Dielectrophoretic microactuation for microparticles manipulation and separation

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2006

Chen, D. (2006). Dielectrophoretic microactuation for microparticles manipulation and separation. Doctoral thesis, Nanyang Technological University, Singapore.

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<https://doi.org/10.32657/10356/6539>

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DIELECTROPHORETIC MICROACTUATION FOR MICROPARTICLES MANIPULATION AND SEPARATION

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2006

Dielectrophoretic Microactuation for Microparticles Manipulation and Separation

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A dissertation submitted to Nanyang Technological University
in fulfillment of the requirements for the degree of Doctor of Philosophy

2006

Abstract

One of the development trends of biomedical devices is towards miniaturization, which aims to integrate the conventional biological processes into automatic operations in a single microchip. Those emerging microfluidic systems such as micro total analysis systems (μ -TAS) or labs-on-a-chip (LOC) are representatives of such a trend. The first step in the operation of these bio-microsystems consists of manipulating and separating the analytes (microparticles) of interest from the background matrix and positioning them into selected locations for subsequent analysis. The use of ac (alternating current) electric fields has been demonstrated as a promising and effective method for this purpose. When subjected to a spatially nonuniform electric field, the microparticles (with different permittivities from the medium) will migrate toward the regions of high field strength or low field strength, depending on the relative polarizabilities of the particles and the surrounding medium. This phenomenon, known as dielectrophoresis (DEP), finds numerous applications in processes involving separation, trapping, selective sorting, and manipulation of individual bioparticles. The main purpose of this work is to study the fundamental phenomena of DEP, and develop DEP-based microfabricated systems for bioparticle separation and manipulation.

To meet the requirements for detailed quantitative modeling and analysis of microscale DEP-based systems, a modeling methodology and computational tool based on a meshless method, i.e. least-square finite difference method (LSFD), was developed and implemented to model the DEP systems and simulate the microparticles' behavior. The feasibility of the developed computational model was demonstrated through the study on a

typical case – the interdigitated electrode system. The LSFD-based modeling environment is well suited for solving DEP problems for the computational ease of free grid/mesh distribution in the computational domain where extremely high gradient of electric field exists, e.g. at edges of the electrodes.

As a straightforward way to study the particle behavior in the nonuniform ac electric fields, planar microelectrode systems and micron-sized polystyrene beads were used in the first part of the study. For high medium conductivity, only negative DEP occurred for polystyrene particles. In general, DEP was found to be frequency dependent. It was found that the latex beads experienced negative DEP at frequencies higher than 10 kHz and experienced positive DEP at lower frequencies. Negative DEP was found to be advantageous over p-DEP of avoiding possible problems associated with surface adhering, interparticle aggregation, and electrical damage. Dielectrophoretic passive levitation height of the latex beads was experimentally measured using a consecutively focusing method and found to agree well with numerical results obtained.

The planar electrodes have a few limitations such as particle escape problems, weak holding force, etc. Hence, the second part of this study focused on the design and development of a 3-D microelectrode system for microparticle manipulation, which presents advantages over the planar electrodes. The 3-D paired microelectrode array was proposed by aligning two layers of microelectrode structure face to face on the top and bottom sides of the microchannel. Such a configuration of the electrode system shows the advantage of strong lateral holding force generated between the dielectrophoretic gates. Depending on the relative strength of the DEP force and hydrodynamic force

acting on the microparticles, the particles can either penetrate the gates or be deflected by the gates. The electrode system was optimized by investigating the factors that influence the performance of the device. Those factors included electrode width, channel height, aligning angle, particle size, etc. The paired microelectrode system was fabricated using the microfabrication techniques. A packaging scheme was proposed, which met the requirements of electrical, optical, and fluidic connection and introduction to the chips.

Finally, the performance of the microfabricated paired electrode array is experimentally validated. Polystyrene particles of various sizes were investigated in the tests. The holding capacity (indicated by threshold velocities) was found to be different for microparticles of different electrical properties or physical sizes. Particle accumulation and separation were successfully achieved with the electrode system. The developed paired microelectrode system has potential applications to manipulate microparticles of different sizes or different dielectric properties.

Acknowledgments

Many people have helped me make my way in the work in the past years. First of all, I would like to sincerely express my gratitude to my supervisor, A/P Du Hejun, for his invaluable supervision and great support in the course of this research. His broad vision has led me to the world of BioMEMS, which I think is the magic part of MEMS. In addition, A/P Du has been always considerate and open-minded, allowing me to make my own mistakes.

My gratitude is then given to Dr. Li Weihua for his valuable suggestions and great help all along. Dr. Li shared his ideas and found-outs, making me save many efforts in the early stage of the project. No doubt, Dr. Li is really a good teacher and helpful friend.

I would like to appreciate the help and kindness from A/P Miao Jianmin in MicroMachines Center. I would also like to thank A/P Thomas Gong Haiqing in BioMEMS Center, for his support in the experiments.

My Thanks also go to those friends in the Centre for Mechanics of Micro-systems (CMMS) and MicroMachines Center (MMC) who pushed me in this or that way. Many thanks go to the staffs in these centers for their great help and excellent service.

Finally, the love, encouragement, and support from my family, especially from my wife are crucial for me to complete this work. To them this thesis is gratefully dedicated.

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CHAPTER 0 Introduction

0.0 Background

One of the trends in developing biomedical devices is towards miniaturization, which aims to make devices into small size (at microscale or nanoscale) by scaling down the system. It has been demonstrated that microscale devices are greatly superior to the conventional ones. They offer the advantages of compact size, smaller sample requirement, disposability, quick analysis time, greater data precision, low cost, etc. Another obvious characteristic of microscale systems is their high surface-area-to-volume (SAV) ratio, which enhances processes that are dominated by surfaces, such as heat transfer. This allows the micro-chambers for polymerase chain reactions (PCR) to ramp temperatures very quickly [1]. A very large SAV ratio makes capillary electrophoresis (CE) more efficient in microchannels by removing excess heat more rapidly. Emerging biomicrosystems are micro total analysis systems (μ -TAS), sometimes called labs-on-a-chip (LOC) [2]. Such systems enable the integration of several different experimental tasks into one process on a single instrumental platform (chip) in combination with automated data analysis.

In general, biomedical microdevices involve working with aqueous solutions flowing through microchannels. Such channels require a small amount of samples and reagents for each process. From the microfluidic point of view, the decrease on the overall size of the system results in laminar flows in microchannels. One consequence of laminar flow is that two or more streams flowing in contact with each other will not mix

except by diffusion [3]. The diffusion between laminar streams in a microdevice has been used for performing assays and sorting particles by size [4, 5]. In addition, the parabolic velocity profile has been widely used in velocity-based applications such as Field-Flow Fractionation (FFF) [6]. The field in microfluidics is rapidly growing and expanding. Currently, there exist no industry standards for microfluidic systems, and the field remains open for exploration.

The detection, analysis, and sample preparation of bioparticles such as cells are of vital importance for further treatments and processing. Such processes are major components towards lab-on-a-chip and microfluidic systems, involving the steps to manipulate fluids and particles. To approach this, a number of methods have been used. These methods usually utilize the differences (e.g. electric, dielectric and optical differences) between the target specimens and their suspending medium. Electric field-based techniques have been widely used for this purpose. Electrophoresis (EP) involves the movements of charged particles in electric fields, and has been successfully used to separate charged bodies from a mixture. However, this is not the case for the uncharged bodies, i.e. bodies with no net charges. To manipulate uncharged bodies (e.g. cells, bacteria, polystyrene beads), other promising techniques involving spatially nonuniform ac (alternating current) electric fields have been used. Among these are conventional dielectrophoresis (cDEP), traveling wave dielectrophoresis (twDEP). Dielectrophoresis refers to the movements of uncharged but polarizable particles in nonuniform ac electric fields [7]. Compared to other available methods, DEP is particularly well suited for manipulation of minute particles in microfluidics because (i)

DEP systems exhibit strong dependencies on a wide range of frequencies. The frequency-dependency arises from the mechanisms of dipole relaxation and interfacial (Maxwell-Wagner) dielectric dispersions that depend on the structure and properties of the particles [8-10]. (ii) From the DEP theory, the requirement to create the DEP force is to create a field gradient. In fact, it can be much easier to meet the requirement for an inhomogeneous (nonuniform) electric field than the requirement for a homogeneous (uniform) one. (iii) Also, DEP systems generally consist of electrode arrays and sample chambers which are amenable to fabricate with microfabrication techniques. (iv) When compared with electrophoresis, where charged particles are manipulated in a dissipative medium [11], DEP has the advantage that the AC fields can avoid problems such as electrode polarization effects and electrolysis at electrodes [12], since no electrophoretic movement occurs in such a field. Even more importantly, the use of AC fields reduces membrane charging of biological cells [13].

Dielectrophoresis has great potentials to impact bioscience in the way of sample preparation, diagnosis, and bioparticle manipulation, when it is combined with the techniques of microfluidics and MEMS (microelectromechanical systems). The heart of a dielectrophoretic device is a channel equipped with an array of electrodes capable of generating a high gradient ac electric field that drives the analytes to the regions of high field strength (positive DEP) or low field strength (negative DEP) depending on whether the analytes are more or less polarizable than the suspending medium. Available micromachining techniques and miniaturization technology enable such an integration of microelectrodes and channels into microfluidic devices. The development of DEP-based

microsystems for effective particle separation and manipulation has attracted great interest in the recent years.

0.0 Objectives and Scope

The previous discussion implies that our ultimate goal is to design and develop a lab-on-a-chip using dielectrophoretic effect to manipulate and separate biological micro-objects such as cells. In more details, the objective of our research is to study the fundamentals of DEP with proper methodologies and develop an integrated device or system capable of automatically manipulating or separating microparticles from a particle/cell/micro-object mixture, and then to analyze and obtain their dielectric properties for detection and diagnosis in bioscience. The device should present the excellent performance and superior features: easy and inexpensive to make, simple to use, small sample volume requirement, quick implementation time, and high reproducibility. This approach will allow us to advance extensive work on analysis, design, fabrication, modeling, and testing of DEP biochip for characterization, manipulation, fractionation, isolation, trapping, and selection of microparticles. The tasks include:

- ◆ Theoretical analysis and numerical simulation of electric field and DEP force in the microelectrode structures.
- ◆ Development of governing equations and models for the behavior of microparticles in nonuniform electric fields.
- ◆ Design and optimization of microelectrode structures suitable for bioparticle manipulation and separation.

- ◆ Microfabrication of the DEP-based microfluidic system containing the microelectrode structures, microchannels etc.
- ◆ Packaging of the microfluidic device, which meets all the requirements for fluid introduction and discharge, electrical connection, and optical observation.
- ◆ Observation and testing of particle behavior. Validation of the device performance by comparing experimental results with numerical results.
- ◆ Characterization and measurement of dielectric properties of microparticles with suitable instrumentations and methodology.

0.0 Outline of the thesis

The primary motivation of this thesis is to develop methodology for modeling and designing DEP-based microfluidic systems and to implement these methods in accomplishing the microsystem containing microfabricated electrode array to demonstrate DEP's suitability in handling various biological particles. The rest of the thesis is organized as follows.

In chapter two, techniques for particle manipulation are summarized. This leads to the review of the art of dielectrophoresis. Conventional DEP (cDEP), traveling DEP (twDEP) and electrorotation are covered. Dielectric modeling of particles and cells used to simplify the analysis by researchers is introduced.

Chapter three introduces a modeling method for the DEP systems based on a novel meshless finite difference scheme. The method has many advantages such as greater applicability and lower computational complexity. The results turn out to be good and

convincing. Although only a specific case, interdigitated electrode array, is investigated in this work, the schemes and methods can be extended to model more complex DEP systems.

In Chapter four, the planar electrode arrays used for DEP applications are demonstrated. Particle behavior in the planar electrode arrays is experimentally investigated. The experimental results can be used to validate the aforementioned modeling environments. In addition, much insight into the phenomena of DEP is gained through the fundamental studies in planar electrode systems.

Chapter five presents the design and optimization of a 3-D microelectrode system – paired electrode array for particle manipulation and separation. The 3-D electrode overcomes some of the limitations existing in the planar structures. The work concentrates on investigating the factors that influence the performance of the device. Finally, a microfluidic system containing the 3-D microelectrode array is proposed for manipulation of microparticles.

Chapter six describes the process flow for fabrication of the 3-D electrode system. The major steps include patterning of the arrays onto two single wafers and bonding of the chips. Various types of arrays are fabricated with some of the parameters varied. A packaging scheme is proposed, which meets the requirements of electrical, optical, and fluidic connection and introduction to the chips.

The performance of the microfabricated paired electrode array is experimentally validated and demonstrated in Chapter seven. Polystyrene particles differing in diameter are investigated in the tests. The holding capacity (indicated with threshold velocities) is

different for microparticles having different electrical properties or physical sizes. Particle accumulation and separation are successfully achieved with the electrode system.

Finally, Chapter eight finishes with conclusions on the thesis as well as directions for future work.

CHAPTER 0 Literature Review

Having chosen DEP as the approach to manipulate microparticles, a review on the state of the art of DEP is presented in this chapter. There are a number of methods for particle manipulation based on different mechanics. DEP applications include particle separation, characterization, fractionation, sorting and trapping etc. These applications are discussed in details. Dielectric modeling of particles is frequently used in analysis to simplify the problem and this topic will also be reviewed and discussed in this chapter.

0.0 Micron-sized particle manipulation

Several methods have been proposed for manipulation of microparticles especially biological cells. These methods can be classified as non-contact manipulation such as laser trapping and electro-rotation by electric fields, and contact manipulation such as mechanical micro-manipulation with vacuum technology. Physical forces used in these cases include acoustic, optical, hydraulic, micromechanical, magnetic and electrical forces.

(1) Acoustic forces: Ultrasonic fields employ acoustical energy to trap particles at potential wells or minima [14, 15], or to levitate small volumes of liquids in air with one or a few cells inside [16]. However, the technique suffers from problems of array-ability and sufficient localization to trap single particles.

(2) Optical forces: Optical tweezers use optical gradient forces to trap particles in the focal points of strongly focused laser beams [17-19]. Optical tweezers can be used to manipulate single particles ranging in size from tens of nanometers right up to tens of

microns in three dimensions and concurrently capture imaging. However, the technique does not scale well, i.e. it requires the same amount of laser beams as that of particles which need to be handled at a time. And the use of laser beams could possibly damage the manipulated cells.

Recent work has been tried to create two- and three-dimensional arrays of optical traps. The array-ability can be realized either by scanning a single beam over different locations [20-22] or by the use of diffractive optical elements (beamsplitter), which use computer-generated holograms to produce holographic optical tweezers (HOTs) [23-25]. HOTs has been used in a number of applications and one may envision its considerable influences in the mesoscopic world as the technique is further developed. However, the work on optical tweezers is preliminary and it emphasizes much on the profound understanding of laser science. In addition, optical manipulation systems use high numerical aperture (and thus high resolution) microscope objective lenses to enhance gradients, which do not cover a large area. Thus it is doubtful to create a large-area (e.g. 1cm x 1cm) array of optical traps.

One way to reduce the optical damage is the helical mode, which focuses the laser beams to a ring (optical vortices/optical spanners) rather than focusing to a point. The dark focus is suitable for trapping reflecting [26], absorbing [27], or low-dielectric-constant [28, 29] objects that would be damaged or repelled by conventional optical tweezers. In helical mode, small particles are drawn to the ring's circumference and thus produce ring-like trapping image. Also, optical tweezers are likely to be applied to the area of lab-on-a-chip and microfluidic devices [19].

(3) Hydraulic forces: Another method to manipulate microparticles is to use hydraulic forces. The most frequently used liquids in these systems are oil and water, which are oil hydraulic micromanipulator and water hydraulic micromanipulator. These devices have been well developed and are commercially available. They are usually used to perform microinjection cooperating with micropipettes and microscope. Alternatively, researchers are trying to apply microbubbles mechanism as actuator to manipulate cells and particles [30, 31]. However, the research in this work is still preliminary.

(4) Mechanical forces: For mechanical manipulation, the performing part of the system is generally of a comparable size to the targeted particles. In this way, microsystem is suitable for manipulating cells and micro-particles. Researchers utilized the compression of cells or micro-particles between two parallel surfaces [32]. Manipulation of particles is accomplished with this method before the further measurement of the mechanical properties of particles. However, the technique does not result in sorting and separation. Microrobots based on conducting polymer's (polypyrrole) electric response have been proposed and developed to grab 100- μm beads and move them around [33]. The application of this work is doubtful because: 1) bioparticles such as cells and bacteria are generally much smaller than the 100- μm beads used in [33]; 2) the method is user-intensive. More generally, mechanical manipulation may cause contact-induced response which may influence the result.

(5) Magnetic forces: Magnetic separation has been considered as a very promising technique in biological applications. The basic concept of magnetic separation in biotechnologies is to bind selectively the bioparticle of interest such as a specific cell or

protein to a magnetic particle and separate it from its surrounding media by applying a magnetic field [34, 35]. The advantages of the technique are: (1) the ease of manipulation of the biomolecules that are immobilized on magnetic particles; (2) a large surface area of immobilization substrate, which results in a high population of target biological molecules and particles. The technique has found various applications such as nucleic acid isolation, protein purification, immunology and isolation of a wide range of specific mammalian cells, bacteria and viruses. Also, a direct magnetic separation method – high gradient magnetic separation (HGMS) has been proposed to separate red cells from whole blood [36-39]. Recently, lab-on-a-chip technology has attracted the interest of researchers to apply magnetic separation technique to the microfluidic devices.

(6) Electrical forces: Electrical forces are well suitable for manipulating biological particles. Two kinds of electrical forces, i.e. Coulomb force and dielectrophoretic (DEP) force, can be used to manipulate microparticles. Coulomb force acts on a particle's charges and induces movement, known as the well known electrophoresis (EP). For biological particles such as cells which are electrically neutral in general, EP may not be applicable for the absence of net charge. In a different manner, DEP force can be used for manipulation of the neutral particles. DEP exerts its effect on the particle's dipole which is induced by the electric field, rather than on its net charge. DEP does not require the particle to be charged in order to manipulate it; the particle must only differ electrically from the medium that it is in. Particle separation, isolation, sorting and trapping have been readily effected by DEP force. DEP-based manipulation has many advantages over the aforementioned forces for manipulating micron-sized particles. First, its rigorous

dependence on the dielectric properties of particles and on a wide range of frequency provides a good means to manipulate particles. Second, DEP devices are amenable to microfabrication, making them easy to be arrayed and thus scale well. Finally, electric fields can be easily generated, addressed and released for the specific manipulation. Because of these advantages, DEP force has been chosen as the subject of study for manipulating particles in this work.

0.0 Dielectrophoresis

A review of the DEP technique is given in this section. Dipole approximation is discussed first and then a more rigorous DEP theory, i.e. multipolar theory is discussed. Applications of DEP including particle separation, trapping, and characterization, etc are summarized.

0.0.0 Dipole approximation

The physics behind the phenomenon of DEP is schematically shown in Figure 2.1. When an electrically charged particle and an electrically neutral particle are placed in a uniform electric field (Figure 2.1a), they behave differently due to different ion/charge dispersion processes. For the former, it attracts ions of opposite polarity around itself, forming the well-known electrical double layer. The symmetry of the distribution of the ions on this double layer is distorted in the presence of the applied electric field, which produces a force on the particle (known as electrophoretic force). For the neutral particle, Maxwell-Wagner interfacial charges are induced at the boundary between the particle and surrounding medium [40, 41], building up a dipole moment. However, the particle

does not feel a net force because each half of the induced dipole moment experiences opposite and equal forces. In Figure 2.1b, the same neutral particle is placed in a nonuniform electric field. Now the two halves of the dipole experience forces of different magnitude and thus a net force is produced. This is the dielectrophoretic force. Depending on the relative polarizabilities of the particle and the surrounding medium, the particle will feel a force which directs it towards the regions of field maxima (termed positive DEP or p-DEP) or regions of field minima (negative DEP or n-DEP). Based on Pohl's theory and fundamental experiments [7], the phenomenon of DEP is summarized as below:

- Particles experience a DEP force only when the applied electric field is nonuniform and the particles (with different permittivities from the medium) are polarisable.
- The force arises from the interaction between the induced dipole moment and the electric field.
- The DEP force does not depend on polarity of the applied voltage and is observed with AC as well as DC excitation; switching the polarity of the field does not affect the direction of the DEP force.
- The dielectrophoretic effect is determined by the combination of permittivities of particles and suspending medium, the particle geometry, and field distribution.
- Particles are attracted to the region of stronger electric field when their permittivity ϵ_p exceed that of the suspension medium ϵ_m , i.e. when $\epsilon_p > \epsilon_m$.
- Particles are repelled from the region of stronger field when $\epsilon_p < \epsilon_m$.
- DEP is most readily observed with particles of size ranging from 1 μm to 1000 μm .

Figure 0.1 Physics of Dielectrophoresis. (a) The behavior of particles (charged and neutral) in the uniform electric field. (b) Net forces that the electrically neutral particles experience in the nonuniform electric field.

The force on the induced dipole exerted by the nonuniform electric field is given by [7, 10]

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

where $\mathbf{p}$ is the dipole moment vector, ∇ is the Del vector, and $\mathbf{E}$ is the external electric field.

The above equation based on the dipole approximation is applicable only when the scale of the electric field nonuniformity is large compared to the particle dimensions [10]. The DEP force can be obtained from Equation (2.1) by substituting the dipole moment with the effective dipole moment. For a homogeneous, isotropic, dielectric sphere, this force is [10]

$$\mathbf{F}_{\text{DEP}} = 2\pi\epsilon_m a^3 \text{Re}[K(\omega)] \nabla |\mathbf{E}|^2 \quad (2.2)$$

where ϵ_m is the permittivity of the surrounding medium, a is the radius of the particle, Re indicates the real part. $K(\omega)$ is the Clausius-Mossotti factor (CM factor), a complex function of the medium and particle complex permittivities. For a homogenous spherical particle, the CM factor is given by

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

with $\underline{\epsilon} = \epsilon - i\sigma/\omega$ ($i = \sqrt{-1}$) is the complex permittivities; ω is the frequency of the electric field. Subscript p and m denote particle and suspending medium, respectively. The complex permittivities are used in the representation because of the fact that the particle and medium exhibit both dielectric (field energy storing) and conduction (energy loss) properties in the presence of the electric field.

Equation (2.2) indicates that DEP force depends on a variety of elements, i.e. particle size, permittivities and conductivities of the particle and suspending medium, and the gradient of electric field square. One can see that the Clausius-Mossotti factor plays an important role in DEP force. The factor represents the whole dielectric properties of the DEP environment from the contributions of the suspending medium and particles. On the other hand, the factor represents the frequency dependence of DEP effect, which is regarded as an excellent feature of the phenomenon. The CM factor comes from solving Laplace's equation and matching the boundary conditions on the surface of the particle [10].

The frequency dependence of DEP force is shown through investigation of the CM factor of a homogeneous sphere, i.e. Equation (2.3). First, the factor can only vary

from +1 (when $\underline{\epsilon}_p \gg \underline{\epsilon}_m$, i.e. the particle is much more polarisable than the suspending medium) to -0.5 (when $\underline{\epsilon}_p \ll \underline{\epsilon}_m$, i.e. the particle much less polarisable than the suspending medium). This means that positive DEP force can be twice as strong as the negative DEP force. Second, at low frequency and high frequency limits, the CM factor reduces to the following forms, respectively

$$\left\{ \begin{array}{l} K = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}, \quad \text{for } \omega \rightarrow 0 \\ K = \frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2\epsilon_m}, \quad \text{for } \omega \rightarrow \infty \end{array} \right. \quad (2.4a)$$

$$(2.4b)$$

Thus, DC conduction governs the low-frequency DEP behavior (2.4a), and the dielectric polarization governs the high-frequency behavior (2.4b); the induced dipole varies between a free charge dipole and a polarization dipole.

The Maxwell-Wagner interfacial polarization indicates these two regimes and the relaxation time is given by [42]

$$\tau = \left(\frac{\epsilon_p + 2\epsilon_m}{\sigma_p + 2\sigma_m} \right) \quad (2.5)$$

The frequency variation of the CM factor comes from the Maxwell-Wagner interfacial response to the electric field. It is due to the competition between the charging processes in the particle and medium, resulting in charge buildup at the particle/medium interface. If both the particle and the medium are non-conducting, there will be no charge buildup in the interface and the CM factor is frequency-independent and is constant. Otherwise, conductivities in the system may cause frequency dispersion in the CM factor due to the polarization in the interface.

Other CM factors can also be derived in the same way. For layered spherical shells

(e.g. model for red blood cells), one solves the Laplace's equation starting from the innermost layer and working outward until the outer layer is reached by satisfying the conditions at the interfaces between every two layers [10], giving the CM factor which has the same form as Equation (2.3) but with an effective permittivity $\underline{\epsilon}'_p$ in place of $\underline{\epsilon}_p$, where $\underline{\epsilon}'_p$ contains the multilayer information of the shells.

For biological cell with a membrane (but without cell wall), this effective permittivity is derived as [10]:

$$\underline{\epsilon}'_p = \frac{\underline{C}_m a \underline{\epsilon}_m}{\underline{C}_m a + \underline{\epsilon}_m} \quad (2.6)$$

where the membrane is described as a shell with a complex surface capacitance $\underline{C}_m = C_m + G_m / j\omega$. C_m and G_m are the surface capacitance and conductance, respectively, given by $C_m = \epsilon_s / \Delta$ and $G_m = \sigma_s / \Delta$, where Δ is the thickness of the membrane, ϵ_s and σ_s are the permittivity and conductivity of the membrane.

The polarity of the CM factor (real part) determines the types of DEP, i.e., positive DEP when the CM factor is positive and negative DEP when the CM factor is negative. Particles with different electrical properties have different frequency dependence and exhibit different behavior. The CM factors for three typical particles are shown in Figure 2.2. For a non-conductive homogenous sphere suspended in water (Figure 2.2a), the CM factor (real part of the CM factor) is constant at ~ -0.5 for the water is much more polarizable than the sphere. The particle will experience negative DEP in the range of frequencies. Now the same sphere, but with a noticeable conductivity of 10 mS/m is suspended in water of low conductivity (Figure 2.2b). At low frequencies, the CM factor

is positive and constant for the sphere is much more conductive than the water. At high frequencies, the CM factor is similar as in (a) since the permittivity of the water (relative $\epsilon_m = 80$) is much higher than that of the particle ($\epsilon_m = 3$). Thus the particle undergoes positive DEP at low frequencies and negative DEP at high frequencies. The CM factor is more complex for cells. Figure 2.2c shows the factor for a yeast cell suspended in a solution with conductivity of 1 mSm^{-1} [43]. Now the factor has two transitions (from positive to negative or negative to positive) because there are two interfaces as the cell is a membrane-bound structure.

Figure 2.2 CM factors (real parts) for different particles. (a) A non-conductive homogeneous sphere suspended in water. (b) A homogeneous sphere with a noticeable conductivity suspended in water. (c) A yeast cell suspended in a solution with conductivity of 1 mSm^{-1} .

