be represented as the total force on a closed-surface by applying the divergence theorem. The total force on a closed-surface is obtained by integrating the dot product of the Maxwell stress tensor and normal vector over the entire enclosed surface, which in indicial form is,

$$F_j^{(\text{surf})} = \oint_S (T_{jk}^{(\text{ave})} + T_{jk}^{(\text{ins})}) n_k dA \quad (\text{B.25})$$

Substituting equations (B.20) and (B.21) into equation (B.25), the time-averaged and instantaneous total force on a closed-surface in individual electric field components are, respectively,

$$F_j^{(\text{s-ave})} = \frac{1}{2} \epsilon \oint_S (E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR}) n_k + (E_{jI} E_{kI} - \frac{1}{2} \delta_{jk} E_{II} E_{II}) n_k dA \quad (\text{B.26})$$

$$F_j^{(s-ins)} = \frac{1}{2} \epsilon \int_V \left(E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR} \right) n_k - \left(E_{jI} E_{kI} - \frac{1}{2} \delta_{jk} E_{II} E_{II} \right) n_k dA \quad (B.27)$$

From the derived solutions, it can be observed that the time-averaged terms are the summation of the real expressions with the imaginary expressions, while the instantaneous terms are the subtraction of the real expressions with the imaginary expressions. As such, the full instantaneous terms consist of real expressions only.

B.2.3 DEP Force in Time Domain

In time dependent condition, the Gauss' law and the conservation of charge form the governing equations in solving for the electric potential. The governing equations are obtained by substituting equation (B.11) into equations (B.7), (B.8) and (B.9),

$$\nabla \cdot (\epsilon \nabla \Phi) = \rho \quad (B.28)$$

$$\nabla \cdot (\sigma \nabla \Phi) = -\frac{\partial \rho}{\partial t} \quad (B.29)$$

These two governing equations provides the potential Φ and charge density ρ as a function of space and time. Integrating this potential would provide the time-dependent Maxwell stress tensor, which is,

$$T_{jk} = T_{jk}^{(ave)} + T_{jk}^{(ins)} = \epsilon \left(E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR} \right) \quad (B.30)$$

The time-dependent dielectrophoretic forces follow as,

$$F_j^{(vol)} = F_j^{(v-ave)} + F_j^{(v-ins)} = \epsilon \int_V E_{jR} \frac{\partial E_{kR}}{\partial x_k} dV \quad (B.31)$$

$$F_j^{(surf)} = F_j^{(s-ave)} + F_j^{(s-ins)} = \epsilon \int_V \left(E_{jR} E_{kR} - \frac{1}{2} \delta_{jk} E_{IR} E_{IR} \right) n_k dA \quad (B.32)$$

B.2.4 DEP Force in Frequency Domain

Under the time-sinusoidal steady-state condition, equation (B.10) becomes,

$$\nabla \cdot \sigma \text{Re}[\tilde{\mathbf{E}}_0 e^{i\omega t}] + \frac{\partial}{\partial t} \nabla \cdot \epsilon \text{Re}[\tilde{\mathbf{E}}_0 e^{i\omega t}] = 0 \quad (\text{B.33})$$

Taking the time derivative and eliminating the common factor $e^{i\omega t}$,

$$\text{Re}[\nabla \cdot \sigma \tilde{\mathbf{E}}_0 + i\omega \nabla \cdot \epsilon \tilde{\mathbf{E}}_0] = 0 \quad (\text{B.34})$$

Expanding equation (B.34) into the real and the imaginary components by substituting the $\tilde{\mathbf{E}}_0$ term from equation (B.5),

$$\text{Re}[(\nabla \cdot \sigma \mathbf{E}_{0R} - \omega \nabla \cdot \epsilon \mathbf{E}_{0I}) + i(\nabla \cdot \sigma \mathbf{E}_{0I} + \omega \nabla \cdot \epsilon \mathbf{E}_{0R})] = 0 \quad (\text{B.35})$$

Substituting the electric field with the potential in equation (B.12), the real and the imaginary components becomes,

$$\text{Real part: } \nabla \cdot (\sigma \nabla \Phi_{0R}) - \omega \nabla \cdot (\epsilon \nabla \Phi_{0I}) = 0 \quad (\text{B.36})$$

$$\text{Imaginary part: } \nabla \cdot (\sigma \nabla \Phi_{0I}) + \omega \nabla \cdot (\epsilon \nabla \Phi_{0R}) = 0 \quad (\text{B.37})$$

These are the two governing equations for solving Φ_{0R} and Φ_{0I} . The frequency-dependent effects on the lossy medium is contained in these equations. Unlike the isotropic medium assumption used in [Green *et al.* 2002], these governing equations can handle a piece-wise system, e.g. a sphere in a fluidic medium.