The crossover frequency at which the force is zero is also shown in the figure. At the crossover frequency, the polarizability of particle and the medium are the same and the particle ceases to experience any DEP force. The real part of CM factor is zero.

Therefore, the crossover frequency is given by

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{(2\sigma_m + \sigma_p)(\sigma_p - \sigma_m)}{(2\varepsilon_m + \varepsilon_p)(\varepsilon_m - \varepsilon_p)}} \quad (2.7)$$

Particles with different crossover frequencies will experience forces of different sign in the frequency window bounded by the crossover frequencies, giving valuable information for manipulation and separation of particles.

The term $\nabla |\mathbf{E}|^2$ in Equation (2.2) represents the spatial nonuniformity of the field magnitude. A more general expression of the DEP force based on dipole approximation is given when the spatial nonuniformities of both the magnitude and phase of the field are taken into account [44, 45]

$$\langle \mathbf{F} \rangle = 2\pi\varepsilon_m a^3 \left\{ \mathbf{Re}[K(\omega)] \nabla |\mathbf{E}|^2 + \mathbf{Im}[K(\omega)] \sum |\mathbf{E}|^2 \nabla \varphi \right\} \quad (2.8)$$

where $\sum |\mathbf{E}|^2 \nabla \varphi$ represents a summation involving the magnitude and phase φ of each field component. $\mathbf{Im}[K(\omega)]$ is the imaginary part of the CM factor. $\langle \rangle$ denotes the time-averaged value.

The above equation indicates that there are two independent force contributions to DEP motion:

1. In-phase component: the first term of right hand side (in the bracket) depends on the real part of CM factor (the induced dipole moment) in the particle and the spatial nonuniformity of the field regions. It also depends on the geometry of particle, e.g. the size of spherical particle. The polarity depends upon where $\mathbf{Re}[K]$ is positive or negative, leading to the positive DEP or negative DEP effect. This is consistent with Pohl's theory and called the conventional dielectrophoresis (cDEP). It is the only DEP force

component that can act when an electrode array is energized by single or dual phase signals.

2. Out-of-phase component: the second term of RHS depends on the imaginary part of CM factor and the spatial nonuniformity of the field phase ($\nabla\varphi$). The effect of this term pushes the particle to move in the same or the opposite direction in which the field is traveling, depending on whether $\text{Im}[K]$ is negative or positive [46, 47]. This effect is termed as traveling wave dielectrophoresis (twDEP). To generate the twDEP force, at least three excitation phases are required.

Similarly, the frequency dependence of the twDEP force is through the imaginary part of CM factor, i.e. $\text{Im}[K]$. For the uniform spherical particle (Eqn. 2.3), this element is zero at high and low frequencies. Different from the real part, $\text{Im}[K]$ can vary from -0.75 to +0.75 depending on the relative polarizabilities of the particle and the medium (Appendix A). In addition, $\text{Im}[K]$ is either positive or negative in the whole range of frequencies. No crossover frequency is found for the twDEP force (Appendix A).

0.0.0 Multipolar theory

The aforementioned approximation for the DEP force is based on the effective dipole moment approach, which is usually adequate for evaluation of the particle response to electric field because the field is moderately inhomogeneous in most cases. However, the dipole approximation may lead to significant errors when the scale of the field nonuniformity is comparable to the size of particle, which easily happens in case that the electric field is generated from microfabricated electrodes. In fact, from a

physics point of view, not only dipole but also arbitrarily high order poles (multipoles) may be induced simultaneously in a particle immersed in the field. The dipole approximation of DEP effect must be revised in the following cases because the influence of the multipoles is considerable:

- Field nulls existing in the electric field: the dipole is zero in the field nulls because it is proportional to the electric field. However, the field gradient near the boundary of nulls may be unexpectedly high due to the variation from zero to nonzero value.
- Strongly nonuniform electric field: this may exist in some domains of the field, e.g. the sharp edges of the electrodes.
- Electric field generated with very small electrodes: it is now possible to produce small electrode structures down to submicron scale with advanced fabrication techniques.
- Electric field of another closely spaced particle: mutual particle interactions occur where particles are so closely spaced that the local field of a particle influences its neighbors. The interactions can be very strong, leading to the changes in the equilibrium structures of particle ensembles (e.g. chain formation and cooperative electrorotation), as well as strong cohesive forces [10].

Dipole approximation is no longer applicable in the above situations because the multipoles become significant. Fortunately, multipolar theory has been recently developed to address the limitations of dipole approximation. Considering the dipole and multipole polarization occur simultaneously in the particle exposed to the electric field,

the general force expression is

$$\mathbf{F}_{\text{DEP}} = (\overline{p}^{(1)} \cdot \nabla) \mathbf{E} + \sum_{n=2}^N \mathbf{F}_n \quad (2.9)$$

The first term on the right hand side describes the force arising from dipole moment $\overline{p}^{(1)}$ induced in the particle; the summation term represents the force due to the multipolar polarization in the particle. Multipoles with order up to N are included in the expression.

The multipolar force can be attained by using an effective moment approach [48-51] or Maxwell tensor method [52]. A compact tensor representation of the force on the n -th general multipole is

$$\mathbf{F}_n = \frac{\overline{p}^{(n)} [\cdot]^n (\nabla)^n \mathbf{E}}{n!} \quad (2.10)$$

Where n refers to the force order ($n=1$ the dipole, $n=2$ the quadropole, $n=3$ the octupole, etc.), $[\cdot]^n$ represents n dot product operations, $(\nabla)^n \mathbf{E}$ represents n vector ∇ operations ($(\nabla)^n \mathbf{E} = \nabla \nabla \dots \nabla \mathbf{E}$). $\overline{p}^{(n)}$ is the multipolar induced-moment tensor.

Thus, one sees that the n -th order force is given by the interaction of the n -th-order multipolar moment with the n -th gradient of the electric field. For $n=1$ the result reduces to the force on a dipole (Equation 2.1).

For sinusoidal excitation ($\mathbf{E}(r,t) = \mathbf{Re}[\mathbf{E}(r)\exp(i\omega t)]$) the time-averaged force arising from the multipolar polarization has the form as [10, 48]

$$\langle \mathbf{F}_n \rangle = \frac{1}{2} \mathbf{Re} \left[\frac{\overline{p}^{(n)} [\cdot]^n (\nabla)^n \mathbf{E}^*}{n!} \right] \quad (2.11)$$

where $\langle \rangle$ and $*$ indicate, respectively, time-averaged and complex conjugate quantities.

A more explicit version for practical application of the time-average force for the i -directed component is [10, 48]

$$\begin{aligned}
 \langle \mathbf{F}_i^{(1)} \rangle &= 2\pi\epsilon_m a^3 \mathbf{Re} \left[K^{(1)} E_m \frac{\partial}{\partial x_m} E_i^* \right] \\
 \langle \mathbf{F}_i^{(2)} \rangle &= \frac{2}{3} \pi\epsilon_m a^5 \mathbf{Re} \left[K^{(2)} \frac{\partial E_n}{\partial x_m} \frac{\partial^2 E_i^*}{\partial x_n \partial x_m} \right] \\
 \langle \mathbf{F}_i^{(3)} \rangle &= \frac{1}{15} \pi\epsilon_m a^7 \mathbf{Re} \left[K^{(3)} \frac{\partial^2 E_n}{\partial x_i \partial x_m} \frac{\partial^3 E_i^*}{\partial x_n \partial x_m \partial x_i} \right] \\
 &\vdots
 \end{aligned} \tag{2.12}$$

for the dipole ($n=1$), quadrupole ($n=2$) and octupole ($n=3$) forces orders, respectively.

The Einstein summation convention has been applied in Eqn (2.12). $K^{(n)}$ ($n = 1, 2, 3, \dots$) represents the CM factor. The multipolar CM factor for a uniform lossy (ohmic loss or dielectric loss) dielectric spherical particle is given by

$$K^{(n)} = \frac{\epsilon_p - \epsilon_m}{n\epsilon_p + (n+1)\epsilon_m} \tag{2.13}$$

It has the same form as the dipolar CM factor ($n=1$, Equation 2.3). The multipolar CM factor has smaller limits, e.g. the quadrupolar ($n=2$) CM factor varies from $-1/3$ to $+1/2$; the octupolar ($n=3$) CM factor varies from $-1/4$ to $+1/3$. We also notice that the multipolar force is proportional to higher order of the particle radius.

0.0.0 DEP-based separation

Separating bioparticles from a particle or cell mixture is a major step for further analysis or diagnostics and is a basic use of DEP force in biotechnology. Generally, the basis of dielectrophoretic separation is linked to differences in polarizabilities of the

populations, i.e. in the different values of the CM factor. Because of the frequency dependence of the CM factor, DEP separation is usually effected in appropriate frequencies, which should be carefully selected in practical operations.

On the other hand, there should be some way of extracting the populations of particles once the separation takes place. That is, the populations should be physically separated with a sufficient distance for extraction to take place. The distance may be in space, where the separated populations are collected at the different outlets of the channels. Also, it may appear as time distance – the different populations reach the single outlet at different times so they are separated.

A. Differential DEP affinity separation

In an appropriate frequency, one kind of particles experiencing p-DEP will be directed to the maxima of the electric field while the other experiencing n-DEP will be repelled to the minima and hence separation takes place. This is called differential DEP affinity separation [53] (or binary separation). In the near-field regime used in DEP, all field maxima are at electrode edges. In practice, the separation process is: once the p-DEP subpopulation is attracted to electrode edges and the n-DEP one is pushed away, a flow of fluid is supplied to wash the n-DEP subpopulation off when the applied field is turning on. The trapping force on p-DEP particles is generally strong enough for retention against hydrodynamic force and n-DEP particles tend to be dragged away by the flow.

It is possible to apply the above method to separate two dissimilar particle types. In

this case, a frequency of field is carefully chosen so that one particle type undergoes p-DEP while the other undergoes n-DEP. This method is extremely effective if the dielectric properties (CM factor) of the particle types are greatly dissimilar. However, it has been found that the method is impractical to separate particles having similar properties because cluster and dipole-dipole interactions occur [54].

Using this method, researchers have separated many types of biological particles, including bacteria from blood cells (e.g. [55], [56]), cancer cells from blood cells (e.g. [55, 57, 58]), normal cells from malaria-infected blood cells [59], CD 34⁺ stem cells from blood [59, 60], live cells from dead cells [61], etc.

B. Traveling wave DEP separation

Another approach to separate mixed particles is using twDEP. The out-of-phase component of particle polarization causes translational motion while the in-phase one is responsible for DEP trapping or repulsion. To load the traveling wave signal used in twDEP, multilayer electrode structure (e.g [62]) and spiral electrode [63] have been proposed by researchers. The former pattern contains bus bars and electrode arrays forming in different layers while bus bars are not necessary in the latter pattern. With careful selection of medium conductivity and permittivity and frequency of the signal, different particle types may experience movement in different directions or in distinct positions, and therefore separation is achieved.

In this way, a number of particles including latex spheres [63], tumor cells [64], yeast [45] and single-celled parasites [65] have been focused at the center of spiral

electrodes, or transported laterally with following collection at opposite ends of the array.

C. Dielectrophoretic Field-Flow Fractionation

Field-flow fractionation (FFF) is a widely used technique in which force fields are applied to particles to place them characteristically at different positions at which the velocities of the flow stream are different [66], and therefore different particles are carried with different velocities in the stream. Typical fields include gravity (sedimentation FFF), temperature gradient (thermal FFF) and viscous properties of the particle in a cross flow (flow FFF) [43].

The application of dielectrophoresis as a force field for FFF (so called DEP-FFF) has been well developed by the researcher (see, e.g. [67-72]). Two modes of DEP-FFF are proposed and developed: steric DEP-FFF and hyperlayer DEP-FFF, in which p-DEP and n-DEP are used as force fields, respectively. For steric DEP-FFF, one of its disadvantages is that the existing of the multi-equilibrium regime causes the particles to move between different equilibrium positions [53].

In the mode of hyperlayer DEP-FFF, particle separation is accomplished by balancing the particle in a certain height above the electrode with the n-DEP force against other forces such as gravitational force that direct the particle to the electrode array. Particles having different density and dielectric properties are levitated to different heights above the bottom surface of a thin chamber, and are carried with the flow stream at different velocities when a parabolic flow velocity profile is generated inside the chamber [72]. The levitation height for a specific particle in this mode is always

single-valued and it is a unique equilibrium problem, overcoming the disadvantages in steric DEP-FFF.

DEP-FFF especially hyperlayer DEP-FFF has been applied successfully to particle separation, including the separation of latex microspheres [67-70], stem cells from tumor cells [71, 73], and blood cell subpopulations (leukocyte subpopulations).

D. Other methods

Another method for particle separation was devised by Markx *et al* [74], so called stepped flow separation, using interdigitated castellated electrode arrays in a channel with two fluid ports – entry and exit. Unlike other methods simply using n-DEP and p-DEP distinctly, this method utilizes both n-DEP and p-DEP simultaneously and allows the formation of cluster at the early stage. In essence, stepped flow separation takes advantage of the fact that p-DEP force is generally stronger than n-DEP force, and therefore particles under p-DEP are more immobilized than that under n-DEP. Although the technique is slow and complex, the repeated action of moving and trapping in this technique likely enables the 100% homogeneous separation. Further more, in theory such a method can separate more than two types of particles in the mixture, provided that appropriate frequencies can be found.

Differing from the above particle separation methods which readily use the dielectric properties of particles, ratchet-dielectrophoresis is another method for particle separation but does not require difference in dielectric properties between particles. Instead, this method utilizes the Brownian motion to effect separation. Two methods, i.e.

thermal and stacked ratchets, were introduced by researchers [75-77]. However, little attention has been paid to ratchet-DEP, probably because of its inherent disadvantages: low efficiency due to the slow process of Brownian motion, poor feasibility, complex structure, etc.

Of course, there are other DEP methods for particle separation, such as the combined use of laser tweezers and DEP, antigen-antibody reaction dielectrophoresis.

0.0.0 DEP-based trapping

Another significant application of DEP is to make particle traps, which has gained great attention from researchers. A general concern about the trapping should be the strength of retention at trapping points and the stability of particle stay at the points. Basically, n-DEP is more suitable for particle traps than p-DEP, because local electric field intensity maxima cannot exist while the existence of field intensity minima is possible for space-charge-free electrostatic fields [31]. In addition, n-DEP has the advantage that particles such as cells move to the region of low field strength, minimizing the risk of biological damage arising from the electric field of high strength. Although rudimentary particle trapping may be made in almost all electrode arrangements, the trap will not be valuable without smart design of the electrode pattern and careful selection of surrounding conditions. Much of the early work used non-microfabricated structures to levitate bubbles or cells using n-DEP [31, 78] or feedback-controlled p-DEP [79, 80], primarily focusing on the validation of dipole theory.

Figure 2.3 n-DEP trapping structures (a) A diagram and photograph of a three-dimensional opposed octopole (from [81]). The octopole consists of two planar quadrupoles on substrates placed apart (and often slightly rotated) from each other. (b) A photograph a planar quadrupole (from [82]). The quadrupole consists of four polynomial electrodes arranged on a substrate.

The reports from the research group at the Institute for Biology at Humboldt University in Germany [83, 84] were the first to demonstrate the use of microscale n-DEP to trap particles. They utilized the (negative) dielectrophoretic field cage (DFC) forming in the opposed octode structure (Fig. 2.3a) [81, 84] to make a strong trap, and later used planar quadrupole electrode configuration (Fig. 2.3b) [82] to trap sub-micrometer particle combined with hydrodynamic force. The advantage of the octopole is the presence of the unique trap having strong holding force because of its three dimensional symmetry. The quadrupole provides a less stable trap at a certain height in the center, due to the levitation effect of n-DEP; in this configuration, the trapped particle of low density is likely over-levitated and escapes under the influence of hydrodynamic force. The combined use of dielectrophoretic field cages and laser tweezers [85] allows simultaneous particle trapping and electrorotation (see section 2.4),

and enables the measurement of field distribution in rotating DFC or measurement of the force exerted by laser tweezers on particle that can hardly be calculated analytically.

Another piece of research work has investigated the particle-size limits of DEP traps. As the particle size decreases, the DEP force decreases with r^3 (dipole approximation, r is the particle radius) while the Brownian motion increases with the inverse of r [86]. One sees that there exists a minimum particle size at which the Brownian motion effect or other forces dominate over or is comparable to the DEP force, making the particle trapping impossible. Early research believed that this limit is 1 μm , that is, DEP could only handle micron-sized particles. Recently, interest was directed to the investigation of sub-micrometer particles (nanoparticles) with DEP. Again, the work done in Humboldt University showed that submicron particles can also be trapped with DEP coupled with electrohydrodynamic force [82]. Using the simple electrode configuration – quadrupole (Fig. 2.3b), they found that 100 nm latex spheres were trapped by p-DEP or n-DEP, and more surprisingly, 14 nm latex beads were levitated and trapped aggregate in the central field funnel by n-DEP. Other work to determine the particle size limits was done by the Morgan's group [86-92]. The forces acting at sub-micrometer particle, most notably electrohydrodynamic flows, were summarized [86, 93]. Their results indicate that trapping of sub-100-nm particles is possible solely with n-DEP forces. In sub-micrometer particle traps, ultra-miniaturized electrodes are necessary to generate a field of higher gradients so as to compensate for reduction of DEP force due to the decreasing particle size. Although the mechanism of sub-micrometer particle traps has not been thoroughly investigated and understood, the

studies on DEP at nano-scale extends its range from micrometer to nanometer particles, and makes us optimistic about trapping and single particle manipulation of bacteria, viruses and some types of macromolecules such as DNA and protein using DEP in ultra-microelectrode systems.

Other research on particle traps concentrates on micron-sized particles. Probably edifying from the original arrangement of quadrupole (Fig. 2.3b), Voldman J. *et al* proposed the extruded quadrupole configuration instead of the planar quadrupole [94, 95]. The extruded quadrupole has a few advantages: stronger holding forces, slow decay of field away from the substrate, lower levitation height and thus lower escape probability. Using this configuration, Voldman investigated the traps of single latex beads (polystyrene bead) and viable cells. Heida T *et al* presented a numerical modeling of trapping in quadrupole electrode using finite element method [96], showing that the flow induced by electro-liquid interface effect and local heating enhances the trapping of the dielectrophoretic force. Using a three-dimensional grid electrode system with ingenious energized sequence and selection [97], a single protoplast cell was captured at the grid intersection by p-DEP then released and moved in the selected direction by n-DEP [97]. Also, a group from Sandia National Laboratory observed the trapping without embedded electrodes, but with arranged arrays of posts standing in the flow, applying an external DC electric field in a certain direction [98].

0.0.0 DEP-based characterization

DEP can also be used for particle characterization. Because of the physics aspect of DEP, it is usually used to determine the physical properties of particles, primarily the

dielectric properties including dielectric constant, permittivities and conductivities, etc. The dielectric properties of colloidal particles and biological cells are usually measured using dielectric spectroscopy [99, 100], with which the bulk permittivity ϵ_s and conductivity σ_s of a suspension are measured to deduce the properties of the particle (ϵ_p and σ_p). The technique is straightforward in principle, but suffers from experimental problems, e.g. electrode polarization, and gives an average or statistic result. An alternative technique for measuring the dielectric properties of a single or a small amount of particles is using AC electrokinetics including electrorotation (ROT, section 2.4) and dielectrophoresis. Generally, electrorotation is more suitable for determination of particle properties while spatial manipulation is performed by DEP. However, DEP can also be applied to measure particle properties, especially in small objects like bacteria, viruses and molecules or exactly spherical particle with few visible internal structures which cannot be investigated by ROT. A number of methods for measuring dielectric properties or spectra of particles based on DEP have been developed over the years, such as crossover frequency method, levitation and impedance method, etc.

The crossover frequency method (zero force method) is based on dipole theory [101, 102]. The technique utilizes the fact that at a particular frequency so called crossover frequency the DEP force acting on the particle vanishes to zero, as it is the critical point between p-DEP and n-DEP. This occurs when the real part of the effective polarisability of the particle is exactly equal to that of the suspending medium. Since the latter is easily known, the effective complex polarisability of the particle can be calculated. In practice, the zero force or the crossover frequency is measured for

changing the conductivity of the suspending medium in sequence (Fig. 2.4). The measurement is usually carried out at low conductivities since the polarization response is insensitive at high conductivities of the medium as shown in Fig. 2.4. Using this technique, both the permittivity and conductivity of a homogeneous solid particle were characterized [103]; the dielectric properties of shelled particles such as cells were measured providing certain assumptions [102, 104]; the dielectric properties of sub-micrometer particles such as sub-micrometer latex sphere [103] and herpes simplex virus-1 [105, 106] were characterized.

Figure 2.4 Theoretical plot showing the range of electrolyte conductivities and frequencies over which a homogeneous dielectric particle experiences p-DEP or n-DEP. The plot was calculated with the following parameters for the particle: $\epsilon_p = 2.55$, $K_s = 1.2$ nS (K_s is the surface conductance of the particle), and with $\epsilon_m = 78\epsilon_0$ [103].

Particle levitation extends the frequency range of measurements. In principle, the dielectric properties are determined from measuring the levitation height of the particle. When a particle experiences n-DEP, it will be repelled from the electrodes, and moves to a stable potential energy minimum where the DEP force is exactly balanced by the

gravitational force. The levitation height provides information of the magnitude of the DEP force as well as the effective polarisability. This technique has been used successfully to measure the dielectric properties of a range of particles [107, 108]. In a divergence and curl free electrostatic field, stable levitation simply using p-DEP is impossible because the density maximum in the field is not allowed. However, using feedback controlled technique, p-DEP levitation becomes possible [10, 79, 80]. The conditions required for feedback controlled p-DEP levitation are that the particle must be stable with respect to radial and axial motions [92].

Other techniques for characterization include measuring the velocity of the particle in the well-defined electric field [109], dielectrophoretic phase analysis light scattering [110, 111] and impedance method [112].

0.0.0 Other applications

The applications of DEP extend from solid particles and biological particles to aqueous and gaseous particles (bubbles). The bubble dielectrophoresis was introduced two decades ago by T. B. Jones [31], but less work has been done on it ever since then due to its limitations and the fact that almost all the systems in chemistry and biology contain aqueous environments. Liquid micro-actuation using DEP was developed also by the group of T. B. Jones [113-118]. They used co-planar electrodes in the mode of open system (with open channels) to actuate liquid motion from a droplet near one end of the gap between electrodes, forming a nanodroplet/microdroplet on the other end. In this way, a small volume of liquid (e.g. water) was actuated and transported to a specific

position in a short time. DEP force is sufficient to overcome the effects of capillary and wetting on the surface. The demonstrated advantage of DEP actuation is the high speed at which liquid volumes can be transported and dispensed. Although the above experiment described the behavior of the de-ionized water (DI water) droplet under dielectrophoretic actuation, one may see the value of the technique if it deals with nanoliter/sub-nanoliter quantities of nutrient-rich, aqueous media containing biological cells and cell components.

On the other hand, DEP can be used to enhance sample mixing. In microscale systems, the flows are laminar, leaving mixing dependent solely on diffusion and therefore time consuming. One way to reduce the time for mixing is to induce chaos in the fluid, which can occur in space, e.g. creating 3D passive geometry and 3D grooves, and occur in time. Researchers used DEP as an external time-varying force exerted onto embedded elements to induce chaotic mixing [119, 120], and the mixing time was dramatically reduced.

As a versatile technique, DEP allows many other applications which are not covered in the above description. DEP is regarded as a promising technique in biomedical science. The long-term goal of DEP study - commercial use is in sight recently as the microfabrication technique comes into use in the field of DEP.

0.0 Dielectric models of particles and cells

For simplicity, a homogeneous spherical particle with constant permittivity and conductivity is frequently used in analysis of DEP force. The assumption simplifies the interaction between the particle and the external field, as the concern only goes to the

surface of the particle. In fact, particles especially biological objects can have various degree of structural complexity, meaning that they cannot be treated as homogeneous spherical particles in such a simple way. A general idea is to find out the equivalent particle, which is a homogeneous spherical or ellipsoidal particle but has equivalent dielectric properties to the original one. Many particles have been modeled this way to simplify the analysis. However, for the particles of complex structures or geometries, it is not easy and in many cases is impossible to work out the equivalent particles analytically. A number of numerical methods such as finite difference method (FDM), finite element method (FEM) and boundary element method (BEM) can be used to model these particles. On the other hand, many relevant types of particles having a layered structure can be dielectrically modeled based on analytical solutions of Laplace's equation, assuming a simplified spherical or ellipsoidal geometry. This can be found in T. B. Jones' book [10].

The schematic process for solving the equivalent particle is briefly shown in Fig. 2.5. A three-layered spherical shell with ohmic loss ($\sigma > 0$) immersed in the suspending medium in uniform field can be dielectrically equivalent to a homogeneous sphere of permittivity $\underline{\epsilon}'_2$ and conductivity σ'_2 , which is given as [10]

$$\underline{\epsilon}'_2 = \underline{\epsilon}_2 \frac{a^3 + 2 \left(\frac{\underline{\epsilon}'_3 - \underline{\epsilon}_2}{\underline{\epsilon}'_3 + 2\underline{\epsilon}_2} \right)}{a^3 - \left(\frac{\underline{\epsilon}'_3 - \underline{\epsilon}_2}{\underline{\epsilon}'_3 + 2\underline{\epsilon}_2} \right)} \quad (2.14)$$

where $\underline{\epsilon}'_3$ is the intermediate complex polarizability which has a similar form with $\underline{\epsilon}'_2$ where $\underline{\epsilon}_2$ and $\underline{\epsilon}'_3$ are substituted with $\underline{\epsilon}_3$ and $\underline{\epsilon}_4$, respectively. The underline denotes the complex polarisability, e.g. $\underline{\epsilon}_2 = \epsilon_2 + \sigma_2 / j\omega$, etc. This can be obtained by solving

the Laplace's equation from the inner layer outwards to the most-exterior, satisfying the boundary conditions at the surface between two adjacent layers. For the electrostatic electric field, the conditions are that both the potential and the normal component of the flux vector must be continuous across the interface.

Figure 2.5 Schematic process of solving the equivalent particle for a multilayer shell. Applying the boundary conditions at each interface between adjacent layers to solve Laplace's equation gives rise to an equivalent homogeneous sphere of radius R_1 , permittivity ϵ'_2 and conductivity σ'_2 [10].

For approximation of biological cells, two simple dielectric models are protoplast and walled cell models [10]. Fig 2.6a shows the simple dielectric model for a protoplast: a conductive fluid (cytoplasm) enclosed by a very thin capacitive layer (membrane). The membrane, which consists of a selectively permeable bi-layer of lipid protein molecules, is thin and quite fragile. It serves a vital function as a two-way conduit for (i) life-sustaining nutrients and regulatory substances required by the cell's metabolism, and (ii) waste materials excreted from the cell. Its dielectric property is characterized by the effective capacitance c_m and conductance g_m . The cytoplasm is extraordinarily complex,

containing the nucleus and vacuole as well as numerous other structures suspended in an aqueous fluid. Its dielectric property is characterized by the effective permittivity ϵ_c and conductivity σ_c . Most mammalian cells have a structure similar to the protoplast model and can be approximated with this model. Assuming that the transmembrane conductance g_m is negligible, the effective permittivity is

$$\underline{\epsilon}'_2 = c_m R \left[\frac{j\omega\tau_c + 1}{j\omega(\tau_m + \tau_c) + 1} \right] \quad (2.15)$$

where $\tau_m = c_m R / \sigma_c$ and $\tau_c = \epsilon_c / \sigma_c$, R is defined in Fig.2.6a.