The calculated potential Φ_{0R} and Φ_{0I} is then used to obtain the time-independent dielectrophoretic force. Substituting equation (B.6) into equation (B.20) and (B.21), and noting that $\langle \cos^2 \omega t \rangle = \langle \sin^2 \omega t \rangle = \frac{1}{2}$ and $\langle \sin \omega t \cos \omega t \rangle = 0$, the time-independent (*time-averaged*) Maxwell stress tensor is,

$$\langle T_{jk} \rangle = \langle T_{jk}^{(\text{ave})} \rangle + \langle T_{jk}^{(\text{ins})} \rangle \quad (\text{B.38})$$

where $\langle T_{jk}^{(\text{ave})} \rangle = \frac{1}{2} \epsilon [(E_{j0R} E_{k0R} - \frac{1}{2} \delta_{jk} E_{10R} E_{10R}) + (E_{j0I} E_{k0I} - \frac{1}{2} \delta_{jk} E_{10I} E_{10I})]$

$$\langle T_{jk}^{(\text{ins})} \rangle = 0$$

Consequently, the time-independent averaged dielectrophoretic force on a volume and a closed-surface are, respectively,

$$\langle F_j^{(v)} \rangle = \frac{1}{2} \epsilon \int_V E_{j0R} \frac{\partial E_{k0R}}{\partial x_k} + E_{j0I} \frac{\partial E_{k0I}}{\partial x_k} dV \quad (B.39)$$

$$\langle F_j^{(s)} \rangle = \frac{1}{2} \epsilon \int_V (E_{j0R} E_{k0R} - \frac{1}{2} \delta_{jk} E_{l0R} E_{l0R}) n_k + (E_{j0I} E_{k0I} - \frac{1}{2} \delta_{jk} E_{l0I} E_{l0I}) n_k dA \quad (B.40)$$

There are two schemes to obtain the dielectrophoretic force on a particle using the Maxwell stress tensor. In the first scheme, the dielectrophoretic force is obtained by taking the average of the closed-surface forces inside and outside the particle [Backstrom 2005]. The second scheme is to take the average of the volume forces inside the particle and the closed-surface forces outside the particle. In both schemes, the value ϵ has to be replaced with either the particle or the medium permittivity.

B.3 Effects of Bounding Wall and Particle Size

A case study is presented here to demonstrate the capability of the formulation. The Maxwell Stress Tensor is used to calculate the electrical force exerted on a sphere bounded by two parallel planar electrodes. This case is simulated as a three-dimensional model.

B.3.1 Geometry and Boundary Conditions

The problem space consists of a sphere with radius r , position at a distance a in a fluidic medium, as shown in figure B.1. The length of the problem space is arbitrarily defined as twice the height. A potential of 1 V is applied to the top edge, and a zero potential is maintained at the bottom edge. The sphere has a relative permittivity ϵ_p of 2.5, and conductivity σ_p of 2.8×10^{-4} S/m. The medium relative permittivity ϵ_m is 80 and medium conductivity σ_m is 1.0×10^{-4} S/m. The applied potential generated an electric field that induces a polarization charge on the sphere surface. This surface charge interacted with the electric field

giving rise to electrical forces acting on the dielectric rod. The problem is setup and solved using a partial differential equation solver (FlexPDE v5.0 Professional). Since the phase angle $\phi = 0$, the boundary conditions on the top plane are $\Phi_{0R} = \Phi_0 \cos \phi = 1$ V and $\Phi_{0I} = \Phi_0 \sin \phi = 0$ V. Similarly, the boundary conditions on the bottom plane are $\Phi_{0R} = \Phi_{0I} = 0$ V.

Figure B.1 Two-dimensional model used to simulate the electric field and dielectrophoretic force. The potential was applied on the left and right edges to generate an uniform electric field.

B.3.2 Simulation results and discussion

The electric field and electrostatic force acting on the sphere are obtained by solving equations B.36 and equation B.37 to find Φ_{0R} and Φ_{0I} with the boundary conditions in figure B.1. For the purpose of comparison, a simulation without the sphere is also performed. The result in figure B.2 shows the electric field distribution when the sphere is omitted. This condition corresponds to the effective dipole moment approach, where the dielectrophoretic force is obtained by taking the product of the equivalent dipole with the gradient of the electric field. Using this approach, the dielectrophoretic force is predicted to be zero throughout the problem space, since the gradient of a uniform electric field is zero. Similarly, the effective multipolar moment approach would predict zero force.

The electric field distribution when the sphere is included in the calculation of the electric field is shown in figure B.3. Due to the existence of the sphere, a non-uniform electric field is generated. The non-uniform electric field give rise to the dielectrophoretic force acting

on the sphere surface. The dielectrophoretic force for different value of sphere radius and position is shown in figure B.4, using the electrical frequency of 10 MHz. The result predicted that the force on the sphere increased significantly when the sphere is near to the wall. The force is small when the particle size is an order of magnitude smaller than the channel thickness and zero when the sphere is at the center of the channel. Figure B.5 shows the changes of the dielectrophoretic force at different frequencies.

Figure B.2 The electrical potential distribution without considering the sphere. Thus, a uniform electric field is generated between the electrode space. Since there is no gradient in the electric field, there is no dielectrophoretic force acting on sphere based on dipole moment method.

Figure B.3 The electrical potential distribution taking into account of the sphere. A non-uniform electric field is generated, which leads to a dielectrophoretic force.

Figure B.4 The dependence of dielectrophoretic force on the particle diameter to microchannel height, a/h , and the particle radius, r . The simulated electrical frequency is 10 MHz. The unit for electrostatic force is Newton (N) if the unit length is in meter (m).

Figure B.5 The dependence of dielectrophoretic force on the separation distance from wall, over different frequencies.

Appendix C

Matlab Scripts

The equation of motion is solved using stiff differential equation solver in Matlab. The codes are shown below. The description of the syntax used in the script below can be found from the Matlab help files or from the entry level Matlab books.