Figure 2.6 Dielectric models for biological cells. (a) Protoplast model (b) Walled model [10].

The complex Clausius - Mossotti factor is given:

$$f_{CM}(\omega) = -\frac{\omega^2(\tau_1\tau_m - \tau_c\tau'_m) + j\omega(\tau'_m - \tau_1 - \tau_m) - 1}{\omega^2(\tau_c\tau'_m + 2\tau_1\tau_m) - j\omega(\tau'_m + 2\tau_1 + \tau_m) - 2} \quad (2.16)$$

Here, $\tau_1 = \epsilon_1 / \sigma_1$ and $\tau'_m = c_m R / \sigma_1$. The low and high frequency limits of the CM factor are

$$\begin{cases} f_{CM} = -0.5, & \text{for } \omega \rightarrow 0 \\ f_{CM} = \frac{\epsilon_c - \epsilon_1}{\epsilon_c + 2\epsilon_1}, & \text{for } \omega \rightarrow \infty \end{cases} \quad (2.17)$$

The low-frequency limit is due to the membrane, which blocks DC current and causes

the cell to behave like an insulating sphere at low frequencies. At high frequencies, the membrane is electrically transparent, making the particle behave like a homogeneous dielectric sphere of permittivity ϵ_c [10].

Walled model shown in Fig. 2.6b consists of three distinct regions: the wall, the cell membrane and the cell interior. The wall is an exostructure that helps provide rigidity to the cell. The membrane and cell interior have the same functions as that in protoplast model. Cells building the plant tissues and many important single-cell microorganisms such as yeasts have the similar structure to walled model and can be approximated by this model.

The wall provides mechanical protections to the fragile membrane, and it is usually modeled as a homogeneous spherically concentric shell of finite thickness with bulk permittivity ϵ_w and conductivity σ_w . The membrane behaves like a very low loss capacitor, blocking low-frequency electric fields and electric current from the interior of the cell. At high frequencies, the membrane becomes electrically transparent, revealing the interior of the cell to possess dielectric permittivity very similar to that of the suspension medium. Similarly, the wall cell can also be modeled with a homogeneous sphere of an effective permittivity $\underline{\epsilon}'_2 = \epsilon'_2 + \sigma'_2 / j\omega$.

On the other hand, in order to investigate electrical response of the particle, electric models are used. A simple electric model for the cell is shown in Fig. 2.7, describing the membrane-covered sphere consisting of the cytoplasm and cell membrane [42, 121]. The cytoplasm of the cell is modeled as a homogeneous medium with some permittivity and conductivity, while the membrane is described as a shell with certain surface electric

properties. The qualitative electric behavior of the cell can be explained with investigation into this electrical model. The conductance of the membrane is usually much smaller than that of the medium and cytoplasm, while the values of the RC pairs of the cytoplasm and extracellular medium are usually similar. At low frequencies, the circuit looks like a resistive divider and DC blocker because of the large resistance of the membrane. Voltage is primarily dropped across the membrane. While at high frequencies, the membrane capacitor contributes more significantly and voltage across the membrane decreases. At last, the model becomes electrically transparent and the membrane voltage saturates.

Figure 2.7 Electric model of the cell. The electrical properties of cell membrane, cytoplasm and extracellular medium are characterized with three resistor-capacitor (RC) pairs with values indicated in the figure. Cytoplasm is also represented with a RC pair. The transmembrane voltage is equivalent to the voltage across the middle capacitor [42, 121].

Quantitatively, the imposed transmembrane voltage is [42]

$$|V_{tm}| = \frac{1.5 |E| R}{\sqrt{1 + (\omega\tau)^2}} \quad (2.18)$$

where ω is the angular frequency of the electric field, and τ is the time constant.

At low frequencies, we have the following equations from Eqn (2.18)

$$\begin{aligned} |V_{tm}| &\approx 1.5 |E| R \\ |E_{tm}| &\approx |V_{tm}| / \Delta = (1.5R / \Delta) |E| \end{aligned} \quad (2.19)$$

where the symbol Δ represents the thickness of the membrane.

The above equation indicates that the transmembrane field (E_{tm}) is multiplied with a factor of $(1.5R / \Delta)$ (~ 1000), which may be quite large since the thickness of membrane is quite small compared to the particle radius. Thus the electric field across the membrane is greatly amplified over that in solution. On the other hand, the electric field in the cytoplasm is not amplified. Although the effects of the large transmembrane field have not been intensely studied, this field may have significant effects on the cell, such as induced current heating and possibility of electrical breakdown.

0.0 Other electromechanics of microparticles

Independent with DEP, several other phenomena may occur in the particles subjected to the external electric field. In many cases, the behavior of the particle is simultaneously affected by some of these phenomena, and one or more of them are dominated. The relevant electromechanics give rise to the other techniques closely related to DEP which can induce microscale manipulations.

Besides the dipole (or multipoles) induced by the field on the particle, the field can also induce torques on the particle. Different from the translational movement caused by the dipole, orientation and rotation can occur with the exerted torques. Orientational torque acts upon a particle having some type of anisotropy due to either intrinsic properties such as crystalline anisotropy or extrinsic factors such as shape (e.g. nonspherical particles) [10]. Both AC and DC electric field can produce orientational torque on the particle. Studying or measuring the particle's orientational behavior can characterize the particle's properties. On the other hand, one can generate rotational torque on the particle by placing it in a rotating electric field. This technique is called electrorotation (ROT) [122]. Electrorotation can be even experienced by a spherical isotropic particle. Electrorotation is a very effective technique which can be used for particle characterization. The electrical properties of the particle can be determined by measuring the rotation rate of the micro-particle as the field frequency changes.

Pearl chains are strings of particles aligned in the field due to the mutual interactions of closely spaced particles [10]. The pearl chains formation is a sophisticated physical process interpreted with the mechanics of dipole-dipole interaction. At certain frequency ranges the induced dipole from neighboring particles will attract each other, forming long chains of particles resembling pearl necklace so called pearl chain.

Electrohydrodynamic forces are another microscale mechanics that occurs in the particle mixture subjected to the electric field. The field induces Joule heating in the suspension, causing the gradients in conductivity and permittivity which in turn give rise to electrothermal forces. In addition, non-uniform AC electric fields produce forces on

the induced charges in the diffuse double layer on the electrodes (at low frequencies), which are referred to as ac electroosmosis [93]. The above effects cause fluid motion, resulting in a viscous drag in the particle or pumping effect on the fluid. Electrohydrodynamic forces can be used to induce either particle or fluid transport [93, 123].

In addition, the techniques for cell micromanipulation with electric field are of great significance, including electroporation (also known as electropermeabilization) and electrofusion [124]. Electroporation is the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane, allowing the transport of molecules, ions, waters and reagents in and out of the cell. Because the mechanism of electroporation is not well understood, the development of protocols for a particular application has usually been achieved empirically, by adjusting pulse parameters (amplitude, duration, number, and inter-pulse interval). Electrofusion uses the same (or similar) electric field to induce two or more cells to fuse or coalesce into one large cell. Usually two cells to be fused into one have to be in contact before the pulsed electric field is applied. Thus, the techniques including DEP, electrorotation and pearl chains can be used together with electrofusion to bring target cells into contact.

0.0 Conclusion

A review on the state of the art of dielectrophoresis as well as microparticle manipulation has been presented in this chapter. Dipolar and multipolar theories have been discussed to address the theoretical aspects of DEP. As a versatile technology, DEP

finds various applications, including particle separation, trapping, characterization, fractionation, and sorting, etc. With the rapid development of micromanufacturing and miniaturization technology, there has been great interest in integrating DEP components into the microsystems or microchips for particle manipulation. In the following chapters of this thesis, the work will focus on the modeling and simulation of DEP force and particle behavior, and the development of DEP systems for microparticles/bioparticles separation and manipulation.

CHAPTER 0 Modeling Methodology and Simulation

Having presented a review on DEP, the modeling method and simulation of DEP are described in this chapter. Because of the complexities of the problems, analytical solutions are inapplicable for most of the DEP systems, except for some simple cases with certain approximations and assumptions. As an alternative and effective option, numerical methods, such as point charge, charge density, finite difference, integral equations and finite element methods, have been used to determine electric fields and DEP forces from electrode arrays [55, 84, 125-127]. In this thesis, a modeling environment based on a meshless method, i.e. least-square finite difference method (LSFD) [128, 129], was developed and implemented to model the DEP systems and simulate the particles' behavior. To demonstrate the feasibility of the developed environment, the work started from modeling and analysis of a typical case – the interdigitated electrode system. Through the discussions in this chapter, it is shown that the LSFD method is well suited for modeling various complex DEP systems.

0.0 Overview

Much of the study on DEP including DEP separation and trapping to date has been carried out in a somewhat qualitative manner. There are a variety of reasons for this: (i) the manipulation of particles using DEP deals with the microscale mechanics, which is difficult to fully understand. Particles suspending in the medium exposed to the electrostatic field may behave unsteadily due to the influences of the operating

conditions. Mutual interactions between the particles in DEP such as subpopulation trap are very difficult to analyze. (ii) In the common experimental conditions, dielectrophoretic operations such as separation and trapping of microparticles are easy to make with many electrode configurations, meaning that the detailed quantitative analysis may not be necessary in such conditions. Even so, one sees that further design of microscale DEP-based systems requires quantitative modeling and analysis, especially for the precise manipulation of micron-sized or submicron-sized particles, since the particle behavior may be extremely sensitive to the conditions in the microfabricated systems.

We go along the general way to study DEP. That is, before any experimental work begins, developing a feasible modeling environment to analyze and predict the phenomena of DEP under certain conditions. Although the calculation used the dipole approximation, multipolar DEP forces can also be accurately calculated with the modeling tools. The greatest advantage of this model environment is its feasibility and expansibility. Based on the case of interdigitated electrode system and particle separation, the model environment can be easily extended to investigate other designs such as polynomial electrode separation system, quadrupole trapping system, etc.

Unless specified otherwise, all simulations performed in this chapter had the following parameters. The solution's (De-Ionized water + saline solution) relative permittivity ϵ_m was 79; the solution's conductivity σ_m was 10 mS/m. Three latex (polystyrene) particles were investigated. Some of their parameters, including radius, density, CM factor K (real and imaginary parts) are listed in the Table 3.1.

Table 0.1 Three subpopulations of latex particles which will be investigated in this work.

Latex Beads	Radius a (μm)	Density ρ (g/cm^3)	$\text{Re}[K]$	$\text{Im}[K]$
#1	2.0	2.0	-0.1	+0.3
#2	3.0	2.0	-0.3	-0.4
#3	6.0	2.0	-0.5	-0.65

0.0 Governing equations

In order to determine the DEP force and particle behavior in the above system, the electric field generated by the electrode array needs to be accurately solved for further calculations. The equations governing the problem can be derived from Maxwell's equations, using the specific conditions in DEP. In this work, phasor notation is used. An arbitrary potential oscillating at frequency ω is defined with phasor notation as

$$\phi(\mathbf{x}, t) = \text{Re}[\tilde{\phi}(\mathbf{x})e^{i\omega t}] \quad (3.1)$$

where $i = \sqrt{-1}$, $\mathbf{x}$ is the position in the field described by coordinates and t is the time.

$\text{Re}[\cdot]$ indicates the real part of the value in the bracket and the tilde $\sim$ indicates the phasor $\tilde{\phi}(\mathbf{x}) = \phi_R(\mathbf{x}) + i\phi_I(\mathbf{x}) = \phi_0 e^{i\theta}$, where ϕ_R and ϕ_I are the real part and imaginary parts of the phasor, respectively; ϕ_0 is the amplitude and θ is the phase.

The electric field is then given by

$$\mathbf{E}(\mathbf{x}, t) = \text{Re}[\tilde{\mathbf{E}}(\mathbf{x})e^{i\omega t}] \quad (3.2)$$

where the vector $\tilde{\mathbf{E}}(\mathbf{x})$ is the corresponding phasor for the electric field given by

$$\tilde{\mathbf{E}}(\mathbf{x}) = -\nabla \tilde{\phi}(\mathbf{x}) = -(\nabla \phi_R(\mathbf{x}) + i\nabla \phi_I(\mathbf{x})) \quad (3.3)$$

For microelectrode structures used in DEP systems, magnetic effects can be neglected when compared with that of electric field since the energy stored in magnetic field is much smaller than the electrical energy [130, 131]. Therefore, Maxwell's

equations can be reduced to the electroquasistatic form [131]

$$\nabla \cdot \mathbf{D} = \rho_q \quad (3.4)$$

$$\nabla \times \mathbf{E} = 0 \quad (3.5)$$

$$\nabla \cdot \mathbf{J} + \frac{\partial \rho_q}{\partial t} = 0 \quad (3.6)$$

where ρ_q is the volume charge density. $\mathbf{J}$ is the current density. $\mathbf{D}$ is the electric flux density or displacement vector.

Eqn (3.4) is the well-known Gauss's equation, (3.5) indicates the curl free (irrotational) electric field, and (3.6) is the charge conservation equation. In the bulk electrolyte with permittivity ϵ and conductivity σ , the electric current density is given by Ohm's law, $\mathbf{J} = \sigma \mathbf{E}$, and $\mathbf{D}$ is given by the coupling equation, $\mathbf{D} = \epsilon \mathbf{E}$. Assuming that the permittivity ϵ and conductivity σ are independent of time and using the relation $\mathbf{E} = -\nabla \phi$, equations (3.4) and (3.6) can be combined as

$$\nabla \cdot ((\sigma + i\omega\epsilon)\nabla \tilde{\phi}) = 0 \quad (3.7)$$

In a homogeneous linear dielectric (uniform permittivity ϵ and conductivity σ), this reduces to Laplace's equation

$$\nabla^2 \tilde{\phi} = 0 \quad (3.8a)$$

For the real and imaginary parts of the phasor, the above equation leads to

$$\nabla^2 \phi_R = 0, \quad \nabla^2 \phi_I = 0 \quad (3.8b)$$

Eqn (3.8) indicates that in the microelectrode systems used in DEP, the field potential generated satisfies the elegant Laplace's equation. Together with appropriate

boundary conditions, the potential can be solved from the differential equations, giving rise to the field distribution. The time-averaged DEP force acting on the particle can then be calculated in the phasor form [86, 132]

$$\langle \mathbf{F}_{\text{DEP}} \rangle = \frac{1}{2} \mathbf{Re}[(\tilde{\mathbf{p}} \cdot \nabla) \tilde{\mathbf{E}}^*] = \frac{1}{2} v \mathbf{Re}[\alpha (\tilde{\mathbf{E}} \cdot \nabla) \tilde{\mathbf{E}}^*] \quad (3.9)$$

Where $\langle \rangle$ denotes time-averaged value. The relationship $\tilde{\mathbf{p}} = v \alpha \tilde{\mathbf{E}}$ is used. v is the volume of the particle and α is the effective polarisability of the particle. The asterisk $*$ denotes the conjugate value of the quantity.

Using vector calculation, the expression (3.9) can be rewritten to

$$\langle \mathbf{F}_{\text{DEP}} \rangle = \frac{1}{4} v \mathbf{Re}[\alpha \nabla (\tilde{\mathbf{E}} \cdot \tilde{\mathbf{E}}^*)] - \frac{1}{2} v \mathbf{Re}[\alpha \nabla \times (\tilde{\mathbf{E}} \times \tilde{\mathbf{E}}^*)] \quad (3.10)$$

The two terms in Eqn (3.10) indicate the two components, cDEP and twDEP force, acting on the particle, which are

$$\begin{aligned} \langle \mathbf{F}_{\text{cDEP}} \rangle &= \frac{1}{4} v \mathbf{Re}[\alpha] \nabla (|\mathbf{Re}[\tilde{\mathbf{E}}]|^2 + |\mathbf{Im}[\tilde{\mathbf{E}}]|^2) \\ &= \frac{1}{4} v \mathbf{Re}[\alpha] \nabla (|\nabla \phi_R|^2 + |\nabla \phi_I|^2), \end{aligned} \quad (3.11a)$$

$$\begin{aligned} \langle \mathbf{F}_{\text{twDEP}} \rangle &= -\frac{1}{2} v \mathbf{Im}[\alpha] (\nabla \times (\mathbf{Re}[\tilde{\mathbf{E}}] \times \mathbf{Im}[\tilde{\mathbf{E}}])) \\ &= -\frac{1}{2} v \mathbf{Im}[\alpha] (\nabla \times (\nabla \phi_R \times \nabla \phi_I)). \end{aligned} \quad (3.11b)$$

The process to solve the problem is summarily described in Figure 3.1. In principle, the particle motion (displacement) is possible to be determined when all the external forces are known. For micron-sized particles suspending in the medium of low conductivity, Brownian motion and electrohydrodynamics forces are negligible when compared with the DEP force [10]. Thus, the particle behavior depends upon the combined effects of DEP force, gravitational force and viscous force.

A numerical approach based on the least-square finite difference scheme was used for solving the above problems, which are discussed in the following sections.

Figure 3.1 Major steps of the modeling environment. Starting from solving electric potential, the electric field is then calculated. Particle motion is determined by coupling the DEP force, gravitational force, viscous force, etc on the particle.

0.0 The least square finite difference method

Traditionally, the finite element method (FEM) can well handle problems of complex geometry. A significant drawback of the FEM is the need to maintain detailed structural information about the computational mesh, comprising all nodal and element-based connectivity and hierarchical data. This may become especially evident in solving the microelectromechanical systems because of the mixed-energy domains governing the behavior of the device.

To rectify some of the shortcomings of the FEM and yet to preserve its ability to accommodate geometric complexity, a group of so-called meshless or mesh free methods

have been proposed in the last two decades. The terms meshless and mesh free refer to the ability of the method to construct functional approximation or interpolation entirely from information at a set of nodes, without any pre-specified connectivity or relationships among the nodes. A number of mesh-less methods have been proposed to date: smoothed particle hydrodynamics method (SPH) [133], the diffuse element method (DEM) [134], the element free Galerkin (EFG) method [135], the reproducing kernel particle method (RKPM) [136], partition of unity method (PUM) [137], the hp-clouds method [138], the finite point method (FP) [139], the meshless local Petrov-Galerkin (MLPG) method [140], and the general finite difference method (GFD) [141] are among the better known methods. One of the key advantages of mesh free methods is the computational ease of adding and subtracting nodes from the pre-existing set of nodes.

Figure 3.2 Diagram of supporting points around the reference point. Supporting points disperse within a circular domain D_0 with a diameter of $2d_0$.

To develop an efficient mesh free method, a weighted least square based finite difference scheme was proposed by Ding H *et al* [128]. It can be viewed as a further

development of the GFD method. The method directly approximates the interested order of derivatives in the governing equations. The approximation of partial derivatives was formulated from the Taylor series expansions and weighted least square technique. The method is simple to apply when compared to integral-based formulation, and much more flexible than conventional FD methods in practical applications.

The method is based on the use of a weighted least-square approximation procedure together with a two dimensional Taylor series expansion. As shown in Figure 3.2, the functional values (e.g. electrical potential) near a reference node, $\bullet$, can be approximated by the functional value and its derivatives at the reference point by using up to 3-order 2-D Taylor series expansion

$$\begin{aligned} \phi \approx \phi_{\bullet} &+ \Delta x \left(\frac{\partial \phi}{\partial x} \right)_{\bullet} + \Delta y \left(\frac{\partial \phi}{\partial y} \right)_{\bullet} + \frac{1}{2} (\Delta x)^2 \left(\frac{\partial^2 \phi}{\partial x^2} \right)_{\bullet} + \frac{1}{2} (\Delta y)^2 \left(\frac{\partial^2 \phi}{\partial y^2} \right)_{\bullet} + (\Delta x)(\Delta y) \left(\frac{\partial^2 \phi}{\partial x \partial y} \right)_{\bullet} \\ &+ \frac{1}{6} (\Delta x)^3 \left(\frac{\partial^3 \phi}{\partial x^3} \right)_{\bullet} + \frac{1}{6} (\Delta y)^3 \left(\frac{\partial^3 \phi}{\partial y^3} \right)_{\bullet} + \frac{1}{2} (\Delta x)^2 (\Delta y) \left(\frac{\partial^3 \phi}{\partial x^2 \partial y} \right)_{\bullet} + \frac{1}{2} (\Delta x)(\Delta y)^2 \left(\frac{\partial^3 \phi}{\partial x \partial y^2} \right)_{\bullet} \end{aligned} \quad (3.12)$$

This equation consists of 9 unknowns, of which there are totally 2 first order, 3 second order, and 4 third order derivatives, respectively. Like conventional FD scheme, these 9 derivatives can be obtained by solving 9 equations. By applying Eqn (3.12) at 9 supporting points within a domain, D_0 , about the reference point, the 9 equations are given

$$\phi_j - \phi_{\bullet} = S_j^T \cdot d\phi, \quad j = 1, 2, \dots, 9 \quad (3.13)$$

where

$$S_j^T = \left[\Delta x_j, \Delta y_j, \frac{1}{2}(\Delta x_j)^2, \frac{1}{2}(\Delta y_j)^2, (\Delta x_j)(\Delta y_j), \right. \\ \left. \frac{1}{6}(\Delta x_j)^3, \frac{1}{6}(\Delta y_j)^3, \frac{1}{2}(\Delta x_j)^2(\Delta y_j), \frac{1}{2}(\Delta x_j)(\Delta y_j)^2 \right] \quad (3.14)$$

$$\mathbf{d}\Phi^T = \left[\left(\frac{\partial \phi}{\partial x} \right)_\bullet, \left(\frac{\partial \phi}{\partial y} \right)_\bullet, \left(\frac{\partial^2 \phi}{\partial x^2} \right)_\bullet, \left(\frac{\partial^2 \phi}{\partial y^2} \right)_\bullet, \left(\frac{\partial^2 \phi}{\partial x \partial y} \right)_\bullet, \right. \\ \left. \left(\frac{\partial^3 \phi}{\partial x^3} \right)_\bullet, \left(\frac{\partial^3 \phi}{\partial y^3} \right)_\bullet, \left(\frac{\partial^3 \phi}{\partial x^2 \partial y} \right)_\bullet, \left(\frac{\partial^3 \phi}{\partial x \partial y^2} \right)_\bullet \right] \quad (3.15)$$

$$\Delta x_j = x_j - x_\bullet, \quad \Delta y_j = y_j - y_\bullet \quad (3.16)$$

The derivative vector, $\mathbf{d}\Phi$, can be obtained as

$$\mathbf{d}\Phi = \mathbf{S}^{-1} \Delta\Phi \quad (3.17)$$

where

$$\Delta\Phi^T = [\phi_1 - \phi_\bullet, \phi_2 - \phi_\bullet, \dots, \phi_9 - \phi_\bullet] \quad (3.18)$$

$$\mathbf{S}^T = [S_1, S_2, \dots, S_9]_{9 \times 9} \quad (11)$$

However, the matrix, $\mathbf{S}$, tends to become ill-conditioned or singular when: (1) one or more supporting points are very close to the reference point; and (2) supporting points are very close to each other. In [128], a scaling matrix $\mathbf{D}$, least-square technique and weighting function $\mathbf{W}$ (Matrix) are applied to overcome these difficulties. The derivative vector $\mathbf{d}\Phi$ is then optimized as

$$\mathbf{d}\Phi = \mathbf{D}(\bar{\mathbf{S}}^T \mathbf{W} \bar{\mathbf{S}})^{-1} \bar{\mathbf{S}}^T \mathbf{W} \Delta\Phi \equiv \mathbf{C} \Delta\Phi \quad (3.19)$$

where

$$\mathbf{D} = \text{diag}(d_o^{-1}, d_o^{-1}, d_o^{-2}, d_o^{-2}, d_o^{-2}, d_o^{-3}, d_o^{-3}, d_o^{-3}, d_o^{-3}) \quad (3.20)$$

$$\mathbf{W} = \text{diag}(W_1, W_2, \dots, W_{SN}) \quad (3.21)$$

d_o is the radius of the domain D_0 ; W_i ($i=1,2,\dots,SN$) is the weighting function whose value is positive and decreases with increasing distance from the reference point.

$$\bar{\mathbf{S}} = \mathbf{S}\mathbf{D} \quad (3.22)$$

In the above optimization, matrix $\mathbf{D}$ is used to overcome the singularity from the first case, while the least-square technique is to avoid the second case [128]. The weighting function $\mathbf{W}$ is introduced to improve the even error-distribution problem; more supporting points ($SN > 9$) are included.

As discussed in [128], the above scheme allows us to approximate the second-order derivatives to the second-order accuracy and the first-order derivatives to the third-order accuracy. The highest order of the derivative of the unknown that can be determined by equation (3.19) depends on the order of the Taylor series approximation. Higher order (e.g. fourth order) approximation needs to be used to obtain higher order derivatives, in which more supporting points are needed.

Unlike mesh generation in conventional FD/FE methods, there is no pre-requirement for the distribution of points in the present method. Point generation in the present method is consequently easier in complex domains as compared with FD/FE methods. In general, there are three grid generation algorithms that can be selected according to the practical application. They are:

- (1) Use the grid generation algorithm of conventional finite difference schemes if the geometry of the domain is very simple, such as rectangles or circles.
- (2) Use the random point generation algorithm if the geometry of the domain is complex and only concerned with Dirichlet boundary condition.
- (3) Use a fast hyperbolic grid or algebraic formulation to produce several layers of locally orthogonal grids near the boundary, and use the random point generation

algorithm to generate other nodes in the rest of the computational domain if the geometry of the domain is complex and Neumann boundary conditions are involved.

The third algorithm is highly recommended as it is much more flexible than the other two when the geometry is complex. In the current problem for the interdigitated electrode DEP system, because the computation domain is rectangle, the first algorithm is used. Higher resolution is required near the edges of the electrodes, thus the density of the point distribution in these areas can be strengthened by adding points into the pre-existing set of points.

0.0 Numerical implementation

The LSFD method is used to solve the DEP problems in this work, including calculation of the electrical potential, the electric field distribution, DEP forces and particle behavior. The calculation starts from solving the electrical potential from the Laplace's equation.

The two items in the left hand of the 2-dimensional Laplace's equation correspond to the third and the fourth items in the derivative vector as indicated in (3.15), respectively. From Eqn (3.19), these two items for each reference point can be represented as

$$\left(\frac{\partial^2 \phi}{\partial x^2}\right)_{\bullet} = \sum_{j=1}^m C_{3j}(\phi_j - \phi_{\bullet}) \quad (3.23a)$$

$$\left(\frac{\partial^2 \phi}{\partial y^2}\right)_{\bullet} = \sum_{j=1}^m C_{4j}(\phi_j - \phi_{\bullet}) \quad (3.23b)$$

where ϕ can be the real part or imaginary part of the potential phasor. m is a number greater than 9 representing the number of the supporting points. C_{3j} represents the entry of Matrix C at 3-rd row and j -th column, and C_{4j} likewise.