The first file is in input for all the parameters.

```
% Equation of motion for sphere under DEP force in microchannel.
% Kua Chin Hock @ August 2006

clear all;

% Microchannel configurations.
global L;
global h;
global Vo;
L = 100e-6;
h = 25e-6;           % channel height, m
Vo = 5;              % amplitude of voltage, V = 1/2Vpp

% Choose files to load.
global filename;      % filename for the gradEsqu
filename = 'gEsqu.dat';
filename = 'gEsqu_inv.dat'; % inverted electrode configuration.
filename = 'gEsqu_inv_6Vpp.dat'; % inverted electrode config + 5V
filename = 'gEsqu_inv_8Vpp.dat'; % inverted electrode config + 15V
filename = 'gEsqu_inv_20Vpp.dat'; % inverted electrode config + 20Vpp
filename = 'gEsqu_inv_H20.dat'; % inverted electrode config + height 20um
filename = 'gEsqu_inv_H30.dat'; % inverted electrode config + height 30um
filename = 'gEsqu_inv_H50.dat'; % inverted electrode config + height 50um
filename = 'gEsqu_inv_W15.dat'; % inverted electrode config + width 15um
filename = 'gEsqu_inv_W20.dat'; % inverted electrode config + width 20um
filename = 'gEsqu_inv_x2.dat'; % inverted electrode config + 2 electrodes
filename = 'gEsqu_inv_x3.dat'; % inverted electrode config + 3 electrodes
filename = 'gEsqu_b2electrode.dat'; % two electrodes at bottom plate
filename = 'gEsqu_top_bot.dat'; % top and bottom electrodes
```

```
% Initial conditions.
global x_loc;
global x_vel;
global y_loc;
global y_vel;
x_loc = 10e-6;    % initial position of x.
x_vel = 0;        % initial velocity in x-direction.
y_loc = 12.5e-6;  % initial position of y.
y_vel = 0;        % initial velocity in y-direction.

% Integration time span.
global tstart;
global tend;
tstart = 0;       % Start time.
tend = 5;         % End time.

% Parameters for particle and medium.
global a;
global V_particle;
global epso;
global epsm;
global CM;
global eta;
global rho_medium;
global rho_particle;
global g;
global m;
a = 2.5e-6;       % particle radius, m
V_particle = 4/3*pi*a^3; % particle volume, m^3
epso = 8.854e-12; % absolute permittivity, F/m
epsm = 78 * epso; % medium permittivity
CM = -0.4;        % Clausius-Mossotti factor
eta = 0.0089;     % fluid viscosity, kg/m/s
rho_medium = 1000; % medium density, kg m^-3
rho_particle = 1040; % particle density, kg m^-3 %1040 glass %1087 yeast
g = 9.81;         % gravitational constant, kg m s^-2
m = rho_particle * V_particle; % particle mass, kg
%Kx = 2;          % wall correction factor in x
%Ky = 5;          % wall correction factor in y

% Output the x- and y-positions and velocities.
global xplot;
global xvplot;
global yplot;
global yvplot;
global tplot;

% calling functions
global coefdragx;
global coefdragy;

if h/a == 2.5
    coefdragx = 'Kx_ha2.5.txt';
    coefdragy = 'Ky_ha2.5.txt';
elseif h/a == 3
    coefdragx = 'Kx_ha3.txt';
    coefdragy = 'Ky_ha3.txt';
elseif h/a == 4
    coefdragx = 'Kx_ha4.txt';
    coefdragy = 'Ky_ha4.txt';
```

```

elseif h/a == 5
    coefdragx = 'Kx_ha5.txt';
    coefdragy = 'Ky_ha5.txt';
elseif h/a == 10
    coefdragx = 'Kx_ha10.txt';
    coefdragy = 'Ky_ha10.txt';
else
    fprintf(['Please choose a h/a value of 2.5, 3, 4, 5, or 10.\n']);
    break
end

global coefKx;
global y_h1;
global h_a1;
global Kx;
global coefKy;
global y_h2;
global h_a2;
global Ky;
coef_ind;
%coef_drag;          %full model
%coefdragx = 'coefKx.txt';
%coefdragy = 'coefKy.txt';
%coefdragx = 'coefKx2.txt'; % Extrapolated Kx.
%coefdragy = 'coefKy2.txt'; % Extrapolated Ky.

xycomp; % Integration involving both x- and y-component.
%xcomp; % Integration considering x-component only.

```

The second files contain the solver. This file calls gradient of electric fields square information, and these files have to be generated from FlexPDE separately and keep in the same folder as the second file.

```

function xycomp

% Load files for gradient of DEP force square.
%-----
global filename;
gEsqu = load(filename); % file containing gradEsqu info
u = reshape(gEsqu(:,1),[51 51]); % x-coordinate
v = reshape(gEsqu(:,2),[51 51]); % y-coordinate
dxEsqu = reshape(gEsqu(:,3),[51 51]); % x-component of gradEsqu
dyEsqu = reshape(gEsqu(:,4),[51 51]); % y-component of gradEsqu

figure
subplot(1,2,1);
plot3(u,v,dxEsqu);
xlabel('X-axis, m'); ylabel('Y-axis, m'); zlabel('dx|E|^2, V^2/m^3')
subplot(1,2,2);
plot3(u,v,dyEsqu);
xlabel('X-axis, m'); ylabel('Y-axis, m'); zlabel('dy|E|^2, V^2/m^3')
%-----

global L;
global h;
global a;
global x_loc;
global x_vel;
global y_loc;
global y_vel;
global tstart;

```

```

global tend;

global xplot;
global xvplot;
global yplot;
global yvplot;
global tplot;

y0 = [x_loc; x_vel; y_loc; y_vel]; % initial condition
options = odeset('Events',@events);

% Accumulate the path of the ball in xplot,yplot.
xplot = [];
xvplot = [];
yplot = [];
yvplot = [];
tplot = [];
while 1
    tspan = [tstart; tend];
    [t,y,te,ye,ie] = ode23t(@f,tspan,y0,options);
    % Accumulate the path.
    xplot = [xplot; y(:,1)];
    xvplot = [xvplot; y(:,2)];
    yplot = [yplot; y(:,3)];
    yvplot = [yvplot; y(:,4)];
    tplot = [tplot; t];
    if isempty(ie) % Extend the interval.
        tstart = t(end);
        y0 = y(end,:);
        if t(end) == tend
            break;
        end
    elseif ie(end) == 1 % Particle hit the start of channel.
        fprintf(['Particle hit the start of channel.\n']);
        break;
    elseif ie(end) == 2 % Particle hit the end of channel.
        fprintf(['Particle hit the end of channel.\n']);
        break;
    elseif ie(end) == 3 % Particle the bottom channel.
        fprintf(['Particle hit the bottom channel.\n']);
        break;
    elseif ie(end) == 4 % Particle hit the top channel.
        fprintf(['Particle hit the top channel.\n']);
        break;
    end
end

figure
plot(xplot,yplot,'.');
title('Particle trajectory in x-y space');
xlabel('X-position, m'); xlim([0 L]);
ylabel('Y-position, m'); ylim([0 h]);

figure
subplot(2,2,1);
plot(tplot,xplot,'.');
title('Particle x-position over time');
xlabel('Time, s');
ylabel('X-directional position, m');

subplot(2,2,2);
plot(tplot,xvplot,'.');
title('Particle x-velocity over time');
xlabel('Time, s');
ylabel('X-directional velocity, m');

subplot(2,2,3);
plot(tplot,yplot,'.');
title('Particle y-position over time');
xlabel('Time, s');
ylabel('Y-directional position, m');

subplot(2,2,4);
plot(tplot,yvplot,'.');
```

```

title('Particle y-velocity over time');
xlabel('Time, s');
ylabel('Y-directional velocity, m');

% System of differential equations.
%-----
function dydt = f(t,y)

    global coefKx;
    global y_h1;
    global h_a1;
    global Kx;
    global coefKy;
    global y_h2;
    global h_a2;
    global Ky;

    global L;
    global h;
    global Vo;
    global a;
    global V_particle;
    global eps0;
    global epsm;
    global CM;
    global eta;
    global rho_medium;
    global rho_particle;
    global g;
    global m;

    gEsq_x = griddata(u,v,dxEsq,y(1),y(3),'nearest');
    Fdep_x = pi * epsm * a^3 * CM * gEsq_x; % Dielectrophoretic force
    A = Fdep_x / m;

    % K_x = griddata(y_h1,h_a1,Kx,y(3)/h,h/a,'cubic')
    K_x = interp1(y_h1,Kx,y(3)/h,'cubic')
    Fdrag_x = 6 * pi * eta * a * K_x; % xx = dx/dt% Fluid drag
    B = Fdrag_x / m;

    gEsq_y = griddata(u,v,dyEsq,y(1),y(3),'nearest');
    Fdep_y = pi * epsm * a^3 * CM * gEsq_y; % Dielectrophoretic force
    Fbuoy = rho_medium * V_particle * g; % Buoyancy force
    Fgrav = rho_particle * V_particle * g; % Gravitational force
    C = (Fdep_y + Fbuoy - Fgrav) / m;

    % K_y = griddata(y_h2,h_a2,Ky,y(3)/h,h/a,'cubic')
    K_y = interp1(y_h2,Ky,y(3)/h,'cubic')
    Fdrag_y = 6 * pi * eta * a * K_y; % yy = dy/dt% Fluid drag
    D = Fdrag_y / m;

    dydt = [ y(2) % y(1)' = y(2)
             A - B*y(2) % y(2)' = A - B*y(2)
             y(4) % y(3)' = y(4)
             C - D*y(4) ] % y(4)' = C - D*y(4)
    % y(1) = x
    % y(2) = dx/dt
    % y(3) = y
    % y(4) = dy/dt
end
%-----

function [value,isterminal,direction] = events(t,y)
global L;
global h;
global a;
value = [ y(1) % Particle hit the start of channel.
          L - y(1) % Particle hit the end of channel.
          y(3) - 1.1*a % Particle hit the bottom channel.
          0
          h - 1.1*a - y(3) ]; % Particle hit the top channel.
% h
isterminal = [1; 1; 1; 1];