Substituting Eqns (3.23a, b) into the Laplace's equation (2D) gives the following relationship

$$\left(-\sum_{j=1}^m (C_{3j} + C_{4j}) \right) \phi + \sum_{j=1}^m (C_{3j} + C_{4j}) \phi_j = 0 \quad (3.24)$$

Repeat the same process to each point in the domain. That is, taking every point as the reference point and its corresponding supporting points in the domain, and then applying the 'LSFD' discretization process. A series of equations of the form of (3.24) will be generated in this way. Using the matrix form for these equations, a general matrix equation is achieved by adjusting coefficients in a corresponding row of the coefficient matrix

$$\mathbf{A} \cdot \boldsymbol{\Phi} = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2n} \\ & & \ddots & \\ a_{n1} & a_{n2} & \cdots & a_{nn} \end{bmatrix} \cdot \begin{bmatrix} \phi_1 \\ \phi_2 \\ \vdots \\ \phi_n \end{bmatrix} = 0 \quad (3.25)$$

where n is the number of the points in the domain, the matrix $\mathbf{A}$ is given by the LSFD process. The quantities ϕ_i ($i = 1, 2, \dots, n$) are the unknowns, i.e. electric potentials here. Notice that $\mathbf{A}$ is a sparse matrix in which every row only has m nonzero entries (m is the number of supporting points).

By applying the boundary conditions into Eqn (3.25), the electric potential distribution in the domain can be solved. After obtaining the potential distribution, the

electric field and the DEP forces can be consequently computed from the derivatives (see Equations (3.15), (3.19)) of the potential.

Figure 3.3 The diagram of the modeling environment based on LSFD meshless method.

The modeling environment based on the meshless method is schematically shown in Figure 3.3. Four basic modules form the bottom tier of the system. Point generation is accomplished in ‘gridding’. The problem domain is defined, and the points are distributed in the domain using the aforementioned grid generation algorithms. The coordinates of the points are stored in the Matrices. Point distribution reflects the geometry property of the domain. After point generation, ‘LSFD’ module applies the least-square finite difference scheme to every point in the domain. The coefficient matrix C (Eqn (3.19)) is obtained. This matrix presents the improvement of LSFD process on the distribution of supporting points around the reference point. Further, the final matrix A in (3.25) is obtained. ‘Load/B.C.s’ module imposes boundary conditions to the system. The imposition of boundary conditions is an important step and varies case by case.

Finally, the problem is solved using the ‘Solve’ module. Results are stored in matrices for postprocessing. Particle behavior is simulated using the results from the solution. ‘User Interface’ enables the user to interact with the respective module in practice.

In order to evaluate the performance of the modeling environment developed, it has been applied to a practical problem – interdigitated electrode DEP systems.

0.0 The dielectrophoretic array

Generally, different electrode configurations generate different distributions of electric field for specific applications. Basically, the electrode system should have the function of generating the required electric field, and be easily fabricated and arrayed. A number of electrode patterns have been proposed and used by various researchers to study DEP, such as interdigitated electrode array (IDA), polynomial electrode, castellated electrode, and three-dimensional cage design (see, e.g. [142-145]). In this chapter, the study is focused on the interdigitated electrode array (Figure 3.4), for its features of compact and easy layout. The model was initially developed with its implementation to this electrode configuration, but also with the capacity to model other electrode systems which are expected to be proposed in this thesis. Like many of the biomedical microsystems, the electrode array sits on the glass substrate and at the bottom of the microchannel containing particle suspension (electrolyte). The width of electrode stripe is d_1 and the spacing (gap width) between adjacent electrodes is d_2 , and the term (characteristic length) d is defined by $d = (d_1 + d_2)/2$. The system will act in the fashion of conventional DEP (cDEP) when it is driven with 180-degree phase shifted voltage supplied to the

electrodes in sequence, while the system will act as traveling wave DEP when a multiphase voltage is applied. For cDEP, the spatial period is $2d$, while twDEP electrode configuration has the period of nd , where n is the number of the phases of the applied signal. This section demonstrates the numerical modeling with the above LSFD-based environment for cDEP. twDEP is discussed in next section.

Figure 3.4 Schematic diagram of interdigitated electrode array. The electrodes are fabricated on the surface of a glass substrate using microfabrication technique. The width of electrode stripe is d_1 and the spacing between adjacent electrodes is d_2 . Driven with 180-degree or 90-degree (or other) shifted phase voltages V_0 , the array can be used for cDEP or twDEP.

0.0.0 General boundary conditions

For the interdigitated electrode array shown in Figure 3.4, the problem reduces to a two-dimensional problem in x - y plane since the dimension of electrode in z direction is much larger than those in x and y directions. To solve the two-dimensional Laplace's equation, the problems for the conventional dielectrophoretic array and the traveling

wave dielectrophoretic array have different unit cells and boundary conditions. However, certain boundary conditions are common to these two configurations, which include:

(1) The potential and electric field both go to zero at heights sufficiently far away from the electrode, i.e. a Dirichlet condition ($\tilde{\phi} = 0$) or Neumann condition ($\partial\tilde{\phi}/\partial n = 0$) can be specified when y goes to infinity.

(2) The microfabricated electrodes are much thinner (thickness $\sim 100\text{nm}$) than their width (tens of μm). Therefore, we ignore the electrode thickness so that the potential on the electrodes is specified at the surface of the substrate, i.e. at $y = 0$.

(3) At the electrode-uncovered interface between the liquid and the substrate (glass), i.e. the gap between two adjacent electrodes, the conditions here can be derived from the fundamental properties of the electrostatic field – curl free and divergence free. The first boundary condition, due to the curl-free condition imposed upon the field, requires that the tangential component of the electric field is continuous across the interface

$$\hat{\mathbf{n}} \times (\tilde{\mathbf{E}}_g - \tilde{\mathbf{E}}_l) = 0 \quad (3.26a)$$

where $\hat{\mathbf{n}}$ is the unit normal vector at the interface. The subscripts g and l indicate the glass and liquid, respectively.

An entirely equivalent statement to this condition in terms of scalar potential is

$$\tilde{\phi}_g = \tilde{\phi}_l \text{ or } \partial\tilde{\phi}/\partial n = 0 \quad (3.26b)$$

Another condition derived from the divergence-free condition describes the continuity of the normal component of the total current (displacement and free current) at the interface

$$\frac{\partial}{\partial t}(\epsilon_g \tilde{\mathbf{E}}_g \cdot \hat{\mathbf{n}}) + \sigma_g \tilde{\mathbf{E}}_g \cdot \hat{\mathbf{n}} = \frac{\partial}{\partial t}(\epsilon_l \tilde{\mathbf{E}}_l \cdot \hat{\mathbf{n}}) + \sigma_l \tilde{\mathbf{E}}_l \cdot \hat{\mathbf{n}} \quad (3.27a)$$

Using phasor calculation, this turns into

$$(\sigma_g + i\omega\epsilon_g)\tilde{\mathbf{E}}_g \cdot \hat{\mathbf{n}} = (\sigma_l + i\omega\epsilon_l)\tilde{\mathbf{E}}_l \cdot \hat{\mathbf{n}} \quad (3.28b)$$

Generally, the component of the electric field in the electrolyte (liquid) is negligible compared to that of the glass for all frequencies because the conductivity and permittivity of the electrolyte are much larger than that of the glass, respectively. Therefore, the boundary condition at this interface can be simplified into Eqn (3.26b), i.e. $\partial\tilde{\phi}/\partial n = 0$.

This condition can be interpreted as the field generated by a 2D charge distribution on the electrodes and these charges will always generate a field tangential to the plane of the electrodes [126]. The approximation is consistent with the zero-thickness-electrode approximation. In order to obtain an analytical expression for the model, the assumption of a linear change in the potential (first-order linear approximation) at the interface between the electrodes was used elsewhere in Ref. [132]. It should be noted that the first-order linear approximation on this boundary may induce considerable inaccuracy in the results, which are discussed in 3.5.5.

The remaining boundary conditions, i.e. those for the sides (left and right) of the problem and for the potential on the electrodes, specifically depend on the electrode configurations and the applied signals.

0.0.0 Complete boundary conditions of cDEP

For cDEP, the phases of electric voltage supplied are depicted in Figure 3.4, with an AC signal of phase 0° and 180° and frequency ω alternately applied to the electrodes.

In this case, the real part and imaginary part of the potential phasor at the electrodes are shown in Figure 3.5. The imaginary part of the potential phasor is zero so that the phasor reduces to its real part. Thus only the real part, ϕ_R , of the potential phasor needs to be solved from the 2D Laplace's equation (for simplification, $\phi = \phi_R$)

$$\frac{\partial \phi^2}{\partial x^2} + \frac{\partial \phi^2}{\partial y^2} = 0 \quad (3.29)$$

Figure 3.5 A schematic diagram of the electrode array with a two-phase signal applied. The vertical broken lines indicate the length (period $\lambda=2(d_1+d_2)$) over which the system repeats. The potential phasor, $\tilde{\phi}$, real part, ϕ_R , and imaginary part, ϕ_I , of potential phasor and the potential ϕ on the electrodes are also shown. From the diagram, the imaginary part of the potential phasor is zero, and therefore the phasor reduces to the real part.

The boundary conditions are repeated for every two electrodes and are symmetric, meaning that the problem space to be considered does not need to be larger than two electrodes. Further investigation into the symmetry of the electrode system leads to more simplified conclusion. As shown in Figure 3.6, the smallest problem space can be reduced to a quarter of the size of the period, which is highlighted by the thicker solid

lines. The vertical edge running through the center of the electrode is a line of even symmetry, i.e. $\partial\phi/\partial n = 0$, while that running through the center of the gap is a line of odd symmetry, i.e. $\phi = 0$. The potential distribution of the whole system can be determined by only solving this basic cell, using the even and odd symmetric properties. In this work, a problem space, which is of a double size of that shown in Figure 3.6, was preferably chosen. This extends the basic cell to cover the whole electrode, and the Dirichlet boundary condition ($\phi = 0$) is used other than the Neumann condition ($\partial\phi/\partial n = 0$) at the sides.

Figure 3.6 The smallest problem space for solving the problem, highlighted with thicker solid lines. The system is symmetric about the vertical line on the left (even symmetry) and on the right (odd symmetry). The repeated dotted cells describe the whole system.

The problem space with complete boundary conditions used in this calculation is shown in Figure 3.7. The space covers an arbitrarily selected electrode with positive voltage applied. The two sides of the space are at the centers of the gaps, imposed with the Dirichlet conditions. The upper boundary is selected sufficiently far away from the

electrode. The exact boundary condition at $y = 0$ is such that the derivative of the potential is zero (Neumann boundary condition) at the gaps while the potential is definite on the electrode. This type of mixed boundary condition leads to the difficulty of obtaining analytical solutions.

Figure 3.7 The problem space for the dielectrophoretic array showing complete boundary conditions. The space covers an arbitrarily selected electrode with positive voltage applied, and two halves of the gap.

0.0.0 Non-dimensional equations

For mathematical simplicity as well as avoiding extreme numbers in numerical calculations, non-dimensionalization is employed by scaling the variables according to typical values. In this work, the potential is scaled by V_0 , and the displacements are scaled by characteristic length d with $d = (d_1 + d_2)/2$. This gives the non-dimensional potential $\phi'_R = \phi_R / V_0$ and the displacements $x' = x / d$, $y' = y / d$. The non-dimensional potentials also satisfy Laplace's equation:

$$\frac{V_0^2}{d^2} \nabla'^2 \phi'_R = 0 \Rightarrow \nabla'^2 \phi'_R = 0 \quad (3.30)$$

where the Del vector operator $\nabla' = \frac{\partial}{\partial x'} \hat{x} + \frac{\partial}{\partial y'} \hat{y}$; $\hat{x}$ and $\hat{y}$ are the unit vectors in x and y directions, respectively.

The relationship between the non-dimensional field-square gradient $\nabla' E'^2$ and the dimensional one is given as

$$\nabla E^2 = \frac{V_0^2}{d^3} \nabla' E'^2 \quad (3.31)$$

The non-dimensional DEP force components are given as

$$F'_{DEP,x'} = (\nabla' E'^2)_{x'} \quad \text{and} \quad F'_{DEP,y'} = (\nabla' E'^2)_{y'} \quad (3.32)$$

And the cDEP force is given by

$$\mathbf{F}_{DEP} = G_{DEP} \frac{V_0^2}{d^3} \nabla' E'^2 \quad (3.33)$$

where the coefficient G_{DEP} is defined as $G_{DEP} = \pi \epsilon_m a^3 \text{Re}[K(\omega)]$.

0.0.0 Weighted LSFD solutions

The first step in the LSFD solving process is to define grid point distribution in the problem space. Nonuniform grid point distribution was used to solve the problem as shown in Fig. 3.8. The grid spacing was gradually and smoothly varied from 0.5 μm to 2 μm . Grid points were distributed more densely near the electrode edges and relatively sparsely in the area away from the electrodes. In practice, the upper boundary was specified at height sufficiently ($h > 16d = 8(d_1 + d_2)$) above the electrodes. The grid points generation was carried out by the module ‘gridding’ in the computer code. The information of the points are recorded in matrices.

Figure 3.8 Nonuniform point distribution for solving the problem.

Figure 3.9 Flowchart for program based on weighted LSFD method.

After grid generation, LSFD method is applied to discretize the governing equation at the grid points. The final form of the linear equations is described. Finally, the problem is solved by applying boundary conditions. The flowchart of the computer program for this process written in Matlab is shown in Figure 3.9. The domain is specified by the user.

For the interdigitated electrode system, the domain is bounded by a rectangle (Fig. 3.7), which is defined by the length and width. Numerical convergence study was carried out to determine the most suitable grid point distribution.

Figure 3.10 The solution of the problem close to the electrode. (a) Contour of the non-dimensional real part, ϕ_R' , of electric potential phasor. (b) Magnitude of the electric field E' .

Results obtained for a representative case with $d_1 = d_2 = 10 \mu\text{m}$ are presented in this section. Different scales are used in getting the non-dimensional values. The contours of the non-dimensional real part, ϕ_R' , of electric potential phasor and the electric field E' ,

are plotted in Figure 3.10a and b, respectively, showing that the potential decreases rapidly with the increase of the distance y away from the electrode. The electric field was calculated by the first derivative of the potential, $\mathbf{E} = -\nabla\phi$, which is given in the derivative vector (Eqn (3.15)). The maximum electric field strength exists near the edges of the electrode, where particles will be attracted when positive DEP takes place. One of the most important features of the field is its symmetry. The field intensity becomes independent of lateral position x' at sufficient heights above the electrode.

Figure 3.11 (a) Logarithmical magnitude of the field-square gradient, $\text{Log}(\nabla' E'^2)$. Note that

DEP force is proportional to $\nabla' E'^2$. (b) Directions of $\nabla' E'^2$.

The magnitude of the field-square gradient is logarithmically plotted in Figure 3.11a. It can be seen that the value of $\nabla' E'^2$ above a certain height of $y' \approx 1$ away from the electrode is a constant against x' across the array. This is also true for the DEP force because the DEP force is proportional to the gradient of the field square. It means that the DEP force for a certain type of particle will be a constant against x' at height of $y' \sim 1$ and above. This feature is precisely what we can make use of to separate different types of microparticles at different heights.

The direction of the vector $\nabla' E'^2$ is plotted in Figure 3.11b. It is observed that at the height $y' \sim 1$ and above, the vectors point straight downward towards the electrode plane. Close to the electrode, the vectors point more and more towards the electrode edges. The pattern of the DEP force is symmetrical about the centre vertical line of the electrode.

Figure 3.12 The magnitude of $\nabla' E'^2$ is plotted versus height.

Figure 3.13 The magnitude of $\nabla' E'^2$ versus the height (from $y'=1$) along the vertical line $x'=0.5$ is fitted as an exponential function.

Since the electric field and the dielectrophoretic force solely depend on the value of y at sufficient heights and the levitation height usually falls into this range of height, the effect of the distance y on the DEP force is investigated, as shown in Figure 3.12. The forces are plotted versus the height above the electrode along two vertical lines, i.e. $x'=0.5$ and $x'=1.0$ (or $x=5\ \mu\text{m}, 10\ \mu\text{m}$). The logarithmic magnitude of $\nabla' E'^2$ is found to become linear above the height of about $y'=1$ (or $y \sim d = 10\ \mu\text{m}$), showing that the value of the DEP force decreases exponentially as the height increases. It implies that above this height, the gradient of field square, $\nabla' E'^2$, can be approximated by an exponentially decreasing function of the height y' , and consequently the DEP force is also an exponential function of the height. Figure 3.13 shows the fitting result of the magnitude of $\nabla' E'^2$ with an exponential function of the form

$$\nabla' E'^2 = A e^{By'} \quad (3.34)$$

with A and B being the constants to be determined by the fitting process. For the case of $x'=0.5$, the constants are calculated to be $A=10.28212 \pm 0.00758$, $B=-3.14811 \pm 0.00065$, that is

$$\nabla' E'^2 = 10.28212 e^{-3.14811 y'} \quad (3.35)$$

This equation as well as the results presented earlier demonstrate that the DEP force acting on the particle converges for any x' provided $y' > 1$ and decays exponentially as the particle moves far away from the electrode plane. However, this conclusion is not applicable for the case where the particle is located very close to the electrodes, i.e. $0 < y' < 1$. The DEP force in this regime behaves more complicated. Figures 3.14a and b show, respectively, the lateral component $F'_{DEP,x'}$ and vertical component $F'_{DEP,y'}$ of the DEP force near the electrodes at different heights. It can be seen that both lateral and vertical DEP forces decrease steadily with increasing height. The vertical force is symmetric with respect to the vertical line at $x'=1$, while the lateral force is anti-symmetric. Notice that the lateral force component can change its sign (so the direction) near the edges of the electrode and the center of the electrode, while the vertical force component remains unchanged in positive or negative sign.

Figure 3.14 Non-dimensional DEP force components in the region near the electrode. (a) x component $F'_{DEP,x'}$. (b) y component $F'_{DEP,y'}$.

These results prove and provide the necessary means for separation or manipulation of microparticles in practical applications. For example, under the condition of negative DEP, the DEP force at sufficient height ($y' > 1$) is independent of the lateral position (x') and expels particles away from the electrode plane. Hence, particles that experience different DEP force will be levitated to different heights where gravity is balanced by the DEP force.

0.0.0 Particle movement

A particle's movement is determined by the combined effects of DEP force, hydrodynamic force, buoyancy, electrohydrodynamic forces and Brownian motion. For micron-sized particles in solution of low conductivity, the last three (buoyancy, electrohydrodynamic forces and Brownian motion) are negligible. The governing equation for the particle movement can be obtained by

$$\begin{aligned} m\ddot{x}(t) &= F_{DEP,x} - 6\pi\eta a\dot{x}(t) \\ m\ddot{y}(t) &= F_{DEP,y} - (\rho_p - \rho_m)vg - 6\pi\eta a\dot{y}(t) \end{aligned} \quad (3.36)$$

where the $(\cdot)$ denote the differentiation with respect to time. m is the mass of the particle. ρ_p and ρ_m are the density of the particle and fluid, respectively. η is the viscosity coefficient of the fluid. a is the radius of the particle. v is the volume of the particle. g is the acceleration of gravity. The terms $-6\pi\eta a\dot{x}$ and $-6\pi\eta a\dot{y}$ represent the Stokes drag on the particle. $F_{DEP,x}$ and $F_{DEP,y}$ are the DEP forces in x and y directions. Since the DEP forces are obtained from numerical method and given in numerical forms, Eqn (3.26) have to be solved in a numerical manner. This was done using a finite difference method (see Appendix B).

The three types of particles and parameters described in section 3.1 were investigated using the electrode spacing $d_1=d_2=10\text{ }\mu\text{m}$. For the particle # 3, the levitation height calculated from the LSFD meshless method is $28.2\text{ }\mu\text{m}$ with applied voltage 5 Volts.

Figure 3.15a shows the lateral (in x direction) movement of latex #3 particle with different initial positions in the solution. It is observed that the particle having an initial

position at the center of the problem space, i.e. $x_0=10\text{ }\mu\text{m}$, has a very small lateral movement due to the symmetry of the field and the DEP force about the centre vertical lines, and the independence of the DEP force with x position. With other initial positions, the particle moves laterally at first but soon reaches to a steady x position.

Figure 3.15 Particle movements. (a) Lateral displacement of the particle with different initial positions. (b) Vertical displacement of the particle with different initial positions.

However, the particle moves drastically in vertical direction (Figure 3.15b) for the DEP force depends on the vertical positions. As expected, the particle stays stably at a

certain height after a short period of acceleration and oscillation. That is the levitation height of the particle. More interestingly, the three types of particles are automatically levitated to different heights (Fig. 3.16), and hence the separation of those particles is achieved. The differences between these heights are around a few micrometers. Thus we can draw a conclusion that different particles experiencing n-DEP will be automatically balanced at different heights respectively and the spatial separation can be realized.

Figure 3.16 Three (#1, #2, #3) particles with different dielectric properties are balanced in different heights. K is the CM factor.

The levitation height depends on a number of parameters, such as applied voltage, frequency, electrode size, etc. Again, for the particle #3 (radius $a = 6 \mu\text{m}$, density $= 2.0 \text{ g/cm}^3$) with the real part of CM factor of -0.5 , the levitation height is shown in Table 3.2 for various applied voltages and various electrode sizes. d is the characteristic width of the electrode.

Table 3.2 Levitation heights of the particle #3 for different voltages and electrode sizes.

	$V_0=2$ V	$V_0=3$ V	$V_0=4$ V	$V_0=5$ V	$V_0=6$ V
	Levitation height (μm)				
$d=10$ μm	22.3	24.8	26.7	28.2	29.3
$d=20$ μm	31.3	36.5	40.1	43.0	45.3
$d=30$ μm	38.3	46.1	51.6	55.8	59.3
$d=40$ μm	40.1	51.1	58.2	64.2	68.1

One sees the trend of the levitation with respect to the variety of the applied voltage and electrode size, as shown in Fig. 3.17. Although larger levitation height is obtained for larger electrode width and electrode gap (larger characteristic width), the particle is less stably levitated for the gradient of the field is smaller there.

Figure 3.17 The levitation heights vary with the applied voltages and electrode sizes.

0.0.0 Comparison study

The above calculations used the exact boundary condition at the bottom boundary, i.e. the Neumann condition $\partial\phi/\partial n=0$, which leads to more accurate results than those obtained using the first-order approximate boundary condition. The latter (Figure 3.18) assumes that the potential on the bottom surface changes linearly at the gaps between the

electrodes. This assumption led to the possible analytical solutions of the Laplace's equation for the interdigitated electrode arrays ([132] & Appendix C). However, considerable errors may be induced in the results because of the use of such an assumption.

Figure 3.18 The first-order approximate boundary condition at the bottom boundary. Linear

potential change is assumed at the inter-electrode gaps.

(a)

(b)

Figure 3.19 Comparison of the numerical and analytical solutions. (a) Comparison of the electric

field. (b) Comparison of the DEP force vector.

The problem was solved again with the LSFD method using the first-order approximate boundary condition. To validate the performance of the developed model, the numerical results obtained with the above approximation were compared with the analytical solutions presented in Appendix C. The logarithmic electric field and the DEP force vector ∇E^2 versus heights along the line $x'=0.5$ ($x=5\text{ }\mu\text{m}$) are shown in Figure 3.19a and b, respectively. Both the numerical and analytical results are shown. The numerical and analytical results agree very well with each other, especially at heights above $y=10\text{ }\mu\text{m}$.

However, one may see that the assumption of linear potential change at lower boundary can cause considerable errors, since the electric field is highly sensitive to the boundary at that area. To compare the numerical solutions obtained using the first-order approximate boundary condition and exact boundary condition, the time-averaged DEP force near the electrode plane, i.e. $y'=0.1$ ($y=1\text{ }\mu\text{m}$) was investigated. The comparison in Figure 3.20 shows that there is a discrepancy between the solutions from different boundary conditions. The difference of the DEP forces is more noticeable near the electrode edges ($x'=0.5, 1.5$), where the electric field and the DEP force are extremely high. Therefore, the force calculated from the approximate boundary condition deviates considerably from that obtained by the exact boundary condition in the area above the gap between the electrodes.

Figure 3.20 Comparison of the numerical time-averaged DEP forces obtained using the approximate and exact boundary conditions. (a) The X-component of the DEP force at $y'=0.1$ ($y=1\ \mu\text{m}$). (b) The Y-component of the DEP force at $y'=0.1$.

3.6 The traveling wave array

The four-phase traveling wave array (Figure 3.4) was simulated using the aforementioned numerical modeling approach in this section. The difference is that the DEP force has two components – in-phase and out-of-phase components. The imaginary part of the potential phasor is nonzero so that its contribution must be taken into account.

3.6.1 Non-dimensional equations

Non-dimensional equations were given in the same manner as in section 3.5.3. Define the non-dimensional potential $\tilde{\phi}' = \tilde{\phi} / V_0$ and the displacements $x' = x / d$, $y' = y / d$. The non-dimensional potentials also satisfy Laplace's equation

$$\frac{V_0^2}{d^2} \nabla'^2 \tilde{\phi}' = 0 \Rightarrow \nabla'^2 \phi'_R = 0, \nabla'^2 \phi'_I = 0 \quad (3.37)$$

Therefore, Eqn (3.11) for the expression of cDEP and twDEP components are rewritten as

$$F_{cDEP} = A_{cDEP} \frac{V_0^2}{d^3} \nabla' (|\nabla' \phi'_R|^2 + |\nabla' \phi'_I|^2) \quad (3.38a)$$

$$F_{twDEP} = -A_{twDEP} \frac{V_0^2}{d^3} \nabla' \times (\nabla' \phi'_R \times \nabla' \phi'_I) \quad (3.38b)$$

where the coefficients A_{cDEP} and A_{twDEP} are defined, respectively, as $A_{cDEP} = \pi \epsilon_m a^3 \text{Re}[f_{CM}]$ and $A_{twDEP} = 2\pi \epsilon_m a^3 \text{Im}[f_{CM}]$ for uniform spheres.

0.0.0 Boundary conditions

The twDEP system with a four-phase signal of amplitude V_0 and angular frequency ω applied is depicted in Fig. 3.4. The voltage is phase-shifted by 90° so that the traveling wave moves in the positive x direction. The electrode system has a wavelength equal to the distance between four electrodes, i.e. $\lambda = 4(d_1 + d_2) = 8d$. Similarly, the real part and imaginary part of the potential phasor at the electrodes are shown in Fig. 3.21. Both are nonzero and have to be solved respectively.

Figure 3.21 A schematic diagram of the electrode array with a 4-phase signal applied for

traveling wave dielectrophoresis. The vertical broken lines indicate the wavelength (period

$\lambda=4(d_1+d_2)=8d$) over which the system repeats. The potential phasor, $\tilde{\phi}$, real, ϕ_R , and

imaginary, ϕ_I , parts of potential phasor, the potential ϕ on the electrodes are shown.