```



```
    direction = [-1; -1; -1; -1];  
end  
  
end
```

Appendix D

Electrohydrodynamics

D.1 Introduction

The applied electric fields in the microchannel would interact with the fluid medium, resulting in fluid flows. There are two fluid flow effects, namely AC electroosmosis and electrothermal effect. These two effects are collectively referred as electrohydrodynamic effect. Such effects had been studied by [Ramos *et al.* 1998, Green *et al.* 2000, Green *et al.* 2000, Green *et al.* 2000, 2001, Castellanos *et al.* 2003, Chen and Du 2006]. Some researchers had used these fluid flow to couple with dielectrophoresis to manipulate particle, like [Zhou *et al.* 2005]. The sections below discuss these effects for the electrode structure used in this study.

D.2 AC Electroosmosis

The AC electroosmosis is generated from the interaction of the electrical double layers on the electrode surfaces with the electric fields. As shown in figure D.1, electrical double layers are formed on the surface of the top and bottom electrodes. The anions and cations experience electrical forces due to the action of the electric fields. These anions and cations would move, and in turn, causes the bulk fluid flow. The direction of the bulk fluid flow is shown in figure D.2.

Fig. D.1 The mechanism of AC electroosmosis. Electrical double layers are formed at the surface of the electrodes. These ions interact with the electric fields, which results in electrical forces acting on the ions. These forces remain in the same direction when the applied voltage changes polarity. (a) First electrical cycle. (b) Second electrical cycle.

Fig. D.2 Illustration of fluid flow due to AC electroosmosis. The segmented arrows represent the motions of the charged ions. These ions move and cause the bulk fluid flow. The dotted-continuous arrows represent the bulk fluid flow.

The experimental results indicate that this fluid flow is strong at electrical frequency below 50 kHz, for medium conductivity of 10 ~ 1000 $\mu\text{S}/\text{cm}$. For instance, the fluid flow was observed to be extending 80 μm from the origin under experimental conditions of frequency of

10 kHz and medium conductivity of 298 $\mu\text{S}/\text{cm}$. The microchannel height was 25 μm . At these frequencies, the body force exerted by the AC electroosmotic fluid flow is typically stronger than the dielectrophoretic force. At frequency less than 10 kHz, the electrodes would generally experience electrolysis.

D.3 Electrothermal Effect

The electrothermal effect is generated due to the temperature gradient in the microchannel. There are two sources of heat, namely the diffusion heating and the Joule heating. The diffusion heating is caused by the heating of the microdevices' substrates by the external heat sources, like microscope lamp. The Joule heating is the conductive heating from the applied voltage. It has been known that the diffusion heating are dominant compared to the Joule heating, as shown by [Green *et al.* 2000].

Assuming that an external light source causes the electrodes to be heated 0.1 K more than the ambient temperature, the temperature profile in the microchannel for the devices used in this study is shown in figure D.3. This temperature profile results in a medium conductivity gradient and permittivity gradient. These gradients give rise to a fluid body force. This body force is also dependent on the electric fields in the system. Figure D.4 shows the direction of the fluid flow at an applied electrical frequency of 2 MHz. The magnitude of the fluid flow is around 2 $\mu\text{m}/\text{s}$.

Fig. D.3 Predicted temperature profile in the microchannel due to the ambient heating and the Joule heating. The array of top electrodes are heated to 300.1 K, while the ambient temperature at other microchannel interface are assumed to be 300.0 K. The unit is Kelvin.

Fig. D.4 Simulated fluid flow velocity. The magnitude of the fluid flow is largest around the energized electrode. The direction of the fluid flow may change depending on the applied electrical frequency. The unit is $\mu\text{m s}^{-1}$. The applied frequency is 2 MHz.

The experimental results indicate that the magnitude of fluid flow due to the electrothermal effect has a much shorter effective range compared to the AC electroosmosis. The effective fluid flow range was observed to be around 20 μm . This observation is consistent with the theoretical prediction, which shows that the fluid flow circulate around region less than 20 μm in the x -direction, as in figure D.4.

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