Figure 3.22 The basic unit cell for solving the problem in four-phase twDEP array, highlighted

with heavy solid lines. Shown are the boundary conditions for real part (a) of potential phasor

and imaginary part (b) of the potential phasor. The repeated dotted cells describe

the whole system.

The smallest problem space can be reduced to a quarter of the size of the period, which is highlighted by heavy solid lines in Fig. 3.22. The basic cell covers two halves of adjacent electrodes and the gap between them. The rest of the problem space is defined by using even and odd symmetry boundary conditions. The whole system can be determined by only solving this basic cell, using the even and odd symmetric properties. With the Neumann condition (Eqn 3.26b) at the lower interface at the inter-electrode gaps, the complete boundary conditions are shown in Figure 3.23.

Figure 3.23 The problem space for the traveling wave array showing the complete boundary conditions.

The 2-dimensional Laplace's equations governing the potential are

$$\frac{\partial \phi_R^2}{\partial x^2} + \frac{\partial \phi_R^2}{\partial y^2} = 0 \quad (3.39a)$$

$$\frac{\partial \phi_I^2}{\partial x^2} + \frac{\partial \phi_I^2}{\partial y^2} = 0 \quad (3.39b)$$

As can be seen from Fig. 3.22, the imaginary part has the same amplitude of that of the real part but shifted $l=\lambda/4$ in the x direction. Thus ϕ_I can be obtained from ϕ_R

$$\phi_I(x, y) = \phi_R(x - \lambda/4, y) = \phi_R(x - l, y) \quad (3.40)$$

Therefore, only ϕ_R needs to be solved directly from the Laplace's equation (3.39a).

0.0.0 Weighted LSFD solutions

Similarly, nonuniform grid point distribution (Figure 3.8) was used in solving the problems. Grid points were distributed more densely near the electrode edges and relatively sparsely in the area away from the electrodes. The problem was solved with $d_1=d_2=d=10\text{ }\mu\text{m}$.

Figure 3.24 (a) The nondimensional real part ϕ_R' , and (b) imaginary part ϕ_I' of the traveling wave potential phasor.

The real part ϕ'_R and imaginary part ϕ'_I of the potential phasor obtained are shown in Figure 3.24. Comparison of the two components shows that one of them is the mirror image of the other about vertical line through the center of the gap. The electric field is shown in Figure 3.25. The field is independent of x' at heights sufficiently far away from the electrode.

Figure 3.25 The logarithmic contour of electric field E' .

The logarithmic magnitude and direction of the vector $\nabla'(|\nabla'\phi'_R|^2 + |\nabla'\phi'_I|^2)$ regarding the conventional DEP (cDEP) force component (Eqn (3.28a)) are plotted in Fig. 3.26. As shown in (a), the magnitude of the vector is constant with x' across the array at sufficient height ($y \sim 1.5d = 15 \mu\text{m}$) above the electrode. The maximum value exists at the electrode edges. As shown in (b), above $y' \sim 1.5$, the vectors point straightly downwards. In the area near the electrode, they direct more and more towards the side edges (at $x' = 0.5, 1.5$).

Similarly, the vector $\nabla' \times (\nabla'\phi'_R \times \nabla'\phi'_I)$ for the twDEP force component (Eqn

(3.28b)) is shown in Figure 3.27. The magnitude of the vector is constant against x' at sufficient height above the electrode, and maximum value exists at the electrode edges. However, the vectors point laterally towards the negative x direction above the height of $1.5d$. Closer to the electrodes, the vectors shows a more complicated pattern around the edges.

Figure 3.26 (a) $\log_{10}$ (magnitude), and (b) vector direction of the vector for the cDEP force

$$\text{component: } \nabla'(|\nabla'\phi'_R|^2 + |\nabla'\phi'_I|^2).$$

Figure 3.27 (a) $\log_{10}(\text{magnitude})$, and (b) vector direction of vector for the twDEP force

$$\text{component: } \nabla' \times (\nabla' \phi'_R \times \nabla' \phi'_I).$$

In Figure 3.28, the forces are plotted versus the height above the electrode along two vertical lines, i.e. $x'=0.5$ and $x'=1.0$ (or $x=5\text{ }\mu\text{m}, 10\text{ }\mu\text{m}$). Above the height of about $y'=1.5$ (or $y \sim 1.5d = 15\text{ }\mu\text{m}$), the magnitude of the DEP force decreases exponentially as the height increases. Similarly, the forces can be approximated by

exponentially decreasing functions of the height y' . By fitting the data, the functions are given as

$$\nabla'(|\nabla'\phi_R'|^2 + |\nabla'\phi_I'|^2) = 0.93332e^{-1.7103y'} \quad (3.41a)$$

$$\nabla' \times (\nabla'\phi_R' \times \nabla'\phi_I') = 0.76915e^{-1.60799y'} \quad (3.41b)$$

Figure 3.28 (a) The magnitude of $|\nabla'(|\nabla'\phi_R'|^2 + |\nabla'\phi_I'|^2)|$ is plotted versus heights. (b) The magnitude of $|\nabla' \times (\nabla'\phi_R' \times \nabla'\phi_I')|$ is plotted versus heights. The values are investigated along two vertical lines: $x'=0.5$ and $x'=1.0$.

The above discussion implies that the actual particle movement comes from the combined contributions of both cDEP and twDEP force components. The former is responsible for the levitation (n-DEP) or trapping (p-DEP) of the particle while the latter gives rise to the traveling wave movement of the particle in positive x or negative x direction dependent on the polarity of the imaginary part of the CM factor, i.e $\mathbf{Im}(K)$.

The particle velocity of the traveling movement caused by the traveling wave DEP force at the levitation height can be calculated from Stoke's formula

$$v_{tw} = \frac{F_{twDEP}}{6\pi\eta a} \quad (42)$$

where η is the viscosity of the fluid.

For example, for the latex bead #3 described in section 3.1, taking the following parameters: $V_{rms}=5$ V, $\mathbf{Re}(K)=-0.45$ and $\mathbf{Im}(K)=-0.55$, then the levitation height is 43.3 μm . At this height, the traveling velocity of particle is 95.6 $\mu\text{m/s}$ in the DI water of viscosity $\eta=1.002\times 10^{-3}$ Pa.s.

0.0 Conclusion

The dielectrophoretic array and traveling wave array were numerically simulated using the developed computational model based on the weighted LSFD scheme. The advantages of the modeling environment are: (1) the ease and flexibility for mesh or grid point generation and distribution. (2) Feasibility of applying the method to model more complex DEP electrode systems.

Since the analytical solutions are usually unavailable for most DEP systems, we

resorted to numerical approaches. With the modeling tool, it is now possible to perform fundamental quantitative analysis and design of various DEP systems.

CHAPTER 0 DEP Microactuation with Planar Microelectrodes

Planar microelectrode systems are widely used in DEP actuation for they are simple and easy to fabricate. Much insight can be gained through the investigation of particle behavior in these electrodes. In this chapter, DEP experiments with planar electrodes such as interdigitated array, castellated array are performed. First, the experimental results can be used to validate the aforementioned modeling environments. Second, fundamental study of particles in planar electrodes provides significant information of particle movements under certain conditions, and gains further insight into the phenomena of DEP. Through these studies, limitations of planar electrodes are well understood, leading to the design of 3-dimensional electrode system for particle manipulation, which will be discussed in the following chapters.

0.0 The planar microelectrode arrays

The commonly used planar microelectrode systems such as interdigitated array, castellated array, and jagged array are shown in Figure 4.1. They are ‘planar’ because they are formed on the flat surface of the substrate and their thickness is negligible compared with other functional elements of the systems. These electrodes are easy to be constructed simultaneously onto one single wafer using surface microfabrication techniques. External signals are connected to the electrode system via the bus bar. Experiments were carried out with these electrodes, with the emphasis on the interdigitated array to demonstrate the phenomena in general. The major difference

among the three types of electrodes is the location of the field minima when they are energized with applied voltages.

Instead of using biological cells in the tests, the commercially available latex beads were used for the fundamental studies. The beads have homogeneous physical properties and many of their parameters are accurately calibrated and measured. This makes the validation tests more straightforward by minimizing the variations in the test parameters which may interfere with the test results. Meanwhile, the tests on the beads can serve as significant reference for the DEP manipulation of living cells.

Figure 0.1 Microfabricated planar electrodes used in experiments. (a) Interdigitated array; (b) Castellated array; (c) Jagged array.

4.2 Materials and methods

4.2.1 Beads and Solutions

Polystyrene beads (cross-linked with 4 to 8% divinylbenzene DVB) with density 1.05 g/cm^3 , in three diameters – $4.3 \text{ }\mu\text{m}$, $9.6 \text{ }\mu\text{m}$ and $16 \text{ }\mu\text{m}$, purchased from Duke Scientific Co. (CA, United States) were used in the investigation. The beads are chemically inert and packaged as aqueous suspensions at 10% solids by weight. Before use, small volumes of suspension containing beads were resuspended at low concentration in stock solutions (deionized water + buffered solution) of appropriate conductivity. A short-time sonication after resuspension was used to accelerate the dispersion and break up temporary agglomerates. All bead solutions were refrigerated after resuspension and used within weeks.

Solutions of conductivities from $\sim 0.1 \text{ mS/m}$ to 100 mS/m were made by taking DI water with 10X phosphate buffered saline (PBS, Fisher Scientific, NJ) added. Appropriate amounts of PBS were added into the DI water, until the nominal conductivity was reached. The conductivity of the solution was measured with a conductivity meter with graphite sensor electrodes (Dist3WP, Hanna Instruments Inc., RI). The solutions were refrigerated and their conductivity was measured before each use.

0.0.0 Microfabrication and Packaging

The planar microelectrodes were fabricated using conventional microfabrication techniques (Figure 4.2a). A 4-inch glass wafer or silicon wafer (with an insulation layer of 800 nm SiO_2 thermally grown on the surface) was used as the substrate. The wafer

was cleaned for 20 minutes in a Piranha solution, then rinsed in DI water and blow dried. Photolithography was performed using the thick positive resist AZ9260 (Clariant, Somerville, NJ) to define the pattern of the electrodes. 50 nm Cr/100 nm Au electrode array of dimensions of $2000 \times 8000 \mu\text{m}^2$ was patterned onto substrate surface using sputtering process followed with liftoff in acetone. The fluidic channel, which is 2000 μm wide, $10^4 \mu\text{m}$ long and 110 μm deep resulting in a volume of $\sim 2 \mu\text{l}$, was formed on the substrate using SU-8 photosensitive epoxy-based resist (MicroChem, Newton, MA, USA). Fluidic access holes of $\sim 0.8 \text{ mm}$ were drilled onto the glass slide by diamond drill bit. For the silicon wafer, the fluidic holes could be attained by etching through the wafer. After dicing, a glass coverslip was then carefully clamped on top of the channel and applied 5-minute epoxy to create a fluidic seal. Two contact pads were now ready for the connection to external electrical power supply. The completed microfabricated electrode arrays are shown in Figure 4.1.

The packaging of the device containing the chip with microelectrodes for test is schematically shown in Figure 4.2b. The slide was mounted on a plastic block with electrical connections on it. The block served as a support for the slide. A spacer with glue on double sides was placed between the slide and the block. The contact pads of electrode system were wire-bonded to the electrical connections, where the external voltage was connected. Fitting and silicone tubing (Cole-Parmer, In. Co, Illinois) attached to the fluidic holes of the slide served as the inlet or outlet for the fluid. In experiments, an appropriate amount of solution containing particles was pumped into the channel via the inlet.

Figure 4.2 (a) Schematic diagram of the planar electrode array with the microchannel. (b)

Schematic of the packaging assembly.

0.0.0 Electrical excitation

Sine wave electrical signals up to 20 MHz and 15 V_{pp} (into 50 Ω) were generated by a Wavetek 90 Function Generator (Wavetek Wandel & Goltermann, Inc., NC, USA). An oscilloscope (Tektronix 2211 digital storage oscilloscope) was used to calibrate the output from the function generator. The electrical output was connected to the electrode system via the contact pads at the edge of the diced chip.

0.0.0 Fluidics

The fluidics components include a custom-built microfluidic connector block, a syringe pump (NE1000, New Era Pump Systems, Inc., NY) and silicone tubings. For the planar

electrode systems, stationary fluids were used. An appropriate volume of stock solution containing particles was pumped into the channel with the syringe pump and a 500 μl luer-lock syringe (Model 750, Hamilton, Reno, Nevada). The volume of the solution delivered was readily controlled through setting the syringe pump from the keypad. An alternative method for liquid delivery was to use a pipette to deliver a measured quantity of liquids into the channel (without cover) and then cover the channel with a glass slip. This method suffered from the problems of liquid leaking and sample contamination, and was not a choice in this work.

0.0.0 Optics

A high-resolution inspection microscope system Olympus MX40 (Olympus Corp, Tokyo, Japan) was used for particle observation. Images and movies were captured and recorded using a Sony digital color video camera.

0.0.0 Crossover frequency measurement

The crossover frequency at which the DEP force switches between being positive and being negative was measured at various conductivities of the medium. Frequencies were varied from low to high values and then high to low values to observe the particles' movements. The points where the particles began to change from being attracted to electrodes to being repelled from electrodes (or vice versa) were recorded as the crossover frequencies. Each measurement was taken 10 times and the averaged value was used.

0.0.0 Levitation height measurement

A particle that experiences negative DEP force will be levitated to a certain height above the electrode where the DEP force is balanced by the gravitational force, which was referred to as passive dielectrophoretic levitation [10]. This height is significant for it is a function of a variety of parameters such as dielectric properties of the particles and medium, frequency of voltage, etc. The levitation heights of the particles were measured by consecutively focusing method - the objective lens of the microscope was firstly focused onto the electrode plane and then onto the levitation plane. Taking the difference of heights between these two planes indicated by the fine focusing control (precision $\sim 1 \mu\text{m}$) of the microscope, the apparent levitation height was given. However, such height is known as the apparent height. Taking the refractive indices of the glass coverslip and aqueous suspending medium into consideration, the physical height is given as [146]

$$h_l = \frac{n_0 - \sqrt{n_0^2 - NA^2}}{n_m - \sqrt{n_m^2 - NA^2}} \times \Delta h \quad (4.1)$$

where h_l is the physical levitation height, Δh is the apparent height obtained from the consecutively focusing measurement. n_0 and n_m are the refractive indices of the air and suspending medium, respectively. NA is the numerical aperture of the objectives used. Under the present conditions, n_0 was taken as 1.0 for the air and n_m was taken as 1.332 for water at 25 °C. NA was 0.80 for the Olympus UPlan Fl 50X objectives. The accuracy of the above scheme was calibrated by measuring the thickness of a glass coverslip with a known thickness of 147.1 μm . The measurement result was 149 μm , showing a good accuracy.

Each levitation height was measured for 10 times and the average values were taken after eliminating the minimum and maximum measurements. Particles located near the boundaries, e.g. channel boundary and electrode ends, were not included in the measurements.

0.0.0 Modeling method

The numerical modeling was performed using the modeling environment described in Chapter 3. Some of the specifications were accordingly changed with reference to the actual values in the experiments.

0.0 Results

0.0.0 Particle behavior as the frequency was varied

A. High frequency

Figure 4.3 shows the behavior of the 4.3- μm beads in a suspending medium of low conductivity (0.3 mS/m) for the characteristic electrode dimension of 30 μm and an applied voltage of 5 $V_{\text{peak-peak}}$. Above a frequency of 5 kHz, negative DEP took place. With the applied voltage off, latex beads were introduced into the channel and randomly dispersed due to the Brownian motion. The beads were settled down on the bottom of the channel by the gravitational force as shown in Figure 4.3(a). Three different movements were observed when an AC voltage of 5 $V_{\text{pk-pk}}$ with various frequencies was applied to the electrode system shown in Figure 4.3(b-d). Particles were levitated to certain heights above the electrodes by negative DEP force, where DEP force was balanced by the

gravitational force, as shown in Figure 4.3b,c (the out-of-focus are the electrodes). However, most particles were horizontally located over the gaps between the electrodes at 10 kHz (Figure 4.3b), while they were aligned over the centers of the electrodes at the levitation heights at 100 kHz (Figure 4.3c). It became more interesting when the frequency was increased to 1 MHz and above. Particles, which were initially settled at the substrate surface by sedimentation, were no longer levitated, and they moved to the gaps between the electrodes and the centers of electrodes (Figure 4.3d). However, the particles originally suspending above the surface could be levitated, or, if the particles were brought into the channel after the electrodes had been energized, most of the particles were found to exhibit stable levitation. To levitate the particles staying at the bottom surface, a lower frequency such as 100 kHz was used to initiate the levitation, then the frequency of 1 MHz (and above) was used. These results indicated that, for the frequencies above 1 MHz, the vertical component of the DEP levitation force was not strong enough to overcome attractive forces between the substrate and the particles.

Figure 4.3 Dielectrophoretic behavior of the 4.3 μm particle at high frequencies. Letter ‘A’ indicates the electrodes of 30 μm . (a) Polystyrene beads were introduced and randomly dispersed in the channel with no voltage applied. (b) The beads were levitated by negative DEP force and aligned over the gaps with 5 $V_{\text{pk-pk}}$ of 10 kHz applying after 2 minutes. (c) The beads were levitated and aligned over electrode centers with 5 $V_{\text{pk-pk}}$ of 100 kHz applying after 2 minutes by lateral force. (d) At higher frequencies, particles were settled at both the gaps between the electrodes and the centers of electrodes.

The above observations indicated that both the vertical and lateral components of DEP forces greatly contributed to the movements of the particles. The former induced stable levitations in a very short time, while the latter continued pushing the particles laterally to the specific locations. Much insight into the lateral movement can be gained by examining the lateral component of DEP force, which is plotted at different heights in Figure 4.4. Numerical results obtained using the modeling environment presented in Chapter 3 were used to plot the figure. The force points to negative x direction when its value is positive and points to positive x direction when it is negative. The directions of the force are indicated by arrows in the figure. Critical points where the force changes its direction are found to be at electrode center, electrode edges, and gap center. At heights

near the electrode plane (Fig. 4.4a,b), the force pushes the particles to locations over the electrode center and gap center. Whether the particles finally stay at the electrode center or gap center depends on their initial locations before the electrodes are energized. As the height is increased, the force no longer changes its direction at electrode edges. The force pushes all the particles only to the electrode center (Fig. 4.4 c,d). The fact that lateral force direction varies with height agrees with the experimental observations as shown in Figure 4.3. Depending on the heights to which the particles were levitated and their initial locations before the voltage was applied, they could be located over the gap centers (Fig 4.3b), electrode centers (Fig 4.3c), or both electrode centers and gap centers (Fig 4.3d). It means that although the lateral component of DEP force is much smaller than the vertical component at the levitation heights, the lateral movement of the particles can still be noticeable. At the stable levitation heights, the lateral force competes against the effect of Brownian motion, and it can be strong enough to overcome the latter in certain circumstances, giving rise to particle movements in the horizontal direction. However, such movements were relatively slow and it took a long time (~ minutes) for the particles to travel to their destinations.

Figure 4.4 Non-dimensional lateral forces $F'_{\text{dep}x}$ at different heights: (a) $y = 1 \mu\text{m}$, (b) $y = 10 \mu\text{m}$, (c) $y = 30 \mu\text{m}$, (d) $y = 50 \mu\text{m}$. The gray bar represents the 30- μm electrode. The arrows indicate the lateral force pointing in positive or negative x direction. It should be noted that the direction of DEP force (indicated by arrows) is opposite to the non-dimensional values plotted in the figure under negative DEP.

B. Low frequency

At low frequencies ($< 5 \text{ kHz}$), particles behaved in a different way. In the range of $1 \text{ kHz} - 5 \text{ kHz}$, they exhibited unstable behavior by oscillating around the electrode edges and over the electrode. Possible contributing factors to this effect include AC electro-osmotic, electrophoretic and electrothermal effects.

At frequencies below 1 kHz , particles were experiencing positive DEP and were

attracted to the electrode edges (Fig. 4.5), where the maximum electric field strength existed. Aggregation of neighbouring particles was apparent when they were exposed to the field for a longer time (>1 minutes). This was due to the dipole-dipole interactions of close-spaced particles.

Attention should be paid to the potential damages to the particles caused by positive DEP. The electric field strength at the edges where particles are trapped is extremely high and hence some kind of electrical damage may be caused. Fig. 4.6 shows the transformation of latex beads under electric field of 100 Hz. Trapped particles were mechanically broken down after a long time exposure (~ 5 mins). Possible contributing factors include the effects of field induced heating and electroporation [124], in which electric field makes micro-pores on the membrane more permeable so that intra-materials can be moved into/out by electroosmosis and diffusion. A positive application of electroporation is to obtain intracellular materials by high electric field.

Figure 4.5 Positive DEP of the beads at the frequency of 500 Hz.

Particle aggregation was observed.

Figure 4.6 At the frequency of 100 Hz of 5 V_{pk-pk}, mechanical breakdown of the particles was caused.

0.0.0 Levitation heights

As discussed above, positive DEP may be hazardous in some cases, for the field at electrode edges where particles are trapped is extremely high. Negative DEP has the advantage that electrical damage is less possible to be imposed on the biological objects by the field. A particle that experiences negative DEP force will be levitated to a certain height above the electrode where the DEP force is balanced by the gravitational force

$$\overline{F}_g + \overline{F}_{DEP} = 0 \quad (4.2)$$

where $\overline{F}_g$ is the gravitational force. $\overline{F}_{DEP}$ is the DEP force.

For a spherical particle of radius a of mass density ρ_2 , suspended in a fluid of mass density ρ_1 , the gravitational force is given by

$$\overline{F}_g = \frac{4}{3}\pi a^3 (\rho_1 - \rho_2) \overline{g} \quad (4.3)$$

where $\vec{g}$ is the gravitational acceleration vector.

The levitation heights were measured using the consecutively focusing method (section 4.2.7), which had a good accuracy when a high magnification (e.g. 1000 times) of the microscope was used.

A. Varying the frequency

The levitation heights measured at different frequencies of the applied voltage 5 V peak to peak were shown in Figure 4.7. In the experiment, the conductivity of the suspension medium was 10.0 mS/m and the 9.6- μm latex beads were used. As expected, the particles were attracted to the electrode edges at frequencies lower than 5 kHz. The levitation height increased as the frequency was increased, till to the maximum levitation at the frequency around 1 MHz. For higher frequencies, the levitation height slowly decreased. The frequency dependence of the levitation height resulted from the frequency dispersion in the CM factor (Eqn 2.3), which in turn influenced the magnitude and sign of the DEP force. On the other hand, for low frequencies, a significant proportion of the applied voltage can drop across the electrical double layer that builds up at the surfaces of the electrodes [147], leading to the reduction of electrical potential into the bulk solution so that the levitation height is also reduced (see section B). For high frequencies, the voltage drop is negligible.

Figure 4.7 The levitation height measured as a function of the frequency of the applied voltage for 9.6 μm latex beads. The electrode width and spacing were 50 μm . The applied voltage was 5 V_{pk-pk} and the conductivity of the suspending medium was 10.0 mS/m.

B. Varying the applied voltage

The levitation was observed at different voltages at the frequency of 1 MHz. The heights were shown in Figure 4.8 versus the applied voltage (peak-peak). The conductivity of the suspending medium was 10.0 mS/m. It can be seen that the levitation height increased steadily as the applied voltage was increased. This agreed with the fact that increasing the voltage resulted in the increase of the electric field strength as well as the magnitude of the DEP force. It was observed that the particles could not be levitated at voltages lower than ~ 1 V_{pk-pk}, indicating that the DEP force generated with such voltages was not strong enough to levitate the particles. Meanwhile, Joule heating is enhanced when an electric field of higher strength is generated. Hence an appropriate voltage should be used in practical applications.

Figure 4.8 The levitation height of the 9.6 μm latex beads at various applied voltages at frequency of 1 MHz. The conductivity of the suspending medium was 10.0 mS/m.

C. Changing the medium conductivity

The 9.6 μm latex beads were suspended in the medium of two different conductivities, i.e. 1.0 mS/m and 10.0 mS/m. The conductivity was easily adjusted by adding PBS solution into deionized water. In the measurement, a frequency of 1 MHz was used. The levitation height of the 9.6 μm latex beads was shown in Figure 4.9. It can be seen that the levitation height for the particles in the 10.0 mS/m solution was a little higher than that in the 1.0 mS/m solution. It was because that the differences of electrical properties between the particles and suspension medium were enlarged when a medium of higher conductivity was used. On the other hand, the small difference of the levitation height for the two conductivities implied that the levitation height only slightly depends on the conductivity of the medium, in agreement with the predictions by equation 2.4. At high frequency, the dielectric polarization instead of DC conduction governs the behavior of

the system.

Figure 4.9 Levitation heights of the 9.6 μm latex beads versus applied voltage of frequency 1

MHz in suspension medium of two different conductivities: 1.0 mS/m and 10.0 mS/m.

D. Different particle diameters

Latex beads with diameters of 4.3 μm , 9.6 μm and 16 μm were investigated. The levitation heights for the three different sizes of particles were shown in Figure 4.10a,b. The conductivity of medium was 10.0 mS/m and the characteristic electrode dimension was 50 μm . It can be seen that the difference of the levitation height for the three types of particles was small. The results implied that the levitation height was independent of particle size. This was due to the fact that both the DEP force and gravitational force are dependent on a^3 (a is the particle radius). However, at certain frequency such as 50 kHz, the difference of height was significant probably because the particles had significantly different effective conductivities at that frequency.

Figure 4.10 Levitation heights of particles of 4.3 μm, 9.6 μm, 16 μm diameters for the characteristic electrode dimension 50 μm. (a) Levitation heights versus the frequency of applied voltage (5 Vpk-pk). (b) Levitation heights versus the magnitude of the applied voltage (1 MHz).

E. Influence of electrode dimension

The electrode dimension influences the electric field distribution, which in turn influences the DEP force. The levitation heights of the 9.6 μm latex beads using the 30 μm and 50 μm electrodes are shown in Figure 4.11a,b as the function of frequency and

magnitude of the applied voltage, respectively. Obviously, the levitation height was higher for the larger electrode (50 μm). Larger electrode generated the electric field to a larger area than smaller one so the levitation height was increased. However, since the dimension of the inter-electrode gap was also increased (provided $d_1 = d_2$), the levitation height may be reduced when the characteristic dimension is increased to a certain value.

Figure 4.11 Levitation height of the 9.6 μm latex beads in 30 μm and 50 μm electrodes. The conductivity of medium was 10.0 mS/m. (a) Levitation heights versus the frequency of applied voltage (5 Vpk-pk). (b) Levitation heights versus the magnitude of the applied voltage (1 MHz).

4.3.3 Crossover frequency

At the crossover frequency, the polarizability of particles and the medium are the same and the particle ceases to experience any DEP force. The crossover frequency is significant as it is one of the means to characterize the effective polarizability of particles since that of the medium can be easily known. The crossover frequency for the 9.6 μm latex beads are shown in Figure 4.12 as a function of the medium conductivity. The crossover frequency was close in a range of conductivities (0.1 mS/m – 3 mS/m), and dropped rapidly at higher conductivities. It was found that the crossover frequency did not exist as the conductivity increased to a certain value (~ 20 mS/m). It indicated that the particles exhibited negative DEP in the whole range of frequencies at such medium conductivity.

Figure 4.12 The crossover frequency as a function of the conductivity of the medium for the 9.6 μm latex beads.

4.3.4 Comparison study

The reliability of the above tests is verified in this section by comparing the experimental results with the numerical calculations. On the other hand, this study in turn demonstrates the feasibility of the numerical method and model presented in Chapter 3. Typically, the comparison is made between the experimental levitation heights and the results obtained from the numerical model in Chapter 3. A comparison between the experimental and numerical levitation heights as a function of the applied voltage is shown in Figure 4.13. In the numerical calculation, parameters identical to the experimental conditions were used. The parameters are: electrode characteristic dimension $d = 50 \mu\text{m}$; bead diameter $= 9.6 \mu\text{m}$; medium conductivity $= 10 \text{ mS/m}$ and frequency of voltage $= 1 \text{ MHz}$. In general, the experimental results agree well with the numerical calculation. The levitation heights increase as the voltage is increased. Most of the numerical values fall into the scattered range of the experimental data (indicated by the error bars). Although a typical case is studied, it is certain that the experimental results agree well with the theory predicted by the numerical model.

Figure 4.13 A comparison between the experimental and numerical levitation heights versus applied voltage.

0.0 Discussions

At the crossover frequency, the particles do not exhibit any DEP movement. The effective polarizability of the particles is equal to that of the suspension medium. Since the latter is easily known, the polarizability of the particles can be characterized. The real part of the CM factor $\text{Re}[K]$ is zero so that the conductivity of the particle is given as

$$\sigma_p = \frac{\sqrt{9\sigma_m^2 + 4\omega_0^2(2\varepsilon_m + \varepsilon_p)(\varepsilon_m - \varepsilon_p)} - \sigma_m}{2} \quad (4.4)$$

where $\omega_0 = 2\pi f_0$ is the crossover frequency.

For example, from Figure 4.12, the crossover frequency was 12.5 kHz for the 9.6 μm latex beads in the suspension medium of conductivity 0.1 mS/m. Using a value of 79 (relative) for ε_m (i.e. $\varepsilon_m = 79$) for water and of 2.55 for ε_p ($\varepsilon_p = 2.55$) for polystyrene from Ref. [148], the conductivity of the latex bead was calculated to be 0.119 mS/m.

The above results and discussion indicated that the particle behavior was affected by a variety of parameters such as the polarizability of the particles and medium, frequency and magnitude of the applied voltage, electrode structures, etc. For other planar electrode structures such as the castellated electrodes and jagged electrodes, electric field distributions such as potential wells and field maxima generated with these electrodes are different. Particles will be collected at potential wells under n-DEP or attracted to field maxima under p-DEP. Figure 4.14 typically shows the 9.6 μm latex beads in the castellated electrodes and jagged electrodes at the frequency of 100 kHz and 1 kHz, respectively. The conductivity of suspension medium was 10.0 mS/m. At 100 kHz (Figure 4.14a, c), the beads were collected to the regions nearer to the locations of low

electric field strength such as center of the electrode surface, electrode bays. Interesting patterns including pearl chains, diamond-shaped aggregations were observed. At 1 kHz, the beads experiencing p-DEP were attracted to electrode bays (Figure 4.14b) or edges (Figure 4.14d). It was demonstrated that electrode structures determine the electric field distribution which in turn influence the movement of the particles.

(a) 100 kHz

(b) 1 kHz

(c) 100 kHz

(d) 1 kHz

Figure 4.14 Particle behavior in the castellated electrodes (a,b) and jagged electrodes (c,d). The medium conductivity was 10 mS/m. Bright areas are the electrodes.

0.0 Conclusion

The dielectrophoretic movement of micron-sized latex beads in planar microelectrodes has been studied in a stationary fluid. In these studies, the conductivity of suspending medium was varied from relatively low value (e.g. 0.1 mS/m) to higher value (e.g. 100 mS/m). For high conductivity, only n-DEP occurred for polystyrene particles. Frequency dependence was that the latex beads experienced n-DEP at frequencies higher than 10 kHz and experienced p-DEP at lower frequencies. Negative DEP has the advantage over p-DEP of avoiding possible problems associated with surface adhering, interparticle

aggregation, and electrical damage. For the interdigitated electrodes, particles were levitated in the bulk suspending fluid by n-DEP. Particles of different dielectric properties were levitated to different heights. In combination with the laminar flow, separation of the particles can be achieved based on the levitation heights, which was referred to as DEP-field-flow fractionation (see, e.g. [70]). In addition, it has been shown that the dielectrophoretic passive levitation is independent of particle size. Although synthetic polystyrene micron-sized particles other than microparticles were investigated, the results provide significant understanding and qualitative conclusions for the dielectrophoretic manipulation of biological bodies.

CHAPTER 0 Design of 3-D Microelectrodes for DEP Microactuation

Planar microelectrodes have been used to manipulate polystyrene spheres in the last chapter. Although the planar electrode structures are sufficient for common usage such as levitation, separation, trapping, etc, there would still be demands for the reconfiguration of the electrode structures for specific applications. In this chapter, a 3-D microelectrode structure is proposed and designed on the basis of the planar electrodes. The 3-D electrode overcomes some of the limitations existing in the planar structures. The design concentrates on investigating the factors that influence the performance of the device. Finally, a microfluidic system containing the 3-D microelectrode array is proposed for manipulation of microparticles.

0.0 Overview

The planar electrodes such as interdigitated array inevitably have the disadvantages which limit their applications. First, in the case of n-DEP, stationary fluids are usually used. A fluid flow will wash away all the particles that are repelled away from the electrodes. Second, the stationary fluid means that only a definite volume (the capacity of the channel) of sample can be processed in an operation. The microchannel usually has a very small capacity ($\sim 1 \mu\text{l}$). It is inappropriate to make sample preparation of moderate volumes (e.g. $100 \mu\text{l}$) using such systems. Third, in the case of DEP field-flow fractionation with planar electrodes on the bottom of the channel, the particles located at low heights still have the problems of adhering to the substrate surface. Particles having

equal dielectric properties (but different sizes) can not be separated with this method since the levitation heights are the same. To overcome these problems, the author resorted to the design of a 3-D electrode which is capable of handling particles in a continuous flow.

All numerical modeling performed in this chapter used the modeling environment described in chapter 3. Exact boundary conditions were used in the simulations. Drag forces were computed using the Stoke's formula in a parabolic flow profile (it is the case for the laminar flow). Unless specified otherwise, the simulations were performed with beads of the following parameters: $a = 5.0 \text{ } \mu\text{m}$, $f = 1 \text{ MHz}$, $V = 5 \text{ V}_{\text{pk-pk}}$, $\rho_p = 1.05 \text{ g/cm}^3$, $\epsilon_p = 2.55$, $\sigma_p = 0.1 \text{ mS/m}$, $\epsilon_m = 79$, $\sigma_m = 10 \text{ mS/m}$. Under these conditions, the particles experienced n-DEP and the real part of the CM factor was $\text{Re}[K] = -0.49$.

0.0 The paired microelectrode array – Introduction

For the planar electrode systems where the electrodes reside on the bottom of the channel, they appear to be as 'open' systems in the y direction when the cover glass is not taken into consideration. The electric field generated around the electrodes and the strength reduces as the height above the electrode is increased. The particles experiencing n-DEP also behave in an 'open' mode, where the particles can move far above the electrodes as possible as they can provided that the electric field is sufficiently strong. This initiates such an idea that finding certain form of scheme to 'close' the systems, so that the particles move in a field of more confined situations. Similar treatments can be found in the literature such as the octode field cages [81], which was optimized against the planar

quadrupole electrodes by aligning two quadrupole electrodes on the top and bottom, respectively.

Figure 0.1 (a) the planar interdigitated electrode system where the electrodes sit on the bottom of the channel, forming the ‘open’ mode in y direction. (b) A second layer of electrodes is placed on the opposite side of the bottom electrode to ‘close’ the system.

Figure 5.2 The electric field and the gradient of the field square versus height (y). (a) In the planar electrode, the values decay solely against the height. The minima are supposed at the infinity. (b) In the paired electrode, the minima are at the centerline of the channel. Different scales were used in the calculation.

The idea originated from the above consideration. Based on the planar interdigitated array (Fig. 5.1a), a second layer of electrodes is placed on the top of the

channel (Fig. 5.1b). With such a treatment, the whole system is enclosed in two layers of electrodes, which is termed as paired electrode array (PEA). Dielectrophoretic gates are formed between the electrode pairs when the array is energized with an AC voltage. The advantages of such an electrode configuration are (i) compared with the planar electrode, the electric field in the area away from the bottom electrode is strengthened by the top one; (ii) most of all, the lateral DEP force is enhanced. Even in the area away from the electrodes, the lateral DEP force is comparable with the vertical force. Particle movements in the system are caused not only by the contribution of the vertical DEP force but also by the lateral one. This results in the possibility of introduction of a flow in the x direction. These features lead to the possible applications which may differ from those in the planar electrodes.

A comparison of the electric field as well as field square gradient generated by the above two electrodes is shown in Figure 5.2. The calculation was performed using $d_1=d_2=10\text{ }\mu\text{m}$ and the channel height was $40\text{ }\mu\text{m}$. Different scales (with units) were used so that the variables (field and field square gradient) were easily plotted in a single graph. The most apparent difference is whether a potential well exists within the channel. In the planar electrode, the values of the two variables decay all the way as the height is increased. For the paired electrode array, the minima of the variables are found at the centerline of the channel while the maxima are at the bottom and top surfaces of the channel.

In this work, efforts were made to design the paired microelectrode array for manipulating micron-sized particles in combination with the flow.

5.3 Theoretical – the threshold velocity

The movement of the particle in the fluid is influenced by the combined effects of DEP force and drag force. Considering a spherical particle in the flow in the presence of DEP force F_{DEP} , the movement of the particle is governed by

$$m \frac{du}{dt} = -\gamma(u-v) + F_{\text{DEP}} \quad (5.1)$$

where m is the particle mass, u is the particle velocity and v is fluid velocity, $-\gamma(u-v)$ is the drag force, γ is the friction factor of the particle in the fluid. For a spherical particle, $\gamma = 6\pi\eta a$ where a is the particle radius and η is the viscosity of the fluid.

The particle velocity is given by the solution of Eqn (5.1), i.e.

$$u = (u_0 - v - \frac{F_{\text{DEP}}}{\gamma})e^{-(\gamma/m)t} + v + \frac{F_{\text{DEP}}}{\gamma} \quad (5.2)$$

where u_0 is the initial velocity of the particle.

The first term on the right hand of the above equation contains the contribution from acceleration. For the spherical particle with mass density of ρ , the characteristic time of the acceleration is $\tau_a = m/\gamma = \frac{2}{9}a^2\rho/\eta$, which is generally much smaller than the time of observation (~ 1 s) for microparticles. For example, taking $a=5$ μm , $\rho=1.05$ g/cm^3 , and $\eta=1.002\times 10^{-3}$ Pa.s for water, the characteristic time is $\tau_a \sim 10^{-6}$ s. It implies that the particle subjected to the DEP force will reach the terminal velocity in an extremely short time, which is unobservable under the normal conditions of observation. Therefore, the particle can be considered to move at its terminal velocity

$$u = v + \frac{F_{\text{DEP}}}{\gamma} \quad (5.3)$$

For a spherical dielectric particle, the time-averaged DEP force on the particle is

given by Eqn (2.2).

Figure 5.3 Forces that particle experiences in the paired microelectrode system.

(a) Cross-section view. (b) Top view.

Negative DEP ($\text{Re}[K] < 0$) takes place when the particle is less polarizable than the suspending medium (it is the case for latex beads at high frequency). In the present microsystem, the particles will be levitated to positions near the centerline of the channel. When a fluid flow is introduced along the channel, the particles also experience a hydrodynamic force. The forces that particles experience subjected to the electric field in the presence of a flow are schematically shown in Fig. 5.3. Both the cross-section view (a) and top view (b) are shown. The electrodes are aligned at an angle θ with reference to the direction of the flow (or the channel wall). The drag force on the particle is given by the Stoke's formula

$$F_{HD} = 6\pi\eta av \quad (5.4)$$

In the x direction, the two force components, i.e. the lateral DEP force and the drag force, are acting on the particle simultaneously and competing against each other. The particle's movement depends on the relative strength of these two forces. From Eqn (5.3), the particles will be deflected from the electrodes as long as $u_{\perp} < 0$, where $u_{\perp}$ is the

velocity component of the particle perpendicular to the electrode, i.e.

$$|F_{\text{DEP}}| > F_{\text{HD}\perp} = 6\pi\eta a v \sin\theta \quad (5.5)$$

On the other hand, the particles will go through the dielectrophoretic gate when $u_{\perp} > 0$. The flow velocity at which the particles begin to penetrate the gate is termed as threshold velocity. Before penetration, the velocity of flow is

$$v < V_{th} = \frac{F_{\text{DEP}}}{6\pi\eta a \sin\theta} \quad (5.6)$$

where V_{th} is the threshold velocity

$$V_{th} = \frac{F_{\text{DEP}}}{6\pi\eta a \sin\theta} \quad (5.7)$$

It implies that the threshold velocity is dependent on a number of parameters, such as the DEP force (which is in turn associated with the dielectric properties of the particles and suspending medium, conditions of the applied voltage, electrode configurations, etc), fluid viscosity η , particles size a , and the aligning angle θ of the electrode. At the velocity which is higher than V_{th} , particles will penetrate the gate and move along with the flow. Different particles have different threshold velocities, meaning that they can be separated with reference to the flowrate.

0.0 Simulation – the electric field, DEP force, and threshold velocity

To understand the electric field and DEP force generated by the dielectrophoretic gate, the system was firstly simulated with a typical case: bottom electrode width = 20 μm , top electrode width = 20 μm , channel height = 75 μm . The boundary condition for solving the electric potential is shown in Fig. 5.4. It reduces to a two-dimensional problem for

the case in which the length of the electrodes is much larger than the width. The problem space (shown by dashed lines) covered half of the top and bottom electrodes. The left boundary was chosen on the line sufficiently far way from the electrode. The potential on the electrodes was specified as the external voltage applied $\pm V_0$, while the Neumann boundary condition $\partial\phi/\partial n = 0$ was used for the rest of the boundaries.

Figure 5.4 Problem space showing the boundary conditions for solving the electrical potential.

The electric potential and logarithmic electric field are shown in Fig. 5.5a and b, respectively. The system is symmetric about the centerline of the channel. The electric field strength becomes more and more independent of the vertical position y in the area sufficiently away from the electrodes. Maximum strength exists at the electrode edges. Similarly, the magnitude of the DEP force is logarithmically shown in Fig. 5.5c. Maximum force exists near the electrodes. Near the electrodes, the DEP force points gradually into the centerline of the channel, and points away from the electrode region, building up the dielectrophoretic gate (Fig. 5.5d). This is exactly what we can make use of – the existence of a force directing away from the gate.

The threshold velocity computed from Eqn (5.7) for a particle of $4.8\ \mu\text{m}$ in radius

is shown in Fig. 5.6. It can be seen that the threshold velocity decreases dramatically with distance away from the bottom and top surfaces of the channel, to the minimum at the centerline of the channel. The height dependence implies that the threshold velocity varies with the height where the particles are levitated.

Figure 5.5 Numerical solution in the region near the electrodes (indicated by the black bars). The following parameters were used: electrode width = 20 μm , channel height = 75 μm ; voltage = 5 V_{rms} , frequency $f = 1 \text{ MHz}$. (a) Electric potential. (b) Logarithmic magnitude of the electric field strength. (c) Logarithmic magnitude of the DEP force. (d) Direction of the DEP force.

Figure 5.6 The threshold velocity plotted as a function of the height within the channel. In the calculation, aligning angle of electrode $\theta = 60^\circ$ was used. The applied voltage is $5 V_{\text{rms}}$ and the particle radius is $4.8 \mu\text{m}$.

0.0 Optimization of electrode system

A variety of factors influence the performance of the dielectrophoretic gate. In the design, a high threshold velocity is preferred so that a wide range of flowrates can be used. For this purpose, further optimization on the electrode structure is performed to improve the performance of the system.

0.0.0 Levitation

Since the threshold velocity is dependent on the vertical locations within the channel, we need to determine the heights to which the particles are levitated. Typically, let the width of the bottom and top electrodes be as $20 \mu\text{m}$. Two channel heights, i.e. $60 \mu\text{m}$ and $100 \mu\text{m}$, are investigated to show the effects of the channel height. The levitation heights are plotted against the applied voltage (peak-peak) in Fig. 5.7a. At sufficiently high voltages ($> 5 V_{\text{pk-pk}}$), the particle is levitated to the centerline of the channel. At low voltages such

as $1 V_{pk-pk}$, the particle is levitated to lower heights. It should be noted that the particle in the 100- μm channel has higher levitation than that in the 60- μm channel. The field generated by the paired electrodes reaches its minimum at the center of the channel, where the particles are supposed to be levitated under n-DEP. However, a thicker channel may result in the reduction of the field strength, which is in turn insufficiently strong to levitate the particles to the channel center, as shown in Fig. 5.7b. In this work, channels of height in the range of $\sim 1 - 5$ times the electrode width were considered. Particles are levitated to the centerline in such channels so that the concern hereafter is only directed to this location.

Figure 5.7 (a) The levitation heights of the latex bead of radius 5 μm plotted versus the applied voltage in the paired electrode system. (b) Levitation height varies with the channel height. The

voltage is 5 V_{pk-pk} . Each levitation height was scaled with the channel height.

5.5.2 Channel dimensions

The rectangular microchannel dimensions including the channel height and width greatly influence the field strength that the dielectrophoretic gate generates. From the magnitude of order analysis, the field strength is inversely proportional to the height of the channel. Therefore, the DEP force is inversely proportional to the square of the channel height. From Eqn (5.7), the threshold velocity is also inversely proportional to the square of the channel height. This means that a channel of a small height is preferred for this purpose. However, the size of the particles limits the minimum size of the channel height. The channel would have a height, which is at least a few times the size of the particles, to avoid clogging and adhering problems expected to encounter in thin channels. For the micron-sized particles used in this work, the possible channel height can be around tens of microns.

The width of the channel influences the performance of the system in the other way. When the channel is wider, the electrodes become longer. A particle has to travel a longer distance from one side of the channel wall to the other. This may be necessary when different particles need a longer travel in the direction of channel width to be separated. When the flow rate is kept constant, a wider channel will result in the decrease of the flow in the channel. In addition, the flow profile in a channel with high aspect ratio will become nearly blunt in the direction of the channel width. The flow is uniform across most of the width-dimension, with the only nonuniformity occurring near the walls. This is advantageous that the particle movement in the fluid is only dependent on the vertical locations in the flow.

5.5.3 Electrode width

The width of the electrode also influences the performance of the system. Generally, the bottom electrode and the top one can be designed to have an equal width (Fig. 5.8a), or different widths (Fig. 5.8b,c). First, the case of (c) is not the option. The large opaque electrode on the top blocks a large part of the observation path from top to bottom under a conventional (upright) microscope. Second, it was found that the force generated by the electrode structure in (b) is less stronger than that generated by the structure with equal electrode width. The nonuniformity of the field generated by the former is less drastic than that produced by the latter. It means that it is not necessary for the bottom and top electrodes to have different width. Therefore, the result turns out to be the simple structure in (a), where one of the electrodes is simply the mirror image (about the centerline of the channel) of the other.

Figure 5.8 Paired electrodes with different widths. (a) The widths of the bottom and top electrodes are the same. (b) The bottom electrode is wider than the top one and (c) is the reverse.

The next issue is to examine the effects of the width of the electrode on the performance of the dielectrophoretic gate when both the bottom and the top electrodes are of the same width. The threshold velocity for the 5- μm (radius) particle is shown in

Fig. 5.9 for various electrode widths (from 10 μm to 100 μm). In the calculation, the channel height was kept constant at 60 μm and the electrode was aligned at an angle of 45° with reference to the flow. When the channel height is constant, the threshold velocity is greatly dependent on the ratio of electrode width to channel height (ratio = d/h , with d being the electrode width and h being the channel height). In general, the V_{th} increases as the width of the electrode is increased, particularly when $d/h < 1$ (i.e. $d < 60$ μm). As the ratio is increased to 1 and above ($d \geq 60$ μm), the threshold velocity is nearly constant. This implies that it is favorable to design the electrodes having a width equal to or larger than the height of the channel.

Figure 5.9 The threshold velocity for different electrode widths. The channel height was 60 μm and aligned at 45° . The ratio of electrode width to channel height was varied from 0.17 to 1.67.

0.0.0 Aligning angle of electrodes

The aligning angle of the electrodes plays an important role on the performance of the

system as indicated by Eqn (5.7). The threshold velocity is higher for small angles. For example, the electrode aligned at 30° has a threshold velocity as high as 1.73 times that of 60° -electrode. However, a small angle means that the electrode has to become long enough to cross the channel from one side to the other. The channel (the width is given) may be too long when the electrode is aligned at a very small angle. Moreover, an extreme case is that V_{th} goes to infinity when $\theta = 0$, i. e. the particles will never penetrate the dielectrophoretic gate in the direction perpendicular to the electrode when the electrode is parallel to the channel.

0.0.0 Particle size

According to Eqn. (5.7), particles of different diameters have different threshold velocities. The DEP force is proportional to the volume of the particle and the levitation height is independent of the particle size (see section 4.3.2). Therefore, from Eqn (5.7), the threshold velocity is proportional to the square of the particle radius. The threshold velocities for two particles with radius of $2\ \mu\text{m}$ and $5\ \mu\text{m}$ are shown in Fig. 5.10 for various applied voltages. The smaller particle has lower values of V_{th} , meaning that it will be the first to penetrate the gate when the flowrate is slowly increased. Thus, we can use a proper range of flowrates to separate and manipulate different particles. One factor limiting the particle size is the channel dimension. The channel has to be designed properly to meet the requirements for handling the micron-sized particles, as discussed in section 5.4.2.

Figure 5.10 The threshold velocities for the 2 μm and 5 μm particles. The smaller particle has lower values of V_{th} . The electrode width was 50 μm and was aligned at 45° . The channel height was 60 μm .

0.0.0 Alignment of electrode pairs

The issue on the alignment of electrode pairs is to investigate how precisely the bottom and top electrodes have to be aligned. In fabrication of the system, the two layers of electrodes cannot be perfectly aligned due to manufacturing errors. Besides the misalignment arising from the unparallel problem of bottom and top planes, three types of possible misalignment are shown in Fig. 5.11. The electrodes may be relatively displaced with a small offset (Fig. 5.11a, b) or slanted with a small angle (Fig. 5.11c). It was found that the deviations arising from such slight misalignment are very small and negligible. For example, when the two electrodes are aligned with a relative offset of 10% of the electrode width, the deviation of the threshold velocity is less than 2%. In this thesis, a mask aligner system (Karl Suss MA6) of accuracy of 5 μm is used. For the

microsystem of the following parameters: channel height = 75 μm , electrode width = 50 μm , and applied voltage = 1 V_{rms} to 5 V_{rms} , the deviation in threshold velocity due to the 5- μm alignment mismatching is $< 1.5\%$. However, we should carefully avoid the rise of slanting the angle α in case (Fig. 5.11c). Since the electrodes may be very long, a small α can induce considerable mismatch at the ends of the electrodes. In practice, precise alignment between the electrode pairs can be achieved by the alignment process in the fabrication stage.

Figure 5.11 The top and bottom electrodes are slightly misaligned. (a), (b) A small offset is observed for the two electrodes. (c) A small angle α between the two electrodes is found.

Figure 5.12 Schematic of the paired microelectrode array containing the electrodes with varying widths and aligning angles (top view). The flow is running from the left to right.

0.0 Final Design

To design an electrode system that is suitable for particle separation and manipulation, all the contributing factors discussed above have to be taken into consideration. Delicately combining these factors gives rise to possible approaches for such applications. For this purpose, a paired microelectrode array of 1x5 was devised as shown in Fig. 5.12. The flow is expected to be running from the left to the right. The array shown contains 5 electrode pairs as indicated by the sequence numbers 1 – 5. From the left to the right, the width as well as the aligning angle of the electrodes was varied in a way so that the threshold velocity for a specific particle is increased at the subsequent electrode pairs as it passes through the dielectrophoretic gates. For particles of different electrical properties or sizes, they can penetrate or be reflected at different gates so that spatial separation occurs. Electrical leads and polarity of applied voltages are shown in the schematic. The voltage is connected to the electrode system via the connection pads located at the edges of the slides. To release the particles trapped near the ends of the electrodes, some kind of voltage switching operation is needed. This is done by manually switching off the respective voltage on the electrode with a switch. On the other hand, the system should have a short fill time so that the reagents are introduced in a timely manner. The fill time is dependent on the void volume of the system and the maximum velocity that the system works. The void volume from the microtubing generally dominates the fill time. Therefore, the tubing having a small inner diameter is preferred for the system.

Table 5.1 Parameters of the final design of the paired microelectrode array.

Electrode No.	1	2	3	4	5
Electrode width (μm)	20	20	40	60	80
Aligning angle	90°	75°	60°	45°	30°
Channel width: 800 μm ; Channel height: 75 μm ; Channel length: 10 ~ 15 mm					

The parameters of the final design for the array are listed in Table 5.1. The first electrode was designed to be as the stopper to initially hold all the particles introduced. The channel has a resulting volume of $\sim 1 \mu\text{l}$.

From the left to the right, the threshold velocities associated with the respective electrode pairs are shown in Fig. 5.13 for the 5 μm particle. The threshold velocity increases at the subsequent dielectrophoretic gate when the particle runs along the channel. The electrode array was designed to have such a series of steps in the threshold velocity in the direction of the flow. The particles can be collected at different locations using a proper range of flowrates.

Figure 5.13 The threshold velocities of the 5 μm particle for the 5 electrode pairs in the electrode array. The applied voltage is 5 V_{pk-pk} . V_{th} increases in the sequence of 1 – 5.

5.7 Conclusion

In this chapter the paired microelectrode array has been proposed and designed over the planar electrodes. The optimization was carried out by investigating the contributing factors that influence the performance of the system. Analytical studies were done through the extensive use of the modeling tools developed in Chapter 3. A paired microelectrode array comprising a series of electrode pairs aligning along the channel was designed. In combination with the fluid flow, the system is capable of separating and manipulating particles that are different in electrical properties or physical sizes.

CHAPTER 0 Array Fabrication and Packaging

In this chapter, the fabrication process of the paired microelectrode array designed in previous chapter is presented. The key steps included patterning of the arrays onto two single wafers and bonding of the chips. Various types of arrays were fabricated with varying parameters. The packaging scheme was proposed, which meets the requirements of electrical, optical, and fluidic connection and introduction to the chips.

0.0 Introduction

The microfabrication techniques used in biomedical engineering enable the miniaturization of the tools and devices for biological and medical applications, such as the micro total analysis systems (μ TAS) and lab-on-a-chip. The miniaturization of these systems presents a few advantages in comparison with the conventional ones. These include minimal sample requirement, ease of use, quick analysis time, greater data precision, low cost and high reproducibility. Another remarkable feature of miniaturized systems owes to the high surface-area-to-volume ratio, leading to a situation where surface effects dominate volume effects.

DEP systems, which comprise microscale functional components such as microelectrodes and microchannels, are usually fabricated using microfabrication techniques. The process usually includes pattern replication, deposition, etching and silicon planar technology. For DEP microsystems where electrodes reside on the surface

of a substrate, surface micromachining is applicable. Surface micromachining involves the fabrication of microscale structures using the deposition and patterning of thin films on a base substrate, typically a single-crystal silicon wafer. Photolithography is the technique used to transfer various patterns defined by the mask into bulk silicon substrates and thin films. In this work, the surface micromachining techniques was used to fabricate the paired electrode array. The major fabrication steps are summarized in Fig. 6.1.

The fabrication runs in such a manner that two parallel processes are carried out as indicated with the left and right columns. The fabrication began with a 4-inch glass wafer (Pyrex 7740) and a silicon wafer with a layer of 1 μm silicon dioxide (SiO_2) thermally grown on the surface as the insulation layer (Fig. 6.1a). The wafers were cleaned in Piranha solution and rinsed in DI water, then blow-dried before the next process started.

In step (b), the electrode layer as well as electrical leads of 25 nm Cr/ 100 nm Au was deposited using magnetron sputtering process, and patterned with lift-off technique. Before the deposition of the electrode layer, 10-minute oxygen O_2 plasma RIE process was applied to remove the residual resist from the development in photolithography. An ultrasonic source was used to accelerate the resist stripping process. Here we utilized Chrome (Cr) to enhance the adhesion of Au or Pt to the substrate and because of its inert biochemical properties.

In step (c), to fabricate the fluidic inlet and outlet of $\sim 500 \mu\text{m}$ in diameter, the silicon wafer coated with microelectrodes on the surface was sent to be etched through using ICP (Inductively Coupled Plasma) deep reactive ion etching. Meanwhile, a

microchannel of 75 μm in height, 800 μm in width was constructed on the glass wafer with the epoxy-based photosensitive resist SU-8. SU-8 was used for it can yield very well defined channel structure.

At this point, the chips were ready for bonding (Fig. 6.1d). Alignment marks were defined on the two chips previously in the microelectrode fabrication step. The electrode face-to-face alignment was performed under an optical microscope. An epoxy-based adhesive with 5-minute curing time was used to seal the structure. The chip was now ready for further processing such as attachment of microfluidic components, bonding of wires, etc.

Figure 0.1 Fabrication process flow of the paired microelectrode array.

0.0 Fabrication

Having overviewed the major steps of the fabrication, more details of the work will be discussed in this section. Unless specified otherwise, all the fabrication work was done in the clean room (class 100) of the MicroMachines Centre (<http://mmc.mpe.ntu.edu.sg/>).

0.0.0 Photolithography

Photolithography is a technique that involves the transfer of patterns on a mask to a photosensitive material (usually referred to as photoresist) by means of ultraviolet light. In general, a thin film of photoresist is deposited evenly on the substrate (e.g. silicon wafer). The film is then exposed to the UV light through a plate (generally referred to as mask) which contains an image of the pattern being created. The resist can be positively or negatively sensitive to the UV light. The masks are usually composed of a patterned thin film of Chrome on quartz. Depending on the resolution, the resist will be required to be sensitive to visual light, ultraviolet light, X-rays, or an electron beam.

In this work, the positive tone photoresist AZ9260 (Clariant Co., NJ) was used because it can yield thick films which are advantageous for the following lift-off process. The coating was performed in an automatic spin coater Suss Microtec Delta 80 (SUSS MicroTec Inc., VT). A 20-minute wafer cleaning was done in Piranha and dehydration was made on a hotplate at 120 °C for 20 minutes. A thin layer of HMDS (Hexamethyldisilazane) was spun onto the wafer to serve as the adhesion promoter. Then a thin film of AZ9260 was spin-coated onto the wafer. The thickness of the layer is

dependent on the speed and the duration setting set on the spin coater. In this work, a layer of AZ9260 with a nominal thickness of 9.91 μm was obtained at a final speed of 1500 rpm. Once the resist was spin-coated onto the wafer, it was pre-baked at 110 °C for 4 mins before it was exposed to the UV light (i-line, 365 nm) in the mask aligner (MA6, Karl Suss). Then the resist was developed in the developer for about 1.5 minutes. The process was completed when the wafer was rinsed thoroughly with DI water and blow-dried in nitrogen gas. Post-bake and hard-bake are not required for the resist AZ9260.

Figure 6.2 General layout of the mask. A variety of arrays with different configurations are addressed in the mask. The alignment marks are located at the center according to the view of microscope of the mask aligner. The left graph is the completed layout of the mask. The right one shows a magnified unit comprising two layers of electrode array, channel and fluidic ports.

The masks used in the photolithography process were designed using the commercial layout software *L-Edit version 11.00* (Tanner EDA, CA). Altogether four

separate masks were used for the complete fabrication of the device. The general layout of the finished mask design is shown in Fig.6.2. The layout defines a number of arrays with different electrode configurations in a single 4-inch wafer. The alignment masks are located at the center of the layout. Larger marks for rough aligning and smaller ones for precise aligning are involved.

0.0.0 Substrate

The most commonly used substrate in MEMS is silicon wafer, owing to its success in the fabrication of integrated circuits. In addition, its excellent electrical properties and mechanical properties enable the design of micromechanical structures. However, silicon may not be the material of choice for biological and medical microsystems. Besides silicon, glass and plastics are the other two options for the material of substrate. Glass wafers provide some unique features, most notably optical transparency, and are widely used in biomedical microsystems. Plastics are extended to the microscale due to the availability of mass production process (e.g. injection molding, embossing and casting). A majority of commercial enterprises are using plastic microdevices, especially for disposable clinical applications for its minimization of sterilization, clogging and drift.

In this work, the 4-inch-diameter 300- μm thick silicon wafer was chosen as the substrate for the bottom electrodes while the 4-inch-diameter 500- μm thick glass (Pyrex 7740) wafer was for the top electrodes. The silicon wafers were used because (i) silicon is much more amenable to deep reactive ion etching. The fluidic ports were easily created by etching through the silicon wafer; (ii) A reflective microscope (Olympus

MX40) was used in the observation. Silicon surface may reflect a larger portion of the incident light than the glass. For the top slide, the glass substrate was used for the requirement of optical access. Wafer pretreatments, including wafer cleaning, insulation layer thermally growing (1 μm SiO_2 for Si wafer), dehydration etc, were performed before further process began.

0.0.0 Electrode array

The electrode arrays were separately patterned on the silicon and glass wafers. Gold has been chosen for the electrode array layer because of its excellent biocompatibility. The complete electrode array (on one wafer) is a structure of double layers, i.e. 25 nm Chrome/100 nm Gold. The chrome was chosen to be used under the gold to serve as an adhesion layer between the silicon/glass and the gold.

In order to obtain well-defined metal lines, magnetron sputtering process followed by lift-off technique was used to form the Cr/Au metal bi-layer. The electrode pattern was defined by the contact lithography with AZ9260 as discussed in 6.2.1. However, more work needed to be done before the lithographic wafer was sent for sputtering, since there could be a thin film of unwanted resist left behind after development. To remove such unwanted resist, an oxygen plasma treatment (so called de-scumming) was performed with a Technics RIE 800II system. The etching was performed for 10 mins at the power of 250 W and an O_2 flow of 20 sccm (standard cubic centimeters per minute). The O_2 plasma etched the resist remained in the exposed area as well as the unexposed resist. Such a treatment ensured a good contact of the metal and substrate in the

uncovered area when the metal was deposited. The effect of the oxygen plasma etching was shown in Figure 6.3. Before the etching, the averaged thickness of AZ9260 was measured to be $10.18\text{ }\mu\text{m}$ (Fig. 6.3a), while after etching the thickness was decreased to $8.86\text{ }\mu\text{m}$ (Fig. 6.3b). A thickness of $\sim 1.3\text{ }\mu\text{m}$ was etched away.

Figure 6.3 The surface profile of the developed resist coated wafer before (a) and after (b) oxygen plasma treatment. The measurements were performed with a Dektak3 profilometer.

The resist profile is crucial for the lift-off process. Lift-off is such a process that a solvent dissolves the photoresist (generally positive tone) underneath the deposited metal, starting from the edge of the unexposed photoresist, and lifts off the metals. For this purpose, straight or reentrant photoresist sidewalls are required to be created. Techniques used to produce such resist profiles include soaking of the resist in chlorobenzene and image-reversal treatment. In this work, overexposure to the UV light was conducted during the photolithography step. The scattering at the refractive interface caused broadening of the radiation profile, which in turn undercut the resist in development. The

undercut profile enabled the solvent entering and dissolving the resist and lifting off the unwanted metal from the edge. The normal exposure time for the 9.91 μm resist is 40 s, while a higher dose of 50 s was used in the present case.

Then the wafer coated with developed resist was sent for deposition of Cr/Au layer with sputtering process. Alternatively, an e-beam evaporator (Edwards Auto 306 Turbo) was used to deposit the metal layer. It was found that the latter suffered from the peeling-off problem in the course of lift-off, whereas no obvious peeling-off was observed for the deposited electrodes obtained from sputtering process. In general, better contact between the deposited thin film and the substrate surface can be formed using sputtering rather than evaporator. To obtain the 25 nm Cr/100 nm Au layer, the basic parameters for the sputtering process were (i) base pressure of the main chamber was 10^{-4} mTorr; (ii) distance between the target and wafer was kept at 100 mm; (iii) Ar gas pressure was 2.3 mTorr; (vi) deposition of 4 mins for Chrome layer and 3 mins for gold layer at DC power of 200 W.

Following the deposition, the acetone lift-off process was performed. The wafer was immersed in acetone to remove the ‘unwanted’ area of the layers. To expedite the lift-off process, ultrasonic agitation was used. To avoid the commonly occurring stiction problem, the wafer was put upside down (with a mechanical support) so that the released metal pieces sedimentated to the bottom of the receptacle rather than the wafer surface. In addition, the wafer would not be dried until the lift off was completely finished. Figure 6.4 shows the electrode arrays fabricated using the above process. Both the bottom electrode on silicon wafer (a) and top electrode on glass (b) are shown.

Figure 6.4 The microfabricated electrode array:

(a) bottom electrode; (b) top electrode with channel.

0.0.0 SU-8 Channel

After forming the electrode layer, the next step is to construct the channel over the top electrodes. SU-8 is an epoxy-based negative photoresist developed by IBM in the 1980's. It is well known for its ability to be patterned with conventional contact lithography into high-aspect-ratio microstructures. The photoresist can be spun with single coats into layers ranging from a few microns to 100s microns thick, and is extremely conformal. In addition, the developed photoresist is quite robust and can be fashioned into structural layers.

SU-8 is a good choice for making channels in biomedical microsystems for a few reasons. First, channels of high depth used as solution containers in biomedical applications can be made but with conformal patterns. Second, SU-8 is inherently adhesive due to its base on epoxy, and leak-free channels are possible to be made with the resist. Finally, SU-8 is transparent and therefore suitable for biomedical applications. For these reasons, SU-8 was chosen to fabricate the channel in this work.

The rectangular channel is of a walled close pattern with a design thickness of 75 microns. The formulation of NATO SU-8 2075 (MicroChem Corp., Newton, MA) was used, which is suitable to form films of thickness ranging from 10s μm to 200 μm . All the SU-8 coating was performed with the Karl Suss Rc5 spin coater. Before the resist was coated, the wafer with electrodes was cleaned with Oxygen RIE process to enhance the adhesion. Adhesion promoter HMDS was not used since it is incompatible with SU-8. To dehydrate the surface, the wafer was baked at 200 $^{\circ}\text{C}$ for 5 minutes on a contact hot plate.

Then SU-8 2075 was spun onto the wafer with a two-step coating cycle. Approximately 4 ml of SU-8 2075 was first poured onto a stationary wafer. Care should be taken to avoid the formation of bubbles during the pouring. Then the spinning speed was ramped up to 500 rpm in 5 seconds and held at this speed for 10 seconds. This low speed was used to spread the resist over the whole area of the wafer. Then the speed was ramped to 3000 rpm at an acceleration of 300 rpm/s and held at the final spin for 30 seconds, resulting in a nominally 75- μm thick film.

Following coating, a two-level soft baking was performed to evaporate the solvent and densify the film. The wafer was first baked on a hotplate at 65 $^{\circ}\text{C}$ for 3 minutes. This lower bake temperature allowed the solvent to evaporate out of the film at a more controlled rate, which resulted in better coating fidelity, reduced edge beads, reduced stress and better resist-to-substrate adhesion. Then the temperature of the hotplate was ramped to 95 $^{\circ}\text{C}$ and the wafer was baked for 9 minutes. The wafer was allowed to cool

to about 30 °C before it was removed from the hot plate in order to minimize any thermal shocks.

The next step is to remove the edge bead. In general, an edge bead is observed at the periphery of the substrate after any spin, particularly for the resist of high viscosity. The existence of the edge bead limits the intimate contact of the sample and photomask in photolithography by producing an airgap. Therefore, the edge bead must be removed prior to pattern exposure. For the SU-8 used in this work, the thickness of edge beads was typically observed to be ~ 15 µm. To remove the edge bead, an automatic edge-bead removal (EBR) system integrated into the spin coater (Suss Microtec Delta 80) was used. AZ EBR solvent 1-Methoxy-2-Propanol acetate (also known as PGMEA, the SU-8 developer) (Clariant Corp., NJ) was dispensed to the edge of the spinning wafer (at 500 rpm) through the nozzle of the system which was precisely positioned by the setting. The same EBR process was run in 3 times to complete the removal. 3 mm of the resist around the periphery of the wafer was removed.

Following the edge bead removal, contact lithography was then performed to define the channel using the mask aligner system (MA6, Karl Suss). Vacuum contact mode was used and the exposure time was 50 s. Following exposure, a post exposure bake (PEB) was performed to selectively cross-link the exposed portions of the film. A two-step baking was also used for the exposed wafer. The wafer was first rested on a hotplate at 65 °C for 1 minute and then baked at 95 °C for 7 minutes. The hotplate was then turned off to allow the sample to cool down slowly.

Development was carried out in SU-8 developer PGMEA (MicroChem Corp., Newton, MA) for several minutes until a clear pattern was observed, then the sample was rinsed in isopropyl alcohol (IPA) for 10 s. Finally, the sample was blow dried with a stream of nitrogen.

The completed SU-8 channel fabricated with the above process is shown in Fig. 6.5. The channel had a well-defined and vertical sidewall profile. The averaged channel height was measured to be 73 μm , close to the nominal value of 75 μm .

Figure 6.5 (a) Photograph of the fabricated SU-8 channel. (b) Dektek3 measurement of the channel. The channel height was measured to be 73 μm .

0.0.0 Fluidic ports

The fluidic inlet and outlet (500 μm in diameter) were created onto the bottom substrate near the two ends of the channel. One of the methods to produce such fluidic ports was

using wet etching (such as HF-based glass etching, KOH-based silicon etching). However, such processes usually result in rough surfaces and nonuniform walls. In this work, the dry etching technology, ICP (Inductively Coupled Plasma) deep reactive ion etching (Deep RIE), was used to etch through the wafer to form the fluidic ports. The primary principle of Deep RIE is the alternated usage of two gas compositions in the reactor. One is responsible for passivation by producing a polymer at the substrate surface, while the other etches the substrate from the bottom. In this way, patterns of high aspect ratio can be achieved at a fast etching rate.

Again, a patterned thin film of AZ9260 (thickness $\sim 10\text{ }\mu\text{m}$) was coated as the etching mask. Before coating, a second bare silicon wafer was attached to the back of the sample wafer as a support using a thin layer of AZ9260 and baked at $200\text{ }^{\circ}\text{C}$ for 1 hour. The second wafer was necessary since the wafer was about to etch through. The etching process was performed in a two-step manner. First, the $1\text{-}\mu\text{m}$ layer of SiO_2 was etched away in a RIE system (Technics RIE 800II) since etching of SiO_2 with Deep RIE will contaminate the chamber. Typically, the sample was etched for 40 mins at 300 W with an O_2 stream of 2 sccm and a CF_4 stream of 15 sccm. The oxide layer was completely etched away by examining the color of the film to be as same as silicon. In addition, the RIE process also etched the resist. By measuring the resist with the profilometer, it was found that a thickness of about $1\text{ }\mu\text{m}$ was etched away. The remaining $9\text{-}\mu\text{m}$ resist was sufficient for masking the Deep RIE process. Then the sample was sent for deep reactive ion etching of silicon with a STS system (Surface Technology Systems plc, Newport, UK). The etching rate was about $3\text{ }\mu\text{m}/\text{min}$ with a gas recipe of $115\text{-sccm SF}_6 + 13\text{-sccm}$

O_2 + 100-sccm C_4F_8 at a cycling of 8 second passivation and 13 second etching. The sample wafer was completely etched through for about one and half hour. After etching, the sample was immersed in acetone to release the attached wafer.

0.0.0 Precise Bonding

At this point, the wafers were ready for bonding. Adhesive bonding was used to bond the developed SU-8 layer (patterned on the glass wafer) to the silicon wafer. Therefore, an epoxy-based adhesive was used. The challenge consisted in precision alignment and complete coverage of the structure during the bonding. For our purpose, alignment marks were defined on the two chips previously in the microelectrode fabrication step. A thin layer of adhesive was first applied on the top surface of the developed SU-8 by a lamination process. The electrode face-to-face alignment was then performed using the Mask aligner Karl Suss MA6 which is of micrometer precision. In our application, a two-part adhesive (Araldite Rapid, Huntsman Advanced Materials (UK) Ltd, Cambridge, UK) with 5-minute curing time was used. After precise alignment, the aligned wafers rested on a horizontal plane (with a certain pressure by weight) at room temperature for 24 hours to attain the maximum bonding strength. The adhesive provided a bond of good coverage, high bonding strength, and small adhesive thickness. Typical thickness of the adhesive layer less than 1 μm was determined by microscopic examination of the cross-section of a sample. The bonded wafers were then carefully cut into slides by a diamond saw. Figure 6.6 shows the graphs of bottom and top slides, and the bonded paired electrode system.

Figure 6.6 (a) Bottom silicone slide with electrodes. (b) Top glass slide with electrodes. (c) The paired electrode system constructed by the adhesive bonding process.

0.0 Packaging

The device had to be in some form of package before it could be used for experiment. The packaging scheme used for the array must meet at least three requirements. First, it allows an accessible interface for the introduction of particle-containing liquids into the microchannel from the outer world of the system. Second, electrical connections for the signal of high frequency but low voltage can be easily attained. Third, observation of the particles in the channel under a microscope is accessible for the packaging.

0.0.0 Microfluidic access

For the microfluidics, the challenge lied in the way to provide an interface for the fluid introduction from the macroscopic scale to the microscale in a leakproof manner. In microfluidics, the commonly used component for fluid delivery is microtubing, and the connections between the fluidic ports and microtubing are generally addressed with

fittings. The microfluidic parts comprising the fittings and microtubings used in this work are shown in Fig. 6.7. Polypropylene fittings (Cole-Parmer, IL, USA) were attached to the ports in the bottom slide using epoxy adhesive. Then FEP (Fluorinated Ethylene Propylene) tubings with inside diameter of 1/32" (0.79 mm) were connected to the system via the fittings. In such a way, the fluid was able to be introduced into the channel without leaking at a moderate pressure.

Figure 6.7 (a) Fluidic ports in the bottom slide. (b) Polypropylene fittings attached to the ports.

(c) Tubings connected to the chip via the fittings.

0.0.0 Electrical connection

The electrical signals were applied to the electrode arrays via the connection to the pads located at the edge of the chip (Fig. 6.8a). The two slides were precisely diced and aligned so that the pads were uncovered by each other when the slides were bonded together. Copper wires were bonded to the electrical pads (using a wire bonder system or conductive adhesive) on one of the slides first. Then the package was turned over for the wire bonding to the pads on the other slide (Fig. 6.8b).

Figure 6.8 (a) Electrical pads located near the edge of the slide;

(b) Copper wires were bonded to the pads.

A homemade printed circuit board (Fig. 6.9) was used to control the status of the signals. The output from the function generator was divided using a solderless breadboard. Two 8-way switches controlled the on/off status of each electrode pair by manually switching the respective switch.

Figure 6.9 The PCB was made for electrical control.

0.0.0 Assembling

Figure 6.10 (a) Schematic diagram of the packaging. (b) Packaging of the DEP-based microfluidic system comprising the 3-D paired electrode array. The DEP chip was mounted on a custom-built mechanical block. Polypropylene fittings and FEP tubings served as fluid delivery.

External electrical signal connected to the system via contact pads.

The completed packaging assembly is shown in Fig. 6.10. A PMMA (polymethyl methacrylate) block with fluidic hole drilled in was used as a mechanical support for the system. The drilling was done with a laser cut system. The DEP chip was mounted on the block using a strongly adhesive double-sided tape. The tubing was then fitted. The wires

leading from the electrical pads were bonded to the connections located at the sides of the block. Now the packaging was ready for external connections such as the electrical signal from the PCB, fluid from the syringe pump etc. One advantage of this package is low profile, in that there are no protrusions above the plane of the top slide, which makes it easy to mount the package under a conventional microscope for observation.

0.0 Conclusion

This chapter described the fabrication process of the paired microelectrode array and the packaging scheme. The process used sputtering deposition followed by lift off process to fabricate electrode on the bottom and top wafer, and SU-8 was used to construct the channel. Then the two slides were precisely bonded face-to-face to form the 3-D paired electrode system. The packaging scheme devised in the work met the stringent requirements for electrical input, fluidic introduction, and optical access. The experimental tests carried out with the system will be addressed in the next chapter.

CHAPTER 0 DEP Microactuation with the 3-D Microelectrode Array

The performance of the microfabricated paired electrode array will be experimentally validated in this chapter. To do this, a fluid flow rather than the stationary fluid containing microparticles was used. Polystyrene particles differing in diameter were investigated in the tests. As described below, the holding capacity (indicated with threshold velocities) is different for microparticles of different electrical properties or physical sizes. Such differences provide us possible means to separate and manipulate microparticles with the 3-D paired microelectrode system.

0.0 Materials and methods

0.0.0 Beads and solutions

All beads and solutions were prepared in the same manner described in Chapter 4. Polystyrene beads with two diameters 9.6 μm and 16 μm were used. The beads were resuspended in low conductivity stock solution before use. The solution was made by deionized water with appropriate amounts of PBS added. The conductivity of the solution was measured with a conductivity meter with graphite sensor electrodes (Dist3WP, Hanna Instruments Inc., RI).

0.0.0 Modeling method

The numerical modeling was performed using the modeling environment described in Chapter 3. Some of the specifications were accordingly changed with reference to the actual values in the experiments.

0.0.0 Laminar flow

The flow generated within the channel was thought to be laminar since the system has a small Reynolds number Re . For the present microelectrode system, Re can be calculated from $Re = \rho_m ul / \eta$, where ρ_m is the mass density of water, l is the characteristic length of the system. u is the velocity of the fluid, which is in the order of 100 $\mu\text{m/s}$. η is the viscosity of the fluid. With $l = 75 \mu\text{m}$ being the channel height, the value of Re is in the order of 10^{-3} .

The generated laminar flow has a parabolic flow velocity profile in the vertical plane (i.e. x - y plane) as shown schematically in Fig. 7.1. The velocity distribution profile within the channel is

$$v(y) = 6v_0 \frac{y}{h} \left(1 - \frac{y}{h}\right) \quad (7.1)$$

where v_0 is the input velocity. h is the channel height and y is vertical location in the channel.

For the present microelectrode system, the particles are levitated to the centerline of the channel where the velocity is expressed by the flowrate indicated by the syringe pump

$$v_{\max} = 1.5Q_{rf} / (w \times h) \quad (7.2)$$

where Q_{rf} is the flowrate generated by the syringe pump. w and h are the width and height of the channel, respectively.

In addition, the velocity in the horizontal plane (i.e. x - z plane) was assumed uniform since the channel had a high aspect ratio.

Figure 0.1 Parabolic flow velocity profile within the channel. Particles levitated to the centerlines of the channel are subjected to the maximum flow velocity.

0.0.0 Measurement of channel height

The principle of the method to measure the physical height of the channel enclosed by the bottom and top slides is indicated in Figure 7.2. The method was in a two-step manner. First, the height h_1 was measured. Then the thickness of the glass chip (h_2) was measured. The height of channel was given as $h = h_1 - h_2$. The measurements of heights h_1 , h_2 were taken with a Logitech CG-10 Precision Electronic Measurement System (Logitech Ltd, Glasgow, UK) which has a precision of $1\ \mu\text{m}$, with h_1 being the difference of probe positions in planes 1 and 3, h_2 being the difference of probe positions of planes 2 and 3. Each height was measured for 10 times at different points and the average value

was taken. Typically, the height of the channel was measured to be $78.1\ \mu\text{m}$, which was within 5% difference from the nominal height $75\ \mu\text{m}$.

Figure 7.2 Measurement of the height of the channel.

0.0.0 Measurement of threshold velocity

The threshold velocity is defined as the flow velocity at which the particles begin to penetrate the dielectrophoretic gate that is formed by the energized top and bottom electrodes (Section 5.3). The threshold velocity was measured in a bi-directional manner, in which the flowrate was gradually increased up to the point where particles began to penetrate the dielectrophoretic gate, and the flowrate was gradually decreased down to the point where particles began to deflect from the gate. The flowrate was readily set and programmed through the keypad of the syringe pump. Each measurement was taken a few times and the average value was used.

0.0.0 Experimental setup

Figure 7.3 (a) Schematic of the experimental setup. (b) Photographs of the setup.

The experimental setup is shown in Fig 7.3. The setup comprised the electrical, fluidic, and optical units. Sine wave electrical signals up to 20 MHz and 15 V_{pp} (into 50 Ω) were generated by a Wavetek 90 Function Generator (Wavetek Wandel & Goltermann, Inc., NC, USA). The fluidics components include a custom-built microfluidic connector block, a syringe pump (NE1000, New Era Pump Systems, Inc., NY), two 500 μ l luer-lock syringes (Model 750, Hamilton, Reno, Nevada), a 4-way

valve (V-101D, Upchurch Scientific, Oak Harbor, WA), and microtubings. The valve allowed the interchanging of syringes without introducing bubbles. A high-resolution inspection microscope system Olympus MX40 (Olympus Corp, Tokyo, Japan) was used for particle observation. Images and movies were captured and recorded using a CCD and SONY ExwaveHAD color video camera (SONY Corp., Japan)

0.0.0 Calibration of the flowrate

The flowrate indicated by the syringe pump was taken to calculate the threshold velocity based on the assumption that the display value accurately represented the real flow velocity. Therefore, the flowrate from the syringe pump had to be calibrated. The calibration was done with the movie captured by the video camera attached to the microscope. The travel of the particles was analyzed with respect to the timescale (indicated by the software). The distance of the travel was previously defined with two marks. In this way, the particle velocity (or flow velocity) was determined. The process repeated for a few times and it was found that the results agreed well with the value indicated by the syringe pump, meaning that it was reasonable to take the displayed values from the syringe pump as the flow velocity.

0.0 Threshold velocity

It has been demonstrated in chapter 4 that the n-DEP force reached its maximum at the frequency around 1 MHz for the latex beads. Therefore, all the studies onwards were carried out at the frequency of 1 MHz. Typically, the threshold velocity was measured

for the 45° and 60 µm electrode pair. The results are shown in Figure 7.4 for the latex beads with diameter of 9.6 µm and 16 µm, respectively. The velocity was calculated from Eqn (7.2) with $V_{th} = 1.5Q_{rf} / (w \times h)$, where Q_{rf} is the flowrate (µl/hour) indicated from the syringe pump, w and h are the width and height of the channel, respectively. Both the numerical and measured values are shown. The numerical modeling used the following parameters: $f = 1$ MHz, $\rho_p = 1.05$ g/cm³, $\epsilon_p = 2.55$, $\sigma_p = 0.1$ mS/m, $\epsilon_m = 79$, $\sigma_m = 1$ mS/m. It can be seen that the numerical and experimental results agreed well with each other. The threshold velocity was higher for the larger particle, and increased as the applied voltages were increased. For lower voltages ($< 1 V_{pk-pk}$), particles were found to be sedimentated to the bottom surface by gravitational force, since the DEP force was insufficiently strong to levitate the particles. In such situations, particles tended to adhere to the bottom surface. Therefore, voltages higher than $2 V_{pk-pk}$ were used.

The diagram of the threshold velocity comprises three regions as indicated by sequence numbers (I), (II) and (III). Flow velocity falling into any one of these regions results in a certain type of particle movement:

(I) Low velocity: A flow velocity lower than both the threshold velocities of the two particles is used. In such a flow, the DEP force is dominant and both the particles cannot pass through the dielectrophoretic gate, resulting in accumulation of the particles (section 7.4). No separation of the particles takes place subjected to such a flow.

(II) Medium velocity: A flow velocity between the threshold velocities of the two particles results in separation of the particles (section 7.5). That is, the particle of smaller size penetrates the dielectrophoretic gate while the other settles at the gate.

(III) High velocity: velocities higher than both the threshold velocities of the particles will wash away all the particles. The dielectrophoretic gate fails to deflect the particles. This is useful in collecting the accumulated or separated particles.

The existence of the three regions of V_{th} gives rise to possible separation and manipulation of particles. Generally, more regions can be found in the diagram of V_{th} when more particles of different properties are examined. Here, particles can differ in sizes or electrical properties.

Figure 7.4 Threshold velocity for the latex beads with diameter of 8 μm and 16 μm , respectively.

Aligning angle is 45° and electrode width is 60 μm . Both the numerical values and measured values are shown.

0.0 Particle trajectories

Figure 7.5 Particle trajectories close to the electrode pair when it was deflected by the electrodes.

Shown are the positions 1 – 6 of the particle captured in sequence of time with an interval of ~ 250 ms. The captured positions are combined into one single photograph using software. The

fluid flow was running from the left to the right. The electrodes were aligned at 60°

and the width was $40\ \mu\text{m}$.

As discussed above, the particles will either penetrate the dielectrophoretic gate or be deflected by the gate depending on the strength of the flowrate. When the particle penetrated the gate, the movement trajectories were observed to be straightforward, with only a slight displacement (few microns) in the direction of channel width when the particle entered to and then exited from the gate. However, the trajectory of a particle was deflected by the electrodes at lower flowrate. The trajectories are shown in Fig. 7.5. Shown are the time-dependent positions of the particle running close to the electrodes subjected to a flowrate of $50\ \mu\text{l/hour}$. The positions (from 1 to 6) were captured in a sequence of time and combined into a single graph using software. As expected, the

particle gradually swerved into the direction parallel to the electrode when it moved close to the electrodes. The particle changed its direction of movement under the combined effects of the flow drag force and DEP force.

0.0 Accumulation of microparticles – Region (I)

All the particles will be deflected by the dielectrophoretic gate at flowrate lower than the threshold velocity. Since the particles have lateral movement along the electrode caused by the hydrodynamic force (section 7.3), particles will be collected at the corner formed by the electrodes and channel wall. Fig. 7.6 shows the particles with diameter of 9.6 μm and 16 μm accumulated by the paired electrode array. A flowrate of 50 $\mu\text{l}/\text{hour}$, resulting in a mean flow velocity of $\sim 231.5 \mu\text{m}/\text{s}$ which fell into region (I) as shown in Fig. 7.4, was used. The electrode width was 60 μm and the electrode was aligned at 45° with respect to the direction of the flow. The flow was running from the left to the right. The particles running with the flow were deflected by the paired electrode structure when they were near the electrode, and continued to move in the direction parallel to the electrode, till to the accumulation area as indicated by 'A' in Fig. 7.6. An aggregate with a triangular shape was formed between the electrode and channel wall when more and more beads ran into that area. The accumulated beads could be released from the accumulation area by turning off the applied voltage or increasing the flowrate.

Figure 7.6 Particles were accumulated by the paired electrode array at the flowrate of 50 $\mu\text{l}/\text{hour}$ and applied voltage of 13 $V_{\text{pk-pk}}$.

0.0 Separation of microparticles – Region (II)

As the flowrate was increased, the particles had the potential to escape from the accumulation area and run through the dielectrophoretic gate. As discussed in 7.2, the smaller particle (9.6 μm) will begin to penetrate the gate first since it has a lower threshold velocity. Choosing a proper flowrate, which is between the threshold velocities of the two particles (Region II in Fig. 7.4), can separate them into two subpopulations. Fig. 7.7 shows the separation of the two particles with the paired electrode system. Two processes, on the basis of a single paired electrode and a couple of paired electrodes, are shown, respectively. At the flowrate of 120 $\mu\text{l}/\text{hour}$ (mean flow $\sim 555 \mu\text{m}/\text{s}$), the particles of 9.6 μm (A) escaped and ran through the gate, while the other particles of 16 μm (B) remained in the accumulation area (Fig. 7.7a). The 9.6- μm particles were carried away by the flow to the external container via the outlet. In addition, a couple of paired electrodes were used to separate the particles, as shown in Fig. 7.7b,c. In order to

increase the threshold velocity for respective electrodes, the first electrode was aligned at 60° and the width was $40\text{ }\mu\text{m}$, while the second one was aligned at 45° and the width was $60\text{ }\mu\text{m}$. At the flowrate of $65\text{ }\mu\text{l/hour}$ (mean flow $\sim 300\text{ }\mu\text{m/s}$), the particles began to separate (Fig. 7.7b). The smaller particles penetrated the first electrode pair while the larger ones settled before the first electrode. However, smaller beads moved forward and were subsequently accumulated at the second electrode pair. Fig. 7.7c shows the separation result of the particles after an application of the flowrate for 5 minutes. The two types of particles were separated and concentrated at different positions in the systems. To collect the particles, switching off the voltage on the second electrode while keeping first electrode on, particles of $9.6\text{ }\mu\text{m}$ will be carried away by the flow to the external container via the outlet. Then particles of $16\text{ }\mu\text{m}$ can be collected by turning off the first pair of electrodes (and the second pair also off). In this way, particles of different sizes were successfully separated. Note that it should also be possible to separate particles of equal size but with different dielectric properties.

Figure 7.7 (a) Particles of 9.6 μm penetrated the gate and were carried away by the flow. (b)

Particles of 9.6 μm penetrated the first gate and stopped at the second gate. (c) After 5 minutes, particles were separated and accumulated at different places.

0.0 Interparticle interactions

In order to collect the accumulated subpopulations, the flow velocity was increased above the threshold velocity while keeping the voltage on. However, it was noticed that

it was hard to release the accumulated particles by simply increasing the flowrate, even to a flowrate much higher than the threshold velocity. The possible factor contributing to this result may be the interparticle interaction. Closely spaced particles subjected to an electric field experience the dipole – dipole interaction [10]. At close spacing, the field-induced interaction can become quite strong, and the mutual force of the interaction can be attractive or repulsive depending on the relative alignment of the particles. In the present application, the accumulated particles were highly condensed due to the combined effects of the DEP force and hydrodynamic force. The accumulation was enhanced by the previously trapped particles, probably due to the dipole-dipole interaction. The accumulated particle aggregate instead of individual particles responded to the flow, meaning that the particles formed an organized structure and behaved en masse due to the strong interactions among them. Fig. 7.8 shows the evolution of the accumulated particle aggregate by varying the flowrate from 50 $\mu\text{l/h}$ to $\sim 500 \mu\text{l/h}$. At the early stage, more and more particles were trapped at the accumulated area. The late-coming particles entered the trap through the front, and the particles located at the front rotated to be balanced (Fig. 7.8a). As the aggregate grew up, the trapped particles aligned to form a stable organized structure (b). Increasing the flowrate can change the shape of the structure. The structure was pushed much closer to the electrode (c). At sufficiently high flowrate, the aggregate began to break down and escape (d). It was found that the release flowrate could be very high. Typically, for the electrode structure with width of 40 μm and aligning angle of 60° , the release flowrate for the 16 μm particle aggregate containing about 30 particles was $\sim 500 \mu\text{l/h}$ with applied voltage of 14

V_{p-p} , which was more than 3 times higher than the threshold velocity (flowrate) of a single particle ($\sim 150 \mu\text{l/h}$). It implied that there was a strong interaction between the accumulated particles. The dipole-dipole interaction vanished once the applied voltage was turned off. The particles located at the channel wall were not taken into consideration since the flow velocity was relatively slow near the wall.

Figure 7.8 Evolution of the accumulated particle aggregate. (a) At early stage, the unstable particles at the front rotated to be self-balanced. (b) The size of the aggregate became larger (flowrate = $50 \mu\text{l/h}$). (c) At higher flowrate of $70 \mu\text{l/h}$, particles were pushed closer to the corner. (d) At sufficiently high flowrate ($\sim 500 \mu\text{l/h}$), the accumulated particles began to escape from the trapped area.

7.7 Conclusion

The performance of the microfabricated paired electrode array was experimentally validated. Particles differing in sizes or dielectric properties have different threshold velocities. For the two latex beads examined in this work, the flowrate was divided into three regions bounded with the threshold velocities. Using a proper range of flowrate, particle accumulation and separation have been successfully achieved with the microfabricated device. The presented paired microelectrode system has potential applications to manipulate particles of different size or different dielectric properties.

CHAPTER 0 Conclusion and Future Work

0.0 Conclusions

Dielectrophoresis is a versatile technique of great potential to impact bioscience. DEP has been demonstrated to have great ability for separation, trapping and characterization of microparticles/bioparticles based on their geometric and dielectric properties. This thesis has been focused on the DEP-based microactuation for micron-sized particles or bioparticles such as polystyrene beads, biological cells. Although polystyrene particles were used in all the verification studies, the results have demonstrated the fundamental facts of DEP phenomena. These results have been used to set up the strategies for dielectrophoretic manipulation of bioparticles (e.g. cells). Generally, a cell can be modeled as a homogeneous spherical or ellipsoidal particle that has equivalent dielectric properties to the original one (section 2.3, [10]). Dielectrophoretic manipulation of cells can be performed in a similar manner to that used for the polystyrene beads. This thesis made efforts to address various issues in developing DEP-based microsystems for microactuation of particles. The research work involved fundamental study, modeling methodology, design and optimization, microfabrication, and experimental tests for DEP-based microactuation. The results achieved are summarized as below:

- For the first time, the meshless LSFD method was introduced to solve DEP problems in this work. The reason to develop such a modeling environment is that analytical solutions are unavailable for most of complex DEP systems, and numerical approaches were demonstrated to produce an accurate approximation. The developed model is useful because of its ease and flexibility for mesh or grid point generation

and distribution in the computational domain. For the electric field used in DEP systems, extremely high gradients of field can be found in specific locations in the domain, e.g. edges of the electrodes, field nulls. The meshless scheme allows feasible point generation in these locations. The feasibility of the modeling environment was validated with the cDEP and twDEP systems, and later it was applied to design and optimize the paired electrode array. With the modeling tool, it is now possible to perform fundamental quantitative studies of various DEP systems.

- Much insight into the DEP behavior was gained with the experiments in planar electrode systems. Lateral movements of the particle in the interdigitated array were observed. Passive dielectrophoretic levitation was thoroughly studied for the micron-sized polystyrene beads. An experimental scheme, consecutively focusing method, was applied to measure the levitation heights under various testing conditions. The levitation heights are significant for characterizing the dielectric properties of particles and for possibly separating microparticles.
- Based on the studies with the planar electrode systems, a 3-D paired microelectrode array was proposed to improve the performance of the existing planar electrode systems. An optimization procedure was performed to design the paired electrode system for achieving high holding capacity (defined by the threshold velocity). The 3-D electrode system overcame the escape problem (due to levitation) encountered in the planar system. A paired electrode array containing electrode pairs with varying aligning angles and widths was devised for particle manipulation.
- The 3-D microelectrode system was fabricated with microfabrication techniques. The

process of constructing the 3-D electrodes by aligning two chips with electrodes may be useful for fabrication of other similar 3-D electrodes. In addition, the packaging scheme employed for the electrode system solved many problems encountered in these systems. The scheme met the requirements for interfacing electrical, fluidic, and optical inputs to a microfabricated device with a package that is easy-to-use and robust.

- Finally, the performance of the 3-D microelectrode system was experimentally validated with latex beads. The diagram of the threshold velocity was divided into a few regions. A proper range of flowrate resulted in the accumulation, separation, collection of the particles. The particles were manipulated in a continuous flow, and hence a great efficiency can be achieved. Particles differing in sizes or dielectric properties can be handled with the paired microelectrode system.

0.0 Future Work

This thesis describes the efforts to develop versatile DEP-based microsystems for manipulation and separation of microparticles. The work has demonstrated the feasibility of the developed systems in a proof-of-concept manner. Based on the achievements to date, several issues related to the topic may be addressed in the future to enrich this work.

First, polystyrene beads were used in the tests. There would be no doubt that the results represent significant progress for handling of bioparticles such as biological cells. As a good demonstration and application, the work may be extended to the bioparticles.

Several significant but fundamentally surmountable challenges exist when the cells other than the latex beads are considered. For example, the cell has much more complex dielectric properties than the bead and presents nonuniformity across its whole body; the cell can be of any geometric complexity other than the spherical, elliptical ones; biophysics of the cell in the AC electric field is complex. To solve all these problems, a general approach is to determine the equivalent dielectric model representing the original one [10].

Second, improvements need to be made in manufacturability. The present method to obtain the 3-D paired microelectrode array demonstrated a feasible one but not an optimized one. A more yield-enhancing scheme would be proposed for the precise alignment and bonding of the two layers of electrodes. In addition, it may be an advantage if the two layers of electrodes can be fabricated into one single wafer so that the bonding process is not required.

Third, the performance of the paired microelectrode system has been demonstrated in Chapter 7 with successful handling of two latex beads. In principle, the system is capable of multi-particles manipulation. When more types of particles are concerned, the diagram of the threshold velocity would comprise more regions. Generally, the number of the regions is $(n+1)$, where n is the number of particle types that are to be handled. With an ingenious scheme, separation of multiple particles can be achieved.

Finally, local heating was not considered in this work. The high electric field generated in the bulk fluid produces thermal dissipation as well. This influences the system in two ways (i) the local temperature of the fluid due to Joule heating may

increase considerably. The high temperature may cause biological damage. (ii) the nonuniformity of the electric field gives rise to the nonuniformity of the thermal dissipation. The latter generates volume forces in the liquid by producing gradients in conductivity and permittivity, which is referred to as electrohydrodynamic force. The induced fluid in turn influences the movement of the particles. For the presented microelectrode system, such effects may be studied in case that the medium has a high conductivity.

Appendix A: Frequency dependence of Traveling wave DEP

The CM factor of a uniform sphere is given by Equation (2.3) which is quoted again as below

$$K(\omega) = \frac{\underline{\varepsilon}_p - \underline{\varepsilon}_m}{\underline{\varepsilon}_p + 2\underline{\varepsilon}_m} \quad (\text{B.1})$$

with $\underline{\varepsilon} = \varepsilon - i\sigma/\omega$ the complex permittivities of the particle and the suspending medium.

The traveling wave DEP (twDEP) arises from the contribution of the out-of-phase component, and the frequency dependence of twDEP resides in the imaginary part of the CM factor (see Equation 2.8). Equation (B.1) reduces to Equation (2.4) at low and high frequencies, meaning that $\text{Im}[K(\omega)] = 0$ and twDEP force is zero at these frequencies.

Substituting the complex permittivities into the above equation, the imaginary part is given as

$$\text{Im}[K(\omega)] = \frac{(\varepsilon_p - \varepsilon_m)(\sigma_p + 2\sigma_m) - (\varepsilon_p + 2\varepsilon_m)(\sigma_p - \sigma_m)}{\omega(\varepsilon_p + 2\varepsilon_m)^2 + (\sigma_p + 2\sigma_m)^2 / \omega} \quad (\text{B.2})$$

At low and high frequencies, the right hand side of Equation (B.2) tends to be zero as discussed above. On the other hand, the above equation reveals that crossover frequency dose not exist for the twDEP. $|\text{Im}[K(\omega)]|$ reaches its maximum value

$$|\text{Im}[K(\omega)]|_{\max} = \frac{1}{2} \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} - \frac{1}{2} \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \quad (\text{B.3})$$

at the frequency of $\omega_{\max} = \sqrt{\frac{\sigma_p + 2\sigma_m}{\varepsilon_p + 2\varepsilon_m}}$.

Depending on the conductivities and permittivities of the particle and medium,

$|\text{Im}[K(\omega)]|$ can vary from the range of -0.75 ($\varepsilon_p \ll \varepsilon_m, \sigma_p \gg \sigma_m$) to $+0.75$ ($\varepsilon_p \gg \varepsilon_m, \sigma_p \ll \sigma_m$).

Appendix B: Solving particle movements by a finite difference method

The particle's movement in the solution subjected to the DEP force and hydrodynamic force is governed by equation (3.36).

Consider the typical motion of a particle with respect to time in the medium with the initial position (x_0, y_0) at (a, b) and zero initial velocity. This is an initial value problem with the following initial conditions ($t = 0$).

$$\begin{aligned} x_0 &= x(0) = a, y_0 = y(0) = b \\ \dot{x}_0 &= \dot{x}(0) = v_a, \dot{y}_0 = \dot{y}(0) = v_b \end{aligned} \quad (\text{B.1})$$

We can approximate the derivatives by the difference method to give

$$\begin{aligned} \dot{x}_n &= \frac{x_{n+1} - x_{n-1}}{2h}, \dot{y}_n = \frac{y_{n+1} - y_{n-1}}{2h}, \\ \ddot{x}_n &= \frac{x_{n+1} - 2x_n + x_{n-1}}{h^2}, \ddot{y}_n = \frac{y_{n+1} - 2y_n + y_{n-1}}{h^2}, \quad n = 1, 2, 3 \dots \end{aligned} \quad (\text{B.2})$$

Here h is the time step.

Substituting the above equations into equation (3.36) produces the numerical solution in the following form

$$\begin{aligned} x_{n+1} &= \frac{h^2 F_{DEP,x}}{M} + \left(2 - \frac{6\pi\eta ah}{M}\right)x_n + \left(\frac{6\pi\eta ah}{M} - 1\right)x_{n-1} \\ y_{n+1} &= \frac{h^2 F_{DEP,y}}{M} + \left(2 - \frac{6\pi\eta ah}{M}\right)y_n + \left(\frac{6\pi\eta ah}{M} - 1\right)y_{n-1} - \frac{h^2(\rho_p - \rho_m)vg}{M} \\ &\quad (n=1, 2, 3 \dots) \end{aligned} \quad (\text{B.3})$$

where $M = m + 3\pi\eta ah$.

It is necessary to estimate x_1 and y_1 as accurately as possible before the recurrence

time integration can be begun. We obtained x_1 and y_1 using the 3-rd order truncated Taylor series as

$$\begin{aligned} x_1 &= x(h_0) = x(0) + h_0\dot{x}(0) + h_0^2/2\ddot{x}(0) + E(h_0^3) \\ &\approx x(0) + h_0\dot{x}(0) + h_0^2/2f(x(0), \dot{x}(0)) \\ y_1 &= y(h_0) = y(0) + h_0\dot{y}(0) + h_0^2/2\ddot{y}(0) + E(h_0^3) \\ &\approx y(0) + h_0\dot{y}(0) + h_0^2/2g(x(0), \dot{x}(0)) \end{aligned} \quad (\text{B.4})$$

where $f(x(t), \dot{x}(t)) = \ddot{x}(t)$, $g(y(t), \dot{y}(t)) = \ddot{y}(t)$, h_0 is the first time step of sufficient small value ($\sim 10^{-6}$ second).

Thus the position (x, y) or trajectory of the particle can be numerically solved by the recurrence relation in (B.3).

Figure B.1 Flowchart for program to calculate particle movement with steps $h_0 < h_1 < h_2 \dots < h_k$.

The flowchart of the program using the above method to determine particle's motion is shown in Figure B.1. Multi-steps ($h_0 < h_1 < h_2 \dots < h_k$) are used in this program in order to reduce the calculation time. Smaller steps ($\sim 10^{-5}$ s) are used in the earlier recurrence because the accuracy of the initial approximation is very import. However, smaller steps lead to longer computational time. For compromise, larger time steps are used in later stage of time integration.

Appendix C: An analytical solution using separation of variables method with the approximate boundary condition

With the approximate boundary condition, the problem can be described as

$$\frac{\partial \phi^2}{\partial x^2} + \frac{\partial \phi^2}{\partial y^2} = 0, \quad (0 \leq x \leq l, 0 \leq y \leq +\infty) \quad (\text{C-1})$$

with the bottom boundary condition in Figure 3.18 and the rest boundary conditions in Figure 3.7.

To solve the equation, ϕ can be assumed to have the product form

$$\phi(x, y) = X(x)Y(y) \quad (\text{C-2})$$

where the function $X(x)$ and $Y(y)$ depend solely on x and y , respectively.

Substituting (C-2) into the Laplace's equation, variables are separated as

$$\frac{X''}{X} = -\frac{Y''}{Y} = -\kappa^2 \quad (\text{C-3})$$

where primes denote differentiation with respect to coordinate. κ is non-negative constant needed to be determined.

Solving (C-3) and using superposition to combine $\kappa = 0$ and $\kappa \neq 0$ solutions (since the

Laplace's equation is linear), we have

$$\begin{aligned}\phi &= (Ex + F)(Gy + H) + (A \cos \kappa x + B \sin \kappa x)(Ce^{-\kappa y} + De^{\kappa y}) \\ &= Ixy + Jx + Ky + M + (A \cos \kappa x + B \sin \kappa x)(Ce^{-\kappa y} + De^{\kappa y})\end{aligned}\quad (C-4)$$

$\kappa \neq 0$

The constants to be determined are $I = EG$, $J = EH$, $K = FG$, $M = FH$, A , B , C , D .

Applying the boundary conditions, the constants in (C-4) can be determined. All the constants are found to be zero except B, C and κ . (C-4) is simplified as

$$\phi = N \sin \kappa x e^{-\kappa y} \quad (C-5)$$

where $N = BC$, $\kappa = \frac{n\pi}{l}$ ($n = 1, 2, 3, \dots$).

Using superposition for $\kappa = \frac{n\pi}{l}$ ($n = 1, 2, 3, \dots$), we have

$$\phi(x, y) = \sum_{n=1}^{\infty} N_n \sin\left(\frac{n\pi}{l} x\right) e^{-\frac{n\pi}{l} y}, \quad (0 \leq x \leq l, 0 \leq y \leq +\infty) \quad (C-6)$$

N_n is coefficient of the half-range sine (HRS) Fourier series and given by

$$\begin{aligned}N_n &= \frac{2}{l} \int_0^l f(x) \sin\left(\frac{n\pi}{l} x\right) dx \\ &= \frac{4lV_0}{d_2 n^2 \pi^2} \left[\sin\left(\frac{n\pi d_2}{2l}\right) + \sin\left(\frac{n\pi(2d_1 + d_2)}{2l}\right) \right]\end{aligned}\quad (C-7)$$

Thus the solution is of the form of infinite series.

The electric field is obtained by taking the first-order derivative of the potential

$$\begin{aligned}\mathbf{E} &= -\left(\frac{\partial \phi}{\partial x} \hat{x} + \frac{\partial \phi}{\partial y} \hat{y}\right) \\ &= \left[-\sum_{n=1}^{\infty} \frac{n\pi}{l} N_n \cos\left(\frac{n\pi}{l} x\right) e^{-\frac{n\pi}{l} y}\right] \hat{x} + \left[\sum_{n=1}^{\infty} \frac{n\pi}{l} N_n \sin\left(\frac{n\pi}{l} x\right) e^{-\frac{n\pi}{l} y}\right] \hat{y}\end{aligned}\quad (C-8)$$

where $\hat{x}$ and $\hat{y}$ are unit vectors.

The DEP force component is

$$\nabla E_{rms}^2 = \nabla(E_x^2 + E_y^2) = 2(E_x E_{x,x} + E_y E_{y,y}) \hat{x} + 2(E_x E_{x,y} + E_y E_{y,y}) \hat{y} \quad (C-9)$$

where the derivatives are

$$\begin{aligned}
E_{x,x} = -E_{y,y} &= \sum_{n=1}^{\infty} \frac{n^2 \pi^2}{l^2} N_n \sin\left(\frac{n\pi}{l} x\right) e^{-\frac{n\pi}{l} y}, \\
E_{x,y} = E_{y,x} &= \sum_{n=1}^{\infty} \frac{n^2 \pi^2}{l^2} N_n \cos\left(\frac{n\pi}{l} x\right) e^{-\frac{n\pi}{l} y}.
\end{aligned} \tag{C-10}$$

The results comprise infinite series. In practice, finite terms from the series can be truncated to approximate the values. The separation of variables method can also be used to obtain the analytical solution for the twDEP array, when the approximate boundary conditions are used.

The above solutions have the following limitations:

(1) As the potential between neighboring electrodes at the lower interface is unpredictable, a linear potential change was assumed. This widely adopted assumption may cause errors or inaccuracy of the solutions. More rigorous boundary condition at this interface needs to be specified.

(2) The problem was solved specifically for the interdigitated electrode system. From the mathematics point of view, the Laplace's equation can be solved by the separation of variables method only when the domain is bounded by constant-coordinate curve. That is, for Cartesian coordinates x, y , the domain (problem space) is a rectangle, bounded by constant- x and constant- y lines. The electrode configuration used in this calculation meets such requirements for the boundary so that the problem can be solved, i.e. the problem is variable separable. For most electrode systems used in DEP, the separation of variables method is inapplicable for the complex geometries of the electrode systems. Analytical solutions are usually impossible for those cases.

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